SCOPOLAMINE IMPAIRS PERFORMANCE ON THE MORRIS WATER MAZE IN BOTH NAIVE AND TRAINED RATS

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Scopolamine, a muscarinic receptor antagonist has previously been shown to impair spatial learning in the rat using a conventional radial arm maze (Stevens, 1981). However it has not been tested on a novel test of spatial learning, the Morris Water Maze (Morris, 1981). In this test rats learn to locate a hidden platform (island) in a square tank (122 x 122 cm) of opaque water, using cues placed around the tank. On each trial the rat was given a maximum of 100sec to find the island. Rats which failed to find the island were placed on it for 5sec at the end of the trial. The following measures were taken using a video-digitising system, linked to an Apple computer (HVS Analysing Ltd): latency to find the island, path length, time in the island quadrant. Male Lister Hooded rats (Olac) weighing approximately 300g were used in both experiments.

In the first experiment the effects of scopolamine, 0.1, 0.5 or 1.0mg/kg i.p. 20min before the first trial on acquisition of this task were examined. Rats received 6 consecutive trials per day for 3 days with the island in the same position for each rat. Scopolamine increased the latency to find the island in a dose-dependant manner. On the third day an additional trial was given with the island removed from the tank. On this trial the time spent in the "island" quadrant was significantly decreased as shown in the Table below. In the second experiment the effects of scopolamine on the ability of trained rats to learn a new island position were examined. Rats which had previously been trained on the water maze to one island position, received 6 trials with a new island position, together with a 7th no-island trial. As in the first experiment scopolamine produced a consistent dose-related impairment of performance, as shown by increased latencies to find the island and increased path lengths. The time spent in the "island" quadrant on the 7th trial is shown in the Table below. These results demonstrate that the Morris Water Maze is a reliable test of spatial learning suitable for pharmacological manipulation.

PERCENTAGE TIME SPENT IN THE ISLAND QUADRANT ON THE NO-ISLAND TRIAL (p value calculated from oneway ANOVAR and protected t-test)

	Time spent in the "island" quadrant (sec)			
	MEAN	SEM	n	p
EXPT. 1. NAIVE RATS				
Control	37.1	2.4	11	
Scopolamine 0.1	34.6	3.3	7	not significant
0.5	27.8	1.2	7	0.004
1.0	25	2.3	7	0.002
EXPT. 2 TRAINED RATS				
Control	43.4	4.7	7	
Scopolamine 0.1	36.9	3.3	7	not significant
0.5	24.3	1.8	7	0.006
1.0	23.7	1.4	7	0.004

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42P

EXPOSURE TO THE SCENT OF AN ISOLATED CONSPECIFIC IS SUFFICIENT TO INDUCE ACUTE ANALGESIA IN MALE MICE

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In a resident-intruder paradigm, persistently attacked intruder mice display a chronic analgesia which is naloxone-sensitive and bidirectionally cross-tolerant with morphine(Rodgers & Randall, 1985a). More recently, we have observed an acute (< 10 min.) analgesia in intruder mice subjected to a defeat experience per se - a reaction that is naloxone-insensitive and which does not display cross-tolerance either to or from morphine(Rodgers & Randall, 1985b). In an initial attempt to further define the specific features of the defeat experience associated with this non-opioid analgesia, it was noted that the reaction was evident even in mice not physically attacked during the test period. The present studies are a follow-up to this preliminary finding.

8-10 week old DBA/2 male mice(Bantin & Kingman, Hull) and 9-10 week old BKW male mice(Bradford University colony) were used. The former served as naive intruders and were group housed, whilst the latter served as experienced residents and were individually-housed. All animals were maintained in a temperature-controlled room ($24\pm1^{o}C$) in which a reversed LD cycle was operative, and all testing was conducted under dim red light during the dark phase of the cycle. In both studies, baseline tail-flick latencies(TFL) were established immediately prior to behavioural testing. In Experiment 1, intruders were assigned to 3 experimental conditions(n=8) in which they were exposed to (1) defeat by an aggressive resident, (2) an unfamiliar clean mouse cage, or (3) the soiled cage of an aggressive resident. Exposure times were identical across groups since they were based upon time to defeat in group 1 (median=39 sec.). In Experiment 2, naive intruders were assigned to 3 conditions (n=7) in which they were exposed to (1) defeat(median time to defeat =50 sec.), (2) a non-aggressive resident or (3) an aggressive resident restrained behind a wire-mesh barrier. In both studies, TFLs were redetermined at 0, 10 and 20 minutes post-exposure. Control animals in each study were simply exposed to repeated tail-flick testing with identical temporal parameters to subjects in the other conditions.

Data were subjected to repeated measures analyses of variance. In Experiment 1, both the time factor($p<0.01$) and the groups x time interaction($p<0.01$) were significant. Further analysis revealed significant analgesia only in the 'defeat' and 'soiled cage' conditions, a reaction which dissipated within 10 minutes of behavioural testing. In Experiment 2, the time factor and groups x time interaction were again significant($p<0.01$) which, upon further analysis, were found to relate to significant acute(<10 min.) analgesia in the 'defeat' and 'non-aggressive resident' conditions only.

Together, these data suggest that mere exposure to the soiled home cage of an unfamiliar isolated male conspecific is sufficient to induce an acute analgesic reaction in group-housed intruder mice. As it has been reported that isolated and dominant mice are physiologically very similar(Brain, 1975), our results imply that acute analgesia may be induced by a pheromonal factor common to isolated male mice. The absence of analgesia in the 'restrained aggressive resident' condition further implies that the influence of scent can be overridden by the intruder's perception of other aspects of the stimulus situation. Finally, on a more general level, our data offer a simple, fast and reliable model with which to study further the mechanisms underlying non-opioid environmental analgesia.

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BIOCHEMICAL PROFILE OF A NEW ATYPICAL ANTIDEPRESSANT IN RODENTS, SR 95191

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SR 95191, 3-(2-morpholinoethylamino)4-cyano 6-phenyl pyridazine, is a new atypical antidepressant drug with monoamine oxidase (MAO) inhibitory and dopaminomimetic activities in rodents (Bizière et al., 1985). The effects of SR 95191 on brain monoaminergic transmissions were therefore studied. Male Sprague-Dawley rats (200-220 g, Charles River, France) were used. SR 95191 was administered orally (base) or i.p. (citrate, doses refer to the salt). Brain MAO-A and B activities were estimated using (^{14}C) serotonin (5-HT) and (^{14}C) phenethylamine (PEA) as substrates. Brain levels of 5-HT and 5-hydroxyindolacetic acid (5-HIAA), dopamine (DA), 3-4 dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-tyramine (3-MT) were measured by HPLC-ED. In addition, extraneuronal DOPAC was estimated by direct measurement in vivo, using carbon fibre microelectrodes and differential pulse voltammetry (Gonon et al., 1980). The neuronal uptake of (^{3}H) monoamines (MA) was measured on rat brain synaptonomes. Finally, the interaction of SR 95191 with the main neurotransmitter binding sites including MA, histamine, acetylcholine and GABA, was studied in vitro on rat brain membranes. Ex vivo, SR 95191 preferentially inhibited brain MAO-A (ED_{50} = 14.2 mg/kg, p.o.). This effect peaked 15 min after treatment then decreased slowly until 24 h. In these conditions, MAO-B was not inhibited by this drug (ED_{50} > 300 mg/kg, p.o.). Thirty min post-treatment, SR 95191 (10 to 300 mg/kg, p.o.) significantly decreased the striatal levels of 5-HIAA (- 22 % to - 32 %) and DOPAC (- 33 % to - 68 %). In contrast, 3-MT levels were strongly enhanced (+ 57 % to + 107 %). The striatal concentrations of 5-HT and DA were slightly enchanced (+ 12 % and + 26 % respectively) at the highest dose tested only (300 mg/kg, p.o.). The direct continuous in vivo monitoring of striatal DOPAC indicated a 60 % decrease of this acidic metabolite, 30 min after administration of SR 95191 (10 mg/kg, i.p.). Within at least 6 h, SR 95191 (150 mg/kg, p.o.) significantly enhanced the levels of 5-HT (+ 48 % to + 80 %) and DA (+ 28 % to + 49 %) in the whole brain. These effects were associated with a concomittant decrease of 5-HIAA (- 18 % to - 29 %) and DOPAC (- 57 % to - 75 %). In these conditions, the time-course of changes in 5-HIAA and DOPAC closely ressembled that of MAO-A activity. In vitro (up to 100 µM) and ex vivo (up to 100 mg/kg, i.p.), SR 95191 (citrate) did not modify the neuronal uptake of MA. Finally, in vitro, SR 95191 (citrate or diHCl, 100 µM) displayed no affinity for the following neurotransmitter receptor sites of the rat brain (^{3}H ligands and particular brain areas used are quoted in parenthesis) : noradrenergic α_1 (WB 4101), α_2 (clonidine) and β(dihydroalprenolol) ; serotonergic 5-HT_1 (5-HT, hippocampus) and 5-HT_2 (spiperone, frontal cortex) ; dopaminergic D_1 (flupenthixol, striatum) and D_2 (spiperone, striatum), histaminergic H_1 (pyrilamine) ; cholinergic muscarinic (QNB, cerebellum) and GABAergic (muscimol). These results clearly indicate that SR 95191 behaves, at least in part, as a moderate, selective and short acting type A MAO inhibitor. Moreover, the dopaminomimetic properties of SR 95191 cannot be related to any affinity for DA receptors but rather to (a) not yet explored mechanism (s).

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IRON-DEFICIENCY INDUCES INCREASED BRAIN MET-ENKEPHALIN AND PAIN THRESHOLD RESPONSE TO OPIATE PEPTIDES

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Distribution of iron in rat and human brain shows stark similarity to neuropeptidergic and dopaminergic loci. Exceptionally high iron concentrations are found in globus pallidus, substantia nigra, caudate nucleus, red nucleus, thalamus and n-accumbens (Youdim, 1985). Rats made nutritionally iron-deficient (ID) have significantly lower brain iron (40-60%) and dopamine D_2 receptor number (Bmax) (50%) (Ashkenazi et al., 1982; Ben-Shachar et al., 1985). The consequence of ID is modified dopaminergic neurotransmission; diminished behavioural response to apomorphine (Ashkenazi et al., 1982), elevated serum prolactin (Barkey et al., 1985), and increased opiate responses to β-endorphin and morphine which could be blocked by naloxone (Yehuda and Youdim, 1984).

The possibility that decreased brain iron, due to ID, may result in the reduction of opiate neuropeptide metabolism has been investigated by measuring the brain concentration of dynorphin B and met-enkephalin. Two weeks after the initiation of nutritional ID in rats (Hb, 8.8±1.2 g/l), dynorphin B and met-enkephalin levels in globus pallidus, caudate nucleus, n. accumbens, habenula, substantia nigra, ventral tegmental area and central grey were unaltered. However, by the end of the 5th week on ID diet (Hb, 5.5±0.5 g/l) significant increases of 50-60% and 80-105% in dynorphin B and met-enkephalin respectively were observed in globus pallidus, caudate nucleus and n. accumbens. Iron-deficient rats have significant elevation of pain threshold response on hot plate test (48°C) confirming our previous studies (Yehuda and Youdim, 1984). In normal circumstances peripheral administration of leu- or met-enkephalin (0.1-3.0 mg/kg) to control rats does not alter the pain threshold. However, ID (30 days) rats show a dose dependent elevation of pain threshold to leu- and met-enkephalins, which could be blocked by naloxone (1 mg/kg)

The mechanism by which ID induces increased brain dynorphin B and met-enkephalin with a consequence of elevated pain threshold responses to opiate peptides is not fully understood. It may be presumed that iron can act as a co-factor for the metal-dependent neuropeptidases which metabolise the opiate peptides and that the activity of these enzymes are reduced in ID. Furthermore, dopamine neurone may exert a tonic inhibition of the firing of some or all opiate neurons (Yehuda and Youdim, 1984). Thus removal of this inhibition as is the case in ID rats may also lead to increased opiate synthesis and less degradation (Tang et al., 1983). Finally it is possible that blood brain barrier is somewhat modified due to iron-deficiency, thus allowing increased uptake of exogenously given opiates by the brain.

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THE EFFECT OF A SINGLE DOSE OF FG-7142 ON MONOAMINE CONCENTRATIONS IN MOUSE CEREBRAL CORTEX

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In previous communications to the Society we reported that both acute and chronic administration of the β-carboline FG-7142 (a benzodiazepine receptor contragonist) produced changes in noradrenergic receptor density in mouse brain (Little et al, 1985a and b). In order to further elucidate these findings we have investigated the effects of a single dose of FG-7142 on brain concentrations of noradrenaline (NA). In addition, for comparison, its effects on dopamine (DA) and 5-HT concentrations were also assessed.

Male CD-1 mice, 30-35 g (Charles River) were given either FG-7142 (40 mg kg^{-1}) or vehicle (Tween-80 1 drop per 10 ml distilled water). Injection volume was 10 ml kg^{-1}. Mice were killed 15 min, 24 hours or 7 days after injection and brains were rapidly removed onto ice. Cortices were dissected and homogenized in 10 vol 0.1 M perchloric acid and 400 μM sodium metabisulphite and spun at 1000 g for 10 min. Monoamine concentrations of the supernatants were assayed by HPLC with electrochemical detection (Davies & Molyneux, 1982; Molyneux & Clarke, 1985).

At 15 min and 24 h after injection there were no significant differences between vehicle and FG-7142 groups for any of the monoamines measured. 5-HT concentrations were unchanged at 7 days also. In contrast concentrations of NA and DA were significantly reduced in the FG-7142 treated animals at this time.

Table 1

		Time after injection		
		15 min	24 h	7 d
NA	Tween	188± 17	182± 15	208± 8
	FG	191± 15	147± 10	152± 3*
DA	Tween	1296±141	1184±200	1619±190
	FG	1233±125	892±161	1175±115**
5-HT	Tween	115± 19	117± 20	146± 21
	FG	100± 12	135± 27	150± 15

Brain concentrations ng g^{-1} (wet wt). Mean ± SEM. n = 7-8. *$p < 0.001$. **$p < 0.05$. Mann Whitney U-test.

If reduced concentrations of neurotransmitters are accompanied by decreased synaptic release then these results are consistent with our previous findings that cortical adrenoceptors are increased 7 days (Little et al, 1985b) but not 24 h after FG-7142. The mechanism by which FG-7142 reduces NA and DA concentrations is presently being investigated including measurement of metabolite concentrations.

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METABOLISM OF LEUKOTRIENE B_4, C_4 AND D_4 IN HUMAN WHOLE BLOOD AND PLASMA

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The leukotrienes (LTs) have potent effects on airways, a negative inotropic effect on the heart, alter the calibre of blood vessels and increase vascular permeability. The cysteinyl containing LTs and the dihydroxy acid LTB_4 can be released from vascular tissue (Piper et al, 1983) and have been detected in the circulation of asthmatics during a severe attack (Zakrzewski et al, 1985). The purpose of this study was to investigate the metabolism of LTs in whole blood and plasma.

Heparinized blood samples (10 ml), obtained from the brachial vein of human volunteers were added to nine centrifuge tubes each containing either LTB_4 - (10^{-8}M or 10^{-7}M), LTC_4 (10^{-8}M) or LTD_4 (10^{-8}M) and incubated in a shaking water bath at 37 °C for different time periods (T_0 - T_{120} min). The reaction was stopped at specific intervals by immersion in ice. Furthermore the conversion of LTC_4 to LTD_4 by γ-glutamyl transferase (γGT) and any protease attack was inhibited by the addition of L-serine Na_2tetraborate (30 mM) and phenyl methyl sulphonyl fluoride (0.1 mM) respectively. Blood samples were then centrifuged (12,000 g for 7.5 min at 4 °C) to obtain plasma. The plasma was extracted in 80% ethanol and centrifuged (12,000 g for 20 min at 4 °C) to remove insoluble material. The supernatant was evaporated to dryness under vacuum, partially purified using Sep-Pak cartridges and millipore filters (0.45 μm) and the LT activity measured by radioimmunoassay using a double antibody technique (Hayes et al, 1983). LT metabolism was also studied in plasma, Hartmanns solution, and incubations performed in the absence of exogenously added material.

Incubation of LTB_4 (10^{-7}M or 10^{-8}M) in whole blood or plasma produced little change in LTB_4 immunoreactivity (IR). In contrast, there is an exponential decline in LTC_4-IR and LTD_4-IR over the reaction period, $T_{\frac{1}{2}}$ was 15 and 16 minutes respectively. Incubation of LTC_4 (10^{-8}M) in plasma also results in a similar decline in LTC_4-IR ($T_{\frac{1}{2}}$ = 19.5 min). There is no basal release of LT-like material and furthermore LT-IR is unaltered during incubations with Hartmanns control. Incubation of LTC_4 (10^{-8}M) in plasma samples containing the γGT inhibitor prevents the fall in LTC_4-IR over the reaction period and suggests an involvement of the glutathione detoxification pathway. Recovery of substrate from whole blood or plasma was - LTB_4 : 23-72%; LTC_4 : 37-92%; LTD_4 48-58% and from Hartmanns control - LTB_4 : 46-90%; LTC_4 : 68-95%, LTD_4 : 48-98%. Our results indicate that in whole blood or plasma, LTB_4 is more resistant to metabolism than LTC_4 or LTD_4. This may be important when considering the actions and interactions of LTs within the circulation and its possible role in regulating these biologically active substances.

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CHARACTERISTICS OF THE UPTAKE OF IRON, COMPLEXED WITH THE PYRONE MALTOL, INTO SLICES OF RAT SMALL INTESTINE

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The absorption of iron from some pyrone and pyridone complexes after intraduodenal administration in rats has been shown to be higher than iron absorption with sulphate, gluconate, fumarate or EDTA complexes (Callingham et al, 1984), the subsequent distribution of the iron within the body organs being qualitatively similar. An in vitro system has now been used to study the mechanisms responsible for iron transfer into the gut wall from a complex with 3-hydroxy-2-methyl-4-pyrone (maltol) and the point at which the dissociation of metal from the complex takes place.

Slices (15-40mg) of rat small intestine were immersed at 37°C in oxygenated 16mM HEPES buffer, pH 7.3 with 10mM glucose, 125mM NaCl, 3.5mM KCl, 1mM $CaCl_2$ and 10mM $MgSO_4$. Oxygen consumption, [^{14}C]glucose uptake, Na content and the ultrastructural appearance of the isolated slices showed that the viability of the slices did not change significantly over the course of 30 min. Initial rates of uptake of ^{59}Fe or [^{3}H]maltol present as the metal:ligand complex (1:4) were measured by dual label counting together with the extracellular fluid markers, [^{3}H]inulin or [^{14}C]mannitol, and compared with uptake of ^{59}Fe from the ferric chelate (1:5) of nitrilotriacetic acid (NTA), a ligand which maintains iron in soluble form but is itself excluded from the intracellular compartment.

Uptake of ^{59}Fe at 0.02 and 0.1mM was much greater from the maltol complex (tissue:medium ratios after 10min at 0.02mM being 1-3 with maltol v. 0.2-0.8 with NTA). With both ligands Q_{10} values for iron uptake were greater than 2. Although not proven that the iron is taken up rather than bound to the tissue surface, such temperature sensitivity would suggest more than just a physical effect. After preloading tissues with Fe:maltol at 0.1 or 1mM, no significant efflux of ^{59}Fe from the tissues was seen within the 30min incubation times. ^{59}Fe uptake from both ligands was saturable with kinetic constants for NTA (Km 149±38μM, Vmax 9.5±2.5pmol.min^{-1}mg^{-1}wet weight) comparable with those found in mouse and human duodenal tissue (Cox & Peters, 1978; Raja et al, 1985). Maltol values were Km 50±10μM and Vmax 13.5±2.2pmol.min^{-1}mg^{-1}wet weight. Neither 1mM KCN, 0.1mM dinitrophenol, 5mM NaF nor the substitution of nitrogen for oxygen inhibited iron uptake with either ligand. Initial uptake of ^{3}H was much greater when given as 0.3mM [^{3}H]maltol than as 0.1mM Fe[^{3}H]maltol (1:3), but in both forms Q_{10} values were 2 or above. Little efflux of ^{3}H from tissues preloaded with 0.1mM Fe:[^{3}H]maltol was seen whereas at 1mM a significant loss was evident. The chemical nature of this [^{3}H]-material has not been identified but recent data suggests it may not be maltol but a phenolic conjugate.

The transfer of iron via the pyrone maltol may thus not be a passive diffusion of the metal-ligand complex but probably involves mechanisms which though not energy-requiring are saturable and temperature sensitive with dissociation occuring at or within the intestinal tissues.

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CARTILAGE BREAKDOWN IN VIVO - A NEW MODEL

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Studies into the mechanisms of breakdown of cartilage implants in rodent subcutaneous (sc) tissues particularly in sc air pouches that bear cavity linings resembling synovial tissue have shown matrix (proteoglycan) loss to be neither influenced by inflammation of the cavity nor by implantation in non-inflated sc tissues (Sin et al, 1984; Sedgwick et al, 1985). We have therefore looked into varying the presentation of inflammation and soft tissue to cartilage and in the present study have investigated the effect of implanting cartilage wrapped with cotton; the later induces a granulomatous inflammation concomitant with the growth of granulation tissue (De Brito and Hanahoe, 1983).

Freshly collected intact Wistar (random bred, 125-175g) rat femoral head cartilage and discs of bovine nasal cartilage with or without cotton were implanted sc in the flanks of ether-anaesthetised rats and mice. After certain time intervals the implants were removed and cartilages assayed for proteoglycan (Farndale et al, 1982).

TABLE 1

Cartilage	Recipient & cotton weight (mg)		Cartilage glycosaminoglycan (μg) 7	14	21	28 days
Rat femoral head	Rat	0	473 ± 25	348 ± 40	160 ± 11	-
		2	354 ± 28(25)*	195 ± 30(44)*	119 ± 14(20)*	-
		5	341 ± 34(28)*	285 ± 35(18)	-	-
	2 + Antigen		-	122 ± 12(65)**+	-	-
	Mouse	0	488 ± 31	398 ± 41	-	-
		2	376 ± 36(23)*	230 ± 44(52)*	-	-
		5	303 ± 52(38)*	163 ± 39(69)**	-	-
Bovine nasal septum	Rat	0	2300 ± 150	2000 ± 125	1990 ± 120	1850 ± 130
		2	2310 ± 125(0)	1960 ± 205(2)	1610 ± 105(19)	1220 ± 225(34)*
	Mouse	0	2410 ± 175	2350 ± 195	2120 ± 165	1960 ± 185
		5	2395 ± 175(0)	2060 ± 160(12)	1465 ± 210(31)*	825 ± 230(58)**

Results are Mean ± SEM for 6-8 animals; * $p<0.05$, ** $p<0.01$ in comparison with cotton free implants, + $p<0.05$ in comparison with 2 mg result, Student's 't' test. () % loss caused by cotton.

Regarding proteoglycan loss the following observations were made (see table). In rats and mice cartilage proteoglycan loss was significantly greater from implants with cotton than from implants without. With bovine cartilage this loss was delayed. In mice loss appeared to be cotton-weight related. Pre-soaking of cotton-cartilage implants with antigen (ovalbumin 0.05%) accelerated the induced breakdown even futher in rats sensitised (De Brito & Hanahoe, 1983) previously to the antigen

The results show that a cotton induced granulomatous response can cause cartilage to breakdown, this effect is neither species (recipient) nor cartilage (implant) specific and it can be increased by an immunological stimulus.

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EFFECT OF ACUTE RENAL FAILURE ON THE PHARMACOKINETICS OF DIBROMOSULPHOPHTHALEIN AND ITS BINDING TO Y AND Z PROTEINS IN RAT LIVER

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Previous work has shown that the hepatic uptake and biliary excretion of organic anions such as BSP and ICG are decreased in rats with acute renal failure (ARF) (Bowmer et al., 1982; Bowmer & Yates, 1984). Both these substances selectively bind to two groups of hepatic cytosol proteins, Y and Z, which may be involved in the transfer of organic anions from plasma to liver (Levi et al., 1969). Consequently reduced binding to these proteins may explain the altered hepatic handling of anions such as BSP and ICG in ARF. This possibility has been investigated using the non-metabolised anion dibromosulphophthalein (DBSP).

ARF was induced in male Wistar rats (Bowmer et al., 1982) and 48h later the plasma disappearance and biliary excretion of DBSP (25 mg kg^{-1}; i.v.) were determined. Y and Z proteins in hepatic cytosol were separated by gel filtration using a Sephadex G-75 column and 0.05 M sodium phosphate buffer, pH 7.4, as the mobile phase (Levi et al., 1969). Amounts of DBSP in peak fractions were calculated by triangulation and binding was expressed as nmol bound mg^{-1} of cytosol protein.

The plasma disappearance of DBSP was biexponential in both groups of rats. Table 1 shows that the rate constants for transport from plasma to liver (k_{12}), liver to plasma (k_{21}) and liver to bile (k_{el}) were all significantly decreased in uraemic rats. In addition, the initial biliary excretion rate, during the first 10 min after dosing, was also reduced in the uraemic group (Table 1). The amount of DBSP bound to the Y fraction from uraemics (0.71±0.06 nmol mg^{-1}; N=8) was less ($p<0.01$) than that from controls (1.15±0.10 nmol mg^{-1}; N=8), however no difference between control (1.07±0.12 nmol mg^{-1}; N=8) and uraemic rats (1.00±0.07 nmol mg^{-1}; N=8) was found in the quantity of dye bound by the Z fraction.

Table 1. Kinetics of DBSP in control and uraemic rats

Parameter	Control (N=8)	Uraemic (N=8)	
k_{12} (min^{-1})	0.648±0.025	0.516±0.029	<0.01
k_{21} (min^{-1})	0.084±0.008	0.055±0.005	<0.05
k_{el} (min^{-1})	0.112±0.007	0.087±0.005	<0.01
Biliary excretion rate 0-10 min ($\mu g\ min^{-1}\ kg^{-1}$)	523±31	329±19	<0.001

All results are mean ± s.e.m.

The hepatic uptake and initial biliary excretion of DBSP were decreased in rats with ARF in a manner similar to that reported for BSP and ICG (Bowmer et al., 1982; Bowmer & Yates, 1984). Although the quantity of DBSP bound to Z protein was not reduced in uraemic rats, binding to Y protein was decreased by about 38%. Of these two proteins, Y protein may have a more important role in hepatic uptake (Theilman et al., 1984) so diminished binding to this particular protein may contribute to altered hepatic uptake of DBSP in ARF.

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50P

REDUCED LOOP-DIURETIC-SENSITIVE 86Rb INFLUX IN RED BLOOD CELLS OF CYSTIC FIBROSIS PATIENTS

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Cystic fibrosis (CF) is the most common lethal genetic disorder of caucasian children having an autosomal recessive mode of inheritance. Clinically, it is characterized by chronic pulmonary disease and pancreatic insufficiency. Although the basic defect has yet to be established, consistent biochemical abnormalities are raised concentrations of sodium and chloride in sweat of these patients. This suggests that an ion transport defect may be present in cells of patients with CF. Recent electrophysiological studies indicate that there may be an abnormality in chloride transport in epithelial tissue from CF patients (Bijman & Quinton, 1983; Knowles et al., 1983; Davis et al., 1985). Loop-diuretic drugs, such as bumetanide, have been used to investigate chloride dependent sodium and potassium co-transport systems in a number of cell systems (Murphy & Ryan, 1983). In human red blood cells, bumetanide (10^{-4} M) was shown to inhibit the chloride-dependent component of potassium transport (Ellory & Stewart, 1982).

We have investigated ^{86}Rb (used as an analogue of K) influx in red blood cells of 16 CF patients and in 16 age and sex matched control subjects. Red blood cells obtained by centrifugation were washed 3 times in medium before incubation. Plasma from CF patients and control subjects was retained and added to incubation media in some experiments in order to investigate the possibility of an inhibitory factor in plasma from CF patients. ^{86}Rb influx experiments were carried out for 30 mins in different media. Total influx was obtained in drug-free medium, while ouabain (10^{-4} M) was used to inhibit the Na-K-ATP'ase component and bumetanide (10^{-4} M) was used to inhibit the loop-diuretic-sensitive component. Haemoglobin concentrations were determined and used to estimate the number of red blood cells per incubation tube. Results were expressed as mmol K l. cell^{-1} h^{-1}.

Total and ouabain-sensitive fluxes were not significantly different in CF red blood cells compared to those from control subjects during incubations without plasma or with plasma from either CF patients or control subjects. However the loop-diuretic-sensitive ^{86}Rb influx was significantly ($p<0.05$) reduced in CF red blood cells (0.51±0.08 mean ± s.e. mean; N=16) compared to control red blood cells (0.86±0.10, N=16) during incubations in homologous plasma. Similar reductions in loop-diuretic-sensitive transport of CF red blood cells were detected during incubations without plasma or with plasma from control subjects.

These findings indicate a defect in the loop-diuretic-sensitive component of ion transport in red blood cells from CF patients. Other studies indicate that loop-diuretic-sensitive transport may be due to a sodium-potassium-chloride co-transport system (Ellory & Stewart, 1982). In our ^{86}Rb influx studies we did not find any evidence for an inhibitory factor in plasma from CF patients. Our findings may be relevant to a recent report of abnormal chloride ion permeability in cultured epithelial cells from CF patients (Yankaskas, J. et al., 1985).

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CHARACTERIZATION OF SENDAI VIRUS PHOSPHOLIPIDS AND FATTY ACIDS - THEIR ROLE IN MEMBRANE FUSION

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The molecular mechanism by which enveloped viruses such as Sendai virus enter host cells is poorly understood. Sendai virus haemagglutinin-neuraminidase (HN) protein is believed to provide the initial attachment to neuraminic acid containing receptors of cellular membranes whereas the fusion (F) protein causes fusion with the target membrane (Scheid et al. 1972). The interaction of the viral envelope with certain glycolipids and phospholipids have been implicated in Sendai virus induced fusion (Huang, 1983). Since protein-lipid interaction is involved in Sendai virus induction of haemagglutination and haemolysis which is believed to be concomittant with fusion, a detailed analysis of Sendai virus phospholipids and their lipid composition of Sendai virus, propagated in chicken eggs, was analyzed by high performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas liquid chromatography (GLC).

Table 1 Phospholipid Composition of Sendai Virus

Phospholipid	Whole [a] Sendai Virus % of Total	Blough & Lawson [b] Whole Virus % of Total
Cardiolipin (CL)	8.9±0.53	-
Phosphatidylinositol (PI)	5.5±0.48	6.4±0.4
Phosphatidylserine (PS)	12.0±0.84	15.0±1.0
Phosphatidylethanolamine (PE)	26.8±1.39	37.0±0.5
Lysophosphatidylethanolamine (LPE)	1.6±0.46	2.0±0.0
Phosphatidylcholine (PC)	37.3±2.80	8.0±0.8
Sphingomyelin (SPH)	8.8±1.10	11.8±0.8
Cholesterol (Chol)	6.7	7.2

a - this work, values represent the mean of 6 analyses of Sendai virus
b - Blough and Lawson 1968.

Analysis of fatty acid methyl esters of the total phospholipids revealed that C18:0 (15.4%) and C18:1 (22.0%) represented the dominant fatty acids. C18:0 also represents 23.5% of the fatty acids in PS; 20.2% in PE; 14.5% in PI; 23.5% in PC; 12.1% in SPH and 10.1% in CL. Notable amounts of 17:1, 18:0, 20:0 and 20:2 hydroxy fatty acids were also observed which were not previously reported. Total saturated and unsaturated fatty acids and the ratio of W6/W3 fatty acids of the whole virus were similar to that of the HN and F proteins. This data constitute the first detailed analysis of the constituent fatty acids of Sendai virus phospholipids. No specific functional role can be ascribed to the observed hydroxy fatty acids but empirical evidence would suggest that they could enhance viral membrane fluidity and fusogenic activity.

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HYPOGLYCAEMIA AND STRESS-INDUCED HYPERINSULINAEMIA IN B.PERTUSSIS INFECTED MICE

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Hypoglycaemia may contribute to the neurological sequelae of B. pertussis infection (Pittman, 1984). We have shown pertussis infected or vaccinated mice to show hypoglycaemia (Furman et al 1981) this being related to hyperinsulinaemia (Furman et al 1985). Our previous work showed hyperinsulinaemia to be most marked when pertussis-infected mice were anaesthetised for blood sampling, a stimulus that produced no change in the plasma insulin in control mice. We have now extended this work to determine if this abnormal insulin secretory response occurs in response to a variety of stressors (anoxia, exposure to CO_2, injection of histamine). All experiments were carried out on HAM-1/CR mice exposed to the stressor (or appropriate control) and bled by decapitation after cervical dislocation. Infected mice received 1 x 10^5 B. pertussis organisms intranasally 14 days before the experiment, control mice receiving the vehicle. Infected animals showed consistent hypoglycaemia relative to controls (e.g. serum glucose, mmol l^{-1}; Infected, 6.3 ± 0.6, control 9.2 ± 0.3) but no significant hyperinsulinaemia (serum insulin, ng ml^{-1}; infected, 1.3 (95% CL 1.1, 1.46) control 0.8 (0.76, 1.1). However, brief (60 sec) exposure to ether vapour resulted in marked hyperinsulinaemia in infected mice but not in controls, thus confirming our previous findings (Furman et al 1981). A very similar response to ether was seen in normal mice pretreated with pertussis toxin (150 ng/mouse 5 days previously). Infected or control mice were exposed to 100% N_2 (15 sec), 100% CO_2 (10 sec) or air (15 sec as control) and then bled after various recovery periods. A brief period of anoxia, whilst not affecting IRI in controls, produced a marked, transient hyperinsulinaemia in infected mice. The highest values (Table 1) were obtained at 60 sec. A similar result was obtained when CO_2 was the stimulus although CO_2 itself caused slight hyperinsulinaemia in controls. (IRI = immunoreactive insulin).

Table 1. Serum IRI (ng ml^{-1}) 60 sec after exposure to gas

	Air	100% N_2	CO_2
Control	0.82 (0.74,0.91)	1.8 (1.4,2.3)	3.5 (2.3,5.4)
Infected	1.31 (1.13,1.52)	21.7 (8.8,53.6)	47.2 (15.9,140.1)

Histamine toxicity in mice is markedly increased by pertussis vaccine, infection or toxin (Pittman et al 1980, Pittman 1984). In control mice histamine (36 mg kg^{-1} I.P.) produced hyperglycaemia without any change in the serum insulin concentration (serum glucose at 15 min; vehicle, 10.8 ± 0.3; histamine, 23.3 ± 0.8 mmol l^{-1}). Infected animals showed no hyperglycaemia in response to histamine and a markedly elevated serum insulin concentration.(5.8 ng ml^{-1} $P < 0.01$).

Thus, various stressors produce hyperinsulinaemia in pertussis-infected or pertussis toxin treated mice. Stress-induced hyperinsulinaemia may be mediated by the known ability of pertussis infection or toxin to convert the normal inhibitory action of adrenaline on insulin secretion to a stimulatory one.

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EVALUATION OF LIPOXYGENASE INHIBITORS *EX VIVO*

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A major problem in studying lipoxygenase inhibitors is the lack of *in vivo* biological models which are leukotriene-dependent. Eicosanoid generation in whole blood has been a useful system for evaluating thromboxane synthase inhibitors and similar systems can be used to profile the inhibitory effects of drugs against 5-lipoxygenase and cyclo-oxygenase *in vitro* (Forder and Carey, 1984; McMillan, Millest, Spruce and Taylor, 1985). We now report the use of *ex vivo* eicosanoid generation in whole blood for evaluating lipoxygenase inhibitors.

Heparinised rat blood was challenged with A23187 (0.1 – 20μg/ml) at 37°C and release of LTB_4 and PGE_2 were quantified using specific radioimmunoassays (Carey and Forder, 1985). For *ex vivo* studies, drugs were prepared as suspensions in carboxymethylcellulose and administered to animals at various times prior to blood collection. For *in vitro* studies, drugs were preincubated with blood for 15 mins. prior to addition of A23187. Incubation of heparinised rat blood with A23187 induced dose-dependent and time-dependent release of immuno-reactive LTB_4 (i-LTB_4), the identity of which was confirmed by co-elution with authentic LTB_4 on reversed phase HPLC. Maximal levels of i-LTB_4, which ranged from 20 to 50ng/ml in different experiments, were generated within 15 minutes and no evidence for subsequent LTB_4 metabolism was evident in this system. Formation of i-PGE_2 followed a similar time course.

Oral administration of BW755C or phenidone to rats 1 hour prior to blood collection produced dose-dependent inhibition of *ex vivo* i-LTB_4 generation. Phenidone was consistently more potent than BW755C (ED_{50} values of 5mg/kg and 30mg/kg respectively). Significant inhibition of i-LTB_4 biosynthesis by either compound was observed within 30 mins. and lasted for up to 6 hours after oral administration. A number of other lipoxygenase inhibitors including quercetin, baicalein, nafazatrom and nordihydro-guaiaretic acid, which are more potent inhibitors of soluble 5-lipoxygenase activity than BW755C, were not orally effective as inhibitors of i-LTB_4 biosynthesis in this system at doses up to 100mg/kg. In contrast all 4 compounds inhibited A23187-induced i-LTB_4 generation from rat blood *in vitro*. All non steroidal anti-inflammatory drugs tested in this system have profiled as selective cyclo-oxygenase inhibitors: for example flurbiprofen exhibited an ED_{50} value against *ex vivo* i-PGE_2 biosynthesis of 0.3mg/kg p.o. but did not significantly inhibit i-LTB_4 biosynthesis at 10mg/kg p.o. Benoxaprofen profiled as a weak cyclo-oxygenase inhibitor (ED_{50} against i-PGE_2 formation = 50mg/kg p.o.) but did not significantly inhibit i-LTB_4 formation at 100mg/kg p.o.

We conclude that *ex vivo* eicosanoid generation provides a useful approach for measuring bioavailability of 5-lipoxygenase and cyclo-oxygenase inhibitors.

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THE VASODILATOR EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN HUMAN SKIN

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Human calcitonin gene-related peptide (CGRP) is a potent vasodilator in rabbit skin and causes a long lasting erythema when injected into human skin (Brain et al, 1985). We have studied the effect of human synthetic CGRP on local blood flow in human skin and compared the response of skin to CGRP with that of other vasodilators. The subjects gave informed consent and ethical approval was obtained.

Human synthetic CGRP (Sandoz) was further purified by HPLC, diluted in 0.9% pyrogen free sterile saline (saline) and injected intradermally (10pmol/50µl) into a predetermined site on the volar surface of the forearm. A second site received 50µl saline. Local blood flow was assessed at each site using a Perimed II laser doppler flow meter (Holloway and Watkins, 1977) and expressed as % increased blood cell flux (number of red blood cells moving in the path of the laser beam, 1-2cu.mm., x mean cell velocity). Readings, taken for two minutes at each site just before and 10 min, 1h, 2h, 3h, and 4h after intradermal injections, were expressed as mean ± s.e.mean of results from four individuals. An uninjected site maintained a stable blood flow throughout (with no greater change than 2.9± 2.0% flux). The saline-injected site exhibited a raised blood flow 10 min after injection (30.2±8.8%flux), which had returned to basal values by 1h (12.0±2.4% flux). At 1h clearly defined local reddening (141.0±20.3sq.mm. area, mean±s.e. mean,n=4) was observed at CGRP-injected sites and blood flow was high (77.7±6.2% flux). Blood flow remained high until 2h after injection (71.0±10.0% flux) and then decreased with time (3h, 53.0±5.7% flux; 4h, 29.8±7.3%flux). This correlated with a gradual return to normal skin colour of the erythematous area.

The visual response to CGRP (10pmol) was compared with that to 5 other vasodilators: vasoactive intestinal polypeptide,VIP (10pmol), substance P (10pmol), PGE_2 (10pmol), PGI_2 (10pmol) and histamine (500pmol). Histamine, substance P, VIP and (in one individual) PGE_2, induced the classical triple response of wheal, flare and local reddening. CGRP and the prostaglandins induced local reddening which was rapid in onset (10 min: CGRP 119.9±30.7sq.mm; PGI_2 72.9±9.8sq.mm; PGE_2 147.5±28.6sq.mm; results expressed as area of erythema, mean s.e.mean, n=4). However, whilst the CGRP-induced local reddening became clearly defined and spread (1h: 149.8±18.0sq.mm; 2h: 160.5±13.0sq.mm), that induced by the prostaglandins faded and had disappeared by 1h (PGI_2) or 3h (PGE_2). VIP, like CGRP, induced a persistent local reddening response that consisted of a small area of erythema which became evident as the wheal and flare subsided. However, the CGRP-induced local reddening was greater in area than that induced by VIP and longer lasting (4h: CGRP 82.0±7.6sq.mm; VIP 5.7±3.5sq.mm).

These results show that CGRP has potent and persistent vasodilator effects in human skin, when compared with other vasodilators. If CGRP is released from nerve endings in vivo, it could have a role in the regulation of vascular tone and blood flow in physiological as well as pathological conditions.

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CARDIOVASCULAR EFFECTS OF HUMAN AND RAT α-CALCITONIN GENE RELATED PEPTIDES IN THE ANAESTHETISED DOG

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Studies of the calcitonin gene led to the discovery of rat and human calcitonin gene related peptides (CGRP) (Rosenfeld et al, 1983; Edbrooke et al, 1985) which can lower blood pressure and increase heart rate in the rat (Fisher et al, 1983; Marshall et al, unpublished observations). In the present experiments the cardiovascular effects of CGRP have been studied in anaesthetised dogs.

Male beagles (12.7 - 17.2 kg), anaesthetised with pentobarbitone sodium (30 mg/kg and 6 mg/kg per hr i.v.) and artificially respired with room air, were prepared for the recording of blood pressure, heart rate, left ventricular pressure, cardiac output and femoral, mesenteric and renal blood flows as previously described (Paciorek & Shepperson, 1985). Bolus injections of peptide (via the femoral vein) were administered in ascending doses at 20 min intervals (3×10^{-11}, 10^{-10} and 3×10^{-10} mol/kg) or 60 min intervals (10^{-9} and 3×10^{-9} mol/kg) to groups of 3 dogs. In control experiments (n = 3) saline vehicle (0.5 ml i.v.) was injected at corresponding times.

Saline administration had no significant effect on blood pressure, heart rate, total peripheral resistance or blood flows. Human and rat CGRP evoked dose dependent falls in mean blood pressure with a maximum after 1½ min (e.g. from 128 ± 5, mean ± s.e. mean, to 74 ± 7 mm Hg and from 150 ± 13 to 85 ± 3 mm Hg for human and rat CGRP 3×10^{-9} mol/kg respectively). At this highest dose a significant decrease in blood pressure was maintained for 60 min and 40 min for human and rat CGRP respectively. Neither peptide had any significant effect on heart rate. The peptides produced falls in toal peripheral resistance, maximal 1½ min after administration (e.g. 49 ± 6% and 53 ± 2% for human and rat CGRP 3×10^{-9} mol/kg respectively) which remained significantly reduced for at least 10 min after 3×10^{-9} mol/kg. The two peptides differed quantitatively in their effects on the heart. The index of cardiac contractility, dp/dt max, was increased over saline controls by rat CGRP in a dose related manner at 1½ min after administration over the dose range 10^{-10} -10^{-9} mol/kg. The maximum effect of the peptide was after 3×10^{-10} mol/kg (29 ± 8%) whereas human CGRP evoked a maximum effect of only 6 ± 2% at this dose. Rat CGRP evoked significant increases in cardiac output compared with saline controls at 3×10^{-10} and 10^{-9} mol/kg of 40 ± 5% and 30 ± 4% respectively 1½ min after administration. Following the highest dose of rat CGRP, 3×10^{-9} mol/kg neither dp/dt max or cardiac output were significantly increased above pre-dose values. Human CGRP did not significantly increase cardiac output. Compared with saline controls rat CGRP increased mesenteric flow at 1½ min after the administration of 10^{-9} mol/kg while human CGRP increased renal flow 1½ min after 3×10^{-10} mol/kg and reduced it between 10 and 40 min after the highest dose of peptide (3×10^{-9} mol/kg).

In conclusion, in the anaesthetised dog, rat CGRP, but not human CGRP, evoked a transient increase in dp/dt max and cardiac output. However, the most marked effect of both peptides in these experiments was a sustained fall in blood pressure unaccompanied by an increase in heart rate.

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HUMAN CALCITONIN GENE-RELATED PEPTIDE IS A POTENT VASODILATOR IN THE PIG CORONARY CIRCULATION

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We have previously reported that calcitonin gene-related peptide (CGRP) is a potent vasodilator in four species, including man (Brain et al, 1985) and has potent positive chronotropic and inotropic actions in rat (Tippins et al, 1984). Preliminary results of our investigations in the pig have been reported elsewhere (Greenwald et al, 1985). We now report further details of the vasodilator actions of CGRP in the pig coronary circulation.

Human α-CGRP, a gift of Dr. J. Pless, Sandoz, Basel, was purified by high pressure liquid chromatography and the identity of the peptide confirmed by fast atom bombardment mass spectrometry. Blood flow was measured in the left descending coronary artery of the anaesthetised pig (the animal was sedated with 2mgkg^{-1} diazepam; anaesthesia was induced with 10mgkg^{-1} ketamine and 6mgkg^{-1} sodium pentobarbitone and maintained with 15% nitrous oxide and 1% halothane in the respired oxygen) at various fixed pressures, as previously described (Greenwald et al, 1985). In addition, left ventricular pressure (LVP), left ventricular dP/dt (LVdP/dt), systemic arterial pressure (SAP), ECG and heart rate were also monitored. CGRP was administered close-arterially to the coronary artery as 10 minute infusions (0.1ml min^{-1}) over the range 10-600 pmol min^{-1}. Before and during each infusion, a pressure/flow relationship was determined at five pressures. CGRP infusions were separated by 30min.

CGRP produced a dose-related increase in flow in the coronary vascular bed from 19±8% at 10pmol min^{-1} to 71±14% at 600pmol min^{-1} (n=3, mean±s.e.m.) from a mean normal value of 20.6±0.8ml min^{-1} at a perfusion pressure of 100mmHg. In all determinations, the gradient of the pressure/flow curves increased with an increase in pressure, indicating a drop in the resistance of the coronary bed. For example, in control responses in one animal, the resistance dropped from 7.3mmHg ml^{-1} min to 4.6mmHg ml^{-1} min when the perfusion pressure was increased from 70 to 140mmHg. These changes correspond to an increase in effective cross-sectional area of the bed of approximately 8%. During drug treatment, the plots of resistance against pressure were similar in shape. The corresponding figures for the drop in resistance were 5.8mmHg ml^{-1} min at 70mmHg and 3.2mmHg ml^{-1} min at 140mmHg with 100pmol min^{-1} CGRP. No changes were observed in LVP, LVdP/dt, SAP, ECG or heart rate during infusions of CGRP.

These results support our suggestion that CGRP is an important regulator of vascular tone and blood flow.

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ACTIONS OF VIP AND PHI ON VASCULAR AND CAPSULAR SMOOTH MUSCLE OF THE ISOLATED, BLOOD PERFUSED DOG SPLEEN

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The structurally homologous polypeptides, Vasoactive Intestinal Peptide (VIP) and Peptide Histidine Isoleucine (PHI), originally isolated from extracts of porcine intestine, share a common mRNA and precursor protein (Itoh *et al*, 1983). Their immunohistochemical localisation within the autonomic nervous system is closely parallel. In the cat spleen VIP-like immunoreactivity has been identified in the innervation to blood vessels rather than the capsular smooth muscle (Lundberg *et al*, 1985). The distribution of PHI in the spleen is unknown.

Both peptides are known to have relaxant effects on various types of smooth muscle. In the present experiments the actions and relative potency of the two peptides were evaluated in the isolated, blood-perfused dog spleen. This organ contains vascular and extravascular (capsular) smooth muscle, both richly innervated by the sympathetic nervous system.

Experiments were performed on dogs anaesthetised with a mixture of chloralose (50 mg/kg) and urethane (500mg/kg). The spleen was isolated, placed in a plethysmograph and perfused with arterial blood. Splenic arterial blood flow (SABF) and perfusion pressure (SAPP) were recorded continuously to indicate changes in vascular tone, whilst spleen volume was also recorded to indicate changes in capsular activity.

VIP and PHI (Bachem) were administered as bolus doses (1pmol - 10nmol) into the splenic artery. The only splenic vascular response observed to each peptide was an increase in SABF, indicating a fall in splenic arterial vascular resistance (SAVR) and vasodilatation (Figure 1). Each peptide caused only very small increases in spleen volume. In individual experiments the dose response curves were approximately parallel with the same maximum (100% increase in SABF). However, the threshold for the vasodilator response to VIP was sometimes below 1pmol whilst that for PHI was 50-100pmol. On a molar basis VIP was at least a 100 times more potent than PHI when assessed by the ratio of doses causing a 50% increase in SABF.

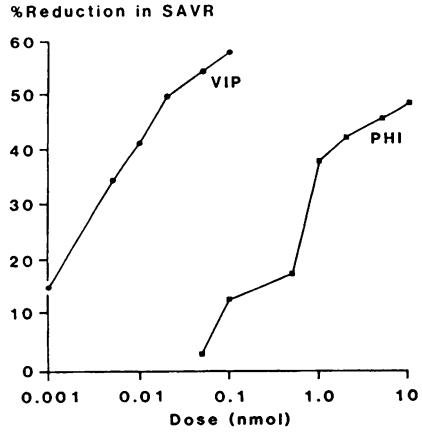

Figure 1 Reduction in splenic arterial vascular resistance (% of control SAVR) in response to i.a. VIP (●) and PHI (■). Spleen weight: 366g

The two structurally related neuropeptides, VIP and PHI, have differing potencies on vascular smooth muscle. However, they both have a differential action, since marked splenic vasodilatation was accompanied by only small changes in spleen volume.

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κ OPIOID RECEPTOR ACTIVATION DECREASES EXCITATORY SYNAPTIC INPUT TO RAT LOCUS COERULEUS NEURONES IN VITRO

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κ binding sites have been observed in the mammalian central nervous system, although little is known of the resultant effects following κ receptor activation. In the guinea-pig myenteric plexus and cultured murine dorsal root ganglion cells, κ receptor activation results in a decreased calcium conductance (Werz and MacDonald, 1984; Cherubini and North, 1985). This is in contrast to μ receptor activation which results in an increased potassium conductance (Morita and North, 1982; Werz and MacDonald, 1984). We have sought to determine the mechanism of action of κ opioids on central neurones by studying the effects of the selective κ agonist U50488 (Von Voigtlander et al, 1982) on neurones of the locus coeruleus in vitro.

Intracellular recordings were made from locus coeruleus neurones maintained at 37°C using the in vitro pontine slice technique (Williams et al, 1984). Slices were superfused (1.5 ml min^{-1}) with oxygenated artificial cerebrospinal fluid and drugs applied in known concentrations in the superfusate. Afferent inputs to the locus coeruleus were stimulated using square wave current pulses (0.1 - 0.2 msec duration at a frequency of 0.033 Hz) with a bipolar stimulating electrode.

Graded excitatory post-synaptic potentials (e.p.s.ps) could be evoked in all locus coeruleus neurones following focal stimulation of the slice surface. U50488 (0.01 - 10μM) produced a concentration-dependent depression of the e.p.s.p. without changes in either the input resistance or resting membrane potential of the post-synaptic neurone. U50488 did not abolish the e.p.s.p.; the maximum depression was produced by a concentration of 1uM and was 38 ± 5% (n=10), the concentration producing 50% maximal response being 85nM. Naloxone (100nM) produced a parallel shift to the right of the U50488 cumulative dose-response curve. The K_d for naloxone against U50488 was 28nM.

In contrast the μ receptor agonists FK 33824 (n=16) and [D-Ala^2,$MePhe^4$, Glyol] enkephalin (DAGO: n=7) produced concentration-dependent membrane hyperpolarisations of the post-synaptic neurone, accompanied by a fall in input resistance. Naloxone (100nM) produced a parallel shift to the right of the DAGO dose-response curve. The naloxone K_d against DAGO was calculated to be 1.5 nM. These K_d values against U50488 and DAGO are consistent with the view that these drugs are interacting with κ and μ receptors respectively (Lord et al, 1977), and that κ receptor activation produces a depression of excitatory synaptic input to locus coeruleus neurones.

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INHIBITORY ACTIONS OF μ AND κ OPIOID AGONISTS ON THE GUINEA-PIG ILEUM: DEPENDENCE ON STIMULUS INTENSITY AND FREQUENCY

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In the neurones of the guinea-pig myenteric plexus μ opicid agonists activate a potassium conductance whereas κ opioid agonists inhibit a calcium conductance (North & Tonini, 1977; Cherubini & North, 1985). The resultant effect of each is to inhibit the evoked release of acetylcholine from nerve terminals. We have sought to determine whether or not the differences in ionic mechanism between μ and κ opioids are manifested as differences in their abilities to inhibit neurotransmission evoked at different intensities or frequencies of stimulation.

Contractions of the guinea-pig longitudinal muscle-myenteric plexus preparation were evoked by electrical field stimulation with square wave pulses of 0.5 msec duration. Two intensities of stimulation were used (a) supramaximal, current intensity being one third greater than that producing a maximal response (b) submaximal, current intensity being adjusted to give a contraction height between 60-70% of that evoked by supramaximal stimulation. The preparation was stimulated at a frequency of 0.1 Hz with alternate supramaximal and submaximal pulses. In those experiments in which the frequency of stimulation was studied, current intensity was supramaximal and the duration of stimulation was always sufficient to produce a maximum response. All electrically evoked contractions were abolished by atropine (1μM).

The equilibrium inhibitions produced by either the μ selective ligand |D-Ala^2, MePhe^4, Glyol^5|enkephalin (DAGO) or the κ selective ligand U50488 were greater when submaximal rather than supramaximal stimuli were applied. DAGO (30nM) inhibited contractions evoked by submaximal and supramaximal stimulation by 75.5± 7.5% (n=4) and 40.0±8.0% (n=4) respectively. The corresponding inhibitions produced by U50488 (10nM) were 85.9±5.8% (n=4) and 50.4±3.2% (n=4).

The inhibitory effect of a maximal concentration of DAGO (300nM) decreased as the frequency of stimulation was increased. The inhibitions were 81.1±5.7% (n=6) at 1 Hz, 23.8±2.9% (n=6) at 3 Hz and 4.2±1.7% (n=6) at 10 Hz. U50488 (300nM) showed a similar frequency dependence; the inhibitions were 79.2±4.3% (n=6) at 1 Hz, 45.1±7.5% (n=6) at 3 Hz and 8.8±2.7% (n=6) at 10 Hz.

Thus μ and κ opioid receptor activation cannot be differentiated on the basis of either stimulus intensity or frequency. The insensitivity to μ and κ opicids of contractions produced by supramaximal stimulation may reflect the recruitment of nerve fibres which do not possess opioid receptors whereas the frequency dependence of opioid action may result from changes in the release process as the frequency of stimulation is increased.

HR is an MRC scholar.

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60P

THE EFFECTS OF μ-, δ- AND κ-OPIOID RECEPTOR AGONISTS AND ANTAGONISTS ON CORTICOTROPHIN RELEASING FACTOR (CRF) SECRETION

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Opiate drugs appear to enhance pituitary-adrenocortical activity in the rat by stimulating specific receptors in the hypothalamus which modify, directly or indirectly, the secretion of CRF (Buckingham & Cooper, 1984). Few attempts have yet been made to classify these receptors pharmacologically. Accordingly, we are studying the effects of selective agonists and antagonists of opioid receptors on the secretion in vitro of CRF. Hypothalami, removed from male Sprague-Dawley rats, were incubated under conditions described previously (Buckingham & Hodges, 1977a). The CRF released into the incubation medium in response to a 10 min stimulation with the agonists was determined according to the method of Buckingham & Hodges (1977b). Antagonists were added, when appropriate, to the incubation medium 10 min (naloxone or the δ-opioid receptor antagonist, ICI 154129) or 90 min (the μ-receptor antagonist, β-funaltrexamine, β-FNA) before the agonists.

The μ-opioid receptor agonists, FK33-824CH(10^{-8}-10^{-6}M) and Tyr-D-Ala-Gly-MePhe-$NH(CH_2)_2OH$(10^{-8}-10^{-6}M), like morphine (10^{-8}-10^{-6}M), caused significant ($P<0.01$, Duncan's test, 5 hypothalami/group) dose-related increases in the release of CRF from isolated rat hypothalami. The κ-opioid receptor agonist, U50,488(10^{-8}-10^{-6}M) was also weakly active in this respect but the δ-opioid receptor agonist, (D-Pen^2, D-Pen^5) enkephalin (2×10^{-10}-2×10^{-7}M), was not ($P>0.05$, analysis of variance, 5 hypothalami/group). The stimulatory effects of morphine and Tyr-D-Ala-Gly-MePhe-$NH(CH_2)_2OH$ were antagonized competitively by naloxone (10^{-7}M) and non-competitively by β-FNA(10^{-9}M). In contrast, the actions of U50,488 were unaffected either by β-FNA or by low concentrations of naloxone (10^{-8}-10^{-7}M) but were reduced significantly ($P<0.01$, Duncan's test, 5 hypothalami/group) by a higher concentration of naloxone (10^{-6}M). The pA_2 values (n=6) for naloxone against morphine, Tyr-D-Ala-Gly-MePhe-$NH(CH_2)_2OH$ and U50,488 were 8.97±0.51, 8.30±0.81 and 5.52±0.46 respectively. The δ-opioid receptor antagonist, ICI-154129 (5×10^{-6}M) did not influence significantly ($P>0.05$, analysis of variance, 5 hypothalami/group) the effects of either the μ- or κ-opioid receptor agonists.

The results suggest that both μ- and κ-opioid receptors are involved in the stimulation of CRF secretion but that δ-opioid receptors are not important in this respect.

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INTRATHECAL AND SYSTEMIC κ OPIOIDS ALTER THE EXCITABILITY OF CONVERGENT NEURONES IN THE RAT DORSAL HORN

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In order to investigate possible functional roles of the kappa sub-class of the opiate receptor in spinal cord function we have studied the effects of ethylketocyclazocine (EKC) and dynorphin (1-13) on the spinal processing of nociceptive information in the rat. Lumbar dorsal horn cells were excited by impulses in both A and C fibre afferents following electrical and natural stimulation of their peripheral receptive fields in the halothane anaesthetized rat. Single unit recordings of neurones receiving A and C fibre inputs were made. Drugs were administered intrathecally (50μl) and systemically (0.1ml bolus injection into the right jugular vein).

Intrathecal EKC was tested on a total of twenty-seven neurones. Low doses of EKC (32nmoles) slightly enhanced C fibre evoked activity, but higher doses produced a dose-dependent, naloxone-reversible inhibition, with an ED50 of 380nmoles. EKC (960nmoles) produced a maximum inhibition of 75± 3% (mean±s.e.) (n=5). Only at these high doses was A fibre activity supressed (40± 12% inhibition). Systemic EKC (2-12mg/kg) produced intrathecal naloxone reversible inhibitions of 7/7 neurones studied. The responses to naturally evoked activity (pinch and touch) were consistent with the changes in electrically evoked activity. In contrast to EKC, dynorphin at all doses (6pmoles-6nmoles) excited 12 neurones and inhibited 7 cells. The opposite effects were of similar magnitude (maximal 60% changes in either direction), and there was no obvious differences between the characteristics of the two neuronal populations. These dual effects are reminiscent of the previously reported actions of another putative kappa agonist, U50488H (Dickenson, *et al*,1985). Both types of effect were relatively insensitive to naloxone antagonism.

The results indicate that unlike morphine, (Dickenson *et al*, 1985) neither intrathecal dynorphin nor EKC (at low doses), putative kappa agonists, produce consistent inhibitions of dorsal horn nociceptive neuronal responses in the rat. Both higher doses of intrathecal EKC and systemic EKC produced naloxone-reversible inhibitions. Binding studies suggest that EKC has similar affinities for both the mu and kappa receptor (Garzon *et al*,1984). Our results therefore suggest that kappa mediated influences are complex but do not necessarily produce antinociception. Behavioural studies on intrathecal kappa agonists are controversial and may be confounded by possible motor actions of these ligands (Yaksh and Noueihed, 1985).

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THE MECHANISM OF THE CONTRACTILE EFFECTS OF PHORBOL ESTERS IN SMOOTH MUSCLE IS NOT PRIMARILY VIA Ca^{2+} CHANNEL ACTIVATION

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Protein kinase C is a Ca^{2+}-and diacylglycerol-dependent enzyme which phosphorylates different sites compared with calmodulin induced phosphorylation. Protein kinase C can be activated directly by phorbol esters, such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Phorbol esters have recently been shown to contract smooth muscle preparations (Rasmussen et al. 1984 ; Danthuluri & Deth, 1984 ; Baraban et al., 1985) implying that protein kinase C is involved in activation processes ; I have tested whether phorbol esters influence activation via voltage-operated Ca^{2+} channels (VOCs).

Ca^{2+}-induced contractions of K^{+}-depolarized taenia preparations from the guinea-pig caecum are a sensitive index of VOC activation (Spedding, 1982). Strips of taenia were set up in 10 ml organ baths in K^{+} (40 mM) Tyrode solution and isotonic contractions in response to Ca^{2+} recorded. Taenia preparations were submaximally contracted with Ca^{2+}, 0.1mM, and incubated for 3 h with TPA, 3μM. TPA did not affect Ca^{2+}-induced contractions, relaxation time following washout or subsequent sensitivity to verapamil, 0.2μM. Thus TPA had no measurable effect in this model of activation of VOCs.

Rings of rat aorta were set up under isometric conditions in Tyrode solution containing 2.7mM K^{+}. Bay K 8644, an activator of VOCs, did not contract rat aorta unless the K^{+} was elevated (> 6mM). TPA 0.03-3μM, caused slow contractions of the aorta (onset 1-5 min, maximal 20-30 min) as reported previously (Danthuluri & Deth, 1984). Submaximal contractions to TPA, 0.3μM, were unaffected by pretreatment with nifedipine 10 nM, verapamil 10μM, diltiazem 10μM, cinnarizine 10μM, or a combination of prazosin 1μm and idazoxan 1μM. However, the contractions were antagonized by W-7, 50-200μM, a calmodulin inhibitor which has also been reported to block protein kinase C activation (Sanchez et al., 1983). The contractions induced by TPA, 0.3μM, were not sustained and waned after 2-4 h. In some preparations rhythmic contractions developed after 2 h which were abolished by verapamil, 1μM, and augmented by Bay K 8644, 1 μM, indicating that VOCs may be activated following prolonged exposure to TPA ; this may be an indirect effect subsequent to membrane depolarization.

These results show that protein kinase C is not directly involved in activation of VOCs, but is involved in contractile events which are unaffected by calcium-antagonists. Thus protein kinase C is probably involved in receptor-mediated activation processes such as intracellular Ca^{2+} release or receptor-operated channels (Baron et al. 1984 ; Baraban et al. 1985). Under certain circumstances, some of the contractile effects of phorbol esters may be sensitive to calcium-antagonists, but these may be secondary to mixed activation processes in a particular smooth muscle.

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DO ADRENALINE-CONTAINING NEURONES FROM THE ROSTRAL VENTROLATERAL MEDULLA EXCITE PREGANGLIONIC SYMPATHETIC CELL BODIES?

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Evidence has recently been accumulated that suggests that a group of tonically active cells in the rostral ventrolateral medulla (R.V.L.) is responsible for maintenance of blood pressure (Ross et al., 1983). Efferent projections from the R.V.L. synapse directly with the preganglionic sympathetic nerve cell bodies in the intermediolateral column of the spinal cord (Ross et al., 1984). Many of the neurones arising from the R.V.L., particularly those from the C_1 area, show immunocytochemical staining for phenylethanolamine-N-methyl transferase (P.N.M.T.), suggesting that adrenaline may serve as the neurotransmitter. We have looked for evidence to support this view.

Experiments were carried out using rats (A.H.A. female, 200-250g) anaesthetised with thiobutobarbitone (140 mg/kg i.p.) and pretreated with atropine methyl bromide (0.5 mg/kg i.v.). Animals were artificially respired with room air (12 ml/kg; 60 strokes/min) and body temperature was maintained at 37-38°C. Blood pressure was recorded via a cannula in the right carotid artery; heart rate was derived electronically from the pressure pulse.

Unilateral injection of L-glutamate (0.6-2 µg) into the R.V.L. (1.7 mm anterior to obex; 1.7 mm lateral from midline; 3.5-4.0 mm ventral from the surface of the medulla) induced transient increases in blood pressure and heart rate. Unilateral stimulation of the same area with a bipolar microelectrode (100 Hz; 2V; 0.5 ms) for periods of 10s. every 10-15 min elicited transient, sub-maximal reproducible increases in blood pressure ($\sim$40 mmHg) and heart rate ($\sim$40 beats/min) that were unaffected by acute adrenalectomy but were greatly reduced by guanethidine (1 mg/kg i.v.). Pretreatment with the P.N.M.T. inhibitor LY 134046 (Fuller et al., 1981), 40 mg/kg i.p. daily for 5 days (last dose 4-6h before testing) did not significantly alter resting blood pressure or heart rate, or the changes produced by R.V.L. stimulation. Although this treatment has been shown to deplete central adrenaline levels very extensively (Fuller et al., 1981) it is conceivable that enough remained to maintain cardiovascular function. Attempts were therefore made to antagonise the effects of locally released adrenaline.

Preganglionic nerves that innervate the heart leave the spinal cord at the C_7-T_2 region. Phentolamine (10 and 100 µg) or propranolol (1, 10 and 100 µg) was injected intrathecally into this region of the spinal cord and the reduction in the tachycardia to R.V.L. stimulation was used as an index of blockade of spinal sympathetic neurotransmission. Phentolamine did not alter the tachycardia to R.V.L. stimulation although the highest dose reduced the accompanying pressor response. This probably reflects leakage into the periphery because pressor responses to phenylephrine (5 µg/kg i.v.) were reduced, by a similar amount, by this dose of phentolamine. In different animals, propranolol, 1 µg, was without effect but 10 and 100 µg reduced the tachycardia to R.V.L. stimulation. However, these doses also reduced the tachycardia to intravenously injected isoprenaline (30 ng/kg), suggesting a peripheral, rather than a spinal site of action of propranolol.

The failure of P.N.M.T. inhibition, and of intrathecal injection of α-and β-adrenoceptor antagonists to inhibit the tachycardia to R.V.L. stimulation (at doses that do not exert peripheral effects), suggest that adrenaline does not subserve a spinal neurotransmitter role, at least in the C_7-T_2 region.

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EFFECTS OF STIMULANTS ON PHOSPHATIDIC ACID LEVELS IN LONGITUDINAL SMOOTH MUSCLE FROM RABBIT AND GUINEA-PIG SMALL INTESTINE

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Increased metabolism of phosphatidylnositol has been detected upon stimulation of smooth muscle by various agents such as carbachol (Jafferji & Michell, 1976; Abdel-Latif & Akhtar, 1976). More recently it has been suggested that PI 4-phosphate (PIP) and particularly PI 4,5-bisphosphate (PIP_2) are cleaved at an increased rate upon receptor activation by a phosphodiesterase to form inositol trisphosphate and diacylglycerol (DAG) (Abdel-Latif et al, 1977; Best, Brooks & Bolton, 1985). DAG is believed to be phosphorylated to form phosphatidic acid (PA) before being reformed into PI.

Longitudinal smooth muscle from rabbit small intestine, preincubated with (^{3}H)-arachidonic acid (AA), showed a concentration dependent increase in incorporation of ^{3}H-AA into PA in response to carbachol (CCh). CCh (10^{-3}M) produced an increase (2-fold) in the level of (^{3}H)-PA within 30s. This effect persisted in the presence of 10^{-3}M EGTA, with no added calcium, but at a reduced level. Other phospholipids present were extracted along with PA by addition of $CHCl_3/CH_3OH/HCl$ (500:250:0:5 v/v). Phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, their lyso-derivatives, and (poly)phosphatidylnositol(s) were isolated by 2 dimensional thin-layer chromatography (t.l.c) (Rouser et al, 1969). No effect of CCh upon levels of (^{3}H)-AA in these substances could be detected.

The increase in labelled PA upon application of stimulants was further investigated using muscle prelabelled with $^{32}PO_4^{3-}$. PA was separated as a single spot by 1-dimensional t.l.c. (Hong & Levine, 1976). CCh produced a concentration-dependent increase in (^{32}P)-PA. 10^{-4}M CCh produced an increase in 5s, which was at a maximum (2-fold) after 2 min. The response to 10^{-4}M CCh was reduced but not abolished in 10^{-8}M calcium. $5x10^{-7}$M atropine inhibited the response to CCh, while 10^{-5}M isoprenaline had no effect on the CCh response.

Muscle from the guinea-pig gave similar results using $^{32}PO_4^{3-}$. In addition, both rabbit and guinea-pig muscle showed a small, but significant rise in (^{32}P)-PA in response to 120mM K^+, and neither showed any response to 10^{-3}M noradrenaline, or to 10^{-4}M 5-hydroxytryptamine. However, 10^{-4}M histamine produced a 2-fold increase in (^{32}P)-PA levels in guinea-pig but not in rabbit, whereas 10^{-4}M substance P produced an increase in (^{32}P)-PA in rabbit, but not in guinea-pig. Both histamine and substance P contract this visceral smooth muscle in rabbit and guinea-pig.

The rapidity of the increased labelling of PA upon application of stimulant suggests its involvement early following receptor activation, although the effect of high K^+ is anomalous. The failure of substance P to produce any increase in (^{32}P)-PA levels in guinea-pig, despite the fact that it produced 100% contraction at lower concentrations than those needed for CCh is also hard to explain.

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SPASMOGENS AND PHOSPHATIDYLINOSITOL TURNOVER IN BOVINE TRACHEAL SMOOTH MUSCLE

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Smooth muscle contraction is related to an increase in cytosolic calcium concentration due to calcium influx across the cell membrane and intracellular mobilisation of calcium stores. A wide variety of agonists stimulate membrane phospholipid breakdown, which in turn is associated with mobilisation of intracellular calcium (Berridge,1981). We have investigated phosphatidylinositol breakdown (PI response) and functional responses in bovine tracheal smooth muscle following administration of carbachol (CCh), acetylcholine (ACh), histamine (H), 5-hydroxytryptamine (5-HT), noradrenaline (NA), leukotriene C_4 (LTC_4) and substance P (SP) and also muscarinic receptor occupancy. PI breakdown was determined using the method of Berridge et al.,(1982). Chopped smooth muscle was incubated with [^{3}H]-inositol and agonist, in the presence of lithium chloride, and the production of inositol-phosphates was measured. Contractile concentration-response curves for each agonist were obtained from tracheal muscle strips mounted isometrically in 10ml organ baths. Muscarinic receptors were identified with [^{3}H]-quinuclidinyl benzylate ([^{3}H]-QNB).

CCh, ACh, H, and 5-HT caused a concentration-dependent increase in PI and contractile responses (see table 1.). There was no PI response after incubation with SP, LTC_4 or NA. There was no response to NA and only small contractions with SP (10^{-5}M) and LTC_4 (2×10^{-7}M) being respectively 6.5% and 2.5% of maximal CCh responses. Pyridostigmine enhanced the PI response to low concentrations of ACh suggesting that there was significant enzymatic degradation, but the maximal response remained similar to that of CCh. Atropine and pirenzepine blocked the cholinergic-induced PI response (IC_{50} = 1.0×10^{-9}M and 3×10^{-6}M respectively). Specific binding of [^{3}H]-QNB was inhibited by CCh (IC_{50} = $3.6.10^{-5}$M) and at low concentrations of CCh GTP 10^{-4}M shifted the competition curve to the right. In contrast to the difference in potency of CCh on PI, functional and binding responses, the potency of atropine and pirenzepine in antagonising these three processes was similar.

Table 1. PI and functional responses of bovine tracheal smooth muscle.

Spasmogen	PI response EC_{50}	Contractile response EC_{50}	n
CCh	38	0.074	12
ACh	7	5	10
Histamine	90	5.7	10
5-HT	13	0.8	10

Concentrations of spamogens causing a half maximal response (EC_{50}) are expressed in μM. Results are expressed as geometric means.

We conclude that contraction of airway smooth muscle by a wide range of spasmogens is related to PI breakdown and that this mechanism may couple receptor activation to modifications of cytosolic calcium; although it appears that only a small proportion of the available receptors need be occupied to produce a response.

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LITHIUM ATTENUATES BOTH 5-HT_1 AND 5-HT_2 RECEPTOR MEDIATED BEHAVIOUR IN THE MOUSE

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Administration to rats of lithium, the widely used prophylactic antimanic agent, decreases binding of [^{3}H]-5-HT and [^{3}H]-spiperone to the presumed 5-HT_1 and 5-HT_2 receptors in the hippocampus (Treiser et al, 1981). The recent availability of behavioural models for both 5-HT_1 and 5-HT_2 receptor function has enabled us to examine possible functional changes in these receptors following lithium administration.

Mice were injected subcutaneously with lithium (10 mmol/kg) in the acute study or with this loading dose followed by 3 mmol/kg twice daily for the longer term studies. 5-HT_2 receptor-mediated function was assessed by measurement of the head-twitch response in the 6 min following injection of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT; see Green & Heal, 1985). 5-HT_1 function was examined by measurement of the locomotor response following injection of RU 24969 (a putative 5-HT_{1B} agonist; see Goodwin & Green, 1985) and the temperature decrease following injection of 8-hydroxy-(di-n-propyl-amino)tetralin (8-OH-DPAT) a putative 5-HT_{1A} agonist (Tricklebank et al, 1984; Goodwin & Green, 1985; Goodwin et al, 1985).

A single injection of LiCl (10 mmol/kg) inhibited the 5-MeODMT head-twitch response 60 min later when compared with NaCl (10 mmol/kg) treated controls. NaCl 11(+3,-0) LiCl: 2.5 (±1), (results shown as median ± interquartile range). After 3 days treatment with LiCl (3 mmol/kg twice daily) there was a modest attenuation with a more marked effect at 14 days (3 days: NaCl; 17(+2,-0), LiCl; 12(+1.5,-0.5), 14 days: NaCl; 14 (+1,-0), LiCl; 6.5(+1,-2.5). Fourteen days treatment with lithium did not alter the activity response to RU 24969 (3 mg/kg): NaCl; 6228 ± 1110 (4), LiCl; 5465 ± 1528 (4) (results shown as automated activity counts/60 min ± S.D. (n)). Three days treatment with lithium did not alter the hypothermia induced by 8-OH-DPAT (0.5 mg/kg s.c.). However, this effect was markedly attenuated by 14 days treatment: NaCl; -1.3 ± 0.3 °C (8), LiCl -0.6 ± 0.3 C (8), results show temperature decrease from basal temperature ± S.D. (n) 20 min after injection. For at least 16 h of the day, plasma Li levels were above the clinically accepted therapeutic minimum (0.4 mM/l).

Assuming that mouse 5-HT_1 receptors can be classified in a similar way to 5-HT receptors in the rat, these data suggest a functional attenuation of 5-HT_{1A} and 5-HT_2 receptor function after longer term lithium treatment but no change in 5-HT_{1B} function.

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SELECTIVE REGULATION OF MAO A BUT NOT MAO B ACTIVITY IN CULTURED ADRENAL MEDULLARY ENDOTHELIAL CELLS BY STEROIDS

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Isolated bovine adrenal chromaffin cells contain primarily monoamine oxidase (MAO) B (Youdim et al., 1984). During culturing of these cells often the presence of other cell types was noted. These cells were isolated by differential plating and identified as true capillary endothelial cells (Banerjee et al., 1985).

Endothelial cells metabolize noradrenaline, dopamine, tyramine and serotonin by a process of uptake and oxidiative deamination via MAO. The Km values of endothelial MAO for the above amines are similar to values previously reported for MAO A in tissues of rat and human (Tipton et al., 1984). However, the Km of endothelial MAO for phenylethylamine is $\simeq$250μM. This value is significantly different from that (25μM) obtained for MAO B in chromaffin cells with this substrate. Clorgyline ($IC50 \simeq 10^{-9}M$) rather than l-deprenyl ($IC50 \simeq 10^{-6}M$) selectively inhibits the endothelial MAO. Substrate specificity and inhibitor sensitivity data indicate that the major form of MAO in this cell is type A.

Chromaffin and endothelial cells were cultured (Youdim et al., 1984; Banerjee et al, 1985) in absence or presence of 1μM of steroids, hydrocortisone, dexamethasone, progesterone, oestradiol and testosterone for 1-24 days. While none of the steroids affected MAO B activity in chromaffin cells, hydrocortisone, dexamethasone and progesterone significantly induced (200-300%) (n=9) and oestradiol reduced (70%) (n=9) MAO A activity in endothelial cells. These changes were maximum by the 8th day of culture and correlated with the incorporation of ^{14}C-leucine and ^{14}C-thymidine into the cell. The Km values of induced MAO for the various amines are unchanged and the induction of MAO activity observed with steroids may be related to the increased synthesis of MAO apoprotein.

The present study confirms previous reports that steroid hormones modulate MAO activity (Holzbauer and Youdim, 1973), but this regulation is related to a change in MAO A activity only. It is well known that endothelial cells either sequest or degrade many vasoactive substances including above amines. The enzyme MAO is essential for metabolism and function of serotonin and noradrenaline (Youdim and Finberg, 1982). Modulation of MAO A activity may be crucial in the known action of these vaso constrictor hormones as releasers of vasodilator substances from endothelial cells (Cocks and Angus, 1983) that in turn act as a physiological antagonist of blood vessel smooth muscle contractile activity.

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EX VIVO INHIBITION OF AMINE OXIDASE ACTIVITIES IN SEVERAL RAT TISSUES BY MDL 72145

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(E)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) is an irreversible inhibitor with selectivity both in vitro and in vivo for the B-form (compared with the A-form) of monoamine oxidase (MAO) in rat brain (Bey et al, 1984; Zreika et al, 1984). Consequently a potential use of MDL 72145 in Parkinsonian therapy has been suggested, based on the clinical efficacy of another MAO-B selective agent, deprenyl. Recently, we reported that MDL 72145, at concentrations similar to or even lower than those producing MAO-B inhibition, is also a potent irreversible inhibitor in vitro of the semicarbazide-sensitive amine oxidase (SSAO) of the aorta (and other cardiovascular tissues) of the rat (Lyles & Fitzpatrick, 1985). Here we have investigated the possibility, by an ex vivo method, that significant inhibition of SSAO may accompany the in vivo inhibition of MAO activities produced by administration of appropriate drug doses to the rat.

Five groups of male rats (140-160 g), each containing 5 or 6 animals, were treated by i.p. injection with MDL 72145 hydrochloride (dissolved in aqueous NaCl, 0.9% w/v) at the following respective doses: 0 (controls), 0.05, 0.1, 1 and 10 mg/kg. Control animals received appropriate volumes of vehicle. Rats were killed 24 h later and the following tissues removed: liver, brain, aorta, heart and lung. Particular amine oxidase activities were assayed radiochemically in appropriate tissue homogenates with the following substrates: 1 mM 5-hydroxytryptamine (MAO-A), 1 mM benzylamine (MAO-B) and 1 μM benzylamine (SSAO).

Mean values for specific enzyme activities were compared in the tissues of drug-treated animal groups with the corresponding mean value in the control animals. The relationship between dose and mean percentage inhibition was plotted in order to estimate those doses (ED_{50}, shown below) required to inhibit enzyme activity by 50% compared with the control group.

Table I Estimated ED_{50} values (mg/kg MDL 72145) for ex vivo inhibition of amine oxidase enzymes

Tissue	MAO-A	MAO-B	SSAO
Brain	1.2	0.31	-
Liver	4.0	0.45	-
Heart	3.5	-	0.60
Aorta	8.9	-	0.33
Lung	4.2	-	0.40

Confirmation of the relatively greater sensitivity of MAO-B compared with MAO-A towards ex vivo inhibition by MDL 72145 is provided by these results. In addition, a similar dose relationship was found for inhibition of both MAO-B and SSAO by the drug.

At present, the physiological importance of SSAO and the implications of its inhibition by drugs remains unknown. However, these pharmacological properties of MDL 72145, or related compounds, could be useful in investigating the significance of recent observations that SSAO is located on smooth muscle cells of the aorta and other rat blood vessels (Lyles & Singh, 1985).

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ENTOPEDUNCULAR INJECTION OF 2-APH OR MUSCIMOL PROTECTS AGAINST PILOCARPINE-INDUCED LIMBIC SEIZURES

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Motor limbic seizures and status epilepticus with widespread neuronal damage within the limbic forebrain follow the administration of pilocarpine (380 mg/kg, i.p.) in rats (1). Modulation of such seizures is produced by pharmacological manipulation in substantia nigra, which is an output relay of the basal ganglia (2). We have studied the influence of the entopeduncular nucleus (EP, another basal ganglia relay nucleus) on pilocarpine-induced seizures by means of intracerebral injections of the excitatory amino acid antagonist, 2-amino-7-phosphonoheptanoate (2-APH), and the potent GABA agonist, muscimol.

Male Wistar rats (280-300 g) were injected with scopolamine methylnitrate (1 mg/kg, s.c.) 30 min prior to pilocarpine (380 mg/kg, i.p.). The rats were observed for a period of 2.5 h and any behavioural changes were noted and scored on a scale of 0-7. For EP studies, rats were injected with 2-APH (5-20 pmoles/side) bilaterally into EP 15 min prior to pilocarpine (380 mg/kg, i.p.). The rats were observed for 3 h and the behavioural score noted. All rat brains were perfusion-fixed and processed both for morphological examination and confirmation of cannulae placements.

Tonic-clonic seizures (score 7) were evoked in all rats injected with pilocarpine within 30 min of injection. Following injection of 2-APH (5 pmoles/side) into EP, 66% of rats developed tremor (score 2) and the remainder showed score 4 (rearing+clonus) 1 h later, compared to vehicle controls (score 5 at 30 min). With 10 pmoles 2-APH the protective effect was more evident, 33% of rats appeared normal (score 0) 2 h after pilocarpine injection, with a mean score of 3 for the rest of the group. The highest dose of 2-APH (20 pmoles) was also effective in protection against pilocarpine-induced seizures. Furthermore, high doses of 2-APH (10, 20 pmoles) injected in EP unilaterally and bilaterally gave equal protection against seizure propagation. However, with 5 pmoles of 2-APH, a bilateral EP injection was required to ensure this protective effect of 2-APH.

Muscimol (50 pmoles) when injected into EP bilaterally or unilaterally provided a strong protection against pilocarpine-induced seizures, with rats displaying a mean score of 2.

In summary, antagonism of excitatory transmission with 2-APH in EP protects against seizures induced by pilocarpine. With high doses of 2-APH, (10, 20 pmoles), protection is also seen after a unilateral injection into EP. Stimulation of GABA receptors in EP (muscimol, 50 pmoles) also produces protection against pilocarpine seizures.

Manipulation of excitatory or inhibitory neurotransmission in EP modulates the severity of pilocarpine seizures. The EP probably plays an important role in the propagation of limbic seizure activity.

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EFFECT OF NMDA ANTAGONISTS ON PAROXYSMAL EVENTS INDUCED BY ZERO MAGNESIUM AND BICUCULLINE

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The appearance of spontaneous paroxysmal events in slices of rat cerebral cortex on removal of the magnesium from the superfusing Krebs medium has been described previously and shown to be sensitive to antagonists of N-methyl D-aspartate (NMDA) (Harrison & Simmonds 1985; Aram & Lodge 1985). Spontaneous events can also be induced by addition of high concentrations of the GABA-A antagonist bicuculline to otherwise normal Krebs medium (Connors 1984). We report here a comparison of the effects of two NMDA antagonists, 2-amino 5-phosphonovalerate (APV) and ketamine, on the frequency of events induced by zero Mg^{2+} and by 50μM bicuculline.

Slices of rat cerebral cortex were prepared as previously described (Harrison & Simmonds 1985) and placed in a two compartment bath so that the slice projected through a greased slot in the barrier separating the two compartments. Removal of the Mg^{2+} from, or addition of bicuculline to, the Krebs medium perfusing the "cortical" end of the slice led to the appearance, within 30 min, of paroxysmal events which had a rise-time of about 50ms to the first negative peak and after-potentials of up to 2s duration. Events were counted over successive 5 min periods and statistical analyses were made by Students t-test.

The frequency of paroxysmal events in zero Mg^{2+} was 1.56 $\pm$ 0.19 events min^{-1} and in 50μM bicuculline was 0.97 $\pm$ 0.09 events min^{-1} (mean $\pm$ sem of 12 ten min control periods prior to antagonist application in 7 and 8 slices respectively) APV (100μM for 10 min) produced significant reductions in the frequency of events ($p<0.05$) during the first 5 min and the frequencies remained significantly reduced for 45 min in zero Mg^{2+} and for 30 min in bicuculline ($n = 5$ in each case). Ketamine (100μM for 15 min) produced a significant decrease in the frequency of events during the first 5 min in zero Mg^{2+} ($n = 7$), but not until the fourth 5 min period in bicuculline ($n = 7$). In both cases the effect of ketamine took more than 2h to recover. The peak effect of each antagonist was measured as the maximum inter-event interval during the 50 min from the start of antagonist application. APV caused a significantly longer silent period in zero Mg^{2+} (23.9 $\pm$ 2.3 min) than in bicuculline (7.3 $\pm$ 0.7 min) (mean $\pm$ sem; $p<0.01$). Ketamine, similarly, caused a significantly longer silent period in zero Mg^{2+} (31.0 $\pm$ 4.6 min) than in bicuculline (7.5 $\pm$ 1.6 min) ($p<0.01$).

Thus, despite the lower frequency of paroxysmal events in bicuculline, APV and ketamine were less effective against bicuculline-induced activity than against that in zero Mg^{2+}. This may reflect a synergism of endogenous GABA-A tone with NMDA antagonists in suppressing paroxysmal events. Alternatively, events evoked by GABA-A antagonists may be less dependent on pathways involving NMDA receptors (see also Thomson & West 1985).

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TEMPERATURE DEPENDENCE OF HISTAMINE-INDUCED INOSITOL PHOSPHOLIPID BREAKDOWN IN GUINEA-PIG CEREBRAL CORTEX

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Histamine H_1-agonists stimulate the breakdown of inositol phospholipids in slices of guinea-pig cerebral cortex by a mechanism that is predominately H_1-receptor mediated (Daum et al., 1984; Carswell et al., 1985). There are, however, indications that the coupling of the response to H_1-receptor activation may be more complex than the currently accepted scheme (Berridge, 1984) would suggest. This has been studied further by comparing the effect of temperature on histamine- and carbachol-stimulated breakdown of inositol phospholipids in guinea-pig cerebral cortex.

Cross-chopped slices of guinea-pig cerebral cortex were preincubated in Krebs-Henseleit medium for 1 hour at 37°C and gassed continuously with O_2/CO_2 (95:5 v/v). The slices were incubated in 15 ml Krebs containing 10 µCi [^{3}H]inositol for 30 min and then thoroughly washed. Aliquots (40 µl) were added to 200 µl Krebs containing 10 mM LiCl and incubated for 15 min at 37°C or 25°C before addition of agonist. The reaction was terminated by addition of chloroform/methanol and the water-soluble [^{3}H]inositol phosphates extracted and separated by anion-exchange chromatography essentially as described by Berridge et al., (1982).

At 37°C both histamine (200 µM) and carbachol (100 µM) produced a significant increase in the accumulation of [^{3}H]inositol 1-phosphate (approximately 3 and 18 fold stimulation over the basal level, respectively, after 45 min incubation). This increase was time-dependent, but whereas the stimulation by histamine showed an initial lag of 5-15 min before increasing linearly up to 45 min and levelling off, that by carbachol was essentially linear from 0-90 min. At 25°C 200 µM histamine produced very little stimulation and the response after 45 min was only 5% of that obtained at 37°C measured at the same time on the same slice preparation. In contrast, carbachol produced approximately 80% of the response obtained at 37°C and the time-course for [^{3}H]inositol 1-phosphate accumulation was virtually the same at both temperatures. Proportionate reductions were seen in the stimulated levels of [^{3}H]inositol bis- and tris-phosphates at 25°C compared to 37°C. Basal levels of all three inositol phosphates were unaffected by the reduction in temperature.

The difference in the temperature-sensitivity of the response to histamine and carbachol may reflect a fundamental difference in the coupling mechanisms between muscarinic and histamine H_1-receptors and inositol phospholipid hydrolysis in guinea-pig cerebral cortex.

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TEMPERATURE DEPENDENCE OF THE BINDING OF A NEW QUATERNARY (^{3}H)-LIGAND TO THE HISTAMINE H_1-RECEPTOR

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[^{3}H]-Mepyramine, the most widely used [^{3}H]-ligand for the histamine H_1-receptor, shows a strong temperature dependence of binding, such that the rate constants for complex formation, k_1, and dissociation, k_{-1}, decline markedly with a decrease in temperature (Wallace & Young, 1983). At 4°C the rate of dissociation is extremely slow and k_{-1} seems to be smaller than would be expected from extrapolation from an Arrhenius plot constructed between 37°C and 15°C. The small increase in the affinity constant, K_a, at 4°C compared to 30°C is also unexpected since k_1 at 4°C seems not to have been slowedv to the same extent as k_{-1}. We have investigated the kinetics of ligand interaction with the H_1-receptor further by examining the kinetics of binding of [^{3}H]-(+)-N-methyl-4-methyl-diphenhydramine ([^{3}H]-QMDP), a quaternary ligand for the receptor. Quaternisation of H_1-antagonists usually leads to a decrease in the affinity for the receptor. However, in the 4-methyl substituted diphenhydramine series the affinity is little changed (Harms et al.,1975).

[^{3}H]-QMDP (83 Ci mmol^{-1}) was prepared by the methylation of the corresponding 3y-amine with [^{3}H]-MeI (Amersham) and purified by high voltage electrophoresis. Binding of [^{3}H]-QMDP to homogenates of guinea-pig cerebellum was measured essentially as described previously (Wallace & Young, 1983), except that the Whatman GF/B filters were presoaked in 0.3% polyethylenimine. Non-specfic binding was measured in the presence of 0.4 uM mepyramine.

[^{3}H]-QMDP bound relatively rapidly and reversibly to the H_1-receptor at 30°C with an affinity, 1.3 x 10^9 M^{-1}, similar to that obtained from histamine-induced contraction of guinea-pig ileal smooth muscle, 1.8 x 10^9. A comparable value of K_a (=k_1/k_{-1}) was obtained when k_1 (6.1 x 10^7 M^{-1} min^{-1}) and k_{-1} (5.8 x 10^{-2} min^{-1}) were obtained from a plot of k_{on}, the observed rate constant of binding, versus the concentration of [^{3}H]-QMDP. Direct measurement of dissociation induced by adding excess non-radioactive QMDP (1 uM) yielded a similar value for k_{-1} (5.2 x 10^{-2} min^{-1}). Both k_{-1} and k_1 (calculated using the measured k_{-1}) declined markedly with temperature. The time taken for the non-specfic binding to reach equilibrium also became slow at lower temperatures (180 min for 1nM [^{3}H]-QMDP at 6°C). Arrhenius plots of ln k_1 and ln k_{-1} versus 1/T were linear for measurements made between 37°C and 6°C (activation energies were 73 and 130 kJ mol^{-1} respectively). The affinities calculated for [^{3}H]-QMDP at 37°C, 30°C and 25°C were in reasonable agreement with values obtained from organ bath studies with the guinea-pig ileum.

There is thus no evidence for any discontinuity in the kinetic properties of [^{3}H]-QMDP as the temperature is lowered. There is no indication that the interaction is anything other than a simple equilibrium.

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PROPHYLACTIC ANTI-ASTHMA DRUGS IMPAIR PLATELET ACCUMULATION WITHIN THE LUNG

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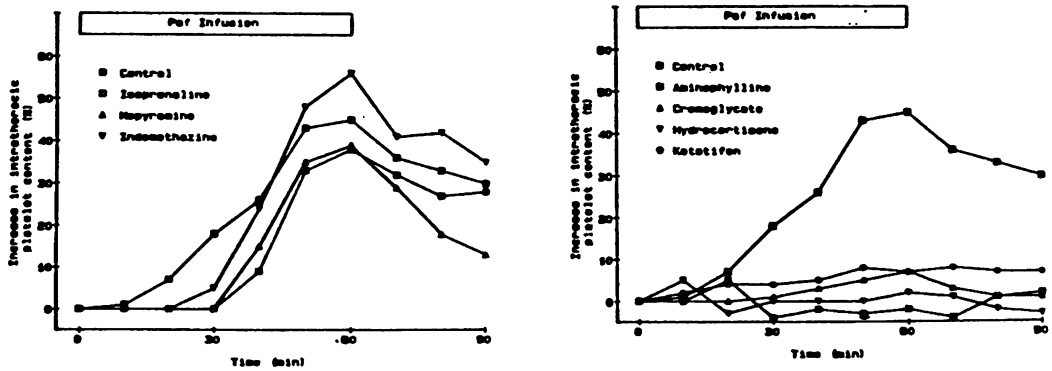
The capacity of PAF to mimic all distinctive features of asthma pathology and the evidence of platelet activation and PAF formation during exacerbation of asthma support the proposition that generation of PAF is central to the pathology of this disease (Morley et al., 1984). Inhalation, infusion or injection of PAF induces an intense and protracted, non-selective hyper-reactivity in guinea-pigs (Mazzoni et al., 1985a) during which there is an emigration of platelets into lung tissue (Lellouch-Tubiana et al., 1985). Since prophylactic anti-asthma drugs impair the development of hyper-reactivity in response to PAF and since this hyper-reactivity depends upon intact platelets (Mazzoni et al., 1985a), we have sought to determine whether platelet accumulation is suppressed by prophylactic anti-asthma drugs.

Accumulation of III-Indium-labelled platelets was determined over a one hour infusion period (Page et al., 1982). PAF (600 ng/kg/hr, n=5) induced a progressive accumulation that was inhibited by the prophylactic anti-asthma drugs: cromoglycate (6.6 mg/kg, n=3), hydrocortisone (6.6 mg/kg, n=3), ketotifen (6.6 mg/kg, n=3) and theophylline (6.6 mg/kg, n=4) but not by mepyramine (2 mg/kg, n=7), indomethacin (2 mg/kg, n=3) or isoprenaline (20 ug/kg, n=5). All drugs were given i.v., as a bolus injection followed by infusion. Effects of anti-asthma drugs upon platelet accumulation accord closely with inhibition of hyper-reactivity, as previously reported in ventilated anaesthetised guinea-pigs (Mazzoni et al., 1985b).



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EFFECTS OF NEUROTENSIN AND TRH ON THE RELEASE OF ENDOGENOUS DOPAMINE FROM RAT CORPUS STRIATUM AND NUCLEUS ACCUMBENS IN VITRO

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The presence of TRH- and neurotensin(NT)-like immunoreactivity in the corpus striatum (CS) and the nucleus accumbens (NAc) and the demonstration of specific receptors in these areas support a role for these peptides as cerebral neurotransmitters (Nemeroff et al, 1980). The actions of NT have been closely associated with dopamine (DA) in these brain regions (Bennett et al, 1981; DeQuidt & Emson, 1983); while TRH has been shown to antagonise many of the effects of NT (Widdowson et al, 1983). The present study compared the influence of the two peptides on endogenous DA release from CS and from NAc.

Male C/D rats (200-300g) housed in groups with ad libitum food and water, were killed by decapitation and the striata and accumbens nuclei rapidly dissected. The tissue was chopped into blocks ($1.0mm^{-3}$) and incubated at $37^{0}C$ in 200 ul of modified Tyrodes solution containing Hepes buffer (5mM), pargyline (350 uM) and l-DOPA (4 uM) for ten 15 min periods. Incubation periods 4 and 8 were carried out in Tyrodes containing 20 mM K+. Synthetic TRH and NT were added to the incubation medium from period 6 to period 10. The concentrations of DA in incubation samples were determined by reverse phase HPLC with electrochemical detection (Mefford, 1980). Stable basal secretion of endogenous DA from both CS (0.95 ± 0.07 ng/mg tissue; n=12) and NAc (0.35 ± 0.03 ng/mg tissue; n=6) with consistent and significant increases of DA output with the two period of K+ stimulation were observed. NT produced a dose-dependent increase in both basal and K+-stimulated DA release from the CS, but not from the NAc. Conversely, TRH elicited a dose-dependent increase only in basal DA output from the NAc and had no effect in the CS. Finally, the effect of NT in the CS was not antagonized by the addition of TRH, nor was TRH activity in the NAc reversed by NT.

Table 1: Mean (±SEM) Change (%) in DA Release from Control

		Basal	K+-stimulated
(1)	Corpus Striatum		
	NT 10uM (n=8)	+45.89±8.03**	+71.30±13.84**
	TRH 50uM (n=8)	- 6.95±6.30	+ 0.60±8.00
	NT 10uM + TRH 50uM (n=8)	+37.88±6.41**	+70.67±8.91**
(2)	Nucleus Accumbens		
	NT 10uM (n=8)	- 3.85±7.30	-12.12±7.78
	TRH 50uM (n=8)	+42.16±10.92*	+ 3.66±2.43
	NT 10uM + TRH 50uM (n=4)	+42.64±11.52*	+ 5.06±4.20

*p<0.05; **p<0.001

The results suggest that NT receptors located on the DA terminals in the striatum and TRH receptors on DA terminals in the NAc modulate the release of endogenous DA in these two brain regions. However, the two peptides do not directly antagonize each others actions on these DA systems.

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ANTIARRHYTHMIC EFFECTS OF DPI 201-106

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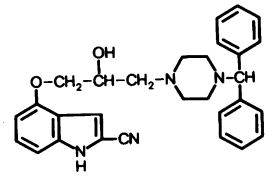
DPI 201-106 increases myocardial force of contraction, lowers heart rate and increases coronary perfusion (Scholtysik et al., 1985).

Chemical structure of DPI 201-106 (DPI)



Prolongation of the open state of sarcolemmal Na^+-channels has been postulated as the main mechanism of the positive inotropic effect of DPI (Buggisch et al., 1985). This action on the Na^+-channels also results in a prolongation in the action potential duration (APD). Since APD prolongation is a characteristic feature of class III antiarrhythmics (Vaughan Williams, 1975), we have investigated DPI for antiarrhythmic activity. In addition its local anaesthetic properties were investigated.

DPI protected rats against aconitine induced ventricular extrasystoles (ED_{50} 0.97 mg x kg^{-1} i.v.) and ventricular tachycardia (ED_{50} 0.36 mg x kg^{-1} i.v.).

Reperfusion-induced arrhythmias were elicited in anaesthetized rats by release of a temporary coronary artery occlusion. DPI infused at a rate of 0.3 mg x kg^{-1} x min^{-1} i.v. completely prevented ventricular fibrillation and mortality, compared to an incidence of 70% and 60% respectively in controls.

In isolated vagus nerves of cats DPI decreased the amplitude of evoked compound action potentials with an EC_{50} of 1.82 x 10^{-5} M as an expression of local anaesthetic activity.

These results demonstrate that DPI has antiarrhythmic activity and possesses local anaesthetic effects. Insufficient information is as yet available to assign DPI to a particular class of the Vaughan Williams (1975) classification. However, the combination of cardiotonic effects with antiarrhythmic activity may represent an interesting pharmacological profile.

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DIFFERENCES IN CONTRACTILE RESPONSE TO ADRENALINE AND NORADRENALINE IN PURE ELECTROLYTE SOLUTIONS AND IN THE PRESENCE OF ALBUMIN

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An improvement of a method (J.H. Christensen et al, 1982) which secures adequate control of oxygen supply and pH in a tissue bath with protein solution has been developed. Rings of the rabbit ear artery were used. Comparison of adrenaline (A) and noradrenaline (NA): A comparison of A and NA in concentrations from 10^{-9} to 10^{-3} M in Krebs-Henseleit (K-H) solutions indicated no difference in the cumulative response (3.26 $\pm$ 1.43 g for A and 3.14 $\pm$ 1.39 g for NA). EC_{50} was 6.3 times higher for NA. The cumulative loss in contractile response during the 25 minutes of each experiment was 4.7% for A and 6.0% for NA (NS). In K-H solutions with 45 g human serum albumin per l (alb.K-H), the maximal cumulative responses were 1.22 $\pm$ 0.56 g for A and 1.44 $\pm$ 0.82 g for NA (NS). The EC_{50} was 6.7 times higher for NA. The cumulative loss of contractile response during the 25 minutes experimental period was 42% for A and 20% for NA ($p < 0.01$).
Comparison of K-H and alb.K-H: The maxima of the cumulative curves in alb.K-H were reduced by 62% ($p < 0.01$) for A and by 43% for NA ($p < 0.01$). The EC_{50} did not differ for A but for NA it was 14 times higher in alb.K-H. The influence of Ca^{++}: Experiments with increasing concentrations of Ca^{++} showed increases in tension from 2 to 6 times the tension observed when no calcium was added. For NA the tension was not increased further by Ca^{++}-concentrations above 2 mM and 10^{-6} M NA in K-H as well as in alb. K-H. By 10^{-3} NA the maximum was reached at 2.5 mM Ca^{++} in K-H and at 3 mM in alb. K-H. For A (10^{-6} and 10^{-3} M) the tension was not increased further by Ca^{++} concentrations exceeding 2 mM in K-H and 3.5 mM in alb. K-H. The addition of albumin decreased the tension response to NA by 25% over the entire Ca^{++} range investigated whereas no evidence of an albumin evoked decrease of the maximal response to A was observed. Conclusions: Catecholamine induced responses to the rabbit ear artery are calcium dependent and A is more potent than NA in pure electrolyte solutions. The better ability of NA to maintain achieved tensions in alb. K-H may indicate that A is more dependent on the Ca^{++}-level than NA is. The data may yield some indirect information on the serumprotein-binding of the two catecholamines. A may well be bound to albumin but the strength of the binding is not sufficient to inhibit the binding to the vessel wall receptor if sufficient Ca^{++} is present. The partial and consistant inactivation of NA in alb.K-H may be due to a serum-protein-binding sufficiently strong to inhibit the binding of NA to the vessel wall.

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EFFECTS OF THE NOVEL ANTI-HYPERTENSIVE AGENT BRL 34915 IN COMPARISON WITH NIFEDIPINE ON RABBIT ISOLATED MESENTERIC ARTERY

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Pharmacological activities of the novel anti-hypertensive agent BRL 34915 have initially been reported (Buckingham et al, 1984). Further studies have now been performed in vitro in order to elucidate the mechanism of action of BRL 34915 on vascular smooth muscle in comparison with the Ca^{2+} channel blocker nifedipine.

Mesenteric arteries were removed from male NZ white rabbits (2 - 2.5 kg) and each artery divided into 4 equal rings. Each ring was suspended in a 10 ml tissue bath containing Krebs-Henseleit solution ($[Ca^{2+}]$ = 2.5 mM; $[K^+]$ = 4.7 mM) at 37°C, and isometric tension recorded. Tissues were contracted by either KCl (30 or 90 mM), noradrenaline (10^{-8} - 10^{-4}M) or electrical field stimulation (0.5 - 20 Hz, 100 v, 0.5 msec). Contractions to field stimulation could be abolished by guanethidine or prazosin and were thus attributable to neuronally released noradrenaline.

BRL 34915, 10^{-8} - 10^{-6}M, produced concentration-related inhibition of contractions elicited by field stimulation, the response to 20 Hz being reduced to 37 ± 6% of control with the 10^{-6}M concentration. BRL 34915, 10^{-7} and 10^{-6}M, produced small concentration-related shifts to the right of the concentration-response curve to exogenous noradrenaline, and reduced the maximum response to 93 ± 4% and 69 ± 12% of control respectively. BRL 34915, 10^{-8} to 10^{-5}M, did not affect contractions to 90 mM KCl yet inhibited contractions to 30 mM KCl to give an IC_{50} value of 3.3 (1.2 - 9.2) x 10^{-7}M.

Nifedipine, 10^{-8} and 10^{-7}M, produced small concentration-related shifts of the frequency-response curve, but 10^{-6}M gave no further shift. At 10^{-6}M nifedipine the response to 20 Hz was reduced to 69 ± 15% of control. Nifedipine, 10^{-8} - 10^{-6}M, had little effect on noradrenaline concentration-response curves other than a depression of the maximum response to 80 ± 6% of control at 10^{-6}M. Nifedipine preferentially inhibited contractions to KCl, with an ID_{50} value of 3.2 (2.1 - 4.8) x 10^{-9}M against 30 mM KCl and 4.6 (3.3 - 6.3) x 10^{-9}M against 90 mM KCl.

The results demonstrate an in vitro pharmacological profile for BRL 34915 which is distinct from that for nifedipine. Hamilton et al (1985) suggested that hyperpolarisation of vascular smooth muscle by increasing K^+ efflux may underlie the actions of BRL 34915. It is postulated that the activity of BRL 34915 depends upon the depolarising nature of the contractile stimulus. BRL 34915-induced hyperpolarisation of vascular smooth muscle may antagonise the effects of neuronally released and exogenous noradrenaline or low K^+ concentrations, but not the depolarisation produced by high K^+ concentrations. The activity of nifedipine however depends on whether the contractile stimulus results in Ca^{2+} influx through voltage operated channels. Thus, in this tissue, nifedipine antagonises responses to KCl but not those to noradrenaline since the latter involve intracellular Ca^{2+} release and influx through receptor operated channels (Kanmura et al, 1983).

Nifedipine was kindly supplied by Bayer.

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STEREOSPECIFIC MECHANISM OF ACTION OF THE NOVEL ANTI-HYPERTENSIVE AGENT, BRL 34915

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BRL 34915, (±)6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl-2H-benzo[b]pyran-3-ol, is a potent new anti-hypertensive agent, structurally unrelated to existing cardiovascular drugs (Ashwood et al, 1984). In conscious, hypertensive animals (rats, cats and dogs) BRL 34915 lowers blood pressure at oral doses (0.01 - 1 mg/kg) 10 to 30 times lower than those required for nifedipine (Buckingham et al, 1984). BRL 34915 has been resolved into its constituent (+)- and (-)-enantiomers and these have been tested for anti-hypertensive activity and for effects in the vasculature in vitro.

BRL 34915 and its (-)-enantiomer were administered intravenously (i.v.) and orally (p.o.) within a dose range 30 - 1000 µg/kg to conscious, spontaneously hypertensive rats. Maximum falls in blood pressure (BP) were observed at the earliest time of recording i.e. 15 min and 1 h following i.v. and p.o. dosing respectively. The doses required to lower BP by 25% were calculated to be 50 µg/kg i.v. and 75 µg/kg p.o. for the (-)-enantiomer and 111 µg/kg i.v. and 210 µg/kg p.o. for BRL 34915. Thus, the (-)-enantiomer was 2.3 fold more potent than the racemate following i.v. administration and 2.8 fold after oral dosing. The similar potency i.v. and p.o. for the two compounds suggests excellent oral absorption. The (+)-enantiomer, however, at doses up to 3 mg/kg i.v. or p.o., produced much smaller changes in BP.

BRL 34915 may produce its blood pressure lowering effect by enhancing outward K^+ conductance in the vascular smooth muscle cell membrane (Hamilton et al, 1985). The mechanism of action of BRL 34915 has been studied using $^{86}RbCl$ as a marker for K^+ efflux in the rabbit isolated mesenteric artery (RIMA). Tissue (c.20 mg) was preloaded with $^{86}RbCl$ (2-5 mCi/mg; c.50 µCi/100 ml in a HEPES-physiological salt solution) for 90 min with the efflux of ^{86}Rb being determined at 3 min intervals over the subsequent 60 min. Drugs being studied were present from 30 to 48 min of the 60 min efflux period. BRL 34915 produced a 110% ± 11 (mean ± sem, n=6) increase in ^{86}Rb efflux rate at 1×10^{-5} mol.litre $^{-1}$. The (-)-enantiomer was slightly more potent in producing a 104% ± 25 (n=6) increase at 3×10^{-6} mol.litre^{-1} whilst the (+)-enantiomer only gave a 30% (±15) increase at 3×10^{-5} mol.litre^{-1}.

The stereospecific effects of the enantiomers have also been demonstrated in RIMA under tension where the contractions due to 3×10^{-2} mol.litre^{-1} KCl were inhibited by the (-)-enantiomer at concentrations greater than 1×10^{-7} mol.litre^{-1}. The (+)-enantiomer, however, was without effect at 1×10^{-5} mol.litre^{-1}.

Thus, BRL 34915 acts by a stereospecific mechanism with the biological activity primarily residing in the (-)-enantiomer. This stereospecificity can be demonstrated in vivo with respect to anti-hypertensive action and in vitro in RIMA and suggests that a specific recognition site exists for the compound which results in a hyperpolarisation of vascular smooth muscle membranes.

The authors are indebted to Dr E. Faruk for preparation of the enantiomers of BRL 34915.

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THE ANTIULCER ACTION OF GABAMIMETICS INVOLVES MORE THAN AN ANTISECRETORY MECHANISM

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The role (either central or peripheral) of GABA in ulceration and gastric acid secretion (G.A.S) is controversial (Bhargava et al, 1985; Goto & Watanabe, 1979; Szabo et al, 1982;Levine et al, 1981;Goto & Debas, 1983). Lloyd & Prouteau (1985) reported that the mixed $GABA_{A+B}$ agonists progabide and SL 75.102 reduce phenylbutazone (PBT) and stress ulcers and in parallel decrease G.A.S. Similar profiles were not observed with either specific $GABA_A$ (THIP) or $GABA_B$ (baclofen) agonists.

In the present study we have enlarged the profile of antiulcer activity of $GABA_A$ (THIP), $GABA_B$ (baclofen) and $GABA_{A+B}$ (progabide, SL 75.102, fengabine) mimetics and examined in parallel the action of these compounds on G.A.S and mucous secretion.

Model	ED_{50} (mg/kg p.o.) for Ulcers, G.A.S. ED_{100} for Mucous Secretion					
	$GABA_{A+B}$ Mimetics			$GABA_A$	$GABA_B$	$AntiH_2$
	Progabide	SL 75.102	Fengabine	THIP	Baclofen	Cimetidine
Stress Ulcers	130	340	10	> 10	EX	15
PBT Ulcers	190	225	200	1.7	EX	3
Shay Ulcers	100	100	NT	10	10	> 100
Ethanol Ulcers	28	13	NT	> 10	BIPH	> 100
G.A.S.	31	55	80	16(T)	0.3(B)	5
Mucous Secretion	200	200	NT	NT	NT	NT

Animals used were female Wistar rats (180-250g)fasted for 20-48h.
EX = exacerbation; (T) = toxic dose; (B) Bell shaped curve; NT = not tested; BIPH = Biphasic response.

The mixed $GABA_{A+B}$ mimetics were active in all four ulcer models whereas THIP was active only versus PBT and pyloric ligation.Baclofen exerted a clear antiulcer action only in the pyloric ligation model whereas it exacerbated stress and PBT ulcers. Bicuculline (2mg/kg i.p.) partially reversed (50%) the antiPBT action of SL 75.102.These results suggest that $GABA_A$ receptor activation reduces PBT ulcers whereas $GABA_B$ receptors modulate pyloric ligation ulcers. $GABA_A$ and $GABA_B$ receptors appear to be antagonistic with regards to G.A.S.

These observations together with the action of $GABA_{A+B}$ mimetics on ethanol ulcers (in which antisecretory agents are inactive viz-cimetidine), suggest that the antiulcer action of $GABA_{A+B}$ mimetics is at most partially related to G.A.S. As these compounds increase gastric mucous production at doses which are within the antiulcer range, this is a probable additional mechanism of action.

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ELECTRICALLY-EVOKED RELEASE OF (^{3}H)-BETAXOLOL FROM RAT ATRIAL SLICES

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The reduction of vasoconstrictor responses to nerve stimulation observed in a variety of preparations after chronic administration of β-blockers (Lewis, 1974 ; Russell et al., 1983) is thought to be due to the tissue accumulation of these drugs and their subsequent release. We have tested whether the cardioselective β_1-adrenoceptor antagonist betaxolol (BTXL) (Cavero et al., 1983) can be accumulated and retained in the rat atria to be subsequently released by electrical stimulation. Atrial slices from ZM/ZD rats (200 - 220 g, Zivic Miller Labs, U.S.A.) sham operated or with stellate ganglionectomy (S-GNCT) (20-28 d) were incubated for 30 min with 0.1 μM ^{3}H-BTXL (S.A. 49 Ci/mmol), or for 15 min with 0.5 μM (±) ^{3}H-NA (S.A. 9.4 Ci/mmol) and perfused in Krebs medium containing 2.6 mM Ca^{2+}. Electrical stimulation (E.S.) was performed in both cases, at 5 Hz, 24 mA, 2 ms for 2 min. Endogenous NA levels in atria were measured by HPLC chromatography with electrochemical detection.

Table 1 : Effects of stellate ganglionectomy on the release of ^{3}H-BTXL and ^{3}H-NA

	^{3}H-BTXL		^{3}H-NA		Endogenous
	E.S.(a)	T (b)	E.S.(a)	T (b)	NA (ng/g)
SHAM	0.71 ± 0.22	48.2 ± 5.2	1.16 ± 0.15	42.6 ± 3.1	1188 ± 191
S-GNCT	0.20 ± 0.10*	39.9 ± 2.2	0.72 ± 0.13*	15.8 ± 1.6**	318 ± 98**
% Change	- 70	- 9	- 38	- 63	- 73

(a) total nCi released above the spontaneous outflow by E.S., T(b) nCi retained per slice at the end of the experiment. ** $p < 0.005$; * $p < 0.05$ vs corresponding sham. Control values are mean ± S.E.M. from 5 - 20 slices per group using at least 5 different animals in each group.

At 0.1 μM, ^{3}H-BTXL was accumulated in the atria with a tissue medium ratio of 12 ± 2.5 ($n = 3$). This phenomenon was temperature-dependent and the accumulation of ^{3}H-BTXL was reduced by 70 % at 0°C. Neither zero Na^+ nor 100 μM ouabain were able to reduce the accumulation of ^{3}H-BTXL. In atria from sham operated animals labelled with ^{3}H-BTXL, the percent of tissue radioactivity released by E.S. represented 2.14 ± 0.19 % ($n = 19$). This value was significantly reduced to 0.15 ± 0.09 % ($n = 7$, $p < 0.001$) in the absence of calcium. Surgical sympathetic denervation by S-GNCT markedly reduced both endogenous NA and the retention of ^{3}H-NA (Table 1), while it produced only a modest reduction on the release of ^{3}H-NA (Table 1). S-GNCT did not affect significantly the retention of ^{3}H-BTXL, while the release of radioactivity by E.S. was markedly reduced (Table 1).

Presynaptic facilitatory β-adrenoceptors in the rat atria (Adler-Graschinsky and Langer, 1975) are not blocked by BTXL, because they are of the β_2-subtype (Langer and Galzin, 1983) ; therefore it is unlikely that BTXL can influence NA release through blockade of these presynaptic β-adrenoceptors. However, the concomitant release of NA and BTXL by sympathetic nerve stimulation, observed under our experimental conditions, may represent a mechanism through which BTXL can reach selectively the innervated postsynaptic β_1-adrenoceptors and reinforce β-adrenoceptor blockade in the heart.

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CYCLIC AMP PHOSPHODIESTERASE IN HUMAN NEUTROPHILS: IDENIFICATION AND STIMULATION IN INTACT CELLS

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We have found recently that pretreatment of human polymorphonuclear neutrophil leukocytes with the selective adenylate cyclase stimulator, forskolin, does not prevent stimulated degranulation unless the cells are treated with cyclic nucleotide phosphodiesterase inhibitors (Nourshargh & Hoult, 1984). This suggested that under normal conditions cyclic AMP is unlikely to play a dominant antisecretory modulator function and that the cells contain active phosphodiesterase (PDE). The present experiments confirm this by direct assay and show that the activity of the enzyme can be augmented by various pretreatments of the cells.

Human neutrophils from healthy non-medicated donors were purified by density gradient centrifugation and hypotonic lysis of contaminating erythrocytes to > 95% purity, resuspended in pH 8.0 40 mM tris buffer containing 4 mM mercaptoethanol, subjected to three cycles freeze-thawing using liquid N_2 and centrifuged at 14,000g for 2 min. The resulting supernatant was used as enzyme source for phosphodiesterase assay: various aliquots were incubated in a final volume of 0.4 ml containing 5 mM $MgCl_2$, 0.1 μCi ^{3}H-cyclic AMP (2.4 nmol per tube) ± cold cyclic AMP where necessary, incubated for 30 min at 30°C and boiled for 45 sec. After cooling 100 μg snake venom 5'-nucleotidase was added, incubated for 20 min and after adding 1 ml methanol, the extent of hydrolysis of cyclic AMP was quantitated by eluting the resulting labelled adenosine from anion exchange columns as described by Thompson et al (1979).

Phosphodiesterase activity was detectable under these conditions in cytosol derived from as few as 0.3 x 10^6 cells and was linearly proportional to amount of cytosol added and to time up to 20-30 min, with rates (pmol cAMP hydrolysed /min/mg protein) of 330-1190, compared to 60 for platelet cytosol prepared in similar fashion, or 0.6 and 33 in the 100,000g membrane pellet and 100,000g cytosol of bovine brain. The enzyme was inhibited by Ro 20-1724, papaverine and isobutylmethylxanthine with ID_{50} values (μM) of 0.45, 1.4 and 25, respectively. In neutrophils treated with 14 μM PGE_1 or PGE_2 or 50 μM forskolin, phosphodiesterase activity was enhanced in a time-dependent manner (peaking 2 to 5 min after addition of stimulant and returning to basal values at 10 min) when assayed in the cytosolic fraction of freeze-disrupted cells. Phorbol myristate acetate (10^{-8}M) had no such effect.

We conclude that human polymorphonuclear neutrophils contain a highly active cyclic AMP phosphodiesterase and that its functional activity within the cells is augmented in response to activation of adenylate cyclase. This may provide a regulatory mechanism for control of cyclic nucleotide metabolism.

S.N. thanks the M.R.C. for a research studentship.

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PROTEIN KINASE C: A REGULATOR OF CALCIUM MOBILISATION AND GRANULE RELEASE IN HUMAN PLATELETS

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The tumour-promoting tigliane ester, 12-O-tetradecanoylphorbol-13-acetate (TPA) is a potent platelet aggregating agent, whose probable site of action is the phospholipid-dependent protein kinase C. TPA is thought to substitute for the physiological activator of protein kinase C, diacylglycerol, which is a product of receptor operated (poly)phosphoinositide hydrolysis (Nishizuka 1984).

The aim of this study was to determine the effect of two activators of protein kinase C (TPA and 12-deoxyphorbol-13-phenyl acetate (12-DOPP)), the inactive 4-β-phorbol, and a selective inhibitor of protein kinase C, 1-[5-isoquinoline sulphonyl]-2-methyl-piperazine (H-7) (Hidaka et al 1984) on human α-thrombin (HαT)-induced elevation of cytosolic free calcium concentration ($[Ca^{++}]_i$) and dense granule release. Prostacyclin washed human platelets loaded with quin 2 (a fluorescent indicator of $[Ca^{++}]_i$) and [^{14}C]-5HT (a dense granule marker) were utilized (Westwick et al 1985).

TPA (1.6-1600nM) or 12-DOPP (20-2000nM) failed to alter basal resting level of $[Ca^{++}]_i$, and produced a limited but dose-related release of [^{14}C]-5HT, as previously reported (Rink et al 1983, Westwick et al 1984) In contrast, HαT (77pM-7.7nM, n=8) produced a rapid and dose-dependent elevation of $[Ca^{++}]_i$ from basal value (88.5±5nM, n=68) to maximum level of >3μM, which preceded but closely paralleled 5HT release. Incubation of platelets with 1.6, 4.8, 16, 48 nM TPA for 1 min reduced submaximal HαT (0.77nM) elevated $[Ca^{++}]_i$ from 200+25nM to 175±27, 35±4, 20±3, 2±2 nM (N=6-8) and the corresponding [^{14}C]-5HT from 49.3±3 to 43±3, 27±5, 18±2, 17±2 respectively. Incubation of platelets with 6, 20 and 60 nM 12-DOPP produced similar dose-related inhibition of HαT-induced elevation of $[Ca^{++}]_i$ and release of 5HT; this effect did not occur with 15μM 4-β-phorbol (n=8). Furthermore, when 1.6-48nM TPA was added at the peak of thrombin-induced elevation of $[Ca^{++}]_i$, a dose-related acceleration of $[Ca^{++}]_i$ to basal values was observed, but not when 4-β-phorbol was added.

Pre-incubation of platelets for 3 min with H-7 (3,10,30,60μM; n=6) did not produce a statistically significant ($p > 0.05$) modulation of either submaximal (0.77nM) HαT-induced elevation of $[Ca^{++}]_i$ or [^{14}C]-5HT release. However, pre-incubation of platelets for 1 min with H-7 (60μM, n=6) prevented the TPA(4.8nM)-induced inhibition of thrombin-induced elevation of $[Ca^{++}]_i$ and 5HT.

TPA has recently been shown to inhibit agonist-induced elevation of $[Ca^{++}]_i$ (MacIntyre et al 1985). We have extended these findings by demonstrating that TPA and 12-DOPP inhibit not only the elevation of $[Ca^{++}]_i$ but also a functional response induced by HαT and that this inhibition is prevented by a selective protein kinase C inhibitor. Thus, protein kinase C may act to limit cellular activation by inhibiting calcium mobilization and granule release.

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ATP ANALOGUES AND THE GUINEA-PIG TAENIA COLI: DOES ENZYMIC BREAKDOWN DETERMINE PHARMACOLOGICAL POTENCY?

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Adenine nucleotides and their analogues have potent pharmacological effects on many tissues and are removed by sequential dephosphorylation by the ectonucleotidases ATPase, ADPase and AMPase (5'-nucleotidase) (Pearson, 1985). As differences in potency could reflect the structure-activity requirements of these ectoenzymes, we compared the rates of dephosphorylation of analogues of ATP, ADP and AMP by guinea-pig isolated taenia coli, with their known ability to induce relaxation of this smooth muscle preparation. The degradation of nucleotides was studied using h.p.l.c. (Hourani et al., 1985).

ATP (Km 125 μM), ADP (Km 100 μM) and AMP (Km 25 μM) were rapidly dephosphorylated, each with a half life for the removal of the terminal phosphate of approximately 20 min. ATP analogues substituted on the purine ring, including 2-chloro-ATP, 2-methylthio-ATP, 8-bromo-ATP and N^6-phenyl-ATP, were all dephosphorylated to the corresponding ADP analogues and at the same rate as ATP itself, although they exhibit widely differing potencies as relaxants (Satchell & Maguire, 1975).

Stereoselectivity of the ectoenzymes towards the ribose sugar was tested by using the unnatural L-ribofuranosyl analogues of adenine nucleotides. L-ATP was degraded to L-ADP (half life=35 min), but further degradation was extremely slow, although the enantiomers of ATP and ADP are virtually equipotent on this tissue (Cusack & Planker, 1979).

Stereoselectivity of the ectoenzymes towards the phosphate chain was tested by using the Rp and Sp diastereoisomers of the phosphorothioate analogues ATP-α-S, ATP-β-S and ADP-α-S. The Rp and Sp isomers of ATP-α-S were degraded to ADP-α-S at the same rate as ATP to ADP, and this lack of stereoselectivity is in contrast to their pharmacological potencies, where Rp-ATP-α-S is more potent than Sp-ATP-α-S and both isomers are more potent than ATP itself (Burnstock et al., 1984). Sp-ADP-α-S was subsequently dephosphorylated to AMPS at the same rate as ADP to AMP, but Rp-ADP-α-S was degraded at only half that rate, and AMPS was resistant to further degradation. Rp-ATP-β-S was degraded to ADP-β-S at the same rate as ATP to ADP, but Sp-ATP-β-S was degraded at less than half that rate, and ADP-β-S was resistant to further degradation. This stereoselectivity of the ectonucleotidases towards ATP-β-S is in contrast to the lack of stereoselectivity for relaxation. ATP-γ-S was resistant to dephosphorylation, yet this analogue is equipotent with ATP as a relaxant (Burnstock et al., 1984).

Our results suggest that the differing potencies of adenine nucleotide analogues at inducing relaxation of the guinea-pig isolated taenia coli cannot be attributed to differences in their rates of degradation.

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2-MeS-AMP-PCP AND HUMAN PLATELETS: IMPLICATIONS FOR THE ROLE OF ADENYLATE CYCLASE IN ADP-INDUCED AGGREGATION?

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Adenosine 5'-diphosphate (ADP) causes human platelets to aggregate (Born, 1962) and inhibits stimulated platelet adenylate cyclase (Haslam & Rosson, 1975). Both of these effects are competitively inhibited by adenosine 5'-triphosphate (ATP) and some of its analogues (Cusack & Hourani, 1982a).

An analogue of ATP, 2-methylthio-adenylyl 5'-(β,γ-methylene-)-diphosphonate (2-MeS-AMP-PCP), which cannot be degraded to the potent agonist, 2-methylthio-ADP, was tested as an inhibitor of ADP-induced aggregation of human platelets. Like other 2-alkylthio analogues of ATP and of AMP (Cusack & Hourani, 1982b), it was a specific but noncompetitive inhibitor. It did not inhibit aggregation induced by 11α,9α-epoxymethano prostaglandin H_2, adrenaline, 5-hydroxytryptamine, arachidonic acid or platelet activating factor. The inhibition by 2-MeS-AMP-PCP of ADP-induced aggregation could not be overcome by high concentrations (100 μM) of ADP.

2-MeS-AMP-PCP, even at a concentration of 100 μM, only achieved about 50% inhibition of aggregation induced by 5 μM ADP, suggesting that it inhibits only one component of the action of ADP. However, it achieved 100% inhibition of the effect of 5 μm ADP on stimulated adenylate cyclase, with the same EC_{50} value (about 0.5 μM) as for inhibition of aggregation.

These results imply that the component blocked by 2-MeS-AMP-PCP may be the inhibition by ADP of adenylate cyclase. A Schild plot was constructed for the inhibition by 2-MeS-AMP-PCP of the effect of ADP upon stimulated adenylate cyclase; a slope of 1.12 was obtained, suggesting that inhibition was competitive. Although the inhibition of adenylate cyclase is not considered to be the mechanism by which ADP causes aggregation (Haslam et al., 1978), our results suggest that it may after all be required for the full expression of platelet aggregation induced by ADP. This interpretation is supported by the finding that 2-MeS-AMP-PCP failed to inhibit aggregation induced by adenosine 5'-(1-thiodiphosphate) (which does not inhibit adenylate cyclase) (Cusack & Hourani, 1981a) but did inhibit aggregation induced by adenosine 5'-(2-thiodiphosphate) (which does inhibit the enzyme) (Cusack & Hourani,1981b).

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FUNCTIONAL β_1- AND β_2-ADRENOCEPTORS IN THE HUMAN HEART: QUANTITATIVE ANALYSIS OF THE REGIONAL DISTRIBUTION

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Evidence is rapidly accumulating that in human heart functional β_1- and β_2-adrenoceptors(R) coexist (Brodde et al.,1983; Heitz et al., 1983; Stiles et al.,1983); the regional distribution of these β-R subtypes, however, has not been determined. Thus, we determined the amount of β_1- and β_2-R in human right and left atrium as well as in right and left ventricular wall obtained from heart transplant recipients who suffered from end-stage heart failure.

Myocardial tissue from all four chambers was obtained from three explanted hearts of heart transplant recipients of the Hannover Medical School Heart transplant program. All 3 patients with end-stage heart failure (NYHA class IV) had the preoperative histologically verified diagnosis "idiopathic congestive cardiomyopathy(COCM)". Cardiac membranes were prepared as recently described (Brodde et al.,1984); the total β-R number was assessed by (-)-^{125}iodocyanopindolol(ICYP) binding (Brodde et al.,1983); concomitantly β_1-R number was assessed by binding with the selective β_1-R radioligand (-)[^{3}H]bisoprolol (Wang et al.,1985). In parallel experiments the amount of β_1- and β_2-R was determined indirectly by non-linear regression analysis of competition curves of the selective β_1-R antagonist bisoprolol (Wang et al., 1985) and the selective β_2-R antagonist ICI 118,551 (Bilski et al., 1983) with ICYP binding.

The highest number of total β-R was found in left atrium (63.4 fmol/mg protein) followed by right atrium (39.5 fmol/mg protein), whereas in right and left ventricular wall total β-R number was slightly smaller. On all four cardiac tissues the number of sites labelled by (-)[^{3}H]bisoprolol(i.e. β_1-R) was less than that labelled by ICYP(i.e. total β-R) indicating that on all 4 tissues β_1- and β_2-R coexist; the β_1/β_2-ratio amounted in the atria to 65/35% and in the ventricles to 75/25%. Identical results were obtained when the β_1/β_2-ratio was calculated from bisoprolol and ICI 118,551 competition curves with ICYP binding. Atrial as well as ventricular β_1- and β_2-R are coupled to the adenylate cyclase since noradrenaline and the β_2-agonist procaterol were capable in activating adenylate cyclase in all 4 cardiac tissues. Compared with data from normal hearts (Brodde et al.,1983; Stiles et al.,1983) in end-stage COCM there seems to be a selective down-regulation of β_1-R (presumably due to elevated plasma noradrenaline levels), while β_2-R number is obviously not altered.

It is concluded that in right and left atrium as well as in right and left ventricular wall of the human heart functional β_1- and β_2-R coexist, which might be involved in the physiological regulation of contractility and/or heart rate.

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VASCULAR α_2-ADRENOCEPTOR FUNCTION IN CONSCIOUS DOCA-SALT HYPERTENSIVE RATS

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Goldstein et al. (1982) observed that SK&F 64139 and SK&F 72223 reduced blood pressure in DOCA-salt and spontaneously hypertensive rats, and suggested that this effect might be attributable to blockade of vascular α_2-adrenoceptors. Similar findings have been made with SK&F 86466 (Hieble et al., 1985). Langer and Hicks (1984) have also suggested that vascular α_2-adrenoceptors may play a pathophysiological role in maintaining hypertension. We have investigated this proposal further by examining the antihypertensive effects of two other selective α_2-adrenoceptor antagonists, RX 781094 (Idazoxan; Chapleo et al., 1981) and Wy 26703 (Lattimer et al., 1982) in conscious DOCA-salt hypertensive rats. The effects of the selective α_1-adrenoceptor antagonist, prazosin, were examined for comparison.

Blood pressure was recorded from a cannula implanted in the aortic arch via the left common carotid artery. Submaximal doses of the selective α_1- and α_2-adrenoceptor agonists, phenylephrine (PE, 0.5 µg) and M-7 (5.0 µg) (Drew, 1980), were both injected into each rat via this cannula at intervals of 10-20 min, before and after intraperitoneal injection of antagonist or saline, to determine the degree of vascular α-adrenoceptor blockade. PE and M-7 produced pressor responses ($\sim$ 35-65 mmHg) that were little affected in individual animals over 2 hours by saline (1 ml/kg i.p.). Prazosin (1 mg/kg i.p.) reduced blood pressure (maximum 40 mmHg, duration > 2 hours) and increased heart rate: pressor responses to M-7 were little affected but those to phenylephrine were greatly reduced. Idazoxan (10 mg/kg i.p.) and Wy 26703 (10 mg/kg i.p.) reduced blood pressure, by a maximum of 40 and 55 mmHg, respectively, and for periods ranging between 60 and 80 min. Heart rate increased after both drugs. Idazoxan almost abolished pressor responses to PE and M-7 for 2 hours after dosing. Wy 26703 greatly reduced the responses to both agonists over the 30 min following dosing; responses recovered progressively over the next 90 min but always lagged behind the recovery of basal blood pressure.

A lower dose of Wy 26703 (1 mg/kg) i.p. slightly reduced resting blood pressure (9 mmHg) but produced a marked, transient (10 min) tachycardia; the pressor response to M-7 was reduced by 47% at this time but recovered rapidly thereafter. Changes in response to PE were small and similar to those in saline treated animals.

Further evaluation showed that, during the 30 min after dosing, Wy 26703 (1 mg/kg) produced a 5 fold shift to the right in the dose response curve to M-7 (1-10 µg i.a.) but had little effect on responses to PE (0.1-1 µg i.a.).

These results show that the hypotensive effect of prazosin is accompanied by a selective reduction in responses to PE. In contrast, the hypotensive effects of Idazoxan and Wy 26703 cannot be attributed to the selective blockade of vascular α_2-adrenoceptors. Furthermore, in the case of Wy 26703, some degree of selective blockade of vascular α_2-adrenoceptors can be produced without much change in blood pressure. These data cast doubt on the potential value of selective α_2-adrenoceptor antagonists as antihypertensive agents.

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EFFECTS OF REVERSIBLE NORADRENERGIC DENERVATION ON α_1-ADRENOCEPTORS OF AVIAN SMOOTH MUSCLE

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The expansor secundariorum (ESM) is a discrete smooth muscle in the avian wing that receives a dense noradrenergic innervation (Bennett and Malmfors, 1970). We have carried out ligand-binding studies on α_1-adrenoceptors in the ESM using [^{3}H]-prazosin after reversible noradrenergic denervation with 6-hydroxydopamine (6OHDA).

To examine the effects of 6OHDA, chicks (White Leghorn X Rhode Island Red cross, 22-25 days old) were injected i.v. with either 6OHDA (100mg/kg in saline + 0.1% ascorbic acid) or with vehicle only (controls). At various times after injection chicks were killed by decapitation, the ESMs removed and homogenized in HEPES-buffered physiological medium (composition mM: NaCl 110, KCl 5.3, $CaCl_2$ 1.8, $MgSO_4$ 0.8, NaH_2PO_4 0.9, glucose 25, sucrose 50, HEPES 20, pH 7.4). Aliquots (0.4ml, 400-600µg protein) of ESM homogenate were incubated with [^{3}H]-prazosin (1.2nM final) in a total volume of 1ml for 30 minutes at 25°C. Bound and free radioligand were separated by filtration over Whatman GF/B filters. Samples were assayed in quadruplicate and non-specific binding defined with 3µM phentolamine.

Table 1 shows specific [^{3}H]-prazosin binding in ESMs from control and 6OHDA treated chicks. Non-specific binding was not altered by 6OHDA treatment.

Table 1

Days after injection	n	Specific [^{3}H]-prazosin bound (fmoles/mg protein, mean ± s.e. mean) Control	6OHDA treated
1	5	20.7 ± 2.9	12.8 ± 1.4*
3	5	18.9 ± 0.6	11.7 ± 0.9***
7	8	21.1 ± 2.3	13.7 ± 1.7*

* $p<0.05$, *** $p<0.001$ by Student's unpaired t-test, compared to control.

Thus 6OHDA treatment produced a 34 to 38% decrease in the apparent number of α_1-adrenoceptors in ESM up to 1 week after injection. By 1 day after treatment there is an almost complete loss of noradrenergic terminals in ESM when assessed by histochemical (Bennett et al., 1970) or functional (unpublished) studies. By 7 days after 6OHDA treatment the ESM is partially re-innervated and maximum nerve-stimulated muscle contraction returns to 40% of control values (unpublished). It is possible that the decrease in the number of α_1-adrenoceptors following denervation reflects a loss of prejunctional receptors. However, since the change in receptor number was not correlated with the time of reappearance of noradrenergic nerve terminals, it is likely that the α_1-adrenoceptors are predominantly located post-junctionally. We have recently identified post-junctional α_1-adrenoceptors which mediate contraction of the ESM.

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THE EFFECTS OF OESTRADIOL ON α_2- AND β-ADRENOCEPTOR FUNCTION IN RAT BRAIN AND IT'S INFLUENCE ON THE ACTIONS OF DESIPRAMINE

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Human platelet α_2-adrenoceptor binding is altered by changes in circulating levels of oestrogen and progesterone (see Metz et al, 1983). Moreover, these authors suggested that this may reflect changed central α_2-adrenoceptor function and that such changes may be linked with the "maternity blues" syndrome. We have, therefore, investigated the effects of repeated administration of oestradiol on α_2- and ß-adrenoceptor function in rat brain, when given either alone or in combination with the antidepressant, desipramine (DMI).

Sexually mature female CD rats (Charles River) initially weighing 100-120 g were used. Oestradiol valerate (100 µg s.c.) or pristane vehicle (200 µl s.c.) were injected once daily starting day 0. Desipramine (DMI; 5 mg/kg i.p.) was injected twice daily starting day 1. Experimental studies were performed 18 h after the final injection of DMI and oestradiol. α_2-Adrenoceptor function was evaluated by measurement of clonidine-induced hypoactivity (sedation) responses (days 5, 10 and 13) and cortical [^{3}H]-idazoxan binding (days 5 and 15). ß-Adrenoceptor binding in cortex was estimated using [^{3}H]-dihydroalprenolol (DHA) (days 5 and 15). The hypoactivity induced by clonidine (0.5 mg/kg) was rated 0-3 on 4 behavioural parameters as described previously (Heal et al, 1983). Cortical membranes were prepared and the specific binding of [^{3}H]-idazoxan (0.4-4 nM) and [^{3}H]-DHA (0.4-4 nM) were defined with 5 µM phentolamine and 200 µM isoprenaline respectively.

In a preliminary experiment, when a dose-response curve to clonidine (0.05-1.0 mg/kg) was constructed for age-matched male and female rats, the latter were much less susceptible to the sedative effects of clonidine. Repeated DMI administration produced a time-dependent decrease in clonidine responses which was maximal at 9-12 days and agrees with the reduction previously reported to occur in male rats (Heal et al, 1983). Although DMI alone produced no alteration after 4 days administration, when this drug was given with oestradiol, the combination produced a marked reduction in hypoactivity. The maximal reduction in clonidine responses was, however, unaffected by oestradiol. Furthermore, oestradiol alone had no effect on hypoactivity after 5 or 10 days administration but did marginally enhance this response after 13 days. DMI administration had no effect on [^{3}H]-idazoxan binding at 4 days and produced a non-significant reduction after 14 days. This reduction became significant ($P < 0.02$) when DMI was given with oestradiol. Oestradiol alone had no effect. [^{3}H]-DHA binding was equally reduced by 4 or 14 days DMI administration. While oestradiol did not alter this reduction, it produced a modest increase in ß-adrenoceptors when given alone for 14 days.

In conclusion, these results show that although repeated oestradiol administration produces modest effects on central noradrenergic function which are the reverse of those induced by antidepressant drugs, paradoxically, it also moderately potentiates some of the possible therapeutic actions of DMI when these drugs are given together.

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PLATELET α2 ADRENORECEPTORS LABELLED WITH THE AGONIST ^{3}H-UK-14,304 AND THE ANTAGONIST ^{3}H-YOHIMBINE

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Most binding studies of platelet α2 adrenoreceptors use antagonist radioligands. Recently ^{3}H-UK-14,304 (5 bromo-6-[2-imidazolin-2-yl-amino]- quinoxaline) was shown to be a good agonist ligand for labelling brain α2 adrenoreceptors (Loftus et al, 1984). Since UK-14,304 is a potent and full agonist in platelet α2 adrenoreceptor mediated aggregation (Grant & Scrutton, 1980), we have used ^{3}H-UK-14,304 as a radioligand in human platelet membranes and compared its binding with that of the antagonist ^{3}H-Yohimbine (^{3}H-YOH). ^{3}H-YOH and ^{3}H-UK-14,304 binding were performed essentially as previously described (Daiguji et al, 1981; Loftus et al, 1984). Saturation assays of ^{3}H-UK-14,304 included 0.1 mM $MnCl_2$.

Binding of ^{3}H-UK-14,304 reached equilibrium by 20 min, was stable for up to 2h at 25°C and varied linearly with protein in the range 50-130 µg per sample.

Drugs displaced ^{3}H-UK-14,304 and ^{3}H-YOH with the same rank order of potency. α2 Adrenoreceptor agonists (UK-14,304, adrenaline, noradrenaline) were more potent in displacing ^{3}H-UK-14,304 (IC_{50} 3-24 nM) than ^{3}H-YOH (58-650 nM) while antagonists (YOH and phentolamine) were more potent at displacing ^{3}H-YOH (6-16 nM) than ^{3}H-UK-14,304 (23-65 nM). IC_{50} values for methysergide, prazosin and propranolol were between 1.4-40 µM. This supports the view that both ligands label α2 adrenoreceptors in the platelet. Saturation analysis showed that the specific binding of both ligands was well fitted to a single population of sites. However ^{3}H UK-14,304 consistently labelled fewer sites (62-65%) than ^{3}H-YOH, in both freshly prepared and previously frozen membranes (Table 1).

Table 1 Binding of ^{3}H UK-14,304 and ^{3}H-YOH to platelet membranes

	Fresh membranes (n=6)		Frozen membranes (n=7)	
	Bmax	K_D	Bmax	K_D
^{3}H-UK-14,304	220±22	0.46±0.04	232±20	1.8±0.1
^{3}H-YOH	350±41	1.8±0.1	357±41	3.8±0.6

Values are means ± s.e.m. K_D=nM. Bmax=fmoles/mg protein

Displacement of ^{3}H-YOH (2.4 nM) by unlabelled YOH indicated a single binding site, with a K_D of about 4.3 nM similar to that from saturation plots and with a slope factor (De Lean et al, 1982) close to unity. However, displacement of ^{3}H-YOH by unlabelled UK-14,304 gave a slope factor of about 0.6 and was best fitted to two binding components, one with a K_D of 2.3 nM, similar to that obtained from saturation analysis, and a second site with a K_D of about 100 nM; there being about twice as many low affinity sites as high affinity sites.

The difference in the number of binding sites labelled by the two ligands reflects ^{3}H-UK-14,304 binding to predominantly high affinity binding sites, whereas ^{3}H-YOH labels the total α2 adrenoreceptor population.

We suggest that ^{3}H-UK-14,304 is a good ligand for labelling high affinity agonist α2 adrenoreceptor binding sites in the platelet.

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TWO COMPONENTS TO THE SPASMOGENIC ACTION OF OXYTOCIN IN THE RAT UTERUS

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A low concentration (0.2 nM) of oxytocin-induced phasic spasms of the isolated uterus of the day 22 pregnant rat. These spasms were sensitive to inhibition by several calcium (Ca) antagonists and were associated with a small increase in ^{45}Ca uptake (Granger et al, 1985a,b). Higher concentrations of oxytocin (2 and 20 nM) did not increase ^{45}Ca uptake. We have now further examined the mechanism of the spasmogenic action of oxytocin and have made comparisons with responses to bradykinin and KCl.

Cumulative concentration-effect experiments were performed with oxytocin, KCl or bradykinin alone and repeated after 40 min incubation in the presence of (+)-cis diltiazem (0.1 to 100 μM). The responses to the three agonists consisted of phasic spasms at low concentrations and predominantly tonic spasms at higher concentrations. The phasic component of the responses to oxytocin and to bradykinin and both components of the response to KCl were inhibited by (+)-cis diltiazem (0.1 and 1 μM). The tonic component of the responses to oxytocin and bradykinin was only reduced by (+)-cis diltiazem at concentrations > 10 μM.

(-)-cis diltiazem was less potent than (+)-cis diltiazem as an inhibitor of Ca^{2+}-induced spasm in a depolarising medium and of the phasic spasms to oxytocin. The two isomers were of similar potency as inhibitors of the tonic spasm to oxytocin.

Oxytocin (0.2 nM) increased the frequency, duration and amplitude of spike activity (measured by the extracellular electrical recording technique of Golenhofen & v.Loh, 1970) in parallel with the enhancement of phasic spasms. With higher concentrations of oxytocin (2 and 20 nM) spike firing was often continuous initially but subsequently often ceased despite the associated tonic contracture. After 40 min incubation in (+)-cis diltiazem (10 μM), oxytocin (0.2, 2 and 20 nM) produced graded tonic spasm without spike activity.

In the term pregnant rat low concentrations of oxytocin produced phasic spasms of the uterus which were sensitive to inhibition by several Ca antagonists, inhibited stereospecifically by diltiazem and associated with spike activity and ^{45}Ca influx. These observations are consistent with this action being related to increased Ca influx via voltage-dependent Ca channels. In addition, at higher concentrations oxytocin produced a tonic spasm which was inhibited by high concentrations of diltiazem in a non-stereospecific manner and was not associated with spike activity or ^{45}Ca influx. These results suggest the tonic spasm is due to another mechanism which does not appear to involve Ca influx.

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EFFECTS OF ROLIPRAM ON GUINEA-PIG VENTRICLES IN VITRO: EVIDENCE OF AN UNEXPECTED SYNERGISM WITH SK&F 94120

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Selective inhibitors of Phosphodiesterase (PDE) III, eg. SK&F 94120 and amrinone, have a positive inotropic action on guinea-pig right ventricles in vitro (Gristwood et al, 1985). Rolipram (ZK 62711), which is currently undergoing development as an antidepressant agent, has recently been described as a selective inhibitor of PDE III (Weishaar et al, 1985) and we have now investigated the effects of this on force of contraction (Fc) and intracellular cyclic AMP concentration ([cAMP]) in guinea-pig isolated right ventricles. In addition we have studied the interaction of rolipram with SK&F 94120.

Guinea-pig right ventricular strip preparations were incubated in Krebs solution (gassed with 5% CO_2 in O_2) at 37°C and electrically stimulated to contract at 1 Hz whilst force of contraction (Fc) was measured. In some experiments intracellular [cAMP] was measured using radioimmunoassay (NEN).

Rolipram at concentrations up to 3.2×10^{-5}M had no effect on Fc and at 1×10^{-4}M caused a small (mean 64 ± 6%) increase in Fc, n=3. Rolipram at 3.2×10^{-5}M, a concentration which did not increase Fc, significantly increased intracellular [cAMP] from 0.51 ± 0.11 to 1.26 ± 0.10 p moles/mg wet wt, n=5.

SK&F 94120 alone caused concentration dependent increases in right ventricular Fc; 15 ± 10% at 1×10^{-5}M, 67 ± 18% at 3.2×10^{-5}M and 134 ± 41% at 1×10^{-4}M, n=3. Responses to SK&F 94120 obtained in the presence of rolipram 3.2×10^{-5}M were significantly larger than control, increases in Fc being; 43 ± 9% at 3.2×10^{-6}M, 181 ± 30% at 1×10^{-5}M, 409 ± 26% at 3.2×10^{-5}M and 578 ± 46% at 1×10^{-4}M, n=3. Although SK&F 94120 has been shown to increase [cAMP] in human ventricle (Cameron et al, 1985), in guinea-pig ventricle SK&F 94120 1×10^{-4}M alone had little measurable effect on intracellular [cAMP]; control 0.89 ± 0.13, treated 1.0 ± 0.18 p moles/mg wet wt, n=3. SK&F 94120 1×10^{-4}M and rolipram 3.2×10^{-5}M in combination, however, caused a marked increase in [cAMP]; control 0.70 ± 0.14, treated 1.86 ± 0.34 p moles/mg wet wt, n=5.

The above data demonstrate a marked potentiation of SK&F 94120 cardiac responses by rolipram. This interaction is not that expected of 2 PDE III inhibitors acting in combination, no synergism was observed between SK&F 94120 and amrinone, another known selective inhibitor of PDE III . This coupled with the dissociation between the increase in [cAMP] and the very weak inotropic action suggests that rolipram has a primary mechanism of action independent of PDE III inhibition.

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POTENTIATION OF ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE BY PLATELET-ACTIVATING FACTOR

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The association between septic shock and gastrointestinal ulceration is well established (Skillman et al., 1970). Recently, a role for platelet-activating factor (PAF) in endotoxic shock has been proposed (Doebber et al., 1985; Terashita et al., 1985). We have therefore investigated the possible role of PAF as a pro-ulcerogenic compound by assessing its effects on the susceptibility of the rat gastric mucosa to damage induced by topically applied 20% ethanol.

Rats (n = 4 to 6 per group) were anaesthetized with sodium pentobarbitone (60 mg/kg i.p.) and an ex vivo gastric chamber was prepared as described previously (Mersereau and Hinchey, 1973). Systemic arterial blood pressure (BP) was measured throughout each experiment, which consisted of six sequential ten-minute periods. At the beginning of each period a fresh solution was added to the chamber. During the first two periods, 0.3 M mannitol bathed the mucosa. In the third period, 20% ethanol (vol/vol) or 0.3 M mannitol was added to the chamber. In the final three periods the bathing solution was 0.05 M HCl in 0.2 M mannitol. PAF (0.3 to 100 ng/kg/min), lyso-PAF (100 ng/kg/min) or vehicle was infused into a femoral vein during minutes 17 to 22. At the end of the experiment the extent of macroscopically-visible haemorrhagic damage was quantified planimetrically (Wallace et al., 1982).

In rats exposed to 20% ethanol alone (vehicle infused i.v.), haemorrhagic damage was limited to the periphery of the chamber and involved only 1.1 ± 0.5% of the total area of the glandular mucosa. The 5-min infusion of PAF caused a dose-dependent increase in the extent of haemorrhagic damage. With a dose of PAF of 1 ng/kg/min there was a significant ($p<0.05$) increase in the area of damage (6.2 ± 1.5%), despite this dose having no significant effect on BP. Infusions of PAF at doses of 10 ng/kg/min or greater caused a rapid, dose-dependent fall in BP. These doses of PAF (10, 30 and 100 ng/kg/min) increased the extent of haemorrhagic damage induced by 20% ethanol to 8.0 ± 3.4, 23.3 ± 4.4 and 81.3 ± 4.2%, respectively. Lyso-PAF (100 ng/kg/min) infusion had no significant effect on the BP or on the extent of gastric damage induced by 20% ethanol (2.5 ± 0.9%). In the series of experiments in which 0.3 M mannitol was added to the chamber in place of 20% ethanol, the 5-min infusion of PAF at doses of 0.3 to 30 ng/kg/min did not cause haemorrhagic damage. However, infusion of PAF at 100 ng/kg/min did produce a significant ($p<0.05$) level of gastric damage (6.9 ± 1.1%).

This study demonstrates that PAF is a potent pro-ulcerogenic compound in the rat gastric mucosa. The hypotensive action of PAF may contribute to its pro-ulcerogenic actions, but does not appear to be the sole mechanism of damage.

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POTASSIUM-, METHACHOLINE- AND HISTAMINE-INDUCED RELAXATIONS OF LONGITUDINAL STRIPS OF GUINEA-PIG TRACHEA

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It is well recognized that in the mammalian trachea smooth muscle is arranged in a transverse manner between the two arms of the C-shaped cartilage rings. In addition, bands of longitudinally-oriented smooth muscle have recently been described in the guinea-pig trachea (Satchell & Smith, 1984). Although the existence of the longitudinal smooth muscle has been known for some time (Nagaishi, 1972) its function is unknown. In contrast to transverse muscle, very little is known about the pharmacology of the longitudinal smooth muscle. In the present study we have examined the responses of isolated tracheal longitudinal strips to drugs.

Trachea were removed from male, English short-hair guinea pigs (400 - 500 g). Two transverse strips (each from 2 cartilage rings) and two longitudinal strips (cartilage-free) were prepared from each trachea and placed in 10 ml baths of oxygenated modified Krebs solution (37° C). Resting loads of 1 g and 250 mg were applied to transverse and longitudinal strips respectively, tissues equilibrated for 1 h and isometric responses to drugs recorded. While KCl, methacholine (MCh) and histamine (Hist) produced concentration-dependent contractile responses of transverse strips, these drugs rather surprisingly, caused relaxation of longitudinal strips. The maximum relaxation to KCl was 89 $\pm$ 8 mg (n = 12) with an EC_{50} of 35 mM. The EC_{50} for KCl in transverse strips was 14 mM. The maximum relaxation produced by MCh (1 mM) in longitudinal strips was 115.5 $\pm$ 8.6 mg (n = 10), although in two preparations MCh caused concentration-dependent contractions of 80 and 120 mg. The EC_{50} for MCh in longitudinal strips (1.62 µM) was similar to that in transverse strips (1.33 µM). Relaxant responses of longitudinal preparations to KCl and MCh were unaffected by 2 µM TTX or 10 µM indomethacin, although 1 µM atropine completely reversed the relaxation produced by 30 µM MCh. Hist induced contractions of transverse strips (EC_{50} = 11.4 µM) and relaxations of longitudinal strips (EC_{50} = 20.2 µM). The maximum relaxation (to 0.1 mM) was 115 $\pm$ 11.2 mg (n = 6) and this was completely reversed by 2 µM diphenhydramine. Isoprenaline (10 nM - 1 µM) caused relaxations of transverse strips, whereas concentrations as high as 100 µM had no effect on longitudinal strips of trachea.

These results are interesting though very unusual in that KCl, MCh and Hist, which normally cause contraction of guinea-pig tracheal transverse smooth muscle, caused relaxation of longitudinal strips. That relaxations were not affected by TTX suggests a direct effect on the smooth muscle. Relaxant responses to MCh are muscarinic since they were blocked by atropine, and those to Hist are mediated by H_1-receptors since they were blocked by diphenhydramine. These observations however, must be viewed with caution. Apparent "relaxations" of longitudinal strips by bronchoconstrictors may be a consequence of lengthening of the tissue due to contraction of the transverse muscle cells present in the preparation. That the EC_{50} for MCh in both preparations was similar suggests this. However it should be borne in mind that longitudinal strips were approximately 2-fold less sensitive to both KCl and Hist than transverse strips.

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EFFECTS OF RESERPINE OR 6-HYDROXYDOPAMINE ON RESPONSES OF MOUSE AND RAT VAS DEFERENS TO FIELD STIMULATION AND DRUGS

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Field stimulation-induced contractions of vasa deferentia from various species are biphasic. The two components of these responses can be separated pharmacologically into an initial non-adrenergic, non-cholinergic (NANC) twitch, that may be purinergic, and a more prolonged adrenergic component (Sneddon et al, 1982; Brown et al, 1983). Whether the two transmitters are released from the same or separate nerves is still doubtful, since in some studies, 6-hydroxydopamine (6-OHDA) removed only the adrenergic component (Brown et al, 1983) but in others, reduced both components (French & Scott, 1983). This study re-examined the effects of 6-OHDA and reserpine pretreatment in mouse and rat vasa, to determine whether any field stimulation-induced contractile response survived and if so whether it resembled the NANC component in its sensitivity to clonidine, morphine and tetrodotoxin (TTX).

Vasa from Wistar rats (200-250 g) and T.O. mice (25-30 g) were mounted in baths containing Krebs solution (36°C), gassed with 95% O_2/5% CO_2. Vasa were stimulated with supramaximal voltage pulses of 0.5 ms duration at various frequencies. Contractions of vasa to agonists and field stimulation were recorded isometrically. Some rats were anaesthetised with pentobarbitone (60 mg/kg, i.p.) and their vasa stimulated by electrodes placed around the vas in situ or by an electrode inserted in the spinal canal, between T_8 and T_9, and exposed from L_5 to the sacrum. Gallamine (5 mg/kg, i.v.) was used to relax skeletal muscle.

Responses of vasa to field stimulation and to stimulation of the spinal nerves were usually biphasic. The NANC component was inhibited by clonidine (10^{-7}M) and morphine (10^{-6}M), unaffected or potentiated by phentolamine (10^{-6}M) and abolished by TTX (3×10^{-7}M). The adrenergic component was less sensitive to clonidine (10^{-7}M) and morphine (10^{-6}M) but was abolished by phentolamine (10^{-6}M) and TTX (3×10^{-7}M). After reserpine pretreatment (1 mg/kg i.p. daily, 4 days), field stimulation produced a monophasic contraction that was unaffected by phentolamine (10^{-6}M) subsensitive to morphine (10^{-6}M) and clonidine (10^{-7}M) and abolished by TTX (3×10^{-7}M). After 6-OHDA pretreatment (2 x 50 mg/kg i.p. day 1 and 2 x 100 mg/kg i.p. day 3), field stimulation produced a monophasic contraction that was unaffected by phentolamine (10^{-6}M), morphine (10^{-6}M), clonidine (10^{-7}M) or TTX (3×10^{-7}M). In anaesthetised rats, pretreated with reserpine or 6-OHDA, there remained a monophasic contraction to in situ field stimulation and to stimulation of the spinal nerves. TTX (0.4 mg/kg, i.v.) abolished the monophasic responses to stimulation of the spinal nerves in reserpine- and 6-OHDA-pretreated rats and to in situ field stimulation in reserpinized rats but did not affect the residual response to in situ field stimulation in 6-OHDA pretreated rats.

These results suggest that in mouse and rat vasa, noradrenaline and the NANC transmitter are contained in the same nerves. The field stimulation-induced monophasic contraction that survives 6-OHDA pretreatment may be non-neuronal, arising from direct stimulation of the supersensitive smooth muscle.

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