Effects of agonists and antagonists at vascular dopamine receptors in the rabbit isolated splenic artery and the mesenteric vascular bed of the anaesthetized dog

G.M. DREW & A. HILDITCH

Department of Pharmacology, Glaxo Group Research Ltd., Priory Street, Ware, Hertfordshire, SG12 ODJ.

The effects of some well established dopamine receptor agonists and antagonists have been examined at the vascular dopamine receptors in the rabbit isolated splenic artery (Crooks & Martin, 1979) and in the mesenteric bed of the anaesthetized dog (Crumley, Hinshaw, Pinder & Goldberg, 1978; Drew & Hilditch, 1980). In both sets of experiments α- and β-adrenoceptors were blocked by phenoxybenzamine (3×10^{-5} mol/l; 2×10^{-5} mol/kg i.a.) and propranolol (1×10^{-6} mol/l; 3×10^{-6} mol/kg i.v.) respectively.

Dopamine (1×10^{-8}–1×10^{-4} mol/l) caused a concentration-dependent relaxation of splenic artery strips contracted with $PGF_{2\alpha}$. In anaesthetized dogs, intra-arterial injection of dopamine (2×10^{-9}–2×10^{-7} mol i.a.) caused dose-dependent increases in mesenteric blood flow. The effects of dopamine were mimicked by epinine, 6,7-ADTN, the N,N-di-n-propyl derivatives of 5,6-ADTN and 6,7-ADTN and by Sandoz 27–403 both '*in vitro*' and '*in vivo*'. In contrast, 5,6-ADTN and N,N-diethyldopamine were weak or inactive in both preparations. SKF 38393 increased mesenteric blood flow in dogs but was almost inactive in splenic artery strips. In the latter preparation, SKF 38393 (1×10^{-5} mol/l) caused a 42-fold shift to the right in the concentration-effect curve for dopamine which suggests that it is a weak partial agonist at dopamine receptors in the splenic artery. The potencies of all agonists, relative to dopamine, are shown in Table 1.

Following intravenous injection, fluphenazine and sulpiride are equipotent antagonists of dopamine in the dog mesenteric vascular bed (Drew & Hilditch, 1980). Fluphenazine also antagonised dopamine in the splenic artery strip ($pA_2 = 6.2$), but sulpiride was without effect at 1×10^{-4} mol/l. Cis α-flupenthixol was approximately ten times more potent than fluphenazine against dopamine '*in vitro*' ($pA_2 = 7.18$) and twenty times more potent '*in vivo*' ($DR_{10} = 6.9 \times 10^{-7}$ mol/kg i.v. compared with 1.2×10^{-5} mol/kg i.v. for fluphenazine, Drew & Hilditch, 1980).

In conclusion, with the notable exception of sulpiride, the sensitivity of the dopamine receptors in the rabbit splenic artery and dog mesenteric artery to agonists and antagonists appear to be similar.

Table 1 Relative potencies of agonists at vascular dopamine receptors in the rabbit isolated splenic artery and mesenteric vascular bed of the anaesthetized dog

	Equipotent concentration	
	Rabbit Splenic Artery	*Dog Mesenteric Vascular Bed**
Dopamine	1	1
6,7-ADTN	0.2 (0.04–0.8)	1.1 (0.8–1.7)
Epinine	0.3 (0.1–0.5)	1.5 (1.4–1.6)
N,N-di-n-propyl 5,6-ADTN	1.1 (0.5–2.3)	1.7 (0.6–5.2)
N,N,-di-n-propyl 6,7-ADTN	3.9 (0.6–14.6)	4.5 (2.3–7.5)
Sandoz 27–403	3.9 (2.4–8.2)	2.4 (1.2–4.4)
5,6-ADTN	> 300	65.7 (41.0–95.0)
N,N-diethyldopamine	> 300	212.7 (162.0–263.0)
SKF 38393	> 300	7.4 (5.0–10.0)

* Drugs given intra-arterially.
Results expressed as geometric means and ranges; $n = 5$ and 3 in splenic arteries and anaesthetized dogs respectively.
5,6-ADTN = 2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene.
6,7-ADTN = 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

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The contribution of sympathetic and parasympathetic nerve mechanisms to the control of heart rate in conscious dogs at rest and during exercise as assessed by using betaxolol and methylatropine

J.M. ARMSTRONG, I. CAVERO & SIMONE FÉNARD

Department of Biology SYNTHELABO (L.E.R.S.), Cardiovascular Group, 58, rue de la Glacière, 75013 Paris, France.

The heart rate of animals and man is modulated by autonomic nerve activity and is in conscious dogs lower than the intrinsic rate set by the main cardiac pacemaker because parasympathetic drive predominates (Lokhandwala, Cavero, Buckley & Jandhyala, 1973). Additionally, it appears that neural sympathetic activity may be suppressed by vagally released acetylcholine acting on sympathetic nerve terminals to reduce the output of the adrenergic transmitter (Vanhoutte & Levy, 1980). We have now studied the relative contributions made by the two divisions of the autonomic nervous system to the cardiac rate of conscious trained dogs at rest and during exercise.

Four mongrel dogs weighing 12–18 kg were trained to run at 6.4 km/h for 5 min periods at intervals on a belt-driven treadmill (Warren & Collins. Mod. P. 2000 E) inclined at a slope of 10%. The electrocardiogram was detected by means of two electrodes placed either side of the shaved chest and connected to a preamplifier coupled to an FM telemetry system. The transmitted signals were detected by a receiver placed 2 meters from the dog and displayed using a pen recorder. The effects of parasympathetic activity were inhibited by intravenous methylatropine (0.5 mg/kg) and those of sympathetic activity inhibited by intravenous betaxolol (0.1 mg/kg) a cardioselective β-adrenoceptor blocking agent of long duration of action (Boudot, Cavero, Fénard, Lefevre-Borg, Manoury & Roach, 1979).

At rest the heart rate of each dog was low (mean $\pm$ s.e. mean of 61 $\pm$ 7 bts/min), indicating that they were well accustomed to the laboratory environment. Within 30 s from the start of exercise the heart rate increased to a maximum mean value of 162 $\pm$ 6 bts/min and after exercise returned to resting values. The values of tachycardia occurring on successive runs were similar. Betaxolol administration neither altered the resting heart rate nor the values developed during exercise.

Methylatropine administration was rapidly followed by an increase in resting heart rate to 246 $\pm$ 15 bts/min but this was not accompanied by any evident change in overt behaviour. The elevated rate was not sustained but declined so that 1 h later it was 190 $\pm$ 11 bts/minute. The gradual fall in heart rate was probably not due to inactivation of methylatropine since an additional dose of methylatropine 60 min after the first did not re-elevate heart rate. In animals pretreated with betaxolol the mean maximum heart rate increase produced by methylatropine was less than in controls by approx. 41 bts/min and thereafter it waned in parallel with that of controls.

After methylatropine the heart rate response to physical exercise was greater (by 74 $\pm$ 13 bts/min, $n = 4$) and the values attained were similar on each successive run made during the subsequent 2.5 h period despite the accompanying decline in resting heart rate. Similarly 2 h after administration of a small dose of methylatropine (0.03 mg/kg i.v.) when resting heart rates were not significantly different from pre-treatment values the tachycardia obtained on exercise was greater by 44 $\pm$ 8 bts/min than that observed in control conditions and was reduced by 23 $\pm$ 6 bts/min after prior administration of betaxolol. When betaxolol was injected 35 min after 0.5 mg/kg methylatropine, the tachycardia produced by exercise was less (by 50 $\pm$ 5 bts/min) than in controls given saline in place of the β-blocking agent.

These findings indicate that in conscious trained mongrel dogs the heart rate recorded at rest is maintained at a low level by parasympathetic activity which is withdrawn on exercise to produce tachycardia. Furthermore, it would appear that during submaximal exercise small quantities of acetylcholine are released from vagus nerves and are sufficient to inhibit sympathetic activity at the cardiac pacemaker.

The latter conclusion is supported by the observation that doses of methylatropine producing minor inhibition of postsynaptic muscarinic receptors can nevertheless augment the tachycardia observed on exercise and this increase was found to be partially mediated via β-adrenoceptor activation.

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Functional and biochemical evidence for the lack of cardiac presynaptic α-adrenoceptor agonist properties in cirazoline (LD 3098)

I. CAVERO, FRANÇOISE LEFÈVRE-BORG & B. SCATTON†

Biology Department SYNTHELABO (L.E.R.S.), Cardiovascular Group and Neurochemistry Unit†, 58 rue de la Glacière, 75013 Paris, France.

Cirazoline (LD 3098) is an imidazoline derivative with potent vascular post-synaptic α-adrenoceptor stimulating properties both under *in vitro* and *in vivo* conditions (Lefèvre, Najer, Cavero & Giudicelli, 1975). It does not produce sedation (Lefèvre, Depoortere & Cavero, 1976) in contrast to clonidine or oxymetazoline which mediate this effect through stimulation of α_2-type adrenoceptors (Cavero & Roach, 1978). Since cirazoline does not stimulate cardiac presynaptic α-adrenoceptors in rats and dogs (Roach, Lefèvre & Cavero, 1978) it was suggested that this compound might be a relatively selective agonist at vascular α_1-adrenoceptors. Dubocovich, Langer & Massingham (1980) have recently reported that cirazoline is a potent agonist at presynaptic α-adrenoceptors in the cat perfused spleen. They noted that cirazoline potentiated blood pressure responses to noradrenaline (NA) in the dog and suggested that the lack of inhibition of sympathetic neural tachycardia (presynaptic α-adrenoceptor stimulation) by cirazoline could be due to increase in end organ responsiveness to NA. Roach *et al.* (1978) reported that the tachycardia to stimulation of spinal cord in the pithed rat was not potentiated by cirazoline when presynaptic receptors were blocked by phentolamine. No increase in heart rate to NA was found during cirazoline infusion when a potentiated pressor response to this catecholamine occurred (Dubocovich *et al.*, 1980). Supersensitivity to noradrenaline in the presence of cirazoline was only obtained in organs having a predominant population of postsynaptic α-adrenoceptors (Massingham, Dubocovich & Langer (1979)).

Spinal dogs were prepared as described by Cavero, Dennis, Lefèvre-Borg, Perrot, Roach & Scatton (1979) for the measurement of coronary sinus venous plasma noradrenaline content. The cardioaccelerator nerve was electrically stimulated (1.0 Hz, 1.0 ms and 7–15 V) to raise heart rate. When a plateau response had been attained, cirazoline was infused (1.0 μg kg^{-1} min^{-1} over 10 min). Noradrenaline content in the coronary sinus venous plasma was measured before, during and after the infusion of cirazoline. In a group of animals the effects of cirazoline on the tachycardia produced by an infusion of NA in the coronary artery perfusing the sinus node area was assessed.

In pithed rats pretreated with atropine (1.0 mg/kg i.v.) and (+)-tubocurarine (5.0 mg/kg i.v.), the thoracic spinal cord was continuously stimulated to obtain a 60–70 beats/min increase in heart rate. The effects of cirazoline, clonidine and oxymetazoline (1.0 μg kg^{-1} min^{-1} over 5 min) on this tachycardia were evaluated in control, or phentolamine (0.5 mg/kg, i.v.) pretreated rats. Cirazoline failed to change the increase in heart rate produced by electrical stimulation of cardioaccelerator sympathetic nerve fibres but oxymetazoline and clonidine produced a fall in heart rate. Cirazoline changed neither baseline heart rate nor the neural tachycardia in animals pretreated with phentolamine to inhibit presynaptic α-adrenoceptors.

In the spinal dog cirazoline failed to modify the baseline heart rate and the tachycardia evoked by an intracoronary infusion of NA. Cirazoline significantly changed neither the tachycardia nor the coronary sinus venous plasma NA content previously increased by electrical stimulation of the cardioaccelerator nerve.

These results confirm our original findings (Roach *et al.*, 1978) that cirazoline, in contrast to clonidine

and many imidazolines, does not stimulate cardiac presynaptic α-adrenoceptors in dogs and rats. The fact that this imidazoline reduces NA release in the cat spleen and rat vas deferens (Massingham *et al.*, 1979) may indicate that cirazoline demonstrates functional organ selectivity. In favour of this hypothesis is the observation that cirazoline does not decrease NA release induced by potassium in rat brain cortical slices or by electrical stimulation in rabbit hypothalamic slices (Langer, personal communication). Whether these findings indicate that presynaptic α_2-adrenoceptors in various organ are not an homogeneous population remains to be assessed.

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Competitive and non-competitive blockade of α-adrenoceptors on rat aorta by prazosin

O.A. DOWNING, K.A. WILSON & V.G. WILSON

Pharmacological Laboratories, Department of Pharmacy, University of Aston, Gosta Green, Birmingham B4 7ET.

The selective α_1-adrenoceptor antagonist prazosin has been reported to be a non-competitive inhibitor of α-adrenoceptors in venous smooth muscle while exhibiting competitive blockade for α-adrenoceptors in arterial smooth muscle of the rat (Cohen, Wiley & Slater, 1979).

The results presented here demonstrate competitive and non-competitive inhibition by prazosin of different components of the contractile response of isolated rat aorta to noradrenaline (NA) or phenylephrine.

Helical strips of descending thoracic aorta were obtained from male Wistar rats (wt. range 180–240 g). These were suspended with a resting tension of 1.0 g in 10 ml baths containing Krebs solution of the following composition (mM): NaCl 118.4; KCl 3.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 2.2; $NaHCO_3$ 24.9 and glucose 10.0 maintained at 37°C and gassed with 5% CO_2 in O_2.

Under isometric conditions, biphasic (fast and slow) contractions of the aortic strip were observed with concentrations of NA above 0.025 μM. Below this concentration only the slow component was observed. Qualitatively similar biphasic responses were seen to phenylephrine which was found to be approximately ten times less potent than NA.

Using NA or phenylephrine as agonist, prazosin (1.0 nM to 25.0 nM) caused a parallel shift to the right, with little or no reduction in the maximum response, of the log dose response curve of the slow phase of the response (measured at maximum development of tension –5 to 15 min after the addition of agonist), but caused a non-competitive depression of the curves for the fast component (measured at 15 s). With concentrations of prazosin of 5.0 nM and above, the fast component was abolished and virtually unsurmountable.

In contrast phentolamine (2.5 and 25 μM) caused similar parallel shifts of the log dose response curves for both the slow and fast components of contractions to NA or phenylephrine. Similarly, yohimbine (2.5 and 25 μM) caused a parallel shift of the log dose response curves for both components of contraction to NA.

Prazosin in concentration of up to 2.5 μM had no

observable effect on contractions of the rat aorta to angiotensin (0.01 to 10.0 μM) or 5-hydroxytryptamine (0.25 to 250 μM).

The above results suggest that prazosin is reducing both components of the contractile response by selective blockade of α-adrenoceptors rather than by any direct interference with calcium mobilisation.

Following adrenoceptor stimulation, the fast component of contraction of rat aorta is believed to be due mainly to release of calcium from intracellular sites, whereas, the slow component is dependant upon entry of extracellular calcium (Godfraind & Kaba, 1972).

The observations with phenylephrine, phentolamine and yohimbine suggest that these components cannot be ascribed to separate or specific α_1 or α_2 stimulation. Therefore, the observation that prazosin non-competitively inhibits the fast component while showing competitive blockade of the slow, seems unlikely to be related to its selectivity for α_1-adrenoceptors.

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Effects of prazosin, phentolamine and yohimbine on the vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation in the rat mesentery

J.C. DOXEY, A.G. ROACH & NARINDER VIRDEE

Reckitt & Colman, Pharmacology Department, Dansom Lane, Kingston-upon-Hull, HU7 8DS.

Antagonism of presynaptic α_2-adrenoceptors increases the amount of neurotransmitter released in response to sympathetic nerve stimulation tending to increase end organ responses whereas postsynaptic α_1-adrenoceptor antagonism tends to reduce these responses (for reviews see Langer, 1977; Starke, 1977). The effects of yohimbine (a selective presynaptic α_2-adrenoceptor antagonist), prazosin (a selective α_1-adrenoceptor antagonist) and phentolamine (a non-selective α_1 and α_2-adrenoceptor antagonist) on the vasoconstrictor responses to exogenous noradrenaline and sympathetic nerve stimulation have been presently studied using the rat isolated perfused mesentery.

Mesenteries taken from male Sprague–Dawley rats (250–300 g) were perfused with Krebs solution (2–4 ml/min) at 37°C, gassed with 95% O_2 and 5% CO_2 and containing cocaine (10 μM) and (±)-propranolol (0.3 μM). Noradrenaline (0.3 and 1.0 μg) was injected into the perfusion fluid and the sympathetic nerves activated by field stimulation (2.0 and 5.0 Hz, 1 ms, supramaximal voltage for 20 s). Responses were obtained before and 30 min after perfusion of the antagonists. Vasoconstrictor responses remained constant in control experiments in which antagonists were omitted.

Prazosin was found to inhibit similarly the vasopressor responses to noradrenaline and nerve stimulation, phentolamine antagonized noradrenaline to a greater extent than stimulation and yohimbine potentiated the responses to nerve stimulation whilst inhibiting noradrenaline (Table 1).

The potentiating effect of yohimbine and the relative lack of effect of phentolamine on stimulation are consistent with a selective and non-selective antagonism of presynaptic α_2-adrenoceptors, respectively. The effectiveness of prazosin against both responses was due to its selectivity for postsynaptic α_1-adrenoceptors. The periarterial nerves appear to possess an active presynaptic α_2-adrenoceptor inhibitory feedback mechanism and the rat perfused mesentery is a suitable isolated vascular preparation to profile antagonists on α_1 and α_2-adrenoceptors.

Table 1 The effects of prazosin, phentolamine and yohimbine on the vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation in rat isolated perfused mesentery

		Noradrenaline (μg)				*Stimulation (Hz)*			
		0.3		*1.0*		*2.0*		*5.0*	
Antagonist	*Antagonist concentration (nM)*	*Control*	*% Change*	*Control response mmHg*	*% Change*	*Control response mmHg*	*% Change*	*Control response mmHg*	*% Change*
Prazosin	0.07	48 ± 8 (*n* = 4)	−53 ± 10	82 ± 11 (*n* = 4)	−41 ± 5	15 ± 5 (*n* = 4)	−46 ± 11	50 ± 1 (*n* = 4)	−43 ± 12
	0.24		−88 ± 7		−80 ± 7		−63 ± 11		−49 ± 14
Phentolamine	2.6	43 ± 6 (*n* = 3)	−82 ± 8	113 ± 16 (*n* = 3)	−37 ± 13	9 ± 1 (*n* = 9)	−6 ± 9	23 ± 1 (*n* = 9)	−24 ± 7
	7.9		−98 ± 2		−72 ± 7		−24 ± 11		−34 ± 7
Yohimbine	260	68 ± 16 (*n* = 4)	−75 ± 15	82 ± 18 (*n* = 4)	−58 ± 17	7 ± 1 (*n* = 6)	+105 ± 44	23 ± 4 (*n* = 6)	+39 ± 14
	790		−96 ± 4		−74 ± 10		+46 ± 24		+4 ± 11

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Prazosin changes the time-course profile of the pressor responses elicited by noradrenaline in the perfused forelimb of spinal dogs

I. CAVERO & FRANÇOISE LEFÈVRE-BORG

Biology Department SYNTHELABO (L.E.R.S.), Cardiovascular Group, 58 rue de la Glacière, 75013 Paris, France.

Pressor responses elicited by either noradrenaline (NA), phenylephrine or various vascular α-adrenoceptor agonists in the pithed rat and other preparations are not similarly inhibited by prazosin (see Cavero & Roach, 1980). Prazosin has been shown to depress particularly the pressor effects of the agonists which mediate vasoconstriction by stimulation of α_1-adrenoceptors (e.g.: phenylephrine).

Recently, Langer, Massingham & Shepperson (1980) reported that a small dose of prazosin did not significantly affect the maximal increase in perfusion pressure produced by i.v. NA in the dog perfused hind limb. These authors did not analyse the time-course profile of this response which, however, they noticed to be shortened after prazosin.

The aim of this communication is to show that prazosin modifies the pressor response to NA if the latter is expressed as a time-course event rather than as a simple peak phenomenon.

Dogs were anaesthetized (pentobarbitone sodium) and artificially respired. The spinal cord and the vagi were severed at the cervical level. Preparation of the forelimb was performed so that the perfused area was mainly constituted by skeletal muscle. A shunt between the brachial artery and the carotid artery was established. A roller pump was interposed in this circuit. The perfusion pressure was maintained at 80–110 mmHg by adjusting the pump rate. Noradrenaline (12.5, 50.0 and 200.0 ng, total dose) was administered as a bolus injection into the brachial artery before and after prazosin (10.0 followed 30 min later by 100.0 μg/kg, i.v.). The animals were given routinely atropine (0.3 mg/kg, i.v.) and decamethonium (0.4 mg/kg + 0.1 mg kg^{-1} h^{-1} throughout the experiment) and some of them were pretreated with propranolol (2.0 mg/kg, p.o., 16 and 1 h before anaesthesia).

The pressor response to NA was evaluated by the peak response (E_{max}), and the area under the pressor effect-time profile (AUC) calculated from the injection time to the moment of 75% reduction of E_{max}.

Prazosin did not significantly change the forelimb perfusion pressure of spinal dogs. The vasoconstrictor pattern of an intra-arterial bolus injection of NA can be described by a two component event: a fast initial phase (from injection time to the moment of peak response) and a second slower phase which may be characterized by the AUC. Prazosin in the studied doses did not significantly modify the magnitude of the response to NA but shortened the terminal phase. For instance, E_{max} for 200.0 ng i.a., NA after 100.0 μg/kg, i.v. prazosin differed by $-3.6 \pm 3.9\%$ ($n = 6$) from the control response. However, AUC was significantly reduced by $35 \pm 7\%$. A similar inhibition was found in dogs pretreated with propranolol.

In conclusion, small i.v. doses of prazosin do not reduce the magnitude of the pressor response to NA (Langer *et al.*, 1980), but alter the phase characterising the decline of this response. These results may be explained by assuming that activation of two pharmacologically distinct types of vascular post-synaptic α-adrenoceptors contribute to the NA pressor response in the skeletal muscle of the dog forelimb. The α_1-adrenoceptors blocked by prazosin are proposed to play an important role in the terminal phase of this pressor response to i.a. NA, whilst its peak response is likely to be predominantly dependent on α_2-adrenoceptors which are not affected by prazosin, at least in the doses used in this investigation.

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The release of an unidentified substance from the guinea-pig isolated atria and the inhibitory action of opiates

A.N.A. WILSON (introduced by J.C. DOXEY)

Department of Pharmacology, Reckitt & Colman, Hull HU8 7DS.

Several workers have shown that the parasympathetic nerves in the hearts of cats, rats and rabbits possess presynaptic opiate receptors (Kosterlitz & Taylor, 1959; Kennedy & West, 1967). The guinea-pig heart, however, has been reported to be devoid of opiate receptors on both the sympathetic and cholinergic innervations (Kosterlitz & Taylor, 1959; Langer, 1977). A reinvestigation using guinea-pig atria confirmed these earlier findings but at the same time revealed that electrical stimulation releases an unidentified substance with positive chronotropic and inotropic properties. The effects of this substance are inhibited by opiates. This action of opiates is reproducible, dose-related and naloxone reversible.

Spontaneously beating atrial preparations were obtained from male guinea-pigs in the weight range 400–800 g. The animals were killed by cervical dislocation and their hearts rapidly removed and placed in cold pre-oxygenated Krebs-Henseleit solution. The atria were set up in a 25 ml organ bath between parallel platinum electrodes under an initial tension of 2 g. The tissues were constantly perfused with Krebs-Henseleit solution at a rate of 6 ml/min and continuous oxygenation was provided with a 95% O_2, 5% CO_2 mixture. All experiments were carried out at 30°C. Both the rate and amplitude of contraction were recorded via an isometric transducer. Field stimulation (3 ms pulses at 0.4–1.0 Hz, 12 V) was applied to the tissues for 30 s every 7–10 min depending upon the time taken for the response to return to control values.

Immediately after stimulation a positive biphasic response was observed on both the amplitude of contraction and rate of atrial beating. The initial part of the response was unaffected by opiates but was inhibited by propranolol hydrochloride in a dose-related manner. A concentration of 300 ng/ml resulting in a complete block of the first phase of the response and also of an equiactive dose of exogenously applied noradrenaline. The second phase of the response was unaffected by propranolol but was inhibited by opiates. Therefore, all experiments relating to the second phase of the response were carried out in the presence of propranolol. Under these conditions normorphine base inhibited the stimulus-induced increased rate of beating in a dose-related manner over the concentration range 0.03–1.0 μg/ml. In 7 experiments a concentration of 1 μg/ml reduced the increase in rate by 75.6% ± 4.4 (mean ± s.e. mean). Naloxone (100 ng/ml) completely reversed this effect of normorphine. Preliminary investigations have also shown that the leucine-enkephalin derivative D-Ala^2-D-Leu^5-enkephalin (BW 180C) is more than a hundred times as potent as normorphine in inhibiting the second phase of the response. The substance responsible for the second phase of the stimulus-induced response has yet to be identified. However, the observation that tetrodotoxin (30 ng/ml) abolished the response implicates a neural site of origin. Such a substance could possibly have a role in the physiological control of the heart.

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Effects of increased pH on contractile force of rabbit isolated papillary muscles

I.W. RODGER & M. SHAHID

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

Aminopyridines have been reported to increase the contractile force of isolated cardiac muscle (Frank, Flom & Ffrench-Mullen, 1978; Yanagisawa & Taira, 1979). The results reported here arose from control experiments that formed part of an analysis of the positive inotropic effect of aminopyridines. Aminopyridines are strongly basic substances and consequently increase the pH of physiological salt solutions. We therefore studied the effects of pH changes produced by other means.

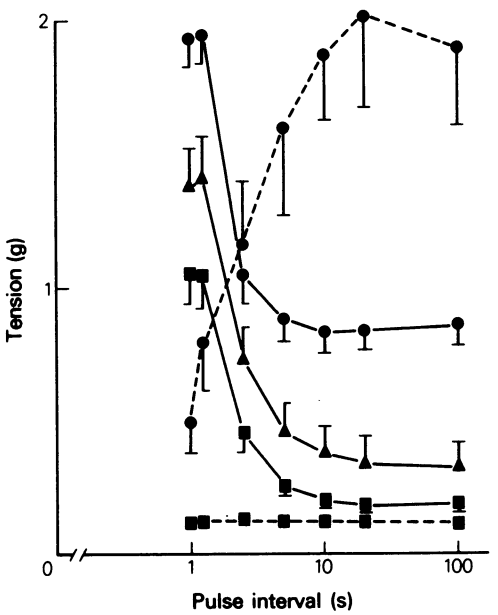

Figure 1 Mean interval-force relationship curves for rabbit isolated papillary muscle performed in (■) physiological salt solution at pH 7, (▲) pH 8 and (●) pH 9. The dotted lines illustrate the effects of verapamil (1×10^{-5} M) on the responses obtained at (■) pH 7 and (●) pH 9. Each point represents the mean (±s.e. mean) of at least 10 preparations.

Papillary muscles from the right ventricles of adult male New Zealand white rabbits were suspended in either Krebs-Henseleit or Tris-buffered Krebs solution at 32°C and their electrically-evoked contractions were recorded by conventional methods. Alterations in the pH of the physiological salt solutions were achieved either by addition of sodium hydroxide or by increasing the ratio of Tris base to hydrochloride. An increase in pH from 7 to 9 produced powerful, graded and reproducible increases in contractile force that were proportionally greater at low (0.05 Hz) than at high driving frequencies (1 Hz). Such inotropic responses were readily reversed to control levels of tension on returning to solutions at pH 7. The combined results are expressed graphically in Figure 1. Propranolol (5×10^{-7} M) was without effect on the inotropic effect induced by a rise in pH. Verapamil (1×10^{-5} M) and nifedipine (1×10^{-6} M) depressed but did not abolish the inotropic effect of increased pH at high driving frequencies but potentiated the effect of low driving frequencies. This inversion of the interval-force relationship curve produced by verapamil is illustrated in Figure 1.

The results suggest that a rise in pH may act to facilitate translocation of membrane Ca^{2+} and/or to increase the release of Ca^{2+} from sources within the cell. They also illustrate the importance of controlling the pH changes in isolated cardiac muscle and suggest that a large component of the inotropic effect of aminopyridines is consequent upon an increase in pH. Although the pH changes studied here are irrelevant in terms of normal physiology, elucidation of the mechanism underlying the inotropic action might provide important information on the functioning of calcium channels and may suggest alternative means of modifying their activity.

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Effect of angiotensin converting enzyme inhibitor on neurogenic vasoconstriction in the pithed rat

D.P. CLOUGH, R. HATTON & SUSAN C. MATTHEWMAN

Bioscience Department II, I.C.I. Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Angiotensin converting enzyme inhibitor (SQ 20881) impairs reflex sympathetically-mediated vasoconstriction in the cat at levels of angiotensin which themselves do not have a direct vasoconstrictor action (Adigun, Clough, Conway & Hatton, 1979; Hatton, Clough, Adigun & Conway, 1980). Since sympathetic efferent nerve activity was not reduced this suggested that a peripheral rather than central action of angiotensin was involved. The present investigation was designed to study the effect of captopril, which is a potent inhibitor of angiotensin converting enzyme (Rubin, Antonaccio & Horovitz, 1978), SQ 20881 and saralasin, on the vascular response to sympathetic nerve stimulation and exogenous noradrenaline in the presence and absence of angiotensin II.

Female Wistar rats were pithed by the method of Gillespie, McLaren & Pollock (1970) and respired artificially. A carotid artery was cannulated for blood pressure measurement and drugs were injected via a jugular vein. The blood pressure response was recorded during stimulation of the sympathetic outflow (lower thoracic-lumbar, at 1 to 30 Hz, 0.5 ms, 60 V) for 10 s and injection of noradrenaline (10 to 500 ng total dose) intravenously.

The pressor response to nerve stimulation at all frequencies was reduced by captopril (1.0 mg/kg i.v., $P < 0.01$) as were those to noradrenaline ($P < 0.01$). A lower dose of captopril (0.1 mg/kg) reduced responses to nerve stimulation but not to noradrenaline. Saralasin (4 $\mu g\ kg^{-1}\ min^{-1}$) also attenuated the pressor response to nerve stimulation and noradrenaline ($P < 0.01$) as did SQ 20881 (10 mg/kg i.v.). The inhibitors themselves did not change the baseline blood pressure. Plasma renin activity (PRA) measured in a separate group was normal (12.55 ± 2.42 ng angiotensin $I\ h^{-1}\ ml^{-1}$) at the time the inhibitors were used.

In animals which had undergone bilateral hephrectomy 18–24 h previously (PRA undetectable), saralasin was now without effect on the vascular responses to nerve stimulation and noradrenaline. Captopril had no effect on the responses to nerve stimulation but there was a small reduction in those to noradrenaline suggesting that the principle effects of the inhibitors were dependent on angiotensin though captopril may have weak additional post-junctional effects.

The results suggest that inhibitors of angiotensin converting enzyme, particularly captopril, may have both pre and post junctional effects on adrenergic neurotransmission which impair neurogenic vasoconstriction by interfering with a peripheral action of angiotensin.

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The effect of captopril on the renal and systemic haemodynamic action of PGI_2 in anaesthetized dogs

LESLEY J. STRACHAN & M.L. WATSON
(introduced by A. UNGAR)

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ.

Systemic infusion of PGI_2 into dogs decreases systemic blood pressure (S.B.P.) (Armstrong, Lattimer, Moncada & Vane, 1977) while intrarenal infusion increases renal blood flow (R.B.F.), urine flow (V) and to a small extent sodium excretion ($U_{Na}V$) and also releases renin from the kidney (Jones, Watson & Ungar, 1980). It is possible that intrarenal formation of angiotensin II from the released renin modulates renal actions of PGI_2 by vasoconstriction and sodium retention. PGI_2 was therefore infused both systemically and intrarenally before and after administration of the angiotensin I converting enzyme inhibitor Captopril.

Nine mongrel dogs were anaesthetized with pentobarbitone sodium. Catheters were inserted into a carotid artery and a jugular vein for recording of S.B.P., collection of blood samples and infusion of fluid containing inulin for measurement of glomerular filtration rate (G.F.R.). Ureters were cannulated for timed urine collections and electromagnetic flow probes placed around each artery for measurement of R.B.F. One renal artery was cannulated for infusion of PGI_2. An initial 10 min control period preceded three systemic infusions of PGI_2 (100 ng/min; 300 ng/min; 500 ng/min). A 5 min recovery period followed each infusion. Three intrarenal infusions of PGI_2 (Table 1) were preceded by a 10 min control period and followed by an 8 min recovery period. Captopril was administered intravenously and the PGI_2 infusions repeated. Results are expressed as mean ± s.e. mean and comparisons made using a paired *t*-test (Table 1).

Captopril significantly decreased S.B.P. (122 ± 4.1 to 103 ± 5 mmHg) and increased R.B.F. (272 ± 39 to 347 ± 44 ml/min). Systemic infusion of PGI_2 caused dose related decreases in S.B.P. which during the 500 ng/min infusions were larger after captopril. Dose related increases in R.B.F. produced by intrarenal PGI_2 infusions were unaffected by captopril. The smaller increases in V after captopril were probably a result of the decreased S.B.P. Changes in $U_{Na}V$, G.F.R. and U_KV in response to PGI_2 were unaffected by captopril.

Captopril did not significantly alter the effects of PGI_2 on S.B.P., R.B.F., or $U_{Na}V$ suggesting that renin released within the kidney by PGI_2 is either not converted to angiotensin II or that the angiotensin II produced is not having a direct effect on renal function.

The gift of PGI_2 from Schering AG, Berlin and captopril from Squibb Ltd. are gratefully acknowledged. This work was supported by grants from M.R.C. and the British Heart Foundation.

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Table 1 The effects of intrarenal PGI_2 infusion on R.B.F. and urine flow before and after administration of captopril (20 mg/kg)

PGI_2 Infusion Rate	*R.B.F. (ml/min)*		*Urine Flow (ml/min)*	
	Before captopril	*After captopril*	*Before captopril*	*After captopril*
Control	270 ± 58	329 ± 58	1.45 ± 0.19	1.37 ± 0.2
50 ng/min	289 ± 59	362 ± 60	1.88 ± 0.02	1.62 ± 0.2
100 ng/min	301 ± 51	379 ± 57	2.53 ± 0.5	1.7 ± 0.25
200 ng/min	335 ± 62	414 ± 74	2.77 ± 0.49	1.68 ± 0.35

$n = 9$.

Inhibition by laevo-propranolol and naloxone of salbutamol-induced depression of white cell and platelet counts in mice

M.P.I. WELLER & D.J.M. WRIGHT
(introduced by J.B.E. BAKER)

Departments of Medical Microbiology and Psychiatry, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF.

A reduction in white blood cell (W.B.C.) and platelet counts consistently follows endotoxin administration in mice (Wright, 1980a). These haematological changes are prevented by β-adrenoceptor antagonists (Wright & Weller, 1980a) and naloxone (Wright & Weller, 1980b). A similar fall in W.B.C. and platelet counts has been found in normal mice after giving the β-adrenoceptor stimulant, salbutamol (Wright, 1980b). The effect of propranolol isomers and naloxone on salbutamol-induced changes are reported.

Six-week old male CBA/CA mice were used in all experiments. All agents were diluted in saline to the appropriate concentration and given intraperitoneally. Mice were divided into groups of six. One group was given salbutamol at an appropriate concentration, one saline and one left untreated, while propranolol or naloxone was given in addition to salbutamol to test groups. The values in the tables were derived from experiments which were repeated once, with the exception of those marked with an asterisk which were repeated twice. Initial salbutamol injections were given either at 9 a.m. or at 5 p.m. White cell and platelet counting methods are described by Wright & Woodrow (1980).

The changes produced by salbutamol were delayed by prior treatment with the β-adrenoceptor antagon-

Table 1 Inhibition by propranolol and naloxone of salbutamol induced depression of white blood cell and platelet counts in mice

Propranolol (25 mg/kg i.p.) Isomer		*Hours after giving Salbutamol (300 mg/kg, i.p.)*	*WBC Count (% of control)*	*Platelet Count (% of control)*
(±)	×1	7	88.4 ± 6.2	95.4 ± 4.5
(±)	×1	7.5	108.3 ± 3.6	100 ± 7
(±)	×1	16	*<12	*<7
(±)	×2	16	*<12	*<7
(±)	×3	16	92.1 ± 4.7	91.2 ± 4.3
(±)	×3	16	79.8 ± 5.2	87.6 ± 7.7
(+)	×1	7	*<12	*<7
(+)	×2	16	*<12	*<7
(−)	×1	7	100.6 ± 5.6	100 ± 8.5
(−)	×2	16	99.4 ± 3	92 ± 8.9
Naloxone (mg/kg, i.p.)				
0.2	×1	7	96.5 ± 4.3	94.5 ± 5.8
0.02	×1	7	91 ± 3.4	92 ± 5.5
0.2	×1	16	41.6 ± 13.9	71.3 ± 7.6
0.02	×1	16	*<12	*<7
0.2	×3	16	87.6 ± 4	89.5 ± 8.1
0.02	×3	16	*<12	*<7

×1 drug given 1 h intraperitoneally prior to salbutamol.
×2 drug given 1 h prior and 4 h after salbutamol.
×3 drug given 1 h prior and 4 h and 8 h after salbutamol.
* Result of two separate experiments.
The mean WBC and the mean platelet count following salbutamol and propranolol or naloxone administration is expressed as a percentage of a time matched normal count. (WBC = 8.9 ± 0.6 × 10^9/l; platelets = 422.5 ± 22.5 × 10^9/l mean value 12 mice at 9 a.m.).
Control Groups:
Between 7 and 16 h after giving salbutamol preceded by saline (as an alternative to propranolol or naloxone), mice showed counts of WBC < 12 and platelets < 7. Mice given propranolol and saline (as an alternative to salbutamol) showed counts that were comparable with the counts observed in normal uninoculated mice. In a separate experiment repeated once only, designed as above (±)-propranolol (5 mg/kg) failed to inhibit the salbutamol effects.

ist (±)-propranolol, but could only be entirely suppressed if repeated (±)-propranolol injections were Given. The salbutamol-induced changes were unaffected by (+)-propranolol but were prevented by (−)-propranolol, suggesting that the haematological changes found after giving high doses of salbutamol are likely to have been the result of a beta-adrenergic activity.

Because naloxone cancels endotoxin-induced changes (Holaday and Fadan, 1978; Wright & Weller, 1980b), the effect of naloxone on salbutamol-induced changes was then examined. Naloxone was found similarly to inhibit the salbutamol-induced changes. These results reveal a new action of naloxone and show how antagonism of naloxone and (−)-propranolol may prevent endotoxin-mediated effects.

A depression in white blood cells and platelets has been found in mice after giving the beta-adrenoceptor agonist orciprenaline, at the low dose of 0.1 mg/kg intraperitoneally. This effect was reversed by naloxone (0.2 mg/kg) like the salbutamol effect described above.

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Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH is a selective ligand for the μ-opiate binding site

H.W. KOSTERLITZ & S.J. PATERSON

Unit for Research on Addictive Drugs, University of Aberdeen.

Evidence for the subdivision of the opiate receptors into μ-, δ- and κ-binding sites has been obtained from a comparison of the binding of tritiated opiates and opioid peptides and their differential inhibition by the unlabelled compounds (Gillan, Kosterlitz & Paterson, 1980; Kosterlitz, Paterson & Robson, 1980). The characterization of the individual binding sites has been complicated by the fact that the ligands interact with more than one site.

The binding characteristics of the enkephalin analogue Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (RX 783006, Reckitt & Colman) in homogenates of guinea-pig brain were determined at 25°C as previously described (Gillan *et al.*, 1980). The specific binding of [^{3}H]-RX783006 (55.9 Ci/mmol) was saturable, the equilibrium dissociation constant, K_D, 0.96 ± 0.16 nM ($n = 4$) and the capacity 2.95 ± 0.32 pmol/g brain. The corresponding values obtained for [^{3}H]-dihydromorphine were 1.66 ± 0.29 nM and 3.8 ± 0.4 pmol/g ($n = 4$) and for [^{3}H]-morphine 0.86 ± 0.18 nM and 2.6 ± 0.2 pmol/g ($n = 3$).

Unlabelled RX783006 has a low affinity for the δ- and κ-binding sites as indicated by the high values of the inhibition constant, K_I. In experiments in which its power to displace competitively the binding of 1 nM [^{3}H]-D-Ala^2-D-Leu^5-enkephalin ($K_D = 0.96$ nM) was tested, a K_I of 820 ± 110 nM ($n = 4$) was obtained. When the effect of unlabelled RX 783006 was tested on the binding of 0.65 nM [^{3}H]-ethylketazocine ($K_D = 0.63$ nM), the displacement curve was biphasic, yielding a K_I of 4.55 ± 1.57 nM ($n = 4$) for 40% of the binding and of 4960 ± 860 nM ($n = 4$) for 60% of the binding. The first of these values can be shown to be due to the fact that, even at these concentrations, [^{3}H]-ethylketazocine binds not only to the κ-binding site but also to the μ-binding site (Paterson, Robson & Kosterlitz, unpublished observations).

Thus, Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH is a selective ligand for the μ-binding site with only low affinities to the δ- and κ-binding sites.

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Effect of temperature on the induction of opiate dependence in guinea-pig isolated ileum

H.O.J. COLLIER, N.J. CUTHBERT & D.L. FRANCIS

Research Department, Miles Laboratories Limited, Stoke Poges, Slough, Berkshire, SL2 4LY.

Little is known about the effect of temperature on the induction of opiate dependence, because this is difficult to vary *in vivo*. The guinea-pig isolated ileum, which develops dependence when incubated with opiates at temperatures ranging from 4 to 40°C (Ehrenpreis, Light & Schonbuch, 1972; Hammond, Schneider & Collier, 1976; North & Karras, 1978; Villarreal & Castro, 1979), offers an opportunity to study this. We have compared the effect of two incubation temperatures on the rate of induction of responsiveness to naloxone in the isolated ileum incubated with normorphine.

Comparable ileal segments from the same animal were incubated for 2 to 6 h with normorphine (30 nM) at 22 or 37°C, as previously described (Collier, Cuthbert & Francis, 1980a, b). Segments were set up in pairs at 37°C in fluid equivalent to the incubation fluid and, 30 min later, challenged with a submaximal but effective dose of naloxone (30 nM). Dependence was measured by comparing the response with a reference response to acetylcholine. After 2, 4 or 6 h incubation at 22 or 37°C, reference responses to acetylcholine and electrical stimulation remained unchanged. After 30 min equilibration in normorphine (30 nM) at 37°C, preparations did not respond to naloxone. Figure 1 gives the effects of incubation at 22 and 37°C on the extent of the response to naloxone. At 2, 4 and 6 h the mean responses to naloxone at both temperatures were significantly ($P < 0.005$) higher than the nil response of controls or the initial response of test segments (0 h). At 2 h, the means at both temperatures were almost identical; but at 4 h they differed significantly ($P < 0.01$) At 6 h the difference was not significant and at 10 h the converging lines for 22 and 37°C met. These findings suggest that

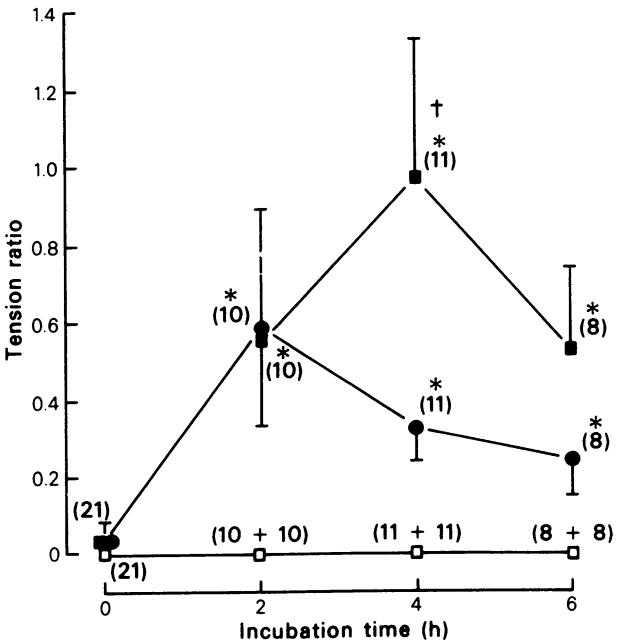

Figure 1 Effect of incubation temperature on induction by normorphine (30 nM) of responsiveness to naloxone in guinea-pig ileal segments. Segments (ca. 10 cm) taken in sets of four from the same animal were incubated for 2, 4, or 6 h in Krebs solution containing (□) hexamothonium (70 μM) with added normorphine (30 nM) at (●) 22°C or (■) 37°C. After incubation, or in the case of fresh control segments, immediately after being prepared, pairs of segments were set up for test at 37°C in fluid equivalent to that used for incubation and, 0.5 h later (0 h), challenged with naloxone (30 nM). The tension ratio is the ratio of the tension elicited by naloxone to that elicited by subsequent challenge with acetylcholine (10 nM). Each point is the mean ± s.e. mean of the results of at least eight experiments (number in parentheses). For significance of differences, by the Wilcoxon matched-pairs signed-rank test, between (a) normorphine and Krebs: *, $P < 0.005$; and (b) 37 and 22°C: †, $P < 0.01$.

two processes may participate in dependence induction.

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Effects of narcotic analgesics on lipase activity *in vitro*

P.D. KELLY & J.R. TRAYNOR

Biochemical Pharmacology Laboratory, Department of Chemistry, University of Technology, Loughborough, Leicestershire, LE11 3TU

In vivo acute and chronic administration of morphine enhances lipase activity. *In vitro* morphine causes a dose-dependent increase in fatty acid release from the rat epididymal fat pad, reaching a maximum effect at 2.5 mm. It has been proposed that morphine may exert its lipolytic effect *via* a low affinity interaction with the plasma membrane adrenaline receptor (Wong, Yeung & Yeung, 1977). Recently the morphine like peptide β-endorphin has been reported to have lipolytic activity in isolated fat cells obtained from rabbits at nearly physiological concentrations (Schwandt, Richter & Wilkening, 1979).

Narcotic drugs have non-specific local anaesthetic properties at high doses (Kosterlitz, Lees & Watt, 1969). Drugs with local anaesthetic properties affect the activity of lipolytic enzymes *in vitro* (Kunze, Nahas, Traynor & Wurl, 1976). In order to determine whether direct enzymatic interactions might be responsible for the observed lipolytic effects of morphine we now report a study of several opiate compounds on the activity of the lipase (EC 3.1.1.3) derived from the mould *Rhizopus arrhizus*. This is a well characterized enzyme which we have previously used as a model (Traynor & Kunze, 1976).

The activity of the lipase was measured using tributyroylglycerol as substrate. The assay consisted of the desired amounts of tributyroylglycerol, added *via* a gas-tight syringe and emulsified in 30 ml of NaCl solution (100 mM) containing the required amount of opiates by stirring for 5 min at 1000 rev/min. The enzyme (10 µg) was added and the liberation of butyric acid, under a stream of nitrogen, was followed at 25°C as a function of time using a pH stat charged with 20 mM NaOH. Kinetic curves were linear for at least 3 minutes. All determinations were made at least in duplicate and reproducibility was found within $\pm 5\%$.

All of the narcotic analgesics examined exhibited bi-phasic dose-response curves against the hydrolysis of emulsified tributryoylglycerol by the mould lipase. At low drug doses a stimulation is obtained, followed at higher doses by an inhibition.

The most potent opiate in causing a stimulation of the lipase-mediated hydrolysis is etorphine. Overall potency of the drugs studied decreases in the order etorphine > buprenorphine > levorphanol > morphine > dextrorphan > codeine. It is noteworthy that levorphanol is some 86 times more potent than its pharmacologically inactive isomer dextrorphan, and also shows a greater degree of stimulation. Indeed all of the drugs show varying degrees of maximal obtainable stimulation. Again the greatest effects is seen with etorphine which causes a 38% stimulation at 50 nM. The non-narcotic thebaine shows no consistent dose-related increase in the hydrolytic activity of the mould lipase.

At higher concentrations all of the opiates had a powerful inhibitory effect on the activity of the lipase towards the emulsified substrate. The levels of different drugs needed to effect inhibition are much closer together than those needed to cause stimulation. Thus

the difference between the narcotically active levorphanol (IC_{50} approx. 5 mM) and its inactive enantiomer dextrorphan (IC_{50} approx. 8 mM) is not marked. Furthermore the non-narcotic thebaine has inhibitory properties equivalent to its analgesically active counterparts.

The results suggest that the observed *in vivo* and *in vitro* lipolytic effects of opiates might in part be caused by a direct stereospecific stimulation of lipase enzyme rather than, or in addition to, mediation *via* a membrane receptor which alters lipolysis through changes in cyclic nucleotide levels.

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Interaction of calcium ions and salmon calcitonin in the production of analgesia in the mouse

R.F.L. BATES, G.A. BUCKLEY, R.M. EGLEN & R.J. STRETTLE

Department of Life Sciences, Trent Polytechnic, Burton Street, Nottingham. Div. Biology, Preston Polytechnic, Lancs.

Calcitonin produces analgesia when injected centrally (Pecile, Ferri, Braga & Olgiati, 1975) by mechanisms independent of the opiate receptor (Braga, Ferri, Santagostino, Olgiati & Pecile, 1978). The actions of opiate drugs are strongly influenced by changes in neuronal calcium ion concentration (Chapman & Way, 1980). We have investigated the effect of changes in brain calcium ion concentration upon calcitonin-induced analgesia.

Abdominal constrictions were induced by i.p. injection of acetic acid (1% in 0.154 mol/l NaCl) in male and female CFLP mice (30 g) after the method of Collier, Dineen, Johnson & Schneider (1968). Intracerebroventricular (ICV) injections of 10 µl/mouse were given by the method of Haley & McCormick (1957). Salmon Calcitonin (SCT), ethylene glycol-bis (β-amino ethyl) N,N′ tetra acetic acid (EGTA) and $CaCl_2$ were dissolved in tris-saline pH 7.35 (Bates, Buckley, Eglen & Strettle, 1980). Control animals received tris-saline alone.

Intracerebroventricular injections of $CaCl_2$ (0.1 µmol/mouse) caused an increase in the frequency of abdominal constrictions. Intracerebroventricular injections of EGTA (0.05 µmol/mouse) decreased the frequency of abdominal constrictions. These findings are similar to those of Schmidt & Way (1980) using the tail-flick test. The ICV injection of an equimolar concentration of $CaCl_2$ together with the EGTA restored the frequency of abdominal constrictions to control values (3.63 ± 0.39 abdominal constrictions/min; mean ± s.e. mean). Intracerebroventricular injection of SCT (10 MRC u/kg) decreased the frequency of abdominal constrictions from 3.24 ± 0.2 to 2.36 ± 0.21 constrictions/min (mean ± s.e. mean). When SCT (10 MRC u/kg) was injected simul-

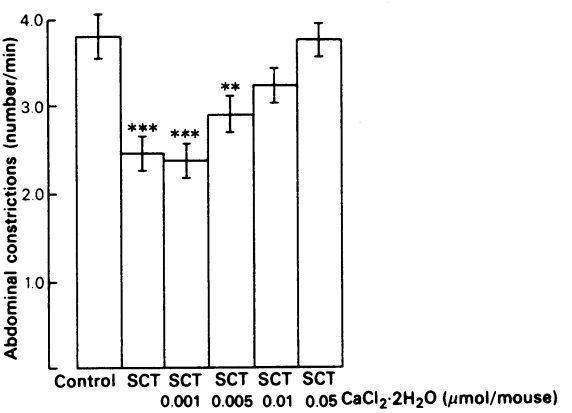

Figure 1 Effect of $CaCl_2.2H_2O$ injected (ICV) simultaneously with SCT (2 MRC u/kg) (ICV), upon abdominal constrictions induced with acetic acid in the mouse. Values are mean ± s.e. mean. $^{**}P < 0.01$ $^{***}P < 0.001$. 10 animals were used in each group.

taneously with EGTA (0.05 μmol/mouse) no additional decrease in the frequence of abdominal constrictions was seen.

The effect of ICV SCT (2 MRC u/kg) upon the frequency of abdominal constrictions was antagonized by the simultaneous ICV injection of $CaCl_2$ (0.001–0.05 μmol/mouse) Figure 1.

In summary, central administration of calcium ions induces hyperalgesia and antagonizes the analgesic effects of EGTA and SCT. Similar findings have been reported (Chapman & Way, 1980) for the interaction of calcium ions and opiate induced analgesia.

The salmon calcitonin was generously donated by Drs. J.W. Bastian and J.P. Aldred, Armour Pharmaceuticals Corp., Kankakee Ill., U.S.A.

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Covalent attachment to dextran to the anti-leukemic enzyme L-asparaginase reduces the type III hypersensitivity to the antigen

P.N.C. ELLIOTT, R.L. FOSTER & T.E. WILEMAN

School of Pharmacy, Liverpool Polytechnic and Department of Science, Birmingham Polytechnic.

The enzyme L-asparaginase from *Erwinia carotovora* has been shown to induce remission in humans from asparagine dependent leukemias. Unfortunately the immunogenicity of this bacterial asparaginase has limited its effectiveness as a tumor inhibitor. In this study the responses to soluble dextran asparaginase conjugates have been determined using the Arthus reaction of sensitized guinea-pigs.

Asparaginase from *Erwinia carotovora* was allowed to react overnight with periodate oxidized dextrans of increasing molecular weight (10,000, 40,000, 70,000 daltons). The enzyme conjugates were stabilized by reduction using sodium borohydride and subsequently purified by extensive dialysis against phosphate buffered saline.

Six female guinea-pigs were sensitized to the native enzyme by two intramuscular doses of the antigen (1 mg). Four weeks after the last injection the hair was removed from the flanks of each guinea-pig following which each animal was injected intravenously with Evans Blue. One h later the antigen solutions, equivalent to 100 μg protein, were given by randomised intradermal injection into the exposed skin as were control solutions containing dextran and saline. Three such injections were administered to each guinea-pig flank allowing all six test solutions to be studied on each animal. The guinea-pigs were studied by impartial observers for localized tissue damage, the intensity of each reaction being scored using a 1–5 rating scale.

Five h following intradermal injection the responses to all three asparaginase conjugates were mutually undistinguishable and scored 46% of the available points. Their score was similar to that obtained in response to dextran (33%). The most severe reactions were those seen to occur in response to the native enzyme which scored 88% of the available points. Tissue damage in response to the enzyme conjugates and to dextran alone was seen to remain unchanged 24 h following intradermal injection and to be completely reversed within 72 hours. In contrast an area of necrosis was seen to develop within 24 h at the site of injection of the native enzyme which failed to heal within 72 hours.

This study shows that covalent attachment of dextran to the surface of asparaginase can markedly reduce the extent of tissue damage that occurs during a type III hypersensitivity reaction to the antigen. Since tissue damage is a direct result of the deposition of immune complexes at the site of injection it is concluded that the bound dextran restricts antibody antigen complex formation probably through steric hindrence or the masking of antigenic sites. In a previous communication (Foster & Wileman, 1979) it was shown that covalent attachment of dextran also increases the circulatory lifetime of the enzyme, this and the above evidence for the conjugates reduced antigenicity suggest that these enzyme conjugates may have therapeutic potential.

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Binding of tritium-labelled 9,11-epoxymethano PGH_2 to human platelets

R.L. JONES, ROMA A. SUTHERLAND & N.H. WILSON

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ.

Thromboxane A_2 (TXA_2) produces rapid and irreversible aggregation of human platelets. A number of stable compounds, including 9,11- and 11,9-epoxymethano (Malmsten, 1976) and 9,11-azo (Corey, Nicolaou, Machida, Malmsten & Samuelsson, 1975) analogues of PGH_2, mimic this action of TXA_2, perhaps by interaction with a common receptor on the platelet. To provide further information on this matter, we have prepared a radio-labelled thromboxane-like agent. Preliminary studies on the binding of this compound to washed human platelets are reported here.

15-Oxo-9,11-epoxymethano PGH_2 was prepared

Table 1 A comparison of displacement of [^{3}H]-9,11-epoxymethano PGH_2 binding and aggregatory actions on human platelets

Compound	*Concentration required for 50% displacement of saturable binding* (μM)	*Aggregatory action on platelets*	*Concentration required for aggregation* (μM)
9,11-epoxy methano PGH_2	0.20	Irreversible	0.54
9,11-ethano PGH_2†	0.89	Reversible only	10–50
11,9-epoxy methano PGH_2	2.1	Irreversible	0.30
9,11-azo PGH_2	1.2	Irreversible	0.073
EP 011†	1.9	Irreversible	0.34
ICI 79939†	0.27	Irreversible	0.72
$PGF_{2\alpha}$	>30	No effect	>100
EP 037†	16	Blocks aggregation	—

The concentration of the labelled ligand was 0.071 μM. Labelled and unlabelled compounds were added simultaneously and incubated for 6 min at room temperature.

* Reversible aggregation wave of 20 units magnitude on chart recorder—mean of at least 3 determinations.

† Racemic compounds.

EP 011 and ICI 79939 are the 17,18,19,20-tetranor-16-*p*-fluorophenoxy analogues of $PGF_{2\alpha}$ and 9,11-etheno PGH_2 respectively. EP 037 is 5-*endo* (6'-carboxyhex-2'Z-enyl)-6-*exo* [O-(p-fluorobenzyl)-oxyiminomethyl]-*bicyclo*[2,2,1] heptane.

from natural PGA_2 (Bundy, 1975). [^{3}H]-Sodium borohydride reduction (Amersham), followed by partition chromatography, afforded 15-^{3}H-9,11-epoxy-methano PGH_2 (13.9 Ci/mmole). Washed human platelets were suspended in a glucose/citrate medium containing Indomethacin (10^{-5} M) to inhibit TXA_2 production, and PGE_1 (1.7×10^{-8} M) to prevent aggregation by the thromboxane mimics. Bound and free ligand were separated by centrifugation at 15,000 *g* for 2 minutes. Binding of [^{3}H]-9,11-epoxymethano PGH_2 (0.071 μM) was complete within 1 min and could be rapidly displaced by non-radioactive 9,11-epoxymethano PGH_2 (1.42 μM). Scatchard analysis yielded a hyperbolic plot indicating two types of binding. The higher affinity site has an equilibrium dissociation constant of 0.88 ± 0.19 nM and a capacity of 444 ± 96 sites per platelet. The lower affinity binding is non-saturable, and may reflect linear uptake of the lipophilic ligand into the platelet.

Displacement of [^{3}H]-9,11-epoxymethano PGH_2 binding by various compounds was studied (Table 1). Those compounds capable of producing irreversible aggregation show high affinity for the saturable binding site whereas $PGF_{2\alpha}$ neither aggregates platelets nor displaces the radio-ligand. 9,11-Ethano PGH_2 produces only reversible aggregation and antagonizes the action of 11,9-epoxymethano PGH_2 suggesting a partial agonist action (Jones & Wilson, 1980): It has high affinity for the binding site. EP 037 antagonizes the aggregation induced by 11,9-epoxymethano PGH_2; dose ratios range from 2 at 2 μM to 20 at 40 μM. Displacement of binding is seen within this concentration range.

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Inhibition of platelet aggregation by ethanol: the role of plasma and platelet membrane lipids

C.G. FENN & J.M. LITTLETON

Department of Pharmacology, King's College, London WC2.

There is epidemiological evidence that moderate ethanol intake (Ricci, G. & Angelico, F., 1979) and intake of dietary unsaturated fat (Dyerberg, J. *et al.*, 1979) play protective roles in coronary thrombosis. Also platelet functions have been significantly correlated with the intake of saturated fat (Renaud *et al.*, 1978). Ethanol (Chin, J. & Goldstein, D., 1977) and incorporation of unsaturated fatty acids (Cooper, R., 1977) both increase the fluid nature of biomembranes. We have considered the interaction of ethanol and different fats on the function of the human blood platelet.

Platelet rich plasma (PRP) from human venous blood (acid citrate:dextrose anticoagulant) was centrifuged on to albumin and passed through a Sepharose CL2B column in the presence of apyrase to prepare 'washed' platelets. in some experiments PRP was incubated with triglycerides (coconut or herring oil), lecithin or cholesterol, previously dispersed by sonication in platelet-poor plasma. 500 μl samples of PRP or washed platelets ($5–7 \times 10^7$ platelets/ml) were studied in a Born RCS aggregometer. Ethanol, when present, was added 2 min before the aggregating agent.

Platelet aggregation induced by arachidonic acid was unaffected or slightly potentiated by ethanol, whereas the response to adrenaline was slightly inhibited by ethanol at concentrations tolerated by man (0–100 mM). ADP-induced platelet aggregation (particularly the second wave) was generally inhibited by ethanol, but there was considerable variation between individuals.

When platelets were aggregated with relatively low concentrations of thrombin, collagen or the calcium ionophore A23187, ethanol (50–100 mM) had a dramatic inhibitory effect often completely abolishing the

response to concentrations of these aggregating agents which had previously produced an 80–100% maximal aggregation.

When PRP was incubated with herring oil, unsaturated acyl chains were incorporated into platelet phospholipids, partly displacing arachidonic acid; incubation with coconut oil increased the proportion of saturated acyl chains in platelet phospholipids. Incubation with cholesterol increased, and with lecithin slightly decreased platelet membrane cholesterol content.

Compared to incubation with herring oil incubation of PRP with coconut oil increased the subsequent responses of platelets to thrombin, collagen and arachidonic acid. Paradoxically the inhibition by ethanol of the thrombin and collagen responses was potentiated by this treatment. Incubation of PRP with cholesterol rather than lecithin enhanced the platelet responses to adrenaline, ADP and arachidonic acid, but had little influence on the inhibitory effect of ethanol on platelet aggregation.

The direct inhibitory effects of ethanol on platelet aggregation seem best explained by inhibition of calcium-stimulated phospholipase activity. Inhibition is relatively specific for some aggregating agents and is affected by the lipid composition, particularly acyl group saturation, of the plasma and platelet membrane. The reason may lie in the physical nature of the platelet membrane or in some interaction at the level of platelet phospholipid metabolism.

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Effects of dock leaf extracts on 5-hydroxytryptamine induced contraction of the rat fundic strip

D.P.DEARNALEY, L. MARSON, R.H. OSBORNE & E. WHITE

Department of Science, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol BS16 1QY.

Emmelin & Feldberg (1947) showed that the stings of the nettle *Urtica urens* contained three smooth muscle stimulating substances, acetylcholine, histamine and a third, later identified by Chesher & Collier (1956) as 5-hydroxytryptamine (5-HT). Subsequently, Brittain & Collier (1956) reported that aqueous extracts from the broad leafed dock plant *Rumex obtusifolius* competitively antagonized the effects of 5-HT on the rat colon and uterus and on the guinea-pig ileum. In this study we have reinvestigated the properties of dock leaf extracts using the rat fundic strip preparation (Vane, 1957) bathed at 37°C in Ringer-Locke solution. Dock leaf extracts were prepared by grinding leaves (10 g) with deionised water (5 ml) in sand. The aqueous extract was lyophilized, stored frozen and redissolved in Ringer-Locke solution (typically, 10 mg/ml) as required.

0.1 mg/ml extract reduced the response to 5-HT (5×10^{-8} M) by 60%. The antagonism was rapid in onset (30 s) and fully reversible within 10 minutes. Log dose response curves were obtained for 5-HT before and after applying extract, curves affected by the extract were shifted to the right along the dose axis and the ED_{50} increased from 4×10^{-8} M to 2×10^{-7} M in the presence of 0.1 mg/ml antagonist and to 2.5×10^{-6} M in the presence of 0.5 mg/ml. By comparison, 10^{-6} M NN-dimethyltryptamine (NN-DMT) increased the ED_{50} from 4×10^{-8} M to 1.6×10^{-7} M but the effect was slow in onset and difficult to reverse. 0.5 mg/ml extract had no effect on ACh induced contractions but occasionally reduced the effects of histamine. The antagonistic activity survived repeated freezing and thawing but exposure to pH 10 and/or temperatures above 80°C caused loss of 5-HT antagonistic properties and the appearance of 5-HT agonist activity within the extract. Log dose response curves obtained for this agonist were shifted to the right in the presence of NN-DMT (10^{-6} M) or 0.1 mg/ml antagonist extract.

Attempts were made to separate active components from extracts containing antagonist and agonist ac-

tivity using descending paper chromatography. A 'spot' (R_F 0.8, pink under ultraviolet light) with 5-HT antagonistic activity was recovered from chromatograms run in butanol:acetic acid:water (4:1:1) but this system appeared to destroy the agonist. Chromatography of agonist extracts using ethyl acetate:methanol:water (100:16.5:13.5) yielded 'spots' with 5-HT agonistic (R_F 0.46, ice blue under ultraviolet light) and antagonistic (R_F 0.77, pink under ultraviolet light) properties. By comparison, 5-HT and NN-DMT had R_F values of 0.43 and 0.79 respectively, were ice blue under ultraviolet light and gave the blue/purple colour characteristic of indoles when treated with Erlich's-reagent. However, as neither the dock leaf antagonist or agonist reacted with Erlich's reagent it appears unlikely that these compounds are indoles.

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Fenfluramine-induced hyperthermia in rats: antagonism by some, but not all, selective inhibitors of 5-hydroxytryptamine uptake

M.F. SUGRUE

Centre de Recherche Merrell International, 16, rue d'Ankara 67084 Strasbourg-Cedex, France.

Fenfluramine elicits a rapid hyperthermia in rats housed in a warm environment. Pretreatment with the 5-hydroxytryptamine (5-HT) uptake inhibitor chlorimipramine attenuates fenfluramine-induced hyperthermia and on the basis of this, and other interaction studies, it has been proposed that the response to fenfluramine is a consequence of the ability of the drug to be taken up into the 5-HT nerve terminal and release the monoamine (Frey, 1975; Sulpizio, Fowler & Macko, 1978). Somewhat surprisingly, fenfluramine-induced hyperthermia was not blocked by the prior administration of the selective 5-HT uptake blocker zimelidine (Pawlowski, Ruczynska & Maj, 1980). The objective of this study was to determine if this lack of effect of zimelidine is common to other selective inhibitors of 5-HT uptake. The compounds investigated were chlorimipramine, citalopram, femoxetine, Org 6582 and zimelidine.

Male Sprague–Dawley rats weighing 180–220 g were used. Rats were placed in a warm room (27–28°C) for 1 h prior to commencing the experiment. The 5-HT uptake inhibitors were injected i.p. 1 h prior to (±)-fenfluramine (7.5 mg/kg, i.p.) and rectal temperature was recorded 1 h after fenfluramine administration. The increase in body temperature elicited by fenfluramine averaged 1.3°C. Fenfluramine-induced hyperthermia was attenuated by pretreatment with chlorimipramine (10 mg/kg), femoxetine (10 mg/kg), citalopram (20 mg/kg) or fluoxetine (20 mg/kg) In contrast, Org 6582 and zimelidine (both 20 mg/kg) were devoid of effect.

When studied for *in vitro* inhibition of [^{3}H]-5-HT uptake into rat hypothalamic synaptosomes the following IC_{50} values (nM) were obtained: citalopram (2.4), chlorimipramine (8.8), femoxetine (14), fluoxetine (16), Org 6582 (75) and zimelidine (250). Whilst it is of interest to note that the two agents which failed to block fenfluramine-induced hyperthermia were the weakest inhibitors of 5-HT uptake *in vitro*, the situation is different *in vivo*. When rats were killed 1 h after the i.p. injection of 20 mg/kg, the subsequent % inhibition (mean ± s.e. mean) of [^{3}H]-5-HT uptake into rat hypothalamic synaptosomes was: citalopram (83.3 ± 0.7), Org 6582 (79.8 ± 0.6), fluoxetine (70.2 ± 1.3), femoxetine (69.7 ± 0.5), zimelidine (67.0 ± 0.5) and chlorimipramine (47.2 ± 0.9). Hence the lack of effect of Org 6582 and zimelidine in the behavioural paradigm cannot be attributed to a failure to adequately inhibit 5-HT uptake.

Fenfluramine-induced hyperthermia is also blocked by 5-HT receptor antagonists such as methergoline, methysergide and mianserin. However, the attenuation by the 5-HT uptake inhibitors of the response to fenfluramine is unlikely to be due to blockade of 5-HT receptors, since all the uptake inhibitors at 0.1 mM had little effect on the high affinity binding of [^{3}H]-5-HT to rat hypothalamic membrane fractions.

The above observations confirm that the ability to block fenfluramine-induced hyperthermia in rats is not a property common to all selective inhibitors of 5-HT uptake. Why Org 6582 and zimelidine are ineffective in this respect remains to be elucidated.

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The effect of some anti-ulcer agents on basal gastric mucosal blood flow and transmucosal flux of hydrogen and sodium ions in the conscious dog

BELINDA J. JOHNSTON & R.L. McISAAC

Academic Surgical Unit, St. Mary's Hospital Medical School, London W2 1PG, England.

Gastric mucosal blood flow (GMBF) is thought to play an important role in acid secretory processes and may also contribute to protection against mucosal injury. For instance, during haemorrhagic shock GMBF is decreased but drugs which prevent this reduction also protect against the ensuing mucosal lesions (Moody, McGreevy, Zalewsky, Cheung & Simons, 1978). We have studied the effect of some anti-ulcer agents on basal GMBF and transmucosal flux of H^+ and Na^+.

Four female dogs with vagally denervated gastric pouches were used. Isotonic hydrochloric acid (100 mM HCl plus 50 mM NaCl) was instilled into the pouch using a continuous loop perfusion and recovered every 30 minutes. H^+ concentration was measured by titration to pH 7.0 with 0.1 M NaOH (Radiometer, Copenhagen) and Na^+ by flame photometry (Corning 450). The H^+ loss or Na^+ gain in the mucosal solution was calculated (as $\mu mol\ min^{-1}$) after subtraction of the H^+ and Na^+ in the original solution. GMBF was estimated by neutral red clearance (NRC) (Knight & McIsaac, 1977). Four anti-ulcer agents were administered during the fourth 30 min period and collections continued for a further three periods. Deglycyrrhinised liquorice (DGL) and carbenoxolone sodium (0.33% and 0.1% solutions respectively) were added to the perfusate for 30 minutes. Cimetidine (10 $\mu mol/kg$ i.v. bolus plus 20 $\mu mol\ kg^{-1}\ h^{-1}$) was given for 30 min and salbutamol (0.2 $\mu g\ kg^{-1}\ min^{-1}$) for 10 minutes. Control experiments were done without drug administration. Experiments were carried out twice in each animal. Means of the second and third periods and fifth and sixth periods were compared using Wilcoxon's matched pairs signed-ranks test (Siegel, 1956).

Hydrogen ion was lost from the perfusing fluid at a pre-drug rate of 4.6 ± 0.45 $\mu mol/min$ (mean $\pm$ s.e. mean, $n = 32$). Na^+ appeared in the perfusate at 2.3 ± 1.19 $\mu mol/min$. None of the drugs had any effect on these rates. In the post-drug period these changes were 5.3 ± 0.58 and 3.5 ± 1.45 $\mu mol/min$ respectively.

Basal NRC in the pouch was 4.6 ± 1.66 ml/min ($n = 8$) in the control experiments but the range between animals was large (1.2 to 8.5 ml/min). Cimetidine had no effect on basal NRC, the pre- and post-drug levels being 4.1 ± 1.81 and 5.0 ± 1.23 ml/min. Salbutamol, DGL and carbenoxolone all significantly increased NRC; Salbutamol: 3.3 ± 0.71 to 6.7 ± 1.16 ml/min ($P < 0.01$), DGL: 4.6 ± 2.11 to 8.6 ± 2.80 ml/min, Carbenoxolone: 5.4 ± 1.22 to 9.9 ± 1.46 ml/min ($P < 0.05$ for both).

These results are for three of the many factors associated with the gastric mucosal barrier: fluxes of H^+ and Na^+ and GMBF. Cimetidine had no effect and might exert its ulcer-healing activity solely by a reduction in acid output although this is disputed (Carter, 1980). The other three agents increased basal GMBF, probably directly in the case of salbutamol and indirectly for DGL and carbenoxolone. This increased GMBF may provide protection against damage by carrying away the H^+ which enters the mucosa.

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The anatomical distribution of ADPase activity in the rabbit aorta

HELEN EMMS & G.P. LEWIS

Department of Pharmacology, Royal College of Surgeons, London.

Exposure of sub-endothelial structures, such as collagen, stimulates the formation of a platelet thrombus, a process which is greatly accelerated by the explosive release of ADP. The destruction or removal of ADP may therefore serve to limit the size of an intravascular thrombus which may otherwise lead to intimal thickening. Heyns, van den Berg, Potgieter & Retief (1974) and Lieberman, Lewis & Peters (1977) showed that human and rabbit aorta were capable of degrading ADP. Furthermore, Cooper, Lewis, Lieberman, Webb & Westwick (1979) demonstrated the presence of this ADPase activity in the vasculature of many tissues and organs of several species and suggested its possible role in the regulation of arterial thrombosis.

In the present experiments we have studied the anatomical distribution of ADPase activity in the rabbit aorta. The experiments have been carried out on live cells isolated from rabbit aortae as described by Peters, Müller & de Duve (1972) and Cooper, *et al.* (1979). The ADPase activity of cells from the whole aortae, which were divided into six sections from the aortic arch to the iliac bifurcation, as estimated by aggregation and by radiochromatography as described by Cooper *et al.* (1979). In each preparation the live cell count was adjusted to 10^4 cells/ml of 0.25% sucrose.

The investigation showed firstly that there is a gradual increase in ADPase activity from the aortic arch to the lower abdominal section of the aorta. The results obtained using radiochromatography as the assay technique correlated well with those obtained by aggregation. The former however was more sensitive and results could be obtained with ADP concentrations of 480, 240, 120 and 60 μM. The majority of samples containing 30 μM ADP were below the limit of sensitivity of the aggregation assay. The inactivation of ADP was dependent on time and substrate concentration. Complete removal of the ADP occurred after incubation with cells from the lower abdominal aorta using concentrations of 240, 120 and 60 μM whereas after incubation with cells from the aortic arch some of the ADP remained even after the lowest substrate concentration.

Secondly using radiochromatography the major metabolite produced from the breakdown of ADP by the aortic arch and upper thoracic aortic cells was AMP; those produced by middle and lower thoracic aortic cells were AMP at high substrate concentrations and AMP, inosine and adenosine at lower ADP concentrations. On the other hand, the lower abdominal aortic cells gave rise to a small amount of ADP and inosine and a high proportion of adenosine at all concentrations of ADP. Adenosine is a potent inhibitor of platelet aggregation (Born & Cross, 1963) and its formation may therefore also serve to control the extent of the thrombotic response.

Atherosclerosis generally has a set pattern of distribution and in rabbits is more severe in the aortic arch and upper thoracic aorta than in the abdominal aorta (Weisbroth, Flatt & Klaus, 1974). The present results therefore suggest a connection between ADPase activity and the incidence of atherosclerosis. In the aortic arch and upper thoracic aorta, where ADPase activity is low there is a high incidence of the disease and conversely in the abdominal regions where ADPase activity is high the incidence of the disease is low.

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Differences in the time-course of tuberculin skin reactions in primary and secondary hypersensitive phases and the influence of vinylidene chloride

R. HICKS & H.A. SAHYOUN

Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, West Yorkshire, U.K.

It was previously reported that vinyl chloride monomer exerted immunosuppressive effects on cell-mediated hypersensitivity in the guinea-pig (Hicks & Sahyoun, 1980). Vinylidene chloride (I.I-dichloroethylene, DCE) was investigated also, in view of its chemical relation to vinyl chloride and the alkylating properties of metabolites of either compound. A part of this study was the comparison of the primary and secondary phases of 'delayed-type' tuberculin hypersensitivity. This revealed that not only was there a more rapid onset of the hypersensitivity stimulated by the secondary sensitizing dose of antigen, but that there were differences in the time-course of skin reactions.

The primary, cell-mediated, immune response was provoked in guinea-pigs by intraperitoneal injection of Freund's complete adjuvant (FCA) (type H37 Ra, Difco) containing 0.5 mg *M. tuberculosis*. Ten weeks later, the same animals received a second identical antigenic stimulus, to provoke a secondary immune response. The state of hypersensitivity to *M. tuberculosis*, during both phases, was assessed by challenging the animals with tuberculin PPD (50 μg, intradermal), at 2, 4, 7, 14, 28, and 56 days after each antigenic stimulus. The time-courses of the provoked skin-reactions were defined by measuring diameter of erythema and skin thickness, from 2 h after the challenge injection to 10 days later. Skin sections from the affected area were taken for histological examination.

Peak reactivity to the PPD challenge was displayed 14 days after the primary antigenic stimulus, but, in contrast, peak reactivity was achieved only 2 days after secondary sensitization. Furthermore, the development of the skin lesions provoked during the secondary phase was more rapid in onset, as indicated by erythema and induration in the first 24 hours. Histological examination revealed that in secondary-phase skin reactions there was greater infiltration of small-lymphocytes and polymorphonuclear involvement included eosinophils as well as neutrophils.

Pre-treatment with DCE (70 mg/kg daily oral dose) prior to primary sensitization (shown to be immunosuppressive using vinyl chloride) did not alter the severity of the hypersensitive state, as indicated by peak skin reactivity. However, the early rapid onset of skin lesions during the secondary phase was inhibited. On the other hand, the later parts of the reactions (after 24 h) were more marked.

It has previously been shown that secondary phase immunity in the mouse developed more rapidly, (Kwast *et al.*, 1977). The present results suggest that, in the guinea-pig, skin reactions display an additional initial component when evoked during the secondary phase. Although DCE appeared not to suppress the state of hypersensitivity, its effect would be consistent with an inhibition or delay of the postulated extra component.

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Methohexitone-tetraethylammonium interactions in the chick biventer cervicis muscle, moving fluid electrode technique and denervation studies

CATHERINE BELL & F.A. WALI
(introduced by R.C. ELLIOTT)

School of Biological Sciences, Brunel University, Uxbridge, Middlesex, UB8 3PH.

Following the demonstration that low concentrations of methohexitone potentiated the contractures of the slow fibre system of the chick biventer cervicis muscle produced by tetraethylammonium (Elliott, 1979), concentration response curves for tetraethylammonium (TEA) alone and for TEA in Krebs Hensleit solution containing methohexitone, 8.8×10^{-5} M (METHO KREBS) were extended to include doses of TEA from 4.76×10^{-4} M to 1.88×10^{-1} M. Concentration response curves were obtained from the cumulative data for six experiments from which the ED_{50} for TEA was calculated as 6.86×10^{-2} M in the controls, and 1.09×10^{-3} M for TEA in METHO KREBS. The ratio of the ED_{50} of the control to the ED_{50} in METHO KREBS was $1:1.59 \times 10^{-2}$.

TEA may act by depolarizing the post-synaptic membrane. Using the moving fluid electrode technique (Fatt, 1950) to simultaneously record depolarization and contracture tension, METHO KREBS potentiated the response to TEA (2.4×10^{-3} M) by $126\% \pm 8.1\%$ for contractures and by $62\% \pm 9.9\%$, $n = 5$ for depolarization. METHO KREBS depressed the response to acetylcholine (ACh) 5.5×10^{-4} M by $52.4\% \pm 1.5\%$ for contracture and by $32.8\% \pm 3.3\%$ $n = 5$ for depolarization. The depolarizations produced by TEA (4.8×10^{-4} to 4.8×10^{-2} M) and by ACh (5.5×10^{-6} to 5.5×10^{-2} M) were plotted against the corresponding contracture tensions and straight line regressions fitted by the method of least squares. TEA produced 4.86 g tension per mV depolarization in the control and 4.43 g/mV in METHO KREBS, these values were not significantly different. The corresponding values for ACh were control 1.56 g/mV and in METHO KREBS 0.72 g/mV, these values were significantly different ($P < 0.002$). The difference between the slopes of the corresponding regressions for TEA and for ACh was highly significant ($P < 0.001$). The contractures and depolarizations produced in METHO KREBS by ACh, by low frequency repetitive indirect stimulation (RS) and by TEA were potentiated by eserine (1.8×10^{-7} M) and depressed by curare (1.27×10^{-5} M). Hemicholinium (2.6×10^{-5} M) reduced the responses to TEA and RS. METHO KREBS had a slight potentiating action ($18\% \pm 2.0\%$, $n = 4$) on the responses to RS within the range 1–20 Hz.

Chick biventer cervicis muscles denervated seven days previously were compared with control muscles from the same chicks. The denervated muscles produced a supersensitive response to TEA which was not potentiated by METHO KREBS. The control muscles showed the usual potentiation of TEA contractures with METHO KREBS. Blaber & Bowman (1962) reported that TEA produced contractures in denervated muscles *in vivo*.

These experiments suggest that TEA may have a direct post-synaptic action in addition to releasing ACh from the presynaptic nerve terminal. TEA appears to produce a proportionately larger contracture tension than ACh for the same amount of depolarization.

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Modification by domperidone of catecholamine effects on circular smooth muscle of the lower oesophageal and pyloric sphincters

B. COSTALL, R.J. NAYLOR & H.A. SAHYOUN

Postgraduate School of Studies in Pharmacology, University of Bradford, U.K.

The ability of domperidone and other neuroleptic agents to facilitate gastric emptying and to prevent gastric reflux may involve an antagonism of dopamine function at dopamine receptors in the gastroesophageal system (Van Neuten, Ennis, Helsen, Laduron & Janssen, 1978). However, recent studies would suggest that the dopamine agonist-antagonist interaction may reflect, additionally or alternatively, an action on α_1-adrenoceptors (Cox & Ennis, 1979; Ennis & Cox, 1980). In the present study we attempt to further characterise the adrenoceptor site(s) of domperidone action in the lower oesophageal (LOS) and pyloric (PS) sphincters.

Male Dunkin-Hartley guinea-pigs weighing 350–450 g were killed by cervical trans-section and segments 0.5 cm in length were taken from the gastroesophageal and gastroduodenal junctions. The segments were cut longitudinally and used as circular muscle preparations. Additionally, the mucosal layer was removed from the pyloric sphincter. The tissues were bathed in 15 ml oxygenated (95% O_2; 5% CO_2) Krebs and Hensleit solution at 37°C containing 100 mg/l ascorbic acid. Tension changes were detected by Grass tension transducers and the response area integrated (Illingworth & Naylor, 1980) in addition to display on a multichannel Grass recorder. One gram tension was applied to the tissues which were allowed to equilibrate for 30–45 min before the addition of drugs.

Isoprenaline (IPNA, 6.2×10^{-7} M) caused relaxation of the LOS whilst phenylephrine (PhE, 8.0×10^{-6} M), noradrenaline (NA, $3.9 \times 10^{-8} - 3.2 \times 10^{-5}$ M) and dopamine (DA, 1.1×10^{-6}–5.6×10^{-4} M) each produced a biphasic response composed of a relaxation phase followed by contraction. Similarly, IPNA relaxed the PS although PhE caused only contraction. NA had a predominantly relaxant effect whilst DA caused a biphasic relaxation/contraction. Domperidone (10^{-5} M) partially antagonised the relaxation phases of the responses to PhE, NA and DA in the LOS and markedly reduced the contractions caused by the three agonists in both the LOS and PS. However, domperidone failed to modify the responses to IPNA. Phentolamine (10^{-6} M) caused changes very similar to those described for domperidone. Propranolol (5×10^{-7} M) markedly reduced the IPNA-induced relaxations in both sphincters, partially inhibited the relaxation responses to NA and DA in the LOS, but failed to modify the ability of NA, DA or PhE to cause contraction in either the LOS or PS.

From this data we would select two points for emphasis. Firstly, that different responses may be obtained from α-agonist action on the two sphincters, although all are antagonized by domperidone. Secondly, that in preferentially antagonising the contractile responses in the pyloric sphincter, domperidone allows the relaxant effects of NA and DA to dominate. We have previously shown that domperidone, *in vitro*, is able to cause changes compatible with increased gastric motility by inhibiting the ability of NA and DA to relax the circular smooth muscle of the stomach and facilitating their contractile effects (Costall, Naylor & Sahyoun, 1980). These combined observations may be relevant to an understanding of the mechanisms by which domperidone acts '*in vivo*' to facilitate gastric emptying.

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Inhibition of vagally-induced gastric acid secretion by atropine and H_2-receptor antagonists in the rat

M.J. DALY, J.J. REEVES & R. STABLES

Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 0DJ.

Gastric secretion evoked by vagal stimulation has been claimed to be dependent on mobilised histamine (Lundell, 1975). In experiments where the vagus was stimulated electrically Ganguly & Gopinath (1979) found a reduction in gastric histamine contents in the rat, and Uvnas-Wallensten & Andersson (1977) showed that gastric secretion in the cat was inhibited by both atropine and the H_2-receptor antagonist metiamide. In contrast Black *et al.* (1972) reported that burimamide, the first H_2-receptor antagonist, did not inhibit vagally-induced gastric secretion in the rat. We have re-investigated rat gastric secretion induced by electrical vagal stimulation, and its sensitivity to different H_2-receptor antagonists and the muscarinic antagonist, atropine.

Female hooded rats (85–125 g), anaesthetized with pentobarbitone, were prepared for perfusion of the stomach (Parsons, 1969) and electrical stimulation of the vagus nerve via a bipolar platinum electrode placed around the lower oesophagus. Gastric secretion was induced by electrical stimulation for 10 s periods every 30 s using square wave pulses of 10 V, 1 ms duration and 4 Hz. Once a constant level of gastric secretion was attained test compound was administered intravenously, each compound being tested at three dose levels using five rats per dose level.

As shown in Table 1, atropine (0.003–0.03 mg/kg) and the H_2-receptor antagonists-burimamide (3–30 mg/kg), cimetidine (0.1–1 mg/kg) and ranitidine (0.03–0.3 mg/kg) produced dose-related inhibition of vagally-induced gastric secretion. Inhibitory ED_{50} values (95% confidence limits) mg/kg i.v. were: atropine 0.0071 (0.0049–0.0095), burimamide 7.3 (4.4–10.8), cimetidine 0.246 (0.198–0.300) and ranitidine 0.071 (0.044–0.102). This order of potency for the H_2-receptor antagonists is similar to that observed for inhibition of histamine-induced secretion (Black *et al.*, 1972; Daly, Humphray & Stables, 1980). Thus the effectiveness of all three H_2-receptor antagonists against vagally-induced secretion is consistent with an involvement of histamine in this response in the rat.

The burimamide was kindly supplied by Dr. M.E. Parsons, Smith Kline and French Ltd.

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Table 1 Inhibition of vagally-induced gastric acid secretion by atropine and H_2-receptor antagonists in the rat

*Dose** (*mg/kg i.v.*)	*% Inhibition (mean ± s.e. mean of five estimates)*			
	Burimamide	*Cimetidine*	*Ranitidine*	*Atropine*
0.003				24.7 ± 7.7
0.01				57.8 ± 8.7
0.03			27.6 ± 4.3	90.0 ± 1.6
0.10		16.4 ± 6.9	63.4 ± 7.9	
0.30		63.4 ± 4.1	79.6 ± 6.9	
1.0		93.4 ± 3.0		
3.0	21.4 ± 6.8			
10.0	68.6 ± 8.4			
30.0	82.4 ± 8.3			

* Refers to the weight of free base.

Hypothermia in rabbits after intrahypothalamic injection of N^6-2′-*O*-dibutyryl adenosine 3′,5′-monophosphate

M.J. DASCOMBE

Department of Pharmacology, Materia Medica & Therapeutics, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT.

N^6-2′-*O*-dibutyryl adenosine 3′, 5′-monophosphate (db cAMP) produces an initial hypothermia followed by hyperthermia in rabbits (Duff, Cranston & Luff, 1972; Philipp-Dormston & Siegert, 1975) and cats (Varagić & Beleslin, 1973; Clark, Cumby & Davis, 1974) when injected into a lateral cerebral ventricle. However, intrahypothalamic administration of db cAMP has been reported to induce only hyperthermia in rabbits (Woolf, Willies, Laburn & Rosendorff, 1975) but hypothermia in cats (Dascombe & Milton, 1975). We have re-examined the effect of intrahypothalamic db cAMP on body temperature in the rabbit and have studied, in addition, the thermoregulatory effects of some other purine nucleotides, including N^2-2′-*O*-dibutyryl guanosine 3′, 5′-monophosphate (db cGMP).

Adult New Zealand White × Full Lop rabbits of both sexes were used. Core temperature was measured by a YSI 401 thermistor inserted about 10 cm past the anus while rabbits were restrained in stocks at an environmental temperature of 21–23°C. Drugs (sodium salts) were dissolved in 1 µl sterile, pyrogen-free 0.9% saline and injected into the preoptic and anterior hypothalamic nuclei (PO/AH) through chronically implanted guide cannulae. Temperature responses were assessed as thermal response indices for up to 5 h after injection (Dascombe & Milton, 1976).

Db cAMP produced a fall in rectal temperature which was dose-dependent between 10 and 400 µg (Table 1). Hypothermia was maximal about 2 h after injection and was associated with heat loss effected by ear skin vasodilatation. After this time rectal temperature returned to control values accompanied by ear skin vasoconstriction. Db cGMP (10–200 µg) also produced a fall in rectal temperature but this effect was less than that of db cAMP. Saline (1 µl), sodium *n*-butyrate (42.5 µg) and sham injections into the PO/AH each caused a rise in core temperature in some rabbits (Table 1) which lasted for more than 5 hours. Hyperthermia was attenuated by paracetamol (50 mg/kg i.p.) and was attributable to tissue damage seen at the injection site *post mortem*.

It is concluded that db cAMP injected unilaterally into the PO/AH can produce hypothermia in rabbits but this response may be reduced or abolished in some animals by the development of a tissue-damage pyrexia.

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Table 1 Effects of db cAMP on rectal temperature in the restrained rabbit

Treatment	*Dose*	*n*	*Thermal Response Index for 2 h (mean ± s.e. mean °C.h)*
None	—	9	+0.06 ± 0.07
Sham injection	—	8	+0.26 ± 0.10
Saline	1 µl	15	+0.21 ± 0.09
Sodium *n*-butyrate	42.5 µg	12	+0.23 ± 0.08
db cAMP	10 µg	4	+0.01 ± 0.15
db cAMP	50 µg	10	+0.08 ± 0.16
db cAMP	100 µg	13	−0.06 ± 0.18*
db cAMP	200 µg	12	−0.46 ± 0.22**
db cAMP	400 µg	7	−0.64 ± 0.37*

Significance of the difference from the response to saline.
* $0.05 > P > 0.025$.
** $P < 0.025$.

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