EFFECTS OF IPSAPIRONE ON BEHAVIOUR OF MICE DURING SOCIAL ENCOUNTERS

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Ipsapirone, an anxiolytic with high affinity for 5-HT_{1A} receptor binding sites (Dompert et al 1985), has been shown in animal studies to inhibit foot-shock induced aggression and to antagonize passive avoidance behaviour (Traber et al 1984). The present studies examine its anxiolytic activity by the alternative procedure of ethopharmacology, a method which has been repeatedly shown to be highly sensitive in the detection of behavioural effects of drugs (Mackintosh et al 1977). To aid in detecting anxiolytic activity, behaviour of injected animals has been examined in encounters with untreated isolated males, many of which were highly aggressive.

Ipsapirone (0.3 and 3.0 mg/kg i.p) was administered to adult male group-housed mice while controls were injected with an equivalent volume of isotonic saline. Behaviour during social encounters with adult isolated males was examined for a 7 min period at 30 min after injection. Behaviour was recorded as a spoken commentary by 2 observers using the check list of behavioural elements and categories described by Mackintosh et al (1977). Body temperature was recorded by rectal thermometer immediately prior to and after each behavioural encounter.

Aggressive behaviour was significantly decreased by ipsapirone (Duration,s., mean$\pm$S.E.; controls 16.4$\pm$5.1; 0.3 mg ipsapirone/kg 1.2$\pm$0.4, 3.0 mg ipsapirone/kg 4.5$\pm$1.3; $P<0.05$ by the Kruskall Wallis test), whereas the elements "head jerk" (Duration,s.: controls 1.1$\pm$0.9; 0.3 mg ipsapirone/kg 4.9$\pm$1.6, 3.0 mg ipsapirone/kg 6.3$\pm$1.4; $P<0.01$) and "scratch" (Duration,s.: controls 1.9$\pm$0.8; 0.3 mg ipsapirone/kg 3.6$\pm$1.1, 3.0 mg ipsapirone/kg 8.6$\pm$2.9; $P<0.05$) were significantly increased in occurrence. Ipsapirone at 3.0 mg/kg induced hypothermia both before (Controls 36.4$\pm$0.2 °C, treated 34.9$\pm$0.3 °C; $P<0.05$) and after (Controls 37.0$\pm$0.2 °C, treated 35.6$\pm$0.3 °C; $P<0.05$) the encounters. Partner mice to the ipsapirone treated animals were characterised by decreases of offensive ambivalence (Duration,s.: partners to controls 24.5$\pm$6.0; partners to mice given 0.3 mg ipsapirone/kg 8.1$\pm$3.3 and to mice given 3.0 mg ipsapirone/kg 9.4$\pm$2.9; $P<0.05$).

These results confirm previous reports of antiaggressive activity by ipsapirone and indicate that it has additional minor behavioural effects including the head-twitch response. Its hypothermic activity, characteristic of a 5-HT_{1A} agonist, was not significantly altered by the stress of a social encounter.

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RITANSERIN AND PROCTOLIN ALTER THE BEHAVIOURAL RESPONSE TO INTRATHECAL 5-METHOXY-N,N-DIMETHYLTRYPTAMINE IN RATS

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Immunohistochemical evidence suggests that a proctolin (Arg-Tyr-Leu-Pro-Thr-OH)-like peptide co-exists with thyrotrophin-releasing hormone (TRH) and 5-hydroxytryptamine (5HT) in bulbospinal raphe neurones which innervate the ventral horn of the spinal cord (Holets et al., 1987). Recent results, obtained by administering drugs intrathecally, suggests that the co-existent peptides and indoleamine interact within these ventral horn neurones, as proctolin pretreatment attenuated the wet-dog shake (WDS) behaviour normally elicited by TRH analogue injection and repeated proctolin injection selectively reduced ventral, but not dorsal, horn TRH and 5HT levels (Fone et al., 1988). The present study further investigates this proposed interaction by examining the effect of proctolin on the behaviours produced by the intrathecal injection of the 5HT agonist, 5-methoxy-N,N-dimethyltryptamine (5MeODMT).

Male Wistar rats (270-320g) were anaesthetised with sodium methohexitone (60mg/kg i.p.) to permit an intrathecal cannula to be implanted along the spinal subarachnoid space (Fone et al., 1987). After a seven day recovery period one group of rats (n=8) received intrathecal injections, at four day intervals, of saline or 5MeODMT (2, 10, 25, 50 and 100 µg), four days later 5MeODMT (25 µg) was given again 30 min after ritanserin (1mg/kg i.p.). A second group of rats (n=8) were given 5MeODMT (25 µg) intrathecally; 30 min after saline (day 7), 30 min after proctolin (10 µg, day 15) and together with proctolin (10 µg, day 11). In both groups the number of WDS and "back muscle contractions" were counted separately for 30 min following saline or 5MeODMT injection. Student's t-test was used for statistical analysis and results are presented as mean $\pm$ s. e. mean.

Intrathecal 5MeODMT produced WDS and back muscles contractions which were both linearly related to $\log_{10}$ dose (from 1 $\pm$ 1 WDS and 19 $\pm$ 4 back muscle contractions in 30 min with 2 µg to 12 $\pm$ 2 and 201 $\pm$ 26 respectively with 50 µg, with no further increase using 100 µg 5MeODMT) and, except for the lowest dose, were significantly different from the effect of saline ($P<0.05$, ANOVAR). Both the number of WDS and back muscle contractions were significantly attenuated ($P<0.001$) by ritanserin pretreatment. In contrast, proctolin pretreatment prevented WDS (0 $\pm$ 0 compared with 6 $\pm$ 3, $P<0.05$) and yet significantly augmented the number of back muscle contractions (135 $\pm$ 17 compared with 96 $\pm$ 10, $P<0.02$) induced by 5MeODMT (25 ug). Neither WDS (9 $\pm$ 3) nor back muscle contractions (119 $\pm$ 16) were altered when proctolin was injected at the same time as 5MeODMT.

Previous work has demonstrated the involvement of the 5HT2-type serotonergic receptor in 5HT drug-induced WDS (Yap and Taylor, 1983). The current study suggests that 5HT2 receptors may be involved in both the WDS and the back muscle contractions produced by intrathecal 5MeODMT, as both behaviours are attenuated by ritanserin pretreatment. Intrathecal proctolin pretreatment, however, had quantitatively opposite effects on these two behaviours suggesting that they are mediated by separate pathways. These result support the proposal that the endogenous proctolin-like peptide in the rodent ventral horn may influence spinal serotonergic neuronal function.

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THE EFFECTS OF THE 5-HT_3 RECEPTOR ANTAGONISTS BRL 43694 AND GR 38032F IN ANIMAL BEHAVIOURAL MODELS OF ANXIETY

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The 5-HT_3 antagonists BRL 43694 (Fake et al., 1987) and GR 38032F (Brittain et al., 1987) have been reported to be as active as diazepam (DZ) in the social interaction (SI) model of anxiety in rats (Tyers 1987). As the degree of this effect could not be confirmed by Johnston & File (1988), these drugs have now been further investigated in this procedure and their profile as potential anxiolytics studied in other animal behavioural tests.

In the SI test based on that described by File (1980), pairs of male hooded Lister rats (Olac, 200-250g) were placed in a brightly lit, unfamiliar (HL/UF) arena and their SI quantitated by an observer blind to their pretreatment. Chlordiazepoxide (CDP) 5mg/kg p.o. typically significantly increased SI by approximately 100% from a control baseline of 60s ($P<0.01$). Increases were similarly observed with both oral BRL 43694 (0.1mg/kg + 83%, $P<0.01$; 1.0mg/kg + 52%, $P<0.05$; 10mg/kg + 42%, $P<0.05$) and GR 38032F (0.1mg/kg + 78%, $P<0.01$; 1.0mg/kg + 60%, $P<0.05$). GR 38032F was inactive at 10mg/kg p.o. In a dimly lit familiar arena (LL/F), baseline SI of 180s was not significantly affected by any drug treatment. At these doses, neither BRL 43694 nor GR 38032F affects spontaneous locomotor activity.

In food-reinforced and water-reinforced conflict tests, no activity was observed by the oral route with either BRL 43694 (0.0005-50mg/kg) or GR 38032F (0.0005-5mg/kg p.o.). Similarly, neither BRL 43694 nor GR 38032F (both 0.005-0.5mg/kg p.o.) demonstrated significant activity in the elevated X-maze test (Handley and Mithani, 1984).

Cynomolgus monkeys (3♂ + 3♀, 2-3kg, Shamrock Farms) were tested in a randomized blind study with either vehicle, DZ (2.5mg/kg p.o.), BRL 43694 or GR 38032F (0.01 and 0.1mg/kg p.o.) for effects on general behaviour. DZ reduced the behavioural scores for anxiety-motivated responses and aggression, eg. -37% $P<0.05$ at 120 min post-dose, but induced sedation and muscle relaxation. BRL 43694 and GR 38032F demonstrated significant, but only weak, anxiolytic effects, eg. BRL 43694 (0.01mg/kg) -13% $P<0.05$ at 90 min and GR 38032F (0.01mg/kg) -19% $P<0.05$ at 150 min, with no sedation or muscle relaxation. In a further study at 1mg/kg p.o., BRL 43694 maintained its anxiolytic activity, but GR 38032F was ineffective in reducing anxiety and, in fact, increased some of the behavioural scores.

These results indicate that BRL 43694 and GR 38032F possess a more limited profile of activity than benzodiazepines in animal behavioural models of anxiety. Only controlled clinical trials will indicate whether a similar situation occurs in man.

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EVIDENCE THAT MCPP AND TFMPP-INDUCED HYPOPHAGIA IS MEDIATED BY SELECTIVE ACTIVATION OF 5-HT_{1C} RECEPTORS

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RU 24969 induces hypophagia in freely feeding rats by an action at postsynaptic 5-HT_{1B} receptors (Kennett et al, 1987). Two other 5-HT agonists, mCPP and TFMPP, reported to have high affinity for the 5-HT_{1B} site, also cause hypophagia (Kennett et al, 1987). However, these latter compounds are also 5-HT_{1C} agonists (Kennett and Curzon, 1988). We have therefore investigated whether mCPP and TFMPP-induced hypophagia might be 5-HT_{1C} mediated.

Male Sprague-Dawley rats were food-deprived for 20h before i.p. injection on the following day with RU 24969, mCPP or TFMPP in saline. 20 min later, food was restored to the animals and subsequent food intake measured by weighing after 1, 2 or 4 h. All three drugs dose-dependently reduced food intake. The effect of antagonists given s.c. 20 min before the above drugs, on these hypophagic responses was then determined.

TABLE 1: Effect of mianserin (2mg/kg) and (±) cyanopindolol (8mg/kg) on the hypophagic response to mCPP, TFMPP or RU 24969

Treatment	2h Food Intake (g)		
Drug:	mCPP	TFMPP	RU 24969
Vehicle + Saline	5.2±0.4	5.3±0.2	6.2±0.3
Mianserin + Saline	4.9±0.3	3.4±0.2	6.2±0.3
Vehicle + Drug	2.6±0.2*	2.0±0.2*	2.6±0.9*
Mianserin + Drug	4.1±0.5^{a}	4.1±0.2^{aa}	3.0±0.8^{+}
Vehicle + Saline	5.8±0.5	5.3±0.4	nd
Cyanopindolol + Saline	5.4±0.3	4.7±0.2	nd
Vehicle + Drug	2.4±0.4*	2.0±0.3*	nd
Cyanopindolol + Drug	4.3±0.3^{a}	5.1±0.6^{aa}	nd

Means ± S.E.M., Significantly different from control * $p<0.01$, from antagonist pretreated, $^{+}$ $p<0.01$ or from drug treated group ^{a} $p<0.05$ ^{aa} $p<0.01$ by Dunnetts test.

The non-specific 5-HT antagonist, metergoline (5mg/kg) and two other antagonists with high affinity for 5-HT_{1C} receptors, mianserin (2mg/kg) (Table 1) and mesulergine (0.2mg/kg) reversed mCPP-induced hypophagia. Mianserin also reversed TFMPP-induced hypophagia (Table 1). The effect of mCPP was unaltered by the 5-HT_{2} antagonists Ketanserin (0.2mg/kg) and ritanserin (0.6mg/kg) or by the 5-HT_{3} antagonist ICS 205 930 (1mg/kg). These results suggest mediation by 5-HT_{1C} receptors. Two 5-HT_{1A}, 5-HT_{1B} antagonists, cyanopindolol (8mg/kg) (Table 1) and (-)propranolol (16mg/kg) also blocked the hypophagic effect of mCPP. As 5-HT_{1A} agonists cause hyperphagia, these results suggest that the hypophagia also requires 5-HT_{1B} receptors. The hypophagic effect of RU 24969, (like that of mCPP) was blocked by cyanopindolol, (Kennett et al, 1987) and also by the 5-HT_{1B} antagonist (-)pindolol (2mg/kg) and metergoline but (unlike mCPP) it was not blocked by the 5-HT_{1C} antagonists mianserin (2mg/kg) (Table 1) or mesulergine (0.2mg/kg). Results as a whole suggest that as RU 24969-induced hypophagia is prevented by 5-HT_{1B} but not by 5-HT_{1C} antagonists it appears to be mediated specifically by 5-HT_{1B} receptors but as mCPP and TFMPP-induced hypophagias are essentially completely prevented by both 5-HT_{1C} and 5-HT_{1B} antagonists they appear to involve a linked 5-HT_{1C}, 5-HT_{1B} mechanism in which the drugs selectively activate 5-HT_{1C} receptors.

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BLOCKADE OF POSTSYNAPTIC 5-HT_1 RECEPTORS ELICITS FEEDING IN SATIATED RATS

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There is considerable evidence that reducing brain 5-HT transmission increases food intake in rats. Thus, it has been shown that activation of pre-synaptic 5-HT_{1A} receptors by 8-OH-DPAT increases feeding (Dourish et al, 1985), whereas stimulation of post-synaptic 5-HT_{1B} receptors by RU 24969 decreases food intake (Kennett et al, 1987). To date, however, there has not been a systematic study of the effects of 5-HT receptor blockade on feeding and, therefore, we examined the effects of eight 5-HT antagonists on food intake in rats.

Male Sprague-Dawley rats (320-380g) were housed individually on a 12:12h light: dark cycle (lights on 08.00h) for at least seven days prior to experiments. Food (Rat/Mouse Standard Pellets, Beekay Feeds) and water were available ad libitum, all testing being carried out in the individual home cages. Metergoline was dissolved in 50µl of 1M glacial acetic acid, made to volume in warm 0.9% saline and bought to pH 4.5-5.0 with 1M NaOH. Ritanserin was dissolved in a 20% DMSO/80% propylene glycol vehicle. All other drugs were dissolved in 0.9% saline. Drugs were injected s.c. between 10.00 and 11.00 am ($n \geq 10$ rats in each treatment group) and food consumption was recorded 2, 4 and 24h post-injection. Data were analysed by one factor ANOVA and Dunnetts multiple range test.

Drug	Dose mg/kg	Minimum Effective Dose to increase Food intake 4h	24h	5-HT Receptor Selectivity
Metergoline	0.3-10.0	3.0**	0.3**	5-HT_1,5-HT_2
Methysergide	0.3-10.0	3.0**	NE	5-HT_1,5-HT_2
Methiothepin	0.01-0.3	0.03*	NE	5-HT_1,5-HT_2
Mesulergine	0.003-3.0	1.0**	NE[a]	5-HT_1,5-HT_2
Mianserin	0.3-10.0	1.0*	NE	5-HT_1,5-HT_2
Ritanserin	0.1-3.0	NE	NE	5-HT_2
ICS 205,930	0.001-10.0	NE	NE	5-HT_3
MDL 72222	0.001-10.0	NE	NE	5-HT_3

* $P < 0.05$, ** $P < 0.01$ compared to controls; NE = No Effect
[a] Significant decrease in 24 h intake ($P < 0.01$) at 1.0 and 3.0mg/kg

The non-selective 5-HT antagonists methiothepin, metergoline, methysergide and mesulergine increased food intake over 2 and 4h. In addition, metergoline increased 24h food intake. All of these compounds have high affinity for 5-HT_1 receptors (Middlemiss et al, 1986). In contrast the selective 5-HT_2 antagonist ritanserin and the selective 5-HT_3 antagonists ICS 205,930 and MDL 72222 had no effect on food intake. Although the antagonists used have varying degrees of selectivity for 5-HT receptor subtypes, the pattern of results suggest that postsynaptic 5-HT_1 receptors play an important role in the control of feeding in rats.

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MEASUREMENT OF BRAIN MHPG LEVELS SUGGESTS THAT CLONIDINE INDUCES HYPOACTIVITY AND MYDRIASIS VIA PRESYNAPTIC α_2-ADRENOCEPTORS

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Clonidine produces characteristic behavioural and biochemical changes in rodents. This α_2-adrenoceptor agonist induces hypoactivity, a distinct form of sedation (Heal et al, 1981), increases pupil dilatation, mydriasis (Heal et al, 1988a) and decreases brain 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations (Sugrue, 1983; Heal et al, 1988b). However, in the CNS, most α_2-adrenoceptors are not presynaptic, but are postsynaptic (Dausse et al, 1982). Although clonidine decreases MHPG by activating presynaptic α_2-adrenoceptors (Sugrue, 1983; Heal et al, 1988b), the synaptic location of the receptors mediating hypoactivity and mydriasis is much less clear. Therefore, dose-response curves have been constructed for the effects of clonidine on brain MHPG levels, hypoactivity and mydriasis in the same mice to determine whether the effects were likely to be mediated via α_2-adrenoceptors with identical synaptic locations.

Adult male C57/Bl/60la mice (Olac) weighing 15-30 g were used. Clonidine (1-3000 µg/kg) was injected i.p. in 0.9% NaCl. Pupil diameter was measured in conscious mice as previously described (Heal et al, 1988a). Hypoactivity was scored 0-3 on five behavioural parameters at 20 min (Heal et al, 1981). Brain MHPG concentrations were also determined at 20 min. Mice were killed and brains (minus cerebellum) were analysed for MHPG content by HPLC with electrochemical detection using the method reported by Heal et al (1988b). Results are presented ± s.e. mean and significance was determined using Student's t-test.

Intraperitoneal clonidine administration to mice altered pupil diameter, hypoactivity scores and brain MHPG concentrations. Pupil diameter (initially 0.42 ± 0.01 mm, n=12) was increased by clonidine with a maximum enlargement of 64% at 500 µg/kg ($P<0.001$, n=11). Clonidine also induced hypoactivity with a maximum score of 10.0 ± 0.4 at 3000 µg/kg ($P<0.001$, n=6). Basal MHPG concentrations (79.8 ± 1.3 ng/g tissue wet wt) were maximally reduced by 47% at 1000 µg/kg ($P<0.001$, n=5). The ED50 values were 53 µg/kg (mydriasis), 100 µg/kg (hypoactivity) and 19 µg/kg (MHPG reduction). In individual mice, the decrease in brain MHPG induced by clonidine (1-3000 µg/kg) correlated with mydriasis ($r=0.79$; $P<0.001$, n=37) and hypoactivity ($r=0.74$; $P<0.001$, n=37). In addition, there was a very good correlation between mydriasis and hypoactivity ($r=0.92$; $P<0.001$, n=37).

The ED50 values obtained for the behavioural and biochemical parameters were all <100 µg/kg which has been suggested to reflect a preferential presynaptic action of clonidine (Anden et al, 1976; Sugrue 1983). Furthermore, since decreases in MHPG reflect presynaptic α_2-adrenoceptor activation (Sugrue, 1983; Heal et al, 1988b), the high correlation between this and the two behavioural parameters suggests that clonidine also induces both mydriasis and hypoactivity by stimulating presynaptic α_2-adrenoceptors.

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THE EFFECTS OF CHRONIC ADMINISTRATION OF OXOTREMORINE AND TETRAHYDROAMINOACRIDINE ON CHOLINERGIC FUNCTION IN THE RAT

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Chronic administration of either cholinergic agonists or acetylcholinesterase (AChE) inhibitors has been shown to decrease muscarinic receptor density in several brain regions (Pedigo & Polk, 1985; Ehlert et al, 1980) and cause behavioural tolerance to cholinergic drugs, although a direct relationship between change in receptor number and tolerance has not been demonstrated (Collins et al, 1987). The present experiments compared the behavioural and biochemical effects of chronically administered oxotremorine (OTMN) and the AChE inhibitor tetrahydroaminoacridine (THA), using two methods of chronic dosing.

Forty-eight Lister Hooded rats (Olac, Bicester) were divided into 3 treatment groups and implanted subcutaneously with Alzet minipumps filled with either THA (0.225 mgh^{-1}), OTMN (0.013 mgh^{-1}) or saline. Another 48 rats were divided into 3 treatment groups and given once daily injections (for 14 days) of either THA (15 $mgkg^{-1}$), OTMN (0.25 $mgkg^{-1}$) or saline. After 2 days of treatment all rats were given a challenge dose of OTMN (0.3$mgkg^{-1}$) and cholinergic effects (lacrimation, tremor, salivation, rearing and chewing) measured. Rats receiving THA by injection were excluded from this challenge since they were still showing marked cholinergic behaviours. On the 14th day of treatment half the rats in each treatment group were taken for the following biochemical assays: [^{3}H]-pirenzepine (1nM) and [^{3}H]-OTMN (1nM) binding in hippocampus and cortex; [^{3}H]-N-methylscopolamine (NMS, 1nM) and [^{3}H]-OTMN binding in brain stem and AChE and choline acetyltransferase activity in all 3 regions. The remaining rats were again challenged with OTMN (0.3 $mgkg^{-1}$) and behavioural changes examined.

No change in either AChE or choline acetyltransferase activity was observed after chronic administration of THA or OTMN. Rats injected with THA or OTMN showed no changes in any of the binding assays listed above. Rats dosed with OTMN via Alzet minipumps showed a decrease in [^{3}H]-NMS in brain stem and [^{3}H]-pirenzepine in hippocampus while those dosed with THA showed a decrease only in [^{3}H]-NMS binding (Table). [^{3}H]-OTMN binding was unchanged in the pump groups. Behavioural tolerance was observed in rats given THA by injection (no cholinergic behaviours observed after 14 days treatment) or minipump (attenuated response to OTMN challenge at day 14 compared to day 2). Behavioural results obtained in OTMN treated rats were equivocal.

In conclusion chronic infusion of OTMN decreased both M_1 and M_2 muscarinic receptor binding while infusion of THA decreased only M_2 receptor binding. In contrast chronic injection of either drug failed to alter either receptor population. [^{3}H]-OTMN binding was unchanged in any treatment group. Nevertheless THA produced behavioural tolerance given by either route. Finally, THA presumably inhibited AChE although this was not demonstrable as it is a reversible inhibitor. Clearly however absolute enzyme levels were unchanged.

	CONTROL	THA	OTMN
[^{3}H]-Pirenzepine (hippocampus)	143 $\pm$ 7	156 $\pm$ 6	131 $\pm$ 5*
[^{3}H]-NMS (brain stem)	150 $\pm$ 15	107 $\pm$ 10*	115 $\pm$ 7*

Values are mean $\pm$ SEM in $fmol.mg^{-1}$ protein. *P <0.05.

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A ROLE FOR HISTAMINE H_3 RECEPTORS IN HISTAMINE-INDUCED HYPOACTIVITY IN THE RAT

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The presence of histaminergic neurones terminating in the n. accumbens suggests that histamine may function in arousal mechanisms. We have reported previously that microinjections of histamine into the accumbens induces a dose related H_1-receptor mediated hyperactivity preceded by a transitory hypoactivity phase (Bristow & Bennett, 1987). The nature of histamine receptors mediating these behaviours has now been further investigated in view of recent evidence for central H_3 autoreceptors (Arrang et al 1987).

Male Wistar rats (300g) were anaesthetized with sodium pentobarbitone (60mg/Kg, ip) and bilaterally implanted with guide cannulae for intra-accumbens injection. Following a seven day recovery period, drug-induced changes in rat activity were monitored using an Actimat Activity meter (Bristow & Bennett 1987). Agonist doses were chosen according to their relative potencies and bilaterally administered in 1 µl. Other rats were pretreated with H_3 antagonists (1 µg, 2 x 0.5 µl) 10 minutes before subsequent histamine administration (20 µg, 2 x 0.5 µl).

Intra-accumbens administration of the highly selective H_3 agonist R_α methylhistamine induced an initial hypoactivity (36% at 10 µg/µl n=6, $p<0.05$) similar to that seen with histamine (see table). Hypoactivity also occurred with N_α methylhistamine and $N_\alpha N_\alpha$ dimethylhistamine (see table) and histamine-induced hypoactivity was reduced significantly following pretreatment with the H_3-antagonists, thioperamide ($p<0.05$, n=9) and ipromidine ($p<0.05$, n=9).

	AGONIST POTENCY	DOSE (µg/µl)	ACTIVITY COUNTS (0-15 mins)
Saline	-	-	259±19
Histamine		20	83±25**
2-Thiazolylethylamine	H1>H2	100	303±43
Dimaprit	H2	20	237±28
N_α-Methylhistamine	H3>H1, H2	10	92±30*
N_α, N_α-Dimethylhistamine	H3>H1, H2	10	105±53**

Results analysed by Wilcoxon signed rank test; ** $P<0.01$, * $P<0.05$ (n=7-10) compared to appropriate saline controls.

In contrast, hyperactivity responses were induced only by intra-accumbens administration of those compounds known to have agonist properties at the H_1 receptor, supporting our earlier studies (Bristow & Bennett, 1987).

In conclusion, the present results suggest that intra-accumbens administration of histamine may cause a transitory hypoactivity as a result of an initial activation of H_3 autoreceptors. This is followed by marked arousal acting via post-synaptic H_1 receptors.

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STRESS CAN REDUCE THE EFFECT OF AN IRREVERSIBLE MAO INHIBITOR IN VIVO

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We have investigated the effect, in rats, of stress on the inhibition of brain monoamine oxidase (MAO) caused by the irreversible MAO inhibitor phenelzine.

Four groups of 6 male Sprague Dawley rats (200g) were studied, with or without 2 hours cold restraint stress, and with or without phenelzine (1mg/kg S.C.) 45 minutes prior to being killed. The brains were then rapidly removed, weighed and homogenised (2% w/v) in cold 100mM phosphate buffer pH 7.4, using an Ultraturrax homogeniser. 20ul samples of homogenate were incubated for 30 min at 37°C with 100ul 100mM phosphate buffer pH 7.4 and 20µl of 2.1mM ^{14}C-tyramine (New England Nuclear Corporation), diluted with unlabelled tyramine, to give a final concentration of 300µM, or 20µl 1.4mM ^{14}C-5HT (New England Nuclear Corporation) diluted with unlabelled 5HT, to give a final concentration of 150µM.

We found no difference in MAO activity in the dilute homogenates of the non-drug treated group between the stress and no stress situation (specific activity ±SEM: 61.8 ± 1.5 and 61.1 ± 1.2 nmoles/mg protein/30 min respectively, for tyramine; 27.9 ± 0.4 and 28.7 ± 0.6 nmoles/mg protein/30 min respectively, for 5HT). As expected phenelzine caused substantial in vivo inhibition of MAO activity. However, when the effect of stress on the drug and saline treated groups were analysed using analysis of variance there was a significant interaction term between the two groups, for tyramine: $F(1.20) = 4.9$, $p<0.05$ and 5HT: $F(1.20) = 10.9$; $p<0.01$, indicating that the effect of stress was different in the drug treated vs normal group of animals.

In Table 1 the results are expressed as the percentage MAO inhibition caused by 1mg/kg phenelzine.

Table 1 Effect of cold restraint stress on inhibition produced by phenelzine (1mg/kg S.C.) on rat brain MAO

		%MAO inhibition (mean ± SE)	
	Substrate n	Tyramine	5HT
No stress	6	36.1 ± 2.0*	38.5 ± 2.2*
Stress	6	24.9 ± 2.8	28.7 ± 1.8

*$p<0.01$ difference from no stress using Student's t test.

From Table 1 it can be seen clearly that phenelzine caused substantially less inhibition in the stressed rats compared to non-stressed rats.

It is well known that reversible MAO inhibitors can prevent MAO inhibition in vivo by a subsequently administered irreversible inhibitor presumably by competition (Green 1984). It is thus possible that these results may be explained by stress-induced generation of an endogenous reversible MAO inhibitor such as tribulin (Glover et al 1981), the effect of which would not be detected in a dilute homogenate. Whatever the explanation, these results indicate that the in vivo efficacy of this drug is dependant upon the state of the subject. The same may be true when irreversible MAO I are used clinically.

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RECEPTOR MEDIATED HYPERPOLARISATIONS OF THE RAT SUPERIOR CERVICAL GANGLION IN VITRO ARE SENSITIVE TO PERTUSSIS TOXIN

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In the central nervous system, hyperpolarisations elicited by baclofen, 5-HT and α_2-agonists have been shown to be sensitive to pertussis toxin (Andrade et al, 1986; Aghajanian and Wang, 1986), which ADP-ribosylates G_o and G_i proteins (Sternweis and Robishaw, 1984). Several different receptors have been demonstrated to mediate hyperpolarisations of the rat superior cervical ganglion: α_2 (Brown and Caulfield, 1979), adenosine (Brown et al, 1979), M2 (Newberry and Priestley, 1987) and 5-HT_{1A} (Gilbert and Newberry, 1987). However little is known of the mechanisms and second messenger systems involved in these responses. It was therefore of interest to determine the effect of pertussis toxin on a number of hyperpolarisations (-) on this preparation. The depolarisation (+) induced by KCl was used as a control response.

D.C. potentials were recorded from superior cervical ganglia, using the grease-gap technique (Newberry and Priestley, 1987), following a 24 hour incubation at 37°C in a Krebs medium with or without 1μg/ml pertussis toxin. All responses were evoked by 1 minute applications of half- to near-maximal concentrations of the agonists shown in Table 1. The responses were recorded in 0.1mM $CaCl_2$ and 0.3μM pirenzepine. Each agonist was tested on 'n' ganglia from different rats.

Table 1

AGONIST	CONC^N (μM)	RECEPTOR	RESPONSE (mean ± SEM, n) in μV CONTROL	PERTUSSIS TOXIN
5-carboxamidotryptamine	0.1	$5HT_{1A}$	-110 ± 20,7	- 28 ± 6,9
(±)muscarine	1.0	M2	-150 ± 20,7	- 5 ± 3,9
(-)noradrenaline	30.0	α_2	-130 ± 10,4	0 ,5
adenosine	100.0	A?	-110 ± 10,6	- 9 ± 4,7
(-)baclofen	10.0	$GABA_B$	- 50 ± 10,7	- 8 ± 4,9
5-hydroxytryptamine	100.0	$5HT_3$	+200 ± 20,7	+300 ± 40,9
potassium chloride	2000	-	+170 ± 10,4	+180 ± 20,5

The small ganglionic hyperpolarisation to baclofen has not been described before. In separate experiments it was found that pertussis toxin was effective against all of the hyperpolarisations at concentrations as low as 1ng/ml.

These results indicate that the different receptor mediated hyperpolarising responses of this preparation share a pertussis-toxin sensitive mechanism(s), probably involving either Gi or Go proteins.

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SOLUBILIZATION OF THE N-METHYL-D-ASPARTATE RECEPTOR FROM RAT BRAIN

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Attempts to solubilize receptors for excitatory amino acids have been hampered by the lack of specific and high affinity ligands for the detection of these receptors in solution. The use of L-[^{3}H]-glutamate has allowed the solubilization of a glutamate binding protein from rat brain, although the binding activity of the protein has no resemblance to any of the pharmacologically defined subtypes of excitatory amino acid receptors (Kuonen & Roberts, 1987). MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine maleate) has been identified as a potent, selective and non-competitive antagonist for excitatory amino acid receptors of the N-methyl-D-aspartate (NMDA) subtype (Wong et al, 1986). We have utilized the fact that [^{3}H]-MK-801 labels a site in the NMDA receptor channel (Foster and Wong, 1987) to investigate the solubilization of the NMDA receptor complex.

Studies were carried out on crude P_2 membranes prepared from pooled cortex and hippocampus from Sprague-Dawley rats. [^{3}H]-MK-801 was incubated with soluble preparations extracted using the following detergents: CHAPS (0.5%); digitonin (0.2%); sodium cholate (1%), sodium deoxycholate (DOC; 0.5%), Triton X-100 (1%), Lubrol PX (0.1%) and N-octylglucopyranoside (0.9%). Binding activity in solution was measured by incubating the extract with [^{3}H]-MK-801 in a final assay volume of 1ml (containing 0.04% DOC, 10mM Tris HCl, 0.2mM EDTA, 50μM L-glutamate, 4% glycerol; pH 7.4) for 24 hours at 4°C and then assayed by filtration through GF/B filters presoaked in 1% polyethylenimine. Of the detergents tested DOC (0.5%) gave the best solubilization (50% protein solubilized), producing a preparation which bound [^{3}H]-MK-801 with an affinity (Kd) of 38 $\pm$ 23nM and site density (Bmax) of 0.78 $\pm$ 0.15pmol/mg protein (mean $\pm$ S.E.M., n = 3). Displacement studies using a number of non-competitive NMDA receptor antagonists gave pKi values for MK-801 and thienylcyclohexylpiperidine (TCP) of 7.49 $\pm$ 0.19 and 7.28 $\pm$ 0.18 respectively and a rank order of: MK-801 > TCP = (-) MK-801 > dexoxadrol = PCP > ketamine = (+) SKF-10047. Quisqualate and kainate at 100μM failed to inhibit [^{3}H]-MK-801 binding. Removal of endogenous glutamate and glycine from DOC extracts by Sephadex G-25 chromatography resulted in almost complete loss of specific [^{3}H]-MK-801 binding. However, the residual binding was enhanced by addition of 30μM L-glutamate and 10μM glycine.

In conclusion, we have solubilized a protein from rat brain which exhibits specific and saturable [^{3}H]-MK-801 binding with the appropriate pharmacological profile for the NMDA receptor. This preparation exhibits modulation by glutamate and glycine in a manner similar to that observed in membranes (Foster & Wong, 1987; Wong et al, 1987) suggesting that association between these sites is maintained after solubilization.

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TACHYKININ RECEPTOR SUBTYPES IN THE RAT FOREBRAIN

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[^{125}I]Bolton-Hunter conjugated substance P ([^{125}I]BH-SP) and [^{125}I]Bolton-Hunter conjugated eledoisin ([^{125}I]BH-ED) have been shown to label different tachykinin receptor subtypes, now termed NK-1 and NK-2/3 respectively (Cascieri, Chicchi and Laing, 1985). These subtypes are differentially distributed in the rat CNS (Danks et al., 1986). We have combined autoradiography with stereotactic surgery to investigate the cellular location of the NK-1 and NK-3 receptor subtypes.

Unilateral lesions were performed (n=4) on male Sprague Dawley rats (150-250g) anaesthetised with Avertin (1 ml/100g body weight). Excitotoxin lesions (ibotenic acid) to the striatum and cortex and 6-hydroxydopamine lesions to the medial forebrain bundle were made with a stainless steel cannula. Undercutting the cortex to separate it from the striatum was achieved using a microknife. In other animals the frontal cortex was removed by suction down to the corpus callosum. Animals were killed four days after surgery and the brains processed for tachykinin receptor localisation according to the method of Beaujouan et al., 1986. The final concentration of each radioligand was 0.1 nM and non-specific binding of [^{125}I]BH-SP and [^{125}I]BH-ED was determined by incubating in the presence of 1μM substance P and 1μM eledoisin respectively. Autoradiographic standards were made using brain paste and [^{125}I]. Standards were apposed to ^{3}H-Hyperfilm (Amersham) together with the brain sections. The resulting autoradiograms were analysed on a Quantimet 920 image analyer (Cambridge Instruments).

Excitotoxin lesions revealed that most (>90%) of the NK-1 binding sites in the striatum and cortex and most of the NK-3 binding sites in the cortex, are located on intrinsic cells. Unilateral 6-hydroxydopamine lesions of the medial forebrain bundle reduced the dopamine content of the ipsilateral striatum by >70% in all animals, but did not significantly alter the amount of NK-1 binding in any forebrain structure. Animals with excitotoxin lesions to the striatum and cortical suction lesions both exhibited an increase in NK-1 binding in cortical layers 1 and 2 ipsilateral to the lesion (as noted by Mantyh and Hunt, 1986, for striatal lesions). However, NK-3 binding in the deeper cortical layers was unchanged by these two lesions. Cortical undercutting did not alter the density of NK-1 or NK-3 binding sites in the striatum or undercut cortex.

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DIFFERENCES IN DESENSITIZATION OF CARDIAC β_1- AND β_2-ADRENOCEPTORS OF KITTEN ATRIA IN VITRO

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In guinea-pigs, atrial β_1-adrenoceptors have been shown by ourselves (Broadley & Herepath 1987) to undergo desensitization after prolonged in vitro exposure to isoprenaline. We have also shown that the β_2-adrenoceptors in the smooth muscle of guinea-pig lung strips undergo desensitization in vitro to a much lesser extent than the β_1-receptors of the atria (Herepath & Broadley 1987). Therefore, the aim of the present study was to examine whether cardiac β_2-receptors undergo in vitro desensitization to a similar extent as cardiac β_1-receptors, as there is a suggestion that, in vivo, they are less prone to desensitization (Brodde et al. 1986).

Kitten left atria were used as a source of cardiac tissue in which both the β_1 and β_2-adrenoceptors are functionally responsive (O'Donnell & Wanstall 1981). Paced left atrial strips (2Hz, 5ms, threshold voltage + 50%) of male kittens (500-750g) were set up in Krebs-bicarbonate solution containing ascorbic acid (1mM) at 37.5°C, gassed with 5% CO_2 in O_2, and the tension responses recorded. After equilibration and incubation with either 10^{-5}M Atenolol or 10^{-7}M ICI 118,551, to block β_1 and β_2-adrenoceptors respectively, a cumulative concentration-response curve to (-)-isoprenaline was constructed. After the final dose (10^{-5}M) the stimulator was turned off and the antagonist removed by washing and replacing the 10^{-5}M isoprenaline. The tissue was then incubated with this concentration of agonist for 4 hours before washing (x 5 in 1 hour), during which the antagonist was replaced. Finally, after the washout, a 2nd curve to isoprenaline was constructed. All pre-incubation curves were corrected from time-matched controls and $n>4$ throughout.

In atria treated with 10^{-7}M ICI 118,551, the β_1-receptors exhibited desensitization as a significant rightward shift ($P<0.05$) of the 2nd curve (EC_{50}, 16 and 180nM) and also as a significant depression of its maximum response to 62.1±3.7%. By comparison, in atria treated with 10^{-5}M Atenolol there was a smaller, though still significant, rightward shift (EC_{50}, 0.43 and 1.4μM), although this time there was no significant depression of the second curve maximum (99.2±5.8%). The smaller degree of shift of the curve for these β_2-receptor-mediated responses was indicated by a significantly lower mean dose-ratio (4.2±0.4) than for the β_1 system (11.3±2.1). The β_1-receptor value was comparable to that obtained with guinea-pig left atria (11.9±1.5) following exposure to isoprenaline under the same conditions, but in the absence of antagonists, where the maximum response was also reduced to 65.2± 2.6%).

Therefore, this study demonstrates that desensitization of the functional responses mediated by both β_1- and β_2-adrenoceptors occurs in kitten left atria following prolonged in vitro exposure to isoprenaline, and that the cardiac β_2-receptors in this tissue appear to be more resistant to these changes than the β_1-receptors.

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REGULATION OF, AND RELATIONSHIP BETWEEN, β-ADRENOCEPTORS, Na-K-ATPase AND PLASMA POTASSIUM

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Adrenaline causes hypokalaemia in experimental animals and man via beta$_2$-adrenoceptors which are probably located on skeletal muscle (Struthers & Reid, 1984). It is suggested that an increase in potassium uptake by skeletal muscle as a result of activation of Na-K-ATPase by beta-adrenoceptor is the mechanism of hypokalaemia (Clausen & Flatman, 1980). The aim of the present study was to investigate the relationship between beta-adrenoceptor, Na-K-ATPase and plasma potassium in heart and skeletal muscle of the rabbit.

The studies were carried out in groups of male rabbits each weighing 2-2.5 kg. Each group was pretreated with either vehicle (0.1% ascorbic acid) or adrenaline (50 nmol/kg.hr) administered for 10 days by osmotic minipumps connected to the femoral vein. At the end of the pretreatment period and 18 hr after removal of the minipumps, adrenaline was infused via the ear vein and arterial blood samples were taken for measurement of serum potassium. The animals were then sacrificed and tissue membranes from gastrocnemius muscles and hearts were prepared for the determination of density (Bmax) and affinity (K_D) of beta-adrenoceptors for the radioligand [^{125}I] cyanopindolol (ICYP) (Elfellah & Reid, 1987) and of Na-K-ATPase receptors for the radioligand [^{3}H]-ouabain (Erdmann & Schoner, 1973).

Acute intravenous infusion of adrenaline (81 nmol/kg.hr) for 15 mins reduced serum potassium level from 3.34±0.09 to 2.85±0.08 mmol/l (mean ± s.e. mean) in control rabbits (n=9) and from 3.2±0.1 to 3.04±0.14 mmol/l in adrenaline minipumps pretreated animals (n=8). Thus the hypokalaemic response to adrenaline was significantly attenuated ($P<0.05$) in adrenaline minipumps pretreated animals. Chronic adrenaline pretreatment reduced the density of beta-adrenoceptors in the skeletal muscle (results from Deighton et al, 1987) and the heart (Table 1). The density of [^{3}H] ouabain binding sites was also reduced in the skeletal muscle and heart from adrenaline minipumps pretreated animals. There was a strong correlation between the densities of ICYP and ouabain binding sites in the heart ($r=0.96$, $n=20$, $P<0.001$). The K_D values for both radioligands were not altered by chronic adrenaline pretreatment.

Table 1 : Effect of chronic in vivo pretreatment of rabbits with adrenaline on the Bmax of ICYP and [^{3}H] ouabain in gastrocnemius muscles and hearts. Results are means ± s.e. mean. n=7-9 rabbits per group.

Bmax	ICYP (fmol/mg protein)		[^{3}H] ouabain (pmol/mg protein)	
Pretreatment	Vehicle	Adrenaline	Vehicle	Adrenaline
Gastrocnemius muscle	43.8±1.0	24.6±1.9*	5.1±1.0	2.2±0.4*
Heart	213 ±31	90.1±3.8*	22.6±3.7	9.5±1.2*

* $P < 0.05$

In conclusion, chronic pretreatment of the rabbit with adrenaline caused desensitisation to the hypokalaemic effect of adrenaline, which was associated with down-regulation of both beta-adrenoceptors and ouabain receptors of the skeletal muscle and heart. Furthermore, there was a strong correlation between beta-adrenoceptor and ouabain receptor densities in the heart.

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5-HT RECEPTORS AND CONTRACTILE RESPONSES TO NERVE STIMULATION IN SHEEP MIDDLE CEREBRAL ARTERIES

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5-Hydroxytryptamine (5HT), present both in platelets and nerves, is a potent vasoconstrictor in the cerebral circulation. Studies in several species have shown high concentrations of 5HT in the perivascular nerves of cerebral arteries (Edvinsson et al.,1984). The object of this study was to characterise the 5HT receptors in sheep cerebral arteries and to examine the effects of several antagonists on nerve mediated contraction of these arteries.

Four ring segments (3-4mm length) were prepared from sheep middle cerebral artery (MCA). The rings were suspended on parallel wires at their optimal resting tension in Krebs solution. Results are expressed as the mean $\pm$ s.e.m.. Cumulative concentration-response curves to 5HT were obtained in the presence of benextramine (3×10^{-6}M). Ketanserin (10^{-9}M, 10^{-8}M or 10^{-7}M) was then added to three of the baths, using the fourth as a control. Following a 30 minute equilibration period a second concentration-response curve to 5HT was obtained. Ketanserin acted as a potent and competitive antagonist of 5HT in the MCA (pA_2=9.19$\pm$0.13, slope of the Schild plot=0.88$\pm$0.06, n=6). Therefore if 5HT is released by nerves in the MCA then ketanserin should antagonise a contraction elicited by nerve stimulation.

Transmural nerve stimulation with parallel silver wire electrodes (70V, 0.3ms width, 8Hz frequency) elicited a contraction which was abolished using tetrodotoxin (10^{-7}M). Trains of stimuli (15 seconds) were repeated at 10 minute intervals until 3 reproducible responses were obtained. This procedure was then repeated following addition of prazosin (10^{-7}M), ketanserin (10^{-7}M) and atropine (10^{-7}M). The order of addition of atropine and prazosin/ketanserin was randomised. Ketanserin was always added directly after prazosin to distinguish any effects of ketanserin on α-adrenoceptors. Each of the three antagonists caused a significant reduction ($P<0.01$) of the plateau phase of the nerve mediated contraction. The effects of the antagonists (expressed as percentage reduction of the initial control response) were: atropine, 26.4$\pm$1.9% (n=9); prazosin, 26.3$\pm$2.8% (n=9) and ketanserin, 17.5$\pm$0.6% (n=7). It was possible to greatly reduce the contractile response by combining the three antagonists (69.2$\pm$4.0%, n=6). The addition of haemoglobin augmented the contractile response (309$\pm$41%, n=4). Addition of indomethacin (10^{-6}M) had no significant effect which suggests that prostanoids do not play a role in the nerve mediated response.

These results suggest that $5HT_2$ receptors mediate the contractile effects of 5HT in the sheep MCA and that a large component of the contractile response to nerve stimulation is mediated by the transmitters acetylcholine, noradrenaline and 5HT.

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FURTHER CHARACTERIZATION OF THE 5-HT_1-LIKE RECEPTORS IN THE CAROTID CIRCULATION OF THE PIG

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Intracarotid infusion of 5-hydroxytryptamine (5-HT) decreases arteriovenous anastomotic (non-nutrient) flow (AVAF), but increases arteriolar (tissue; nutrient) flow (TF) in the anaesthetized pig (Saxena & Verdouw, 1982). These effects are mediated by 5-HT_1-like receptors, since they are mimicked by 5-carboxamidotryptamine and blocked by methiothepin, but not by ketanserin or MDL 72222 (Saxena et al., 1986). In an attempt to further characterize these 5-HT_1-like receptors, we studied the effects of 8-OH-DPAT and ipsapirone (TVX Q 7821), two drugs which show a high affinity for 5-HT_{1A} binding sites (Peroutka, 1986).

As described earlier (Saxena & Verdouw, 1982; Saxena et al., 1986), the radioactive microsphere technique was used to measure the partition of carotid blood flow (CBF) into AVAF and TF in 18 young pigs (18-26 kg) during pentobarbital anaesthesia. The animals were given 10 min intracarotid infusions of physiological saline or increasing doses of either 8-OH-DPAT or ipsapirone. As shown in Table 1 8-OH-DPAT dose-dependently decreased CBF and AVAF, whereas at the highest dose TF increased. In contrast, neither ipsapirone nor saline caused any significant changes. Arterial blood pressure did not change with any of the treatments.

TABLE 1. Effect of 8-OH-DPAT and Ipsapirone on CBF and Its Distribution.

		Baseline	Dose (μg/kg/min)			
			0.3	1	3	10
Saline (n=6)	CBF	108 ± 17	108 ± 20	115 ± 20	123 ± 22	124 ± 23
	AVAF	87 ± 18	89 ± 20	95 ± 21	100 ± 23	102 ± 24
	TF	20 ± 3	19 ± 2	20 ± 3	22 ± 3	22 ± 3
8-OH-DPAT (n=6)	CBF	115 ± 13	110 ± 12	101 ± 13	87 ± 15*	77 ± 16*
	AVAF	79 ± 10	74 ± 9	65 ± 10*	47 ± 10*	25 ± 7*
	TF	36 ± 4	36 ± 4	36 ± 5	40 ± 7	52 ± 10*
Ipsapirone (n=6)	CBF	141 ± 14	152 ± 17	162 ± 19	155 ± 19	153 ± 20
	AVAF	106 ± 11	118 ± 14	125 ± 17	122 ± 18	119 ± 20
	TF	35 ± 7	34 ± 7	36 ± 6	34 ± 6	34 ± 5

Data in ml/min; mean ± s.e. mean; *, $p < 0.05$ vs. baseline.

We conclude that though 8-OH-DPAT mimics the effects of 5-HT in the carotid vascular bed, the other putative 5-HT_{1A} agonist, ipsapirone, does not. It is not known whether this contrast is due to a difference in the 5-HT_1-like receptor subtype or in the efficacy of the two compounds.

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MODULATION OF STIMULATION-EVOKED [^{3}H]-NORADRENALINE RELEASE FROM RABBIT ISOLATED EAR ARTERY

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Adrenaline may be implicated in the development of essential hypertension by enhancing stimulation-evoked noradrenaline release through activation of presynaptic facilitatory β-adrenoceptors (Majewski & Rand, 1984). While presynaptic inhibitory α-adrenoceptors are found in most tissues (Starke, 1987), the occurrence of presynaptic facilitatory β-adrenoceptors is uncertain in several tissues (Misu & Kubo, 1986). Although the rabbit aorta, a large elastic artery, does not possess presynaptic β-adrenoceptors (Abrahamsen & Nedergaard, 1986), it might be possible that small muscular arteries of the rabbit contain these adrenoceptors. Thus, Majewski & Rand (1981) found presynaptic facilitatory β-adrenoceptors in the rabbit ear artery. In contrast, Hope et al. (1976) were unable to demonstrate β-adrenoceptors in this tissue. The aim of the present study was to investigate the presence of presynaptic α- and β-adrenoceptors in the rabbit ear artery.

We developed a set-up to study the modulation of stimulation-evoked [^{3}H]-noradrenaline release from isolated strips of the rabbit ear artery. Ear arteries were cut open longitudinally into rectangular strips, mounted vertically and loaded with (-)-[^{3}H]-noradrenaline (10^{-7}M) for 30 min. After wash-out, the tissue was subjected to electrical-field stimulations (S_1-S_8; 150 pulses; 3 Hz; 225 mA; 0.5 msec) through platinum electrodes situated on either side along the whole length of the strip. Cocaine (3×10^{-5}M) plus corticosterone (4×10^{-5}M) were present from the onset of the wash-out and throughout the experiment.

Adrenaline (10^{-9}-10^{-6}M) and the selective α_2-adrenoceptor agonist clonidine (10^{-9}-10^{-6}M) decreased the stimulation-evoked ^{3}H-overflow concentration-dependently. The inhibitory effect of adrenaline (10^{-8}-10^{-6}M) was antagonized by the selective α_2-adrenoceptor antagonist, rauwolscine (10^{-6}M).

Isoprenaline (10^{-9}-10^{-6}M) did not alter the stimulation-evoked ^{3}H-overflow. This was also the case in the presence of either (1) rauwolscine (10^{-6}M); (2) rauwolscine (10^{-6}M) plus the selective phosphodiesterase inhibitor ICI 63 197 (2-amino-6-methyl-5-oxo-4-n-propyl-4,5-dihydro-s-triazolo 1,5-a pyrimidine; 3×10^{-5}M); or (3) rauwolscine (10^{-6}M) plus forskolin (10^{-6}M), an activator of adenylate cyclase.

Rauwolscine (10^{-7}-10^{-6}M) and phentolamine (3×10^{-7}-3×10^{-5}M) concentration-dependently increased the stimulation-evoked ^{3}H-overflow by up to 209% and 227%, respectively. Higher rauwolscine concentrations (3×10^{-6}-10^{-5}M) increased stimulation-evoked ^{3}H-overflow to a lesser extent.

In some experiments the rabbit ear artery strips were loaded with (-)-[^{3}H]-noradrenaline (10^{-6}M) for 60 min and after a wash-out period subjected to electrical-field stimulations with the same stimulation variables (5 Hz; 300 pulses) as used by Majewski and Rand (1981). Neither cocaine nor corticosterone was present. Isoprenaline (10^{-7}M) alone or in the presence of phentolamine (10^{-6}M) had no effect on stimulation-evoked ^{3}H-overflow. Phentolamine (10^{-6}M) enhanced the ^{3}H-overflow up to 173%.

We conclude that postganglionic sympathetic nerve endings in the rabbit ear artery possess presynaptic inhibitory α_2-adrenoceptors, but not presynaptic facilitatory β-adrenoceptors.

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EFFECTS OF HYPOTHYROIDISM ON ISCHAEMIA AND REPERFUSION INDUCED ARRHYTHMIAS AND ADRENOCEPTOR RESPONSES IN RATS

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Changes in α-adrenoceptors have been implicated in the genesis of ischaemia and reperfusion induced arrhythmias (Sheridan et al., 1980). Since there is increased responsiveness to α-adrenoceptor stimulation in rats made hypothyroid with 6-propylthiouracil (PTU) (Chess-Williams and Critchley, 1987) the present study was designed to examine arrhythmias under these conditions.

PTU was administered in drinking water (0.4 mg ml^{-1}) to male Wistar rats for 8 to 10 weeks. Some rats were then anaesthetised with sodium pentobarbitone and subject to coronary artery occlusion for either 25 min (ischaemia) or 5 min followed by reperfusion. The other rats were killed and tissues removed for in vitro functional studies. Similar experiments were performed in age-matched control rats.

In isolated papillary muscles set up in a Krebs solution at 37°C the maximum driving frequency (determined at 2 x threshold voltage) was reduced ($P < 0.05$) in tissues from hypothyroid animals (10.7 ± 0.5 Hz) compared with control animals (13.2 ± 0.7 Hz). In the same tissues, inotropic responses to isoprenaline and phenylephrine were obtained in the presence of desipramine (1μM) and metanephrine (10μM) to inhibit amine uptake. Isoprenaline EC_{50} values were similar in control (43.9 (20.2 - 95.5)nM) and PTU-treated animals (36.0 (5.7 - 228.7)nM), but phenylephrine responses obtained in the presence of propranolol (1μM) were potentiated in hypothyroid animals. The phenylephrine EC_{50} value was reduced ($P < 0.01$) from 21.6 (9.9 - 47.3)μM to 3.5 (1.3 - 9.5)μM and the phenylephrine maximum response relative to that of isoprenaline was increased ($P < 0.001$) from 27.0 ± 4.6% to 70.9 ± 9.6%.

In anaesthetised control rats, prior to coronary artery occlusion, heart rate was 436 ± 12 beats min^{-1} and arterial blood pressure was 126 ± 6/91 ± 6 mmHg (n = 18). A marked bradycardia was observed in the hypothyroid rats, 256 ± 6 beats min^{-1} ($P < 0.001$) and systolic but not diastolic blood pressure was also significantly ($P < 0.05$) lower, 107 ± 4/86 ± 3 mmHg (n = 20).

Although there was no significant difference in the total number of ischaemia induced ventricular premature beats, the incidence of ventricular fibrillation (VF) was reduced from 80% in controls (n = 10) to 36% in hypothyroid rats (n = 11). Mortality was also reduced from 50% to 0% ($P < 0.001$). Reperfusion induced VF and mortality were completely absent in hypothyroid rats (n = 9) whereas 75% of the controls had VF following reperfusion and 50% died in terminal VF (n = 8).

Whether the marked reduction in VF observed in hypothyroid rats is related to the bradycardia, the prolongation of the effective refractory period, increased α-adrenoceptor responsiveness or some other factor remains to be investigated. It is certainly clear from the above results that in this situation where α-adrenoceptor sensitivity is enhanced, VF is markedly reduced.

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SYNERGISTIC ACTIONS OF 5-HT AND ARACHIDONIC ACID IN PLATELET AGGREGATION: EFFECT OF 5-HT BLOCKERS

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It is well documented that arachidonic acid (AA) can cause platelet aggregation mainly by the formation of potent proaggregatory and a vasoconstrictor agent; thromboxane-A_2 (TXA_2). In addition, 5-HT is also thought to play a role in platelet aggregation. It has recently been demonstrated that synergism between the contractile effects of platelet released 5-HT and TXA_2 can occur in human blood vessels (Young and Moulds, 1984). However, the role of 5-HT in platelet aggregation is clearly not understood. In this report a selective potentiating effect of 5-HT in AA-induced platelet aggregation is described and a possible mechanism of action is explored.

Blood was obtained from normal adult volunteers who had not taken any aspirin-like drug for 10 days. Platelet-rich plasma (PRP) was prepared by centrifugation at 260 xg and aggregation was monitored turbidmetrically on a dual-channel Chrono-Log aggregometer. All aggregations were carried out at 37°C with PRP having platelet counts of 2-2.5 x 10^8/ml. Each drug was tested at 3-4 concentrations in triplicate. The results showed that 5-HT alone upto 500 uM concentration did not induce aggregation ($P<0.001$; n=47). AA (50-1500 uM concentration) showed a dose-response aggregation in PRP. The sub-threshold concentration of AA was 70 ± 9 uM (n=70). When 5-HT (0.2 mM) and AA in subthreshold concentration were added together or succesively, a marked potentiation of aggregation was obtained ($P<0.001$ n=34). In order to assess the specificity of 5-HT and AA combination-induced aggregation, we tested the effect of various 5-HT receptor blockers against platelet aggregation. The mean values ± S.E.M. (uM) for inhibiting platelet aggregation by 50% (IC50) for various drugs are given in Table 1.

Table 1: INHIBITION OF ARACHIDONIC ACID + 5-HT INDUCED PLATELET AGGREGATION (IC50* (uM) ± S.E.M.)

Drug	Aggregating Agent: Arachidonic Acid (AA)	Aggregating Agent: Arachidonic Acid Plus 5-HT	Relative potency against AA+5-HT-induced aggregation
Cyproheptadine	550 ± 1	0.83 ± 0.05**	660,000
Ketotifen	105 ± 5	0.05 ± 0.01	2,100
Imipramine	390 ± 2	0.13 ± 0.03	3,000
Amitriptyline	340 ± 0.5	0.36 ± 0.02	950
Ergometrine	225 ± 3	1.33 ± 0.2	170
Methysergide	N.I.	2.50 ± 0.50**	400,000
Pizotifen	230 ± 1	0.90 ± 0.02	3,000
Indomethacin	14 ± 1.0	6.15 ± 0.05**	2,276
Nordihydroguaiaretic acid (NDGA)	196 ± 6	6.15 ± 0.02**	31,869

N.I. Not inhibited; * Concentration producing 50% inhibition of aggregation. ** Concentration expressed as nM. Statistical significance was assessed by students-t-test.

As shown in Table 1, cyproheptadine, methysergide, ketotifen, pizotifen, imipramine and various other drugs completely inhibited aggregation induced by 5-HT and AA together in a dose-dependent fashion ($P<0.005$). These drugs also inhibited AA-induced aggregation but only at extremely higher concentrations of the drugs. The data given in Table 1 also show that 170-660,000 times higher doses of 5-HT blockers are needed to inhibit AA-induced aggregation as compared to their inhibitory potency on 5-HT plus AA-induced aggregation. It is important to note that indomethacin and NDGA (inhibitors of AA-metabolism), abolished the potentiating effect of 5-HT on aggregation. These data suggest that increased formation of AA metabolites may be one of the factors responsible for the potentiating effect of 5-HT.

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LOW-DENSITY LIPOPROTEINS BUT NOT HIGH-DENSITY LIPOPROTEINS INHIBIT EDRF & NITRIC OXIDE MEDIATED RELAXATION OF RABBIT AORTA

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Low-density lipoproteins (LDL), a known risk factor in coronary heart disease were recently shown to inhibit EDRF-mediated relaxation of intact rabbit aortic rings (Andrews et al 1987). Since the activity of EDRF is attributed to the release of nitric oxide (NO) from endothelial cells, we examine here the possibility that (1) LDL inhibits NO evoked relaxations, and (2) high density lipoproteins (HDL), a protective factor for atherosclerosis, have different effects to LDL.

LDL (density 1.006-1.0063 g/ml) and HDL (density 1.063-1.21 g/ml) were prepared from human plasma by the method of Chung et al (1980). Total HDL was further purified by heparin-sepharose chromatography (Weisgraber & Mahley, 1980) to remove LDL contaminants. The procedures for organ bath studies were previously described (Andrews et al.1987). Endothelium-denuded or intact transverse rings were prepared from the thoracic aortae of 6-month old New Zealand White rabbits, contracted with serotonin and relaxed with one of the following relaxants: acetylcholine (ACh) or NO generated in situ from acidified sodium nitrite (Furchgott et al. 1987). After washout, the tissues were incubated for 30 min with LDL at a 2mg/ml protein concentration or HDL (0.5-2.0 mg protein/ml) with or without a contraction/relaxation cycle. The tissues were again washed after 30 min and the cycle of contraction/relaxation repeated.

1 μM ACh evoked in precontracted intact aortic rings a relaxation of 76 ± 2.6% (mean + s.e. mean, n = 4). In the presence of LDL and after washout, the relaxation was inhibited by 89 ± 2% and 84 ± 3% respectively, whereas HDL had no effect. Nitric oxide, generated 'in situ' from 1μM acidified sodium nitrite, induced a 68 ± 3% relaxation in endothelium-denuded rings. Relaxations were inhibited in the presence of LDL by 64 ± 4% and were reversed after washout of the lipoprotein. In contrast, in the presence or after removal of HDL, relaxations were similar to controls. The inhibition of NO/EDRF relaxations is therefore confined to LDL.

Inhibition of NO-induced relaxations may result from an effect of LDL on guanylate cyclase or a direct inactivation of NO. The latter possibility may contribute to the inhibition of ACh-evoked relaxations in intact aortic rings. This inhibition, unlike that of NO induced relaxations, continues after lipoprotein removal. LDL is therefore likely to be transported to the subendothelial space where it can interact with NO; a process which may account for the inhibition of EDRF-mediated relaxations in atherosclerotic arteries (Verbeuren et al. 1986). Additionally, an inhibition of NO generation in the endothelium remains a possibility.

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DO EXCITATORY NEURONAL PROSTANOID RECEPTORS EXIST IN THE GUINEA-PIG ILEUM?

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Prostaglandin (PG) E_2 contracts guinea-pig isolated ileum (GPI) via an action at EP_1-receptors on the smooth muscle (Kennedy et al 1982), an effect blocked by such antagonists as AH6809 (Coleman et al 1985). However, PGE_2 also augments cholinergically-mediated contractions of GPI by increasing neurotransmitter release (Ehrenpreis et al 1973, Kadlec et al 1978). We have therefore investigated the role of endogenous prostanoids on cholinergic neurotransmission and the site of action of exogenously administered PGE_2 in this tissue.

Segments of proximal ileum were obtained from Dunkin-Hartley guinea-pigs (400-500g) of either sex. Strips of longitudinal muscle with myenteric plexus intact were prepared essentially as described by Paton and Zar (1968) and were mounted in organ baths containing modified Krebs solution at 37°C, gassed with 5% CO_2 in O_2, and containing AH6809 (10μM) to prevent EP_1-receptor mediated direct smooth muscle contraction by PGE_2. The preparations were placed under a resting tension of 1g, and were field-stimulated via two platinum electrodes using pulses of supramaximal voltage (25V), current duration (CD) of 1ms and frequency of 0.1 or 0.2Hz. The resulting contractions could be abolished by tetrodotoxin (0.52μM) and by atropine (0.3μM).

Indomethacin (IM, 0.3-100μM) caused slow (30-60 min) concentration-related suppression of electrically-induced contractions with an EC_{50} of 8μM (n=4) and a maximum inhibition of 83 ± 8% (mean ± s.e.m.). In the presence of 3 and 30μM IM, which produced 41±2% and 63±4% inhibition of responses respectively, addition of PGE_2 (0.1-100nM) caused concentration-related potentiation of contractions in all experiments. Although the EC_{50} value of PGE_2 was little affected by the presence of IM, being 6.3±1nM (-IM), 4.0±0.2nM (+IM, 3μM) and 3.5±0.6nM (+ IM, 30μM) (n=17), the degree of potentiation was most marked in IM (30μM)-treated and least in untreated preparations. Current duration (0.01-3ms)- contractile effect curves were next constructed in the absence and then in the presence of IM (30μM). The presence of IM caused a small rightward shift and a marked depression (62±5%, n=7, inhibition of the maximum) of the CD-effect curves. Subsequent addition of PGE_2 reversed this IM-induced inhibition. Thus PGE_2 (0.1-10nM) caused a concentration-related steepening and leftward shift of the CD-effect curves, 3nM PGE_2 completely reversing the IM-induced effect, and 10nM PGE_2 even causing a small enhancement (15±8%, n=4) of the CD-effect curve maximum.

These data suggest that in addition to smooth muscle EP_1-receptors, GPI contains excitatory neuronal prostanoid receptors and that endogenous prostanoids may play a role in mediating contractions of GPI to field stimulation.

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NEW ASPECTS OF ARACHIDONIC ACID METABOLISM IN HUMAN PLACENTA

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Prostaglandins (PGs) particularly of the E series are considered to serve an important role in the control of utero-placental haemodynamics (Mitchell et al, 1982). It has been demonstrated that human placenta synthesizes PGs from arachidonic acid (AA) but the complete pathways of AA metabolism in human placenta have not been determined. In this study it was found that human placental tissue, when incubated with [^{14}C] AA generates several lipoxygenase products of which two have been identified as 12-hydroxy-5,8,10,14 eicosatetraenoic acid (12-HETE) and 5-hydroxy-6,8,11,13-eicosatateraenoic acid (5-HETE).

Human term placentae were collected at the end of uncomplicated pregnancies. Pieces (2-3g) of placental tissue were cut and homogenized in phosphate buffer (50 mM, pH 7.4). The homogenate (1000 xg supernatant fraction containing 1.5 mg of protein) was incubated with 0.45 uCi of [1-^{14}C] AA (spc. act. 58.4 mCi/mmol, Amersham). After incubation the radioactive lipoxygenase metabolites and unchanged AA were extracted with ethylacetate. The organic phase was evaporated, redissolved in 40 ul ethanol and applied quantitatively to silica gel G TLC plates. The plates were developed in ether/petroleum ether (boiling range 20-40°C)/acetic acid (50:50:1 by vol). Zones containing radioactivity were located and quantified by use of Berthold TLC linear analyzer Model (LB511) equipped with chromatography data system. In separate experiments, the lipoxygenase metabolites of the above reaction were separated by straight phase high performance liquid chromatography (HPLC) on a u Porasil (3.9 mm x 30 mm) column eluted at 3 ml/min with a linear solvent gradient from 0.5% to 1.5% isopropanol in hexane containing 0-1% acetic acid over a 30 min period. Metabolites were monitored by assay of radioactivity in the fractions and were identified on the basis of retention times on HPLC.

The results showed that incubation of [1-^{14}C] AA with placental homogenate converted the radioactive AA into atleast two radiolabeled metabolites. The chromatographic mobility of the main product (Rt 0.62) (conversion of AA, 3-6%) was identical with aunthentic 12-HETE. The minor product (Rf 0.30) (conversion of AA -.1-0.3% was found to be 5-HETE. Further confirmation of the identity of these products was obtained by HPLC. These results are indicative of the presence of lipoxygenase activity in the human placenta. An apparent Km of the enzyme of 3.0 uM for AA was estimated from the lineweaver-Burke double-reciprocal plot of 12-HETE formation. The enzymic reaction was also found to be linear both with respect to time of incubation and amount of placental homogenate. The effects of ETYA, nordihydroguaiaretic acid (NDGA) and indomethacin on the formation of 12-HETE are presented in Table 1.

Table 1: EFFECTS OF VARIOUS COMPOUNDS ON 12-HETE FORMATION BY HUMAN PLACENTA

Test Compound	Concentration (uM)	Mean percentage inhibition of 12-HETE production ± S.E.M. (n=7)
ETYA*	0.1	47.0 ± 1.5
	1.1	65.0 ± 6.5
	11.1	87.3 ± 0.2
Nordihydroguaiaretic acid (NDGA)	6.6	39.0 ± 0.5
	50	53.3 ± 5.2
	500	89.0 ± 1.0
Indomethacin	5.3	11.2 ± 8.7**
	26.3	3.5 ± 2.2
	132.0	16.2 ± 4.3

* 5,8,11,14-Eicosatetraynoic acid (ETYA); ** Stimulation.

These results are indicative of the existence of lipoxygenase enzymes that can act on AA in human placenta. Since lipoxygenase products are potent chemotactic and vasoactive agents it is not unreasonable to suggest that lipoxygenase activities and HETE formation in placenta may serve to regulate recruitment of leukocytes and/or macrophages during pregnancy and parturition.

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ILOPROST-A POTENT EP_1- AND IP-RECEPTOR AGONIST

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A problem encountered in the study of prostacyclin-sensitive (IP) receptors in blood platelets and smooth muscle is the chemical instability of the standard agonist prostaglandin (PG)I_2. Although many stable analogues of PGI_2 have been synthesised, increased stability has usually been associated with loss of potency. However, the carbacyclin analogue iloprost (ZK36374) appears to combine chemical stability with high IP-receptor agonist potency. Although iloprost has no vasoconstrictor (TP-agonist) activity (Schrör et al., 1981), it does exhibit potent agonist activity on some but not all PGE_2-sensitive (EP-) receptor containing preparations (Dong et al., 1986).

We have previously classified EP-receptors into EP_1, EP_2 and EP_3 subtypes (Coleman et al., 1987). Therefore, we have evaluated both iloprost and PGI_2 on smooth muscle preparations containing: EP_1-(guinea-pig fundus and ileum), EP_2-(cat trachea, guinea-pig ileum circular muscle, trachea and dog saphenous vein) and EP_3-(guinea-pig vas deferens), as well as FP-(dog iris) and IP-(rat and human blood platelets) receptors. The methods are as described by Brittain et al. (1985), Coleman (1987) and Coleman et al. (1987). Iloprost was approximately equipotent with PGI_2 on the IP-receptor containing tissues where PGI_2 is the most potent natural prostanoid. They were also approximately equipotent on the FP-, EP_3- and all but one (cat trachea) of the EP_2-receptor containing preparations, being at least 70-fold weaker than the most potent natural prostanoid, $PGF_{2\alpha}$ (dog iris) and PGE_2 (EP-receptor containing preparations). However, on guinea-pig fundus and ileum, iloprost was approximately equipotent with PGE_2 (mean equipotent concentration, EC (95% C.L.) = 0.8 (0.5-1.4) and 1.4 (0.2-11) respectively, n>4) whereas PGI_2 was substantially weaker (EC=20 (10-45) and 82 (51-132) respectively, n>5). Although on cat trachea, iloprost was relatively potent (EC ~1-10, PGE_2=1, n=6), its activity was variable, and of modest degree (i.e. 26-53% PGE_2 maximum). Furthermore, 30μM iloprost did not antagonize subsequent responses to PGE_2 on this preparation. We next examined the effect of the EP_1-receptor blocking drug, AH6809, against both iloprost and PGI_2 on guinea-pig fundus. AH6809 caused concentration-related antagonism of both agonists, yielding pA_2 values of 6.9 (6.5-7.4) and 6.9(6.5-7.3) with Schild slopes of 1.0(0.7-1.4) and 0.7(0.5-0.9) (n>5) against iloprost and PGI_2 respectively, these being similar to the values for AH6809 against PGE_2 and $PGF_2\alpha$ on this preparation (Coleman et al., 1985), consistent with an action at EP_1-receptors.

We conclude therefore, that while iloprost, like PGI_2, is a potent IP-receptor agonist, it is also a potent EP_1-receptor agonist, being at least as potent as PGE_2 and at least 20-fold more potent than PGI_2. The unexpected high potency of iloprost on cat trachea may result from the presence of a small and variable population of EP_1- and/or IP-receptors in addition to the predominant EP_2-receptors in this preparation.

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COMPARISON OF THE SURVIVAL OF EDRF AND NITRIC OXIDE (NO) WITHIN THE ISOLATED PERFUSED MESENTERY OF THE RAT

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Several agents release endothelium derived relaxing factor (EDRF) from cultured endothelial cells (Cocks et al., 1985; de Nucci et al., 1988) and the EDRF released has been identified as nitric oxide (NO, Palmer et al., 1977). Release of EDRF in the effluent from perfused vascular beds has not been shown, although it has been demonstrated within them (Furchgott et al., 1987). We investigated the responses of the isolated perfused mesenteric bed of the rat to endogenously generated EDRF, to exogenous NO and the survival of these in the effluent from the preparation.

The isolated perfused mesenteric bed was prepared by the method of McGregor (1965) using heparinised rats (100U/100g i.p.). The mesenteric bed was perfused at a constant flow of 5ml/min with oxygenated (95%O_2/5%CO_2) Krebs' solution at 37°C containing indomethacin (5 μM). Vascular tone was increased by an infusion of noradrenaline (NA, 0.5-500 μM) or U46619 (0.03-1.5 μM) through the mesentery to give a perfusion pressure rise from 15-22 mmHg to 50-80 mmHg. The effluent from the mesenteric bed superfused a bioassay cascade of 4 de-endothelialized rabbit aorta strips (RbA) which were also contracted by the NA or U46619. Hyoscine (0.3 μM) was infused over the tissues (OT) throughout. Compounds were administered as bolus injections either thorugh the mesenteric bed (TM) or OT. In some experiments, superoxide dismutase (SOD, 10U/ml) was infused TM.

Acetylcholine (ACh 1.5pmols-300nmols), NO (1-30nmols) and glyceryltrinitrate (GTN, 120-200pmols) produced dose related vasodilatations (n=13). SOD had no effect on the basal perfusion pressure or on the vasodilatation induced by ACh, NO or GTN (n=8). GTN injected TM produced similar relaxations of the RbAs to the same doses injected OT (n=6). In contrast, NO when given TM needed 3.3±0.6 times the dose OT to give similar relaxation of the RbAs. SOD TM significantly increased the survival of NO so that when given TM only 1.4±0.4 times the dose of NO used OT was needed ($p<0.05$). Thus, vasodilator doses of NO in the mesenteric bed also induced relaxations of the superfused RbAs. However, doses of ACh which gave equivalent vasodilator effects in the mesentery did not cause relaxations of the RbAs (n=13). For example 1nmol NO given TM produced vasodilatation in the mesentery and sufficient survived to cause relaxation of the RbAs. A dose of ACh which gave vasodilatation equivalent to 30 nmol NO in the mesentery still did not cause relaxation of the RbAs, even in the presence of SOD (n=3).

This data shows that EDRF is not released into the perfusing medium of the mesenteric bed after stimulation with ACh in a quantity sufficient to be detected by the RbAs, although doses of exogenous NO, equivalent in terms of mesenteric bed vasodilatation to much smaller doses of ACh, partially survive passage through the bed. This suggests the release of EDRF in this system is abluminal rather than adluminal or that the endothelial cells offer a permeability barrier to exogenous NO.

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OBSERVATIONS ON THROMBOXANE RECEPTOR-MEDIATED CONTRACTION OF GUINEA-PIG AND HUMAN AIRWAYS BY OTHER PROSTANOIDS

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Prostaglandin (PG) D_2 is the predominant cyclooxygenase product of human lung mast cells (Lewis *et al.*, 1982). The contractile responses of guinea-pig and human airways to PGD_2, 9α,11β-PGF_2, $PGF_{2\alpha}$ and the thromboxane (TX) A_2-mimetic U-46619 have previously been reported (Beasley *et al.*, 1987). We have made further studies on the relative potencies of these agonists, and also the TXA_2-mimetic ONO-11113, as an aid to classifying the receptor type(s) mediating prostanoid-induced contraction of the airways. As the affinities of competitive antagonists form a more reliable basis for receptor classification than agonist potency studies, we have also studied the effects of four prostanoid antagonists, BW245C, EP092, GR-32191 and ONO-11120.

Human airways of 4mm diameter, dissected from lung tissue obtained from patients undergoing surgery for bronchial carcinoma, or tracheae from male Dunkin Hartley guinea-pigs, killed by asphyxiation with CO_2, were cut into spirals and suspended in 5ml tissue baths containing Krebs solution with 3 μM indomethacin at 37°C, gassed with 95% O_2: 5% CO_2. Using isometric recording, cumulative concentration-response curves were constructed for each prostanoid in the absence and presence of one of the antagonists. Each antagonist was left in contact with the tissue for 15 minutes before re-testing the agonist. At least three concentrations of each antagonist were tested to enable the construction of Schild plots.

The prostanoid EC_{50} values (nM) (95% confidence limits and number of experiments) in human bronchus were: U-46619 4.7 (2.1-10.4: n=13), 9α,11β-PGF_2 840 (530-1340: n=16), $PGF_{2\alpha}$ 1470 (770-2790: n=13) and PGD_2 3940 (2110-7350: n=13) and in guinea-pig trachea were: ONO-11113 2.7 (1.2-6.4: n=6), U-46619 5.8 (3.9-8.9: n=48), $PGF_{2\alpha}$ 343 (134-879: n=38), PGD_2 370 (106-1292: n=56) and 9α,11β-PGF_2 453 (207-993: n=41). Using PGD_2 as the agonist, the pA_2 values (95% confidence limits) of antagonists in the human bronchus were: BW245C 5.5 (5.3-5.7: n=3) and EP092 7.2 (6.9-7.5: n=6) and in guinea-pig trachea were; BW245C 6.3 (5.6-6.9: n=6), EP092 7.4 (7.0-7.8: n=5), GR32191 8.8 (7.7-9.9: n=7) and ONO-11120 7.1 (6.7-7.5: n=4). The pA_2 values obtained using U-46619, ONO-11113 or 9α,11β-PGF_2 did not differ significantly from those seen with PGD_2. However, when $PGF_{2\alpha}$ was used as the agonist, BW245C gave a pA_2 value of 6.2 (5.8-6.6: n=3) in human bronchus and 5.4 (5.1-5.7: n=5) in guinea-pig trachea, these values being significantly ($P<0.05$) different from those for PGD_2. The pA_2 value for EP092 against $PGF_{2\alpha}$ of 8.1 (7.6-8.6: n=5) in the guinea-pig trachea was also significantly ($P<0.05$) different from that against PGD_2. None of the antagonists altered concentration-response curves to methacholine.

Our data suggests that the contractile effects of PGD_2, $PGF_{2\alpha}$, 9α,11β-PGF_2 and the TXA_2-mimetics are mediated through similar receptor populations, in which thromboxane receptors are likely to predominate. However, we cannot exclude the possibility that $PGF_{2\alpha}$ in particular may act in part through stimulation of another receptor sub-population.

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PYROGENIC IMMUNOMODULATORS INCREASE THE ACCUMULATION OF INOSITOL PHOSPHATES IN MONOCYTES

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It is well established that exogenous pyrogens exert their effects on a variety of immunocompetent cells which are thought to be involved in the pathogenesis of fever. In particular peripheral blood monocytes (Mø) and other mononuclear phagocytes are stimulated to release interleukin 1 (IL1) which is thought to be the endogenous mediator of fever. IL1 subsequently stimulates the biosynthesis of prostanoids from a variety of other cell types. There is little information, however, on the underlying molecular events which initiate the biosynthesis of these mediators. Recently much attention has been paid to the role of phosphatidylinositol (PI) metabolism and the production of insitol trisphosphate in the transduction of extracellular signals into intracellular responses. It is therefore possible that pyrogenic immunomodulators exert their effect through the metabolism of PI. In the present study we investigated the effects of lipopolysaccharide (LPS), polyinosinic:polycytidylic acid (Poly I:C), muramyl dipeptide (MDP) and IL1 as well as the protein synthesis inhibitor anisomycin on the accumulation of inositol phosphates (IP) in rabbit Mø.

Adherent cells (monocytes), prepared on percoll density gradients followed by adherence to plastic culture dishes over 2 hours at 37 °C, 5% CO_2 100% humidity, were labelled with [H^3]-inositol in RPMI 1640 medium for 20 hours. Cells were then incubated in RPMI 1640 with the immunomodulators in the presence of LiCl for 2 hours after which chloroform/methanol was added and the total IPs in the aqueous layer was estimated by ion exchange on formate-form Dowex-1 columns as described by Berridge, (1984).

LPS, Poly I:C and MDP all increased the IP level above control (expressed as 100%) in a concentration-dependant manner. The concentrations which gave a maximal increase within the incubation period were 1 µg/ml, 100 µg/ml and 100 µg/ml respectively which resulted in significant IP increases of 228%, 172% and 178% (S.D.s of 2%, 1.5% and 2.5% respectively, n = 4, $P < 0.001$). No stimulation was observed if incubations were carried out for less than 1 hour. In separate experiments anisomycin (5 µg/ml) reduced the LPS-stimulated increase from 157% to 123%, (S.D.s of 2.5% and 1.5% respectively n = 4, $P < 0.001$). IL1-α (4 µg/ml) increased the IP level to 126% within 10 minutes of incubation and 167% after 30 minutes,(S.D.s of 2% and 3.5% respectively, n = 4, $P < 0.001$). Anisomycin (5 µg/ml) had no effect on the IL1-stimulated increase in IP level.

In view of the longer period required by the exogenous immunomodulators before a significant increase in IP level was observed, and the inhibition by anisomycin, it would appear that the increases in IP levels in response to in particular LPS require a protein mediator. A likely candidate being IL1 as a rapid increase in the IP level was observed. In conclusion these results suggest that the increases in IP levels in response to the exogenous immunomodulators do not occur directly and that this is not a likely mechanism by which they trigger the biosynthesis of IL1. It would appear more likely that this is a possible signal transduction mechanism by which IL1 exerts its vast array of actions including possibly prostanoid production.

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THE RELATIONSHIP BETWEEN THE EDRF, THE SMOOTH MUSCLE INHIBITORY FACTOR FROM THE BRP AND NITRIC OXIDE

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EDRF and the smooth muscle inhibitory factor (IF) extracted from the bovine retractor penis muscle (BRP) have several features in common. Both relax smooth muscle, have a short half life, the inhibitory effect of both is abolished by haemoglobin and borohydride and both appear to act by stimulating guanylate cyclase. Experimental evidence that EDRF is NO has recently been published (Palmer et al, 1987) and it has been suggested that the IF may also act through nitric oxide (Furchgott, 1988; Martin et al, 1988) liberated by exposure to acid. If both EDRF and the IF act through NO then the sensitivity of different smooth muscles to all three stimuli should show the same ranking order and factors which modify the effects of NO should similarly change the effects of EDRF and the IF.

We have examined the effect of these three stimuli together with sodium nitroprusside on four smooth muscles; the endothelium-free rabbit aortic strip, the BRP, the guinea-pig trachea and the rat anococcygeus. The effect of haemoglobin solution and an equivalent volume of intact RBCs has also been examined. NO solutions were made by dissolving an appropriate volume of gas in saline deoxygenated by gassing with helium for 1 hour. EDRF was liberated by acetylcholine from a 6 cm length of rabbit aorta perfused at 4 ml/min with oxygenated Krebs solution containing indomethacin 5×10^{-6} M. The perfusate leaving the aorta passed over the test muscles in a chamber immediately above the donor aorta.

The aortic strip and the BRP contracted with 10^{-5} M ACh + 10^{-5} M 5-HT were sensitive to the relaxant effect of EDRF. Dose-response curves showed maximum release of EDRF at an ACh concentration of 5×10^{-6} M. Neither the rat anococcygeus nor the guinea-pig trachea responded to EDRF in the concentrations reached in these experiments. The IF and NO produced a similar ranking order of sensitivity; the aortic strip was most sensitive, the BRP only a little less so. The IF and NO at high concentrations did relax the rat anococcygeus and the IF produced a small relaxation of the guinea-pig trachea.

Haemoglobin binds NO. We, therefore, mixed both NO and acid-activated IF with a 10^{-5} M solution of haemoglobin and, after 10 mins, separated the haemoglobin by filtration through an appropriate ultrafiltration membrane. The relaxant properties of the NO solution were completely lost and those of the activated extract considerably reduced. Nitric oxide passes through membranes with ease. If NO is the active principle in acid activated extracts of the IF, then exposure to RBCs should still allow the NO to bind to haemoglobin. This was tested by exposing a solution of NO and of acid-activated inhibitory factor to a concentration of RBCs equivalent to 10^{-5} M haemoglobin and, after 10 mins, separating the RBCs by centrifugation. When the supernatants were assayed the relaxant properties of the NO solution were completely abolished but there was no loss of activity in the solution of IF. These results are consistent with NO as the mediator of relaxation for both the IF and the EDRF but, if so, the NO in the IF is bound in a way which makes it unable to diffuse through the erythrocyte membrane.

H.S. is a University of Glasgow Postgraduate Scholar

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DIVALENT CATION INFLUX IN HUMAN NEUTROPHILS IS STIMULATED BY F-MET-LEU-PHE, PAF AND LEUKOTRIENE B_4

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When fura-2-loaded human neutrophils are stimulated with f-met-leu-phe (10^{-10} - 10^{-6}M), PAF (10^{-12} - 10^{-7}M) or leukotriene B_4 (10^{-11} - 10^{-6}M), a rise in cytosolic free calcium concentration ($[Ca^{2+}]_i$) is observed. In the absence of external Ca^{2+} with EGTA (1mM) added, $[Ca^{2+}]_i$ rises rapidly from a resting level of 50-80nM to a transient peak of around 1uM which then declines to baseline; this is presumably due to release of Ca^{2+} (by inositol (1,4,5)-trisphosphate) from an internal store which then becomes depleted. Subsequent addition of Ca^{2+} to restore the extracellular concentration to 1mM, in the continued presence of agonist, then results in a further rise in $[Ca^{2+}]_i$ to a maintained elevated level. In the presence of external Ca^{2+} (1mM), the initial transient peak in $[Ca^{2+}]_i$ is followed by a maintained elevation.The maintained stimulated elevation in $[Ca^{2+}]_i$ is dependent on external Ca^{2+} and is blocked by Ni^{2+} (5mM; a known Ca^{2+} antagonist), and is therefore assumed to be the result of a stimulated Ca^{2+} influx. However, measurement of cytoplasmic ion concentrations can only provide indirect evidence for ion movements across the plasma membrane. Such differences in $[Ca^{2+}]_i$ responses observed in the presence or absence of extracellular Ca^{2+} may simply reflect a requirement for external Ca^{2+} to more completely discharge the internal store. An alternative approach is to add a different divalent cation extracellularly (whose source would then be defined) with properties that can distinguish it from Ca^{2+} (Hallam & Rink, 1985). When Mn^{2+} binds to fura-2, the fl rescence is quenched at all excitation wavelengths. At an excitation wavelength of 360nm, fura-2 fluorescence is insensitive to changes in $[Ca^{2+}]$ but is still quenched by Mn^{2+}; this allows detection of changes in $[Mn^{2+}]$ independently of changes in $[Ca^{2+}]$. In the presence of extracellular Mn^{2+}, f-met-leu-phe, PAF and leukotriene B_4 all stimulate quenching of intracellular fura-2 fluorescence which is caused by stimulated entry of Mn^{2+}. Similarly, addition of extracellular Mn^{2+} in the continued presence of agonist, after $[Ca^{2+}]_i$ has peaked and declined to baseline, results in a stimulated quench in fluorescence due to Mn^{2+} entry. Simultaneous monitoring of fluorescence at 340nm excitation shows changes in $[Ca^{2+}]_i$ (although the Mn^{2+} quench is superimposed). This stimulated Mn^{2+} entry, like the Ca^{2+} entry, is blocked by Ni^{2+} (which does not itself enter cells). Use of Mn^{2+} with fura-2-loaded neutrophils therefore allows demonstration of an agonist-stimulated divalent cation influx.

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INFLUX OF DIVALENT CATIONS INTO THE CYTOPLASM OF HUMAN ENDOTHELIAL CELLS CAN BE INDEPENDENT OF RECEPTOR STIMULATION

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When human umbilical vein endothelial cells are stimulated with thrombin, histamine or ATP, the cytoplasmic free calcium concentration, $[Ca^{2+}]_i$, increases due to both discharge of Ca^{2+} from intracellular stores and influx of Ca^{2+} across the plasma membrane (Hallam & Pearson, 1986). Further direct evidence that thrombin causes the influx of divalent cations (ie Mn^{2+}) has also been reported (Hallam, 1987). Here, we show the results of experiments designed to examine the dependence of stimulated divalent cation influx on both the state of the intracellular Ca^{2+} store and receptor occupation using monolayers of fura-2 loaded human umbilical vein endothelial cells.

In the absence of extracellular Ca^{2+}, with 1mM EGTA, a maximally effective dose of histamine, 100uM, increases $[Ca^{2+}]_i$ from the basal level of 90-110nM to a peak of around 2uM that rapidly declines back to the basal level. Subsequent addition of histamine or thrombin has little further effect on $[Ca^{2+}]_i$. This is because the intracellular store of Ca^{2+} has been depleted by stimulation with histamine. Addition of 20uM mepyramine after histamine followed by incubation in the presence of 1mM extracellular free Ca^{2+} for 2-3 min. restores the response to thrombin indicating that the intracellular stores have now refilled. Addition of extracellular Ca^{2+} under these conditions causes a transient elevation in $[Ca^{2+}]_i$ that returns to basal levels and is presumably caused by influx of Ca^{2+} into the cytoplasm which is then curtailed when the intracellular store is replenished. The influx of divalent cations can be confirmed using extracellular Mn^{2+} showing that quenching of intracellular dye by stimulated Mn^{2+} entry can occur if Mn^{2+} is added after the cells have been stimulated with histamine and then treated with mepyramine.

The observation that divalent cation influx can refill an intracellular store in the absence of receptor-stimulation is not totally novel. This mechanism was first proposed for parotid acinar cells but in the parotid refilling occurs without any increase in $[Ca^{2+}]_i$ suggesting that influx is direct into the intracellular store (Putney, 1986; Merritt & Rink, 1987). Here we have clearly shown influx of Ca^{2+} and Mn^{2+} into the cytoplasm of endothelial cells that is sensitive to the state of the intracellular store. The mechanism for this sensitivity in the absence of receptor stimulus remains obscure.

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INDOMETHACIN ENHANCES PROTEOGLYCAN LOSS FROM ARTICULAR CARTILAGE IN ANTIGEN-INDUCED ARTHRITIS

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Indomethacin is one of a large group of non-steroid anti-inflammatory drugs which is widely used in the treatment of arthritis. Whilst giving symptomatic relief by the reduction of pain and swelling, there is little or no evidence that these drugs arrest the underlying destruction of joint tissues. Moreover, there have been suggestions that non-steroid anti-inflammatory drugs actually exacerbate the chronic degenerative processes. We have now investigated the effects of indomethacin on joint swelling, leucocyte infiltration and loss of proteoglycan from articular cartilage during the development of antigen-induced arthritis in rabbits.

Groups of New Zealand White rabbits (2.5-3.0 kg) were sensitised to ovalbumin in Freund's complete adjuvant and arthritis was induced by injection of antigen (5mg) into the knee joint of one hind limb (Dumonde and Glynn, 1962). Animals were dosed orally with indomethacin (1mg/kg x 3/24hr) or vehicle alone, the first dose being administered 1hr before antigen challenge. Joint diameters were measured with calipers at regular intervals and animals were killed after 1,4,7 or 17 days. Joint fluids were collected by washing with saline and leucocyte counts were determined. Synovial tissues were removed for histological examination and the proteoglycan content of articular cartilage was measured as previously described (Pettipher et al., 1986). Lymphocytes were counted in 10 bands of tissue, 250 microns wide, running from surface to deep fibrous tissue at 2.5 mm intervals along the tissue surface.

Antigen challenge caused an increase in joint diameter of 5-7 mm which peaked at 2 days and was maintained for up to 17 days. Similarly, leucocyte numbers in joint washes increased from less than 10^4 cells/ml to $2\text{-}5\times10^7$ cells/ml after 24hr and remained high for the duration of the arthritis. In vehicle-treated animals there was a progressive loss of proteoglycan from articular cartilage with a mean reduction of 36.2% ± 2.6 (mean ± s.e. mean, n=8) after 17 days. Indomethacin reduced joint swelling by up to 50% but had no effect on leucocyte numbers in joint washes. In indomethacin-treated animals the proteoglycan loss from articular cartilage was significantly ($p<0.05$) greater than in control animals, reaching 51.4% ± 6.2 (n=6) after 17 days. Also, at 17 days, synovial tissues from indomethacin-treated animals contained significantly ($P<0.05$) higher numbers of lymphocytes (98 ± 16, n = 15) than control tissues (52 ± 10, n = 19).

These results confirm the observation that indomethacin reduces joint swelling in antigen-induced arthritis in the rabbit (Blackham et al., 1974) and this is consistent with the inhibition of cyclo-oxygenase. We have now shown, however, that cartilage degradation is enhanced by indomethacin treatment and this may be associated with increased lymphocyte recruitment to joint tissues. It is possible that inhibition of the cyclo-oxygenase product PGE_2, which suppresses cytokine release (Kunkel et al., 1986), may result in increased production of interleukin-1 which in turn stimulates greater tissue degeneration (Pettipher et al., 1986).

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INHIBITION OF METOPROLOL METABOLISM BY CHLOROQUINE AND OTHER ANTIMALARIALS

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Polymorphic control of the metabolism of metoprolol (M) has been demonstrated in Caucasians and is of the debrisoquine (D)-type (McGourty et al, 1985). More recently the role of genetic polymorphism in the metabolism of M and D has been investigated in a Nigerian population (Iyun et al, 1986) but, in contrast to the data for Caucasians, bimodality in the distribution of the log M/α-hydroxyM (HM) and D/4-hydroxyD (HD) urinary ratios was not apparent. Furthermore, the median values of both ratios were significantly higher in Nigerians than in Caucasians. It has been suggested that treatment with antimalarials, particularly chloroquine (C), may contribute through inhibition of metabolism, to these ethnic differences in M and D oxidation. Accordingly, we have tested the ability of C and other antimalarials to impair the metabolism of M in rat and man.

Microsomes prepared from male Wistar rat livers or from human livers obtained from renal transplant donors were incubated with M and inhibitor at 37^{o} and pH 7.25. Anaesthetised whole rats were dosed intraperitoneally with C or an equivalent volume of saline. Ten minutes later M was injected either intravenously or intraperitoneally and serial blood samples (0.1 ml) were taken from a cannula in the carotid artery. M, HM and O-desmethylM (ODM) were assayed by HPLC.

C was found to be a potent and apparently competitive inhibitor of HM and ODM appearance in rat liver microsomes. Mean values for inhibition constants were 0.12 μM (range 0.11 to 0.13 μM) for HM and 0.24 μM (range 0.18 to 0.34 μM) for ODM. Corresponding IC_{50} values at a substrate concentration of 20 μM were 0.36 μM (range 0.23 to 0.48 μM) for HM and 0.52 μM (range o.31 to 0.74 μM) for ODM. C was the most potent of the antimalarials studied, inhibitory effect decreasing in the order C > quinine > quinidine = primaquine > mefloquine. C was also found to be an inhibitor of M oxidation in human liver microsomes, although its inhibitory effect was less than that in the rat and displayed wide interindividual variability in the 3 livers examined. The values of IC_{50} obtained were 39, 615 and >1250 μM for ODM appearance at a substrate concentration of 25 μM. Treatment of whole rats with C (50 mg/kg) decreased significantly the apparent systemic clearance of M (10 mg/kg) by 53% (mean $\pm$ sem: control = 0.11 $\pm$ 0.03; treatment = 0.053 $\pm$ 0.012 $l.min^{-1}.kg^{-1}$, $p < 0.05$). C also decreased the clearance of M (40 mg/kg) following intraperitoneal injection and in a dose-dependent fashion from 0.079 $\pm$ 0.016 at 2.5 mg/kg to 0.048 $\pm$ 0.010 $l.min^{-1}.kg^{-1}$ at 40 mg/kg.

Since high hepatic concentrations of C are attained in vivo (Prouty and Kuroda, 1958), the findings suggest that C may inhibit M oxidation in man. Thus antimalarial treatment could contribute to differences in the distribution of the M/HM ratio between Caucasians and Nigerians.

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SUBSTRATE DEPENDENCE OF TRIIODOTHYRONINE-INDUCED CHANGES ON RAT HEPATIC UDP-GLUCURONOSYLTRANSFERASE

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The elimination of oxazepam is increased in hyperthyroid subjects, which suggests an increase in the activity of UDP-glucuronosyltransferase (GT) (Scott et al., 1984). Male Wistar rats treated with 3, 3', 5-triiodothyronine (T_3, 0.55 mg/kg) showed a 400% increase in GT activity towards 4-nitrophenol, whilst activity towards bilirubin was decreased by 80%. The aim of this study was to investigate the dose and substrate dependence of T_3 effects on hepatic GT activity in the rat.

Male Sprague Dawley rats (200 - 250g, Charles River UK Ltd) were injected i.p. with T_3 (5-200 ug) daily for seven days. Liver microsomes were prepared and GT activity towards phenolphthalein, 4-nitrophenol and 1-naphthol was measured as described by Morrison and Hawksworth, 1984, bilirubin was measured by the method of Van Roy et al., 1968 and testosterone by a modification of the method of Dutton et al., 1981. Enzymes were activated using either 0.025% Triton X-100 in the microsomal suspension or, for the bilirubin assay, CHAPS (1:1, detergent: protein). Statistical analysis was by one way ANOVA with significance assigned using Dunnett's test.

Serum T_3 concentrations showed a non-linear increase over the dose range studied, whereas serum T_4 levels were suppressed below the limit of detection (30 nmol/l) in all treated animals. GT activity towards phenolphthalein peaked at a dose of 20 ug T_3, whereas activity towards 4-nitrophenol and 1-naphthol increased to a maximum at a dose of 120 ug. Activity towards bilirubin and testosterone decreased with increasing doses of T_3 to a minimum of 200 ug.

Hepatic GT activity (nmoles glucuronide formed/mg. protein/min) in male Sprague Dawley rats following treatment with varying doses of T_3

T_3 dose (ug)	0	5	20	40	120	200
Phenolphthalein	1.4 ± 0.1	2.1 ± 0.1*	2.6 ± 0.3*	1.1 ± 0.2	-	1.3 ± 0.1
4-nitrophenol	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.2	1.8 ± 0.2	2.9 ± 0.2*	2.1 ± 0.4*
1-naphthol	12.8 ± 0.4	16.3 ± 0.9	15.8 ± 0.7	16.9 ± 1.0*	17.4 ± 0.6*	14.4 ± 0.8
Bilirubin	0.27 ± 0.05	0.18 ± 0.01*	0.18 ± 0.01*	0.09 ± 0.01*	0.08 ± 0.01*	0.06 ± 0.02*
Testosterone	9.6 ± 0.3	6.4 ± 0.3*	5.4 ± 0.9	4.2 ± 0.4*	5.0 ± 0.4*	3.5 ± 0.5*

* $p < 0.05$ compared with control. Data are mean ± SEM (n = 5)

The increase in phenolphthalein GT and the decrease in bilirubin GT was also observed in the 'native' enzyme preparation (data not shown) which suggests a commensurate increase or decrease in the corresponding enzyme protein, rather than activation or inhibition of activity due to changes in the lipid environment.

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METABOLISM OF TAMOXIFEN IN RATS AND MICE: A COMPARISON WITH PATIENTS UNDERGOING BREAST CANCER THERAPY

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Tamoxifen (TAM) is a non-steroidal antioestrogen used for breast cancer therapy (Jordan 1986). The investigation of TAM and other antioestrogens have relied heavily on carcinogen-induced rat tumour models and currently the athymic mouse implanted with human breast cancer cells. There are however only a few studies that compare and contrast the metabolism of TAM in laboratory animals with the clinical situation (Robinson and Jordan 1987). Although TAM itself is an active antioestrogen, metabolities such as 4-hydroxytamoxifen (4-OH-TAM) and N-desmethyltamoxifen (N-Des-TAM) have been implicated in its antioestrogen action *in vivo* (Robinson and Jordan 1987). Clearly the production of different metabolites in laboratory animals might cloud the interpretation of antitumour experiments.

Human serum was obtained from patients taking TAM 10 mg, 30 mg, or 120 mg twice daily for more than three months. TAM and metabolites were determined in serum by high performance liquid chromatography (Brown et al. 1983). The principal metabolite, N-Des-TAM, was usually at twice the concentration of TAM. Only trace amounts of 4-OH-TAM were observed.

In animal experiments the levels of TAM or metabolites were determined after single or multiple oral administrations of TAM. Mice (ovariectomized 25-30 gm ICR) were given a 5 mg TAM suspension in 0.1 ml of peanut oil and serum determinations made on groups of at least 3 animals at 3, 6, 12, 24, 48, 72, and 96h. For daily administrations groups were sacrificed 24h after the last dose for up to 10 doses. The same procedure was used for rats (ovariectomized 35-50 gm Sprague Dawley) with a dose of 7.5 mg TAM in suspension.

In mice peak levels of TAM (707±262 ng/ml) were reached by 6h after single dose administration. A novel metabolite, 4-hydroxy-N-desmethyltamoxifen (4-OH-N-Des-TAM) was also identified by comparison with the retention times of standards. TAM and metabolites were cleared to unmeasurable levels by 72h. Following multiple doses, circulating levels reached a plateau after 4-5 days. In contrast to the human, levels of 4-OH-TAM were equivalent to those of TAM and N-Des-TAM.

In rats 4-OH-TAM was a minor metabolite and N-demethylation appears to be the predominant metabolic route. Peak levels of TAM occurred 3-6h after single dose administration whereas maximum levels of N-Des-TAM were not observed until 24-48h. TAM and metabolites levels were unmeasurable by 96h. Steady state levels after repeated dosing with TAM were achieved within a similar time in rats and mice. However severe toxic effects, resulting in marked weight loss, occurred in rats treated with TAM for 4-7 days.

The metabolic pattern determined in the rat and mouse, when compared to the patient profile of metabolites, suggests the rat may be of greater relevance when studying the antitumour action of TAM *in vivo*. The adoption of the athymic rat as a host for human breast tumours may therefore provide an improved model over the athymic mouse in this respect.

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THE EFFECT OF C-AMP ON STEROID METABOLISM IN ISOLATED, PERMEABILISED RAT HEPATOCYTES

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It is known that many hormones can affect hepatic steroid metabolism and some of these are thought to act by altering the generation of the second messenger, c-AMP. The enzyme principally responsible for the metabolism of steroids is the mixed function oxidase with cytochrome P-450 as an integral part, which can be phosphorylated by the c-AMP-dependent protein kinase (PKa) to alter its activity (Pyerin et.al.,1984).

In isolated hepatocytes it has been shown (Berry & Skett, 1988) that 8-bromo-c-AMP can mimic the effects of adrenaline in decreasing steroid metabolism and that forskolin, which directly activates adenylate cyclase, has the same effect. The data suggests that adrenaline acts via a β_2-adrenoceptor to increase intracellular c-AMP and, thus, activate PKa. PKa, perhaps by phosphorylating cytochrome P-450, inhibits steroid metabolism. A large increase in c-AMP (>10-fold), however, actually stimulated steroid metabolism but later than the inhibition.

To study this biphasic, dose-dependent effect of c-AMP , it is necessary to know how much c-AMP has entered the cell. We have approached this problem by using hepatocytes cultured in hormone- and serum-free medium (Hussin & Skett, 1986), electropermeabilised by the method of Knight & Baker (1982) . During permeabilisation a known concentration of c-AMP is included in the buffer and, thus, it is assumed that this concentration of c-AMP is to be found in the cells. The cells are left for varying lengths of time and assayed for steroid metabolising capacity (Hussin & Skett, 1986). To ascertain if protein kinase or protein synthesis were involved in the action of c-AMP, K-252a (protein kinase inhibitor) and cycloheximide (protein synthesis inhibitor) were added with the c-AMP.

	16α-HYDROXYLASE (% of control)	
ADDITION	1h	2h
None	100 ± 8	100 ± 6
c-AMP	57 ± 5 *	134 ± 7 *
c-AMP + K-252a	97 ± 9	92 ± 9
c-AMP + Cycloheximide	69 ± 12*	96 ± 10

Results for $5x10^{-5}$M c-AMP at 1h and $5x10^{-3}$M c-AMP at 2h are given in the table.Low concentrations of c-AMP gave inhibition of steroid metabolism at 1h, whereas higher concentrations gave stimulation at 2h. Both effects were blocked by K-252a whereas only the stimulatory effect was blocked by cycloheximide.

It would appear that c-AMP has two effects on steroid metabolism in isolated hepatocytes; one to inhibit acutely enzyme activity by a process involving phosphorylation and, two, at a later stage to stimulate protein synthesis and, thereby stimulate steroid metabolism.

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TIMOLOL REDUCES INTRAOCULAR PRESSURE IN THE ISOLATED, ARTERIALLY PERFUSED BOVINE EYE

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It is well known that timolol lowers intraocular pressure (IOP) in man and animals by reducing the rate of aqueous humour formation. However, the mechanism of this effect is obscure since there is much evidence that classical β-adrenoceptor blockade is not directly involved (Sears, 1984); e.g. while timolol and other β-blockers inhibit cyclic AMP synthesis, drugs such as cholera toxin and forskolin, which promote cyclic AMP production, also lower IOP by decreasing the rate of formation of aqueous humour. The technique described here demonstrates that timolol can lower IOP in the isolated, arterially-perfused eye.

Fresh bovine eyes from the abattoir were trimmed of fat and extraocular muscle. One long posterior ciliary artery was cannulated proximal to its point of entry into the sclera. The eye was placed on a 37° jacket and perfused with a modified Krebs solution containing dextran (3%, w/v; mol. wt. approx. 40,000) and bovine serum albumin (0.5%, w/v), together with (mM) NaCl, 92; KCl, 6; $MgSO_4$, 1.2; $CaCl_2$, 2.2; $NaHCO_3$, 25; NaH_2PO_4, 3.7; glucose, 11.6; ascorbic acid, 0.05. The pH of this solution was adjusted to 7.2 by bubbling with O_2 containing CO_2 (5%) prior to addition of dextran and albumin. Arterial perfusion at 37° was commenced under a constant pressure of 30 mm Hg and perfusate emerging from the veins was collected into a beaker whose weight was continuously recorded. After 20 min the perfusion pressure was raised to 45 mm Hg. The anterior chamber was cannulated and connected to a H_2O manometer to read IOP. Effects of timolol on IOP could be seen 5-10 min after bolus injection (3-100 μl) into the perfusate. Injection of successively higher doses of drug at 20 min intervals gave a cumulative dose-response curve. Drug effects were compared with data from control perfusions in which saline was injected at 20 min intervals.

At the end of each experiment, aqueous humour from both posterior and anterior chambers was sampled. Assay of dextran as total hexose (Dubois et al., 1956) in these samples gave a measure of any leakage of macromolecules through the "blood-aqueous barrier". Breakdown of this barrier due either to mishandling of the tissue or to a drug effect could thus be detected.

The initial IOP (mean ± SEM) in control eyes was 7.02 ± 0.31 mm Hg rising slightly to 7.16 ± 0.31 mm Hg during a 100 min perfusion. Timolol (3-300 nmol) produced a dose-dependent fall in IOP from 7.43 ± 0.16 to 6.49 ± 0.15 mm Hg ($n = 11$, $P < 0.001$). There was no significant change in perfusion rate which had a pre-drug value of 1.64 ± 0.12 $ml.min^{-1}$ and remained at 1.54 ± 0.20 $ml.min^{-1}$ after the final dose of timolol. Neither was there a significant effect of timolol on the concentration of hexose in the aqueous humour of either chamber at the end of the perfusion.

The ability of timolol to lower IOP in the isolated eye proves that its effect is not dependent on sympathetic nerve activity nor on circulating catecholamines. This is additional evidence that timolol's ocular effect may not be mediated by β-adrenoceptors. The IOP values recorded using this technique are lower than in vivo largely because venous pressure is virtually zero. The advantages offered by this preparation lie in the convenience of an isolated tissue and the rapid effect of intra-arterial administration. In addition, the use of high mol. wt. solutes protects vascular integrity, a factor ignored in previous ocular perfusion methods (Kodama et al., 1985).

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A NEW TECHNIQUE FOR QUANTITATIVE MEASUREMENT OF STIMULATED ENDOGENOUS DOPAMINE RELEASE IN BRAIN SLICES USING VOLTAMMETRY

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Stimulated neuronal dopamine release can be measured in the rat brain in vivo using fast cyclic voltammetry (Millar et al., 1985). We report here the application of this technique to brain slices in vitro and the first quantitative evaluation of drug effects on stimulated dopamine release in real time.

Slices of rat corpus striatum, 350μm thick, were superfused with oxygenated artificial cerebrospinal fluid at 32°C. Dopamine release was monitored with a carbon fibre microelectrode located 75μm below the surface of the slice. Release was evoked by a parallel bipolar electrode placed 200-400μm from the recording electrode. Stimulation parameters were optimised to a single square pulse of 0.1ms width, amplitude 20V, applied every two minutes. These parameters, and the separation between stimulating and recording electrodes, exclude the possibility of direct excitation of nerve terminals. Reproducible evoked release of dopamine could be obtained over a period greater than 9 hours, and concentration-response curves to agonists for inhibition of release could be obtained.

Evidence that the electrochemical signal recorded was dopamine released as a consequence of the activation of nerve fibres accrued from the following observations:

(i) The release was reversibly abolished by tetrodotoxin (10^{-7} M) or by the omission of calcium from the incubation medium.

(ii) Voltammograms obtained during electrical stimulation were indistinguishable from those of exogenous dopamine.

(iii) The magnitude of the electrochemical signal was increased up to 4-fold by the selective dopamine uptake blocker GBR 12909 (Heikkila & Manzino, 1984) over the range $3x10^{-8}$-$3x10^{-6}$M, and the rate of decay of the signal was reduced. Concentrations of desipramine which selectively block noradrenaline and 5HT uptake ($5x10^{-9}$-$5x10^{-7}$M) did not affect the responses.

(iv) The electrochemical signal was abolished by Ro 4-1284 ($5x10^{-7}$M), a monoamine depleter (Shore, 1976).

(v) The dopamine receptor agonist N,N-dipropyl-5,6-ADTN (0.1-1.2μM) concentration-dependently decreased the signal.

This work demonstrates that the stimulated release of endogenous dopamine can be measured in striatal slices using fast cyclic voltammetry at carbon fibre microelectrodes. This new technique has potential applications in the characterization of presynaptic receptors throughout the CNS.

P. Palij is a Glaxo/SERC CASE scholar.

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EFFECTS OF UPTAKE INHIBITORS ON STIMULATED DOPAMINE RELEASE AND UPTAKE IN THE NUCLEUS ACCUMBENS STUDIED BY FAST CYCLIC VOLTAMMETRY

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Much research into schizophrenia has focussed upon the nucleus accumbens (Matthysse 1981). This has led to several studies of the action of antipsychotics in this nucleus and its afferents. However, other factors controlling dopamine (DA) function, particularly uptake, have received much less attention. The present study sought therefore to investigate the effects of some DA uptake blockers upon the stimulated release and uptake of DA in the nucleus accumbens.

All experiments were performed in chloral hydrate-anaesthetised male Sprague Dawley rats (250-350g). A carbon fibre microelectrode was implanted into the nucleus accumbens (Acb) while reference (Ag/AgCl) and auxiliary electrodes were placed on the skull. Electrical stimulation (50 Hz, 100-110μA r.m.s., 2s train) of the median forebrain bundle (MFB) was used to evoke release of DA in Acb. The concentration of DA in the extracellular fluid (ECF) was monitored using fast cyclic voltammetry (FCV) with 50ms intervals between successive measurements (Stamford et al, 1986b). The overflow and subsequent reuptake of DA was measured before and 30 min after the intraperitoneal administration of various DA uptake blockers. The results are shown in Table 1.

Drug	Dose (mg/kg)	Peak DA overflow (% of pre-drug values)	Rate of DA uptake (% of pre-drug values)
Saline		98.8 ± 2.9	97.5 ± 2.6
Cocaine	10	212.2 ± 42.8*	101.8 ± 8.2
GBR 12909	10	220.4 ± 57.7*	98.4 ± 9.3
Nomifensine	10	267.8 ± 36.7*	68.8 ± 5.2*
Benztropine	20	130.8 ± 10.2*	111.2 ± 9.0
Bupropion	50	151.4 ± 23.9	61.4 ± 5.0*
L-DOPA	200	178.2 ± 20.1*	109.8 ± 4.2

All values are mean ± s.e.m. n=5/6. *P < 0.01 vs saline (Mann Whitney U test).

Cocaine, GBR 12909, nomifensine and benztropine and L-DOPA (given as a positive control) increased the overflow of DA into the ECF. However, only nomifensine and bupropion blocked its subsequent reuptake. There was no correlation between drug effects upon overflow and uptake, indicating that potentiation of DA overflow is not solely the result of uptake inhibition. Other mechanisms (e.g. increased synthesis or mobilisation of stored DA) may contribute to effects on DA overflow. It is also possible that the removal of DA from the ECF is not due to neuronal uptake but is the result of a separate low affinity high capacity uptake system, as previously reported for the striatum (Stamford et al, 1986a). The calculated rates of in vivo DA uptake (1.61 ± 0.15μM/s ≡ 13.5 ± 1.3 nmol/min/g (Woodward et al, 1967), mean ± s.e.m., n = 36), are consistent with this interpretation.

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ENHANCED STRIATAL RESPONSE TO D-AMPHETAMINE AS REVEALED BY INTRACEREBRAL DIALYSIS FOLLOWING SOCIAL ISOLATION IN RATS

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Isolated rats demonstrate many behavioural disturbances such as increased locomotor activity and exploratory behaviour and deficits in both learning and spatial memory tasks. Isolates have also been reported to show enhanced oral behaviours in response to tail-pinch stimulation and to display an increase in the intensity of amphetamine-induced behavioural stereotypies (Sahakian et al, 1975). As both oral behaviours elicited by tail-pinch and amphetamine stereotypy are dependent on the functional integrity of the nigro-striatal dopamine projection, this pathway is implicated in the development of the isolation 'syndrome'.

Striatal dopamine function was investigated *in vivo* in both isolated and grouped rats using the intracerebral dialysis technique in conjunction with High Performance Liquid Chromatography with electrochemical detection (Ungerstedt, 1984). Extracellular dopamine, and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) together with 5-hydroxyindoleacetic acid (5-HIAA) were measured in the striatum under baseline conditions and following injection of either d-amphetamine (2 mg/Kg S.C.) or saline.

Lister hooded rats were caged either individually or in social groups of 6 at weaning (21 days) and for the duration of the experiment. Isolates were spontaneously hyperactive and gained weight more rapidly when compared with grouped animals. When the rats were mature (350g) they were anaesthetised with halothane and stereotaxically implanted with a dialysis probe (Zetterström et al, 1983) which was perfused with physiological saline (2µl/min). Basal extracellular concentrations of dopamine did not differ between the two groups and was estimated to be 3.5×10^{-8} M based on *in vitro* recovery experiments. As expected, treatment with d-amphetamine greatly increased the concentration of dopamine in the perfusate from both groups of animals. However, this increase was significantly enhanced in the isolates (maximum increase= + 1500%; n=6) compared with grouped animals (maximum increase= + 1100%; n=6). Consistent with this finding, there was a significantly greater decrease in the perfusate DOPAC concentration in the isolates ($p<0.05$). HVA was decreased to an equal extent in both groups and 5-HIAA concentration was not significantly affected. These results suggest that the social environment to which an organism is exposed during development can affect central presynaptic dopaminergic function.

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EVIDENCE THAT NICOTINE DOES NOT EVOKE THE RELEASE OF VESICULAR DOPAMINE FROM STRIATAL SLICES

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Previous studies have shown that nicotine can stimulate the release of dopamine (DA) from nerve terminals of the nigro-striatal and mesolimbic DA systems although the mechanisms involved in this effect remain controversial (Sakurai et al 1982; Rowell et al 1987). In this study reserpine pretreatment has been used to examine the effects of nicotine on the release of vesicular DA.

Slices prepared from the striatum of male Sprague-Dawley rats were preincubated at 35°C in Krebs Ringer bicarbonate solution with ^{3}H-DA (2×10^{-8}M) for 60 minutes in the absence of monoamine oxidase inhibitor or for 5 minutes in the presence of pargyline (1×10^{-5}M). The slices were then transferred to perfusion cells and superfused (0.5 ml/min) with Krebs solution, pargyline being added to the superfusion fluid for the slices preincubated with the inhibitor. After a period of washing (40 min) the superfusate was collected in 4 minute fractions and the tritium release into each calculated as a fraction of the radioactivity remaining in the tissue. The effects of reserpine (1×10^{-6}M) were examined by preincubating the slices with the drug for 30 minutes prior to incubation with ^{3}H-DA in fresh reserpine-free medium.

The addition of KCl (2×10^{-2}M) increased ($P<0.01$) tritium overflow from 4.2 ± 0.2 to 16.1 ± 2.2 percent ($N = 7$) for slices incubated in the absence of inhibitor and from 5.0 ± 0.6 to 16.9 ± 0.8 percent ($N = 4$) for slices preincubated in the presence of pargyline. Preincubation with reserpine decreased ^{3}H-DA uptake ($P<0.01$) by slices incubated in the absence of pargyline from 165 ± 15 x 10^{-15} moles/mg tissue to 62 ± 8 x 10^{-15} moles per mg tissue ($N = 8$) and abolished the response to KCl. In slices incubated with ^{3}H-DA in the presence of pargyline, the reduction in DA uptake evoked by reserpine (180 ± 11 to 150 ± 11 x 10^{-15} moles per mg tissue) was not statistically significant. Pretreatment with reserpine also failed to reduce the response to KCl measured in the presence of pargyline. Nicotine (1×10^{-6}M) increased ($P<0.05$) the fractional release of tritium from 5.9 ± 0.5 to 7.1 ± 0.8 percent in slices incubated with pargyline whereas its effect measured in the absence of the inhibitor (an increase from 5.3 ± 0.5 to 5.8 ± 0.8 percent) was not statistically significant. Reserpine had no significant effect on the increased secretion of radioactivity evoked by the administration of nicotine.

It is concluded that the data appear most consistent with the hypothesis that the principal mechanism by which nicotine evokes the secretion of DA from striatal slices is by displacement from an extra-vesicular compartment rather than stimulation of exocytosis.

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REGULATION OF ENDOGENOUS DOPAMINE RELEASE FROM RAT STRIATAL SLICES BY D-2 AUTORECEPTORS : EFFECTS OF FORSKOLIN AND N-ETHYLMALEIMIDE

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Striatal dopamine (DA) release is known to be regulated by presynaptic autoreceptors having the pharmacological characteristics of D-2 receptors (Starke et al 1983; Herdon et al 1987). Although some exceptions have been described (e.g. Memo et al 1986), most functions of D-2 receptors are thought to be mediated by decreasing intracellular cyclic AMP levels via their inhibitory coupling to adenylate cyclase (Kelly & Nahorski; 1986); however, it is not known whether the action of the DA release-modulating receptors involves this mechanism. Under conditions where these receptors are largely occupied by endogenous DA, their activity can be assessed indirectly by measuring the increase in DA release caused by antagonists such as sulpiride, so this test has been used to examine the effect of increasing intracellular cyclic AMP levels by stimulating adenylate cyclase activity with forskolin on the functioning of the autoreceptor.

Endogenous DA release from superfused striatal slices was measured using HPLC with electrochemical detection as described previously (Herdon et al 1987; Herdon & Nahorski 1987). Two 4min periods of electrical field stimulation (3Hz,2msec, 30mA) were applied 40min apart (S1&S2); spontaneous release was measured before each stimulation. Drugs were added 20mins before either S1 or S2 and remained present for the rest of the experiment. Results are presented as mean ± SEM (n = 6-8) of S2/S1 ratios normalised to percentages of the relevant control values. Sulpiride (1μM) alone (S2) produced a large increase in electrically-evoked DA release (277±32%); it also increased spontaneous release (172±10% of control). These findings are similar to those reported previously (Herdon & Nahorski 1987) and suggest substantial occupation of the autoreceptor by endogenous DA. Forskolin (10μM) alone (S2) also significantly ($p<0.05$) increased evoked DA release (147±15%) and spontaneous release (140±10% of control); however, the effects of sulpiride and forskolin did not appear to be additive. In the presence of forskolin (S1&S2), the effect of sulpiride (S2) was significantly ($p<0.05$) reduced to only 149±10% of control for evoked release and 136±7% for spontaneous release.

These results could suggest an action of D-2 autoreceptors via inhibition of cyclase. In order to test this possibility more directly we used the alkylating agent N-ethylmaleimide (NEM) which has been shown to inactivate the G proteins which are involved in coupling inhibitory receptors such as α-2 adenoceptors to adenylate cyclase and mediating their effects on transmitter release (Allgaier et al 1986; Fredholm & Lindren 1987). Preincubation of striatal slices with NEM (30μM for 30mins) produced a 2-3 fold increase in spontaneous release and a 50% increase in S1 evoked release but reduced S2/S1 ratios to 71±7% of control values. However, preincubation with NEM did not alter the ability of sulpiride to increase evoked DA release (263±38% of control for NEM-treated vs. 198±42% for untreated). Although the effectiveness of NEM in inactivating G proteins was not tested directly, these findings suggest that D-2 autoreceptors may not regulate DA release via a direct inhibitory effect on adenylate cyclase; instead, another possibility is that an interaction between the effects of D-2 receptor activation and elevated cyclic AMP may occur at a level distal to adenylate cyclase.

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A SELECTIVE INCREASE IN MITOCHONDRIAL SUPEROXIDE DISMUTASE IN PARKINSONIAN SUBSTANTIA NIGRA

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Excessive lipid peroxidation provoked by toxic free radical species may kill dopamine neurones in Parkinson's disease since parkinsonian substantia nigra contains higher basal levels of malondialdehyde than found in control subjects (Dexter et al, 1986). This is accompanied by reduced levels of catalase and glutathione peroxidase which normally protect against toxic oxygen species (Kish et al, 1985). Another protective enzyme, superoxide dismutase (SOD) exists as two isoenzymes - a mitochondrial manganese dependent form and a cytosolic copper-zinc dependent form (Weisiger and Fridovich, 1973). We now report the activity of the isoenzymes of SOD in the substantia nigra and cerebellum of patients with Parkinson's disease and age-matched controls.

Tissue was obtained from 11 patients with Parkinson's disease (mean age 75.3 $\pm$ 1.9 years) and from 13 controls (mean age 65.5 $\pm$ 6.4). Average time between death and removal of brain tissue was 19.2 $\pm$ 2.4 h for parkinsonian patients and 20.9 $\pm$ 1.9 h for controls. Tissue samples were homogenised in 20 vol 0.32 M sucrose buffer and centrifuged at 100,000 g for 60 minutes to separate the particulate and cytosolic fractions. SOD activity in the homogenate, supernatant and pellet was assayed by the procedure of Misra and Fridovich (1972), based on the ability of SOD to inhibit the auto-oxidation of adrenaline. When measuring SOD in the particulate fraction, incubation with 5 mM potassium cyanide ensured that only the cyanide insensitive manganese form was detected.

The activity of both the cytosolic and mitochondrial forms of SOD was greater in the substantia nigra than the cerebellum in both control and parkinsonian tissue (Table 1). In the cerebellum no difference was observed in the activities of the cytosolic and mitochondrial forms of SOD between Parkinson's disease and control brain tissue. However, in the substantia nigra of parkinsonian patients the activity of the mitochondrial manganese dependent form of SOD was higher than in control patients.

Table 1 Activity of SOD in parkinsonian and control brains

Brain area	Disease category	n	U/g wet weight		
			Total SOD	CuZn SOD	Mn SOD
S. Nigra	Control	13	1343 $\pm$ 19	1074 $\pm$ 19	167 $\pm$ 4
	PD	11	1393 $\pm$ 42	1072 $\pm$ 34	224 $\pm$ 14*
Cerebellum	Control	13	961 $\pm$ 17	727 $\pm$ 20	128 $\pm$ 3
	PD	11	931 $\pm$ 32	739 $\pm$ 15	120 $\pm$ 4

Values are expressed as mean $\pm$ SEM. * $p < 0.05$ compared to control tissue.

The increase in mitochondrial SOD in parkinsonian substantia nigra may reflect the induction of a protective mechanism against further damage by oxygen radicals. Alternatively, the increased activity of SOD may in itself cause toxic damage through the accumulation of the hydrogen peroxide formed.

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HYPOXIA AND ENDOTHELIUM-MEDIATED CHANGES IN THE PHARMACOLOGICAL RESPONSIVENESS OF SHEEP CIRCUMFLEX CORONARY ARTERIES

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We have previously shown that hypoxia modifies the mechanical and pharmacological responsiveness of sheep coronary artery rings (Kwan et al, 1988). We have now investigated whether some of these effects are mediated by the endothelium.

Sheep isolated coronary artery rings are suspended under their optimal resting tension (1.5 g) in Krebs' solution. This was either bubbled with $95\%O_2$-$5\%CO_2$ (pO_2=520±50 mmHg) or $95\%N_2$-$5\%CO_2$ (hypoxia) (pO_2=45±2 mmHg). Cumulative concentration-response curves were constructed before and after the introduction of hypoxia. The endothelium of the artery was removed by gentle rubbing of the lumen with a wooden stick and confirmed by histological examination.

In endothelium-intact preparations, under resting tension, introduction of hypoxia caused a sustained contraction and re-introduction of O_2 caused a transient relaxation. In pre-contracted (40 mM KCl) arteries, hypoxia caused a similar response except that it was preceeded by a transient relaxation. In contrast, in endothelium-deprived arteries, hypoxia always caused relaxation (whether under resting tension or pre-contracted). These results show that the hypoxic-induced contraction is endothelium dependent.

In 95% O_2, removal of the endothelium caused a leftward shift of the concentration-response curves of 5-HT (EC_{50}:0.51±0.08 vs 2.95±0.80 μM) and U46619 (EC_{50}:0.14±0.04 vs 0.30±0.33 μM). Endothelium removal also significantly increased the maximum tension development with 5-HT (176±47 vs 43±8 g cm^{-2}) and K^+ (152±18 vs 81±6 g cm^{-2}) but not that with U46619. The removal of the endothelium also markedly depressed the vasodilating effects of adenosine on the pre-contracted (by 40 mM KCl) artery (EC_{50}:0.70±0.10 vs 0.15±0.01 mM ; maximum vasodilating effects: 28±9 vs 87±16 g cm^{-2}). In the endothelium-rubbed preparations and in the presence of hypoxia, there was a significant decrease in the maximum active tension development with 5-HT (10±5 vs 176±47 g cm^{-2}) and K^+ (91±8 vs 152±18 g cm^{-2}). The contractile effect of U46619 on the endothelium-deprived preparations, under hypoxia, was completely abolished. However, in the presence of the endothelium we have previously shown that hypoxia increases responses to 5-HT and U46619.

It is concluded that in the sheep coronary artery, hypoxia reduces the basal release of a relaxant substance or increases the release of a constrictor substance from the endothelium. There are a number of posssible explanations for the altered pharmacological responsiveness under conditions of hypoxia and endothelial denudation which include modification of the release of vasoconstrictor and/or vasodilator mediators.

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INHIBITION OF THE INOTROPIC EFFECTS OF DPI 201-106 BY ITS METHYL-INDOLE DERIVATIVE (BDF 8784) AT THE SODIUM CHANNELS

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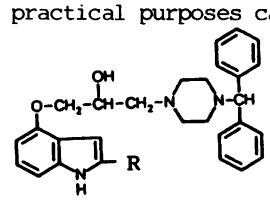
Racemic DPI 201-106 (inset, DPI) is a novel cardiotonic agent, the S-enantiomer of which activates cardiac Na^+-channels (Scholtysik et al., 1985). Kohlhardt et al. (1986) have demonstrated that DPI modulates cardiac Na^+-channels in a voltage-dependent manner. Recently, Romey et al. (1987) have found DPI to interfere with the binding of (^{3}H)-batrachotoxinin A 20 -α-benzoate (^{3}H-BTX) to specific binding sites at the Na^+-channels in guinea pig brain membranes. Whilst studying chemical derivatives of DPI we synthetized the methyl analogue which carries a CH_3- instead of the CN-group in the indole moiety of DPI, which for practical purposes carries the code BDF 8784 (BDF). In isolated, electrically driven right ventricular papillary muscles of guinea pigs (1 Hz, 35°C in Krebs-Henseleit solution containing 1.8 mM Ca^{++}) BDF itself had no influence on force of contraction at 0.1 and 1 µM, but caused a negative inotropic effect (20-30%) at 10 µM. Preincubation of untreated organs for 90 min with 0.1 µM BDF already caused a slight rightward shift of the concentration response curve (crc) of DPI.
At 1 µM BDF caused an even more pronounced rightward shift of the crc for DPI, but the maximal inotropic effect was suppressed.
Pretreatment with the negative inotropic concentration of 10 µM BDF completely abolished the positive inotropic effect of DPI in such a manner that DPI concentrations of up to 100 µM were ineffective. Insolubility of DPI prevented investigation of higher concentrations. In conventional electrophysiological studies on guinea pig papillary muscles BDF 0.1 - 1 µM had no influence on action potential duration (APD), but 10 µM depressed dV/dt max from an average of 215 to 170 V/s. BDF 0.1 µM did not affect the electrical actions of DPI, but 1 µM BDF reversed the inotropic and APD-prolongating actions initiated by 1 µM DPI. 10 µM BDF completely abolished the mechanical as well as electrical actions of DPI.



R = CN : DPI 201-106

R = CH_3 : BDF 8784

In receptor binding studies using (^{3}H)-BTX and guinea pig membranes, we have compared the interactions with DPI, BDF and the enantiomers of BDF. The IC_{50}-values for displacement of (^{3}H)-BTX from the putative Na^+-channels were 458 and 460 nM for racemic DPI and BDF, respectively. The values for S- and R-BDF were 693 and 510 nM, respectively. The enantiomers of BDF were equipotent in reversal of the inotropic actions of DPI. Thus BDF and DPI have a common binding site at the putative Na^+-channels labeled by (^{3}H)-BTX.
In addition, the antagonism of DPI by BDF is not enantioselective. The antagonism of DPI by such a closely related derivative is similar to the interactions of the 1,4-dihydropyridines at the voltage-dependent calcium channels, and may indicate a receptor site for DPI at the Na^+-channels. In conclusion, BDF inhibits the inotropic and APD-prolongating actions of DPI by an interaction at the Na^+-channels.

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CROMAKALIM (BRL 34915) INHIBITS NORADRENALINE-EVOKED VASOMOTION IN SHR ISOLATED MESENTERIC VASCULAR BED

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Rhythmic contractions and relaxations have been observed in small arteries, arterioles and venules by many workers (e.g. Clark & Clark, 1943; Wiedeman, 1957; D'Agrosa, 1970; Colantuoni et al, 1984) and such vasomotion may be an important determinant of peripheral vascular resistance (Funk et al, 1983). In the present paper we have characterised some of the features of vasomotion produced by noradrenaline infusions in SHR isolated mesenteric vascular bed perfused at 4 ml min^{-1} (37°C) with a Krebs solution of the following composition (mM): NaCl, 118.0; $NaHCO_3$, 25.0; D-glucose, 11.1; KCl, 4.72; NaH_2PO_4, 1.13; $MgCl_2$, 1.12; $CaCl_2$, 2.56; (±)-propranolol, 10^{-6}M. The perfusate was gassed with 5% CO_2 in O_2. Upto 5 repeat 20 min infusions of noradrenaline (1.57 µg min^{-1}), given 30 min apart, produced fairly reproducible increases in baseline perfusion pressure and usually, though not invariably, oscillatory pressure changes of variable frequency and amplitude which could be persistent or intermittent in different tissues. The increases in baseline pressure and the oscillatory pressure changes were abolished by 5.5 - 6.0 min perfusion with a medium devoid of Ca^{2+} and containing EGTA (2 mM). Prior perfusion for 5.5 - 6.0 min with medium lacking Ca^{2+} and from which EGTA was omitted, allowed an initial, though unsustained, response to noradrenaline infusion. Equilibration (25.5-26.0 min) of tissues with diltiazem (10^{-6}, 10^{-5}M) resulted in noradrenaline responses which were of a similar character, though smaller, than those observed in the absence of the drug. Equilibration (25.5-26.0 min) of tissues with cromakalim (BRL 34915; 10^{-6}, 10^{-5}M), however, resulted in severely reduced noradrenaline responses. Invariably the oscillatory pressure changes were greatly diminished or absent and increases in baseline perfusion pressure, where these occurred, were intermittent. In other experiments in which sequential log dose-pressor response curves to bolus injections of noradrenaline (0.1 - 100 µg) were constructed, concentrations of cromakalim upto 10^{-5}M scarcely influenced the transient increases in perfusion pressure.

The results demonstrate the dependence of noradrenaline-induced vasomotion in SHR mesenteric vascular bed on extracellular Ca^{2+}, though Ca^{2+} entry may occur largely via channels insensitive to blockade by diltiazem. Cromakalim's ability to inhibit the oscillatory pressure changes may be due to suppression of rhythmic firing of vascular pacemaker cells resulting from the opening of membrane K^+ channels. Such a proposal has already been made to account for cromakalim's inhibitory effect on phasic contractions of rat isolated portal vein (Hamilton et al, 1986).

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EFFECT OF HYPOXIA UPON NORADRENALINE INDUCED CALCIUM ACCUMULATION IN RAT ARTERIES

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Hypoxia differentially reduces noradrenaline (NA) induced contractions of different rat arteries in vitro (Downing et al, 1986). The depression of contractions of rabbit aorta to NA during hypoxia is coincident with a reduction of Ca^{2+} entry (Ebeigbe, 1982). We have investigated whether differences in Ca^{2+} uptake between vessels in hypoxia might account for the differential effects observed on contraction.

5-7mm circular preparations of thoracic aorta (AO), superior mesenteric artery (MA) and femoral artery (FA) were dissected from male Wistar rats (200-250g). Tissues were mounted in 20ml baths containing physiological salt solution (PSS) ($CaCl_2$=2.5mM) at 37°C and gassed with 5%CO_2 in O_2 (pO_2=395mmHg). After 1hr equilibration, reproducible contractions to NA (1μM) were obtained (normoxic response). After washout and recovery, tissues were gassed with 5%CO_2 in N_2 for 30min (pO_2=59mmHg) and a further response to NA obtained (hypoxic response). To measure Ca^{2+} uptake, tissues were incubated for 1hr in 5ml phosphate free PSS gassed with 5%CO_2 in O_2. In "hypoxic" experiments, tissues were then gassed with 5%CO_2 in N_2 for 30 minutes before transfer to 2ml hypoxic PSS containing ^{45}Ca (2.5μCi/ml) for 5 min. After a 2min exposure to NA (1μM) they were transfered to a 50mM solution of $LaCl_3$ for 5min at 21°C. Experiments were also performed under normoxic conditions. Tissues were then blotted and weighed. ^{45}Ca was determined by liquid scintillation counting after overnight extraction into 2ml EDTA solution (5mM). Results are summarized in Table 1.

TABLE 1: Ca content of rat arteries under different conditions.

	NORMOXIA		HYPOXIA	
	Unstimulated	NA Stimulated	Unstimulated	NA Stimulated
AO	90.1±7.2 (8)	146.4±9.4 (8)*	99.6±5.2 (8)	109.8±6.5 (8)
FA	109.6±18.3 (6)	176.1±30.1 (5)*	163.7±25.8 (6)*	120.4±6.2 (6)#
MA	80.8±21.3 (5)	172.5±13.9 (5)*	157.2±25.3 (5)*	242.4±38.8 (5)*#

Results are mean±sem, μM/Kg wet weight. Symbols indicate values significantly different (p>0.05) from: *unstimulated-normoxia; # unstimulated- hypoxia.

In AO, hypoxia significantly decreased NA stimulated ^{45}Ca accumulation and contractile responses to NA were reduced by 39.7±6.5% (n=10). In MA, hypoxia significantly increased unstimulated ^{45}Ca accumulation. However, addition of NA still resulted in a significant increase in calcium content as compared with hypoxic controls. Contractile responses of MA to NA were reduced by only 5.4±4.7% (n=15) in hypoxia. In FA, resting ^{45}Ca accumulation was also significantly increased in hypoxia. In NA stimulated vessels, calcium content was significantly decreased compared to hypoxic controls. Contractile responses of FA to NA in hypoxia were transient and the mean contraction over 8 min was reduced by 30.0±7.9% (n=19).

The effects of hypoxia on NA stimulated ^{45}Ca accumulation correlate well with effects on contractility: in AO and FA, both accumulation and contractility were reduced in hypoxia whereas in MA neither were affected. The decrease in stimulated ^{45}Ca content of FA during hypoxia may underlie the transient nature of the contractile response in hypoxia and might involve changes in ^{45}Ca efflux.

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IS INTRACELLULAR CALCIUM ELEVATED IN DISPERSED ARTERIAL SMOOTH MUSCLE CELLS FROM HYPERTENSIVE RABBITS?

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Perinephritis hypertension in the rabbit is associated with increased pressor responses to alpha adrenergic agonists and enhanced responses to calcium antagonists (Hamilton et al, 1987). In addition, mesenteric arteries from these animals show a specific increase in basal $^{45}Ca^{2+}$ uptake while altered responses to drugs acting on calcium channels have been observed in aorta from animals with perinephritis hypertension (Dong and Wadsworth, 1987). Thus the calcium regulatory system in arterial smooth muscle may be altered in this model of hypertension.

Rabbits were made hypertensive by wrapping one kidney in cellophane and removing the contralateral kidney. The mean arterial pressure (MAP) of hypertensive and sham operated rabbits was measured via an arterial cannula inserted under local anaesthesia.

Dispersed cells were prepared from carotid arteries of animals with perinephritis hypertension and arteries of sham operated control rabbits. The isolated cells were prepared by a modification of the method of Warshaw et al, 1986. Arterial sacs were inflated with a solution containing elastase and collagenase in a low calcium Krebs' buffer. The sacs were incubated for 2.75 hours at 37°C and cells were harvested by gentle inflation and deflation. The resultant cell suspension was concentrated by centrifugation (100 g for 3 min) and then incubated with 100 uM Quin 2-AM at 37°C for 1 hour. After loading cells were washed to remove excess dye and intracellular free calcium was measured in a fluorescence spectrometer with an excitation of 339 nm and emission of 492 nm. Results are expressed as mean ± SEM.

The cell suspension contained 0.2-0.5 x 10^6 cells/ml consisting mainly of smooth muscle with some endothelium. The smooth muscle cells were present as small groups or individual cells, were partly relaxed, and excluded Trypan blue. In the sham operated group (MAP = 85 ± 3 mmHg) the intracellular free calcium concentration was 102 ± 10 nM (n=11). In the hypertensive group (MAP = 127 ± 5 mmHg) the intracellular free calcium concentration was not significantly different from the sham group at 118 ± 8nM (n=10). A regression of intracellular free calcium and MAP showed no significant correlation (r = 0.17).

This study has shown no change in resting intracellular free calcium in arterial smooth muscle cells from rabbits with perinephritis hypertension. It is possible that either agonist-induced changes in intracellular free calcium or responsiveness to calcium underlie functional changes observed in arteries from hypertensive rabbits.

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DO ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS FAIL TO CAUSE REFLEX TACHYCARDIA IN THE CAT?

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The failure of captopril to cause reflex tachycardia is surprising as it lowers blood pressure by causing vasodilation (Rubin and Antonaccio, 1980). This seems to be a general property of ACE inhibitors. The mechanism of this action has been difficult to investigate as ACE inhibitors are only weakly effective in lowering blood pressure in animals with normal circulating renin concentrations (Natoff, 1987). To ensure an adequate fall in blood pressure cats were injected daily for 5 days with frusemide (40 mg per animal, i.p.) and kept on a salt free diet to stimulate the renin angiotensin system. The effect of acute administration of captopril and ramiprilat, the active diacid of the prodrug ramipril, on central sympathetic tone and blood pressure was then investigated in these animals.

Cats were anaesthetised with α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (12 mg) and paralysed with vecuronium bromide. Simultaneous recordings were made of brachial arterial pressure (BP), heart rate (HR), femoral arterial conductance (FAC) and thoracic preganglionic sympathetic nerve activity (PSNA) as described previously (Ramage, 1984). Using a retroperitoneal approach whole renal nerve activity (RNA) was also recorded. Nerve activity was tested to ensure that it was under baroreceptor modulation by its response to injections (i.v.) of sodium nitroprusside (2μg kg^{-1}), noradrenaline (0.5 μg), angiotensin (A) I (150 ng kg^{-1}) and II (0.5 μg kg^{-1}). After these tests the above variables were recorded for 20 min before injection of either captopril or ramiprilat dissolved in 0.1 N NaOH. Changes from pre-injection values were measured ten minutes after the injections of either captopril (1 mg kg^{-1}) or ramiprilat (0.1 mg kg^{-1}) and are shown in table 1. Injections of vehicle alone caused only minor changes.

Table 1

Drug	ΔBP mmHg	ΔHR beats min^{-1}	ml	ΔFAC (x10^{-3}) mmHg^{-1} min^{-1}	ΔPSNA %	ΔRNA %
Captopril	-33 ± 6***	+7 ± 6		+33 ± 13*	+28 ± 6*	+47 ± 12**
Ramiprilat	-20 ± 3***	+30 ± 8**		+9 ± 8	+69 ±17*	+60 ± 11**

Compared with vehicle *p < 0.05 **p < 0.01 ***p < 0.001 ; (n=5) ± s.e.mean.

The pressor responses to noradrenaline and AII were unaffected by administration of these ACE inhibitors. However, captopril and ramiprilat caused a 60% and 57% reduction respectively in the pressor response to AI. In the captopril experiments gallamine (2 mg kg^{-1}, i.v.) failed to increase heart rate indicating there had not been an increase in vagal tone.

The fall in blood pressure caused by captopril and ramiprilat was associated with an increase in central sympathetic outflow. The failure of heart rate to rise in response to a large fall in blood pressure, associated with an increase in sympathetic outflow, suggests that captopril may be having an action at the sympathetic neuroeffector junction. The tachycardia induced by ramiprilat indicates that the above peripheral action of captopril is unlikely to be related to ACE inhibition. Further studies with other ACE inhibitors are being undertaken to clarify these observations.

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RAT CALCITONIN GENE-RELATED PEPTIDE (CGRP) CAUSES CAROTID VASODILATATION IN CONSCIOUS, UNRESTRAINED RATS

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In man, there are reports of facial flushing following intravenous administration of CGRP (Howden et al 1988) and atrial natriuretic peptide (ANP; Richards et al, 1985), indicating that both peptides might influence carotid blood flow. We have calculated changes in common carotid vascular resistance in response to infusion of CGRP (0.6 nmol/h) or ANP (3.7 nmol/h) in conscious, male Wistar rats (n = 8). Animals were anaesthetized (sodium methohexitone, 60 mg/kg I.P.) and had miniaturized pulsed Doppler probes (Haywood et al, 1981) implanted around both common carotid arteries. The probe wires were led subcutaneously to the back of the neck where they were soldered into a micro-connector held in a harness worn by the rat. Animals were left at least 7 days after operation, housed singly in their home cages with free access to food and water. Thereafter, healthy animals were briefly re-anaesthetized (as above) and had catheters implanted in the abdominal aorta and jugular vein. The next day continuous recordings of mean arterial pressure (MAP), heart rate (HR) and left and right carotid mean Doppler shift (DS) signals were made before, during and after 1 h infusions of CGRP or ANP; the peptides were given in random order separated by at least 1.5 h. Changes in vascular resistance (%) were calculated from the MAP and mean DS signals (Haywood et al, 1981). CGRP and ANP infusions were matched for their effects on MAP after 60 min infusion; the results are summarized in the table.

Table 1. Changes in MAP, HR, DS and common carotid vascular resistances after 60 min infusion of CGRP or ANP. Values are mean (s.e.m.).

			Carotid DS (%)		Carotid Resistance (%)	
	MAP(mmHg)	HR(b/min)	Left	Right	Left	Right
CGRP	-16(3)*	+45(11)*	+54(11)*	+56(19)*	-42(5)*	-38(10)*
ANP	-16(6)*	-6(10)*	-25(6)*	-26(6)*	+19(10)*	+26(13)*

*denotes $P<0.05$ for change relative to baseline (Wilcoxon test).

Under our experimental conditions CGRP caused carotid hyperaemia associated with flushing in the ears, whereas ANP caused a reduction in the common carotid flow signal. Our observations indicate that an increase in flow produced by CGRP is directed through the external carotid vascular bed, at least. It remains to be determined if CGRP also influences internal carotid blood flow.

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BRATTLEBORO RATS HAVE NORMAL CARDIAC BAROREFLEX SENSITIVITIES THAT ARE UNAFFECTED BY CENTRALLY ADMINISTERED VASOPRESSIN

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In contrast to earlier reports (Gardiner & Bennett, 1982), Imai et al (1984) have claimed that vasopressin (AVP) deficient Brattleboro rats have markedly suppressed baroreflex sensitivities (BRS) that are normalized by central administration of AVP. We have investigated the effects of AVP administered either into a lateral cerebral ventricle (i.c.v.) or into the cisterna magna (i.c.) on BRS of Brattleboro and control Long Evans rats.

Male Long Evans and Brattleboro rats were prepared with guide cannulae for i.c.v. and i.c. injections (Harland et al, 1987). On the day before the experiment the animals were anaesthetised (sodium methohexitone, 60 mg/kg i.p.) and catheters placed in the abdominal aorta via the caudal artery and in a jugular vein. Continuous recordings of systolic, diastolic and mean arterial blood pressure (MAP) and heart rate were begun following overnight recovery (17-18 hr). BRS were assessed from the slope of the regression lines relating MAP to pulse interval during i.v. infusion of methoxamine (0.4 mg/ml, 0.2 ml/min) or following a bolus i.v. injection of sodium nitroprusside (100 ug/ml, 0.1 ml). Doses of AVP which had no effect on MAP or heart rate were administered i.c.v. or i.c. 5 min before assessment of BRS. The results are summarised below.

Table 1 Effect of i.c.v. and i.c. AVP on BRS [median (range)]of conscious rats

		BRS (ms/mmHg)	
Long Evans	n	methoxamine	nitroprusside
control	8	1.22 (0.75 - 1.53)	0.72 (0.4 - 1.6)
i.c.v. AVP, 300 pg		*1.6 (1.13 - 2.2)	0.75 (0.45 - 1.45)
control	9	1.19 (0.88 - 1.56)	0.73 (0.4 - 1.29)
i.c. AVP 300 pg		*1.55 (0.96 - 2.4)	*1.55 (0.4 - 2.34)
Brattleboro			
control	8	0.9 (0.63 - 1.54)	0.9 (0.4 - 1.21)
i.c.v. AVP, 300 pg		0.74 (0.5 - 1.63)	1.01 (0.5 - 1.79)
control	8	1.01 (0.65 - 1.52)	1.04 (0.52 - 1.47)
i.c. AVP 100 pg		0.73 (0.4 - 1.6)	0.96 (0.54 - 2.67)

* $P<0.05$ compared with respective control (Wilcoxon test).

In Long Evans rats AVP increased BRS under three of the four conditions tested. However, BRS of Brattleboro rats which were not reduced relative to Long Evans rats, were not modified by i.c.v. or i.c. AVP. Since Brattleboro rats show increased cardiovascular effects compared to Long Evans rats with higher doses of AVP administered centrally, (Harland et al, 1987), the lack of effect of AVP on BRS of Brattleboro rats in the present study is not likely to be due to an absence of central AVP receptors.

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CHARACTERISATION OF κ-OPIOID RECEPTORS IN THE GUINEA-PIG ILEUM LONGITUDINAL MUSCLE-MYENTERIC PLEXUS PREPARATION

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The receptors mediating opioid effects in the guinea-pig ileum have been shown to correspond to u and k on the basis of agonist and antagonist potency in the isolated tissue preparations (Hutchinson et al., 1975; Lord et al., 1977). The biochemical characterisation of the kappa receptor in the guinea-pig ileum longitudinal muscle-myenteric plexus preparation (LM-MYP) has mainly involved radioligand binding studies using the non-selective opioid [^{3}H]bremazocine ([^{3}H]BMZ) under suitable conditions to suppress binding to u- and δ-receptors (Corbett et al., 1985). However the nature of the remaining [^{3}H]BMZ binding sites although they are presumed to be kappa, has yet not been clearly defined. [^{3}H]U69593 is a ligand which has been reported to have the highest selectivity for the kappa receptor (Lahti et al., 1985). This study therefore compared the binding characteristics of [^{3}H]BMZ and [^{3}H]U69593 in the LM-MYP.

Equilibrium binding studies were performed at 25°C using LM-MYP membrane homogenates and either [^{3}H]U69593 alone or [^{3}H]BMZ in the presence of a 300 fold excess of unlabelled [D-Ala^2,MePhe^4,glyol^5]-enkephalin (DAGOL) and [D-Ala^2,D-Leu^5]-enkephalin (DADLE) to eliminate interaction with u- and δ-sites respectively.

The specific binding of both radioligands was saturable and represented binding to a homogeneous population of binding sites. The equilibrium dissociation constant, K_D (nM), and the apparent density of binding sites, B_{max} (fmol/mg protein) were 0.11 + 0.01 and 119.4 + 7.3 respectively for [^{3}H]BMZ and 3.9 + 1.0 and 84.7 + 5.0 respectively for [^{3}H]U69593. Thus [^{3}H]U69593 only labelled a proportion (70%) of the sites labelled by [^{3}H]BMZ. We have previously reported a similar observation in the guinea-pig cerebral cortex (Hall et al., 1987) and suggested that it might represent evidence for a heterogeneity for kappa binding sites. However, the association and dissociation kinetics for [^{3}H]BMZ were single exponentials indicating that the binding represented a simple bimolecular reaction. Affinity values were in good agreement with saturation studies. The kappa opioid nature of both [^{3}H]U69593 labelled sites and the total sites labelled by [^{3}H]BMZ under "kappa-selective" conditions was confirmed in competition studies by the high affinity of compounds such as PD117302, U69593 and dynorphin (1-8) and the low affinity of DAGOL, DADLE and the δ-selective [D-Pen^2, D-Pen^5]-enkephalin. A close correlation was obtained between the affinity (K_i) values for each compound against either radioligand.

In conclusion, the characteristics of both [^{3}H]BMZ and [^{3}H]U69593 binding in the LM-MYP are consistent with occupation of the kappa$_3$ receptor. However, the identity of the additional sites labelled by [^{3}H]BMZ requires further investigation.

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EFFECTS OF ERGOMETRINE IN RAT UTERUS AND INVOLVEMENT OF 5-HT RECEPTORS

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Ergometrine is a spasmogen of isolated rat uterus, although the effects are inconsistent (Sund, 1963). Ergometrine has also been described as an antagonist of 5-hydroxytryptamine (5-HT) in this tissue (Sund, 1963; Ichida et al., 1983). 5-HT receptors in the rat uterus have been tentatively classified as of the 5-HT_2 type (Bradley et al., 1986). Recently, potent competitive antagonists at 5-HT_2 receptors, such as ICI 169,369 (Blackburn et al., 1987), have become available. The aim of this study was to clarify the mechanism of action of ergometrine in the rat uterus.

Uteri, from rats treated 18-24 h previously with 17β-oestradiol benzoate (100 μg kg^{-1}), were mounted in Krebs' solution in tissue baths at 30°C. Concentration-effect curves were constructed to spasmogens before and after 30 min incubation with modifying agents.

Ergometrine (30 nM - 1 μM) induced phasic spasm; concentrations > 1 μM did not usually induce tension development. 5-HT, acetylcholine (Ach) and potassium chloride (KCl) induced spasm which was phasic at low concentrations but maintained at higher concentrations. Methysergide (1 and 10 nM) antagonised ergometrine and 5-HT but did not modify responses to Ach or KCl. ICI 169,369 (10 nM - 10 μM) produced parallel rightward shifts without depression of maximum of concentration-effect curves to 5-HT (pA_2 8.0 ± 0.3, slope -1.2 ± 0.1, n=8) and to ergometrine (pA_2 8.5 ± 0.2, slope -1.0 ± 0.1, n=8). ICI 169,369 (10 nM - 10 μM) did not antagonise Ach.

The initial phasic spasms to ergometrine were not maintained and, therefore, it was possible to assess the antagonist action of ergometrine. Ergometrine (1 and 10 μM) produced rightward shifts of the concentration-effect curves to 5-HT (230 and 2,600 fold, respectively, n=7) with some depression of maximum. Ergometrine did not antagonise Ach or KCl.

These results suggest that in rat uterus ergometrine is both 1) an agonist at receptors with characteristics of 5-HT_2 receptors and 2) a selective antagonist at 5-HT receptors. The partial agonist action of ergometrine in this tissue could explain previous inconsistencies.

We thank Sandoz and ICI for gifts of methysergide and ICI 169,369 respectively.

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EVALUATION OF MYO-INOSITOL(1,4,5)TRISPHOSPHOROTHIOATE, A NOVEL PHOSPHATASE-RESISTANT ANALOGUE OF MYO-INOSITOL(1,4,5)TRISPHOSPHATE

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Myo-inositol(1,4,5)trisphosphate ($InsP_3$), an immediate hydrolytic product of receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate, acts as a second messenger to release calcium from intracellular stores of many cells (Berridge & Irvine, 1984). To date, there has been a lack of specific drugs to interfere with this signalling system and we report here the biological activity of a novel synthetic analogue myo-inositol(1,4,5)trisphosphorothioate ($InsP(S)_3$) designed to be resistant to metabolism but retaining activity.

Specific binding of ^3H-$InsP_3$ to rat cerebellar membranes was performed as previously described (Willcocks et al. 1987). Release of non-mitochondrial bound $^{45}Ca^{2+}$ was evaluated in saponin-permeabilised rat pituitary tumour GH_3 cells using a modification of the technique described by Gershengorn et al. (1984). We have recently described the synthesis of DL-$InsP_3$ and DL-$InsP(S)_3$ (Cooke et al. 1987a, 1987b).

Permeabilised GH_3 cells were loaded with 150 nM $^{45}Ca^{2+}$ in the presence of 5 mM ATP and 2 µg/ml oligomycin. $InsP_3$ or $InsP(S)_3$ were added at 20 min and free and bound $^{45}Ca^{2+}$ separated by centrifugation through silicone oil at 1 min. DL-$InsP_3$ released about 30% of bound $^{45}Ca^{2+}$ with an EC_{50} of 1 µM whereas DL-$InsP(S)_3$ although 3-4 fold weaker was nevertheless a full agonist on the release of Ca^{2+}.

[^3H]$InsP_3$ stereospecifically labels a site on cerebellar membranes that possesses properties of an $InsP_3$ 'receptor' (Willcocks et al. 1987). Synthetic DL-$InsP_3$ potently displaced binding from these sites with an IC_{50} of $2.6 \pm 0.28 \times 10^{-7}$ M. The phosphorothioate analogue DL-$InsP(S)_3$ although somewhat weaker (IC_{50} $1.3 \pm 0.15 \times 10^{-6}$ M), nevertheless displaced all the specific binding of [^3H]$InsP_3$ with a slope close to unity. In a further series of experiments the resistance of $InsP(S)_3$ to a specific Ins(1,4,5)P_3 5-phosphatase obtained from human erythrocytes was established. Incubation of $InsP_3$ with 5-phosphatase (20 µg protein, 30 min, 37°) resulted in a marked 20-fold shift to the right in the displacement curve of ^3H-$InsP_3$ binding to cerebellar membranes. However, an identical incubation of $InsP(S)_3$ resulted in a totally unaltered activity in this system.

These data suggest that the novel synthetic analogue $InsP(S)_3$ is a full agonist of intracellular Ca^{2+} release, a potent displacer of ^3H-$InsP_3$ binding to cerebellar membranes yet is resistant to 5-phosphatase attack. Such a compound offers considerable potential in the investigation of phosphoinositide-linked receptor responses.

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STIMULATION OF α_1- BUT NOT OF α_2-ADRENOCEPTORS CAUSES HYDROLYSIS OF PHOSPHOINOSITIDES IN THE FEMORAL VEIN OF THE RAT

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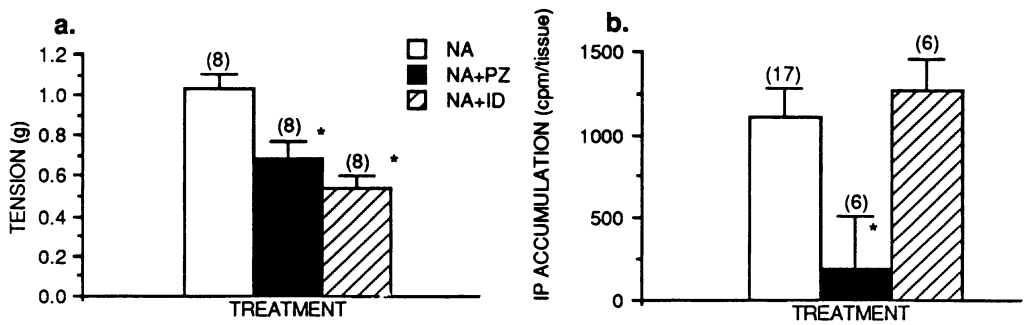
In many tissues, receptor occupation is associated with hydrolysis of phosphoinositides. This hydrolysis results in the formation of the two intracellular messengers inositol 1,4,5-trisphosphate, which releases intracellular calcium, and diacylglycerol, which activates protein kinase C (see Berridge & Irvine, 1984). Chiu and co-workers (1987) have shown that stimulation of the α_1-adrenoceptors of the rat aorta induces such hydrolysis of phosphoinositides. In the present study we have examined changes in inositol phosphate levels associated with stimulation of the α_1- and α_2-adrenoceptors of the femoral vein of the rat, a vessel which contains both postjunctional α-adrenoceptor subtypes (Downing et al, 1986).

Both femoral veins were removed from Male Wistar rats (200-300g), cleaned of adherent tissue and cut open to form flat sheets. The vessels were incubated in 2ml of physiological salt solution (PSS) containing myo-^{3}H-inositol (30μCi/ml) at 37°C gassed with 95% O_2/5% CO_2 for 3 hours. Tissues were then washed and placed into 1ml PSS containing 10mM LiCl, and noradrenaline (NA)(10^{-5}M) was added. Tissues were incubated for a further 1hr and then 3ml $CHCl_3$:CH_3OH:HCl (200:100:1) was added. After 20min, the mixture was centrifuged at 500g for 5min, the upper aqueous phase was removed, added to 3ml H_2O and applied to a Dowex chromatography column. Inositol phosphates were eluted as described by Berridge (1983). Tritiated inositol phosphate content was assessed by liquid scintillation counting. In some experiments, prazosin (PZ, 10^{-8}M) or idazoxan (ID, $5x10^{-6}$M) was added 15 min before NA. To study contraction, 5mm rings of femoral veins were mounted under 0.5g tension between 2 fine steel wires in PSS at 37°C gassed as above. NA was added after a 60 min equilibration period and antagonists were added 30min before addition of agonist. Results are summarised in Fig 1.

Figure 1. The effects of antagonists on a) contractile responses to NA and (b) inositol phosphate accumulation in the presence of NA.



*significantly different from NA, $p<0.05$. Results are mean±sem of (n) experiments

These observations provide evidence that while the contractile response of the femoral vein of the rat to NA is blocked by antagonists of both α_1- and α_2-adrenoceptors, it is only the α_1-mediated response that is linked to hydrolysis of phosphoinositides.

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IS ALLERGIC AIRWAY HYPERREACTIVITY OF THE GUINEA-PIG DEPENDENT ON EOSINOPHIL ACCUMULATION IN THE LUNG?

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Dunkin-Hartley guinea-pigs (300-350 g) recieved cyclophosphamide (100 mg/kg i.p.) 1 day prior to sensitisation. Animals were sensitised by i.p. injection of an emulsion of $Al(OH)_3$ (2 mg), pertussis vaccine (0.25 ml) and 1 ug (group B) or 10 ug (group C) of ovalbumin (OA) per animal and were boosted on weeks 3 and 6. Two weeks after the last boost, sensitized and control (group A) animals were exposed to an aerosol of OA (0.1 %) for 1 hour. At 24, 48 and 72 hours after exposure, basal airway resistance (R_1, cm H2O/l/sec), basal dynamic compliance (C_{dyn}, ml/cm H2O), and histamine reactivity were measured in aneasthetised guinea-pigs. Subsequently animals were killed, lungs were lavaged with 6 x 10 ml of Ca^{2+} and Mg^{2+} free Tyrode, containing BSA (0.5%) and EDTA (20 mM). Lavage fluid was centrifuged (200g; 10 min) and the cell pellet was resuspended in 1 ml of Tyrode. Total cells were counted in a haemocytometer and differential cell counts were made from smears stained by Leishman's. Each time point comprises observations from 5 animals.

TIME AFTER OA CHALLENGE (HOURS)	R_1 BASAL	C_{dyn} BASAL	ΔR_1 HISTAMINE (1.8 ug/kg)	TOTAL EOSINOPHIL (x 10^6)
A) Naive guinea-pigs				
24	123± 9	0.7±0.04	52±26	1.9±0.6
48	117± 7	1.0±0.1	84±37	2.6±0.7
B) 1 ug OA used for sensitization				
NO CHALLENGE	136± 6	1.4±0.2	122±65	4.2±1.6
24	127± 5	1.1±0.1	175±59	23.2±5.4*
48	130± 7	1.0±0.1	163±76	41.6±2.2*
72	112± 7	1.0±0.1	108±29	36.0±5.2*
C) 10 ug OA used for sensitization				
NO CHALLENGE	146± 4	1.1±0.1	182±25	3.2± 0.7
24	145± 4	0.7±0.1	514±130*	38.6± 2.3*
48	144±13	1.2±0.4	360±90*	31.0±11.3*
72	138± 5	1.2±0.2	155±28	23.6± 3.8*

* *indicates a significance level of at least P<0.05 (t-test) relative to no challenge.*

Sensitisation caused minimal changes in R_1 and C_{dyn} in both groups (B and C). Exposure to OA induced a pronounced eosinophil accumulation in all sensitized animals, however in animals from group B no noteworthy hyperreactivity was observed whereas animals from group C exhibited marked hyper-reactivity at 24 and 48 hours. There was no correlation between airway hyperreactivity and eosinophil counts for individual animals in group C (R=0.32) as well as group B (R=0.17). These results caution against the presumption that accumulation of eosinophils is paralleled by changes in airway reactivity.

NORADRENALINE AND SALBUTAMOL INHIBIT HISTAMINE-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS IN BOVINE TRACHEAL SMOOTH MUSCLE

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β_2-adrenoceptor agonists are potent and therapeutically important bronchodilators (Barnes, 1987). Many agents, such as histamine (HA) and carbachol (CCh), which contract visceral smooth muscle stimulate the hydrolysis of inositol containing membrane phospholipids resulting in the formation of the two second messengers inositol-(1,4,5)- trisphosphate and diacyglycerol, which respectively mobilise intracellular calcium and activate protein kinase C (Abdel-Latif, 1986). In this study we have investigated the effect of β_2-adrenoceptor agonists on the accumulation of inositol phosphates elicited by HA and CCh in bovine tracheal smooth muscle.

Slices of bovine tracheal smooth muscle were incubated for 75 min at 37°C in Krebs-Henseleit solution containing [^{3}H]-myo-inositol (0.4 μM) under an atmosphere of O_2/CO_2 (95:5). The prelabelled slices were then transferred to tubes containing Krebs-Henseleit solution, LiCl (5mM) and, where appropriate, antagonist drug for 30 min (total volume 300 μl). Agonists were then added in 10 μl of medium and incubations were terminated 45 min later by addition of 10% (w/v) perchloric acid. [^{3}H]-inositol phosphates (IP) were then separated by anion exchange chromatography (Hill & Kendall, 1987).

HA and CCh produced concentration-dependent increases in total [^{3}H]-IP accumulation (HA EC_{50} = 38 ± 9 μM, n=9: CCh EC_{50} = 3.5 ± 1.2 μM, n=6) in slices of bovine tracheal smooth muscle. The responses to HA and CCh were inhibited competitively by mepyramine (50nM) and atropine (50nM) respectively indicating the involvement of histamine H_1- and muscarinic receptors. The maximum response to HA in this tissue was 35 ± 1 % (n = 3) of that to CCh. Noradrenaline (NA, 100 μM) alone produced a small (6 ± 1 % of the response to 100μM HA ,n=4) increase in [^{3}H]-IP accumulation but in addition produced a significant ($p < 0.05$) 55 ± 5 % inhibition of the inositol phosphate response to 0.1 mM HA (6 experiments). NA did not, however, inhibit the CCh-induced response (3 experiments). Phentolamine (1μM) abolished the response to NA alone but did not attenuate the inhibitory effect of NA on HA-induced [^{3}H]-IP accumulation (3 experiments).

The β_2-agonist salbutamol produced a dose-related inhibition of HA-induced (100 μM) [^{3}H]-IP accumulation at concentrations below 5 μM (IC_{50} = 0.5 ± 0.2 μM, maximal inhibition 66 ± 3 %, n= 11). At higher concentrations a small upturn in the dose-response curve was usually observed. A similar inhibition of HA-induced [^{3}H]-IP accumulation was observed with isoprenaline. This inhibitory effect of salbutamol (1 μM) was antagonised by propranolol (2 μM, 6 experiments) but not by phentolamine (2 μM, 3 experiments).

These results suggest that a mechanism exists whereby β-adrenoceptor stimulation can reduce the [^{3}H]-IP formation induced by HA in bovine tracheal smooth muscle. This effect may contribute to the bronchodilator actions of β_2-adrenoceptor agonists in airway smooth muscle.

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ARE THERE DOPAMINERGIC NERVES IN GUINEA-PIG LUNG?

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Dilator receptors for dopamine (DA) have been reported in the pulmonary circulation of some species (Eyre, 1973; Gorman, 1987) and intravenous infusion of DA has been found to cause hypoxaemia, perhaps through altering ventilation-perfusion balance (Marin et al. 1979). The presence of dilator receptors for DA has been shown in several systemic vascular beds to be correlated with the presence of sympathetic vasomotor nerves that appear to be dopaminergic (see Bell, 1987) and dopamine has been found in lung tissue (Aviado & Sadavongvivad, 1970). In this study we have looked at the neurochemical evidence for the neurotransmitter function of DA in the lung of the guinea pig.

Adult male guinea-pigs were killed with an overdose of sodium pentobarbitone and the lungs, hearts and spleens were removed. Total tissue catecholamines were measured by oxidative electrochemistry after separation by HPLC. Histochemical demonstration of catecholamines was carried out by fluorescence microscopy and tyrosine hydroxylase and dopa decarboxylase were localized by immunohistochemical methods (see Harris et al. (1986) for details).

In atrium and spleen, which are thought to contain only noradrenergic sympathetic nerves, the DA represented $1.7\pm0.6\%$ and $0.7\pm0.2\%$ respectively of the total catecholamines whereas in lung this value was markedly higher, $4.2\pm0.6\%$ ($p<0.01$, $N=7$). This result suggested the presence of an additional tissue store of DA in lung. Tissue samples taken from guinea pigs treated with 6-hydroxydopamine (200 mg/kg i.p., 24h earlier) showed a marked depletion of both noradrenaline and DA in lung and no non-neuronal cells with catecholamine fluorescence were seen by microscopy, indicating that the excess DA was neuronally localized. The immunohistochemical techniques localised tyrosine hydroxylase in a pattern similar to that seen for catecholamine fluorescence, i.e. perivascular plexuses but virtually no innervation of airways. A small proportion of the perivascular nerves was also immunoreactive for dopa decarboxylase and these fibres were distributed to both bronchial and pulmonary vessels.

As the presence of immunohistochemically demonstrable dopa decarboxylase activity in terminal axons is characteristic of dopaminergic, but not of noradrenergic, neurones (see Harris et al, 1986), our biochemical and immunohistochemical results both indicate that the intrapulmonary vasculature of guinea-pig lung receives a dual sympathetic innervation by dopaminergic and noradrenergic nerves.

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SUBSTANCE P AND PERIPHERAL INFLAMMATORY HYPERALGESIA

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Substance P is widely distributed in the central and peripheral nervous system, and its physiological effects have been extensively studied (see Pernow, 1983). Its putative role as a neurotransmitter of noxious sensory information is of particular interest. The present study describe the possible participation of substance P in peripheral infalmmatory hyperalgesia.

Male Wistar rats (150-170g) were used. Hyperalgesia was measured by a modification of the Randall-Selitto test (Ferreira et al., 1978).

Substance P injected directly into the footpad of a hindpaw induced hyperalgesia both time- and dose-dependent, the potency of substance P being approximately similar for up to 1 hour after injection (ED_{50} and 95% confidence limits being 1.3 (1.1-1.6) and 1.5 (1.3-1.7) µg/paw at 30 and 60 min respectively) and increased markedly with time thereafter at 4 h the ED_{50} being 5.8 (5.2-7.0) µg/paw.

Exposure of the paw to multiple injections of sub-threshold doses of substane P increased the potency and time course of the hyperalgesic response in the paw on subsequent exposure of that paw to a challenging dose of substance P. Thus, 5 h after the last of 3 injections of 10 ng/paw (given at 15 min intervals), challenge of the paw to a previously sub-threshold dose of substance P (0.5 ng/paw) now induced pronounced hyperalgesia.

Substance P-induced sensitisation was inhibited by prior exposure of the paw to the substance P antagonist ($D.Arg^{1}, D.Pro^{2}, D.Trp^{7,9}, Leu^{11}$) substance P (5 µg/paw).

Multiple injections of sub-threshold doses of prostacyclin (4 x 0.5 ng/paw given at 10 min intervals) or PGE_2 (6 x 100 ng/paw twice daily over 3 days) also produced prolonged hyperalgesia to a challenging low dose of prostacyclin (0.5 ng/paw). Furthermore, the sensitising effects of these regimes with prostacyclin and PGE_2 were also antagonised by the substance P antagonist.

It is suggested that in some peripheral inflammatory processes substance P can be released and can sensitise nociceptors to different mediators. This effect can also be associated to some conditions of chronic pain.

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CHANGES IN PLASMA CORTISOL, CATECHOLAMINES AND PAIN THRESHOLDS IN SHEEP WITH A CHRONICALLY PAINFUL CLINICAL CONDITION

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The evaluation of pain in animals has produced many difficulties in quantification. In an attempt to examine this problem we have measured a series of parameters in sheep suffering from apparent clinical pain and compared them to the values in normal control animals.

In a group of control sheep, the plasma cortisol was measured, over several hours, by radioimmunoassay (RIA Kit, Amersham, U.K.) and plasma catecholamines monitored using h.p.l.c. with electrochemical detection (Hjemdahl et al 1979). The perception thresholds of a mechanical stimulus to the leg and a thermal stimulus to the ear were also measured (Nolan et al 1987).

As a model of a seemingly painful condition we looked at sheep with an infectious lesion of the foot, known as foot rot (FR). This is a clinical condition which produces a chronic lameness of the affected limb which is apparently very painful, but can be alleviated by relatively simple treatment. The severity of the foot rot was scored by an independent observer and blood samples taken for cortisol and catecholamine determination. Pain threshold values were also assessed. The affected limb was subsequently nerve blocked using 0.25% bupivacaine, and the plasma factors and mechanical threshold remeasured. Sheep were then treated by foot paring and local and systemic antibiotic administration until the foot rot was alleviated and rescored by the same observer. Blood sampling and the measurement of perception thresholds was then repeated.

Sheep with foot rot showed a significant reduction in plasma cortisol levels, compared to controls. (Control 1.72 ± 0.28µg/100ml, n=10; FR 0.91 ± 0.16 µg/100ml, n=6, $p < 0.05$). Plasma noradrenaline and adrenaline were elevated in sheep with foot rot, (noradrenaline, control 4.74 ± 0.66 nM, n=10; FR 8.07 ± 0.58, nM, n=5; $p < 0.01$: Adrenaline, control 2.03 ± 0.22 nM, n=10; FR 4.78 ± 0.56 nM, n=5, $p < 0.01$).

The lame sheep showed no significant differences in thermal threshold, compared to control, either before or after treatment. (Control 54.6 ± 1.4°C, n=8; FR 54.7 ±1.6°C, n=5; FR post treatment 54.6 ± 1.2°C, n=6).

The mechanical threshold was 4.35 ± 0.18 Newtons in control sheep. This was reduced in sheep with foot rot both before and after treatment (FR 2.89 ± 0.37 N, n=6, $p < 0.01$, FR post treatment 2.27 ± 0.53 N, n=6, $p < 0.01$). The nerve block did not alter the mechanical threshold, which was still reduced (2.32 ± 0.27, n=6), however nerve block of the affected limb did result in an increase of plasma cortisol levels and a decrease of plasma catecholamines towards normal.

These preliminary studies would indicate that in animals experiencing clinical pain, the plasma cortisol levels are depressed, the plasma amines are elevated and that the mechanical pain threshold is reduced.

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EFFECT OF AMITRIPTYLINE ON THE SECRETION AND UPTAKE OF HISTAMINE AND 5-HYDROXYTRYPTAMINE FROM RAT PERITONEAL MAST CELLS

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Recent reports (Theoharides et al, 1982; Carraway et al, 1984) indicate that amitriptyline ($1x10^{-4}$M) inhibits the secretion of histamine but not that of 5-HT, from rat peritoneal mast cells, stimulated by compound 48/80 (1.0 $\mu g\ ml^{-1}$). These results suggest the existence of separate secretory mechanisms for the two amines from the mast cell. However, Berlin and Enerback (1986) observed that amitriptyline ($1x10^{-4}$M) inhibited the secretion of histamine and 5-HT to the same extent. In light of the conflicting nature of previous reports, and the importance of differential release of mast cell amines, we have investigated the effects of amitriptyline upon both the secretion and uptake of histamine and 5-HT by rat peritoneal mast cells.

Mixed peritoneal cells were obtained by lavage from female Wistar rats (300-350g) and the mast cells purified (90%) over bovine serum albumin (38%). Purified cells were incubated in HEPES buffered Locke's solution (pH 7.0) with compound 48/80 for 15 min at 37°C, in the presence or absence of amitriptyline ($1x10^{-4}$M: 5 min preincubation). The supernatant was assayed fluorimetrically for the presence of histamine and 5-HT (Cohen et al, 1987). Results are expressed as percentage amine release corrected for their respective spontaneous release in the presence or absence of amitriptyline.

In secretion experiments compound 48/80 (0.1-30.0 $\mu g\ ml^{-1}$) released significantly more histamine than 5-HT, such that the dose response curve was steeper and the maximum histamine release greater than that for 5-HT (Cohen et al, 1987). In the presence of amitriptyline ($1x10^{-4}$M) the histamine dose response curve was shifted to the right by approximately 0.2 log units and maximal histamine output (3.0 $\mu g\ ml^{-1}$ compound 48/80) was reduced from 72% to 64%. In contrast, amitriptyline had no significant effect upon the secretion of 5-HT. Concentrations of amitriptyline less than $1x10^{-4}$M were without effect upon histamine secretion and concentrations greater than this were cytoxic.

In uptake experiments, purified rat peritoneal mast cells were incubated with exogenous histamine (0.1-3.0 $\mu g\ ml^{-1}$) and 5-HT (0.02-0.6 $\mu g\ ml^{-1}$) alone and in mixtures in a ratio of 5:1 as found in vivo (Cohen et al, 1987): the concentrations of amine chosen reflected those measured in secretion experiments. Aliquots of mast cells ($5x10^{5}$ cells cm^{-3}) were incubated for 15 min at 37°C with the two amines in the presence or absence of amitriptyline ($1x10^{-4}$M: 5 min preincubation). Although similar amounts of histamine and 5-HT were removed, the cells showed a preference for 5-HT. For example in the presence of 200 ng ml^{-1} 5-HT, the cells removed 50 ng of amine, whereas for histamine, a concentration of 1.0 $\mu g\ ml^{-1}$ was required for 50ng uptake into cells. In the presence of amitriptyline, 5-HT (200 ng ml^{-1}) uptake was inhibited by approximately 60%, while histamine (1.0 $\mu g\ ml^{-1}$) uptake increased by approximately 50%. In addition histamine uptake was still apparent at 4°C, while 5-HT uptake was almost completely inhibited.

These results suggest that the apparent reduction in histamine secretion in the presence of amitriptyline may be explained by alteration of amine uptake, rather than differential inhibition of histamine and 5-HT secretion.

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WHAT IS GADDUM'S "SUBSTANCE R"?

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In 1953 Gaddum published a highly cited paper in which he described for the first time, the superfusion technique. Less well known is that in this paper Gaddum also reported the production by the perfused rat intestine of a factor with powerful oxytocic activity. The results obtained by Gaddum suggested that this factor was a protein. We have confirmed Gaddum's initial findings and have carried out further experiments to characterise what he termed, "substance R".

To generate substance R, male Sprague-Dawley rats were first anaesthetised with ether and Heparin (1000 u) was administered via a tail vein to prevent clotting during the perfusion. Following a laparotomy the small intestine was ligated at duodenal level distal to the pancreas at the ileo-coecal junction. The superior mesenteric artery was cannulated and all other vessels except the hepatic portal vein were ligated. The mesenteric bed was then removed, suspended in a heated jacket and perfused with Tyrode's solution (37^{o}C, 0.4 ml/min). Effluent was collected for consecutive 20 min periods and aliquots of fluid were assayed for oxytocic activity on segments of stilboestrol-primed rat uterus superfused with de Jalon's solution (30^{o}C, 5 ml/min).

No activity was detectable during the first 20 min of collection. However, oxytocic activity was released in increasing amounts thereafter reaching a maximum after 3-4 h and was sustained up to 6 h. Uterine contractions were delayed in onset (30 sec) compared to carbachol or bradykinin and were not blocked by the administration of atropine (10^{-6}M) or methysergide (5.7×10^{-7}M) to the isolated organ. Oxytocic activity in the perfusate was non-dialysable (m.w. cut off 10-15 KDa) and dialysed samples did not contract isolated guinea-pig ileum or rat fundic strip. The material was stable when frozen (-20^{o}C) for at least one month.

The characteristics of this oxytocic principle were very similar to those reported by Gaddum for substance R and are also similar to those of kallikrein, as observed by Gaddum himself. To explore the similarity between the two substances we used desalted rat urine as a source of kallikrein and compared its properties with substance R. Incubation (37^{o}C, 30 min) of substance R or kallikrein with heat-treated rat serum (60^{o}C, 3 h) plus captopril (4.6×10^{-4}M) resulted in the generation of kinin in both instances. Superfusion of the rat uterus with aprotinin, (50 -100 KIU/ml), a plasma and tissue kallikrein inhibitor (Trautschold et al, 1967), reduced the oxytocic activity of substance R and urinary kallikrein. However, soya bean trypsin inhibitor (100 µg/ml), a plasma kallikrein inhibitor (Werle & Maier, 1952) blocked the action of substance R without affecting responses to urinary kallikrein.

We conclude that Gaddum's substance R has kininogenase properties but is unlikely to be a glandular kallikrein. It is being further characterised in our laboratory.

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