ROLE OF M_1 MUSCARINIC RECEPTORS IN K^+ FACILITATION OF PHOSPHATIDYL INOSITOL HYDROLYSIS

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We have recently shown that ambient K^+ concentrations of 20 and 50mmol/l facilitate carbachol stimulated phoshatidylinositol hydrolysis (PI) in the rat cerebral cortex (Candy et al, 1985). This facilitation by K^+ of PI hydrolysis was blocked by atropine indicating that the effect is mediated through muscarinic receptors (Candy et al, 1985). In this communication we report the effect of K^+ on both carbachol stimulated polyphosphoinositide metabolism and the sub-type of muscarinic receptor mediating the response.

Miniprisms (350x350µm) prepared from rat cerebral cortex were preincubated in Krebs Henseleit buffer (KHB) with myo-(2-^{3}H) inositol for 2h for both the antagonist and metabolism studies. In the antagonist experiments miniprisms were washed with excess buffer and incubated for a further 20mins in KHB in the presence or absence of 50nmol/l pirenzepine. 50µl aliquots of miniprisms were then incubated with 250µl KHB containing 10mmol/l Li^+ for 1h in the presence and absence of drugs and raised $[K^+]$ (K^+ was substituted for Na^+). 50nmol/l pirenzepine, antagonised the K^+ facilitation of carbachol stimulated PI hydrolysis (Table 1). The effect of muscarine 10^{-4}mol/l was similarly potentiated by K^+ 18mmol/l and the potentiated response was partially inhibited by 50nmol/l pirenzepine.

Table 1.

Effect of pirenzepine on K^+ facilitated carbachol stimulated PI hydrolysis

	Additions		
$[K^+]$mmol/l	0	Carbachol 10^{-4}mol/l	Carbachol 10^{-4}mol/l + pirenzepine 50nmol/l
6	0.050 ± .007	0.065 ± .012	0.056 ± .007
18	0.058 ± .014	0.173 ± .042	0.096 ± .027

(Results are expressed as dpm in inositol phosphate fraction/dpm in inositol phosphate and phospholipid fractions: means ± SD, $p<.01$ for effect of pirenzepine and K^+ on carbachol induced hydrolysis, 2-way ANOVA).

The tritiated inositol phosphates produced after a 10min incubation of prelabelled miniprisms with 18mmol/l K^+ and 1mmol/l carbachol were studied using ion-exchange chromatography according to the method of Berridge et al, 1983. Raised ambient $[K^+]$ together with carbachol elicited an increase in the labelling of inositol tris-, bis- and mono-phosphates.

These results show that K^+ facilitation of carbachol stimulated PI hydrolysis can be antagonised by a concentration of pirenzepine that is within the range for selective blockade of M_1 muscarinic receptors (Hammer et al, 1980), suggesting that the response is mediated through M_1 receptors. In addition K^+ induced facilitation of PI hydrolysis leads to an increase in inositol tris-, bis- and mono-phosphates indicating that the primary event may be the formation of inositol trisphosphate, a similar effect to that of carbachol alone (Berridge et al, 1983).

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POST-GANGLIONIC NEURONAL MUSCARINIC RECEPTORS IN TRANSMURALLY STIMULATED GUINEA-PIG TRACHEA

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Neuronal muscarinic receptors have been shown to attenuate bronchoconstriction induced by pre-ganglionic vagal nerve stimulation in cat and guinea-pig (Fryer & Maclagan, 1984). Gallamine was a specific antagonist and pilocarpine an agonist for these receptors. These drugs produced potentiation and inhibition of vagally-induced bronchoconstriction respectively, without altering the post-synaptic sensitivity to ACh. The following experiments were carried out to determine whether these neuronal muscarinic receptors have a pre- or post-ganglionic location by testing the effects of gallamine and pilocarpine on tracheal tube preparations stimulated at different loci in vivo and in vitro.

Guinea-pigs were anaesthetised with urethane (1.5 g kg^{-1} i.p.) and a cannula inserted in the thoracic portion of the trachea, low in the neck, taking care to preserve the tracheal blood and nerve supply. The animals were paralyzed with suxamethonium and artificially respired through the thoracic tracheal cannula. Blood pressure, heart rate and pressure in the thoracic trachea were recorded as described previously (Fryer & Maclagan, 1984). The cervical tracheal segment, with blood and nerve supplies intact, was tied at both ends to form a tube filled with saline. Changes in tracheal tube pressure were measured to indicate contraction of the trachealis muscle. Atropine-sensitive contractions of the tracheal tube could be induced by stimulation (30 V, 15 Hz, 0.2 ms, for 3 sec.) of the distal ends of the cut cervical vagi (pre-ganglionic) or by transmural stimulation in the presence of hexamethonium (post-ganglionic). In all experiments pretreatment with guanethidine (4 mg kg^{-1} i.v.) 30 minutes before the start of the experiment, prevented effects of sympathetic nerve stimulation.

Gallamine potentiated the contractions of the tracheal tube induced by both pre- and post-ganglionic stimulation in a dose dependent manner. A 2 to 3 fold potentiation occurred at 10 mg kg^{-1} gallamine i.v. The opposite effect occurred with pilocarpine (5-50 μg kg^{-1}) which inhibited contractions of the tube induced by transmural stimulation.

In some experiments, the tracheal tube was isolated, bathed in Krebs' solution and transmurally stimulated (Coleman & Levy, 1974). Both pilocarpine and McN A343, inhibited the contractions induced by post-ganglionic stimulation in vitro. This inhibition could be reversed by the subsequent addition of gallamine (10 μM).

Transmural stimulation of smaller conducting airways was not possible in vivo so DMPP (25 μg kg^{-1} i.v.) was used to stimulate the parasympathetic ganglia in anaesthetised animals. In these experiments bronchoconstriction was recorded as a rise in pressure from the thoracic tracheal cannula. After guanethidine pre-treatment DMPP produced an atropine-sensitive bronchoconstriction which was potentiated approximately 5 fold by gallamine (10 mg kg^{-1} i.v.).

These results demonstrate that inhibitory muscarinic receptors are present in the post-ganglionic, parasympathetic nerves innervating both the cervical trachea and the smaller conducting airways in the guinea-pig; a situation analogous to that found in the intestinal innervation (Dzieniszewski & Kilbinger, 1978). However, the possibility that muscarinic receptors may also have a ganglionic or pre-ganglionic location cannot be excluded.

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THE MECHANISM OF ACTION OF CHOLINERGIC ANTAGONISTS ON BOVINE CHROMAFFIN CELLS

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Ascher et al (1979) have demonstrated that the cholinergic blocking action of hexamethonium and tubocurarine on rat submandibular ganglion cells is voltage-dependent and probably results from a block of the ACh ion channel. In contrast the cholinergic blocking action of trimetaphan is voltage-independent, a result compatible with trimetaphan blocking the cholinergic receptor (Ascher et al, 1979). The neuromuscular blocking drugs pancuronium and vecuronium produce ganglion block at relatively high doses (Durant et al, 1979). Using the patch clamp technique we have investigated the actions of pancuronium, vecuronium and the ganglion blocker trimetaphan on cultured bovine chromaffin cells which possess cholinergic receptors pharmacologically similar to those of vertebrate autonomic ganglia (Fenwick et al, 1982) in an attempt to determine the site of action (cholinergic receptor or ion channel) of these compounds.

Bovine chromaffin cells were isolated, cultured and used 1-7 days after plating (Fenwick et al, 1982). The voltage clamped "whole cell" configuration of the patch clamp technique was used (Hamill et al, 1981) and all experiments were performed at room temperature (20 - 22°C).

ACh (100 μM) pressure applied from a modified patch pipette evoked an inward current on all cells tested (372 $\pm$ 45 pA, mean $\pm$ S.E., n = 12, holding potential -60 mV). The ACh-induced current amplitude was linearly related to holding potential in the range -20 mV to -100 mV, increasing with membrane hyperpolarisation. Bath application of the blocking agents with the cell membrane potential clamped at -60 mV produced a decrease of the ACh-induced current of 61 $\pm$ 4% (mean $\pm$ S.E., n = 5), 60 $\pm$ 9% (mean $\pm$ S.E., n = 5) and 80 $\pm$ 5% (mean $\pm$ S.E., n = 4) for pancuronium (10 μM), vecuronium (10 μM) and trimetaphan (3 μM) respectively. The blocking action of the antagonists reached equilibrium in 3 - 5 minutes and was reversible on washout.

For both pancuronium (10 μM) and vecuronium (10 μM) the cholinergic blocking effect was clearly voltage-dependent increasing e fold for a 27 $\pm$ 4 mV (mean $\pm$ S.E., n = 4) and 31 $\pm$ 6 mV (mean $\pm$ S.E., n = 4) hyperpolarisation respectively. There was no significant difference in the voltage sensitivity of the blocking effect for these agents. In contrast the blocking effect of trimetaphan (3 μM) showed no consistent dependence on membrane holding potential (-20 mV to -100 mV). In preliminary experiments neither pancuronium (10 μM) or vecuronium (10 μM) had any effect on ACh-induced outward currents recorded at +60 mV.

In conclusion pancuronium, vecuronium and trimetaphan antagonise the ACh-induced current in bovine chromaffin cells. The antagonism produced by trimetaphan is consistent with an action of trimetaphan to block the receptor recognition site, whereas the voltage-dependence of the pancuronium or vecuronium-induced cholinergic block suggests that these agents act primarily to block the cholinergic ion channel with little or no effect on the cholinergic receptor.

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M1 MUSCARINIC RECEPTOR SELECTIVITY OF McN-A-343 MAY NOT BE DUE TO RECEPTOR HETEROGENEITY

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McN-A-343 selectively stimulates the muscarinic receptors (mAChRs) present in the sympathetic ganglia and neural tissue but has little effect on the mAChRs present in smooth muscle (Roszkowski, 1961). Since M1 mAChRs are thought to be located on neural tissue and show selective affinity for pirenzepine (Hammer & Giachetti, 1982), McN-A-343 has been proposed as an M1 selective agonist. However, Mitchelson (1984) has reported that in the taenia caeci, pirenzepine antagonises the contractile responses to McN-A-343 and exhibits an affinity consistent with activation of M2 receptors. The aim of this study was to measure the potency and affinity of McN-A-343 at a range of mAChRs to determine if any selectivity could be demonstrated.

The agonist potency was assessed at the following guinea-pig tissues in vitro: ileum, atria (paced and non-paced), trachea and bladder. These tissues exhibit affinities towards pirenzepine characteristic of the M2 mAChR (Eglen & Whiting, 1985). Antagonist affinity was assessed at ileal and atrial receptors using carbachol as the agonist.

M1 receptor function was studied using the pithed rat in which stimulation of mAChRs in sympathetic ganglia produces an increase in blood pressure (Hammer & Giachetti, 1982). The affinity of McN-A-343 at cortical and myocardial membranes was assessed using ligand binding techniques. EDTA-washed membranes (Cheung et al, 1982) were assayed according to the method of Hammer et al (1980).

McN-A-343 exhibited no degree of agonism at any of the tissues studied. However, a hypertensive effect was observed in the pithed rat, in response to McN-A-343 (10, 30 and 100 $\mu g.kg^{-1}$ elicited 7%, 68% and 154% rises in blood pressure respectively). This selectivity between mAChRs present in smooth muscle and sympathetic ganglia has been observed by other workers (e.g. Mutschler and Lambrecht, 1984).

The affinities observed with the agonist at muscarinic binding sites present on cortical and myocardial membranes were similar (Ki cortex = 9.0×10^{-6} mol. $litre^{-1}$, 0.96 nH; Ki myocardium = 6.0×10^{-6} $mol.litre^{-1}$, 0.84 nH). This indicates that, in terms of affinity, McN-A-343 did not exhibit any appreciable degree of selectivity between M1 and M2 receptors. In addition, the affinities at ileal and atrial mAChRs were also very similar (pA_2 atria = 4.75; pA_2 ileum = 4.46; slopes not significantly different from unity) and these values were similar to those observed in the ligand binding experiments.

These studies indicate that McN-A-343 is an agonist at M1 and M2 receptors and appears not to be selective in terms of affinity. The apparent selective action of McN-A-343, which has been previously reported, may be just a reflection of a high receptor reserve in the ganglia as opposed to that in most other tissues studied. Therefore, a response to McN-A-343 should not be considered to be indicative of the presence of M1 receptors.

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α-ADRENOCEPTOR MEDIATED CONTRACTILE EFFECTS OF ADRENALINE IN CAT AIRWAYS

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The existence of α-adrenoceptors that mediate contraction of airway smooth muscle has been described in various mammalian species including man (Fleisch et al, 1970; Kneussl and Richardson, 1978). However their contribution to the overall neuronal and hormonal control of airway calibre is still unclear and in some instances, the functional presence of these receptors can only be demonstrated either in diseased tissue or following prior exposure to contractile agonists such as 5-HT and histamine (Barnes et al, 1983). In the present study we have investigated the nature of adrenaline-induced contraction of the cat isolated trachealis muscle in situ together with concomitant changes in intrathoracic airway calibre.

Male mongrel cats (2-3.5 kg) were anaesthetised initially with halothane (5%) and then chloralose (60-80 mg/kg i.v.), placed in a dorsal recumbent position, and mechanically ventilated (27 strokes/min of 15 ml of laboratory air/kg body wt) through a low cervical tracheostomy. A segment of cervical trachea approximately 2cm in length and containing 8 cartilage rings was prepared for measurement of changes in tension under isometric conditions as has been described previously (Lees and Payne, 1985). The load applied across the preparation was fixed at 10g equating to a resting tension of 5-6 g/cm longitudinal length. Pulmonary inflation pressure (an index of intrathoracic airway calibre) was measured via a lateral port in the tracheostomy circuit. Catheters were placed in both femoral veins and the left carotid artery for administration of test substances, and in a femoral artery for measurement of b.p./heart rate.

Bolus injections of adrenaline (0.001 - 1.0 μg/kg) into the left cranial thyroid artery via the carotid catheter had no effect on pulmonary inflation pressure but caused dose-dependent decreases in trachealis resting tension of up to 2.1 ± 0.3 g/cm (n=5). Following β-adrenoceptor blockade with propranolol (2 mg/kg i.v. + infusion of 20 μg/kg/min i.v.) these relaxant effects of adrenaline were converted into small, but initally dose-related increases in trachealis tension reaching a maximum of 0.4 ± 0.2 g/cm, thereafter declining as the dose-range was extended to 3 and 10 μg/kg i.a. At the same time adrenaline (0.3 - 10 μg/kg i.a.) provoked dose-related increases in pulmonary inflation pressure of up to 1.5 ± 0.3 cm H_2O). In the tracheal segment the contractile effect of adrenaline following propranolol was antagonised (dose-response curve shifted rightward approx. 20 fold) by additional pretreatment with either the α_1/α_2 antagonist phentolamine (3 mg/kg i.v., n=5) or the α_2 antagonist yohimbine (1 mg/kg i.v., n=5) but not by the α_1 antagonist prazosin (1 mg/kg, n=5). In contrast concomitant adrenaline-induced increases in pulmonary inflation pressure following propranolol were inhibited strongly by all three α-adrenoceptor antagonists.

These results suggest that in cat trachea the α-adrenoceptor subtype mediating adrenaline-induced contraction in vivo is predominantly of the post-junctional α_2 subtype as in canine trachea (Barnes et al, 1983). At the same time α-adrenoceptor mediated bronchoconstriction of intrathoracic airways appears to result from a combination of α_1 and post-junctional α_2 subtype activation although precise anatomical interpretation is necessarily complicated because of the possible involvement of a vascular component in this latter response.

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EFFECTS OF ALFUZOSIN AND IDAZOXAN ON NERVE MEDIATED CONTRACTIONS IN THE RABBIT ISOLATED URETHRA

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Alfuzosin, a selective α_1-adrenoceptor antagonist (Cavero et al., 1984a, b), blocks α_1-adrenoceptor mediated contractions in rabbit isolated bladder preparations and the increase in urethral pressure to hypogastric nerve stimulation in the anesthetised dog (Cavero et al., 1985). The present study describes the effects of alfuzosin on contractions evoked by either field stimulation or exogenous noradrenaline (NA) in rabbit isolated urethra preparations.

Rings of urethra (5 mm long), obtained from male rabbits (3-4 kg) were prepared according to the methods of Andersson et al. (1983), and mounted in Krebs' bicarbonate at 37°C containing propranolol (1 µM), and subjected to a 2 h equilibration at 1 g tension. Thereafter, contractions were evoked by electrical field stimulation (50 V, 2 ms, 1-48 Hz for 20 s), using electrodes placed on the tissue holder and in close proximity to the muscle.

Frequency contractile response curves ((E_{max} 3.7 $\pm$ 0.2 g, n=18) and responses to NA (30 µM; E_{max} 2.4 $\pm$ 0.2 g, n=14) were reproducible when repeated at 30 min intervals. The effects of electrical stimulation were abolished by tetrodotoxin (TTX, 1 µM), and strongly reduced (75-80%) by guanethidine (3 µM, n=4). Neither of these treatments antagonised the effects of NA. Stimulation-evoked responses were also significantly inhibited by atropine (0.1-10 µM), alfuzosin (0.1-1 µM), idazoxan (0.3 µM), or the combination of alfuzosin (1 µM) with atropine (1 µM). Alfuzosin (1 µM) or idazoxan (0.3 µM) significantly antagonised the contractions evoked by NA but not by carbachol (30 µM). Alfuzosin (1 µM) plus atropine (1 µM) and idazoxan (0.3 µM) did not however completely abolish the responses evoked by field stimulation.

In conclusion, as previously shown by Andersson et al. (1983), neurogenic contractions of the isolated rabbit urethra can be evoked by field stimulation. These responses were partially reduced by idazoxan or atropine, and greatly inhibited by guanethidine, alfuzosin, or alfuzosin plus atropine. However, they were virtually abolished after blockade of α_1/α_2-adrenoceptors and muscarinic receptors.

The results indicate that the male rabbit urethra is innervated by the sympathetic and parasympathetic nervous systems through α_1/α_2 adrenoceptors and muscarinic receptors, respectively. Endogenously released NA can evoke contraction in this preparation by postsynaptic α_1- and probably α_2-adrenoceptors.

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α_1- AND α_2-ADRENOCEPTOR-MEDIATED EFFECTS OF NORADRENALINE IN RAT GASTRIC FUNDUS

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α-adrenoceptor-mediated responses in rat gastric fundus include post-junctional α_1-adrenoceptor-mediated relaxation and pre-junctional α_2-adrenoceptor-mediated inhibition of cholinergic nerve-induced contractions(Dettmar et al, 1984, 1985; Verplanken et al, 1984). It has also been suggested that the pre-junctional $\alpha_2$adrenoceptors in rat gastric fundus are atypical with characteristics of both α_1- and α_2-adrenoceptors (Verplanken et al, 1984). The present experiments were carried out to assess the relative contribution of α_1- and α_2-adrenoceptors in the inhibitory effect of noradrenaline on cholinergic nerve-induced contractions.

Strips of rat gastric fundus were suspended in Krebs medium containing neostigmine (1 μM), propranolol (2 μM), guanethidine (5 μM), ascorbic acid (30 μM), EDTA (30 μM), cocaine (3 μM) and hydrocortisone (30 μM). Tone was induced by $BaCl_2$ (1-2 mM). Electrical field stimulation (EFS) was carried out via $Ag/AgCl_2$ ring and hook electrodes (1 ms pulse width, 0.5 Hz, supramaximal voltage).

In low tone EFS produced cholinergic nerve-induced contractions. Noradrenaline (0.03-1 μM) produced a dose related inhibition of these contractions which was partially reversed by idazoxan (0.03-1 μM) and prazosin (0.03-1 μM). The effect of idazoxan was much greater than that of prazosin and the effects of the two antagonists were additive (Table 1).

Table 1. Effects of idazoxan and prazosin on noradrenaline-induced inhibition of nerve-induced contractions in rat gastric fundus.

	% control responses	
	idazoxan first	prazosin first
noradrenaline 1 μM	8 ±2	7 ±1
+ idazoxan 1 μM	47 ±10[a]	-
+ prazosin 1 μM	-	20 ±2[a]
+ combination	59 ±9[b]	57 ±3[c]

significant differences: [a] from noradrenaline alone; [b] from noradrenaline + idazoxan; [c] from noradrenaline + prazosin; paired t test, $p<0.01$, $n=8$.

In raised tone, noradrenaline produced post-junctional α_1-adrenoceptor-mediated relaxation in the same concentration range as the inhibition of cholinergic nerve-induced responses, suggesting that the prazosin sensitive component of the inhibition is post-junctional.

In conclusion, noradrenaline inhibits cholinergic excitatory contractions in the rat gastric fundus partly by an action on inhibitory pre-junctional α_2-adrenoceptors and partly by an action on inhibitory α_1-adrenoceptors which are probably post-junctional.

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DIFFERENT DISPLACEMENT PROFILES OF α_2-ADRENOCEPTOR AGONISTS AND ANTAGONISTS VERSUS (^{3}H)-RAUWOLSCINE BINDING IN RAT CEREBRAL CORTEX

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Despite earlier results to the contrary (Cheung et al., 1982) a number of authors have recently suggested that ^{3}H-rauwolscine binds specifically to two sites on rat cortical membranes (Asakura et al., 1985). In this laboratory we have shown that the inclusion of spiperone (300nM) in the incubation buffer markedly attenuates the low affinity component of ^{3}H-rauwolscine binding; the remaining high affinity site retains the characteristics of an alpha$_2$-adrenoceptor (Broadhurst et al, in press). We have now examined the displacement of ^{3}H-rauwolscine from rat cortical membranes by a number of standard alpha$_2$-adrenoceptor agonists and antagonists in the presence and absence of spiperone. In each experiment ^{3}H-rauwolscine (1-2nM) was incubated (in the presence and absence of 300 nM spiperone and test drug) with rat cortical membranes (approx 0.4mg protein/ml) in 20mM HEPES buffer (pH 7.4) for 30 minutes at 25°C. The reaction was terminated by filtration (GF/B filters). Displacement curves were analysed by computerised non-linear curve-fitting (Allfit).

The displacement of ^{3}H-rauwolscine by all the test drugs in the absence of spiperone resulted in shallow inhibition curves with Hill slopes significantly less than unity (Table 1). Computer analysis revealed that all of the competition curves were best resolved into two components. The inclusion of spiperone (300nM) in the incubation buffer had little effect upon the displacement of ^{3}H-rauwolscine by clonidine, UK 14304, idazoxan and phentolamine whereas the displacement curves for rauwolscine and yohimbine were markedly steepened in the presence of spiperone. In the presence of spiperone the displacement curves generated by rauwolscine and yohimbine, but not the other test drugs, were best resolved into a single component.

Table 1. The effect of spiperone on ^{3}H-rauwolscine displacements

Conditions	Control		Spiperone	
Drug	IC_{50} (nM)	Slope	IC_{50} (nM)	Slope
Idazoxan	11.9±1.7	0.66±0.03*	11.4±4.2	0.68±0.06*
Rauwolscine	16.8±1.5	0.68±0.05*	14.5±2.2	1.16±0.20
Yohimbine	17.9±0.3	0.71±0.02*	20.7±5.0	1.06±0.17
Clonidine	74.2±27.1	0.54±0.07*	45.8±20	0.66±0.07*
UK 14304	75.7±11.5	0.60±0.10*	68.7±17.2	0.44±0.04*
Phentolamine	120±19.3	0.54±0.05*	94.3±12.4	0.65±0.01*

Values = mean ± SEM of 3 or more experiments. *$p < 0.05$, Students t test)
Phentolamine and idazoxan, but not rauwolscine or yohimbine, have been found to exhibit some functional intrinsic (agonist) activity at alpha$_2$ sites. This phenomenon, therefore, may well prove to be of importance in the preliminary detection of agonist activity in putative alpha$_2$-adrenoceptor antagonists.

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EFFECTS OF DESIPRAMINE AND YOHIMBINE ON PLASMA CATECHOLAMINES AND CORONARY LIGATION INDUCED ARRHYTHMIAS IN THE ANAESTHETISED RAT

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The effects of the uptake-1 blocker desipramine and the α_2-antagonist yohimbine on coronary ligation induced arrhythmias and plasma catecholamines (CA) have been studied in the anaesthetised male rat (Clark et al, 1980).

Drug or vehicle (saline) was administered i.v. 5 min before occlusion of the left coronary artery. Occlusion was maintained for 20 min and arrhythmias occurring during that period as premature ventricular contractions (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF) evaluated. In a separate study plasma samples obtained 3 min after coronary occlusion were assayed for noradrenaline (NA) and adrenaline (ADR) by alumina extraction and HPLC analysis (Eriksson and Persson, 1982).

Table 1. Effects of DMI and YOH on blood pressure (BP), heart rate (HR) and arrhythmias induced by coronary ligation.

Drug (mg/kg)	n	Δ BP (mmHg)	Δ HR (beats/min)	Total PVC	Incid. VT(%)	Incid. VF (%)	Deaths (%)
Saline	38	-1(2)	+6(3)	765(127)	92	29	11
DMI, 0.1	12	+18(4)t	+67(5)t	627(184)	83	33	8
DMI, 0.5	12	+13(2)t	+42(7)t	182(48) m	42x	Ox	O
DMI, 2.5	13	-8(5)	-22(8)t	765(171)	31x	Ox	O
YOH, 1.0	12	-17(5)t	-10(9)	222(107)m	33x	Ox	O
YOH/DMI, 1.0/0.1	12	+27(5)t	+115(8)t	343(150)m	33x	Ox	O

t $P<0.05$: paired t-test (5 min. post-drug v pre-drug). m $P<0.05$: Mann Whitney U-test (v control). x $P<0.05$: Chi squared test (v control). Data expressed as mean (s.e. mean).

DMI, 0.1 mg/kg, and YOH, 1.0 mg/kg, did not affect plasma CA when administered alone. When given together they caused an increase ($P<0.01$) in plasma NA, from 4.5 ± 0.7 to 10.1 ± 1.7 pmol/ml (n=6), without affecting plasma ADR. The antiarrhythmic action of YOH persisted in the presence of DMI despite the large increase in HR (Table 1). The antiarrhythmic doses of DMI (0.5 and 2.5mg/kg) reduced plasma ADR with no effect on plasma NA. The reduction in ADR from 12.7 ± 3.2 to 6.2 ± 1.7 pmol/ml (n=6) obtained with 2.5mg/kg was significant ($P<0.05$).

These results suggest that the antiarrhythmic effects of DMI and YOH *in vivo* are not directly related to changes in plasma CA and haemodynamic alterations. Both DMI (Tamargo et al, 1979) and YOH (Northover, 1983) have class I antiarrhythmic activity. However, the reduction in circulating ADR produced by DMI cannot be ruled out as a contributory factor in its antiarrhythmic action.

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THE EFFECT OF GRADED INCREASES IN HAEMOGLOBIN-OXYGEN AFFINITY ON MYOCARDIAL FUNCTION IN ISOLATED RABBIT HEARTS

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Pretreatment of anaesthetised dogs with BW12C, (a substituted benzaldehyde that induces a dose dependent increase in haemoglobin oxygen affinity, Beddell et al, 1984) significantly increases myocardial infarct size in an experimental occlusion/reperfusion model (Allan et al, 1984). The mechanism by which BW12C exerts this action is presumably due to a reduction in oxygen delivery to the myocardium during coronary reperfusion with a consequent impairment of reperfusion salvage. To further investigate the effects of impaired oxygen delivery on cardiac function we now report the effects of graded increases in haemoglobin oxygen affinity, induced by BW12C, on myocardial function in isolated rabbit hearts perfused with erythrocyte-enriched buffer.

Isovolumically beating rabbit hearts, paced at 180 beat min^{-1} were perfused via the aorta at 37°C with erythrocyte enriched modified Krebs-Henseleit buffer (Allan et al., 1985). Left ventricular pressure (LVP), left ventricular dP/dt (LVdP/dt max), and coronary perfusion pressure (CPP) were monitored continuously. Samples of pulmonary effluent were collected for the measurement of blood gases, lactate concentration and measurement of the oxy-haemoglobin dissociation curve (ODC).

Hearts were perfused for thirty minutes with normal erythrocytes, thirty minutes with erythrocytes with a left-shifted ODC of 10 ± 0.5 mmHg (1mM BW12C), 19 ± 0.7 mmHg (2mM BW12C) or 22 ± 0.5 mmHg (4mM BW12C) from a control p50 of 24.2 ± 0.9 mmHg.

Maximum changes induced in the measured parameters during perfusion with left-shifted erythrocytes (measured at 30 min of perfusion) are shown in the table below.

BW12C* (mM)	LVP (mmHg)	LV dP/dt $mmHgsec^{-1}$	Venous PO_2 mmHg	Myocardial lactate Production% $\frac{\text{Arterial-Venous}}{\text{Arterial}} \times 100$
1	-25±3	-75±95	-7.4±2.0	+18±24
2	-12±5**	-320±125**	-14.9±4.0**	+52±31**
4	-11±3**	-233±57**	-24.7±7.0**	+142±48**

* n=4-5 ** $p < 0.05$ from control values

On return to normal erythrocytes, all parameters returned towards control values. BW12C did not exert direct pharmacological effects (at the free concentration 10^{-3}M existing in the erythrocyte-enriched buffer) on hearts (n=4) perfused solely with Krebs-Henseleit buffer indicating that BW12C induced decreases in myocardial function are attributable to impaired oxygen supply.

Thus alterations in haemoglobin-oxygen affinity can affect myocardial function in vitro and in vivo. Agents like BW12C represent novel useful tools in studying the effects of altered haemoglobin oxygen affinity on tissue function.

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THE EFFECTS OF (±) 3-PPP AND ITS ENANTIOMERS AT PERIPHERAL NEURONAL DOPAMINE RECEPTORS IN THE CAT HEART

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(±)3-(3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP) is reported to be a selective agonist at dopamine autoreceptors in the CNS (Hjorth et al., 1981). However its enantiomers act as either agonists or antagonists at central dopamine receptor sites (Hjorth et al., 1983). We have therefore examined (±) 3-PPP and its enantiomers at peripheral neuronal dopamine receptors (DA_2) in the cat heart (Ilhan and Long, 1975).

Electrical stimulation (0.5Hz; 1ms; 10v) of the cardiac accelerans nerve of the chloralose anaesthetised cat increased resting heart rate (55 ± 9 beats/min). Dopamine (0.3-10 µg/kg i.v.) produced only a small inhibition (< 10%) of the stimulation evoked tachycardia. In contrast (±) 3-PPP (10-300 µg/kg i.v.) produced a more marked inhibition (up to 35%) of the tachycardia. When the neuronal catecholamine uptake process ($uptake_1$) was blocked with desmethylimipramine (DMI; 1 mg/kg i.v.) the extent of the tachycardia was increased (69 ± 3 beats/min) and the inhibitory effect of dopamine potentiated. However under these conditions the inhibitory effect of (±) 3-PPP was significantly reduced (fig. 1).

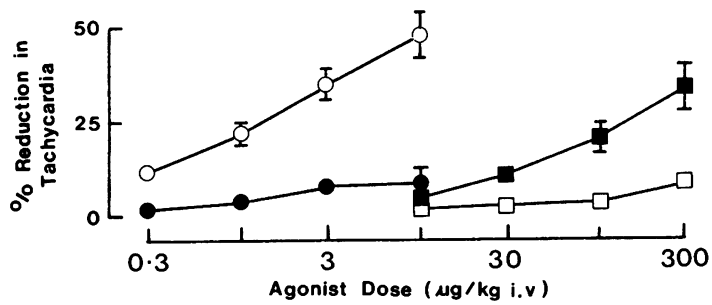

Fig. 1. The effect of dopamine or (±) 3-PPP before (●, ■ respectively) or after (○,□) DMI (1 mg/kg i.v.) Results shown are group means ± s.e. mean (n=4).

In order to determine why DMI should inhibit the effects of (±) 3-PPP, we examined the effects of the enantiomers of (±) 3-PPP. (+) 3-PPP (10-300 µg/kg i.v.) had little effect on resting heart rate whereas (-) and (±) 3-PPP both produced small increases in rate (<5 beats/min). DMI did not alter these effects. (-) 3-PPP (10-300 µg/kg i.v.) had little effect on the stimulation-evoked tachycardia either before or after administration of DMI. (+) 3-PPP (10-300 µg/kg i.v.) produced a dose-related inhibition of the tachycardia which was unaffected by DMI. The effects of (+) 3-PPP were shown to be mediated via neuronal dopamine receptors because its effect, like that of dopamine, was abolished by haloperidol (0.1 mg/kg i.v.). In further experiments, the inhibitory effects of (+) 3-PPP (10-300 µg/kg i.v.) on the stimulation-evoked tachycardia was relatively unaffected by the simultaneous administration of the same doses of (-) 3-PPP before administration of DMI but were markedly reduced by (-) 3-PPP after the inhibition of $uptake_1$ by DMI.

These findings are consistent with the hypothesis that the (-) enantiomer of (±) 3-PPP is removed by $uptake_1$ and allows the inhibitory effect of (+) 3-PPP at neuronal dopamine receptor sites, to occur. After inhibition of $uptake_1$ by DMI the (-)-enantiomer is not removed and acts as an antagonist of the (+)-enantiomer.

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CARDIOVASCULAR PROFILE OF QUINPIROLE, A SELECTIVE AND SPECIFIC DA_2-DOPAMINE RECEPTOR AGONIST, IN RATS

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Quinpirole (LY 171555), the levo-isomer of LY 141865 (Tsuruta et al., 1981) has been reported to decrease blood pressure in the anaesthetized monkey (Hahn and MacDonald, 1984). These effects are due to stimulation of DA_2-dopamine receptors since they were blocked by sulpiride. In this communication we have evaluated the mechanisms responsible for the hypotensive and bradycardic effects of quinpirole in rats.

Male rats (Sprague Dawley) weighing 200-250 g were anaesthetized with pentobarbitone (60 mg/kg i.p.) placed under artificial respiration and prepared for the measurement of aortic blood pressure and heart rate. In intact rats the effects of several doses of quinpirole (2.5-40.0 µg/kg/min i.v.) infused over 15 min were studied. Subsequently, the 10.0 µg/kg/min dose of quinpirole was further evaluated in rats pretreated with either i.v. ranitidine (0.5 mg/kg), haloperidol (0.3 mg/kg), S-sulpiride (0.3 mg/kg), SCH 23390 (0.12 mg/kg), chlorisondamine (0.5 mg/kg), (-)-timolol (0.3 mg/kg), idazoxan (0.3 mg/kg) or bilateral vagotomy. Vasoconstrictor responses to cirazoline, BHT-920, angiotensin II, 5-HT and electrical stimulation (60 V, 1 msec, 0.25-2 Hz for 30 sec) through the spinal outflow were studied in pithed rats pretreated with either saline or quinpirole. The compound was also studied in pithed rats in which a tachycardia of 100 beats/min was evoked by electrical stimulation of preganglionic or postganglionic cardioaccelerator nerve fibers (Cavero and Thiry, 1985).

Quinpirole produced dose-related decreases in blood pressure which reached a maximum at the end of the 15 min infusion and which were sustained for a further 30 min. The hypotensive effect (-42 $\pm$ 3 mmHg; n=5) evoked by quinpirole (10.0 µg/kg/min i.v. for 15 min) was completely blocked by S-sulpiride or haloperidol but not vagotomy, ranitidine, timolol or SCH 23390. Quinpirole did not change blood pressure in either pithed or chlorisondamine-pretreated rats. In pithed rats quinpirole antagonised only the pressor responses evoked by electrical stimulation of the spinal cord without affecting vasoconstriction elicited by cirazoline, BHT-920, 5-HT or angiotensin II. In intact rats, the hypotensive action of quinpirole (10.0 µg/kg/i.v. over 15 min) was accompanied by a decrease in heart rate which attained a maximum (119 $\pm$ 12 beats/min, n=5) 30 min after the end of the administration. This bradycardia was entirely blocked by haloperidol and partly antagonised by either bilateral vagotomy or timolol. Quinpirole significantly inhibited the tachycardia evoked by electrical stimulation of pre- or postganglionic cardioaccelerator nerve fibers. This effect was not modified by idazoxan but blocked by S-sulpiride.

In conclusion, quinpirole is a very selective and specific agonist of cardiovascular DA_2-dopamine receptors which are present on sympathetic nerve terminals of the resistance vessels and cardiac accelerator fibers in rats and which mediate an inhibition of neurally released noradrenaline.

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PERIAQUEDUCTAL GREY MATTER AS A SITE OF CENTRAL INHIBITION OF INTESTINAL MOTILITY IN THE RAT

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Neurotensin (NT), a tridecapeptide isolated from hypothalamic extracts but also detected in the gut, is peripherally involved in the regulation of gastric acid secretion and gastrointestinal motility following the ingestion of food, especially fat.Moreover, NT centrally administered was found to produce antinociception, hypothermia and reduced locomotory activity so suggesting that the peptide may play a modulatory role in the central nervous system. At this regard, we were able to demonstrate (Parolaro et al., 1983) that NT, like to morphine, intracerebroventricularly (i.c.v.) administered to rats, elicits a block of intestinal transit (tested by charcoal meal) linearly related to the log of administered doses, without inducing neither analgesia (tested by tail flick) nor hypothermia (at room temperature). The peptide results on molar basis 50 times more activity than morphine and pretreatment with naloxone does not antagonize this intestinal effect so suggesting a non opiate mechanism.

In the present work we examined the effect of local injections of NT into the various areas of the periaqueductal gray matter (PAG) on rat intestinal motility simultaneously evaluating behavioural and vegetative effects. NT was able to produce a full inhibition of intestinal transit at doses one third the maximal i.c.v.. At difference of morphine, this effect was localized only in the dorsal region of the PAG, regardless the anterior posterior dimension considered and was obtained without any evident behavioural effect. Moreover, to elucidate the possible nervous patwhay mediating this effect, the possible role of vagus nerve and serotoninergic system was studied respectively performing subdiaphragmatic vagotomy and electrolytic or chemical lesions into the Raphe magnus.

The existence of central non opiate peptidergic system modulating the intestinal propulsion is suggested.

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EVALUATION OF INHIBITORS OF THE RENIN-ANGIOTENSIN SYSTEM IN THE CONSCIOUS MARMOSET (CALLITHRIX JACCHUS)

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A conscious, chronically-cannulated marmoset preparation has been developed, which is suitable for the in vivo evaluation of inhibitors with specificity for human renin, since the enzyme-substrate reaction in this laboratory-bred primate is apparently homologous to that in man (Hofbauer et al, 1983). The chronic implantation of intravascular cannulae in the conscious animal obviates the use of anaesthesia during experiments. Anaesthetics stimulate renin release (Keeton & Campbell, 1980) and can modify the responsiveness of the cardiovascular system to drugs.

Adult marmosets of either sex (body-weight, approx. 300g) are surgically-anaesthetised (1 ml kg^{-1}, Saffan Anaesthetic Injection, Glaxo), and polythene cannulae (PP50) implanted into the left femoral vein and right femoral artery. Each cannula is passed subcutaneously to exit at the side of the tail approximately 3 cm from the base. The cannulae are filled with a sterile heparin solution (250 iu ml^{-1}) and sealed with stainless-steel plugs. After recovery, the preparation can be used to obtain stable direct arterial blood pressure and heart rate records in the conscious animal for periods up to 5h without the use of sedative drugs. Since the preparation can be used repeatedly, it enables multiple dose studies or cross-over design experiments to be performed in the same animals.

Dose-responses to i.v. administered angiotensin I (AI, 100- 1,600 ng kg^{-1}), angiotensin II (AII, 50- 800 ng kg^{-1}) and human renal renin (0.05- 0.8 G.U. kg^{-1}) have been obtained in this marmoset preparation (n=6). The dose-response curves to AI and AII were similar, except that AII was approximately twice as potent as AI. The dose-response curve to human renin was shallower and the response to each dose was more prolonged. We have demonstrated the renin inhibitory activity of the decapeptide human substrate analogue H142 (Szelke et al, 1982) and the angiotensin-converting enzyme inhibitory activity of captopril in the preparation. Intravenous infusion of H142 (0.1 mg kg^{-1} min^{-1} for approx. 40 min) reduced the submaximal pressor response to i.v. human renal renin from 98 ± 12.0 (mean ± s.e. mean, n=6) to 6 ± 3.5 mm Hg.min (area under response curve) after 10 min of infusion. Full recovery of the response occurred within 15 min of the end of the infusion. H142 did not affect the submaximal pressor response to i.v. AI. Oral administration of captopril (2.5 mg kg^{-1}) reduced the submaximal pressor response to i.v. AI from 67 ± 7.9 (n=4) to 6 ± 2.6 mm Hg.min. The response recovered partially to 14 ± 3.8 mm Hg.min 1.5h later. The submaximal pressor response to i.v. AII was unaffected by captopril. After acute sodium depletion by i.v. frusemide (5 mg kg^{-1}), an i.v. infusion of H142 (0.1 mg kg^{-1} min^{-1} for 25 min) reduced BP from 114 ± 5.4 (n=6) to 102 ± 6.1 mm Hg ($p<0.01$, paired t-test) after 10 min of infusion. The BP returned to the pre-infusion level within 10 min of the end of the infusion. In similar experiments using conscious sodium-deplete marmosets, i.v. captopril (1 mg kg^{-1}) reduced BP from 112 ± 5.4 (n=6) to 100 ± 7.1 mm Hg ($p<0.01$) within 10 min, and the BP stabilised at this reduced level for >2h.

We conclude that this small laboratory-bred primate preparation will be useful for investigating the cardiovascular effects of drugs, particularly inhibitors of the renin-angiotensin system with specificity for the human renin enzyme.

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CARDIOPROTECTIVE ACTIVITY IN VITRO OF L-ARGININE METHYLESTER DURING CARDIAC HYPOXIA AND REOXYGENATION

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L-Arginine methylester (L-Arg-Me) is an inhibitor of exocellular proteases (Troll et al., 1954) and during anoxia and reoxygenation there is an increase of lysosomal fragility (Decker et al., 1980). We decided to investigate whether L-Arg-Me possesses a cardioprotective action in a model of hypoxia without substrate followed by reoxygenation with substrate (glucose).

To accomplish these aims isolated guinea-pig hearts were perfused for 30 min with a medium gassed with N_2:CO_2 (97:3%) without glucose and for the next 30 min with a classic Tyrode solution gassed with O_2:CO_2 (97:3%); the following parameters were studied: ECG, tension, basal tone, LDH release in the effluent (Antonini et al., 1983). The administration of L-Arg-Me was started at the beginning of the hypoxic phase and continued throughout the experiment.

L-Arg-Me exerts its action both in hypoxic and reoxygenation phases although results show that it prevails during reoxygenation. In fact, 7.5 and 10 mM L-Arg-Me abolish ventricular fibrillation and reduce ventricular tachycardia during hypoxia and reoxygenation, while 5 mM L-Arg-Me abolishes ventricular fibrillation and reduces ventricular tachycardia only during the reoxygenation phase. Besides all three doses increase the recovery of normal electrical activity at the end of the experimental period, but only 7.5 and 10 mM L-Arg-Me reduce LDH release in coronary effluent both during hypoxia and reoxygenation. The effects of L-Arg-Me are dose-dependent and equimolar concentrations of L-Arginine do not mimic any effects of L-Arg-Me. The treatment of 10 mM L-Arg-Me does not prevent the increase of calcium tissue concentration, observed during reoxygenation. During hypoxia and reoxygenation there is an increase of lysosomal fragility as measured as the ratio between non-sedimentable and total cardiac cathepsin D (Barret, 1970) in the tissue. In the L-Arg-Me-treated hearts this increase is abolished.

Our results suggest a cardioprotective effect of L-Arg-Me and this is not due to an anesthetic-like activity because this compound failed to produce any anesthetic -like activity as measured with the Bianchi test (Bianchi, 1956). An antiarrhythmic effect was found in a model of legature of coronary and reperfusion (Pagella et al., 1984). As a matter of fact, L-Arg-Me is a stabilizer of lysosomes and this effect could explain the cardioprotective action of this compound.

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ANALYSIS OF ENDOGENOUS NORADRENALINE OUTFLOW FROM CARDIAC SYMPATHETIC EFFERENT FIBRES

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A more physiological approach to study the sympathetic regulation of neurotransmitter overflow in the mammalian heart is provided by isolated organ with functional innervation intact (Dart et al., 1983; Fuder et al., 1983). For this purpose the influence of the sympathetic nervous system in regulating myocardial function and noradrenaline (NE) release has been investigated in vitro on isolated hearts with the cardiac post-ganglionic fibers attached. Isolated rat hearts were perfused retrogradelly via the aorta (at a constant rate of 4.5 ml/min) while heart rate, force of contraction and coronary perfusion pressure were continuously recorded. One of the main nerves emerging from the right stellate ganglion was dissected and prepared for electrical stimulation with a bipolar platinum electrode. Before and after the stimulation period, samples of coronary effluent perfusate were collected from the cannulated pulmonary artery for catecholamine assay using an HPLC apparatus coupled with electrochemical detection.

In basal conditions, no NE was detected in the coronary effluent. The electrical stimulation of the cardiac nerve (8V, 3 msec, at 2.5-20 Hz, for periods of 1 min) produced an increase in heart rate and force of contraction, a decrease of perfusion pressure, while measurable amounts of NE were released in the perfusate. Such amount of released NE was related to the rate of electrical stimulation. Addition to the perfusion medium of 10^{-6} M desipramine (DMI), an inhibitor of NE neuronal reuptake, or 10^{-6} M yohimbine (YOH), a alpha$_2$ adrenergic receptor blocking agent, resulted in a marked enhancement of NE release during nerve stimulation. However, in the absence of electrical stimulation, both DMI and YOH failed to affect NE release and myocardial performance. Finally, 3×10^{-7} M propranolol (PROP), added to the perfusion medium after DMI (DMI+PROP), was able to abolish the response on heart rate, force of contraction and coronary perfusion pressure elicited by nerve stimulation, while the beta-blocker did not modify the overflow of endogenous NE as obtained with DMI alone.

The above results show the utility of this isolated organ nerve preparation for the analysis of myocardial metabolism of catecholamines and its modification by various pharmacological agents.

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BINDING OF ^{3}H-NITRENDIPINE DURING HYPOXIA AND SUBSEQUENT REOXYGENATION

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^{3}H Nitrendipine binding (^{3}H-Nit) has recently proved to be useful tools for studying the binding sites believed to exist on the calcium channel (Glossmann and Ferry, 1983). During reoxygenation which is preceded by hypoxia there is an alteration in the handling of calcium (Nakanishi et al., 1982). Consequently we have studied some characteristics of ^{3}H-Nit in cardiac membrane isolated from hypoxic and reoxygenated hearts. A previous report indicates that taurine exerts a cardioprotective effect in a model of hypoxia and reoxygenation (Franconi et al., 1985) and we investigated the binding of ^{3}H-Nit in taurine treated hearts.

Isolated guinea-pig heart was submitted to hypoxia in the absence of substrate for 30 min and reoxygenated in the presence of substrate for 30 min as described in Franconi et al., 1985. Binding assay was performed on ventricular tissue treated as in Williams and Lefkowitz, 1978. 40 µg of protein was incubated in a final volume of 0.125 ml of Tris-HCl (50 mM) pH 7.5 at a temperature of 25°C under sodium vapour lighting and bound ^{3}H-Nit was determined by liquid scintillation counting of radioactivity trapped on Whatman GF/B filters after rapid vacuum filtration. Specific ^{3}H-Nit binding was defined as the binding displaceable by cold nitrendipine (0.1 µM) and was approximately 70-80% of total binding with 1 nM of ^{3}H-Nit.

Displacement of ^{3}H-Nit by nifedipine (10^{-11} - 10^{-7} M) indicates that the ^{3}H-Nit binding is specific. The binding is protein dependent. Kinetic analysis (Weiland and Molinoff, 1981) give the following constant rate for association and dissociation. 1.82×10^8 $M^{-1} \times min^{-1}$ and 0.026 min^{-1} respectively being K_D 0.14 nM.

The hypoxic perfusion does not modify the ^{3}H-Nit time course, but it is statistically reduced when it is performed with material obtained from hypoxic and reoxygenated hearts. Taurine (20 mM) does not modify the binding of ^{3}H-Nit, but when the membranes were obtained by hypoxic and reoxygenated hearts treated with 10-20 mM taurine, the ^{3}H-Nit binding was practically identical to that of control heart.

In conclusion the ^{3}H-Nit binding in control situation appears similar to that described by others (Bellemann et al., 1981). Our data indicate that during reoxygenation there is a decrease in ^{3}H-Nit binding and taurine treatment prevents this decrease.

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EFFECT OF AMILORIDE ON VASCULAR SMOOTH MUSCLE CONTRACTION AND INHIBITION OF SODIUM-CALCIUM EXCHANGE

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The correlation between Na^+ and vascular smooth muscle tension is well known. Conditions leading to a net gain of Na^+ by the cell(i.e. removal of external K^+ or treatment with cardiac glycosides)induce an increased tension in arterial strips. Replacement of external Na^+ by choline or sucrose induces reversible contractures of arterial muscle. These contractures are associated with a net gain of Ca^{2+} by the tissue as a result of a decrease of Ca^{2+} efflux and an increase of Ca^{2+}influx. In fact it has been observed in arterial muscle that the muscle tension depends on the ratio $[Ca^{2+}]_o/[Na^+]_o$ in analogy to what was previously found for cardiac muscle(Blaustein,1977). These observations led to the conclusion that Ca^{2+} transport across the sarcolemma, by means of Na^+/Ca^{2+} exchange, plays a critical role in the maintenence of vascular smooth muscle tone.

Taking into account the above reported observations we considered worth investigating the effect of amiloride, a potassium sparing diuretic known as inhibitor of passive Na^+ transport, on vascular smooth muscle contraction. In fact it has been recently shown that amiloride inhibits Na^+/Ca^{2+} exchange in erythroleukemia cells (Smith et al.,1982), synaptic membranes (Schellenberg et al.,1983) and cardiac sarcolemmal vesicles (Floreani and Luciani,1984; Debetto et al.,1985).

Helical strips of guinea pig aorta were vertically suspended in an organ bath filled with physiological solution at 35°C and hung to an isometric transducer connected to a pen recorder. The preparation was allowed to stabilize for 90 min before strating the experiments. On the basis of Ozaki and Urakawa' observations(1979) we studied the effect of amiloride on guinea pig aorta under three different experimental conditions: 1)low-Na^+ medium(25 mM); 2)KCl-free medium; 3)incubation with ouabain(0.02 mM). All these conditions produced the appearance of persistent contracture, reaching its plateau within 120-180 min. The contracture was readily reversible when the physiological salt solution was readmitted. The above described modifications of the medium are considered to produce an influx of Ca^{2+} through the Na^+/Ca^{2+} exchange mechanism. Amiloride(0.05-0.10 mM)induces a 60-80% reduction of the developed tension. No significant modification was observed on the rate of appearance of the contracture. Under the same experimental conditions the Ca^{2+} blocking agent diltiazem does not affect the development of the contracture. The concentrations of amiloride that reduce the development of tension in guinea pig aortic strips are in the same range as those that increase the cardiac contractility. In guinea pig atria spontaneously beating on left atria electrically driven at 1 Hz (Floreani and Luciani,1984).

Cardiac sarcolemmal vesicles isolated from beef heart have been used to directly test the effect of amiloride on Na^+/Ca^{2+} exchange. 0.005-0.5 mM amiloride has been found to inhibit the Na^+/Ca^{2+} exchange in these preparations.

We can conclude that the effect of amiloride observed in guinea pig aorta, when Ca^{2+} influx id mediated by the Na^+/Ca^{2+} exchange(low-Na^+ medium)can be ascribed to inhibition of these antiport mechanism.

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DEFIBROTIDE'S THROMBOLYTIC ACTIVITY ON THROMBI OF DIFFERENT AGES IN THE RABBIT

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Experimental thrombosis was induced by insertion of a collagen coated nylon thread into the femoral vein of rabbits to study the time-course of thrombus evolution. Thrombi were followed from 1 hour till to 10 days and evaluated by their weights, protein contents and gross histological examinations. During the first 7 hours the thrombi grew in a near linear fashion ($R^2 = 0.85$), attaining their maximum weights and protein contents of 14.4 ± 0.6 mg and 11.7 ± 0.5 mg respectively between the 6th and 7th hour. Thereafter their weights and protein contents decreased very slowly to 3.1 ± 0.2 mg and 2.3 ± 0.2 mg respectively on the 10th day. From the histological point of view the younger thrombi appeared constituted mainly by red cells and loose fibrin network, looking like coagula. As they grew older a reorganization took place with proliferation of a granulation tissue and a decrease of fibrinous hemorrhagic depots.

Defibrotide (Niada et al., 1981; Mussoni et al., 1979; Porta et al., 1984; Bonomini et al., 1985) was i.v. administered under different schedules of treatment (i.v. bolus twice a day for 6 days, single infusion for 3 or 6 hours or single i.v. bolus + single infusion) to rabbits with thrombi of different ages. Defibrotide displayed a dose-dependent thrombolytic activity under all these experimental conditions.

Defibrotide was compared with Urokinase, Heparin, Dipyridamole, PGI_2, SP54, ASA and Ticlopidine. Urokinase and Dipyridamole had thrombolytic activity, while PGI_2, SP54 and Ticlopidine were modestly active. Heparin and ASA were ineffective. PGI_2, Ticlopidine and Dipyridamole at the doses tested induced strong blood pressure decreases. It is well known that Urokinase can cause a generalized degradation of plasma coagulant proteins, including fibrinogen, giving rise to a hemorrhagic state (Bell, 1976). On the contrary high doses of Defibrotide did not cause a generalized lytic state (unpublished results). Heparin was ineffective and this result is in agreement with the general thought that Heparin stops only thrombus formation. Moreover it has been shown that some thrombi can extend during Heparin theraphy (Marder et al., 1977).

In previous experiments it was found that Defibrotide increased tissue plasminogen activator in the rat (Mussoni et al., 1979). Defibrotide showed also a noteworthy dose-dependent profibrinolytic activity in different animal species (Porta et al., 1984). Moreover concomitant to profibrinolytic activity an increase both in total plasmin activity and in plasminogen levels, also after repeated injections, was found in the rabbit (Porta et al., 1984). While fibrinogen and α_2 antiplasmin normal levels were unmodified (unpublished results) in the rabbit, those abnormally high of FDPs were normalized both in animals (Porta et al., 1984) and man (Bonomini et al., 1985). The thrombolytic activities of Defibrotide, here reported, could be attributed almost in part to its profibrinolytic activity and to enhanced vascular PGI_2 production (Niada et al., 1981).

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PARTICIPATION OF OPIATE PEPTIDERGIC, GABAERGIC AND PROSTAGLANDIN MECHANISMS IN THE CARDIOVASCULAR EFFECTS OF CLONIDINE

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INTRODUCTION

Clonidine, an α_2-adrenergic stimulating drug, determine a dose-dependent hypotensive effect both in animals with normal arterial pressure and with spontaneous or DOC-hypertension (Donatelli and Marmo 1981). The arterial hypotension is associated with sinus bradycardia. The aim of the present investigation was to evaluate the participation of opiate peptidergic, GABAergic and prostaglandin mechanisms in the cardiovascular effect of clonidine on dogs and rats.

RESULTS

1. In anaesthetized dogs, clonidine administered orally (0.01, 0.1, 1 and 10 mg/kg) determined a dose-dependent hypotensive effect with sinus bradycardia. A pretreatment with naloxone (0.1 mg/kg i.m. 15 min before) partially ($P < 0.05$) reduced the hypotension induced by clonidine. Naloxone also reduced ($P < 0.05$) the bradycardia induced by clonidine.

2. In freely moving and anaesthetized rats either with normal arterial pressure or with spontaneous or DOC induced hypertension, i.c.v. (III ventricle; 2.5 mcg/an) or i.v. (0.1 mg/kg) administration of bicuculline 15 min before clonidine (1-5 mcg/an i.c.v. or 10 mcg/kg i.v.) reduced the arterial hypotension either in normotensive or hypertensive rats.

3. In conscious rats, with normal arterial pressure or with spontaneous or DOC-hypertension, an oral administration of NSAID (acetylsalycilic acid 25 mg/kg or flurbiprofen 5 mg/kg) 2 hours before clonidine (0.01-0.1 mg/kg orally) reduced the arterial hypotension with sinus bradycardia. In anaesthetized rats with normal arterial pressure or with spontaneous or DOC-hypertension an oral administration of flurbiprofen (5 mg/kg) 2 hours before reduced the arterial hypotension and sinus bradycardia of clonidine (20 mcg/kg/min for 30 min i.v.).

DISCUSSION

Our results suggest that also the peptidergic and GABAergic system is involved in cardiovascular effects of clonidine, an α_2-adrenergic stimulating agent. Also our experiments suggest that the hypotensive and bradycardic effects of clonidine in normotensive and hypertensive rats may in part be secondary to stimulation of prostaglandin biosynthesis.

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ALTERED CARDIOVASCULAR RESPONSIVENESS IN THE AGED RAT

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In this study we have examined the effects of ageing on cardiovascular responsiveness to alpha- and beta-adrenoceptor stimulation in anaesthetised or pithed rats.

Old male Sprague-Dawley rats (21-24 months, 500-700 g) and weight-matched young animals (5-7 months) were anaesthetised with pentobarbitone sodium and ventilated with 100% O_2. Some animals were pithed by the method of Gillespie et al. (1970) and the cardioaccelerator nerves were stimulated via the pithing rod. In all animals, the carotid artery was cannulated for blood pressure recording and the jugular vein was used for the administration of drugs.

In anaesthetised rats, resting diastolic blood pressure (DBP) was 121.8±5.2 mmHg (n=16) and 92.2±7.3 mmHg (n=20) and resting heart rate (HR) was 420±12 min^{-1} and 388±7 min^{-1} in young and old, respectively (for both HR and DBP, $P<0.05$ between groups). Noradrenaline (NA) (1 µg/kg) produced a rise in DBP of 39.1±4.6 mmHg (n=12) in young and 34.0±3.5 mmHg (n=11) in old rats (no significant difference). However, in the presence of prazosin (1 mg/kg) to eliminate $alpha_1$-adrenoceptor mediated responses, NA (1 µg/kg) was significantly more potent in young than in old, producing rises in DBP of 49.5±9.2 mmHg (n=4) and 14.8±1.8 mmHg (n=4), respectively ($P<0.01$). The beta-adrenoceptor agonist isoprenaline (10 ng/kg) produced a rise in HR of 32.9±4.7 min^{-1} (n=8) in young and 25.4±4.8 min^{-1} (n=8) in old rats, respectively (no significant difference).

In pithed rats, resting DBP was 35.3±3.3 mmHg (n=6) and 27.2±1.5 mmHg (n=7) ($P<0.05$) and resting HR was 308±12 min^{-1} and 291±14 min^{-1} (no significant difference) in young and old, respectively. Isoprenaline (10 ng/kg) produced a rise in HR of 95.8±9.8 min^{-1} (n=5) in young and 85.4±12.3 min^{-1} (n=6) in old rats (no significant difference). The cardioacceleration evoked by a single stimulus to the pithing rod electrode was 22.3±4.0 min^{-1} (n=7) in young and 15.8±3.5 min^{-1} (n=6) in old rats, respectively (no significant difference).

In conclusion, we are able to demonstrate a reduced responsiveness of vascular $alpha_2$-, without change in vascular $alpha_1$- or cardiac beta-, adrenoceptors in old rats. The most surprising finding is that there is no aged-related reduction in beta-adrenoceptor responsiveness in these rats, in contrast to the situation in man (Vestal et al., 1979).

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FCE 22509, A STABLE CARBOPROSTACYCLIN ANALOGUE: ANTIAGGREGANT, CARDIOVASCULAR AND RESPIRATORY ACTIVITY

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FCE 22509 | 5-(Z,E)-13,14-didehydro-20 methyl-carboprostacyclin | in vitro inhibits platelet aggregation a) in human PRP induced by ADP (IC_{50}=5.9 ng/ml compared to 2.2 ng/ml for PGI_2), collagen (IC_{50}=25 vs 3.6 ng/ml), epinephrine (IC_{50}=43 vs 13 ng/ml) and Ca^+ ionophore (IC_{50}=57 vs 14.5 ng/ml); b) in rat PRP induced by ADP (IC_{50}=7.7 vs 1.5 ng/ml) and c) in guinea pig PRP induced by ADP (IC_{50}=2 vs 0.34 ng/ml) and PAF (IC_{50}=0.36 vs 0.14 ng/ml for PGI_2). FCE 22509 inhibits ex vivo ADP-induced platelet aggregation in rat PRP (ED_{50}=260 mcg/kg s.c. and ED_{50}=45.2 mcg/kg i.v. vs 45.7 mcg/kg i.v. for PGI_2) and in vivo in the mouse reduces mortality induced by collagen + adrenaline (ED_{50}=350 mcg/kg s.c.).FCE 22509 deaggregates platelet clumps formed on rabbit Achilles tendon bathed with heparinized cat blood with an ED_{50}=7.6 mcg/kg i.v. vs 1.6 mcg/kg i.v. for PGI_2.

FCE 22509 is less potent than PGI_2 in lowering mean systemic arterial pressure (MSAP) in conscious normotensive (ED_{25}=11.5 vs 2.1 mcg/kg i.v. and 1.03 vs 0.5 mg/kg p.o.) and in spontaneously hypertensive rats (ED_{25}=4.8 vs 2.3 mcg/kg i.v. and 0.75 vs 0.49 mg/kg p.o.). Heart rate (HR) was increased but not dose-dependently. In the open chest anaesthetized dog, the hypotensive effect of FCE 22509 is again weaker than that of PGI_2 (-30.0% and 50.0% respectively at 0.4 mcg/kg i.v.). and, like PGI_2, it does not modify HR and mean pulmonary arterial pressure (MPAP). In the open chest anaesthetized cat FCE 22509 infused at the same dose as PGI_2 (0.2 mcg/kg/min x 15 minutes) is equipotent to PGI_2 in inhibiting ex vivo platelet aggregation induced by ADP but, unlike PGI_2, does not lower MSAP and MPAP.

FCE 22509 orally administered to the guinea pig caused slight, short-lasting, not dose-related flushing, less than that induced by PGI_2 and far less than that by nicotinic acid.

Intravenous FCE 22509 in the spontaneously breathing anaesthetized dog caused, like PGI_2, a dose-dependent increase in respiratory rate, closely correlated with a decrease in pause time, and in respiratory flow, with no significant changes in tidal volume, inspiratory and expiratory time, transpulmonary pressure, lung compliance and airway resistance. These modifications in respiratory rate and flow were probably related to the marked decrease in systemic arterial pressure (1).

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CENTRAL NERVOUS SYSTEM (CNS) CONTROL OF ARTERIAL BLOOD PRESSURE IN STZ DIABETIC RATS

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Despite frequent descriptions of central nervous system (CNS) specific lesions in the diabetes, the existence of a diabetic encephalopaty is still questioned. Aim of this study was to evaluate how CNS controls arterial blood pressure in streptozotocin (STZ) induced diabetes. In order to do this we examined cardiovascular responsiveness to cerebral stimulation of cholinergic and gabaergic systems in one and three week diabetic rats treated or not with insulin. We also evaluated acethylcholine (Ach) and GABA concentration in several brain areas. Male rats were made diabetic by an intravenous (iv) injection of STZ (40 mg/Kg) and 7 or 21 days after experiments were carried out in freely moving animals with chronically implanted intracerebroventricular (icv) and intraarterial cannulae. Citrate buffer (pH 4.5) treated rats were used as controls. Icv injection of carbachol (125, 250, 500 ng) or physostigmine (1.25, 2.5, 5 mcg), in one week STZ rats produced a dose-dependent increase in mean arterial pressure (MAP) and heart rate (HR) which resulted to be more pronounced in diabetic than in control animals. No changes were observed in three week diabetic animals. Icv injection of ethanolamine-O-sulphate (5, 10, 20, 40 M) and muscimol (0.5, 1, 5 mcg), caused a dose-dependent decrease in MAP and HR significantly higher in one and three week animals than in controls. Pressor responses following angiotensin II (100, 200 ng) did not show differences between control and diabetic animals, thus rouling out an impairment of peripheral nerve conduction. Ach brain concentration was modified only within one week, while GABA brain content was impaired both in one and three week animals. Daily insulin treatment was able to revert all these cardiovascular and biochemical changes.
Our data strongly suggest that STZ-induced diabetes might produce a diabetic encephalopaty which is reverted by insulin treatment.
Key words: Central Nervous System - Arterial blood pressure - Streptozotocin induced diabetes.

SK&F 94120 A SELECTIVE PDE III INHIBITOR POTENTIATES ISOPRENALINE INOTROPIC BUT NOT CHRONOTROPIC RESPONSES IN THE GUINEA-PIG HEART

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There is evidence that theophylline and 3-isobutyl-1-methylxanthine (IBMX), which are non-selective phosphodiesterase (PDE) inhibitors, potentiate both the positive inotropic and chronotropic actions of isoprenaline in the guinea-pig heart in vitro (Broadley and Wilson, 1980). SK&F 94120 has recently been shown to be a very selective inhibitor (IC_{50} 1.1 x 10^{-5}M, Reeves personal communication) of PDE III from guinea-pig ventricular myocardium (Gristwood et al., 1985) and we have now investigated the effects of this on guinea-pig myocardial isoprenaline responses in vitro.

Cumulative concentration-response curves (CRC) to isoprenaline (Iso) were obtained in electrically driven (at 1 Hz) right ventricular strips and spontaneously beating right atria, both before and after exposure of tissues to SK&F 94120. Tissues were incubated in Krebs solution at 37°C. Responses were measured as absolute increases in rate or force of contraction and geometric mean EC_{50} values calculated. In the absence of SK&F 94120, repeated Iso CRC for both tissues were similar to initial CRC.

In right ventricle strips, SK&F 94120 at 1 x 10^{-5}M caused a mean (± s.e. mean) 25% ± 4% increase in force of contraction (n=6) and produced a parallel displacement of the repeated Iso CRC to the left with no significant change in the maximum response. The EC_{50} value for the repeated Iso CRC of 1.0 x 10^{-9}M (0.7 - 1.45 x 10^{-9}M, 95% confidence limits) was significantly lower than that for the initial CRC of 4.0 x 10^{-9}M (3.0 - 5.2 x 10^{-9}M) indicating a 4 fold increase in Iso inotropic potency.

In right atria SK&F 94120, also at 1 x 10^{-5}M, caused a 12% ± 1% increase in rate (n=4) but had no effect on the Iso CRC, the initial and repeated EC_{50} values being 3.1 x 10^{-9}M (1.8 - 5.6 x 10^{-9}M) and 3.6 x 10^{-9}M (1.1 - 12.3 x 10^{-9}M) respectively.

The effects of SK&F 94120 on Iso inotropic and chronotropic responses were also studied in guinea-pig isolated working hearts. Iso at 1 x 10^{-9}M which was administered before and after incubation of hearts with SK&F 94120 at 3.16 x 10^{-5}M (n=3) caused increases in sinus rate of 22 ± 12 beats/min and 14 ± 2 beats/min respectively (difference not significant) and increases in dLVP/dt max (an index of left ventricular contractility) of 176 ± 91 mmHg/sec and 530 ± 115 mmHg/sec respectively (difference significant).

The above data indicate that SK&F 94120 potentiates the inotropic but not chronotropic actions of Iso in the guinea-pig heart. Both right atrial rate and ventricular force responses to Iso are believed to be mediated via increases in intracellular cyclic AMP, thus, it is not clear why SK&F 94120 unlike IBMX and theophylline does not potentiate Iso right atrial rate responses. The difference may be related to the selectivity of PDE inhibition by SK&F 94120.

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SPECIFIC INHIBITION OF TYPE III PHOSPHODIESTERASE ACTIVITY: COMPARISON WITH POSITIVE INOTROPIC POTENCY

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SK&F 94120 is a novel potent inotropic agent which also possesses vasodilator activity (Coates et al, 1985). SK&F 94120 has previously been shown to selectively inhibit a 'low Km' type III phosphodiesterase (PDE) activity from guinea-pig cardiac ventricle with a K_i of 5.4 μM (Gristwood et al, 1985). Inhibition of type III PDE was suggested to be the primary mechanism of action of SK&F 94120 as a positive inotropic agent.

We have obtained a superior preparation of type III PDE from the cat cardiac ventricle which, unlike the guinea-pig enzyme, gives a single 'low Km' activity possessing simple Michaelis-Menten kinetics. K_i values have been measured for a range of positive inotropic agents believed to work via type III PDE inhibition. Their respective K_i values have been compared with their effects as positive inotropes as measured by the concentration which caused a 50% increase in the force of contraction in the guinea-pig ventricular strips (see table).

The rank order of potency as type III inhibitors i.e. Milrinone > CI 914 > Fenoximone > SK&F 94120 > Amrinone compares well with the order of potency as positive inotropes i.e. Milrinone > CI 914 > SK&F 94120 > Fenoximone > Amrinone.

This correlation of the potency of type III PDE inhibition and positive inotropic effect is taken as further evidence that the primary mechanism of action of these compounds, as positive inotropic agents, is via type III phosphodiesterase inhibition.

	K_i (type III) μM	$E_{50\%}$ (ventricle strip) μM
Milrinone	0.8	3.4
CI 914	1.4	4.2
Fenoximone	3.0	8.8
SK&F 94120	4.4	6.0
Amrinone	19.4	15.0

Comparison of K_i and $E_{50\%}$ for a range of positive inotropic/type III inhibitors

Coates, W.J. et al. Br. J. Pharmac. 84, 22P.
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ALTERATIONS IN BRAIN DOPAMINE RECEPTOR FUNCTION FOLLOWING CHRONIC ADMINISTRATION OF L-DOPA, BROMOCRIPTINE OR PERGOLIDE TO RATS

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Chronic L-DOPA therapy in Parkinsonian patients results in a loss of therapeutic response and the onset of motor abnormalities (Marsden & Parkes, 1976; 1977). Chronic L-DOPA or dopamine agonist treatment may alter brain dopamine function. We now investigate the effect of oral administration via drinking water of L-DOPA plus carbidopa or bromocriptine or pergolide to rats for up to 12 months on brain dopamine function.

Male Wistar rats (250 $\pm$ 2 g at the start of the experiment; Bantin & Kingman) received one of the following: a) distilled water, b) L-DOPA (target dose 200 mg/kg/day) plus carbidopa (target dose 25 mg/kg/day), c) bromocriptine (target dose 5 mg/kg/day), d) pergolide (target dose 0.5 mg/kg/day) continuously for 12 months. Target doses were based on those used to control Parkinson's disease on a mg/kg bases multiplied by an arbitary factor of five times.

Administration of pergolide (0.36-0.51 mg/kg/day) enhanced locomotor activity after 1,3 and 12 months but not at 6 months (Table 1). Administration of L-DOPA (153-173 mg/kg/day) plus carbidopa (19-22 mg/kg/day) reduced locomotor activity after 6 months drug intake but not at other times. Bromocriptine (3.3-4.6 mg/kg/day) treatment did not alter locomotor activity. Apomorphine (0.06-1.0 mg/kg/sc) -induced stereotyped behaviour was enhanced after 1,3,6 and 12 months administration of pergolide and after 1 month intake of bromocriptine but not at later times. Stereotyped behaviour was unaltered by L-DOPA plus carbidopa administration. Specific ^{3}H-spiperone (0.03-1.0 nM; defined using 10^{-5}M ($\pm$)-sulpiride) binding (Bmax) to striatal membranes was not altered by L-DOPA plus carbidopa or bromocriptine treatment but was decreased at 6 and 12 months of pergolide treatment; no effect was observed at 1 and 3 months. Following 12 months drug administration, specific ^{3}H-spiperone binding to mesolimbic (tuberculum and accumbens) membrane was not altered by any drug treatment (Table 1).

Table 1 Effect of 12 month administration of dopamine agonist on spontaneous locomotor activity, apomorphine(0.06-1.0 mg/kg sc) induced stereotypy and specific ^{3}H-spiperone binding comparison with age-matched controls

Treatment	Locomotor activity (counts/20 min)	Apomorphine stereotypy ED_{50}(mg/kg)	^{3}H-Spiperone binding Bmax (pmol/g) Striatum	Mesolimbic
Control	303$\pm$27	0.15$\pm$0.02	20.1$\pm$0.5	7.65$\pm$0.67
L-DOPA/carbidopa	380$\pm$29	0.15$\pm$0.03	17.6$\pm$2.0	6.42$\pm$0.43
Bromocriptine	342$\pm$16	0.13$\pm$0.02	17.9$\pm$1.0	6.63$\pm$0.45
Pergolide	473$\pm$39*	0.06$\pm$0.02*	13.6$\pm$0.7*	6.28$\pm$0.24

* $P < 0.05$ compared to age-matched control animals

Chronic administration of L-DOPA plus carbidopa or bromocriptine produced little or no change in brain dopamine function during 12 months treatment. Pergolide treatment appears to produce behavioural supersensitivity as shown by enhanced locomotor activity and stereotyped behaviour but this is accompanied by a decrease in striatal, but not mesolimbic, dopamine D-2 receptors.

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SPECIFIC INHIBITION OF D_1-MEDIATED cAMP FORMATION BY D_2, MUSCARINIC AND OPIATE RECEPTOR STIMULATION IN RAT STRIATUM

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It is now thought that a variety of neurotransmitters can induce a receptor-mediated inhibition of adenylate cyclase activity in several cells. In the rat striatum, stimulation of dopamine D_2, muscarinic cholinergic and opiate receptors inhibit the increase in cyclic AMP accumulation evoked by dopamine D_1 receptor stimulation (Stoof & Kebabian, 1981; Olianas et al. 1983; Minneman, 1977). In this study we have investigated the specificity of these effects by comparing the ability of a D_2 specific agonist (N-n-propyl-di-β(3-hydroxyphenyl)ethylamine; RU24926), carbachol and morphine to inhibit cyclic AMP formation due to D_1 receptor stimulation in striatal slices with their ability to suppress cyclic AMP accumulation induced by other receptor agonists and cholera toxin.

Rat striatal slices (0.35 x 0.35 mm) were incubated in Krebs buffer containing 1 mM IBMX for 90 min. 20 µl Aliquots were then incubated in the presence or absence of agonist for a further 20 min before estimation of tissue cyclic AMP content. Where necessary, tissue was preincubated with antagonist for 5 min.

Neither RU24926, carbachol nor morphine (all 10^{-8}–10^{-5} M) affected basal levels of cyclic AMP in striatal slices. The D_1 specific agonist, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SKF38393; 1 µM) increased cyclic AMP levels from 12.99 ± 0.84 to 22.48 ± 1.45 pmoles/mg protein, an effect abolished by the D_1 antagonist 7-chloro-2,3,4,5-tetrahydro-3-methyl 5-phenyl-1H-3-benzazepine-7-ol (SCH23390; 1 µM) but unaffected by the D_2 antagonist (-)-sulpiride (10 µM). The stimulation of cyclic AMP produced by SKF38393 (1 µM) was inhibited in a dose-dependent fashion by RU24926, carbachol and morphine (all 10^{-8}–10^{-5} M). Maximum inhibition of between 70-95% of the SKF38393 response was observed in each case. These effects were in turn antagonised by (-)-sulpiride (10 µM), atropine (1 µM) and naloxone (1 µM) respectively. However, RU24926, carbachol and morphine (all 1 & 10 µM) did not inhibit cyclic AMP accumulation produced by isoprenaline (10 µM), vasoactive intestinal polypeptide (0.25 µM), PGE_1 (1 µM), 2-chloroadenosine (100 µM, in the absence of IBMX) or unilateral intrastriatal injection of cholera toxin (4 µg 20-24 h previously).

We have also investigated whether the inhibitory effects of D_2 and muscarinic receptors on D_1-stimulated cyclic AMP formation are mediated by Ni, the GTP binding protein associated with inhibition of cyclase. Initial results indicate that prior intrastriatal injection of the Ni-inactivating agent, pertussis toxin (2 µg 20-24 h previously) at least partially reverses the ability of RU24926 (1 µM) and carbachol (10 µM) to inhibit D_1-stimulated cyclic AMP formation.

These results indicate that the inhibition of striatal cyclic AMP accumulation induced by D_2, muscarinic and opiate receptor stimulation is specific for D_1 receptors and is probably mediated by pertussis toxin sensitive Ni protein. Whether such mechanisms relate to the ability of these cyclase inhibiting agonists to modulate motor behaviour remains to be established.

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SCH 23390 LOSES ITS HIGH AFFINITY FOR [^{3}H]-PIFLUTIXOL BINDING SITES IN PIG SOLUBILISED STRIATAL MEMBRANES

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SCH 23390 is a novel benzazepine derivative with high affinity for D1 dopamine receptors (Hyttel, 1983). Here we report on the characterisation of [^{3}H]-piflutixol binding sites in solubilised pig striatal membranes with particular reference to the affinity of SCH 23390 for these sites.

Sow striata were obtained within 10 min of slaughter at the local abattoir and stored at -70°C. A P2 pellet was prepared according to the method of Woodruff & Freedman (1981). For binding studies in the native membrane preparation, the P2 pellet was resuspended in 10 vol Tris-HCl/120 mM NaCl, pH 7.4. 100-200 µg protein were incubated with 1 nM [^{3}H]-piflutixol (11.8 Ci/mMol) in the presence and absence of drugs for 45 min at 25°C in Tris-HCl/120 mM NaCl, pH 7.4, at a final incubation volume of 500 µl. Specific binding was defined by 10 µM cis-flupenthixol. The incubate was filtered through Whatman GF/C filters and washed with 35 ml ice-cold buffer. Bound radioactivitiy was assayed by liquid scintillation spectrophotometry. The P2 pellet was solubilised by resuspension and thorough homogenisation in 10 vol 10 mM CHAPS (3-3-cholamido-propyl-dimethylammonio-1-propane sulphonate), 0.72 M NaCl in Tris-HCl pH 7.4. The homogenate was centrifuged at 100,000g for 60 min. 250 µl of the soluble fraction was incubated with 0.01-5 nM [^{3}H]-piflutixol (11.8 Ci/mMol) in the presence and absence of drugs for 90 min at 25°C in Tris-HCl pH 7.4 at a final incubation volume of 1.5 ml. Specific binding was defined by 10 µM cis-flupenthixol. 300 µl 10% charcoal, 2% dextran precipitated the unbound ligand and 1 ml of the supernatant was assayed for radioactivity by liquid scintillation spectrophotometry. Protein content was estimated by the method of Lowry (1951) using bovine serum albumin as standard.

Saturation analysis in the soluble fraction indicated that [^{3}H]-piflutixol bound to a single, saturable high affinity site; K_D 0.34 ± 0.02 nM, Bmax 170 ± 28 fmol/mg protein, Hill coefficient 1.28 ± 0.21 (n=3). The K_D calculated from kinetic data was 0.25 nM. Binding was linear with respect to protein concentration, inactivated at 50°C with a t1/2 of 1.3 min, and inhibited by the sulphydryl alkylating agent N-ethylmaleimide with an IC_{50} of 5.8 x 10^{-4}M (n=2). The potency of dopamine receptor agonists and antagonists at the binding site was: fluphenzaine, 14 nM; cis-flupenthixol, 33 nM; (+)-butaclamol, 36 nM, trans-flupenthixol, 1.1 µM; domperidone, 1.1 µM; (±)-sulpiride, 1.5 µM; spiroperidol, 1.6 µM; apomorphine 5.8 µM; SCH 23390, 19.3 µM; ADTN, 39.8 µM, (n=2). Of a range of other drugs tested, (promethazine, GABA, atropine, cimetidine, nomifensine, noradrenaline and ketanserin), only promethazine displaced a significant proportion of [^{3}H]-piflutixol binding at a concentration less than 1 µM with an IC_{50} of 602 nM. SCH23390 was tested against [^{3}H]-piflutixol bound to the native membrane preparation and was found to have an IC_{50} of 1.8 nM (n=2). The IC_{50} for cis-flupenthixol in the membrane was 4.6 nM (n = 3).

The low affinity of SCH23390 for the [^{3}H]-piflutixol binding site in the soluble fraction could be explained by the loss of a component, essential for its high affinity, upon solubilisation.

We are grateful to Dr. J. Hyttel (H. Lundbeck & Co A/S) for a gift of [^{3}H]- piflutixol.

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NOMIFENSINE INCREASES STIMULATED DOPAMINE RELEASE IN VIVO BY AN ACTION ON THE DOPAMINE STORAGE POOL: VOLTAMMETRIC EVIDENCE

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Nomifensine (10 - 30 mg/kg) causes stereotypy and increases striatal HVA in rats, indicating elevated dopamine (DA) release. Both effects can be abolished by pretreatment with reserpine, but not alpha methyl-p-tyrosine (AMPT), (Braestrup, 1977). In the present study the effect of nomifensine on electrically stimulated DA release was examined in vivo after pretreatment with AMPT or Ro 4-1284, a fast-acting reserpine-like drug (Pletscher, 1977).

Striatal DA release was measured using high speed cyclic voltammetry in chloral hydrate-anaesthetised rats as previously described (Millar et al, 1985). Ten stimulations of the median forebrain bundle (10 s train, 50 Hz sine waves, 80 - 100 µA r.m.s.) were performed, 20 minutes apart. Nomifensine maleate (20 mg/kg i.p.) was given after the 4th or 7th stimulation. AMPT (250 mg/kg i.p.) or Ro 4-1284 (1 mg/kg i.p.) was given after the 4th stimulation. The results are shown in Figure 1 (below).

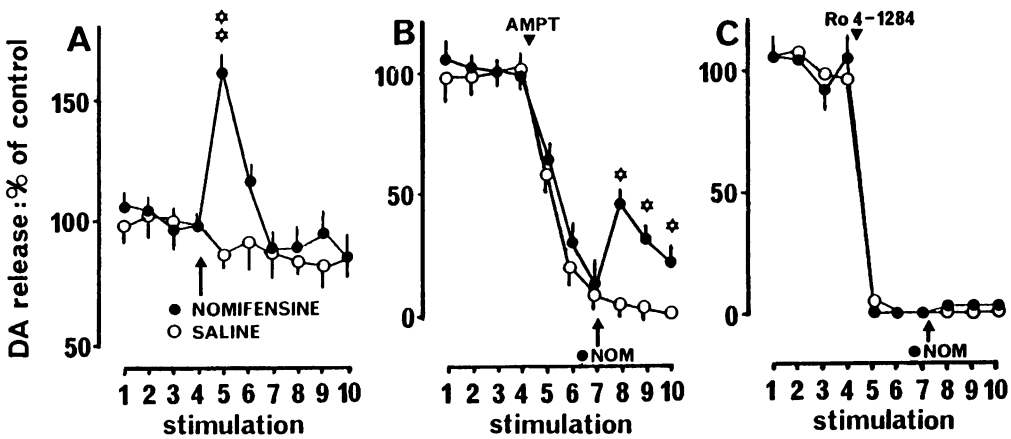

Figure 1 : Stimulated DA release. Means ± s.e.m. (n = 4/5)
✡ P < 0.05 ✡✡ P < 0.02. Mann Whitney U test.

Nomifensine increased stimulated DA release by 60% (Fig. 1A). AMPT decreased release by 95 - 100% but did not prevent the response to nomifensine (Fig. 1B). Ro 4-1284 abolished DA release. Nomifensine was unable to restore release after Ro 4-1284 (Fig. 1C).

AMPT blocks DA synthesis but leaves tissue stores largely intact. Ro 4-1284 disrupts granular DA storage (Shore, 1976). Fig. 1 shows that nomifensine requires intact granular DA storage to enhance impulse-dependent release. This is consistent with the behavioural and biochemical actions of the drug.

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CENTRAL HIGH AFFINITY BINDING OF [^{3}H]-TYRAMINE

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Tyramine (TA) is a 'trace amine' that is unevenly distributed in mammalian neural tissues. No definite functional role for the p- and m-isomers of TA has been as yet demonstrated, though both have been implied in the pathogenesis of depressive illness and schizophrenia. Based upon electrophysiological (Jones,1984) and behavioural (Stoof et al.,1976) studies, many of the neuronal effects of trace amines would be triggered by specific receptors in the CNS.

Optimum conditions for the binding of ^{3}H-p-tyramine (^{3}H-TA) to partially purified rat brain membranes were here ascertained. The specific binding of ^{3}H-TA (0.5-32 nM) was defined as that displaced by 10 μM p-TA; it was saturable, reversible, and unevenly distributed in the brain. Most significant densities of TA binding 'sites' were detected in dopamine (D)-rich regions such as the striatum and the hypothalamus (Table 1).

Table 1 Regional distribution of ^{3}H-TA binding in the rat brain

	B_{max} (fmoles/mg protein)	K_D (nM)	N
Striatum	1659 ± 69	11.5 ± 0.8	10
Hypothalamus	243 ± 48	10.3 ± 1.3	4
Cortex	151 ± 43	11.4 ± 1.5	4

Binding of ^{3}H-TA (4 nM) to striatal membranes was potently displaced by D, with an IC_{50} value of 18 nM. D2-receptor antagonists such as (+)butaclamol, haloperidol, domperidone, spiperone and sulpiride, and the D2-agonist apomorphine had a low affinity (IC_{50} 750 nM-100 μM)for ^{3}H-TA 'sites'. The binding of TA was not related to transport processes inasmuch as nomifensine, a specific inhibitor for the carrier of D uptake, imipramine, and the 5-HT uptake inhibitor citalopram were weak displacers (IC_{50} 150 nM-15 μM) of ^{3}H-TA binding.
Surgical hemitransection of the nigro-striatal bundle, thus, degeneration of D nerve endings, did dramatically (by over 70%) decrease the number of ^{3}H-TA 'sites', compared with controlateral non-lesioned striata, a finding suggesting a presynaptic location of these 'sites'. Based upon Seeman's (1982) nomenclature for D receptors, ^{3}H-TA binding 'sites' share all properties of a D3 receptor, as having nM affinity for D and μM affinity for butyrophenones, as being sulpiride-insensitive and, probably, presynaptically located.

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FUNCTIONAL ACTIVITY OF GABA RECEPTORS IN MYENTERIC PLEXUS FROM MORPHINE TOLERANT GUINEA-PIGS

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GABAergic system has been reported to be affected in the morphine dependence. Myenteric plexus of ileum longitudinal muscle is a good model to study $GABA_A$ and $GABA_B$ receptors (Giotti et al., 1983). Responsiveness of guinea-pig longitudinal muscle to the activation of $GABA_A$ and $GABA_B$ receptors was studied in preparations isolated from morphine-tolerant animals. For this purpose morphine pellets were implanted in guinea-pig following Gibson and Tingstad (1970) or Blasig et al. (1973).

Activation of $GABA_A$ receptors with GABA 10^{-6} - 10^{-3} M in non-stimulated preparations caused a dose-related contraction that did not differ from tolerants to controls. On the contrary, inhibition elicited by (-)-baclofen and GABA 10^{-6} - 10^{-3} M in electrically stimulated strips was about 80% lower in tolerants than in controls. Hyporesponsiveness to $GABA_B$ receptor activation began 12 h after pellet implantation and was maximal on the third day. The above effect was evident also in ileum from morphine-dependent animals with a withdrawal syndrome induced by naloxone. The specificity of this effect was demonstrated by the fact that adenosine inhibition was almost unchanged in morphine-dependent animals in comparison with controls.

In conclusion, our findings indicate a lower functionality of $GABA_B$ receptor in this model while $GABA_A$-mediated effects were unmodified. It must also be pointed out that $GABA_B$ receptor activation may play a role in the prevention of abstinence syndrome.

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THE INFLUENCE OF PIRENZEPINE ON GASTRIN AND SOMATOSTATIN PORTAL LEVELS IN DOGS

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Gastrin and somatostatin release seem to be regulated by non-cholinergic excitatory neurons on G cells and cholinergic inhibitory neurons on D cells (Martindale et al., 1982; Schubert et al.,1982). On isolated rat stomach, the selective antimuscarinic drug pirenzepine (PZ) has been found to reverse carbachol-induced gastrin stimulation and somatostatin inhibition (Sue et al.,1983) but no data are available about the effects of PZ on gastrin and somatostatin secretion in vivo. The present study was designed to assess the action of PZ on gastrin and somatostatin portal levels in dogs.

Six mongrel dogs (14-22 Kg) were anaesthetized with sodium thiopental, laparatomized and the portal vein was cannulated. The dogs were continuously infused i.v. with bethanechol (BT) 160 µg/kg/h; after 30 min, when BT-induced somatostatin inhibition was maximal, PZ 1 mg/Kg was injected i.v. as a bolus. Blood samples were drawn from the portal vein under basal conditions and every 10 min for 90 min during BT and BT+PZ administration. Gastrin and somatostatin concentrations were measured by RIA.

As shown in Figure 1, at the 30th min of BT infusion, gastrin levels were not significantly different, the trend being towards an increase, while somatostatin levels were significantly lower. PZ produced a significant decrease in gastrin levels throughout the experiment and a marked increase in somatostatin levels, with maximal enhancement at the 30th min following its injection (Figure 1).

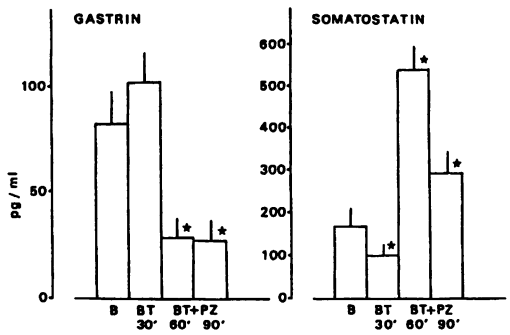

Figure 1-Effects of BT and BT+PZ on basal (B) gastrin and somatostatin concentrations from dog portal vein. Each column represents the mean value of 6 experiments ± s.e.mean vertical lines. Student's t test for paired data: *P < 0.05 for BT vs B and BT+PZ vs BT.

The presents results indicate that gastrin and somatostatin release from dog stomach are regulated by muscarinic receptors. PZ-induced somatostatin enhancement may be mediated by the suppression of inhibitory muscarinic influences on D cells. The high increase in somatostatin concentration may account at least partly for the simultaneous decrease in gastrin secretion.

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REGULATION OF RESTING ACID SECRETION IN THE RAT ISOLATED FUNDUS

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Mechanisms responsible for the spontaneous acid secretion have been evaluated in the isolated gastric fundus from the immature rat by the use of different compounds acting as stimulants or inhibitors of gastric secretion. Among the stimulatory compounds, the order of potency was: pentagastrin $\geqslant$ isoprenaline $>$ bethanechol $>$ dibutyryl cAMP $=$ histamine $>$ theophylline, pD_2 values were 7.60, 7.13, 5.54, 4.61, 4.03 and 3.01, respectively. The maximum responses to bethanechol, dibutyryl cAMP and theophylline were twice as high as those to the other compounds. Basal acid secretion was not affected by the H2-receptor antagonists cimetidine (10^{-4}M), ranitidine (10^{-4}M) and mifentidine (10^{-4}M) but it was markedly reduced by oxmetidine (10^{-5}M-10^{-3}M) and, to a lesser extent, by compound SKF 93479 ($3x10^{-5}$M-$3x10^{-4}$M). Basal acid secretion was not modified by a series of compounds acting at different sites like anticholinergics, proglumide, tetrodotoxin, propranolol, morphine, aminoguanidine, 5-hydroxytryptamine, prostaglandin E1 and dichlorophenamide. The H^+/K^+-ATPase inhibitor, omeprazole ($3x10^{-7}$M-10^{-5}M) and KSCN ($3x10^{-4}$M-$3x10^{-2}$M) were able to abolish basal rates of secretion, whereas somatostatin (10^{-6}M-10^{-5}M) induced only a partial inhibition (20-25%). The calcium ionophore A23187 induced a marked stimulatory effect in the range $3x10^{-6}$M-$4x10^{-5}$M, whereas verapamil ($3x10^{-4}$M), nifedipine ($3x10^{-4}$M) and trifluoperazine (10^{-3}M) were without effect. Reduction of calcium concentration (1.2 mM *vs* 2.4 mM) in the bathing media did not change the basal acid output; on the contrary, incubation of the stomachs in a calcium-free medium caused a 50% reduction in basal rates of secretion.
The above data suggest that the regulation of rat basal secretion "in vitro" does not seem to involve the common pathways (histamine H2-, cholinergic- or gastrin- receptors) responsible for the acid production "in vivo". The effect of the inhibitory compounds points out the importance of mechanisms active at a very peripheral step in the acid secretory process.

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μ AND δ BUT NOT κ OPIOID AGONISTS MEDIATE GASTRIC SECRETORY EFFECTS IN THE DOG

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There is now considerable evidence implying the control of gastric secretion by endogenous opioids (see Rozè and Dubrasquet,1983).The variety of effects including both excitatory and inhibitory secretory responses may reflect the multiplicity of opioid receptor subtypes which could be involved in the regulation of gastric secretion (Feldman and Li,1982).In the present study we examined the effects of the proposed mu agonist dermorphin (DER),delta agonist D-Ala^2-D-Leu^5-enkephalin (DADLE) and kappa agonist dynorphin 1-13 (DYN) on gastric acid secretion in conscious dogs.

Six mongrel dogs (14-22 Kg) chronically fitted with both gastric fistulae (GF) and Heidenhain pouches (HP)(4 in each experiment) were used.In a first series of experiments DER,DADLE or DYN were infused in 30 min step equimolar doses of 2-4-8-16-32 nM/Kg/h under basal conditions.In a second series of experiments DER,DADLE or DYN were administered by i.v. infusion of 30 min during the secretory plateau induced by pentagastrin 1 μg/Kg/h.Acid output was calculated in mEq H^+/15 or 30 min.The significance of differences was calculated by using Student's t test for paired data.

DER caused a dose-dependent increase in acid secretion under basal conditions:maximal increase occurred at the dose of 16 nM/Kg/h (GF=5.634±0.673;HP=0.665±0.076 mEq H^+/30 min).Under the same conditions neither DADLE nor DYN had any significant effects on acid secretion.The excitatory responses to DER were significantly ($P<0.05$) prevented by both naloxone 0.11 μM/Kg/h and the peripheral opioid antagonist N-methyl levallorphan methanesulphonate (SR 58002C) 16.5 μM/Kg/h.The addition of DER to submaximal stimulation with pentagastrin caused a significant increase in acid secretion from both GF (+38%; $P<0.05$) and HP (+145%; $P<0.05$).Simultaneous yet opposite effects were obtained with DADLE 16 nM/Kg on pentagastrin-stimulated secretion with a significant inhibition of GF (-53%; $P<0.05$) and a significant enhancement of HP secretion (+103%; $P<0.05$).Both naloxone and SR 58002C significantly ($P<0.05$) inhibited acid output stimulated by pentagastrin and completely prevented the stimulatory effect of DER on acid secretion.On the contrary,these opioid antagonists failed to affect either the inhibitory or the excitatory responses of DADLE.No significant effects were observed for DYN 16 nM/Kg on pentagastrin-stimulated secretion from either GF or HP.

The present results suggest that peripheral mu receptors are mainly involved in mediating the excitatory effects of opioids on dog gastric secretion.On the contrary, selective activation of delta receptors seems to mediate both inhibitory and excitatory effects on acid secretion,as also observed in the case of the alpha-2 agonist clonidine (Soldani et al.,1984).Further,these data indicate that opioid effects on gastric secretion are not mediated by kappa receptors.

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EFFECT OF CALCIUM ANTAGONISTS WITH DIFFERENT SITE OF ACTION ON THE DUODENAL MUSCLE

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Several calcium antagonists, described to interfere with calcium ions at different levels, have been tested on isolated duodenum from rats and rabbits, in order to investigate the role of calcium in the duodenal muscle contractility. Nifedipine, verapamil and diltiazem, as calcium-entry blockers, and the calmodulin-antagonist, trifluoperazine, have been used. Moreover, some experiments have been performed in calcium-free, calcium-free-EGTA and high K^+-calcium-free solutions.

Rabbit duodenum. The spontaneous phasic activity of the rabbit duodenum was markedly reduced after 10 min exposure to a calcium-free medium and actually abolished by a further addition of EGTA. The same inhibitory effect was obtained after administration of the calcium antagonists which differred from one another only for their potency: IC_{50s} for nifedipine, verapamil, diltiazem and trifluoperazine were 1.6×10^{-11}M, 4.6×10^{-9}M, 4.7×10^{-8}M, and 6.9×10^{-6}M, respectively. The inhibition induced by the calcium-entry blockers was overcome by small concentrations of the novel calcium-agonist, Bay K8644 but not by acetylcholine, whereas no recovery was obtained in the case of trifluoperazine either with acetylcholine or Bay K8644.

Rat duodenum. On this preparation, which was virtually devoid of spontaneous motility, the effects of the different calcium antagonists have been evaluated on drug-induced tonic contractions. The contractile activity of $BaCl_2$, which was found to be independent of extracellular calcium, was antagonized by all the compounds examined: IC_{50s} for nifedipine, verapamil, diltiazem and trifluoperazine were 2.8×10^{-13}M, 3.3×10^{-9}M, 2.6×10^{-8}M and 1.4×10^{-6}M, respectively. The concentration-response curve to $CaCl_2$ in high K^+-calcium-free solution, was competitively antagonized by nifedipine and verapamil (pA_2 values = 12.3 and 8.17, respectively) and non-competitively by trifluoperazine.

From these results it was concluded that calcium ions play a fundamental role in the duodenal contractility of rats and rabbits; moreover, the high potency shown by the compounds interfering with the transmembrane calcium influx suggests a high dependence of the duodenal motility on the availability of calcium ions at extracellular level.

THE RELEASE OF LEUKOTRIENES FROM LARGE AND SMALL AIRWAYS OF GUINEA-PIG AND MAN

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The early discovery that slow reacting substance of anaphylaxis (SRS-A) could be generated from guinea-pig (GP) and human lung fragments following immunological challenge (Orange, 1974) paved the way for further experiments which have contributed to the mounting evidence implicating SRS-A in the pathophysiology of respiratory disease. We have examined the differential release of the cysteinyl-containing leukotrienes (LTs) and LTB_4 from large and small airways of GP and man. The effects of cyclo-oxygenase inhibition on LT release following immunological challenge or stimulation with Ca ionophore A23187 were also investigated.

GP lung parenchyma (GPP) and trachea (GPT) were weighed, chopped and pre-incubated (37°C, 30 mins) with or without indomethacin (1μg/ml). Tissues from GPs sensitized to ovalbumin (OA) were then challenged (OA 50μg/ml) and incubated for 15 mins. Tissues from unsensitized GPs were stimulated with A23187 (0.5 - 5μg/ml) and incubated for 60 mins. Human bronchus (HB) and parenchyma (HP) were weighed, chopped and stimulated with A23187 (5μg/ml) and incubated for 60 mins. LTB_4- and LTD_4-like materials were quantitated by bioassay on GP lung strip and ileum (Samhoun & Piper, 1984).

Sensitized GPT released LTB_4 (88±13 pmol/g) but no detectable levels of LTD_4. GPP released similar amounts of LTB_4 (131±25 pmol/g) and LTD_4 (168±66 pmol/g). Indomethacin significantly increased the generation of LT-like material from the GPT and GPP. A23187 (5μg/ml) released LTB_4 and LTD_4 from the GPP, whereas the GPT released LTB_4 (314±50 pmol/g) but no detectable LTD_4. In the presence of indomethacin, the amount of LT released from the parenchyma was increased, unlike the LT release from the trachea. HP released LTB_4 (415±59 pmol/g) and LTD_4 (34±9 pmol/g) after A23187 (5μg/ml) stimulation. By comparison the bronchus released LTD_4 (27±9 pmol/g) and relatively low levels of LTB_4 (27±9 pmol/g). Indomethacin had no effect on LT release from the human tissues.

After bioassay, the samples were partially purified using Sep-Pak C_{18} cartidges and subjected to reverse phase high pressure liquid chromatography (RP-HPLC). The LTD_4-like material generated from the GP tissues consisted of a mixture of the cysteinyl-containing LTs, the biological activity of which co-chromatographed with synthetic LTD_4 and LTE_4. When the incubation period was shortened to between 5 - 10 mins, LTC_4 could also be identified.

In conclusion, LTB_4 is the predominant LT generated from the GPT; no detectable levels of the cysteinyl-containing LTs are released, whereas levels of LTB_4 and LTD_4 released by the GPP are similar. In contrast to the GPP, the HP is found to preferentially release LTB_4, and the HB releases comparatively low levels of LTs.

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NOVEL PROSTAGLANDIN ENDOPEROXIDE ANALOGUES WHICH BLOCK THROMBOXANE RECEPTORS AND MIMIC PROSTACYCLIN

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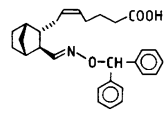
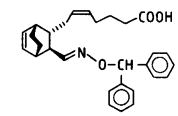
We have developed a number of prostaglandin endoperoxide analogues which specifically block thromboxane receptors in smooth muscle and platelets (Jones et al, 1982; Armstrong et al, 1985). Several members of the series were found to show a much broader inhibitory action on the human platelet. Our investigations of two of these compounds, EP 035 and EP 157, are presented in this communication.

EP 035 

EP 157 

In human PRP EP 035 (3 μM) and EP 157 (0.5 μM) completely suppressed maximal aggregation responses induced by ADP, PAF, thrombin and the TXA_2 mimetic, 11,9-epoxymethano PGH_2. Rises in cyclic AMP were associated with the inhibitory effects; EP 035 and EP 157 at 25 μM gave maximal increases of 13.4 ± 0.6 and 17.0 ± 1.4 (s.e.m. n=12) times the basal level. Exposure of the platelets to PGE_1 (2.5 μM) markedly reduced the effects of EP 035 and EP 157 on cyclic AMP accumulation; PGD_2 (2.5 μM) was considerably less effective. Since PGE_1 desensitizes the PGI_2-sensitive adenylate cyclase system but not the corresponding PGD_2-sensitive system (Miller & Gorman, 1979), it appeared that EP 035 and EP 157 might mimic the action of PGI_2.

In further experiments the actions of EP 035 and EP 157 were compared with two stable PGI_2 analogues, iloprost (Casals-Stenzel et al, 1983) and 6a-carba PGI_2 (Whittle et al, 1980), on plasma-free preparations of human platelets.
(a) Inhibition of aggregation induced by 11,9-epoxymethano PGH_2 (1 μM indomethacin). Equipotent molar ratios: iloprost = 1.0 (IC_{50} = 0.04 - 0.18 nM), 6a-carba PGI_2 = 26 ± 6 (n=4), EP 157 = 49 ± 12 (n=6), EP 035 = 276 ± 26 (n=4).
(b) Activation of adenylate cyclase in platelet homogenates. Half-maximal activation was achieved with 9.5 nM iloprost. 6a-Carba PGI_2, EP 157 and EP 035 were 23, 84 and 240 times less effective respectively.
(c) Displacement of [^{3}H] iloprost (20 nM) binding to platelet membranes (method of Leigh & MacDermot, 1985). Ratios of IC_{50} values were: iloprost = 1.0 (IC_{50} = 117 nM), 6a-carba PGI_2 = 7.5, EP 157 = 13.7, EP 035 = 24, PGD_2 >> 100.

Displacement of [^{3}H] 9,11-epoxymethano PGH_2 (100 nM) binding to washed platelets was also measured (Armstrong et al, 1983). IC_{50} values for EP 035 and EP 157 were 7.5 and 4.0 μM respectively, well above concentrations used in (a).

On thromboxane-sensitive smooth muscle preparations EP 035 and EP 157 specifically blocked the contractile action of 11,9-epoxymethano PGH_2. Dose ratios (n=4) were: rabbit aorta, EP 035 10 μM = 26.3 ± 3.8, EP 157 10 μM = 19.5 ± 2.5; dog saphenous vein, EP 035 2.5 μM = 38.0 ± 7.2, EP 157 2.5 μM = 28.5 ± 4.3.

These data strongly support the idea that EP 035 and EP 157 inhibit aggregation by acting as agonists at PGI_2 receptors on the human platelet.

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EFFECT OF ACTINOMYCIN D ON PROSTAGLANDIN $F_{2\alpha}$ OUTPUT FROM THE GUINEA-PIG UTERUS SUPERFUSED IN VITRO

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The outputs of $PGF_{2\alpha}$, PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 from the guinea-pig uterus superfused in vitro increase 21.9-, 1.8-, 2.9- and 1.2-fold, respectively, between Days 7 and 15 of the oestrous cycle (Poyser & Brydon, 1983). Oestradiol acting on a progesterone-primed uterus causes this relatively specific increase in output of $PGF_{2\alpha}$, the uterine luteolytic hormone (Poyser, 1983a). Endometrial phospholipase A_2 (PLA_2) and $PGF_{2\alpha}$ synthesizing capacity increase 1.5- and 2.5-fold, respectively, between Days 7 and 15/16 (Downing & Poyser, 1983; Poyser, 1983b), indicating that increases in enzyme activity alone cannot account for the 21.9-fold increase in $PGF_{2\alpha}$. It is proposed that oestradiol released from the guinea-pig ovary after Day 10 "switches on" endometrial $PGF_{2\alpha}$ synthesis from Day 11 by "activating" PLA_2 in some way. The role of fresh protein synthesis in this process has been studied.

Actinomycin D (20 μg) or 0.5 ml saline (control) was injected into each uterine horn of anaesthetized guinea-pigs on Day 10 (n=5). The animals were killed and blood was taken for progesterone measurement by radioimmunoassay (RIA). Both uterine horns from each animal were superfused separately with Krebs' solution at 5 ml min^{-1} (Poyser & Brydon, 1983). After 60 min, the outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ were measured by RIA in nine 10-min samples collected consecutively. A23187 (1 μg/ml) was added during the collection of samples 4, 5 and 6 from one horn. Results are mean ± s.e. mean. Plasma progesterone levels were 0.21 ± 0.04 and 4.13 ± 0.52 ng ml^{-1}, basal $PGF_{2\alpha}$ outputs were 0.093 ± 0.015 and 0.018 ± 0.005 ng min^{-1} 100 mg $tissue^{-1}$, and maximum A23187-stimulated $PGF_{2\alpha}$ outputs were 0.287 ± 0.079 and 0.053 ± 0.007 ng min^{-1} 100 mg $tissue^{-1}$, in the control and actinomycin D-treated guinea-pigs, respectively. This study was repeated with, additionally, both groups of animals receiving 10 μg oestradiol benzoate s.c. daily on Days 11 to 14. Plasma progesterone levels were 0.11 ± 0.02 and 3.31 ± 0.80 ng/ml^{-1}, uterine weights were 0.905 ± 0.064 and 0.580 ± 0.032 g, basal $PGF_{2\alpha}$ outputs were 0.348 ± 0.115 and 0.085 ± 0.017 ng min^{-1} 100 mg $tissue^{-1}$, and maximum A23187-stimulated $PGF_{2\alpha}$ outputs were 0.879 ± 0.231 and 0.260 ± 0.044 ng min^{-1} 100 mg $tissue^{-1}$ in the control and actinomycin D-treated animals, respectively. In neither study was PG synthesis re-directed to PGE_2 or 6-keto-$PGF_{1\alpha}$. Actinomycin D treatment significantly ($P < 0.01$) prevented the increase in uterine $PGF_{2\alpha}$ synthesis and release, thus preventing corpus luteal regression. These effects were not reversed by oestradiol administration. The inhibition of the uterotrophic response of oestradiol by actinomycin D showed that protein synthesis had been prevented. These studies suggest that fresh protein synthesis is involved in the process by which oestradiol acting on a progesterone-primed uterus switches on uterine $PGF_{2\alpha}$ production. Since changes in enzyme activity are only secondary events in the process, this finding raises the question as to whether the ovarian steroids cause the synthesis of a "lipostimulin" which activates PLA_2 in the endometrium and is therefore the primary stimulus for $PGF_{2\alpha}$ synthesis. The increases in PG levels in the ovary and brain in response to LH and pyrogen and which are necessary for ovulation and fever, respectively (see Poyser, 1981; Townsend et al, 1984) are also prevented by protein synthesis inhibitors, suggesting that production of a "lipostimulin" may be involved in the induction of PG synthesis by a variety of stimuli.

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MODULATION OF EICOSANOID FORMATION IN HUMAN LUNG PARENCHYMA

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Normal human lung parenchyma responds to a challenge with the Ca^{++}-Ionophore A23187 with a marked formation of arachidonate metabolites. LTE_4 is the SRS-A component that is formed in larger amounts (2-3 nmols/g fresh tissue) together with smaller amounts of LTD_4 and negligible quantities of LTC_4. LTB_4 is also found in the incubating medium following A23187 stimulus but not immunological challenge. Addition of reduced glutathione (10 mM, GSH) prevents LT interconversion and only LTC_4 and LTB_4 can be detected. In a similar way addition of cysteine to the incubates (10 mM, CYS) inhibits the transformation of LTD_4 to LTE_4 and in these experimental conditions SRS-A like activity is due to a mixture of LTC_4 and LTD_4 only. Several cyclo-oxygenase products are also released from human lung parenchyma including PGE_2, 6-keto-$PGF_{1\alpha}$, TXB_2 and PGD_2. This last metabolite is released in larger amounts and considering its marked bronchoactive properties may contribute to exacerbate the effects of the released LTs. Preincubations of lung fragments with the GSH-sequestering agent 1-chloro-2, 4-Dinitrebenzene (CDNB, 25-0.1 μM) causes a preferential inhibition of sulfidopeptide leukotriene biosynthesis due to lack of substrate for GSH S-Transferase compared to that of LTB_4 and PGD_2 as well; in fact GSH, plays a key role not only at the level of SRS-A formation but also at the earlier steps leading to the oxidative events involved in arachidonic acid metabolism.

The purine nucleotide adenosine was also tested for its capacity to influence leukotriene or prostanoid synthesis in human lung. At concentrations of 10^{-4} - 10^{-5} M, adenosine seems unable to influence A23187 challenge, whereas preliminary experiments seem to suggest a capacity of the purine nucleotide to blunt PGD_2 and LT formation at least at 10^{-4} M.

Finally a number of antiinflammatory agents reportedly able to inhibit 5-lipoxygenase, e.g. benoxaprofen, were also tested for their ability to interfere with arachidonic acid metabolism: at 10^{-5} M a 50% inhibition of PGD_2 formation was observed, whereas even at 10^{-4} M the capacity of the drug to affect SRS-A formation was negligible.

ANTILIPOLYTIC AND ANTIINFLAMMATORY ACTION OF A NEW NICOTINIC ACID DERIVATIVE IN THE RAT

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L_{44-0} is a new nicotinic acid ester (N-oxide of 2 terbutyl-cycloexyl-phenyl-nicotinate) that retained the action of the parent compound on lipid metabolism.
The antilipolytic activity of L_{44-0} is present when the adipocytes are stimulated either by norepinephrine, through cAMP, or by fast which is indipendent of adenyl cyclase activation. The effect of L_{44-0} after a single administration persists for at least 6 hours. During the inhibition of lipolysis the drug modifies the arachidonic acid metabolism in the endothelial cells, reducing the formation of PGI_2 in the small arteries impinging the adipose tissue as well as in the thoracic aorta. This inhibition appears to be more pronounced since it lasts, in the small arteries, more than 24 hours.
Since the mechanism of action of a majority of non-steroidal antiinflammatory drugs has been explained as an inhibition of arachidonate cyclooxygenase we studied the activity of L_{44-0} on two phlogenic responses evoked by carrageenan-oedema and by the subcutaneous implantation of polyester sponges soaked in carrageenan. The nicotinic acid derivative (100 mg/kg) is able to reduce the formation of oedema similarly to indomethacin (10 mg/kg) 3 hours after the carrageenan inhection into the plantar aponeurosis of the rat hind paw. Concentrations of TXB_2, PGE_2 and 6-keto-PGF_1 in inflammatory exudates, measured 6 hours after the sponge implantation, by radioimmunoassay, were significantly reduced by three day treatment with L_{44-0} (100 mg/kg). In the same experimental conditions nicotinic acid was uneffective. Preliminary observations of the gastric mucosa of the treated rats, indicate that L_{44-0} induces no or very weak gastric irritancy when compared with indomethacin, suggesting that the ulcerogenic effects of many non-steroidal antiinflammatory drugs are not completely related to their inhibition of prostaglandins biosynthesis.

EFFECT OF L_{44-0} ON ACUTE INFLAMMATION INDUCED BY CARRAGEENAN SOAKED SPONGES

CONDITIONS	TXB_2 ng/ml EX.	PGE_2 ng/ml EX.	6-keto PGF_1 ng/ml EX.	Volume of Exudate ml
Carrageenan	25.9 ± 5.6 (14)	30.0 ± 5.8 (14)	5.6 ± 1.1 (14)	4.1 ± 0.3 (15)
Car + Indomet.	0.9 ± 0.2 (12) p 0.001	0.9 ± 0.2 (13) p 0.001	0.4 ± 0.1 (13) p 0.001	3.9 ± 0.3 (14)
Car + L_{44-0} (100 mg/kg)	8.1 ± 1.9 (14) p 0.001	10.9 ± 3.1 (14) p 0.01	2.4 ± 0.5 (14) p 0.02	2.8 ± 0.1 (15) p 0.001

SPECIFIC BINDING OF LEUKOTRIENE C_4 (LTC_4) TO MEMBRANES FROM HUMAN LUNG PARENCHYMA AND BRONCHI

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Leukotriene C_4 (LTC_4), one of the major components of the slow reacting substance of anaphylaxis (SRS-A), is a potent constrictor of human airways (Dahlén et al. 1980), acting mainly at the periphery (Drazen et al. 1980).

Aim of our study was to investigate whether human lung parenchima and bronchi possess specific binding sites for LTC_4.

Binding assay was performed at 4°C for 15 min, in the presence of 20 mM Serine borate, an inhibitor of γ-glutamyl-transpeptidase, which converts LTC_4 to LTD_4.

In membranes from human lung parenchima, ^3H-LTC_4 binding was specific, rapid (equilibrium was reached in 15 min), reversible and saturable (B_{max}=32-41 pmol/mg prot.). $CaCl_2$ enhanced binding of LTC_4 at equilibrium by increasing its affinity (Kd = $1.9x10^{-7}$ and $3.6x10^{-8}$ M, in the absence and presence of 10^{-2} M $CaCl_2$). The other cysteine-containing leukotrienes and the SRS-A antagonist FPL 55712 competed for the binding sites labelled by ^3H-LTC_4, being however less potent than LTC_4 (IC_{50}s = $2.2x10^{-6}$, $2.4x10^{-5}$ and $2.4x10^{-5}$ M, for LTD_4, LTE_4 and FPL 55712, respectively, in the presence of 10^{-2} M $CaCl_2$). The binding sites were sensitive to heat and probably possessed a protein moiety, being inactivated upon trypsinization.

In membranes from human bronchi, as well, ^3H-LTC_4 binds in a dose-related fashion, rapidly and reversibly. Similarly to what observed in lung parenchima, Ca^{++} increased specific binding, and LTD_4 and FPL 55712 displayed a much lower affinity than LTC_4. Smaller caliber bronchi seem to possess a higher binding level for ^3H-LTC_4 than larger airways, in agreement with the proposed preferential action of leukotrienes on peripheral airways.

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SPECIFICITY OF ENHANCEMENT OF SALBUTAMOL RESPONSES BY CYCLO-OXYGENASE INHIBITION IN THE RAT ISOLATED UTERUS

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Inhibition of acetylcholine (ACh)-induced tone in the rat uterus by β-adrenoceptor agonists is enhanced after cyclo-oxygenase blockade (Boyle & Ohia, 1985). The inhibition produced by relaxants in smooth muscles which possess no intrinsic tone may be influenced differentially by the mechanisms whereby tension is increased (Daniel, 1982). The cyclo-oxygenase substrate arachidonic acid is also a substrate for lipoxygenase, and products from this pathway may be involved in uterine motility (Carraher, Hahn, Ritchie & McGuire, 1983). Thus this study investigated whether the enhancement of β-adrenoceptor responses seen after cyclo-oxygenase inhibition was dependent on the motor agent used and whether lipoxygenase inhibition affected the relaxations in the four phases of the oestrous cycle.

Virgin female Wistar rats weighing (180-250g) were used and the preparation was set up as described previously (Boyle & Ohia, 1985). Tone was induced with either ACh or potassium chloride (KCl, $5x10^{-2}M$). Since KCl produced a biphasic contraction with an initial transient phase and a secondary sustained phase lasting for more than one hour, cummulative dose response curves were constructed to the relaxants 15 min into the sustained phase until maximum inhibition of the KCl-induced tone was obtained. The cyclo-oxygenase inhibitor, flurbiprofen (FBF, $10^{-6}M$), and the inhibitor of both cyclo-oxygenase and lipoxygenase, BW 755C ($10^{-5}M$), when used, were present in the Tyrodes solution and had no effect on KCl or ACh responses.

As with ACh-induced contractions, salbutamol (SAL, $1.5x10^{-11}$-$5x10^{-6}M$) produced dose dependent relaxations of KCl-induced tone in proestrus, oestrus, metoestrus and dioestrus. FBF increased SAL inhibitory responses and produced a significant shift in the dose response curves to the left. For instance in proestrus, SAL pD_2 values were increased significantly from 9.77 ± 0.30, n=6 before, to 10.66 ± 0.09, n=6 ($P<0.01$) after FBF.

BW 755C enhanced SAL relaxation of KCl-induced tone and shifted dose response curves to the left to the same degree as occurred with FBF.

When inhibition of ACh-induced tone was produced by histamine acting via H_2-receptors (HIS, 10^{-7}-$10^{-4}M$, in the presence of H_1-receptor antagonist mepyramine, $5x10^{-8}M$), FBF produced no enhancement in any phase of the oestrous cycle.

The FBF enhancement of SAL inhibition in uteri stimulated with both KCl and ACh suggests that its effect is not dependent on the motor agent used. Since BW 755C had no greater effect than FBF, it is likely that products of the lipoxygenase pathway were not involved in β-adrenoceptor mediated relaxation. Inhibition induced by stimulating HIS H_2-receptors was not affected by FBF and therefore enhancement of SAL responses is specific to β-adrenoceptor activation. The hormonal state of the animal did not appear to be of major importance because the same effects were observed in all four phases of the oestrous cycle. Rubanyi & Vanhoutte (1985) reported similar potentiation of β-adrenoceptive mechanisms by cyclo-oxygenase inhibition in vascular smooth muscle.

S.E.O. is an ORS award scholar.

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ISOLATED AND VENTILATED GUINEA-PIG LUNG: A METHOD FOR STUDYING DRUG INTERACTION WITH CYCLOOXYGENASE AND LIPOXYGENASE PATHWAYS

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Lipoxygenase as well as cyclooxygenase pathways of arachidonic acid metabolism are involved in antigen-induced bronchoconstriction in ovalbumin-sensitized guinea-pig lungs. In the present investigation we characterized thromboxane (TX) and leukotriene (LT) release from normal and anaphylactic isolated and perfused guinea-pig lung by radioimmunoassay (RIA) of TXB_2 and LTC_4 and LTB_4, respectively. Isolated lungs obtained from ovalbumin-sensitized animals were perfused and ventilated (Preziosi et al, 1959) and challenged with 0.1% ovalbumin solution. Biologic activity of perfusates was tested on isolated guinea-pig ileum. A sustained contraction of the smooth muscle was observed only with perfusates obtained from lungs which underwent bronchoconstriction during ovalbumin exposure. Pretreatment with LT antagonist FPL55712 reduced or abolished the contractile response of ileum. LTC_4 RIA measurement was performed by two different antisera (AS): AS1 (Hayes et al, 1983) and AS2 (Aehringhaus et al, 1982) showing a different cross-reactivity with LTD_4 and LTE_4. LTC_4-like immunoreactivity (LTC-LI) in perfusates of anaphylactic lungs ranged from 0.2 to 3.0 ng/ml with AS1 and 0.2-3.9 ng/ml with AS2. No immunoreactive material was detected in perfusates collected under basal conditions (no antigen challenge) or in those from ovalbumin-challenged lungs which did not show bronchoconstriction. LTB_4 was measured by RIA (Salmon et al, 1982). In 11 anaphylactic lung perfusates TXB_2 release averaged 13.7 ± 7 (SD) ng/min, LTC-LI 6.4 ± 4.3 ng/min and LTB_4 0.56 ± 0.3 ng/min. Statistically significant correlations did exist between TXB_2 and LTC-LI ($r = 0.723$, $p < 0.01$), TXB_2 and LTB_4 ($r = 0.732$, $p < 0.01$) and LTC-LI and LTB_4 ($r = 0.90$, $p < 0.01$). Sulindac sulfide at 1, 10 and 50 μg/ml concentration in lung perfusing medium reduced LTC-LI release by 8 %, 61 % and 89 %, respectively, LTB_4 release by 0 %, 57 % and 79 %, respectively, and TXB_2 release by 22 %, 86 % and 93 %, respectively. TX-synthase inhibitor OKY 046 at 10 μg/ml concentration reduced TXB_2 release by 83 %, without affecting LTC-LI or LTB_4 levels. Sulindac treatment in a dose-dependent fashion and OKY 046 significantly reduced ovalbumin-induced bronchoconstriction. In a second series of experiments a chemical guinea-pig lung challenge was induced by an aerosol solution containing formaldehyde 10 ppm given for 10 min to perfused and ventilated lungs obtained from normal guinea-pigs. The same lungs were given a control aerosol containing medium alone before treatment. LTB_4 release resulted unaffected ($0.05 \pm 0.02 \rightarrow 0.06 \pm 0.02$ ng/min $\pm$ SD, p= NS), while TXB_2 release increased from 0.62 ± 0.4 to 2.66 ± 0.7 ng/min ($\pm$SD, $p < 0.01$). LTC-LI concentrations were unmeasurable. In conclusion: 1) LT-induced TXA_2 production may be, at least in part, responsible for bronchospasm in anaphylactic guinea-pig lung; 2) drugs interacting with arachidonic acid metabolism may affect bronchoconstriction induced by antigen exposure; 3) xenobiotics acting by non-immunologic mechanisms on bronchial mucosa may stimulate the cyclooxygenase pathway; 4) isolated and ventilated guinea-pig lung represents a useful experimental model for studying pharmacological and toxicological actions on arachidonic acid metabolism.

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SYNTHESIS OF ARACHIDONATE PRODUCTS BY RAT AND HUMAN COLON. EFFECTS OF INTESTINAL SECRETAGOGUES

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Introduction. We have recently demonstrated that rat colon converts [1-^{14}C]-arachidonic acid into both lipoxygenase and cyclo-oxygenase products, and that this metabolism is stimulated by phenolphthalein (Capasso et al, 1984). Human colon forms similar products (Bennett et al, 1981), and we have now investigated the effect of several intestinal secretagogues on the metabolism of exogenous arachidonic acid by rat and human isolated colon.

Methods. Colon from operation specimens (human) or from male Wistar rats were cut finely and suspended in Krebs solution to give 20 mg/ml. Aliquots of 5ml were pre-incubated (20 min, 37^{o}C) with test compounds (ricinoleic acid, phenolphthalein, picosulphate, sulfosuccinate, mannitol; 100-500 µg) and then homogenised and further incubated for 1 hour with [1-^{14}C]-arachidonic acid (0.1 µCi, 34 nM). The labelled products were extracted and chromatographed on silica gel thin layer plates together with authentic standards.

Results. Human colonic muscle was more active than the mucosa in metabolising the arachidonic acid. The chromatography results indicated that the main products formed were prostaglandins, 5-HETE and LTB_4. Ricinoleic acid was the most potent stimulant of metabolism in both tissue layers. Phenolphthalein, picosulphate and sulfosuccinate were also active, but mannitol had no effect. Similar results were obtained with segments of rat colon. The cyclo-oxygenase inhibitor indomethacin 1 µg/ml inhibited the increase in cyclo-oxygenase products, and the dual inhibitor BW755C 1µg/ml also reduced the formation of the lipoxygenase products and prostaglandins, both in control tissues and those treated with ricinoleic acid or phenolphthalein.

Conclusions. Arachidonic products have diarrhoeagenic actions, and our results add to the evidence that they contribute to the action of some intestinal secretagogues.

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IS A REDUCTION IN TISSUE CYCLIC AMP RESPONSIBLE FOR LEUKOTRIENE D_4-INDUCED TENSION DEVELOPMENT IN GUINEA-PIG LUNG PARENCHYMA?

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It is now recognised that the activity of adenylate cyclase in many cell types is under dual stimulatory and inhibitory control by both hormones and guanine nucleotides. An increase in adenylate cyclase activity, with the subsequent elevation in tissue cyclic AMP, is generally believed to be the mechanism by which drugs acting at β-adrenoceptors mediate smooth muscle relaxation. Considerably less is known, however, about drugs which inhibit adenylate cyclase activity and, thus, reduce the level of cyclic AMP within the cell. With the possible exception of the α_2-adrenoceptors coupled to adenylate cyclase in human platelets, little evidence is available to suggest that drug-induced inhibition of adenylate cyclase activity represents a ubiquitous signal transduction mechanism in eucaryotic cells. We present here the results from preliminary studies which suggest that a reduction in tissue cyclic AMP maybe the primary event by which leukotriene (LT) D_4 elicits contraction of guinea-pig lung parenchyma (GPLP) in vitro.

Sub-pleural parenchymal lung strips prepared from male Dunkin-Hartley guinea-pigs were set up for the recording of isometric tension in 6 ml tissue baths containing oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution (KHS) maintained at 37^0C. Cyclic AMP was measured in tissues frozen in liquid N_2 either before or at pre-determined times after drug addition using a competitive protein binding assay. The concentration of thromboxane B_2 (TXB_2) released from the lung tissue was measured in the KHS by RIA at the same time points as cyclic AMP. TXB_2 and cyclic AMP levels are expressed as fmol and pmol/mg protein respectively. Values in the text refer to the mean ± s.e.m. of 6 observations. Differences between means were assessed using non-parametric statistical analyses and considered significant when $P < 0.05$.

LTD_4 (200 nM) elicited a biphasic contraction of GPLP (2.9 ± 0.3 mN); an initial fast (phasic) response followed by a slowly developing, well sustained, contracture which persisted for several hours even after LTD_4 was washed out. Surprisingly, the contractile response was succeeded by a time-dependent, 16 fold, increase in the release of TXB_2 from the tissue (from 143 ± 47 to 2237 ± 539 after 15 min, $P < 0.05$). Thus, the time taken for half the maximum response to be elicited (ET_{50}) was significantly ($P < 0.05$) greater for the formation of TXB_2 (ET_{50}: 2.31 ± 0.03 min) than for the generation of tension (ET_{50}: 1.35 ± 0.09 min). Additionally, LTD_4-induced contractions of GPLP were accompanied by time-dependent increases in the level of tissue cyclic AMP which peaked after approximately 5 min drug contact (from 7.4 ± 0.4 to 50.1 ± 3.6, $P < 0.05$) thereafter decaying. Pre-treatment of tissues with flurbiprofen (8 μM for 60 min) abolished the initial fast contractile response induced by LTD_4 without affecting the slow tonic contracture. Consistent with its proposed mechanism of action flurbiprofen abolished the release of TXB_2 from the lung. Under these conditions contractions induced by LTD_4 were now preceded by a rapid reduction in the level of cyclic AMP (from 9.1 ± 0.5 to 4.4 ± 0.7 after 30 s, $P < 0.05$).

These data illustate that LTD_4 contracts GPLP by both direct and indirect mechanisms, the latter involving the generation of constrictor cyclo-oxygenase product(s). Additionally, these data suggest that the rapid reduction in tissue cyclic AMP which preceded the contractile response may represent the primary intracellular event by which LTD_4 initiates contraction of GPLP. In support of this hypothesis Andersson et al (1982) have published similar results for the effects of LTC_4 on guinea-pig trachea. It is possible, therefore, that receptors for LTD_4 are negatively coupled to adenylate cyclase in GPLP and that the receptor-mediated attenuation of the enzyme's activity is associated with, or is responsible for, the observed LTD_4-induced contracture. Studies are now being conducted to assess this possibility.

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EFFECT OF THE MIXED CYCLO-OXYGENASE/5-LIPOXYGENASE INHIBITOR, BW755C IN A MODEL OF YEAST-INDUCED INFLAMMATION

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Cyclo-oxygenase inhibitors have been used for many years as analgesic/anti-inflammatory drugs. However, since the discovery of the pro-inflammatory leukotrienes, attention has focussed on the development of mixed cyclo-oxygenase/5-lipoxygenase inhibitors. In the present study we report the effects of the mixed inhibitor 3-amino-1-[3-trifluoromethyl)-phenyl]-2-pyrazoline, BW755C, in a model of yeast-induced hyperalgesia and oedema in the rat as previously described (Carey and Haworth, 1985). BW755C was administered i.p., 30 min before injection of yeast.

In controls, hyeralgesia developed over 2-4hr post-injection while oedema was observed at 0.5hr and gradually increased thereafter. BW755C at 100mg kg^{-1} inhibited hyperalgesia throughout the 2-4hr period (max 3hr, 97 ± 17%, $p<0.001$) Student's t-test, n=6). Oedema was inhibited at 0.5hr (64 ± 6%, $p<0.001$) and this was sustained up to 4 hr (62 ± 4%, $p<0.001$). LTB_4 and PGE_2 were also inhibited in 4hr paw exudate (85 ± 2% and 70 ± 8%, $p<0.001$ respectively). At 50 mg kg^{-1}, similar but more variable effects were observed on hyperalgesia (max 3 hr, 88 ± 31%, $p<0.02$) and paw exudate eicosanoids, (PGE_2, 67 ± 16%; LTB_4, 57 ± 17%, $p<0.02$) but inhibition of oedema was reduced (0.5hr, 24 ± 10% not significant; 4hr, 25 ± 7%, $p<0.05$). At 25 mg kg^{-1}, BW755C prolonged the onset of hyperalgesia until 3 hr (55 ± 12% inhibition, $p<0.01$), but by 4hr had no effect. No significant effect was observed on oedema or paw exudate LTB_4 but PGE_2 was inhibited (78 ± 3%, $p<0.01$). At 10 mg kg^{-1} there was no significant effect on any parameter. Approximate ID_{50}s (mg kg^{-1}) for BW755C were 40 for 4 hr hyperalgesia, 80 for 0.5hr and 4 hr oedema and 19 and 40 for paw exudate PGE_2 and LTB_4 respectively. These findings are in general agreement with those described in a carrageenin sponge-implant model (Salmon et al., 1983).

The effects of BW755C contrast with those of NSAIDs since the latter inhibit hyperalgesia and exudate PGE_2 but have little effect on LTB_4 and paw oedema. The inhibition of early oedema by BW755C may be unrelated to 5-lipoxygenase inhibition as before 2hr, LTB_4 is not elevated in paw exudate (Haworth and Carey, 1985). While inhibition of cyclo-oxygenase may account for the analgesic properties of BW755C, 5-lipoxygenase inhibition may also contribute to this activity. Indeed, LTB_4 has been reported to induce hyperalgesia (Levine et al., 1984).

In conclusion, our results demonstrate the use of the yeast inflamed paw model to study the efficacy and bioavailability of potential mixed cyclo-oxygenase lipoxygenase inhibitors.

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ROLE OF ARACHIDONIC ACID METABOLITES IN PULMONARY β-ADRENOCEPTOR DESENSITIZATION: IN VITRO AND EX VIVO STUDIES

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The role of arachidonic acid (AA) and/or its metabolites in ß-adrenoceptor desensitization has been studied in rat lung parenchyma both from a functional and biochemical point of view as previously described (1) (2). In vitro perfusion of rat lung with isoprenaline (ISO) induces a dose and time dependent desensitization of ß-adrenoceptors in this tissue as shown by the shift to the right of the ISO dose response curve on lung parenchymal strips paralleled by the loss of ^{3}H-dihydroalprenolol binding sites and adenylate-cyclase activity. The cyclo-oxygenase inhibitor indomethacin (INDO) prevents the ß-adrenoceptor desensitization as indicated by the restored ISO relaxing capacity and adenylate-cyclase activity, at least after the milder desensitization procedure performed. In addition to this, perfusion of rat lung with AA results in reduced functional responsiveness of lung parenchymal strips and adenylate-cyclase activity to ISO without modifying ^{3}H-dihydroalprenolol binding sites. INDO prevents the ß-adrenoceptor desensitization induced by the prostaglandin precursor.

Further results indicate that desensitization of rat lung ß-adrenoceptors can be achieved after in vivo administration of ISO. The ISO dose response curve on lung parenchymal strips taken from in vivo treated rats is shifted to the right, and adenylate-cyclase activity and ^{3}H-dihydroalprenolol binding sites are reduced. Moreover in vivo INDO treatment prevents the onset of tachyphylaxis to ISO.

These data indicate that arachidonic acid metabolites are involved in lung ß-adrenoceptor desensitization although they do not seem to combine directly to ß-adrenoceptors.

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PHOSPHOINOSITIDE METABOLISM AND CYTOSOLIC FREE Ca^{2+} IN LEUKOTRIENE B_4 (LTB_4)-INDUCED ACTIVATION OF HUMAN NEUTROPHILS

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LTB4-induced activation of human neutrophils is initiated by combination with distinct receptors located on the surface membrane (Goldman & Goetzl, 1984). However, the mechanisms that link LTB4-receptor occupancy to human neutrophil activation remains unknown. Studies using other agonists and/or cells isolated from other species indicate that neutrophil responsiveness is regulated by the actions and interactions of two second messenger molecules: cytosolic free Ca^{2+} ($[Ca^{2+}]i$) and 1,2-Diacylglycerol (DAG) (Di Virgilio et al, 1984; Lew et al, 1984) that are produced as a consequence of receptor-mediated phosphoinositide metabolism (Nishizuka, 1984). In the present study we examined the role of phosphoinositide metabolism and changes in $[Ca^{2+}]i$ in LTB_4-induced aggregation of, and enzyme secretion from, human neutrophils.

Neutrophils were isolated from the blood of healthy adult donors by gelatin sedimentation followed by hypotonic lysis of contaminating erythrocytes. $[Ca^{2+}]i$ was determined by using quin 2 (White et al, 1983). Phosphoinositide hydrolysis was monitored, indirectly, as changes in $[^{32}P]$-Phosphatidate (-PtdA) and $[^{32}P]$-Phosphatidylinositol (-PtdIns) in cells pre-labelled with $[^{32}P]$-PO_4 (Dougherty et al, 1984). Neutrophil aggregation was monitored photometrically and release of β-N-acetylglucosaminidase (NAG) was monitored fluorimetrically (McMillan et al, 1980).

Resting $[Ca^{2+}]i$ in human neutrophils was 133±6nM (mean ±S.E., n=20). LTB_4 (25pM-25nM) elevated $[Ca^{2+}]i$ to around 250-500nM and elicited aggregation and release of NAG but did not increase the levels of $[^{32}P]$-PtdA or $[^{32}P]$-PtdIns. Mean EC_{50} values (±S.E., n=3-5) were 0.9±0.3nM for elevation of $[Ca^{2+}]i$, 30±13nM for aggregation and 59±11nM for NAG release.

LTB_4-induces elevation of neutrophil $[Ca^{2+}]i$ at concentrations at least 30 fold lower than elicit neutrophil functional responses (aggregation and NAG release). Moreover, LTB_4-induced elevation of neutrophil $[Ca^{2+}]i$ is not accompanied by phosphoinositide hydrolysis, at least as monitored by changes in $[^{32}P]$-PtdA or $[^{32}P]$-PtdIns. This is in accord with the findings of Volpi et al (1984) using rabbit neutrophils. These results indicate that elevation of $[Ca^{2+}]i$ alone cannot mediate human neutrophil functional responses to LTB_4. Consequently there must exist other mediator(s) that act alone, or in concert with $[Ca^{2+}]i$, to promote human neutrophil activation. Both the elevation of $[Ca^{2+}]i$ and the formation of these putative other mediator(s) in response to LTB_4 apparently occur independently of inositol phospholipid metabolism.

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DIFFERENTIAL Ca^{2+} SENSITIVITY OF ARACHIDONATE LIBERATION EVOKED BY COLLAGEN AND IONOMYCIN IN FURA2-LOADED HUMAN PLATELETS

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Human platelets were labelled with [^{3}H]-arachidonate and loaded with fura-2 (a new fluorescence [Ca^{2+}] indicator (Tsien, Rink and Poenie, 1985) by incubating them at 37°C for 45min with 2μCi/ml [^{3}H]-arachidonate and 1μM fura-2 acetoxymethyl ester respectively. The cells were resuspended in Hepes-buffered saline, 1mM [Ca^{2+}], at 37°C, and $[Ca^{2+}]_i$ measured from the fluorescence (ex 340nm, em500nm). 3 min. after addition of agonists, reactions were quenched with ice-cold methanol, and the lipids extracted and analysed by thin-layer chromatography.

The mean resting $[Ca^{2+}]_i$ was 112nM±4nM (S.E. n=20). $[Ca^{2+}]_i$ was raised to increasing levels by increasing concentrations of the Ca ionophore ionomycin. Up to 1μM $[Ca^{2+}]_i$ there was no measureable arachidonate release. As $[Ca^{2+}]_i$ was increased to 2 to 5μM a substantial release, up to 4 times basal, occurred with concommitant loss of label from phosphatidyl choline. Presumably these changes reflected activation of Ca-calmodulin-dependent phospholipase A_2. With collagen a different picture was seen. In platelets from drug-free donors 10μg/ml collagen typically produced a 3-fold increase in free arachidonate, mostly from phosphatidyl choline, but about 25% from phosphatidyl inositol, but $[Ca^{2+}]_i$ rose only to 430±28nM (SE, n=16). With 10μM indomethacin to block the formation of prostaglandin endoperoxides and thromboxane, 10μg/ml collagen raised $[Ca^{2+}]_i$ barely at all (to 125±6nM SE, n=12); but still caused a 3-fold release of arachidonate this time very largely from phosphatidyl choline.

This result confirms previous findings with quin2-loaded platelets suggesting that prostaglandin endoperoxides and thromboxane are mainly responsible for collagen-evoked rises in $[Ca^{2+}]_i$ (Rink and Hallam 1984) and shows that collagen can evoke release of arachidonate from phosphatidyl choline essentially independent of any rise in $[Ca^{2+}]_i$.

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EFFECT OF THE EP_1 RECEPTOR ANTAGONIST, SC19220 IN A MODEL OF YEAST AND PGE_2 INDUCED HYPERALGESIA AND OEDEMA

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Prostaglandins of the E series induce hyperalgesia by sensitisation of peripheral nociceptors to other stimuli. They are implicated in yeast-induced hyperalgesia as this is reduced by NSAIDs (Winter and Flataker, 1965). Develoepment of hyperalgesia is temporally associated with elevation of paw exudate PGE_2 (Carey and Haworth, 1985) and the latency to onset is shortened by exogenous PGE_1/E_2 (Tyers and Haywood, 1979). In contrast, PGs appear to have only a minor role in yeast-induced oedema since its rapid onset (max 0.5hr) is not associated with elevation of exudate PGE_2 and NSAIDs have little effect. In this study, we have investigated the effect of the EP_1 receptor antagonist, 1-acetyl-2-(8-chloro 10,11-dihydrobenz[b,f][1,4] oxazepine-10-carbonyl) hydrazine, SC-19220, (Kennedy et al., 1982) in the rat yeast-inflamed paw model. Hyperalgesia and oedema induced by subplantar injection of 0.1ml brewer's yeast (20% w/v) or yeast + PGE_2 ($10\mu g\ ml^{-1}$) were measured at 0.5 to 4hr and paw exudate eicosanoids at 4 hr post-injection, as previously described (Haworth and Carey, 1985). SC-19220 was dosed orally 30 min before subplantar injection.

In yeast-injected controls, onset of hyperalgesia was delayed (2-3hr) whilst injection of yeast + PGE_2 shortened the latency of onset to 0.5hr. In both cases oedema was maximal at 0.5hr and thereafter remained relatively constant. When dosed at $100mg\ kg^{-1}$, SC-19220 prolonged the onset of yeast-induced hyperalgesia until 4hr when its extent was inhibited by 67 ± 24% ($p<0.02$, Student's t-test). Oedema was also reduced over the 2-4hr period (max 4hr, 31 ± 10%, $p<0.05$) and paw exudate PGE_2 and LTB_4 were significantly inhibited (81 ± 2%, $p<0.05$ and 88 ± 3%, $p<0.01$ respectively). At 50 and $25mg\ kg^{-1}$ there was no significant effect on hyperalgesia or exudate eicosanoids but at the higher dose oedema was reduced at 4hr (41 ± 8%, $p<0.01$) . Yeast + PGE_2 induced hyperalgesia was unaffected at 0.5hr by $100mg\ kg^{-1}$ SC-19220 but thereafter inhibition increased to a maximum at 2hr (75 ± 17%, $p<0.01$) and was sustained at 3-4hr. Oedema (max inhibition 4hr, 42 ± 6%, $p<0.001$) and paw exudate eicosanoids (PGE_2, 50 ± 6% and LTB_4 53 ± 10%, $p<0.02$) were also reduced but no effect was observed at 50 and $25mg\ kg^{-1}$. In addition to effects on hyperalgesia and oedema SC-19220 induced behavioural effects at 100 and $50mg\ kg^{-1}$ but not at $25mg\ kg^{-1}$.

The inhibitory action of SC-19220 on hyperalgesia and oedema may not be entirely attributable to EP_1 receptor antagonism since reduction of paw exudate PGE_2 and LTB_4 and behavioural side-effects may also have contributed. These findings indicate the requirement for other selective EP_1 receptor antagonists to evaluate their potential as novel analgesic and anti-inflammatory agents.

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BAY K-8644 INCREASES CALCIUM UPTAKE IN THE PRESENCE OF ISOPRENALINE AND KCL IN RABBIT CARDIOMYOCYTES

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The dihydropyridine BAY K-8644 [methyl 1,4-dihydro-2,6-dimethyl -3-nitro-4 -(2-trifluromethylphenyl)-pyridine-5-carbxoylate] is a positive inotropic agent acting as an agonist on the calcium channel (Schram et al , 1983). The beta-adrenoceptor agonist isoproterenol augments slow calcium action potentials in rabbit cardiomyocytes (Vogel et al , 1985) by increasing the time individual channels spend in the open state (Bean et al , 1984). The aim of this investigation was to determine the effect of BAY K-8644 on calcium accumulation into rabbit cardiomyocytes under basal conditions and in the presence of isoproterenol and potassium.

Myocytes were isolated using a modified Langendorf preparation and suspended in Krebs' solution (NaCl, 100mM ; $CaCl_2$, 1.3mM). Cells were incubated with $^{45}Ca^{2+}$ (1uCi/ml) for 5 mins at 37°C. Incubations were carried out in Krebs' buffer (1.3 mM $CaCl_2$) in the presence of different concentrations of BAY K 8644 and isoproterenol. The cardiomyocytes were washed in a Hepes-choline chloride buffer ($LaCl_3$ 500uM). $^{45}Ca^{2+}$ content was determined by liquid scintillation counting and was used as a marker for total calcium accumulation.

We have previously demonstrated that on activation of beta-1 adrenoceptors there is an increase cAMP accumulation in heart cardiomyocytes producing a subsequent increase in calcium accumulation. In a non-depolarising buffer (5mM KCl) isoproterenol increased calcium accumulation (18.2±2.7 nmoles/mg at 100nM) , but BAY K-8644 (300nM) did not modify calcium accumulation. Cardiomyocytes do not significantly accumulate $^{45}Ca^{2+}$ on depolarisation with 40mM KCl (Disilets et al , 1982). As BAY K-8644 has been proprosed to activate the calcium channel favouring the influx of calcium we used this compound to study calcium accumulation in the presence of KCl and isoproterenol. BAY K-8644 (300nM) potentiated calcium accumulation on depolarisation with 10mM KCl (11.5±1.6 nmoles/mg) and 40mM KCl (84±21 nmoles/mg). The stimulation of calcium accumulation induced by BAY K-8644 (300nM) in a buffer containing 10mM KCl (1.3mM $CaCl_2$) was potentiated by 320% in the presence of 100nM isoproterenol. This enhancement of calcium accumulation was above the basal calcium accumulation stimulated by isoproterenol alone , which is believed to be due to an increased capacity of sarcoplasmic reticulum for calcium. The effect of isoproterenol was mimicked by dibutyryl cAMP (100nM) and antagonised by propranolol (1uM). Rod shaped cardiomyocytes were quiescent in a Krebs buffer containing 40mM KCl (2.6mM $CaCl_2$) in the presence and absence of 300nM BAY K-8644 or 50nM isoproterenol alone. However , in the presence of both 300nM BAY K-8644 and 50nM isoproterenol the number of rod shaped cells that contracted and the rate of contraction increased. This possible calcium overload (Altshuld et al , 1985) augmented by isoproterenol was mimicked by dibutyryl cAMP (50nM) and antagonised by propranolol (1uM).

These results indicate that the dihydropyridine BAY K-8644 acts as a calcium agonist on depolarisation with KCl stimulating calcium accumulation into the rabbit cardiomyocytes. Isoproterenol increases basal calcium accumulation and potentiates the action of BAY K-8644 on slow calcium channels via an increase in cAMP levels. The dual effect of isoproterenol may result in a possible calcium overload in depolarised cardiomyocytes.
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ANALYSIS OF THE POSITIVE CHRONOTROPIC ACTION OF 5-HT ON THE ISOLATED GUINEA-PIG ATRIA

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5-hydroxytryptamine (5HT) receptors have been reported to exist in at least three classes: $5HT_1$, $5HT_2$ and $5HT_3$ (or M receptors). The actions of 5HT on the myocardium may be exerted via a direct action on $5HT_1$ receptors, which has been reported for the cat (Conner et al, 1985; Saxena et al, 1985), by an indirect action on $5HT_3$ receptors present on nerve endings (Fozard, 1984) which has been reported in the isolated, perfused rabbit heart, or finally by a primarily direct action (Trendelenburg, 1960).

The aim of the present study was to analyse the action of 5HT on the isolated atria of the guinea-pig, a preparation which has yet to be classified with regard to the action of 5HT.

Paired atria were isolated from Dunkin-Hartley guinea-pigs (female, 250 g b.w.) and suspended under 1.0 g preload in Krebs-bicarbonate buffer, pH 7.4, 37°C. Pargylline (1×10^{-5} mol.litre^{-1}) and normetanephrine (5×10^{-6} mol.litre^{-1}) were routinely added. After one hour equilibration the preparations developed a spontaneous rhythm and cumulative concentration-response curves were constructed to 5HT and isoprenaline. The following antagonists were examined using 5HT as the agonist: methysergide, ketanserin, MDL 72222, phentolamine, atenolol, pindolol and hexamthonium. Antagonist equilibration time was 45 min and three concentrations of each antagonist were examined in each tissue.

Positive chronotropic responses to both 5HT (EC_{50} = 3.6×10^{-6} mol.litre^{-1}) and isoprenaline were observed (EC_{50} = 8.5×10^{-9} mol.litre^{-1}). The maximum response obtained with 5HT was approximately 40% of that to isoprenaline. The response to 5HT was unaffected by 1×10^{-6} mol.litre^{-1} methysergide, ketanserin, MDL 72222 or phentolamine but at 1×10^{-5} mol.litre^{-1} a slight, but significant rightward shift was observed with all 4 antagonists. Hexamethonium, 1×10^{-4} mol.litre^{-1} was without effect on the response to 5HT. Atenolol (1×10^{-7} to 1×10^{-5} mol.litre^{-1}) produced a concentration-dependent depression in the maximum resulting in complete abolition of the response to 5HT at 1×10^{-5} mol.litre^{-1}. Similar effects were observed using pindolol (1×10^{-8} to 1×10^{-6} mol.litre^{-1}).

In summary, the positive chronotropic effects of 5HT appear not to be mediated through 5HT receptors since the response was unaffected, except at high concentrations, by methysergide, ketanserin and MDL 72222. The lack of effect of phentolamine indicates that α-adrenoceptors were not involved in the chronotropic response to 5HT. The lack of effect of hexamethonium indicates that this response was not mediated through ganglionic nicotinic receptors.

The ability of atenolol and pindolol to antagonise the response to 5HT at concentrations which would be expected to antagonise β_1-adrenoceptors shows that the effect is mediated by direct β_1-receptor stimulation.

In conclusion, therefore, the positive chronotropic response of the guinea-pig atria to 5HT is mediated through β_1-adrenoceptors. These results are in general agreement with those reported by Trendelenburg (1960) for the isolated guinea-pig atria but are in contrast to those observed in the cat heart and the isolated perfused rabbit heart.

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