EFFECTS OF ETOXADROL ON AMINO ACID EXCITATION OF MAMMALIAN CENTRAL NEURONES

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In previous communications to this Society, ketamine (Anis et al., 1982a) and phencyclidine (Anis et al., 1982b) two dissociative anaesthetics, have been shown to selectively block excitatory responses of central mammalian neurones to N-methyl-aspartate (NMA). In order to see whether this property is common to other dissociative anaesthetics we have examined the effects of etoxadrol [d-2-ethyl-2-phenyl-4-(2-piperidyl)-(1,3-dioxolane hydrochloride)] on excitation of spinal neurones by electrophoretically administered amino acids in pentobarbitone-anaesthetised rats and cats. The neurophysiological effects of etoxadrol anaesthesia are similar to those of ketamine (Tang and Schroeder, 1973), the two compounds having related but somewhat different structures.

On 23 spinal neurones in the rat, etoxadrol HCl (5-25 nA, 50 mM in 150 mM NaCl; 5-50 nA, 25 mM in 175 mM NaCl) reduced responses to NMA by 77 ± 20%, those to quisqualate by 17 ± 18%, and those to kainate by 9 ± 21%.

On 8 spinal neurones in the cat similar administration of etoxadrol reduced responses to NMA by 78 ± 30%, those to quisqualate by 5 ± 9% and those to kainate by 10 ± 14%.

Ketamine, when compared with etoxadrol on 4 cat neurones, showed a similar degree of selectivity but was 2-5 times less potent. Unlike ketamine, ejection of etoxadrol especially with higher currents was found to reduce action potential amplitude, and therefore it seems unlikely that etoxadrol will be a useful tool for further electrophoretic studies on the NMA receptor.

When given intravenously, etoxadrol (4 mg/kg) reduced responses of spinal neurones to NMA more than those to quisqualate or kainate.

The results are compatible with the hypothesis that an action of the dissociative anaesthetics at the NMA receptor-ionophore complex may underlie their anaesthetic/analgesic properties. It is of particular interest both for this hypothesis and the role of NMA receptors in synaptic transmission that etoxadrol selectively reduces flexor reflexes in cats and nociceptive reflexes in rats (Tang and Schroeder, 1973).

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INHIBITION BY α_2-ADRENOCEPTOR ANTAGONISTS OF CALCIUM-DEPENDENT ELECTRICALLY-EVOKED RELEASE OF (^{3}H)-GABA FROM CEREBRAL CORTEX SLICES

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Two subtypes of α-adrenoceptors have been clearly defined, based on pharmacological selectivity for agonists as well as antagonists. In addition to the postsynaptic localization of α_2-adrenoceptors in the CNS (Morris et al, 1981), presynaptic α_2-adrenoceptors are involved in the modulation of the release of noradrenaline (Langer, 1980) and serotonin (Frankhuysen and Mulder, 1980 ; Göthert et al, 1981). The purpose of the present studies was to investigate whether α_2-adrenoceptors are involved in the modulation of (^{3}H)-GABA release in vitro.

Rat cerebral cortex slices (0.4 mm thickness), were incubated with 135 nM (^{3}H)-GABA (S.A. 29.3 Ci/mmol) during 10 min and continuously superfused with fresh Krebs' medium during 89 min. Two periods (S_1, S_2) of electrical stimulation (3 msec, 24 mA, 5 Hz during 2 min), were applied 49 min and 73 min after the beginning of the superfusion. Drugs were added 8 min before S_2. The effects of the drugs on (^{3}H)-GABA release were measured by the S_2/S_1 value : ratio of the fractional release elicited by S_2, in the presence of the drug, over the fractional release elicited by S_1 in its absence.

The electrically-evoked release of (^{3}H)-GABA was almost entirely calcium-dependent (S_2/S_1 = 0.95 $\pm$ 0.03, n=16 under control conditions, and S_2/S_1 = 0.19 $\pm$ 0.04, n=3, $P<0.001$, in the absence of calcium before S_2).

The depolarisation-induced release of (^{3}H)-GABA was inhibited in a concentration-dependent manner by yohimbine with no alteration of the spontaneous outflow of radioactivity. The maximal inhibitory effect was attained at 10 μM yohimbine (S_2/S_1=0.19 $\pm$ 0.05, n=5, $P<0.001$). Rauwolscine (10 μM) also inhibited the electrically-evoked release of (^{3}H)-GABA (S_2/S_1=0.49 $\pm$ 0.08, n=3, $P<0.001$). Yet, exposure to pseudoyohimbine 10 μM did not reduce the release of (^{3}H)-GABA. The electrically-evoked release of (^{3}H)-GABA was also inhibited by 10 μM phentolamine (S_2/S_1=0.61 $\pm$ 0.03, n=6, $P<0.001$) and the α_2-adenoceptor antagonist RX781094 (Chapleo et al., 1981) (1 μM : S_2/S_1=0.86 $\pm$ 0.03, n=6, $P<0.05$; 10 μM : S_2/S_1=0.66 $\pm$ 0.01, n=3, $P<0.001$) but not by prazosin 0.1 μM.

In contrast to the effects of the α_2 adrenoceptor antagonists, the electrically-evoked release of (^{3}H)-GABA was not affected by clonidine (0.1, 1.0 and 10 μM) or M7 (2-dimethylamino 5-6 dihydroxytetralin) (0.1 and 1 μM). Noradrenaline (1 and 10 μM) elicited a significant increase in the spontaneous outflow of radioactivity but did not affect the electrically-evoked release of (^{3}H)-GABA. Clonidine (1 μM) did not antagonize the inhibitory effect of yohimbine (3 μM).

In rats pretreated with reserpine (2.5 mg/kg, i.p., 24 h before the experiment) yohimbine (10 μM) inhibited the electrically-evoked release of (^{3}H)-GABA to the same extent (70%) as in control animals.

It is concluded that several α_2-adrenoceptor antagonists inhibit the electrically-evoked release of (^{3}H)-GABA through a mechanism which does not involve α_2-adrenoceptors, or endogenously-released noradrenaline. Caution is therefore required when using α_2-adrenoceptor antagonists with the aim of affecting selectively noradrenergic neurotransmission.

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RX 781094 ANTAGONIZES THE INHIBITION BY α_2-AGONISTS OF (^{3}H)-NA AND (^{3}H)-5HT RELEASE WHILE ENHANCING ONLY THE RELEASE OF (^{3}H)-NA

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Release-modulating α_2-adrenoceptors are present on noradrenergic nerve terminals, both in the central and in the peripheral nervous system (for review, see Langer 1980). Presynaptic, inhibitory α_2-adrenoceptors are also present on the serotoninergic nerve fibres of the rat brain cortex (Göthert et al 1981). The physiological role of the α_2-autoadrenoceptor on noradrenergic nerve endings is reflected by the increase in transmitter release which is obtained with α_2-adrenoceptor antagonists, while a possible physiological role for the α_2-adrenoceptor located on 5HT nerve endings remains an open question (Göthert et al, 1981). The aim of the present experiments was to compare the effect of a new selective antagonist of α_2-adrenoceptors RX 781094 (Chapleo et al 1981)on the electrically evoked release of ^{3}H-NA or ^{3}H-5HT in the central nervous system.

Rabbit or rat hypothalamic slices were labelled with ^{3}H-(±)-NA or ^{3}H-5HT (creatinine sulphate) respectively and superfused with Krebs' solution.Two periods (S_1 and S_2) of electrical stimulation were applied with an interval of 44min and drugs were added either 40 or 20min before S_1, or 20min before S_2. The parameters of stimulation were 5Hz, 26mA, 2 msec during 2 min for ^{3}H-NA and 3Hz, 20 mA, 2 msec during 2 min for ^{3}H-5HT.

In rabbit hypothalamic slices prelabelled with ^{3}H-NA, and in the presence of 10 µM cocaine, exogenous NA (0.1µM) added before S_2, significantly inhibited the stimulation-evoked release of ^{3}H-transmitter (S_2/S_1=0.48 ± 0.07 n=4 $p<0.005$ when compared to the corresponding control S_2/S_1=1.11 ± 0.06 n=13). This effect was selectively blocked by yohimbine (0.1µM). Exposure to RX 781094 (0.1 to 30µM) 20min before S_2, increased in a concentration-dependent manner the electrically-evoked release of ^{3}H-NA. When cocaine (10µM) and RX 781094(1µM) were added to the medium 40min before S_1 and remained present throughout the experiment, the ratio S_2/S_1 was 0.93 ± 0.05 n=4. In the presence of cocaine and RX 781094 there was a significant shift to the right for the concentration-effect curve to NA. In rat hypothalamic slices prelabelled with ^{3}H-5HT, and in the absence of cocaine, exposure to NA (0.03 and 0.3 µM) significantly inhibited the electrically-evoked release of ^{3}H-5HT (S_2/S_1=0.78 ± 0.03 n=6 and S_2/S_1=0.27 ± 0.06 n=8 respectively for 0.03 and 0.3µM NA, $p<0.05$ and 0.001 respectively when compared to the corresponding control value S_2/S_1=0.92 ± 0.06 n=8). This inhibitory effect of NA was completely antagonized by 0.1µM phentolamine and by 0.1µM RX 781094. These results demonstrate the presence of presynaptic α_2-adrenoceptors on serotoninergic nerve endings of the rat hypothalamus. Nevertheless, in contrast with the results obtained with ^{3}H-NA release, exposure to RX 781094 in concentrations up to 1µM, had no significant effect by itself on the stimulation-evoked release of ^{3}H-5HT.

It is concluded that unlike the presynaptic α_2-adrenoceptors involved in the negative feed-back mechanism that modulates the release of NA, the presynaptic α_2-adrenoceptors on 5HT nerve endings in the hypothalamus do not play a physiological role in neurotransmission because selective α_2-antagonists do not increase by themselves the electrically-evoked release of ^{3}H-5HT.

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THE EFFECT OF β-ADRENOCEPTOR AGONISTS ON (^3H)-OVERFLOW FROM RAT CORTEX SLICES PREVIOUSLY INCUBATED WITH (^3H)-MONOAMINES

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The β_2-adrenoceptor agonist salbutamol exhibits antidepressant actions in man, (see Lecrubier et al, 1980) although the mechanisms involved remain unclear. Previous pharmacological and behavioural studies have indicated that salbutamol facilitates neurotransmission in serotinergic and noradrenergic systems in rat brain (Hallberg et al, 1981; Ortmann et al, 1981) possibly through an action at central β-adrenoceptors.

We have investigated the effects of some β-adrenoceptor agonists on the electrically evoked overflow of (^3H) from rat occipital cortex slices after previous incubation with (^3H)-5-Hydroxytryptamine (5-HT) or (^3H)-(-)-Noradrenaline (NA). The method has been detailed previously (Hagan & Hughes, 1981). Table 1 shows means ± s.e.mean for the ratios of resting and of evoked fractional overflows in 2 stimulation periods (S_2/S_1) obtained using some beta agonists, after the slices had been pre-incubated with (^3H)-5HT . Exposure before S_2 to salbutamol (1.0μM) or isoprenaline (0.5μM) significantly enhanced the stimulation evoked overflow of (^3H) from these slices. This enhancement appeared to be more marked with stimulation at a lower frequency and was antagonised by 0.5μM timolol. Salbutamol had no significant effect on evoked (^3H) overflow from slices pre-incubated with (^3H)-(-)-NA. None of the β-adrenoceptor agonists tested had any effect on resting (^3H) overflow for either (^3H)-monoamine.

Table 1 Means ± s.e. mean for the ratio of the fractional overflow of (^3H) (resting or evoked) in S_2 to that in S_1, from rat cortex slices previously incubated with (^3H)-5HT

Drugs (μM) Applied before S_2	n	Fractional (^3H)-Overflow S_2/S_1	
		Resting	Evoked
None (control)	25	0.79 ± 0.01	1.01 ± 0.02
Salbutamol (1.0)	11	0.79 ± 0.02	1.29 ± 0.06*
Isoprenaline (0.5)	5	0.81 ± 0.02	1.18 ± 0.06*
Terbutaline (1.0)	5	0.80 ± 0.02	1.08 ± 0.08
Rimiterol (1.0)	5	0.81 ± 0.02	1.09 ± 0.06

*Significant difference from control $P < 0.05$

This effect of salbutamol may be mediated by facilitatory β-adrenoceptors modulating 5-HT release in rat cortex, but the lack of effect of rimiterol and terbutaline would suggest that these are not typical β_2-adrenoceptors. The reported ability of salbutamol to induce subsensitivity of β-adrenoceptor coupled adenylate cyclase is consistent with a noradrenergic mechanism in the aetiology of depression. The observation that salbutamol enhances the release of 5-HT from rat cortical slices provides an alternative explanation for the antidepressant effect which is compatible with an indoleamine hypothesis of depression.

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EFFECTS OF N-DESMETHYLCLOMIPRAMINE ON THE UPTAKE AND BRAIN LEVELS OF BIOGENIC AMINES

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The tricyclic antidepressant clomipramine undergoes extensive metabolism following oral administration in man yielding a number of breakdown products the major one of which is N-desmethylclomipramine. Using animal models this metabolite has been found to possess antidepressant activity, being more potent in this respect than its parent compound (Harries & Luscombe, 1980). To investigate the possible mechanism by which N-desmethylclomipramine exhibits this activity we have carried out in vitro studies to determine whether the metabolite either interferes with monoamine uptake into cerebral nerve endings or whether it affects brain concentrations of these amines. These experiments have been carried out because the most significant neurochemical effect of the clinically useful tricyclic antidepressants is their ability to inhibit the uptake of one or more of these monoamines and this may be directly related to their ameliorative action in depressive illness.

The effects of N-desmethylclomipramine on ^{14}C-labelled biogenic amine uptake were investigated using synaptosomes prepared and purified according to the method of Gray & Whittaker (1962). Cerebral cortex was used for the study of NA, whole forebrain for 5-HT and the corpus striatum for DA. Desmethylclomipramine was found to be a particularly powerful inhibitor of NA uptake the IC_{50} value being 0.24 μM compared with a value of 6.6 μM for its parent compound. The metabolite also inhibited 5-HT uptake although being less powerful in this respect (IC_{50} 0.032 μM) than clomipramine (IC_{50} 0.005 μM). With regard to dopamine uptake although N-desmethylclomipramine (IC_{50} 5.2 μM) was of a similar potency to clomipramine (IC_{50} 6.8 μM) it was considerably less effective than the potent dopamine uptake inhibitor nomifensine (IC_{50} 0.16 μM). Comparative IC_{50} values for imipramine and desipramine were 4.6 μM, 8.0 μM (NA); 0.019 μM, 0.35 μM (5-HT); 23.0 μM, 17.5 μM (DA), respectively.

To investigate the effects of acute dosing on brain levels of biogenic amines drugs (5,10 and 15 mg kg^{-1}) were injected (i.p.) into groups of 5 male rats. One hour later NA, 5-HT, DA and the associated metabolites 5-HIAA, DOPAC and HVA were extracted from brain homogenates by the method of Earley & Leonard (1978). Both N-desmethylclomipramine and its parent compound induced significant ($p<0.05$, Student's 't' test) elevations of 5-HT levels which were dose related. At 15 mg kg^{-1} increases of 222% and 209%, respectively, compared to controls were observed. In contrast, neither drug caused significant changes in brain levels of other amines. Imipramine and desipramine likewise raised brain 5-HT levels whilst failing to influence other brain amines or associated metabolite concentrations.

The present study has shown that not only is N-desmethylclomipramine a potent inhibitor of NA uptake, but it is also able to elevate brain 5-HT levels following acute treatment. While it seems that the former effect may be associated with the long term adaptive changes believed to be linked with antidepressant activity the significance of raised 5-HT levels remains to be defined.

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FURTHER ANALYSIS OF THE IN VIVO BINDING TECHNIQUE USING BRAIN DIALYSIS

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A technique of brain dialysis has recently been described (Ungerstedt et al,1981) where it has been possible to recover endogenously released dopamine and amino acids in awake and anaesthetized rats. This technique may also be a useful method for studying binding as described previously by Forster &Ungerstedt(1982)

The surgical procedure was identical to that described earlier (Forster &Ungerstedt,1982). After the viability of the dialysis tube had been assessed, ^{3}H-haloperidol ($14.2 Ci.mMol^{-1}$)was perfused at a speed of $2\mu l.min^{-1}$, either alone or in combination with cold drug. The following experiments were performed. Firstly, ^{3}H-haloperidol was perfused for 1h in order to establish steady-state conditions, after which time varying concentrations of cold drug was added to the perfusing medium (the amount of radioactivity remained constant). Secondly, after labeling for 1h the perfusing medium was replaced by Ringer solution and perfusion continued for 30min before changing the medium to varying concentrations of cold drug. Finally, a combination of ^{3}H-haloperidol and cold drug was used to perfuse from the onset of the experiment. The results were expressed as % cpm of a similar volume of standard (i.e. 5µl of perfusion medium taken prior to perfusion). Samples were taken at 5 or 10min intervals and where appropriate total brain cpm was assessed at the termination of the experiment.

There was a significant, dose-related increase in the amount of radioactivity appearing in the perfusate when cold drug was perfused together with ^{3}H-haloperidol 1h after the initial labeling period. The highest concentration of cold haloperidol (1mM) produced a 25% increase in the amount of radioactivity in the perfusate. In the second series of experiments, cold haloperidol similarly caused a concentration-related increase in perfusate radioactivity. Likewise, cold dopamine possessed the same characteristics but only at high concentrations was a significant ($P<0.05$) increase in radioactivity demonstrated. Finally, as the concentration of ^{3}H-haloperidol was decreased in the presence of a constant concentration of cold haloperidol the time-association curve was displaced to the right in a concentration-dependent manner. Thus implying that a longer period was necessary for steady-state conditions to be achieved with decreasing amounts of ^{3}H-haloperidol.

These results further support that the technique of brain dialysis may be a useful method for studying <u>in vivo</u> binding characteristics and experiments are progressing in these laboratories to pursue this hypothesis.

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Ro 15-1788 BLOCKS THE POTENTIATION OF INHIBITION PRODUCED BY BENZODIAZEPINES

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The central effects of benzodiazepines are mediated through the γ-aminobutyric acid (GABA) receptor. One of the difficulties in determining any physiological role for the benzodiazepine receptor has been the lack of a specific benzodiazepine antagonist. A recently synthesised imidazodiazepine, Ro 15-1788, appears to block the specific binding of benzodiazepine to its receptor (Hunkeler et al, 1981). The present study aimed to test this compound as a specific antagonist of the benzodiazepine receptor by application to an in vitro preparation containing an extensively studied GABA inhibitory system (Scholfield, 1982).

Slices of guinea-pig olfactory cortex were placed in a recording bath perfused with Krebs solution. The membrane conductance of these cells was monitored using intracellular recording micro-electrodes (Scholfield, 1982). Bath application of the GABA analogue, muscimol, increased the membrane conductance. The effect of muscimol was roughly doubled by diazepam (1 μmol/l) while Ro 15-1788 (1 μmol/l) prevented this potentiation. Ro 15-1788 had no effect on muscimol applied alone nor on the passive membrane properties and the action potential in concentrations up to 10 μmol/l.

Further studies were made on the summed potentials recorded with gross extracellular electrodes on the pial surface. Stimulating the lateral olfactory tract, generates a monosynaptic surface EPSP. A polysynaptic EPSP follows generated from the soma of these neurones deep within the slice. This deep EPSP is partly shunted out by the synaptic inhibitory conductance whereas the surface EPSP is generated out on the dendrites and is less affected by these events. The amplitude of the deep EPSP was used to monitor the benzodiazepine-induced potentiation of inhibition. Two stimuli 200 ms apart were applied, the first generated an inhibitory conductance and the second timed to fall within its time-course. The amplitude of the second deep EPSP was measured in the presence of various concentrations of diazepam. Diazepam dose-depression curves were plotted for Ro 15-1788 concentrations.

Ro 15-1788 produced parallel shifts in the dose-response curves to the right by three-fold at 0.01 μmol/l and by 300 fold at 10 μmol/l. Ro 15-1788 also blocked flurazepam, chlordiazepoxide and clonazepam. At 10 μmol/l Ro 15-1788 there was sometimes a small depression of the second deep EPSP whereas the other potentials were unaffected, up to 100 μmol/l. Ether, halothane, pentobarbitone and ketamine also potentiate inhibition (Scholfield, 1980) but were unaffected by Ro 15-1788 up to 100 μmol/l.

These results show that Ro 15-1788 appears to be a reasonably specific and potent antagonist of this central action of benziodiazepines.

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EFFECTOR MECHANISMS INVOLVED IN HYPOTHERMIA FOLLOWING i.c.v. HISTAMINE ADMINISTRATION TO THE CONSCIOUS RESTRAINED RAT

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Stimulation of H1 receptors, located in the rostral hypothalamic thermoregulatory centre, is believed to lower the thermoregulatory set point leading to increased heat loss and/or reduced heat gain, whilst stimulation of H2 receptors, located close to the third ventricle, is believed to activate the efferent heat loss pathway directly (Lomax and Green, 1979). It may be expected that lateral ventricular histamine administration would stimulate both sets of receptors resulting in hypothermia. Whilst the selective H2 agonist 4-methylhistamine has been shown to cause hypothermia associated with an increased tail temperature (Cox et al, 1976), no such effect has been reported for histamine and the effector mechanisms involved in histamine induced hypothermia are unknown. The following study examined the effect of i.c.v. histamine administration on rectal temperature and two effector systems, tail heat loss and electromyographic activity.

Male Sprague-Dawley rats (260-280g) with chronic indwelling right lateral ventricular guide cannulae were placed in open restraint boxes and EMG activity, rectal temperature and the temperature at the base of the tail recorded. After 1 hour rats were injected i.c.v. with 10µl saline (pH corrected) or 10µl histamine acid phosphate. Three readings prior to treatment were averaged and used as control values. Readings obtained after treatment were expressed as change from control values and measured in °C for temperature changes and percent of control values for EMG changes. The table below shows the results expressed as areas under the curve.

Table 1 The effect of intracerebroventricular histamine administration on the rectal temperature (ΔTR), tail temperature, (ΔTT) and electromyographic activity (ΔEMG), of conscious rats restrained at 16±2°C

Dose (nmole)	0	11.25	45	180
ΔTR	5.5±10.8	2.8±6.5	-37.3±12.3*	-63±15.5*
ΔTT	-0.1±8.2	-4.9±4.4	-9.7±4.1*	-0.7±6.1*
ΔEMG	908±241	169±1414	-1581±263*	-1813±613*
n =	6	6	6	6

Values are $\bar{x}$±s.e.m. *=P<0.05 (Mann Whitney U test)
ΔTR is the rectal thermal response index from 0-72 mins in °C min
ΔTT is the tail thermal response index from 0-48 min in °C min
ΔEMG is the EMG activity response index from 0-48 mins in %min

These results show that in the conscious restrained rat i.c.v. administration of 45 and 180 nmole histamine causes hypothermia associated with reduced muscle activity. Both high frequency, low amplitude EMG activity and voluntary movement seemed to be suppressed, the rats appearing sedated. No evidence was obtained to suggest that the hypothermia was associated with increased tail heat loss. It remains uncertain whether these results represent a specific thermoregulatory effect or are associated with sedation.

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EFFECT OF 3-ISOBUTYL-1-METHYLXANTHINE ON HISTAMINE RECEPTOR MEDIATED cAMP ACCUMULATION IN GUINEA-PIG HIPPOCAMPAL SLICES

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Histamine stimulates the accumulation of cAMP in slices of guinea-pig hippocampus and cortex (Palacios et al, 1978; Smellie et al, 1979). Palacios et al (1978) have demonstrated clearly that the stimulation in hippocampus is mediated by both H_1- and H_2-receptors. H_2-receptor mediated accumulation can be potentiated by H_1-receptor stimulation but H_1-receptor activation alone gives no accumulation. Thus H_2-receptor antagonists can abolish completely the response to histamine, but H_1-receptor antagonists block only the H_1-receptor mediated component of the accumulation. cAMP is catabolized in tissues by hydrolysis, catalysed by one or more of the phosphodiesterase class of enzymes (Wells and Kramer, 1981). We report here the effect of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) on histamine-stimulated cAMP accumulation in hippocampal slices. The experimental procedure of Palacios et al (1978) was used. Incubations, in triplicate or quadruplicate, were usually for 15 minutes.

Histamine stimulated the accumulation of cAMP as reported by Palacios et al (1978) but IBMX (100 µM) did not significantly ($p > 0.1$) potentiate the maximal response (Table 1), although the response to a lower (3×10^{-6}M) agonist concentration was potentiated (approx. 75%, $p < 0.01$). In some experiments, using a shorter incubation time, IBMX reduced the response to 10^{-3}M histamine. Dimaprit, a specific H_2-receptor agonist (Parsons et al, 1977) also stimulated the accumulation of cAMP, as reported by Palacios et al (1978); IBMX (100 µM) potentiated the accumulation, resulting in a significant ($p < 0.01$) increase in maximal activation (Table 1). The potentiation by IBMX of H_2-receptor mediated stimulation was confirmed by experiments in which the H_1-receptor antagonist mepyramine (1 µM) was included in incubations containing histamine as agonist; the residual H_2-receptor mediated accumulation was potentiated significantly ($p < 0.01$) by IBMX (Table 1).

Table 1 Effect of IBMX on cAMP accumulation in guinea-pig hippocampal slices

	cAMP (pmoles per 0.125 mg wet weight of tissue)				
Agonist	Basal	10^{-5}M	3×10^{-5}M	10^{-4}M	10^{-3}M
Histamine (HA)	0.20 ± 0.03		3.97 ± 0.46		4.57± 0.49
" + IBMX	0.52 ± 0.04		4.50 ± 0.14		5.18± 0.32
HA + mepyramine	0.12 ± 0.03	0.85 ± 0.09		1.05 ± 0.14	1.07± 0.07
" " + IBMX	0.15 ± 0.03	1.44 ± 0.17		1.91 ± 0.07	2.02± 0.13
Dimaprit	0.0	0.22 ± 0.04		0.95 ± 0.20	0.64± 0.05
" + IBMX	0.16 ± 0.09	0.83 ± 0.04		1.65 ± 0.10	1.60± 0.14

It was also shown that IBMX alone caused little or no increase in cAMP levels in the absence of agonists, that IBMX added just prior to terminating incubations containing stimulated slices did not elicit a potentiation, and that exogenous cAMP added just prior to termination of the incubations was recovered, either in the absence or presence of IBMX. It is concluded that IBMX prevents at least partially the catabolism by phosphodiesterase during the incubation of cAMP accumulated intracellularly in response to H_2-receptor agonists. The failure of IBMX to potentiate the response to histamine may be due to a secondary action, e.g. a concomitant inhibition of the H_1- response.

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EFFECT OF DOPAMINE AND M7 ON THE ELECTRICALLY-EVOKED RELEASE OF (^{3}H)-NORADRENALINE FROM THE RABBIT HYPOTHALAMUS

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Inhibitory presynaptic α_2-adrenoceptors are present on noradrenergic nerve endings in the rabbit hypothalamus (Galzin et al 1982). Dopamine (DA) receptor agonists were also shown to inhibit the stimulation-evoked release of ^{3}H-noradrenaline(NA), nevertheless, a direct inhibitory effect of DA itself could not be demonstrated under these experimental conditions (Galzin et al., 1982). The aim of the present experiments was to reexamine this question by comparing the effects of DA on ^{3}H-NA overflow under various experimental conditions, with those of M7, which possesses both α_2- and DA-receptor agonist properties (Shepperson and Langer, 1981; Costall et al, 1981).

Rabbit hypothalamic slices were labelled with ^{3}H-(±)-NA and superfused with Krebs' solution as described by Galzin et al (1982). Two periods (S_1 and S_2) of electrical stimulation (5Hz, 26mA, 2msec, 2min) were applied with an interval of 44min and drugs were added either 40min before S_1 or 8 and 20min before S_2.

When hypothalamic slices were superfused in 0.65mM Ca^{++}-Krebs medium, DA (0.1-1µM) when added 8min before S_2, decreased in a concentration-dependent manner the stimulation-evoked release of ^{3}H-NA, without affecting the spontaneous outflow of radioactivity. Under the same experimental conditions the DA-receptor antagonist S-sulpiride (1µM) antagonized the inhibitory action of DA (S_2/S_1=0.94 ± 0.04 n=4 $p<0.005$ when compared with the ratio for DA 0.3µM alone S_2/S_1=0.39 ± 0.12 n=4). When the α_2-adrenoceptor antagonist RX 781094(1µM) was added 40min before S_1 in the presence of cocaine(10µM), the ratio S_2/S_1 was 0.96 ± 0.03, n=5. Under these experimental conditions, the inhibition of ^{3}H-NA overflow induced by DA was not affected by the α_2-antagonist. Consequently DA inhibited the release of ^{3}H-NA by activating DA$_2$ receptors, and had no effect on the presynatic α_2-adrenoceptor. When DA was added 20min instead of 8min before S_2, or when the Ca^{++} concentration was raised to 1.3mM, exposure to DA failed to inhibit the stimulation-evoked release of ^{3}H-NA. It appears that a rapid desensitization of the presynaptic DA receptor occurs on noradrenergic nerve endings, similar to that reported for DA on the inhibitory DA autoreceptor modulating DA release in the rabbit striatum (Arbilla et al., 1982). When M7 (2 dimethylamino 5-6 dihydroxytetralin) was added to the superfusion medium 20min before S_2, it decreased in a concentration-dependent manner the stimulation-evoked release of ^{3}H-NA. Exposure to S-sulpiride(1µM) antagonized only partially the inhibition of ^{3}H-NA overflow induced by M7. When the α_2-antagonist yohimbine (0.1µM) was added to the medium, it produced only partial antagonism of the inhibitory effect of M7. When the two antagonists S-sulpiride and yohimbine were added simultaneously, the concentration-effect curve for M7 was then clearly shifted to the right.

These results confirm that noradrenergic nerve endings in the rabbit hypothalamus are endowed with both presynaptic inhibitory α_2 and DA$_2$ receptors, and that drugs like M7 inhibit noradrenergic neurotransmission by acting on both α_2- and DA$_2$ receptors in noradrenergic neurons.

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DOPAMINE RECEPTOR BLOCKADE AFTER DISCONTINUATION OF SIX MONTHS CONTINUOUS TREATMENT WITH I.M. FLUPHENAZINE DECANOATE

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Wistedt et al (1981) found fluphenazine and elevated prolactin in the plasma of schizophrenic patients for several months after ceasing chronic treatment with i.m. fluphenazine decanoate (FPZ-d). In animals given FPZ-d for 6 months we noted behavioural evidence for dopamine (DA) receptor blockade over several months after terminating treatment (Waddington & Gamble, 1981) which we characterise here.

Male Sprague-Dawley rats (500-600 g) were given i.m. injections into alternate rear leg muscles at 2-3 week intervals (0.2ml of 25 mg/ml FPZ-d or oil vehicle alone); at various times after the last depot injection completing 6 months of treatment they were challenged with the DA agonist apomorphine (APOM) and resultant stereotyped behaviour assessed (Waddington & Gamble, 1980). With 0.15 mg/kg s.c. APOM, stereotypy remained markedly antagonised ($p < 0.001$) for 17 weeks after treatment cessation. At 5 months after discontinuation, responsivity at 15 min after APOM was less prominently attenuated but blocked at 25-40 min ($p < 0.001$). At 6 months after discontinuation, responsivity at 15 min after APOM was weakly influenced while still significantly antagonised ($p < 0.05$) at 25-40 min. With 1.0 mg/kg APOM, at 2 weeks after discontinuation a characteristic syndrome of perimeter hyperlocomotion and corner sniffing and rearing was seen in 100% of FPZ-d animals (0% in controls, N=8-10) with occasional episodes of licking; at 8 weeks after cessation, the syndrome was again seen in 100% of FPZ-d animals, with 90% now also showing licking (0% in controls, $p < 0.01$). At 5 months after cessation, only 40% of FPZ-d animals showed the syndrome (0% in controls, NS).

Responsivity to low doses of APOM remained antagonised over 5-6 months following discontinuation of 6 months treatment with FPZ-d. With a high dose of APOM a hyperlocomotion syndrome was enhanced in a characteristic way, as noted with 6 months treatment with oral FPZ (Waddington et al, 1982); this, together with potentiation of licking, endured for at least 2 months following FPZ-d discontinuation. Thus the functional state of DA receptor blockade may be interpreted differently depending on the dose of APOM used. We suggest that responses to the lower dose are the most relevant clinically (Wistedt et al,1981), with the DA receptor blocking action of FPZ-d being exerted for several months beyond cessation of chronic treatment. Whatever the basis of this effect may be, the concordance between clinical and animal data suggests that modification of conventional FPZ-d treatment regimens might be appropriate in some schizophrenic patients (Waddington, 1982).

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DOPAMINE RECEPTOR SUPERSENSITIVITY PRODUCED BY REPEATED NEUROLEPTIC ADMINISTRATION CORRELATES WITH INCREASED D-2 RECEPTORS

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Repeated neuroleptic administration for a few weeks, followed by some days drug withdrawal, enhances apomorphine-induced stereotyped behaviour, increases striatal ^{3}H-spiperone binding (D-2 receptors) but causes inconsistent changes in dopamine stimulated adenylate cyclase (D-1 receptors) (Muller & Seeman 1978). We compare the effects on cerebral dopamine receptors of repeated administration of three neuroleptics with differing D-1 and D-2 receptor selectivity, namely sulpiride (D-2), haloperidol (D-2$\gg$D-1) and cis-flupenthixol (D-2$\equiv$D-1).

In two separate studies male Wistar rats (150±10 g at the start of the experiment) were treated for 21 days with saline (0.5 ml ip), haloperidol (5 mg/kg ip), cis-flupenthixol (2.5 mg/kg ip) or sulpiride (2 x 100 mg/kg ip), followed by a 3 or 4 day drug withdrawal period. Apomorphine (0.0625-1.0 mg/kg sc 15 min previously)-induced stereotyped behaviour was enhanced to an equivalent degree by all three neuroleptic treatments (Table 1). The number of striatal and mesolimbic ^{3}H-spiperone (0.05-1.0 nM) binding sites (D-2) (Bmax) (as defined using 3×10^{-5} M sulpiride) was increased by all neuroleptic treatments to tissue from saline treated control animals (Table 1). The dissociation constant (K_D) was unchanged. The number of striatal and mesolimbic ^{3}H-piflutixol (0.08-1.8 nM) binding sites (D-1) (as defined using 10^{-6} M cis-flupenthixol in the presence of 3×10^{-5} M sulpiride) was not altered by pretreatment with haloperidol or sulpiride (Table 1). cis-Flupenthixol administration increased striatal, but not mesolimbic, ^{3}H-piflutixol binding when compared to tissue from saline treated control animals. The dissociation constant (K_D) for ^{3}H-piflutixol binding was not altered by neuroleptic treatment. Dopamine (1-1000 uM)-stimulated adenylate cyclase activity in striatal and mesolimbic tissue preparation was not increased by neuroleptic treatment, except by cis-flupenthixol which increased striatal cyclic AMP formation (Table 1).

Table 1 Alterations in apomorphine (0.25 mg/kg sc)-induced stereotypy and striatal (ST) and mesolimbic (ML) ^{3}H-spiperone and ^{3}H-piflutixol binding and dopamine (50 uM)-stimulated adenylate cyclase (AC) activity caused by repeated neuroleptic administration

Drug treatment	Stereotypy score	Bmax (pmoles/g tissue) ^{3}H-spiperone ST	^{3}H-spiperone ML	^{3}H-piflutixol ST	^{3}H-piflutixol ML	AC (pmoles/2.5 min/2 mg tissue) ST	AC ML
Saline	1.4±0.2	17.8±0.2	3.4±0.3	70±5	35±3	32.4±5.3	25.4±4.5
Haloperidol	3.0±0.2*	29.0±2.3*	5.8±0.3*	83±4	42±3	36.5±5.5	28.2±7.8
cis-Flupenthixol	3.1±0.2*	27.8±1.2*	5.5±0.4*	86±5*	41±6	52.7±7.5*	27.7±3.7
Sulpiride	3.1±0.2*	25.5±1.5*	5.1±0.4*	77±5	39±2	32.6±8.3	28.4±4.2

* $p < 0.05$ compared to saline treated animals

Repeated neuroleptic treatment of rats enhanced apomorphine-induced stereotyped behaviour which correlated with increased D-2 binding sites in striatum and mesolimbic tissue. No correlation with change in D-1 binding sites or dopamine-stimulated adenylate cyclase was apparent.

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LESIONS OF THE TECTO-SPINAL PATHWAY AND DOPAMINE DEPENDENT BEHAVIOURS

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It has previously been demonstrated that large lesions of the superior colliculus cause decreased apomorphine-induced stereotypy and increased amphetamine-induced locomotor behaviour in the rat (Redgrave, Dean, Donohoe & Pope, 1980). Associated with these changes we have observed an increased dopamine turnover in the nucleus accumbens but not in the corpus striatum (Dawbarn & Pycock, 1982). In this study we have extended this investigation by lesioning the tectospinal outflow at the level of the dorsal tegmental decussation (DTD). The results suggest that this system is necessary for the expression of dopamine-mediated stereotypy and that an inhibitory pathway runs from the superior colliculus through, or near to, the DTD to the ventral tegmental area (VTA).

Female Porton rats ($\sim$200g) received either large bilateral electrolytic lesions of the superior colliculus (Dawbarn & Pycock, 1982) or small midline electrolytic lesions of the DTD (co-ordinates from the atlas of König & Klippel. A : 1.4, L : 0, V : -1.3). An equal number of sham-operated animals were prepared which acted as controls in both behavioural and biochemical experiments. All tests were performed ten days post-operatively. Animals with DTD lesions were tested for apormorphine stereotypy (0.5 - 5 mg/kg, s.c.) and amphetamine hyperactivity (1-5 mg/kg, i.p.) as previously described (Dawbarn & Pycock, 1982). A second group of animals with DTD lesions were killed and the striata and nucleus accumbens were dissected out. The tissue was homogenized and the dopamine metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were isolated on Sephadex G10 columns and assayed fluorimetrically (Westerink & Korf, 1976). Dopamine turnover was estimated after synthesis inhibition with α-methylparatyrosine (250 mg/kg, 2 hr) in striatum and nucleus accumbens tissue from another group of animals with DTD lesions. Dopamine was assayed using electrochemical detection after separation with high pressure liquid chromatography (Mefford, 1981). A further group of DTD lesioned animals together with a group of collicular lesioned animals were killed and the ventral tegmental area was dissected out and assayed for glutamic acid decarboxylase (GAD) activity.

Animals with lesions of the DTD showed attenuated stereotypy responses to apomorphine and enhanced locomotor responses to amphetamine. In biochemical assays both HVA and DOPAC were significantly elevated (by 95% and 65% respectively) in nucleus accumbens. Dopamine turnover was found to be significantly increased in nucleus accumbens (from 0.13 to 0.29 ng/mg/hr) yet decreased in striatum (from 0.21 to 0.13 ng/mg/hr) of DTD lesioned animals. Furthermore GAD activity was significantly decreased in the VTA taken from animals with either superior colliculus or DTD lesions.

The results demonstrate that an apparently intact tectospinal pathway is necessary for the expression of apormorphine-induced stereotypy in the rat. It is thought that an afferent pathway from the superior colliculus passes through, or close to, the DTD to the VTA (Phillipson, 1979). Lesions of either the superior colliculus or DTD cause a fall in GAD activity in the VTA (by 34% and 40% respectively), suggesting this pathway to be Gabaergic in nature, and presumably relieve inhibition on mesolimbic dopamine neurones, resulting in enhanced amphetamine hyperactivity and increased dopamine turnover in the nucleus accumbens.

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THE EFFECT OF NICOTINE ON THE DEVELOPMENT OF CORTICAL SLOW POTENTIALS ASSOCIATED WITH DISCRIMINATION IN THE RAT

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Negative slow potentials (SPs), similar to the human Contingent Negative Variation (Walter et al, 1964), can be recorded from the frontal cortex of rats during the interval between an auditory stimulus (S_1) and delivery of a food reinforcement (S_2) (Pirch, 1977). Pirch (1977a) also demonstrated that rats could be taught to generate differential SPs to two auditory cues, one which signalled food reinforcement and the other which signalled no reinforcement. The magnitude of the SPs to the rewarded tone was significantly greater than the magnitude of the SPs to the unrewarded tone. The present study investigated the effect on the SP responses of rats treated with nicotine prior to training them in a discrimination paradigm.

(Rats (n=4) were implanted with non-polarizable electrodes as described previously (Ebenezer, 1982). EEGs were recorded on a.c. amplifiers (time constant 15s).

The rats were given three habituation sessions to tone S_a (1400Hz, 100msec) and tone S_b (400Hz, 100msec). Over the next 15 training sessions S_a was followed 2s later with a food reinforcement while S_b was not reinforced. The rats were injected with nicotine (0.4mg/Kg, s.c.) 10 min prior to each training session. S_a and S_b were presented in pseudo-random order. Inter-trial intervals varied between 30 and 90s. Thirty trials were given to a rat in a single session (15 trials with each tone) and only one session was conducted per day. Artefact-free trials were averaged off-line on a PDP8 computer and the area of the SPs to each tone calculated.

SPs developed to both tones. The SP responses to the two tones showed no significant difference for the first 3 training sessions. On the subsequent 12 sessions the SP area to the rewarded tone was significantly greater than the SP area to the unrewarded one. It was also found that while the SP area to S_a increased with successive sessions, the SP area to S_b tended to decrease. When saline was administered to these rats instead of nicotine there was no significant differentiation in the SP areas to the two tones. Further, the mean SP area to S_a after saline was significantly reduced (reduced by 30%, $p < 0.05$, 3d.f. 2T) compared with the value obtained with nicotine on the previous day, while the mean SP area to S_b after saline was slightly (but not significantly) increased compared with the area obtained with nicotine.

The results from this study indicate that rats can learn to form differential SPs to two tones under nicotine, but that this "learned" response may be disrupted in the absence of nicotine (i.e. if nicotine is replaced with saline). It is suggested that the rats trained under nicotine in this study maintained an optimal level of attention which enabled them to adequately discriminate between the two tones. This was reflected in their ability to generate differential SPs to S_a and S_b. However, when nicotine was withdrawn there was a decrease in the level of attention of these rats which resulted in an inability of the rats to significantly discriminate between the two tones, with consequent undifferentiated SPs to S_a and S_b.

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STRUCTURE-ACTIVITY RELATIONS AMONG STEREOTYPY-INDUCING DOPAMINE AGONISTS

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Ernst (1965) first established the involvement of dopamine (DA) receptors in some behavioural effects of apomorphine, and some years later a DA sequence was recognized in the structure of apomorphine and in several ergolines such as bromocriptine, lergotrile, lisuride and pergolide. The existence of four DA receptors, the corresponding stereo-selective structures of this biogenic amine and the conformation of DA at the dopamine receptor have been extensively discussed by Komiskey et al (1978) and Seeman (1980).

Two types of DA agonists may be recognized (i) directly acting DA, epinine, ADTN, apomorphine, bromocriptine, lysergide (and other ergolines) and piribedil (ii) the indirectly acting agonists such as p-tyramine, amphetamine, methamphetamine, phemetrazine and phentermine, but also some atypical compounds like amantadine and nomifensine.

However, the active sequence common to all these structures has not yet been completely elucidated; we now report a structure-activity investigation.

The selected animal was the rabbit, for the reasons already outlined by Mignot & Savini (1980). The stereotypies (foot-shocks) and sudden turnings were counted after giving the drug by I.V. route. The results are shown in Table I.

Table I Stereotypy and turning behaviour in the rabbit

DRUGS	N	DOSE	STEREOTYPIES (foot-shocks) with ± SEM	TURNINGS (rotations) with ± SEM
apomorphine	23	5 µM/kg	87.7 ± 12.1	11.1 ± 4.4
bromocriptine	10	20 µM/kg	60.0 ± 10.4	11.8 ± 2.5
methoxamine	12	20 µM/kg	95.7 ± 13.8	0.9 ± 0.4
amphetamine	12	50 µM/kg	17.0 ± 5.7	78.9 ± 16.3
metamphetamine	23	50 µM/kg	31.7 ± 7.9	130.5 ± 18.9
ethylamphetamine	10	25 µM/kg	68.7 ± 17.1	60.2 ± 9.0
ephedrine	10	150 µM/kg	12.6 ± 3.6	0.8 ± 0.3
phenylethylamine	10	150 µM/kg	24.1 ± 4.1	11.4 ± 2.8

(a foot-shock means the rabbit thumps the cage floor with its hind limbs)

Benzamphetamine at 25 µMol/kg produced tonico-clonic convulsions. The following drugs were inactive : dopamine, noradrenaline, adrenaline, norfenefrine, phenylephrine, p-tyramine, synephrine, hordenine, metaraminol, α-methylnoradrenaline, isoxsuprine, ritodrine, phenylpropanolamine, phenylethanolamine, pseudoephedrine.

Some general conclusions have been drawn : (i) the active sequence is phenylisopropylamine (amphetamine) (ii) this sequence is included in the chemical structure of apomorphine (iii) α-CH3 group is essential for bioavailability (iv) one-OH on the phenyl ring inhibits penetration into the CNS (v) blocking of -OH groups by methyl groups increases the activity (methoxamine) (vi) catecholamines and analogues do not cross the blood-brain barrier (vii) the β-OH groups seem not to play an important role.

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EVIDENCE AGAINST ENDOGENOUS OPIOID INVOLVEMENT IN DEFENSIVE BEHAVIOURS IN RATS

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It has recently been proposed that endogenous opioid mechanisms may function to prevent the disruption of innate defense responses by noxious stimuli (Bolles & Fanselow, 1980). In support, it has been found that naloxone enhances both shock-induced defensive freezing (Fanselow & Bolles, 1979) and defensive fighting (Fanselow et al, 1980; Gorelick et al, 1981), and that such effects may be attributable to increased perception of the shock. However, we have previously reported that fighting in response to footshock actually prevents the analgesic reaction observed in individually-shocked animals and that naloxone is without effect on this phenomenon (Rodgers & Deacon, 1981). The current study was conducted in view of this apparent anomaly, the absence of dose-response data on naloxone's effect on defensive fighting and the exclusive use of one opiate antagonist in previous reports.

96 adult male hooded Lister rats were weight-matched and pairs randomly allocated to treatment conditions:- saline; 0.1, 1.0 or 10.0mg/kg of either naloxone hydrochloride (Endo Labs. Inc) or diprenorphine hydrochloride (Reckitt & Colman). Twelve pairs were tested in the saline condition and six in each drug condition. All injections were performed i.p. in a volume of 1ml/kg, ten minutes before testing. Pairs were subjected to 60 footshocks (1mA intensity; 0.5sec. duration; 6/min.) and responses recorded as fight, threat or escape/avoidance. Statistical evaluation (ANOVA; Dunnett's test) revealed a biphasic influence of naloxone on fighting, with low dose facilitation ($p<0.025$) and high dose inhibition ($p<0.05$); a dose-dependent inhibition of threat postures by naloxone ($p<0.01$); a dose-dependent enhancement of escape/avoidance by naloxone ($p<0.025$). The only significant effect of diprenorphine was an inhibition of threat postures, observed at 1mg/kg ($p<0.025$.

In a control study, the influence of both antagonists on electric shock thresholds was determined. 48 naive animals were randomly allocated to treatment conditions (as above). Following injection, individual animals were placed in the test chamber where they received 6 series of shocks (0.5sec. duration), delivered at 15 sec. intervals to the grid floor. Series were administered in alternating ascending and descending order with intensities ranging between 0.1 and 1mA in ten steps. The intensity at which animals emitted the jump response was noted for each series and an overall mean calculated to give an index of electric shock threshold for each subject. ANOVA failed to reveal a significant drug effect on this measure of pain reactivity ($F(6,41)=0.74$,NS).

These studies suggest that naloxone's effect on defensive fighting cannot readily be attributed to alterations in shock threshold and, furthermore, cast doubt upon the involvement of endogenous opioid mechanisms in defensive behaviour in rats.

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NON-PEPTIDE MORPHINE-LIKE COMPOUNDS IN VERTEBRATE BRAIN TISSUE AND FOOD

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Evidence has accumulated over the past six years of the presence of non-peptide morphine-like compounds (MLC) in the cerebrospinal fluid (Shorr et al. 1978), brain tissue (Gintzler et al. 1976, Killian et al. 1981, & Blume et al., 1977) and milk from human and other vertebrate species. The compound isolated from milk was demonstrated by a variety of physical, chemical, immunological and pharmacological tests to be identical to morphine (Hazum et al. 1981). The dietary origin of the morphine was also postulated (Hazum et al. 1981). It was therefore speculated that since morphine is retained within lactating animals and excreted in milk, and that specific receptors for morphine are present within the ileum (μ receptors?), morphine is an essential dietary factor with unknown physiological roles. We report here the isolation of MLC's from the brain of sheep, rats and rabbits using four methods:

i) By exhaustive methanolic extraction of lyophilised brain homogenates using a Soxhlet apparatus.
ii) Dialysis of brain homogenates against dilute aqueous ammonia (1% by volume)
iii) Using specific morphine antibodies coupled to magnetizable solid phase cellulose particles (MSP) and elution by methanol of the MSP phase after incubation with brain homogenates.
iv) Ultrafiltration of brain homogenate tissue in buffer.

Methods i) - iii) yielded, after concentration and/or reconstitution into a phosphate-bovine serum albumin medium (0.02M, pH 7.4), extracts which showed immunological activity to morphine specific antibodies in the presence of tritiated dihydromorphine label. Analysis of neat ultrafiltrate from iv) failed to yield detectable MLC. In addition, the residues after evaporation of the methanolic extracts from i) were subjected to exhaustive extraction with ethanol/chloroform (3/1 by volume) and sodium carbonate solution (10mM, pH 9.0). The organic phase after evaporation was demonstrated to contain morphine-like activity (Ω 3ng/g of sheep brain tissue) and morphine-like activity was also observed in the aqueous extract (Ω 40ng/g). Dilution of the MLC's from organic and aqueous extracts from sheep and rabbit brains followed by radioimmunoassay using morphine standards gave displacement curves which were superimposable with diluted morphine standards when normalized over the same concentration range, and over the linear part of the curve, identical linear regression equations. The assay showed no appreciable cross-reactivity to N-dealkyl codeine (<1%) and leucine- and methionine-enkephalins (<0.3%). The MLC obtained by method i) and after pH 9 extraction into organic phase was chromatographed on thin layer plates (silica gel G, 0.1mm thickness) using benzene: dioxan: 880 ammonia: ethanol (50 : 40 : 55) and the band having an R_f value identical to morphine (R_f = 0.11) was removed together with other bands. All the detectable immunological activity found in extracts from these bands was associated with the band at R_f = 0.11. Food pellets, fed to the rats and rabbits whose brains were examined in the above studies, were powdered and extracted as in method i). The resulting extracts also were observed to possess morphine-like activity (5 - 15ng/g).

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(^{3}H)-SULPIRIDE BINDING TO GUINEA-PIG RENAL CORTEX MEMBRANES

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In the guinea-pig kidney dopamine is a potent vasodilator of renal blood flow in vivo and in vitro it stimulates a specific adenylate cyclase (Marcou et al., 1982). The benzamide sulpiride is a potent antagonist of the vasodilator response, yet it is totally without effect in inhibiting the ability of dopamine to stimulate the adenylate cyclase. This dichotomy of action is typical of the effects of sulpiride in central nervous tissue. In rat striatal preparations specific sulpiride binding sites have been identified and are well characterised (Woodruff & Freedman, 1981). This report is concerned with an examination of renal binding sites for the benzamide.

Crude membrane preparations were made by the method of Rosenblatt et al. (1980). Aliquots (approximately 0.5 mg protein) were assayed for binding by incubation with 20 nM (^{3}H)-sulpiride (S.A. 26.2 Ci per mmol) at 25 °C for 10 min. Specific binding was assessed by the addition of 1 µM S-(-)-sulpiride to half of the tubes, and free and bound ligand separated by rapid centrifugation. The bound fraction was dissolved in protosolve (1 ml) and assayed by liquid scintillation spectrometry, corrections for background, quench and efficiency being made. The buffer was tris-Krebs, pH 7.3. Under these conditions specific binding was about 31% of the total. Dopamine content was measured by spectrofluorimetry (Earley & Leonard, 1978) and renal vasodilation and adenylate cyclase as described by Marcou et al. (1982).

Specific (^{3}H)-sulpiride binding to renal cortex membranes was dependent on time, temperature and protein concentration (linear 200-700 µg). The binding was saturable (range 0-100 nM) and Scatchard analysis of the data revealed a single binding site with a Kd 20 nM and a Bmax of 177 fmol per mg protein. The distribution of specific binding was highest in the cortex as were dopamine levels, dopamine-induced vasodilatation and the dopamine-stimulated adenylate cyclase. Dopamine levels were similar in the medulla but (^{3}H)-sulpiride binding and dopamine-stimulated adenylate cyclase were less, and the pelvic region was devoid of activity.

Dopamine antagonists of all classes were potent in displacing the binding (Ki values - fluphenazine 0.01; spiroperidol 0.18; (+)-butaclamol 0.24; cis-flupenthixol 0.48; haloperidol 1.17; S-(-)-sulpiride 9.96 and chlorpromazine 15.87 nM). The binding site showed stereospecficity since cis-flupenthixol was 18 X more active than trans-flupenthixol, S-(-)-sulpiride was 13 X more active than R-(+)-sulpiride and (-)-butaclamol was inactive. Dopamine agonists were generally less potent than antagonists (ADTN 12.23 and dopamine 698 nM) and non-dopaminergic agents such as prazosin, propranolol, (-)-noradrenaline, histamine and GABA were inactive.

The results demonstrate that specific binding sites for (^{3}H)-sulpiride can be identified in guinea-pig renal cortex. The characteristics of binding, the distribution and the pharmacological profile suggest that the site is of a dopaminergic nature. Furthermore it appears to resemble striatal (^{3}H)-sulpiride binding sites.

(^{3}H)-sulpiride was a gift from Chemitechna.

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CARBACHOL-METHOHEXITONE INTERACTIONS AT THE CHICK NEURO-MUSCULAR JUNCTION: MOVING FLUID ELECTRODE TECHNIQUE

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The effect of methohexitone on the depolarization and contracture responses produced by carbachol (CCh) and acetylcholine (ACh) in the isolated chick biventer cervicis (BVC) nerve-muscle preparation was studied using the moving fluid electrode technique (Fatt, 1950).

Carbachol (5.5μM-0.5mM) produced contracture and depolarization responses which were concentration-dependent. These responses were greatly reduced by methohexitone (88μM). The mean ED 50s for the contractures produced by CCh in the control Krebs solution and in methohexitone were 36±0.1μM and 210±0.1μM, n=6, respectively. The mean ED 50s for the depolarizations produced by CCh were 81±0.4μM and 360±0.3μM, n=6, respectively.

Acetylcholine (5.5μM-11mM) produced contracture and depolarization responses which were concentration-dependent. These responses were greatly reduced by methohexitone (88μM). The mean ED 50s for the contractures produced in the control Krebs solution and in methohexitone were 240±0.2μM and 2.3±0.1mM, n=6, respectively. The mean ED 50s for the depolarizations were 840±0.3μM and 3.7±0.1mM, n=6, respectively. These actions were reversible upon washing out the methohexitone but recovery took about 1 h.

Methohexitone had little effect on the relationship between the depolarization and contracture responses produced by CCh and ACh, and shifted the concentration-response curves to the right in a non-competitive manner. The calculated slopes for the responses produced by CCh and ACh in the control Krebs solution were 1.3 g mV^{-1} and 1.52 g mV^{-1}, and in methohexitone were 1.18 gmV^{-1} and 1.09 g mV^{-1} respectively. The intercepts on the Y axis were 0.53 g, 0.11 g and 0.43 g, 0.12 g respectively, and the corresponding correlation coefficients were 0.94, 0.93 and 0.92, 0.91.

Eserine(0.77μM) had little effect on the contractures produced by CCh (55μM) (control 1.6±0.06 g, test 1.9±0.05 g, n=6), while it greatly potentiated the contractures produced by ACh(550μM) (control 1.43±0.03 g, test 3.8±0.08 g, n=6).

The present results are in agreement with those obtained by Marshall (1971), that CCh acts postsynaptically, and are in conflict with those obtained by Chiou & Long (1969), since eserine failed to significantly potentiate the responses produced by CCh. Carbachol acts via nicotinic postsynaptic ACh receptors and produces depolarization and contracture responses which are greatly reduced by methohexitone. Thesleff (1956), found that pentobarbitone reduces the responses produced by iontophoretically applied ACh and CCh in the frog sartorius muscle. Since Adams (1976) has shown that barbiturates block open but not closed endplate channels at the frog sartorius muscle, it is possible that methohexitone, in the chick muscle, blocks the depolarizations produced by CCh and ACh in the manner suggested by Adams (1976) in the frog muscle.

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CARBACHOL - ADRENALINE INTERACTIONS ON VULNERABILITY TO VENTRICULAR FIBRILLOFLUTTER IN THE RAT ISOLATED HEART

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Lubbe et al (I978) have shown that adrenaline reduces the ventricular fibrillo-flutter threshold in rat hearts, and implicated cyclic AMP as the arrhythmogenic mediator. It has been suggested that this effect of adrenaline may theoretically be antagonised by elevated levels of cyclic GMP (Opie et al, I979). Therefore the present study investigated the interaction of carbachol, a known stimulant of myocardial cyclic GMP levels, and adrenaline on electrical stability of the isolated rat heart.

Ventricular fibrilloflutter thresholds were assessed by a method described previously(Daugherty & Woodward, I98I)with the exception that a train of extra stimuli was applied instead of a single pulse. A train of pulses(70 msec duration, 200 Hz, 2msec pulse width)was placed I0 msec after the peak of the R wave. Extra-stimuli were applied every I5 sec with increments of I mA. The fibrillo-flutter threshold was taken as the current required to produce irregularities on two successive stimulations. A control value was obtained before and after each concentration of drug and statistical analyses performed on the mean of these two values against that obtained in the presence of the drug.

Carbachol produced a concentration-related decrease in fibrilloflutter threshold which was significant at concentrations higher than I x 10^{-6}M. It has been speculated that some of the effects of carbachol on ventricular myocardium are due to catecholamine release(for review see Higgins et al, I973). However, atenolol(I x 10^{-5}M)did not influence the effects of carbachol. Atropine(Ix10^{-6}M) appeared to antagonise the effects of carbachol. Atropine(Ix10^{-6}M)appeared to antagonise the effects of carbachol, although these data are complicated by the significant($p<0.05$)decrease in control values. In agreement with Lubbe et al (I978)adrenaline(3x10^{-7}M)decreased fibrilloflutter threshold and this effect was potentiated in the presence of carbachol.

Table I. Effect of carbachol on the fibrilloflutter threshold (mA). Results are expressed as mean±s.e. mean.

	n	CONTROL	DRUG	CARBACHOL					
				CONTROL	$1x10^{-7}$M	CONTROL	$1x10^{-6}$M	CONTROL	$1x10^{-5}$M
	6			10.7±0.9	10.8±1.2	10.3±0.4	7.5±0.7	12.3±1.4	5.4±0.4
ADRENALINE ($3x10^{-7}$M)	9	8.4±0.7	5.5±0.7	8.0±0.8	5.9±0.8	8.4±0.8	5.1±0.6	8.5±0.8	3.6±0.5
ATENOLOL ($1x10^{-5}$M)	6	13.2±1.7	13.8±3.5	11.7±1.3	12.4±2.7	12.8±1.5	7.4±1.1	12.3±2.5	5.0±1.0
ATROPINE ($1x10^{-6}$M)	6	11.9±1.7	14.5±2.5	11.5±1.8	8.5±2.0	11.3±1.5	8.8±1.6	7.8±1.0	8.7±1.4

These results do not support the speculation of Opie et al(I979), and show a synergistic effect of adrenaline and carbachol on electrical stability in the rat heart.

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COMPARISON OF THE CARDIAC EFFECTS OF IQB-M-81 AND LIGNOCAINE

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2-(hexohydrazepine)-N-(2,6-dimethylphenyl)acetamide (IQB-M-81) is a a new primary β-amino anilide with local anesthetic properties which is chemically related to lidocaine (Moratalla et al 1982). In the present study the effects of IQB-M-81 and lidocaine were compared on the electrical and mechanical activity in isolated rat atria and guinea-pig papillary muscles.

Spontaneously beating right atria and left atria driven at 3Hz were suspended in Tryode solution (34ºC). Slow contractions were elicited by adding isoprenaline (10^{-6}M) to high potassium (27 mM) Tyrode solution. The maximum following frequency and the effective refractory period were measured as previously described (Tamargo, 1980). Guinea-pig papillary muscles were perfused with Tyrode solution and stimulated at a basal rate of 1 Hz. Intracellular action potentials were recorded with glass microelectrodes.

Cumulative addition of IQB-M-81 or lidocaine (10^{-7}M - 10^{-4}M) to spontaneously beating right atria produced a dose-dependent decrease in rate, contractile force, work index and maximum following frequency, and prolonged the sinus node recovery time. In isolated left atria both drugs decreased contractile force, prolonged the effective refractory period and decreased atrial excitability. At concentrations higher than 10^{-6}M, IQB-M-81 caused a more significant depression of all these parameters than did lidocaine ($p < 0.05$). IQB-M-81 and lidocaine did not modify the slow contractions elicited in depolarized atria.

IQB-M-81 did not alter the positive chronotropic and inotropic responses to isoprenaline and did not reduce the positive inotropic effect of raised calcium concentration.

In guinea-pig papillary muscles, IQB-M-81 at concentrations higher than 10^{-6}M caused a significant decrease in action potential amplitude, overshoot and maximum rate of depolarization, and shortened the action potential duration and the effective refractory period. The resting membrane potential was not significantly reduced until the concentration of IQB-M-81 was raised to 10^{-5}M. Similar results were obtained in the presence of lidocaine but the change were significant only at concentrations between 10^{-5}M and 10^{-4}M.

Thus, the new primary β-amino anilide IQB-M-81 exerted on isolated rat atria and guinea-pig papillary muscles a direct cardiodepressant effect similar to, but more potent than, that of lidocaine.

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PLATELET-ACTIVATING FACTOR, U44069 AND VASOPRESSIN STIMULATE PHOSPHATIDYL INOSITOL TURNOVER IN HUMAN BLOOD PLATELETS

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Platelet activation (shape change, aggregation, degranulation) is dependent upon agonist-induced elevation of the cytosolic free calcium ion (Ca-f) concentration (Rink et al., 1981). The biochemical mechanisms that regulate Ca-f are not fully understood but in platelets, as in other systems, it has been proposed that agonist-induced stimulation of phosphatidyl inositol (PI) turnover and the consequent formation of phosphatidic acid (PA) result in the elevation of Ca-f (Berridge, 1981).

Agonists such as ADP, collagen and thrombin stimulate platelet PI turnover (Gerrard et al., 1981). However human platelets contain distinct receptors for, and are activated by, several agonists including adrenaline, vasopressin, TxA_2 and platelet -activating factor (PAF). In the present study we compared the effects of these four agonists on PI turnover in human platelets. Plasma-free suspensions of platelets ($5x10^9$-10^{10} cells/ml) in phosphate-free buffer (150mM NaCl; 4mM KCl; 1mm $MgCl_2$; 10mM dextrose; 5mM Na Hepes, pH 7.4) were prepared essentially as described by Moncada et al. (1982). Platelets were incubated (37°C; 90 min) with carrier-free [^{32}P]-PO_4 (25-30µCi/ml final concentration) before resuspension in 1.5 volumes of phosphate-free buffer. Agonists (<10µl) were added to 0.4ml samples of [^{32}P]-labelled platelets at 37°C for 30 sec prior to extraction of phospholipids according to method B of Lloyd et al. (1972). Phospholipids were separated by two dimensional thin layer chromatography (Yavin & Zutra, 1977) visualized by iodine vapour and/or autoradiography, scraped into scintillation vials and counted by liquid scintillation. Release of platelet dense granule contents (ADP, 5HT) was measured using an isotope ([^{14}C]-5HT) prelabelling technique.

PAF (0.18nM-1.8µM), the TxA_2-mimetic U44069 (3.3nM-33µM) and vasopressin (1nM-1µM), but not Adrenaline (5nM-25µM), induced concentration dependent formation of [^{32}P]-PA. The EC_{50} and maximum extent of stimulation of [^{32}P]-PA production were respectively:- PAF (15nM and 10 fold); U44069 (80nM and 8 fold); vasopressin (45nM and 8 fold). Because [^{32}P]-PA represents a large fraction of the total platelet PA whereas [^{32}P]-PI represents a small fraction of the total platelet PI, stimulated production of [^{32}P]-PA was not always associated with a detectable increase in production of [^{32}P]-PI. Agonist-induced stimulation of platelet PI turnover could not be attributed to release of endogenous agonists, such as ADP or 5HT, because over the concentration range tested U44069 and vasopressin induced <2% release of the platelet dense granule marker [^{14}C]-5HT. Similarly PAF (0.18nM-180nM) released <2% [^{14}C]-5HT but PAF (1.8µM) released 9% [^{14}C]-5HT.

It has been proposed that receptors for agonists can be differentiated on the basis of the transduction mechanism that links receptor occupancy to biological response (Berridge, 1981). The results of the present study indicate that receptors for PAF, TxA_2 and vasopressin on platelets are coupled to stimulation of PI turnover whereas receptors for adrenaline on platelets are coupled to a different transduction mechanism, presumably adenylate cyclase.

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SELECTIVE INHIBITION OF PAF-INDUCED HUMAN PLATELET AGGREGATION BY VERAPAMIL AND METHOXYVERAPAMIL

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Exogenous 1-O-alkyl-2-acetyl-sn-glyceryl-3 phosphorylcholine, also known as platelet activating factor (PAF) is a potent stimulant of platelet aggregation and degranulation. PAF is synthesized and/or released by platelets in response to certain agonists (Chignard et al., 1979) and thus could function as an endogenous mediator of platelet activation. It is generally agreed that cytosolic free calcium ions serve as intracellular mediators of platelet functional change. There is evidence that PAF induces platelet activation by combining with specific receptors (Shaw & Henson, 1980). Although the mechanisms that link receptor occupancy to biological effect are not fully understood PAF has been reported to stimulate calcium influx in rabbit platelets (Lee et al., 1981). Therefore, in the present study we examined the effects of the calcium "antagonists" verapamil, methoxyverapamil and nicardipine on human platelet aggregation induced by PAF, vasopressin and ADP.

Platelet aggregation was measured photometrically in 0.17ml samples of citrated platelet rich plasma. To eliminate the contribution of endogenous PGG_2, PGH_2 and TxA_2 to aggregation responses, all studies were performed on PRP obtained from donors who had ingested aspirin (600mg) 24 hours previously. Drugs or vehicle (in control samples) were preincubated in PRP for 2 min at 37°C before the addition of agonists. Using concentrations of agonists that induced submaximal aggregation responses the effects of the calcium antagonists are summarised in table 1.

Table 1 Inhibition of Platelet Aggregation by Calcium "Antagonists".
I_{50} value (mean ± SE of n observations)

Agonist	Verapamil(μM)	Methoxyverapamil(μM)	Nicardipine(μM)
PAF	22±3.5 n=7	9±3 n=7	168±32 n=5
ADP	163±25 n=7	172±22 n=4	> 200 n=3
Vasopressin	89±14 n=7	130±36 n=3	> 200 n=3

Verapamil and methoxyverapamil were more potent inhibitors of aggregation induced by all three agonists than was nicardipine. Moreover both verapamil and methoxyverapamil were more potent inhibitors of aggregation induced by PAF than of aggregation induced by ADP or vasopressin ($P<0.001$).

In the present study we report that verapamil and methoxyverapamil are much more selective inhibitors of PAF-induced human platelet aggregation than of aggregation induced by vasopressin or ADP. The concentrations of verapamil which inhibit PAF-induced aggregation are similar to those reported to block adrenaline induced human platelet aggregation (Ikeda et al., 1981) and to suppress adrenaline induced calcium influx into human platelets (Owen & Le Breton, 1981). These results suggest that activation of human platelets by PAF may involve a calcium translocation process, sensitive to verapamil and methoxyverapamil but not to nicardipine. Studies are in progress to characterise the role of extracellular calcium and calcium influx in PAF-induced human platelet activation.

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DDAVP (1-DESAMINO-8-D-ARGININE-VASOPRESSIN) AS AN ANTAGONIST OF VASOPRESSIN IN RENIN RELEASE AND PLATELET AGGREGATION

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The presence of D-arginine in 1-desamino-8-D-arginine vasopressin (DDAVP) preserves the antidiuretic potency but unlike vasopressin (AVP) does not contract smooth muscle cells. The pressor response to AVP depends on an increase in cytosolic Ca^{2+} and has been postulated to be mediated by a subtype (V_1) of AVP receptor. In contrast the antidiuretic response is mediated via an increase in [cyclic-3,5-AMP] and may be mediated by a different type (V_2) of receptor (Mitchell, 1976). The aim of this study was 1) to assess the nature of the AVP receptor involved in renin release and platelet aggregation, 2) to determine the effects of DDAVP, on these responses.

Rabbit kidney cortex slices were incubated as described by Churchill, (1981). AVP 10^{-10}-10^{-7} mol/L causes a dose dependent inhibition of renin release; 10 to 80%. In a calcium free Ethylene glycol-bis-(β-aminoethyl ether)-N,N-tetraacetic acid (EGTA) 3×10^{-3} mol/L containing medium AVP did not inhibit renin release. Verapamil 5×10^{-5} mol/L blocked the inhibitory effect of AVP on renin release. DDAVP up to 10^{-5} mol/L had no effect on basal renin release but competitively blocked the effect of AVP. AVP causes aggregation of platelet without a change in cellular [cyclic-3,5-AMP] (Haslan, 1972). Platelet rich human plasma was prepared from heparinized plasma. AVP induced aggregation was absent in the presence of EGTA 3×10^{-3} mol/L and verapamil 5×10^{-4} mol/L. DDAVP in a concentration up to 10^{-5} mol/L had no agonist activity for platelet. AVP induced a dose dependent increase in platelet aggregation, ED_{50} 1.2×10^{-8} mol/L. DDAVP blocked the responses of AVP on platelet aggregation. The results on platelet confirm the study of Thomas (1982).

In-vivo experiments were performed in 6 male un-anaesthetised rabbits. They were infused either with AVP or DDAVP 4×10^{-10} mol/kg over two minutes. After AVP blood pressure increased 22 ± 8mmHg ($\bar{X} \pm S.D.$), heart rate decreased 55 ± 7 beats/min and renin fell 4.2 ±0.7 pmol AngI.ml^{-1} h^{-1}. After DDAVP blood pressure decreased 8 ± 2mmHg, heart rate increased 58 ± 10 beats/min and renin increased 11 ± 4 pmol AngI.ml^{-1} h^{-1}.

Inhibition of renin release in the rabbit kidney slice preparation and platelet aggregation are both calcium dependent effects (V_1-receptor), DDAVP competitively blocks both effects of AVP. The in-vivo effects of DDAVP, a fall in blood pressure and an increase in both heart rate and renin could be explained by a competitive blockade of endogenous AVP. Recently it has been reported that DDAVP increase prostacyclin release from the rabbit aorta (Belch, 1982). Prostacyclin induced vasodilation may also explain the fall in blood pressure and rise in heart rate and renin after DDAVP.

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PREFERENTIAL ANTAGONISM OF α_2-RECEPTOR AND VOLTAGE OPERATED Ca^{2+} CHANNELS BY DILTIAZEM IN RAT PORTAL VEIN

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Post-synaptic α_2-receptor agonists increase phasic activity in rat portal vein without causing contracture (Hicks, 1982) and these effects are dependent on extracellular Ca^{2+}. Since α_2-receptor mediated pressor effects in pithed rats (Van Meel et al, 1981) and contractile effects in dog saphenous vein (Langer & Shepperson, 1981) are antagonised by calcium entry blocking drugs, it was decided to study the effects of diltiazem on phasic responses in the rat portal vein.

Portal veins from male Sprague-Dawley rats (250-350g) were incubated at 37°C under 0.5g tension in physiological salt solution (PSS), containing propranolol 10^{-7} M and ascorbate 10^{-4} M. Isometric recordings of phasic activity were quantified as changes in frequency x amplitude of phasic waves ($\Delta fa/fa$; Hicks, 1982).

Cumulative response curves were obtained to Ca^{2+} (0.03-2.5mM) after incubation in Ca^{2+}-free PSS (15 min) before, or after, addition of submaximal doses of either the α_2-receptor agonist UK14304 (18μM); phenylephrine (PE, 2.6μM) or K^+ (20mM). Agonist stimulated Ca^{2+}-dose response curves were repeated after 30 min incubation with diltiazem 10^{-9}-10^{-7} M. Diltiazem competitively antagonised K^+, or UK14304 stimulated Ca^{2+}-responses, but caused a non-competitive antagonism of PE-induced responses at 10^{-8} or 10^{-7} M. Antagonism of non-stimulated Ca^{2+}-responses was only observed at 10^{-7} M. PE-stimulated phasic activity was antagonised by prazosin (10^{-9} ; $5x10^{-8}$ M) but not by yohimbine (10^{-7} M). K+ stimulated Ca^{2+} dose-responses were not susceptible to α-receptor blockade.

The results demonstrate that diltiazem is a potent antagonist of voltage or α_2-receptor mediated phasic activity at concentrations which do not influence normal myogenic or α_1-receptor induced phasic activity and strongly suggest that different Ca^{2+}-gating mechanisms exist in this preparation.

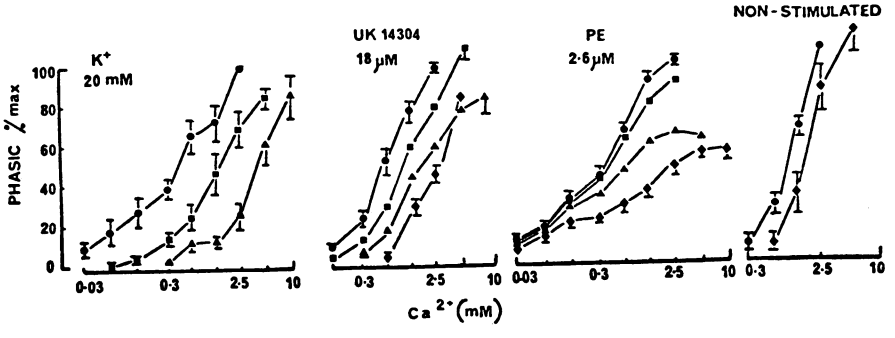

Figure 1. Effects of diltiazem on K^+, UK14304, PE or non-stimulated Ca^{2+} dose-response curves for $\Delta fa/fa$. Control (● ; n = 8); diltiazem 10^{-9}M (■ ; n= 8); 10^{-8}M (▲ ; n=6); 10^{-7}M(◆ ; n=6).

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EFFECTS OF SOME DRUGS AND OF ALLOXAN-DIABETES ON THE RESPONSES OF RAT MESENTERIC ARTERIES TO CATECHOLAMINES

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Over recent years there have been several reports showing increased vascular reactivity to pressor agents in experimentally induced diabetes. No clear explanation for such an effect has emerged although a number have been suggested, including degeneration of sympathetic vasoconstrictor nerves (Jackson & Carrier, 1981), inhibition of non-neuronal uptake of catecholamines by increased concentrations of circulating steroid hormones (Cseuz et al, 1973), changes in the receptors (Cseuz et al, 1973) and changes in the availability of calcium (Owen & Carrier, 1980).

In an attempt to investigate these possibilities further, use has been made of the perfused isolated mesenteric artery preparation described by McGregor (1965). The arterial bed of the rat mesentery was perfused through a cannula in the superior mesenteric artery with Krebs-Henseleit solution at 37°C gassed with 95% O_2 and 5% CO_2. Evaporation was prevented by covering with a sheet of plastic sealing film. The rate of flow was kept constant at 2.0 ml min^{-1} with an LKB Microperpex peristaltic pump and the perfusion pressure monitored with a Washington PT400 transducer. Perfusion pressures in the absence of drugs were in the range of 20-40 mm Hg. Drugs were administered in 0.2 ml of saline solution. Male Wistar or Sprague-Dawley rats of 220-455g were made diabetic by a single i.v. injection of 50 mg kg^{-1} alloxan and used following measurement of urinary glucose 3-5 days later, together with their age-matched controls.

Parallel log dose-response curves were obtained in both diabetic and control rats which showed that adrenaline (ADR) was 4-8 times more potent than noradrenaline (NA) as a pressor agent, while the sensitivity to both amines appeared to increase with body weight up to about 400g. Preparations from diabetic animals were more sensitive to the pressor action of both ADR and NA when compared with their age-matched controls. Phentolamine (10^{-7}M) moved the log dose-response curves to the right in a parallel manner while practolol (10^{-7}M) had no effect in either diabetic or control preparations. However (±)-propranolol (10^{-7}M) in preparations from control animals produced a significant shift of the log dose-response curves to the left to the same extent for ADR and NA. When such preparations were exposed to cocaine (10^{-6}M) and corticosterone (3×10^{-5}M) propranolol caused a very small additional shift to the left. This was found for both amines even though the initial shift due to cocaine and corticosterone was greater with ADR than with NA. The K_i of (±)-propranolol for neuronal uptake of NA in the perfused rat heart was found to be 1×10^{-5}M.

From these results it would appear that β_1 receptors if present, do not influence the effects on perfusion pressure of either ADR or NA. It is however, surprising that propranolol is equally effective in potentiating the pressor responses to both amines. It seems unlikely that ADR and NA can be equipotent on β_2 receptors or that their neuronal uptake can be equally affected. At present no clear conclusion is possible. However in the mesenteric artery preparations from the alloxan diabetic rat there is a significant increase in sensitivity towards both ADR and NA but propranolol causes little or no further augmentation of this pressor effect.

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POSSIBLE HETEROGENEITY OF DOPAMINE RECEPTORS IN CORONARY VASCULAR SMOOTH MUSCLE OF THE ANAESTHETIZED DOG

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Dopamine receptors have been sub-classified on the basis of the pharmacological differences that exist between them. Thus, in the anaesthetised dog, dopamine receptors located in the smooth muscle of the mesenteric vascular bed were designated dopamine-1 (D-1) sub-type to distinguish them from those present on peripheral sympathetic nerves of the heart and nictitating membrane. The latter were designated dopamine-2 (D-2) receptors (Shepperson et al., 1982). Dopamine-sensitive sites have also been identified in the smooth muscle of the coronary bed of the dog and their stimulation leads to vasodilatation (Goldberg 1972, Woodman et al.,1981). We have now studied the coronary arteriolar dilating activity of some dopamine-like agents and their susceptibility to inhibition by antagonists at D-1 and D-2 receptors.

Mongrel dogs of either sex and weighing 12-25 kg were anaesthetised with intravenous pentobarbital (35 mg/kg and then continuous perfusion of 6 mg/kg/hr, iv). Animals were artificially respired. Recordings were made of aortic blood pressure and heart rate. Coronary arterial blood flow was recorded using an electromagnetic flow probe placed around the circumflex artery after thoracotomy at the 5th intercostal space. Agents were injected either intra-arterially distal to the flow probe head in volumes of 0.05-0.2 ml or into the cannulated femoral vein. Adrenoceptors were blocked with intravenous phenoxybenzamine (10 mg/kg) and propranolol (1 mg/kg and 0.25 mg/kg/hr). Atropine (1 mg/kg, iv) and chlorisondamine (1 mg/kg, iv) were also given.

The baseline coronary flow was 40 $\pm$ 4 ml/min (n=16). Intra-coronary-arterial injections of dopamine (1-20 µg) increased blood flow by approximately 10-40 ml/min and was equipotent with LY 141865 (trans-[+]-4,4a,5,6,7,8,8a,9-Octa-hydro-5-propyl-2H- pyrazolo [3,4-g] quinoline dihydrochloride) a selective D-2 agonist (Tsuruta et al., 1981). These two compounds were more potent than intra-arterial DPDA (N,N-di-n-dipropyl-dopamine, 20-200 µg) and pergolide (20-200 µg). The increase in flow produced by DPDA was comprised of an initial rapid phase followed by a secondary increase that developed more slowly. Increases in flow were not associated with changes in systemic blood pressure (initially 127 $\pm$ 4 mmHg, n=16) or heart rate (initially 116 $\pm$ 4 b/min, n=16). The D-1 antagonist bulbocapnine (3 mg/kg, iv) or the non-selective antagonist (+)-butaclamol (0.3 mg/kg, iv) markedly inhibited the flow-increasing effects of dopamine but did not inhibit the effect of LY 141865 or pergolide. The D-2 antagonist (S)-sulpiride (0.3 mg/kg, iv), when injected at a dose previously shown to block presynaptic dopamine-receptors (Shepperson et al 1982), did not antagonise the effects of dopamine, pergolide or LY 141865. Ergometrine, (0.5-2.0 mg/kg, iv) by itself, increased coronary blood flow and after the flow had returned to control values it then inhibited responses produced by dopamine, pergolide and LY 141865.

These findings suggest that dopamine increases blood flow in the coronary vascular bed by stimulation of primarily D-1 receptors. The effect of pergolide and LY 141865 appears to be more susceptible to blockade by ergometrine than the other antagonists used. Therefore, the coronary vascular smooth muscle of the dog contains sites sensitive to pergolide and LY 141865 but which appear to differ from D-1 and D-2 subtypes because they are not blocked by either bulbocapnine or (S)-sulpiride.

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A COMPARISON OF THE EFFECTS OF SODIUM NITROPRUSSIDE WITH THOSE OF ACIDOSIS ON RAT VASCULAR MUSCLE

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Ito et al (1978) have demonstrated hyperpolarization of the rabbit portal vein by sodium nitroprusside (NP) coincident with relaxation. Since Siegel and Schneider (1981) have presented evidence which suggests that membrane hyperpolarization is the causal link between acidosis and vascular muscle relaxation we considered it worthwhile to compare the vascular relaxant effects of acidosis with those of NP.

Longitudinal preparations of the portal vein (PV) and ring preparations of aortae were obtained from rats (wt 200-280g) and suspended under 0.5 and 2.0 g tension respectively in Krebs solution maintained at 37°C and pH 7.5 by gassing with 5% CO_2 in O_2. Variations in the pH of the Krebs over the range 7.5 to 7.0 were achieved by increasing the CO_2/O_2 ratio.

Lowering the pH of the Krebs solution bathing the PV selectively reduced the spontaneous activity being less effective in reducing contractions to noradrenaline (NA) (3μM) or KCl (60mM) (Table 1). NP had a broadly similar profile of action, whereas neither verapamil nor diazoxide selectively reduced spontaneous activity (Spont. act.) (Table 1).

Table 1 Percentage reduction (mean ± s.e.mean, n>6) of contractions of PV by low pH, low $|K^+|_o$ and drugs.

	pH		low $\|K^+\|_o$	NP		Diazoxide	Verapamil
	7.2	7.0	(2.5mM)	10μM	100μM	100μM	1μM
Spont. act.	95 ± 10	100 ± 0	64 ± 6	80 ± 5	96 ± 4	99 ± 7	52 ± 7
NA (3μM)	28.5 ± 6	45.5 ± 15	28 ± 10	45 ± 3	55 ± 3	86 ± 3	63 ± 3
KCl (60mM)	17 ± 9	34 ± 8	-	5 ± 3	3 ± 5	80 ± 10	77 ± 5

Lowering the external potassium concentration ($|K^+|_o$) from 5.9mM to 2.5mM, which might be expected to produce hyperpolarization (Siegel et al 1978), also selectively reduced spontaneous activity. Lowering the pH from 7.5 to 7.0 caused a significantly smaller reduction of KCl (60mM) responses on aorta (12 ± 5%, n=6) compared with PV (Table 1) ($P < 0.05$). This difference between the effectiveness of acidosis to inhibit KCl contractions of PV and aorta was more apparent with lower concentrations of KCl; using 30mM KCl the % reductions to pH 7.0 were 25 ± 8, (n=6) and 79 ± 13 (n=6) for the aorta and PV respectively. This difference in sensitivity to acidosis may reflect a steeper votage/tension relationship in the PV. NP was far more effective in reducing contractions of the aorta than those of PV to KCl (60mM); 1μM NP caused a maximally obtainable reduction of 61 ± 11% (n=11) of aorta, while concentrations of up to 100μM were virtually ineffective on PV (Table 1).

Selective suppression of spontaneous activity of PV may indicate membrane hyperpolarization. If the main causal link between action of NP or acidosis and vascular relaxation is hyperpolarization then the mechanism of each is likely to be different.

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THE RESPONSE OF THE SUPERFUSED RAT ANOCOCCYGEUS TO ADENOSINE 5'-TRIPHOSPHATE: AN EFFECT INVOLVING MORE THAN ONE MECHANISM

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The response of the isolated anococcygeus muscle of the rat to adenosine 5'-triphosphate (ATP) was studied in the superfused preparation. Paired preparations from Sprague-Dawley rats (200-300g) were superfused with McEwen's solution, 5ml/min, at 35 ± 1°C, gassed with 5% carbon dioxide and 95% oxygen and responses recorded isometrically.

ATP (0.02 - 200μmol) produced a dose-related contraction of the anococcygeus and the response could be divided into two components; an initial rapid, phasic response followed by a slow, tonic contraction. Dose-response curves for ATP were obtained before and after 30min superfusion with antagonist. From these curves, the responses of the tissue to 20μmol ATP are summarized in table 1; these were representative of the results obtained with all doses of ATP. Control curves, repeated in the absence of antagonist, were unaltered. The P_2-purinoceptor antagonist, 2-2'pyridylisatogen tosylate (PIT, 5 and 25μM), preferentially reduced the tonic response in a concentration-dependent manner, leaving the phasic contraction almost unaffected. The ATP-induced contraction of the rat anococcygeus has been claimed to be mediated by prostaglandins (Burnstock et al, 1978) therefore, the effect of prostaglandin synthesis inhibitors was investigated. Indomethacin (0.56 and 1.4μM) and flurbiprofen (0.5 - 4μM) also showed a selective effect by reducing the tonic contraction while having little effect upon the phasic response.

Table 1. Response of the superfused rat anococcygeus to 20μmol ATP.

Treatment	Conc μM	n		Control	Treated	% Control
				g ± s.e.mean		
PIT	25	5	Phasic	2.3 ± 0.29	1.6 ± 0.26	73
			Tonic	2.4 ± 0.53	0.0	0
Indomethacin	1.4	6	Phasic	2.5 ± 0.38	1.9 ± 0.21	78
			Tonic	2.5 ± 0.44	0.0	0
Flurbiprofen	4	5	Phasic	2.9 ± 0.24	2.4 ± 0.17	85
			Tonic	3.3 ± 0.37	0.1 ± 0.08	2
McEwen's	-	10	Phasic	2.7 ± 0.28	2.6 ± 0.25	94
			Tonic	2.8 ± 0.31	2.7 ± 0.30	99

Therefore, it would seem that the ATP-induced contraction of the rat anococcygeus is only partly mediated by products of arachidonic acid metabolism and that PIT inhibits the response by an action similar to that of indomethacin and flurbiprofen. The phasic contraction of the anococcygeus to ATP may not be due to stimulation of a P_2-purinoceptor.

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THE EFFECTS OF HYPEROXIA AND HYPOXIA ON THE RESPONSES OF SMOOTH MUSCLE TO NERVE STIMULATION AND TO DRUGS

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In rat anococcygeus, in vitro, contractile responses to noradrenaline (NA) are biphasic under normoxic conditions (O_2 tension = 60-100 mmHg). An initial rapid peak declines within 1 min to a level which remains steady for 5 min. At higher oxygen tension (720 mmHg; 95%), the maintained component increases to exceed the early peak, leaving a monophasic contraction and masking the biphasic nature of the response. These effects of NA are mimicked by other phenylethanolamines. Chemically distinct agonists, which, in other circumstances, activate α_2-adrenoceptors, e.g. xylazine, act through an α_1-adrenoceptor, as does NA, but produce phasic contractions. Thus, in anococcygeus, α-adrenoceptor activation by exogenous drugs can initiate contraction by more than one pathway and the apparent significance of each will vary according to the oxygen tension (McGrath, 1982).

The present objective was to examine the response to field stimulation of the intramural nerves to see how these were modified by hyperoxia and to find whether the adrenergic nerve-induced contraction corresponded to any of the various effects of exogenous agonists. An effect on the inhibitory nerve response emerged, which was pursued on the bovine retractor penis (BRP) in which such a response is dominant.

Isometric tension of rat anococcygeus or BRP was recorded in vitro at 37°C (Gillespie, 1972) in Krebs' bicarbonate saline continuously gassed with mixtures containing O_2 = 0-95%:CO_2 = 5.0-5.8%: balance N_2.

The contraction to NA (1 nM-100 μM) was highly dependent on the O_2 tension. Above 40% the maintained component increased until at 75% it equalled the early peak and at 95% exceeded it. Taking the O_2 tension above atmospheric to a maximum of 2 atmospheres (in a hyperbaric chamber) resulted in only a small further increase in the maximum contraction: the full toxicity of O_2 had thus developed by 95%.

The contractions to field stimulation (2-20 Hz,20s) were not markedly altered in height across the range of O_2 tensions 8-95%. However, there was a small increase on changing from 95% to 16%. Since this could result from loss of the inhibitory nerve response, the experiment was repeated in the presence of guanethidine (1-100 μM) with the tension elevated by guanethidine, carbachol or xylazine. The relaxations to field stimulation were attenuated at 16% compared with the 95% O_2 at which they have been studied previously.

In BRP, which attains tone spontaneously and exhibits inhibitory nerve responses, (i.e. relaxation), without the addition of tone-producing drugs, the inhibitory nerve responses were rapidly and completely lost on changing from 95% to 0% O_2. concurrently, the preparations lost tone, which is thus shown to be a function of O_2 tension. However, the loss of tone could not, by itself, explain the loss of the response. Conversely in 0% O_2 nerve-induced contractions were increased.

Thus the adrenergic motor nerve response corresponds to the initial phase of the response to exogenous NA and can be demonstrated with equal facility at zero, normal or elevated O_2 tensions. In contrast, the relaxation to inhibitory nerve stimulation, in anococcygeus or BRP, is absent in the absence of oxygen, and increases in amplitude as the O_2 content of the gas mixture is increased from zero to 95%.

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LACK OF FUNCTIONAL ADRENERGIC TRANSMISSION IN THE VAS DEFERENS OF IMMATURE RATS

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Previous studies showed that in the rat vas deferens, functional adrenergic transmission did not develop until 5-6 weeks of age although the tissue responded to exogenous noradrenaline (NA) from 3 weeks, the earliest age tested (MacDonald & McGrath, 1980). In the present study the delay in functional adrenergic transmission was investigated in male Wistar rats approximately 4 weeks old.

Unlike the biphasic responses of adult rats (McGrath, 1978), the responses to single pulse (0.5 ms) field stimulation of prostatic and epididymal portions from the vasa of immature rats were monophasic. Corynanthine (3 μM) had either no effect or caused a small reduction in the responses, indicating a small adrenergic component. This was confirmed by cocaine (3 μM) which potentiated the responses. This potentiation was removed by corynanthine (3 μM). The adrenergic response could be obtained separately by employing nifedipine (10 μM), which blocks the non-adrenergic response of the rat vas deferens but leaves the adrenergic component intact (French & Scott, 1981). Nifedipine (10 μM) almost completely abolished the responses, leaving only small responses which were potentiated by cocaine (3 μM) and removed by corynanthine (3 μM), confirming that they were adrenergic. In contrast to its lack of effect on adrenergic nerve-induced responses, nifedipine (10 μM) antagonized the contractile effects of exogenous NA in the immature rats. The contractile effect of exogenous NA is probably analogous to the prolongation of responses seen after cocaine in adult rats (MacDonald & McGrath, 1982). Some prolongation of nerve-induced responses was seen in immature rats after cocaine (3 μM).

Electron microscopy (Tranzer & Richards, 1976) revealed varicosities containing mainly small granular vesicles with some large granular vesicles. No qualitative differences between immature and adult rats as regards number and type of vesicles or in neuromuscular distances could be distinguished. Thus electron microscopy confirmed that adrenergic nerves containing NA are present and that close neuromuscular distances occur at 4 weeks of age.

In conclusion, there appears to be only a small adrenergic component in the nerve-induced responses of vasa deferentia from sexually immature rats at a time when (1) adrenergic nerves and neuromuscular junctions appear mature, (2) the vasa are fully capable of responding to exogenous NA and (3) the non-adrenergic nerve-induced response is present. The lack of adrenergic transmission may be due to either (a) insufficient transmitter output or (b) lack of post-junctional receptors or the activation mechanism for neuronally-released NA.

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POSTJUNCTIONAL α-ADRENOCEPTOR MEDIATED FACILITATION OF THE NANC RESPONSE IN RAT VAS DEFERENS

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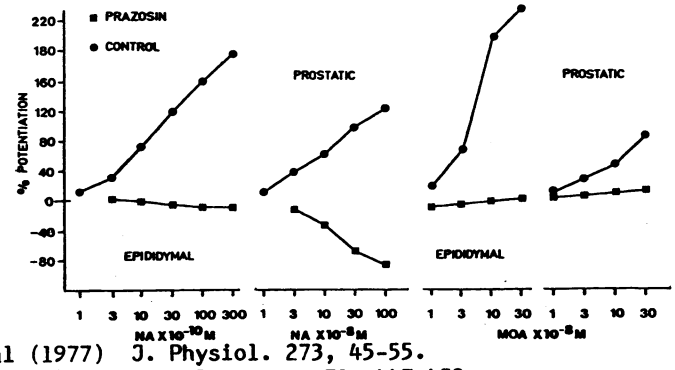
The twitch response of rat vas deferens to single pulse stimulation of the intramural nerves has two components - an initial non-adrenergic, non-cholinergic (NANC) phase and a subsequent adrenergic phase (Anton et al, 1977). The NANC component is dominant in the prostatic end of the tissue at all times (McGrath, 1978) and in the epididymal end when the frequency of stimulation exceeds 0.05 Hz (French & Scott, unpublished). This latter effect may be attributed to feedback inhibition of noradrenaline (NA) release. The adrenergic phase is absent in vasa taken from reserpinised rats. It has been reported that α-adrenoceptor blockers can reduce the size of but not eliminate the twitch responses to repetitive stimulation in the prostatic end of the tissue (Doggerell, 1981).

The nature of this inhibition was investigated, using epididymal or prostatic ends of vasa deferentia taken from rats previously (18h) treated with 5 mg/kg reserpine. The tissues were stimulated with a single shock every 5 min in Krebs Henseleit solution containing 2.7×10^{-5}M EDTA, 1×10^{-4}M ascorbic acid, and 1×10^{-5}M cocaine. 5×10^{-8}M rauwolscine was also added to inhibit presynaptic α_2 receptors which are involved in the regulation of NANC transmission (French & Scott, 1981). In the presence of either NA or methoxamine (MOA) at subcontractile concentrations there was a dose-related increase in the size of the twitches in both ends of the tissue. The preferential α_1 adrenoceptor antagonist prazosin completely antagonised the NA- and MOA-induced potentiation in the epididymal end of the tissue. However, in the prostatic end of the tissue, while the effects of MOA were also antagonised, NA caused a decrease in the size of the twitch responses in the presence of prazosin. This reduction may be due to the action of NA on presynaptic α_2 receptors in the presence of a concentration of rauwolscine which was too low. Increase of the rauwolscine to 5×10^{-6}M reversed this negative action of NA. Prazosin had no antagonistic action if the rate of stimulation was less than 0.01 Hz, perhaps since at this rate, sufficient time elapses between each stimulus to allow uptake and diffusion processes to reduce NA to ineffectual concentrations.

In rat vas deferens, therefore, the peak of responses to repetitive stimulation at rates above 0.05 Hz, although NANC in origin, may be potentiated by the presence of small amounts of NA released by previous pulses. It may be that it is this potentiation which is antagonised by α-blockers, rather than the NANC response itself.



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EFFECTS OF PHENTOLAMINE AND RESERPINISATION ON STIMULATION INDUCED DECAY OF MOTOR TRANSMISSION IN RAT VAS DEFERENS

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Repetitive stimulation with long trains of electrical stimuli is known to cause inhibition of motor transmission in rodent vas deferens and the transmission-failure has been attributed to a decline in noradrenaline release (Chang & Chang, 1965; Swedin, 1971; Farnebo & Malmfors, 1971). We present evidence here which indicates that the failure of transmission in rat vas deferens, following repetitive stimulation, is associated specifically with the failure of non-adrenergic transmission.

All experiments were performed on isolated preparations of prostatic end (P.E.) and epididymal end (E.E.) of rat vas deferens. The isolated preparations, suspended in Krebs-Henseleit solution containing propranolol, 2×10^{-6}M, at 37°C were stimulated transmurally with trains of electrical stimuli (90 pulses, 1ms, 10Hz) once every min. Electrical stimulation evoked a biphasic (P-1 and P-2) contractile response; both phases of the response were abolished by tetrodotoxin, 2×10^{-7}g/ml and were therefore neurogenic.

Following a 30min period of electrical stimulation of P.E. preparations (n=6), the mean % inhibition ± s.e. mean of P-1 and P-2 responses was 52.2 ± 1.8 and 15 ± 7.6 respectively. The corresponding values for E.E. preparations (n=6), following an identical treatment were 19.7 ± 2.2 and 22.9 ± 4.4 for P-1 and P-2 responses respectively.

Treatment with phentolamine, 5×10^{-6}M virtually abolished P-2 responses; P-1 responses of E.E. preparations were reduced to 22.8 ± 4.5% of their control values (n=4). P-1 responses of P.E. preparations were augmented by phentolamine (114 ± 7.2%; n=4). A 30 min period of electrical stimulation caused a greater inhibition of the motor response in phentolamine-treated preparations, compared to the untreated preparations; values for mean % inhibition ± s.e.m. in phentolamine-treated P.E. and E.E. preparations were respectively 70.5 ± 2.8 and 59.7 ± 0.9.

Electrical stimulation of vasa from reserpine-treated animals (reserpine phosphate 10mg/kg s.c. 48h before and 10mg/kg i.p. 24h before the experiment) evoked monophasic contractile responses. Repetitive stimulation for 30 min caused a sharper and more intense inhibition of transmission which, unlike responses of non-reserpinised vasa, remained unaffected by phentolamine, 5×10^{-6}M. The mean % inhibition of transmission in vasa from reserpine-treated animals (n=3), following 30 min of electrical stimulation was 88.9 ± 2.7 and 90.2 ± 3.5 respectively for P.E. and E.E. preparations. The corresponding values in phentolamine-treated preparations (n=3) were 89.7 ± 2.4, and 90.5 ± 2.8.

The results support the view that electrical stimulation induced decay of motor transmission in rat vas deferens is caused by a specific failure of non-adrenergic transmission. It is important that while evaluating actions of drugs with adrenergic overtones on rat vas deferens, the concept of the failure of non-adrenergic motor transmission on repetitive stimulation must be borne in mind in order to avoid misinterpretation of experimental findings.

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STIMULATION OF α_2-ADRENOCEPTORS BUT NOT OF SYMPATHETIC NERVES CONSTRICTS CAT ISOLATED PERFUSED MIDDLE CEREBRAL ARTERIES

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In vitro studies using spiral strips or cylindrical segments of cerebral blood vessels to investigate the effects of nerve stimulation and of biogenic amines have yielded inconsistent results (reviewed by McCalden, 1981). The present communication therefore describes the effects of electrical stimulation and of drugs with α-adrenoceptor agonist and antagonist activity in a perfused cerebral artery preparation, which represents a closer approach to the in vivo situation. Recently Skärby et al (1981), using cylindrical segments of cat middle cerebral artery, suggested that smooth muscle adrenoceptors were of the α_2-subtype although yohimbine appeared to act in a non-competitive manner.

Cats were anaesthetised with pentobarbitone and the brain rapidly removed. The proximal 5 mm of each middle cerebral artery was isolated, cannulated and perfused and superfused with oxygenated Krebs solution (37^{0}C, pH 7.6) at a rate of 5 ml/min. Within 30 min the perfusion pressure (PP) had fallen to less than 10 mmHg and thereafter remained essentially constant. The maximum constrictor response of the vessels to KCl (127 mM) was measured as an increase in PP of 108 $\pm$ 4 mmHg (n=70). Noradrenaline (NA; 0.001-100 μM) increased PP in a concentration-dependent manner (pD_2=6.8 $\pm$ 0.1, maximum=22 $\pm$ 4% of KCl, n=12). The presence of cocaine (4 μM) and propranolol (1μM) increased the maximum NA response to 35 $\pm$ 4 % of KCl (n=27). Adrenaline was equipotent with NA in increasing PP whereas dopamine and phenylephrine were approximately 100 times less potent. The effects of the selective α_2-adrenoceptor antagonist RX 781094 (Chapleo et al., 1981) were invesigated. Whereas responses to NA up to 1 μM were reduced by RX 781094 (0.1 μM), prazosin (0.3 μM) did not affect NA responses up to 1 μM but reduced those to 10 and 100 μM of NA. It may be concluded that in the middle cerebral artery of the cat, both α_1- and α_2-adrenoceptors are present but that vasoconstrictor responses are predominantly mediated by those of the α_2-subtype.

If tone was induced in the vessels (using either NA, serotonin or $PGF_{2\alpha}$), perarterial electrical stimulation (0.3 msec pulses, 15 V, 1-20 Hz) produced frequency-dependent decreases in PP, abolished by tetrodotoxin (1 μM). In the absence of tone, either in the presence or absence of cocaine and propranolol, stimulation was ineffective . Increasing the voltage or pulse width produced increases in PP which were resistant to tetrodotoxin and to reserpine pretreatment, and were thus probably due to direct smooth muscle stimulation.

The apparent absence of a sympathetic vasoconstrictor response together with the predominance of smooth muscle α_2-adrenoceptors is of interest in view of the suggestion of Langer et al (1980) that postjunctional α_2-adrenoceptors are mainly extrajunctional in vascular smooth muscle. The stability and sensitivity of the perfused vessel preparation used in this study suggests that it may yield more meaningful results for in vitro quantitative analysis of contractile mechanisms in the cerebral vasculature, than other methods.

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APAMIN REDUCES α-ADRENOCEPTOR-INDUCED HYPERKALAEMIA IN GUINEA-PIGS

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Adrenaline (i.v.) causes a transient rise in the concentration of potassium in the plasma of most mammals. The additional potassium comes from the liver, probably as a consequence of an α-adrenoceptor-mediated increase in the potassium permeability of the parenchymal cell membranes. Recent work with isolated hepatocytes has shown that the effect on permeability can be blocked by nanomolar concentrations of apamin, a toxin from bee venom (Burgess, Claret & Jenkinson, 1981). Apamin might, therefore, be expected to reduce the hyperkalaemia elicited by α-adrenoceptor agonists; this has now been tested.

Male guinea-pigs were anaesthetised with pentobarbitone sodium (25 mg/kg), fentanyl (0.2 mg/kg) and droperidol (20 mg/kg), all i.p. The left carotid artery was cannulated for blood pressure recording, and an airway was placed in the trachea for pump-aided respiration, if needed. A potassium-sensitive electrode consisting of a thin valinomycin-containing PVC membrane (see Band, Kratochvil & Treasure, 1977), formed on the end of a 15 cm length of PVC tubing (1.5 mm O.D.), was inserted in the right jugular vein and its end positioned in the right side of the heart or in the suprahepatic portion of the vena cava. Drugs were given via the left jugular vein, in a solution containing 145 mM NaCl and 4 mM KCl.

(-)-adrenaline bitartrate (0.7 - 70 μg/kg) and the α_1-selective agonist (±)-amidephrine mesylate (14 - 140μg/kg) given at 7 - 8 min intervals produced transient increases in plasma potassium (maximal after 40 sec, and falling thereafter to below the resting value which was reestablished after 3-4 min). These were consistent over several hours, and were about 2 times larger in the vena cava as compared with the heart. Amidephrine was studied in most detail; 34μg/kg and 70μg/kg increased the potassium concentration in the vena cava by 0.93 ± 0.04 (mean ± s.e. mean, n=33) and 1.42 ± 0.10 (n=19)mM respectively above its resting value of 4.41 ± 0.10 (n=25)mM. With the largest doses of adrenaline, plasma potassium increased by as much as 4 mM. The maximum increase with amidephrine was about half this.

Apamin reduced the hyperkalaemic response to amidephrine and adrenaline in a noncompetitive manner. The degree of block, expressed as the percentage reduction (which did not vary significantly with the dose of amidephrine), came to 55 (2 expts), 59 ± 12 (n=4) and 70 ± 5 (10)% with apamin at 10, 40 and 200 μg/kg respectively. In similar experiments with adrenaline, apamin at 200 μg/kg reduced the hyperkalaemia by 59 ± 5% (n=4). In both cases, raising apamin to 400 μg/kg caused no further reduction. These doses of apamin were without effect on the action of adrenaline and amidephrine on systolic and diastolic BP. There was, however, a small dose related increase in the mean baseline pressure (from 35.9 ± 1.2 to 44.3 ± 1.3 mm Hg with 200 μg/kg).

These experiments show that apamin reduces the hyperkalaemic response to adrenaline and amidephrine by about two-thirds. The apamin-resistant component requires further study.

Rachel Coats is an MRC scholar.

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THE DISTRIBUTION OF β-ADRENOCEPTOR SUBTYPES IN GUINEA-PIG AIRWAYS

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Although $beta_1$ and $beta_2$ adrenoceptors have been directly identified in peripheral lung of the rat (Barnett et al, 1978), functional studies in the guinea-pig suggest that whilst catecholamine-induced smooth muscle relaxation can be mediated by both subtypes in the trachea, relaxation of lung strips is solely mediated by $beta_2$ adrenoceptors (Zaagsma et al, 1979). In the present study, the distribution of beta adrenoceptor subtypes in different regions of guinea-pig airways has been determined by both radioligand binding and functional studies.

Binding studies were performed using either ^{3}H-dihydroalprenolol or ^{125}I-cyanopindolol in homogenates of guinea-pig trachea, bronchi and parenchyma prepared as described previously (Carswell & Nahorski, 1982) in 50 mM Tris-HCl (pH 7.8) using conventional filtration assays. The dissociation constant (K_D) for both radioligands was identical in each area, but there were significant regional differences in maximal binding capacities (B_{max}). The highest density of beta adrenoceptors was found in the parenchyma (366 ± 31 fmol/mg protein, n = 3), then the bronchi (196 ± 14 fmol/mg protein, n = 3) and the lowest in the trachea (46 ± 4 fmol/mg protein, n = 3). Displacement curves of the radioligands with selective $beta_1$ and $beta_2$ antagonists analysed using computer-assisted curve fitting, indicated that both drugs labelled a heterogeneous population of receptors, consisting of approximately 85% of the $beta_2$ subtype and 15% the $beta_1$ subtype. There was no significant variation in the relative proportion of these two subtypes between the regions of the airway.

Experiments were also carried out on isolated tracheal spirals and lung strips, and the agonists isoprenaline (ISO), noradrenaline (NA) and terbutaline (TER) (in the presence of uptake and alpha adrenergic receptor blockers) all produced concentration-related relaxations of both preparations. PA_2 values for selective and non-selective beta antagonists against each agonist were then calculated (when possible) from Schild plots. In the trachea, both subtypes appeared to mediate relaxation as the PA_2 value of, for example, atenolol ($beta_1$-selective) varied substantially depending upon the agonist used (Table 1) whilst in lung strips taken from the same animals, this variation was reduced, suggesting the relaxation is mediated predominantly by $beta_2$ adrenoceptors.

TABLE 1 PA_2 values for timolol (non-selective) and atenolol ($beta_1$-selective) calculated from Schild plots.

		Timolol		Atenolol	
		PA_2	Slope	PA_2	Slope
Tracheal Spirals	ISO	8.98 ± 0.15	1.04 ± 0.09	5.06 ± 0.07	0.96 ± 0.03
	NA	9.2 ± 0.09	0.91 ± 0.02	6.96 ± 0.06	0.60 ± 0.01
	TER	9.1 ± 0.12	1.13 ± 0.11	5.01 ± 0.06	0.92 ± 0.05
Lung Strips	ISO	9.23 ± 0.13	1.07 ± 0.01	5.50 ± 0.10	1.10 ± 0.08
	NA	9.38 ± 0.13	1.07 ± 0.08	6.06 ± 0.06	0.56 ± 0.11
	TER	9.30 ± 0.35	1.10 ± 0.19	5.50 ± 0.15	1.06 ± 0.12

These functional studies therefore agree with those of Zaagsma et al (1979), but there is an obvious discrepancy with the receptor labelling studies. It will be important to establish the functional significance of $beta_1$ in the lung periphery.

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CHARACTERIZATION OF THE RAT LIPOLYTIC β-ADRENOCEPTOR USING NOVEL β-ADRENOCEPTOR AGONISTS

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Lands et al (1967) found that the lipolytic response of rat white adipose tissue to a series of sympathomimetics correlated with activity on rabbit heart but not guinea-pig lung and thus classed the response as β_1. Subsequent workers however suggested that the lipolytic receptor is of the β_2 type (Jolly et al, 1978) or has both β_1 and β_2 characteristics (De Vente et al, 1980). The rat lipolytic β-adrenoceptor has now been further characterized using two examples from a series of arylethanolamines which exhibit novel agonist selectivity.

BRL 28410 (4-[(2-hydroxy-2-phenylethyl)amino]propyl]-benzoic acid) and BRL 35113 (4-[2-[(2-hydroxy-2-(3-trifluoromethylphenyl)ethyl)amino]propyl]-benzoic acid) were compared with standard β-adrenoceptor agonists on the following responses: increase in rate of rat isolated spontaneously beating right atrium (β_1), increase in tension of rat isolated paced (1 Hz,3m sec) left atrium (β_1), relaxation of guinea-pig intrinsic tone tracheal zig-zag preparation (mainly β_2), relaxation of rat K^+-depolarized uterus (β_2) and glycerol production of rat white adipocytes.

dl-Isoprenaline (non-selective between β_1- and β_2-adrenoceptors) was equiactive on atria, uterus and trachea but less active on lipolysis (Table 1). Fenoterol and salbutamol (β_2 selective) were more active on uterus and trachea than on atria, and less active still on lipolysis. Prenalterol (β_1 selective) was much more active on atria than uterus or trachea and had little agonist activity on lipolysis. In contrast to the standard β-agonists, BRL 28410 and BRL 35113 showed some absolute selectivity for lipolysis. Relative to isoprenaline selectivity was pronounced.

Table 1 Molar EC_{50} values (50% isopren.max.). Intrinsic activities in parentheses

AGONIST	LIPOLYSIS	R.A.RATE	L.A.TENSION	UTERUS	G.PIG TRACHEA
Isopren.	1.2×10^{-8} (1)	1.3×10^{-9} (1)	4.1×10^{-9} (1)	3.1×10^{-9} (1)	2.1×10^{-9} (1)
Fenot.	4.5×10^{-7} (1)	4.8×10^{-8} (1)	6.7×10^{-8} (1)	9.9×10^{-9} (1)	3.4×10^{-9} (1)
Salbut.	9.5×10^{-6} (0.86)	9.8×10^{-7} (0.94)	3.2×10^{-6} (0.90)	2.2×10^{-8} (1)	2.8×10^{-8} (1)
Prenalt.	(0.08)	2.9×10^{-8} (0.82)	6.2×10^{-8} (0.91)	7.5×10^{-4} (0.54)	(0.46)
BRL28410	3.4×10^{-6} (0.86)	8.7×10^{-5} (0.69)	1.9×10^{-4} (0.68)	1.0×10^{-5} (0.82)	1.8×10^{-5} (0.98)
BRL35113	8.7×10^{-7} (0.88)	(0.44)	(0.11)	1.3×10^{-5} (0.86)	2.1×10^{-5} (0.93)

Antagonist data are also difficult to explain on the basis of the present β_1-β_2 classification. The pA_2 values for dl-propranolol on lipolysis were 6.7, 6.4 and 6.0 with isoprenaline, fenoterol and BRL 28410 respectively as agonist. The corresponding pA_2 values on left atrial tension were 8.7, 8.2 and 8.7. Similar results were found using selective antagonists. The pA_2 values for practolol (β_1 selective) were 1.7-2.5 log units lower (depending on lipolytic agonist) on lipolysis than atrial stimulation and pA_2 values for ICI 118551 (β_2 selective) were 2.8-3.2 log units lower on lipolysis than tracheal relaxation.

In conclusion the rat white adipose tissue β-adrenoceptor differs from that in heart or lung. No β-adrenoceptor antagonist selective for lipolysis is available, but the agonists BRL 28410 and BRL 35113 display a novel selectivity for this response. These compounds are being evaluated as anti-obesity and anti-diabetes agents.

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MUSCARINIC RECEPTOR SUBTYPES AND RESPONSES TO McN A-343 AND PIRENZEPINE

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McN A-343 (4-m-chlorophenylcarbamoyloxy)-2-butynyltrimethyl ammonium chloride) is a selective agonist of ganglionic muscarine receptors (Roszkowski 1961) and pirenzepine a selective antagonist of different subclasses of muscarinic receptors whose activity in binding studies has correlated well with its known pharmacological activities (Hammer et al 1980).

As an extension of these findings the tabulated doses of atropine and pirenzepine were determined, each being the dose which reduced the specified responses by 50% (mean of 5 determinations). (A) Doses of the antagonists by injection into the lingual artery for inhibiting responses of the nictitating membrane elicited by 10 µg McN A-343 by the same route in chloralose anaesthetised cats; (B) doses i.v. for inhibiting the pressor response to 300 µg/kg McN A-343 i.v. in pithed rats; (C) doses i.v. for inhibiting the bradycardia in pithed rats caused by supramaximal stimulation of the right vagus for 30 sec. at 10 Hz. In each test the antagonist preceded the stimulus by 2 minutes.

	(A) Nictitating membrane µg i.a.	(B) Pressor µg/kg i.v.	(C) Bradycardia µg/kg i.v.
Pirenzepine	0.033	4.1	172
Atropine	0.029	7.7	3.2

Atropine and pirenzepine are similarly potent in inhibiting the ganglionic receptors (involved in A and B) whereas in inhibiting the cardiac receptors concerned with the bradycardia response (C) only atropine retains high activity.

The abilities of antimuscarinics to compete with the 'in vitro' binding of the specific marker ligand N-methyl-scopolamine to membranes of sympathetic ganglia and atria provided further characterisation of these receptors. Atropine had similar affinities for muscarinic sites of either tissue (KI 1.1 and 3.2 nM for ganglion and atria, respectively). In contrast pirenzepine in sympathetic ganglia recognised two sites: the first binds the drug with high affinity (KI 11 nM) while the second has a lower affinity for the compound (KI 280 nM). Binding of pirenzepine to atrial tissue was characterised by low affinity (KI 620 nM). The results of the present study suggest heterogeneity of muscarinic receptors within the same tissue (ganglia) and between tissues. They add to the information concerning heterogeneity of muscarinic receptors (Birdsall et al, 1978; Hulme et al, 1978; Hammer et al 1980) and indicate that, by the classification of Goyal and Rattan (1978), the high affinity receptors of the ganglia are of the M_1 type whereas the low affinity receptors of the atria may correspond to the M_2 type.

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EXCITATORY TRANSMISSION TO THE BLADDER OF THE FRUIT-EATING BAT (Eidolon helvum)

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Nerve stimulation of the urinary bladder elicits contractions which are not blocked by atropine (Langley & Anderson, 1895, Krell et al, 1981). Evidence has however been adduced to implicate another excitatory transmitter (Ambache & Zar, 1970, Burnstock et al, 1972), for this resistance. This observation was therefore examined, for the first time, in the bat, a flying mammal that roosts in millions on our University Campus.

Isolated in vitro preparations of the bat's detrusor from about 100 animals underwent intermittent electrical field stimulation at 15Hz, 0.5ms delivered for 5s and at regular intervals of 120s. The electrically-induced contractions (EIC^S) comprised a short latency and about 8-9s duration. Such responses were frequency-dependent (0.5-15Hz), reaching maximum at 15Hz. The EIC^S were resistant to blockade by hexamethonium or pentolinium but were abolished by Tetrodotoxin (1.5×10^{-7}M). Atropine (10^{-7}-10^{-5}M) produced a 40-45% decline in the amplitude of the EIC^S and the atropine-resistant component was not potentiated by physostigmine. Guanethidine (4×10^{-6}M), phentolamine (10^{-6}M) and propranolol (3.4×10^{-6}M) augmented the EIC^S weakly (ca 5-10%) while noradrenaline ($0.1\text{-}3.10^{-5}$M), adrenaline (10^{-6}M) and isoprenaline (3.6×10^{-6}M) produced an inhibition of the twitches. Lower doses of noradrenaline however enhanced the EIC^S.

The present observations suggest that a non-adrenergic and non-cholinergic motor transmission co-exists with the atropine-sensitive transmission. The adrenoceptors seem to subserve only a modulatory role to this transmission.

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ANTAGONISM BY METOCLOPRAMIDE OF AN INHIBITORY RESPONSE TO CLONIDINE IN RAT ISOLATED STOMACH MUSCLE

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The effects of Metoclopramide (Mcp) on gastrointestinal responses to catecholamines are not clear. In guinea-pig ileum Mcp selectively antagonises inhibition of cholinergic-induced contractions caused by dopamine, noradrenaline or clonidine (Spedding, 1981), but has no effect on various gastric responses to catecholamines in the absence of cholinergic activity (Costall et al, 1981). We have now examined the effects of Mcp on adrenoceptor-mediated inhibition of cholinergic contractions in rat isolated stomach muscle.

Strips of gastric fundus were cut parallel to the longitudinal muscle (Bennett et al, 1980). Consistent, approximately equal isotonic contractions were obtained every 10 min with either acetylcholine (ACh; 20s contact 10-40% maximum contraction) or electrical field stimulation (EFS; Crema et al, 1968; bipolar 0.5ms rectangular pulses; 5Hz rate; 20s train; maximum current 1A/electrode [80-120V/cm]). Contractions to EFS could be blocked with atropine 1μg/ml or tetrodotoxin 0.2μg/ml, indicating cholinergic activation. Cumulative concentrations of adrenoceptor agonists were tested against contractions to EFS or ACh. Effective concentrations of clonidine or isoprenaline which reduced contractions to EFS by 25% (median ED25's: 0.001 and 0.003μg/ml respectively) were obtained. ED25's for phenylephrine (median: 0.32μg/ml) were obtained with ACh, since large concentrations (1-10μg/ml) were required to reduce contractions to EFS. Unlike isoprenaline, clonidine did not reduce contractions to ACh. The effects of antagonists on these ED25 values were then determined.

Responses to clonidine were inhibited by the α_2-antagonist yohimbine 0.1μg/ml, the α_1-antagonist prazosin 0.1μg/ml or with Mcp 1 & 10μg/ml; only prazosin 0.1μg/ml antagonised the effect of phenylephrine (Table 1). In contrast to propranolol 0.4μg/ml, Mcp 10μg/ml did not increase the ED25 for isoprenaline ($P>0.05$, n=6). The antagonists alone had no effect on the cholinergic contractions, except Mcp which increased contractions to EFS (McClelland & Sanger, 1982).

Table 1: Multiple Increases in ED25 (Medians with semiquartile ranges in parenthesis) required after addition of antagonist (P = Wilcoxon matched pairs test; NS = $P>0.05$; n = number of preparations)

Drug μg/ml		with clonidine	P	n	with phenylephrine	P	n
Mcp	1	10.8 (2.8-140)	<0.02	8	not tested		
Mcp	10	210.0 (9->5222)	0.02	8	1.5 (1.0-6.8)	NS	7
Yohimbine	0.1	246.0 (12-365)	0.01	8	0.9 (0.6-2.0)	NS	6
Prazosin	0.1	283.0 (7-1079)	0.01	8	>21.1 (14->25)	<0.05	6

Phenylephrine may activate α_1-adrenoceptors located post-junctionally to the cholinergic neurone; the response was unaffected by Mcp. Clonidine reduced contractions to EFS but not to ACh, and this was antagonised by Mcp, yohimbine or prazosin. Each of these antagonists could therefore block α_2-adrenoceptor-mediated responses, or neural α_2- and α_1-adrenoceptors may be activated by clonidine in rat stomach.

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INDUCTION OF SKIN ARYL HYDROCARBON HYDROXYLASE BY 3-METHYL-CHOLANTHRENE

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Mammalian skin possesses monooxygenase activity which is inducible by polycyclic hydrocarbons (Damen & Mier, 1982). However, the induction of skin monooxygenase activity is substantially less than that of hepatic monooxygenases after systemic administration of substances such as 3-methylcholanthrene (3MC) (Vizethum et al, 1980), whereas multiple topical administration of polychlorinated biphenyls markedly induces hepatic monoxygenases (Bickers et al, 1974). The present study was designed to explore the potential for induction of skin aryl hydrocarbon hydroxylase (AHH) by single doses of 3MC administered intraperitoneally (i.p.) and topically.

Groups of 6 female hairless mice were treated with varying doses of 3MC administered i.p. in corn oil, or applied topically in acetone. 21 hours after dosing animals were *killed* by cervical dislocation and liver and skin microsomes prepared, after homogenisation, by differential centrifugation. Microsomal AHH activity was estimated fluorimetrically (Nebert & Gelboin, 1968). Dose-response curves were prepared for each route of administration. Response was measured as the induction ratio (IR) of AHH activity in treated animals compared to controls receiving the vehicle alone.

Basal skin AHH activity (2.18 ± 0.58 pmoles/min/mg microsomal protein) was less than 3% that of basal liver activity (80.7 ± 19.4 pmoles/mg/min). 5×10^{-6} moles 3MC i.p. increased hepatic AHH activity 8-fold but skin activity less than 3-fold. Increasing the dose to 3×10^{-5} moles did not enhance induction in either organ. 10^{-6} moles 3MC applied topically induced skin levels 7-fold, but did not induce hepatic levels. Increasing the dose to 10^{-5} moles resulted in a hepatic IR of only 1.6. However, subsequent i.p. injection of 10^{-6} moles 3MC increased liver microsomal AHH activity to maximal levels.

We conclude that skin AHH is at least as inducible as liver AHH by 3MC, and that systemic access of 3MC after topical challenge may be limited by dermal absorption, or presystemic cutaneous metabolism.

A.E.R. is an MRC postgraduate student.

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EFFECT OF ALTERED PROTEIN CONCENTRATIONS ON THE ELIMINATION OF PROPRANOLOL FROM THE ISOLATED PERFUSED RAT LIVER

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Free drug concentrations and hence pharmacological activity of drugs that are highly extracted by the liver will be determined by liver blood flow, the unbound fraction of drug in blood and the intrinsic clearance of free drug (Wilkinson & Shand, 1975). Many of these drugs bind to α_1-acid glycoprotein (α_1-AGP) in addition to albumin (Piafsky et al, 1978; Grossman et al, 1982 and Routledge et al, 1980). The aim of this study was to investigate the effects of lowered albumin and/or increased α_1-AGP on both total and free concentrations of propranolol.

Livers from male Sprague Dawley rats (300 ± 9 g) were perfused with a semi-synthetic medium in re-circulating mode (Hems et al, 1966). Circulating protein concentrations were altered from 'normal' (albumin 40g l^{-1}, α_1-AGP 500 mg l^{-1}) to 'low albumin' (albumin 20g l^{-1}, α_1-AGP 500 mg l^{-1}) or 'high' α_1-AGP (albumin 40g l^{-1}, α_1-AGP 1500 mg l^{-1}). Propranolol was added to the system via the reservoir (0.193 µmol) to simulate intravenous dosing or via the portal vein (1.93 µmol) to simulate oral dosing. Samples were removed from the reservoir over a 60 min period and assayed for total propranolol by the method of Di Salle et al, (1973). For each measurement of total drug concentration the percentage drug bound was measured using equilibrium dialysis (M.S.E. Dianorm).

The results obtained after intravenous administration are shown in Table 1.

Table 1

	Cl_T (ml min^{-1} g^{-1} liver)	Cl_F (ml min^{-1} g^{-1} liver	$T^{1}/_{2}{}_T$ (min)	$T^{1}/_{2}{}_F$ (min)	% drug bound
'Low Albumin'	0.67 ± 0.02	1.95 ± 0.16	6.7 ± 0.6	6.4 ± 1.0	63 ± 1*
'Normal'	0.71 ± 0.03	2.33 ± 0.16	6.2 ± 1.1	5.8 ± 1.3	69 ± 1
'High α_1-AGP'	0.70 ± 0.05	4.11 ± 0.16*	5.8 ± 0.8	6.2 ± 0.8	82 ± 1*

* P <0.001 using non-paired Student's t test
P refers to differences from 'normal' (mean ± s.e.; n=4)

Propranolol protein binding was significantly decreased in the 'low albumin' perfusate and significantly increased in the 'high α_1-AGP' perfusate. However, this altered binding resulted in only a slight decrease in the systemic clearance of free drug (Cl_F) in the 'low albumin' system but a significant increase in the systemic clearance of free drug in the 'high α_1-AGP' system. The clearance of total drug (Cl_T) in both systems was not significantly different from the 'normal' group. After portal administration the total clearance of propranolol is lower in the 'high α_1-AGP' system (1.08 ± 0.12 ml min g^{-1}) compared with 'normal' (2.94 ± 0.27 ml min g^{-1}) or 'low albumin' (2.91 ± 0.55 ml min g^{-1}). However, the free drug clearances are similar in the three systems. These results suggest that raised α_1-AGP concentrations, as seen in various disease states (e.g. rheumatoid arthritis, Crohn's disease) are more important in determining the pharmacodynamic response to propranolol after intravenous dosing than oral dosing.

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SELECTIVITY OF INHIBITION OF LIGNOCAINE METABOLISM BY β-ADRENOCEPTOR ANTAGONISTS

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The beta-adrenoceptor antagonists propranolol and metoprolol have been shown to inhibit oxidative drug metabolism in animals and man (Topham, 1970; Bax et al, 1981). The effect of propranolol, the more lipid soluble drug, was significantly greater than that of metoprolol. We have subsequently demonstrated a strong linear correlation between the log octanol/pH 7.4 buffer partition coefficient (Kp) of 10 beta-adrenoceptor antagonists and inhibition of lignocaine (L) metabolism in rat liver microsomes (Deacon et al, 1981). This study has been extended: (1) To include a further three beta-adrenoceptor antagonists, viz. penbutolol, pamatolol and pindolol. (2) To investigate the effect of beta-adrenoceptor antagonists on individual routes of L metabolism. (3) To determine the effect of pre-treating the animals with beta-adrenoceptor antagonists on the ability of their liver microsomes to metabolise L.

Partition coefficients were measured at 37°C by the method of Woods and Robinson (1981). Microsomal incubations were performed as described by Deacon et al (1981). Unchanged L, 3-hydroxylignocaine (3OHL), monoethylglycinexylidide (MEGX) and glycinexylidide (GX) were measured by glc and hplc. In the pre-treatment studies 0.34 mmoles/kg per day of propranolol, alprenolol or metoprolol were given orally for 5 days. The liver microsomes were isolated at 18h after the last dose at which time residual unchanged concentrations of beta-adrenoceptor antagonists were < 0.01-0.4μM.

(1) A strong linear correlation between log Kp and inhibition of L disappearance was observed (r^2=0.84; $p<0.001$). (2) The formation of 3OHL was inhibited to a greater degree by the more lipid-soluble beta-adrenoceptor antagonists (e.g. pamatolol 44%, propranolol 94% inhibition) whereas the presence of these agents enhanced theappearance of MEGX. (3) Pre-treatment with beta-adrenoceptor antagonists also inhibited L metabolism in the order propranolol>alprenolol>metoprolol, with a selective effect on aromatic hydroxylation. The last observation is consistent with the influence of propranolol on its own metabolism reported by Schneck & Pritchard (1981), who showed that propranolol pretreatment in rats impaired its subsequent hydroxylation but spared metabolism via N-dealkylation. They also produced evidence suggesting that the inhibition was caused by a covalently-bound metabolic intermediate of propranolol. Further studies are indicated to determine whether the mechanism of inhibition seen when beta-adrenoceptor antagonists are co-incubated with lignocaine is the same as when they are given as a pre-treatment and to investigate the role of covalently bound species.

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ACETYL HYDRAZINE AND HYDRAZINE: A PHARMACOKINETIC STUDY IN THE RAT

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The anti-tubercular drug isoniazid is metabolised in vivo to the toxic compounds hydrazine and acetyl hydrazine (Lautenberg et al, 1981; Beever et al, 1982). The chronic toxicity of isoniazid may, in part result from the action of these metabolites. To assess the amount formed and the possibility of accumulation we have studied the kinetics of hydrazine and acetyl hydrazine in the rat.

Male Sprague-Dawley rats were employed in the study. Compounds were administered i.p., blood was collected into Sorensens glycine buffer pH 3. Quantitative determinations were carried out by a stable isotope dilution technique based on GC/EIMS.

Hydrazine (peak level 3.5 μg/ml, at 3.0 h) and acetyl hydrazine (peak level 9.8 μg/ml, at 4.5 h) could be detected after dosing rats with isoniazid (100 mg/kg). Acetyl isoniazid, a major metabolite of isoniazid also gave rise to these compounds although the time course for hydrazine production was altered. The peak level (1.7 μg/ml) occurred at 5.0 h after dosing with 100 mg/kg of acetyl isoniazid. Two typical time courses of hydrazine and acetyl hydrazine decay after separate administration of the two compounds (1 mmol/kg) to two rats are shown in Fig. 1. The peak concentrations occurred within 0.5 h followed by a rapid α removal phase (hydrazine, $t_{\frac{1}{2}}$ = 0.49±0.19 h, n=5; acetyl hydrazine, $t_{\frac{1}{2}}$ = 1.04±0.34 h, n=5) and and a slower β removal phase (hydrazine, $t_{\frac{1}{2}}$ = 5.99±0.43 h, n=5; acetyl hydrazine $t_{\frac{1}{2}}$ = 10.74±1.87 h, n=5). The pharmacokinetics are further complicated by the observation that hydrazine and acetyl hydrazine readily interconvert in the rat.

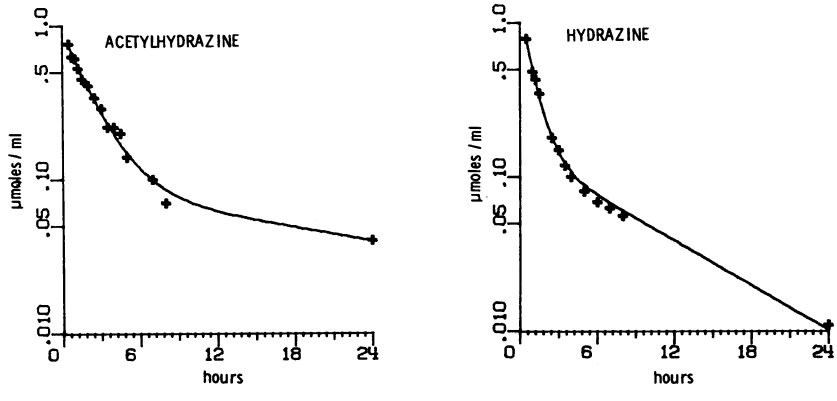

Fig.1 Two of the decay profiles for acetyl hydrazine and hydrazine after dosing rats with 1mmol/kg of each compound.

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SECRETION OF SALICYLIC ACID IN BREAST MILK: OBSERVATIONS AND PREDICTIONS

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There are numerous reports of drugs that transfer from maternal blood into breast milk but attempts to predict milk concentration from plasma concentration in the human are lacking.

Six volunteers who had been breast feeding for 2-8 months each took soluble aspirin tablets BP 600mg by mouth; paired samples of blood and breast milk were taken at 30 minute intervals for 4 hours. Measurements were made of milk pH, total salicylic acid concentration in plasma and milk by HPLC, and binding of salicylic acid to milk and plasma proteins by ultrafiltration. The ratio of unbound salicylic acid in milk (M) and plasma (P) was calculated as:

$$\frac{M}{P} = \frac{1 + 10^{(pHM - pKa)}}{1 + 10^{(pHP - pKa)}}$$

The following results were obtained:

Volunteer	Total concentration in plasma / Total concentration in milk	Milk pH (range)
	mean ± SEM (n=paired observations)	
1	45.26 ± 4.46 (n=8)	7.02 - 7.44
2	31.07 ± 2.05 (n=5)	7.51 - 8.04
3	34.66 ± 11.05 (n=3)	7.21 - 7.56
4	31.67 ± 1.97 (n=5)	7.12 - 7.34
5	13.76 ± 1.21 (n=7)	7.06 - 7.97
6.	14.58 ± 2.50 (n=6)	not measured

Binding of salicylic acid to milk proteins was 37.2 ± 1.96% and to plasma proteins was 80.33 ± 1.45%. Predictions of concentrations of salicylic acid in milk based on observed total plasma concentrations, binding and observed milk pH correlated poorly with observed total milk concentrations. According to the relationship (above) the major determinant of transfer of salicylic acid into milk is milk pH. Variation in pH of expressed milk suggests that the observed pH may differ from that at the site of transfer within the breast. The milk pH which gave the best agreement between observed and predicted values was calculated. In 4 individuals the sums of squares of residuals between predicted and observed points was 16197 calculated using observed milk pH and was 0.6517 when milk pH was assumed to be 6.0. In the other 2 volunteers the sum of squares of residuals between predicted and observed points was 2844 calculated using observed milk pH and was 2.11 when milk pH was assumed to be pH 6.5. pH of excreted milk varies and may not reflect the pH within the breast at the point of drug transfer which must be further defined for accurate prediction of drug transfer into milk.

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EFFECT OF DAUNORUBICIN AND AMITOXANTRONE IN VIVO ON HEPATIC DRUG-METABOLISING ENZYMES AND INVOLVEMENT OF LIPID PEROXIDATION

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Daunorubicin is an effective anthracycline antitumour agent although its clinical use is restricted by a cardiotoxic side-effect. Metabolic reduction of the anthracyclines to generate free radical intermediates resulting in lipid peroxidation is implicated in this cardiotoxicity (Myers et al., 1977). Amitoxantrone is one of a group of anthracycline based alkylaminoanthraquinones undergoing clinical trials and is of interest because it is a synthetic derivative and appears to be less cardiotoxic than daunorubicin (Cheng et al., 1979). In this study we have compared the effects of a single dose of these two drugs on hepatic cytochrome P450 and glutathione-S-transferase activity since lipid peroxidation is also implicated in drug induced damage to drug-metabolising enzymes.
Daunorubicin hydrochloride and amitoxantrone were dosed to two groups of male Swiss F1 generation mice (body weight 25±2g) at 20mg kg^{-1} and 25 mg kg^{-1} respectively. Control mice were dosed on each occasion with isotonic saline vehicle. After 48h the mice were sacrificed and cytochrome P450 determined in liver microsomes (100,000g pellet) as described by Gorrod et al. (1975). Glutathione-S-transferase activity in the cytosol (100,000g supernatant) was determined essentially as described by Habig et al. (1974). Lipid peroxidation was quantitated by determination of the thiobarbituric acid-malondialdehyde complex (Bernheim et al., 1948). Values are the mean ± se of the mean, of four experiments.

Cytochrome P450 in daunorubicin-treated mice was 720 ±48pmol mg^{-1}, this was significantly less than 932 ± 61 pmol mg^{-1} ($p < 0.05$) for control animals. Similarly, for amitoxantrone treated mice cytochrome P450 was significantly less at 995 ± 65 pmol mg^{-1} compared to control animals at 1268 ± 59pmol mg^{-1} ($p < 0.05$). Glutathione-S-transferase activity in daunorubicin treated mice was 15.3 ± 1.2µmol min^{-1} compared to 21.7 ±1.8 µmol min^{-1} ($p < 0.05$). In amitoxantrone treated mice glutathione-S-transferase at 18.2 ± 1.1 µmol min^{-1} was not significantly different to control animals at 18.6 ± 0.7 µmol min^{-1}. Lipid peroxidation of liver microsomal fraction in daunorubicin treated mice was significantly greater being 0.29 ± 0.03 nmol mg^{-1} compared to 0.20 ± 0.01 nmol mg^{-1} ($p < 0.05$) for control animals. In amitoxantrone treated mice lipid peroxidation was not significantly different being 0.275 ± 0.02 nmol mg^{-1} compared to 0.27 ± 0.02 nmol mg^{-1} for control animals.

The results show that both daunorubicin and amitoxantrone significantly reduce the content of cytochrome P450 in mouse liver. However it is only daunorubicin treatment that significantly increases lipid peroxidation in liver microsomes and reduces cytosolic glutathione-S-transferase activity. These results are consistent with reports of adriamycin (14-C-OH-daunorubicin) mediated microsomal enzyme destruction (Mimnaugh et al., 1981; Marchand & Renton, 1981). It appears that both daunorubicin and amitoxantrone are liable to compromise the drug metabolising capabilities of the liver, especially when administered in combination with drugs that depend on certain routes of metabolism as a means of detoxification. However, amitoxantrone may be less detrimental in this respect than daunorubicin.

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EFFECT OF 6 KETO PROSTAGLANDIN E_1 ON GASTROINTESTINAL AND REPRODUCTIVE SMOOTH MUSCLE IN VITRO

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6 keto prostaglandin E_1 (6K PGE_1) is a biologically active metabolite of prostacyclin (PGI_2) produced by rabbit liver and human platelets (Wong et al, 1980; 1981;). 6K PGE_1 is a potent vasodilator and inhibits platelet aggregation but there have been no reports of its spasmogenic activity or ability to inhibit nerve mediated contractions of smooth muscle. We have now studied the effect of 6K PGE_1 on human platelet aggregation, longitudinal smooth muscle of rat stomach strip, guinea-pig and rabbit ileum and on electrically induced contractions of the guinea-pig vas deferens.

Isolated, smooth muscle preparations were suspended in warmed, gassed (95% O_2 : 5% CO_2) Krebs' solution and contractions recorded isometrically using Gross FTO3 transducers connected to a Devices pen recorder. Guinea-pig vas deferens were field stimulated with parallel platinum electrodes connected to an SR1 square wave stimulator (0.1Hz, 1msec, 150v). Human platelet aggregation to ADP (5-10μM) was determined turbidometrically using a Payton dual channel aggregometer.

PGI_2, 6K PGE_1 and PGE_1 inhibited ADP induced platelet aggregation with ID_{50} values of 0.3 ± 0.07ng/ml (n=22), 6.4±1.7ng/ml (n=8) and 35.9±2.1 ng/ml (n=11) respectively. 6K PGE_1 was of similar potency to PGI_2 on the guinea-pig ileum (ED_{50} = 22.7±2.9ng/ml, n=8 and 18.4±4.4 ng/ml, n=6 respectively) but slightly less potent on rabbit ileum (ED_{50} = 228.8±17.0 ng/ml, n=6 and 141.5±21.5 ng/ml, n=6 respectively). On the rat stomach strip 6K PGE_1 had almost 3x the activity of PGI_2 (ED_{50} values 13.8±2.5 ng/ml, n=9 and 36.8±4.1 ng/ml n=10, $P < 0.01$ Student's t test). 6 keto $PGF_{1\alpha}$ produced contractions of all three preparations only at high concentrations (1-10μg/ml).

6K PGE_1 like PGE_1 and PGE_2 inhibited the twitch response of the field stimulated guinea-pig vas deferens. ID_{50} values were 0.78±0.05ng/ml, n=6, 0.30±0.05ng/ml, n=6 and 0.28±0.03ng/ml, n=6 respectively. High doses of PGI_2 (> 20ng/ml) also produced inhibition but this spontaneously reversed before washout unlike the other prostaglandins, presumably due to hydrolysis of PGI_2 in the bath.

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THE SOURCE OF PROSTAGLANDINS AND THROMBOXANES IN EXPERIMENTAL INFLAMMATION

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Prostaglandins and thromboxanes generated by arachidonate cyclo-oxygenase, are present in inflammatory exudates (Higgs and Salmon, 1979) but it is not clear from which tissues they are derived. Platelets and leukocytes, as well as the inflamed tissues are possible sources of cyclo-oxygenase products and we have now investigated the effects of thrombocytopaenia and neutropaenia on the synthesis of thromboxanes and prostaglandins in experimental inflammation.

Thrombocytopaenia was induced in male rats (180-220g) following the intravenous injection of rabbit anti-rat platelet serum (Ubatuba and Ferreira, 1976). Neutropaenia was induced in similar groups of rats following intraperitoneal injection of methotrexate (2.5 mg/kg) on three consecutive days (Willoughby and Spector, 1968). Inflammatory exudates were collected from thrombocytopaenic or neutropaenic rats 6-24 h after the subcutaneous implantation of carrageenin-impregnated polyester sponges. Thromboxane $(TX)B_2$, prostaglandin $(PG)E_2$ and 6-oxo-$PGF_{1\alpha}$ concentrations in exudates and serum from the same animals were assayed by specific radioimmunoassay (Higgs and Salmon, 1979). Total and differential leukocyte counts in whole blood and exudates were estimated using conventional methods.

Inflammatory exudates collected at 6 h contained 82.5 ± 6.8 ng TXB_2/ml (mean ± s.e. mean; n=54), 59.7 ± 4.1 ng PGE_2/ml, 29.3 ± 4.5 ng 6-oxo-$PGF_{1\alpha}$/ml and 6.83 ± 1.4 x 10^6 leukocytes/ml (n=14). 24 h exudates contained 18.9 ± 4.1 ng TXB_2/ml (n=23), 89.8 ± 13.5 ng PGE_2/ml, 36.6 ± 5.6 ng 6-oxo-$PGF_{1\alpha}$/ml and 63.6 ± 6.4 x 10^6 leukocytes/ml (n=30). Serum from control animals contained 174.8 ± 8.6 ng TXB_2/ml (n=119).

Methotrexate induced a selective neutropaenia and reduced the numbers of leukocytes in 6-24 h inflammatory exudates to less than 5% of control values. Serum TXB_2 concentrations were not significantly changed in neutropaenic animals but methotrexate reduced TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ concentrations in 6 h exudates by 60-95%. After 24 h however, the concentrations of cyclo-oxygenase products in sponge exudates from neutropaenic rats were the same as or higher than control values. Anti-platelet serum induced a selective depletion of circulating platelets and TXB_2 was undetectable (<5.0 ng/ml) in serum from thrombocytopaenic animals. The reduction of circulating platelets had no effect on total leukocyte numbers in 6 h inflammatory exudates and concentrations of TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ in these exudates were not significantly different from control values.

These results indicate that platelets are the source of thromboxanes in clotting blood but do not contribute to cyclo-oxygenase activity in carrageenin-induced inflammation. Neutropaenia leads to a decrease in cyclo-oxygenase activity in the first 6 h of experimental inflammation without affecting TXB_2 synthesis in serum. This suggests that migrating leukocytes are a major source of TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ in acute inflammation. Neutropaenia does not, however, decrease cyclo-oxygenase products in 24 h exudates at a time when total leukocyte accumulation is normally high. Local tissues are, therefore, the most likely source of prostaglandins and thromboxanes during the later stages of this response.

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INCREASED AORTIC PROSTACYCLIN AND 'RCF' RELEASE IN SPONTANEOUSLY HYPERTENSIVE RATS

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The various neural, vascular, renal and humoral differences between Wistar-Okamoto spontaneously hypertensive rats (SHR) and age- and weight-matched normotensive control rats (NTR), and the relevance of the rat model to human essential hypertension have been evaluated in recent reviews (Tippodo & Frohlich, 1981; McGiff & Quilley, 1981). Alterations in the prostaglandin (PG) system include increased aortic prostacyclin production (Pace-Asciak et al,1978) and decreased renal PG metabolising enzyme activity (Ahnfelt-Ronne & Arrigoni-Martelli,1977; Pace-Asciak, 1976; Armstrong et al, 1976).

We have attempted a comprehensive comparison of the PG system in SHR and weight-matched NTR by measuring (a) aortic and caecum PGI2 release (by platelet bioassay and RIA for 6-keto PGF1α), (b) plasma levels of prostaglandin 'reciprocal coupling factor' (RCF), which inhibits microsomal PG synthesis and enhances 100,000 g cytosolic PG inactivation (see Moore & Hoult, 1980b), (c) organ levels of PG synthetase and PGDH activity (in terms of microsomal conversion of arachidonate to bioassayable or immunoassayable PGs and conversion of [^{3}H]-PGF2α to metabolites, respectively, see Moore & Hoult, 1980a).

In 380g SHR, aortic PGI2 release was increased (31.5 ± 4.2 v. 16.8 ± 1.4 ng/mg/10 min, $P < 0.05$). This was confirmed ($P < 0.001$) by RIA of 6-keto PGF1α, which was also released in larger amounts from caecal fragments of SHR (6.13 ± 0.73 v. 5.69 ± 0.74 ng/mg/15 min, n.s.). Immunoreactive PGE2 and PGF2α were released from both tissues in much smaller proportions (ca. 3% of the total in aorta and 22% in caecum), but amounts were greater from SHR. Plasma RCF activity was greater in SHR according to both the synthesis-inhibition test ($P < 0.01$) and the activation of PG breakdown ($P < 0.05$), plasmas tested at 1% and 5% v/v, respectively. Microsomal PG synthesis in colon, kidney and lung was increased in SHR, whether measured by bioassay or RIA, and the usual order of abundance of products was i6KF1α > iE2 ≈iF2α. Similar conclusions were obtained when assaying PG 'levels' from extracted homogenates. Finally, the PGDH activity in these organs was colon >> lung ≈ kidney and was lower in SHR than NTR.

These results show that the adaptations of the PG system in SHR include elevated vascular PGI2 synthesis, elevated tissue PG synthesis coupled to a decrease in PGDH activity (c.f. the 'reciprocal changes' described previously by Moore & Hoult,1980b, in other rat models), and release of RCF. Similar results were obtained in experiments using 160 g rats.

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EFFECTS OF ADRENOCEPTOR AGONISTS ON BRADYKININ AND HISTAMINE-INDUCED PLASMA PROTEIN EXTRAVASATION IN GUINEA-PIG SKIN

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Plasma protein extravasation (PPE) is influenced by both blood flow and microvascular permeability (Williams & Peck, 1977). Local administration of either alpha-or beta-adrenoceptor agonists inhibits cutaneous PPE induced by inflammatory mediators in the guinea-pig (Beets & Paul, 1980; Kenawy et al., 1978). Alpha-adrenoceptor agonist inhibition of PPE appears to be a result of reduced blood flow to the inflammatory site (Beets & Paul, 1980). Since beta-adrenoceptor agonists do not reduce blood flow in guinea-pig skin, it has been inferred that these compounds act on vascular endothelium to oppose the permeability increasing action of inflammatory mediators (Beets & Paul, 1980; O'Donnell & Persson, 1978). This study presents a further investigation of the proposed effects of adrenoceptor agonists.

Cutaneous PPE responses in male Dunkin-Hartley guinea-pigs (500-550 g) were quantitated in terms of extravasation of circulating (125I)-human serum albumin and expressed as equivalent ul of blood (Beets & Paul, 1980). PPE was induced by intradermal injection (0.1 ml/site) of bradykinin (BK), BK plus prostaglandin E2 (PGE2) or histamine (HIST) and the inhibitory effects of concomitantly administered isoprenaline (ISO) or phenylephrine (PHE) were evaluated.

The PPE response to 0.5 ug BK was not significantly different from that to 0.5 ug HIST measured in the same animals whilst the PPE response to 0.1 ug BK was significantly greater than that to 0.1 ug HIST ($p < 0.025$; n=6; t-test). These results confirmed that the log dose - PPE response slopes were different for BK and HIST. PHE (6 nmol/site) reduced equivalent responses to 0.5 ug BK and 0.5 ug HIST to levels which were not significantly different (n=6). ISO (0.5 nmol/site) reduced the response to 0.5 ug BK significantly less than that to 0.5 ug HIST ($p < 0.05$; n=5). PHE (2 nmol/site) produced significantly greater inhibition of the BK (0.5 ug) response than did ISO (5 nmol/site) ($p < 0.05$; n=6). However, in the same animals in the presence of the vasodilator PGE2 (2 nmol/site), PHE was no more inhibitory than ISO.

Thus, the extent of the inhibitory effect of ISO, but not PHE, on PPE is dependent upon the inflammatory mediator and is essentially unchanged in the presence of a vasodilator substance. These results are compatible with alpha-adrenoceptor agonists inhibiting PPE by a reduction in blood flow and beta-adrenoceptor agonists by an anti-permeability effect. The quantitatively greater inhibitory effect of ISO on HIST-induced PPE may be a result of the different slopes of the mediator log dose-response curves. Other possible explanations are currently being investigated.

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PEPSTATIN IS NOT A SPECIFIC RENIN INHIBITOR IN VIVO

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The conversion of renin substrate to angiotensin 1 by renin in vitro is inhibited by pepstatin. Pepstatin also inhibits the pressor responses to renin injections in vivo and has a hypotensive action in animal models with high endogenous renin concentrations.(Gross et al.,1972, Miller et al, 1972, Scholkens and Jung 1974). Experiments investigating the specificity of pepstatin for renin have not been widely reported. However, Lazar et al, 1972, observed a non-specific effect of pepstatin on the blood pressures of nephrectomised rats at a dose producing only partial inhibition of the pressor response to injected renin. This report led us to further investigate the specificity of pepstatin for renin in vivo.

In these studies we have measured the in vivo inhibition of the pressor responses to renin, angiotensin **II**, vasopressin and phenylephrine by pepstatin in rats anaesthetised with Inactin (120 mg/kg i.p.) and treated with pentolinium (20 mg/kg i.p.).

The partially purified renin was obtained from hog kidneys. This contained no angiotensin 1 because of the extraction procedures used. A rise in mean blood pressure of 34.6 $\pm$ 4.3 mmHg (n = 4) was produced by an intravenous injection of 0.3 international hog units/kg. Captopril (1 mg/kg i.v.) inhibited this pressor response by 92.1 $\pm$ 4.0%. Hence it is likely that renin was the only pressor agent in the extract. The effects of pepstatin on the pressor responses to renin and angiotensin II were then compared. During a 25 minute pepstatin infusion of 70 µg/kg/min the pressor response to renin was inhibited by 40.1 $\pm$ 3.8% (n = 7) and was significantly different from control experiments infusing pepstatin vehicle ($p < 0.001$). The pressor response to angiotensin II was not significantly inhibited. However, at an infusion rate of 150 µg/kg/min, when the pressor response to renin was inhibited by 68.8 $\pm$ 6.7% (n = 6, $p < 0.001$), the pressor response to angiotensin II was also inhibited (29.2 $\pm$ 3.6%, n = 6, $p < 0.005$). In addition baseline blood pressure was lowered by 14.4 $\pm$ 3.0 mmHg (n = 12, $p < 0.01$).

Finally the effects of pepstatin on the pressor responses to vasopressin and phenylephrine were compared to its effects on angiotensin II. As in the first experiments pepstatin 70µg/kg/min did not significantly inhibit the pressor response to angiotensin II, neither did it inhibit the pressor responses to the other agonists. At 150 µg/kg/min, pepstatin inhibited angiotensin II (45.3 $\pm$ 5.0%, n = 6, $p < 0.001$) and in addition inhibited the pressor responses to phenylphrine (63.7 $\pm$ 4.6%, n = 6, $p < 0.001$) and vasopressin (27.4 $\pm$ 6.4%, n = 6, $p < 0.02$).

Hence at a dose of 70 µg/kg/min pepstatin specifically inhibited the pressor response to renin. However, at 150 µg/kg/min, which still produced only partial inhibition of the pressor response to renin, pepstatin also inhibited the pressor responses to angiotensin II, phenylephrine and vasopressin. We conclude that pepstatin is not a specific renin inhibitor in vivo.

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THE INFLUENCE OF NALTREXONE ON PAIN LATENCIES DURING THE OESTROUS CYCLE OF RATS

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Recently, it has been reported that pituitary concentrations of endogenous opioids change during the oestrous cycle of rats (Kumar et al, 1979) and that opiate agonists and antagonists influence the release of gonadotrophins (Bruni et al, 1977). As it is known that endorphins produce analgesia under a variety of circumstances (Bodnar et al, 1977) and that the pituitary-gonadal axis plays an important role in mediating opiate-induced analgesia (Pinsky et al, 1975), it may be expected that, in a situation where gonadotrophins are changing phasically (such as during the oestrous cycle), there may also be phasic alterations in nociception. The present study investigated whether a) pain latencies vary over the oestrous cycle and, if so, b) they are modified at any stage of the cycle by the opiate antagonist, naltrexone.

30 adult virgin female hooded-Lister rats (230-280g) were individually housed, under reversed lighting, for 14 days prior to the start of experimentation. On test days, animals were weighed and then injected with either saline, 0.5mg/kg or 1mg/kg naltrexone hydrochloride (n=10/gp). All injections were performed i.p. in a volume of 1ml/kg. 30 minutes post-injection, tail-flick latencies were established, following which all animals were subjected to saline lavage. This process was repeated at the same time daily, until all animals had been tested at each stage of the oestrous cycle.

Analysis of variance revealed no overall significant effects of test condition ($F(2,27) = 0.586$), stage of cycle ($F(3,81) = 1.99$) or any significant condition x stage of cycle interaction ($F(6,81) = 0.650$). These data suggest that pain latencies do not vary over the oestrous cycle and that naltrexone is without effect, irrespective of stage.

These findings are important in two main respects:- (1) they indicate that the reported changes in endorphin levels across the cycle are unrelated to naltrexone-sensitive analgesia mechanisms and (2), they exclude the possibility that the recently-reported hyperalgesia following copulatory experience in female rats (Hendrie and Rodgers, 1982) is an artefact of alterations in pain sensitivity across the cycle.

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