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EVIDENCE FOR TWO TYPES OF EXCITATORY RECEPTOR FOR 5-HYDROXYTRYPTAMINE IN DOG ISOLATED VASCULATURE

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1 As part of an investigation into the mode of action of anti-migraine drugs, a study of the excitatory receptors for 5-hydroxytryptamine (5-HT) has been carried out in a range of isolated vascular preparations from the dog.

2 5-HT contracted the dog isolated femoral artery and saphenous vein over the concentration-range 1.0×10^{-8} to 5.0×10^{-6} mol/l.

3 In the femoral artery methysergide and cyproheptadine were potent, competitive and specific antagonists of the contractile responses to 5-HT, with pA_2 values of 8.52 and 8.55 respectively.

4 In the saphenous vein, methysergide was only a weak antagonist of 5-HT. In addition, it was an agonist over the concentration-range 5.0×10^{-8} to 1.0×10^{-5} mol/l. Cyproheptadine was a weak and unsurmountable antagonist of contractile responses to 5-HT and methysergide.

5 Contractile responses to 5-HT and methysergide in the saphenous vein were not antagonized by morphine (3.0×10^{-5} mol/l), indomethacin (5.0×10^{-5} mol/l), phentolamine (5.0×10^{-7} mol/l), propranolol (1.0×10^{-6} mol/l), atropine (1.0×10^{-6} mol/l), mepyramine (1.0×10^{-6} mol/l) or cimetidine (1.0×10^{-5} mol/l).

6 In the external carotid and lingual arteries the pattern of activity obtained with methysergide and cyproheptadine was the same as that in the femoral artery, while in the auricular artery the pattern of activity was the same as that in the saphenous vein.

7 The results are consistent with the hypothesis that there are two types of receptor mediating 5-HT-induced vasoconstriction in dog vasculature. One type, characterized by the pattern of activity obtained in the femoral artery, is like the previously described 'D-receptor'. The other type, characterized by the pattern of activity obtained in the saphenous vein, has not been described before. The verification of this hypothesis requires the identification of a specific antagonist of 5-HT and methysergide in the saphenous vein.

Introduction

Methysergide was first evaluated clinically as an anti-migraine drug because it was shown to be a potent antagonist of the peripheral actions of 5-hydroxytryptamine (5-HT) (Sicuteri, 1959). Methysergide is indeed a highly potent antagonist of the excitatory responses to 5-HT in various types of smooth muscle (Fanchamps, Doepfner, Weidmann & Cerletti, 1960; Gagnon, 1972; Apperley, Humphrey & Levy, 1976), but its mode of action in migraine has been questioned by Saxena (1974) who showed that methysergide, administered intravenously to the anaesthetized dog, potently constricted the common carotid arterial bed but had little effect on other vascular beds. We thought it important to investigate the mechanism of this remarkable selective vasoconstriction.

Our finding that methysergide potently contracted the dog isolated saphenous vein strip prompted a detailed comparison of the 5-HT receptor in the saphenous vein with that in the dog femoral artery, in which methysergide is a potent antagonist of 5-HT. The investigation was then extended to other isolated vascular preparations of the dog. The relevance of our findings to the classification of receptors for 5-HT and to the mode of action of methysergide in migraine is discussed.

Preliminary accounts of these results have been presented to the British Pharmacological Society (Apperley, Humphrey & Levy, 1977; Feniuk, Humphrey & Levy, 1977) and to the 3rd International Symposium on Neuro-effector Mechanisms, July 1978.

Methods

Beagle dogs of either sex, body weight 7 to 12 kg, were anaesthetized with barbitone sodium (300 mg/kg

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i.p.) after induction with thiopentone (25 mg/kg i.v.) and pentobarbitone (60 mg i.v.). Vessels were then removed and prepared for measurement of tension or perfusion pressure *in vitro* as described below.

Preparations

Vascular strips Saphenous veins and femoral, external carotid and lingual arteries were cut spirally into strips as described previously (Furchgott & Bhadrakom, 1953). Each strip was suspended by a cotton thread in a 20 ml organ bath containing a modified Krebs solution (Apperley *et al.*, 1976). Contractions were recorded isometrically using a Statham Microscale Accessory (Model UL5) attached to a Statham Universal Transducing Cell (Model UC3). Strips were 1 to 2 cm in length at an applied resting tension of 400 to 500 mg.

Perfused isolated auricular artery Right and left intermediate auricular arteries were carefully cleared of all connective tissue and perfused intraluminally with Krebs solution at a constant flow rate (2 to 6 ml/min) using a peristaltic pump (HR flow inducer, Watson-Marlow Ltd.). The flow rate was adjusted to produce a perfusion pressure in the absence of drugs of 15 to 25 mmHg. The perfusate was continually recirculated such that a constant volume of Krebs was maintained in the organ bath (15 ml). The volume of Krebs in the tubing outside the bath was less than 0.2 ml. Drugs were added to the Krebs solution in the organ bath. Changes in intraluminal pressure were recorded with a pressure transducer (Bell and Howell Ltd., type 4-422-0001) attached to a side arm of the perfusion cannula.

Experimental design

The sensitivity of many isolated vascular preparations to agonists increases over the first few hours after setting up. Therefore, a period of 2 to 3 h was allowed between the setting up of the preparations and the start of the experiment proper. During this period up to three 'priming' doses of potassium chloride (3×10^{-2} mol/l) were administered at intervals of about 30 min.

Determination of pA_2 values in femoral artery and saphenous vein pA_2 values were determined as previously described by Apperley *et al.* (1976). Briefly, four preparations from the same vessel were set up, one in each of four organ baths. A single agonist was administered in a cumulative dosing schedule to each strip. The agonist was then washed from the baths and after 30 min different concentrations of a single antagonist added to three of the baths whilst the fourth acted as a control. After 30 min contact with the antagonist, agonist concentration-effect curves were redetermined in all four strips. Agonist concentration-ratios were calculated as before (Apperley *et al.*, 1976) for each concentration of antagonist, correction being made for any spontaneous change in sensitivity as judged from the control strip. The sensitivity of the control strips to 5-HT varied by less than 2 fold during the experiment; the sensitivity to methysergide either increased or decreased by up to 2 fold, although occasionally as much as 4 fold changes in either direction occurred.

Antagonism was judged to be competitive and the pA_2 value assumed to correspond to the affinity constant only when the antagonist caused parallel displacement to the right of the agonist concentration-effect curve with no reduction in the maximum response and when a Schild plot gave a linear regression with a slope not significantly different from unity. In some instances (e.g. Figure 4) cyproheptadine caused a reduction of the maximum and of the slope of the agonist concentration-effect curve; in the text we have referred to this as unsurmountable antagonism.

The antagonism of responses to methysergide by phentolamine in saphenous veins was not always clearly concentration-dependent. For this interaction, therefore, an approximate estimate of the pA_2 was obtained using the Gaddum equation (Gaddum, 1957); this estimate was the mean derived from all the methysergide concentration-ratios at each effective concentration of phentolamine.

Antagonism of 5-hydroxytryptamine by methysergide in saphenous vein In the saphenous vein methysergide was found to have agonistic actions of its own and also to be a weak antagonist of 5-HT. However, in initial experiments it was noticed that contractile responses to methysergide were smaller in strips that had been exposed previously to 5-HT. Therefore, in order to examine both the agonistic and antagonistic actions of methysergide in the same preparation for evidence of partial agonism the following experimental protocol was used. A cumulative concentration-effect curve was constructed to 5-HT. The 5-HT was then washed from the bath and the preparations left for 90 min. A different concentration of methysergide was then added to each test bath and left in contact for 30 min. 5-HT concentration-effect curves were then redetermined in the presence of methysergide. Under these conditions the desensitizing effect of 5-HT on contractile responses to methysergide was less evident while the potency of 5-HT itself increased by only a little over 2 fold.

Effects of methysergide and cyproheptadine in a variety of vascular preparations In order to compare the effects of methysergide and cyproheptadine in the

femoral artery and saphenous vein with their effects in other vessels, the following study was carried out. Individual strips were set up as described above and a cumulative concentration-effect curve constructed to either 5-HT or methysergide. The agonistic activity of either compound was quantified as the maximum contractile response produced (in g tension or mmHg) and as the concentration required to produce 25% of its own maximum response (EC_{25}). The agonist was washed from the bath, cyproheptadine (1.0×10^{-7} mol/l) added and after 30 min the agonist concentration-effect curve was redetermined. The agonist concentration-ratio was then calculated at the EC_{25} value. No corrections were made for spontaneous changes in sensitivity to the agonist, but the results for the femoral artery and saphenous vein, which were included for comparison, were consistent with the more detailed study. pA_2 values were estimated from the log of the affinity constant derived from the Gaddum equation (Gaddum, 1957).

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate, mol. wt. 405.4 (Koch-Light); methoxamine hydrochloride, mol. wt. 247.7 (Burroughs Wellcome); methysergide bimaleate, mol. wt. 469.5 (Sandoz); cyproheptadine hydrochloride, mol. wt. 350.9 (Merck, Sharp and Dohme) and phentolamine methane sulphonate, mol. wt. 377.5 (Ciba). Stock solutions of 5-hydroxytryptamine, methysergide and cyproheptadine were made up in distilled water and dilutions made with isotonic saline (0.9% w/v NaCl). Stock solutions of phentolamine and methoxamine were made up in isotonic saline and dilutions made with isotonic saline.

Results

Dog femoral artery strip

Agonists 5-HT (1.0×10^{-8} to 5.0×10^{-6} mol/l) and methoxamine (1.0×10^{-7} to 2.0×10^{-4} mol/l) contracted the dog femoral artery, producing maximum tension changes (mean ± s.e. mean) of 0.57 ± 0.06 g ($n = 20$) and 0.71 ± 0.07 g ($n = 20$) respectively. 5-HT ($EC_{50} = 1.95 \pm 0.10 \times 10^{-7}$ mol/l) was about 20 times more potent than methoxamine ($EC_{50} = 4.03 \pm 0.33 \times 10^{-6}$ mol/l).

Methysergide (1.0×10^{-8} to 1.0×10^{-6} mol/l), cyproheptadine (1.0×10^{-8} to 1.0×10^{-6} mol/l) and phentolamine (1.0×10^{-8} to 1.0×10^{-5} mol/l) had no agonistic activity.

Antagonists Methysergide, cyproheptadine and phentolamine were investigated as antagonists of 5-HT. The results are summarized in Table 1. Methysergide and cyproheptadine were potent and competitive antagonists of 5-HT (Figure 1). Both drugs were also weaker antagonists of methoxamine. Conversely, phentolamine was a potent and competitive antagonist of methoxamine and a weaker antagonist of 5-HT.

Dog saphenous vein strip

Agonists 5-HT (1.0×10^{-8} to 5.0×10^{-6} mol/l) and methoxamine (1.0×10^{-7} to 2.0×10^{-4} mol/l) con-

Table 1 Interactions between agonists and antagonists in dog femoral artery strip

		pA_2 (30 min) against	
Antagonist		*5-Hydroxytryptamine*	*Methoxamine*
Methysergide	pA_2	8.52 (8.03–9.01)	5.77 (5.45–6.09)
	slope	0.88 (0.56–1.20)	1.07 (0.82–1.32)
Cyproheptadine	pA_2	8.55 (8.39–8.71)	7.10 (6.93–7.26)
	slope	1.18 (0.96–1.40)	1.64 (1.39–1.89)
Phentolamine	pA_2	6.55 (6.15–6.95)	7.70 (7.25–8.15)
	slope	1.03 (0.85–1.21)	0.98 (0.72–1.23)

Each value is the mean (95% confidence limits) of 4 to 8 estimates.

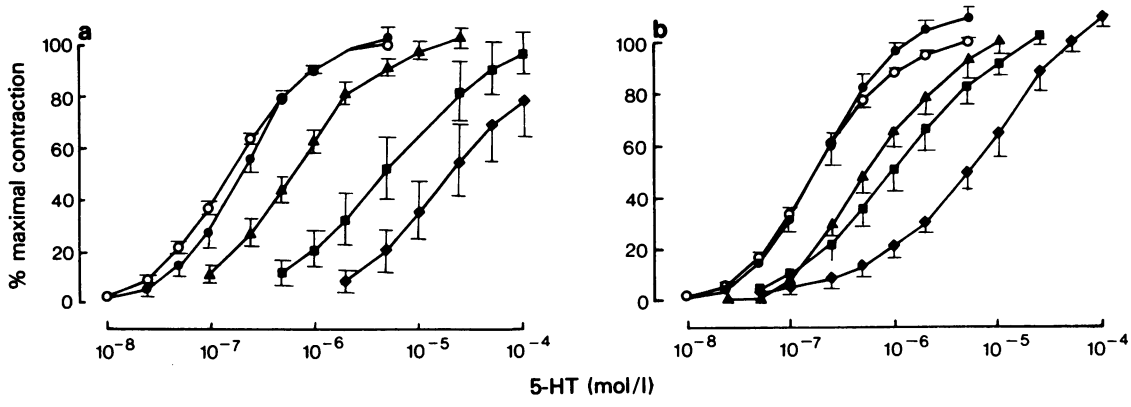

Figure 1 Dog isolated femoral artery strip. Effects of methysergide (a) and cyproheptadine (b) on contractile responses to 5-hydroxytryptamine (5-HT). Both the first (○) and second (●) control concentration-effect curves to 5-HT were obtained in the absence of antagonist (see text). Methysergide concentrations examined were 1.0×10^{-8} mol/l (▲), 1.0×10^{-7} mol/l (■), and 1.0×10^{-6} mol/l (◆); cyproheptadine concentrations were 2.5×10^{-9} mol/l (▲), 1.0×10^{-8} mol/l (■) and 5.0×10^{-8} mol/l (◆). Ordinate scale represents the response as a percentage of the maximum response obtained in the first concentration-effect curve. Each value is the mean of six and four estimates for methysergide and cyproheptadine respectively; vertical lines show s.e. mean.

tracted the dog saphenous vein, producing maximal tension changes of 1.52 ± 0.23 g ($n = 24$) and 2.14 ± 0.23 g ($n = 20$) respectively. 5-HT (EC_{50} $6.29 \pm 0.16 \times 10^{-8}$ mol/l) was about 50 times more potent than methoxamine ($EC_{50} = 3.31 \pm 0.65 \times 10^{-6}$ mol/l).

Methysergide (5.0×10^{-8} to 1.0×10^{-5} mol/l) contracted the saphenous vein, in contrast to its complete lack of agonistic activity in the femoral artery (Figure 2). Methysergide produced a maximal tension change of 0.69 ± 0.09 g ($n = 23$) at about 1.0×10^{-5} mol/l and was about 10 times less potent than 5-HT

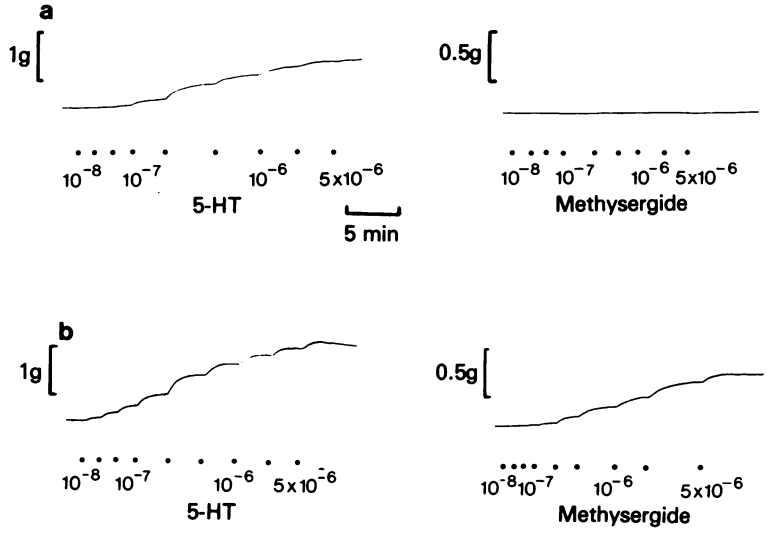

Figure 2 Experimental record of contractile responses to 5-hydroxytryptamine (5-HT) and methysergide in dog isolated femoral artery strip (a) and saphenous vein strip (b). Agonist concentrations were increased cumulatively from 1×10^{-8} to 2.5×10^{-8}, 5×10^{-8}, 1×10^{-7}, 2.5×10^{-7}, 5×10^{-7}, 1×10^{-6}, 2×10^{-6} and 5×10^{-6} mol/l in each case. Note that methysergide contracted the saphenous vein but not the femoral artery.

($EC_{50} = 5.89 \pm 0.93 \times 10^{-7}$ mol/l). High concentrations of methysergide ($> 1.0 \times 10^{-5}$ mol/l) caused the strips to relax, producing 'bell-shaped' concentration-effect curves. Cyproheptadine (1.0×10^{-8} to 1.0×10^{-6} mol/l) and phentolamine (1.0×10^{-8} to 1.0×10^{-5} mol/l) had no agonistic activity.

Antagonists Methysergide, cyproheptadine and phentolamine were investigated as antagonists of 5-HT and methoxamine. The results are summarised in Table 2.

Low concentrations of methysergide (up to 1.0×10^{-7} mol/l) had little contractile activity and had little or no 5-HT antagonistic activity (Figure 3). This contrasts with the femoral artery where marked antagonism of 5-HT was produced by similar concentrations of methysergide; for example, methysergide (1.0×10^{-7} mol/l) produced a 5-HT concentration-ratio of 33 in the femoral artery (Figure 1). Higher concentrations of methysergide contracted the saphenous vein (see above) and also weakly antagonized responses to 5-HT (Figure 3). Methysergide was a weak competitive antagonist of methoxamine in the saphenous vein (Table 2) as in the femoral artery.

The results obtained with cyproheptadine in the saphenous vein were also very different from those obtained in the femoral artery. In the saphenous vein, cyproheptadine was a weak and unsurmountable antagonist of 5-HT, whereas in the femoral artery it was a potent and competitive antagonist of 5-HT (compare Figure 4 with Figure 1). Cyproheptadine was also a weak and unsurmountable antagonist of methysergide-induced contractions in the saphenous vein (Figure 5a). On the other hand, cyproheptadine was a competitive antagonist of methoxamine in the saphenous vein, although a relatively weak one (Figure 5b and Table 2). Its potency against methoxamine in the saphenous vein was similar to that in the femoral artery (Table 1).

In the saphenous vein, phentolamine was a potent, competitive antagonist of methoxamine and a weaker, competitive antagonist of 5-HT (Table 2). Furthermore, phentolamine produced parallel displacements of methysergide concentration-effect curves over a concentration-range similar to that required to antagonize 5-HT responses. However, the antagonism of methysergide was not always concentration-dependent and in three out of five experiments no estimate of the slope of the Schild plot could be made. The interaction between phentolamine and methysergide is undoubtedly complicated by the additional actions of the higher concentrations of methysergide, as shown by its unusual 'bell-shaped' concentration-effect curve (see above). This was not investigated further.

Effects of various antagonists and of indomethacin on contractile responses to 5-hydroxytryptamine and methysergide Morphine (3.0×10^{-5} mol/l), indomethacin (5.0×10^{-5} mol/l), atropine (1.0×10^{-6} mol/l), propranolol (1.0×10^{-6} mol/l), mepyramine (1.0×10^{-6} mol/l) and cimetidine (1.0×10^{-5} mol/l)

Table 2 Interactions between agonists and antagonists in dog saphenous vein strip

		pA_2 (30 min) against		
Antagonist		*5-Hydroxytryptamine*	*Methysergide*	*Methoxamine*
Methysergide	pA_2	<7.0*	—	5.63 (4.00–7.03)
	slope	—	—	1.18 (0.64–1.72)
Cyproheptadine	pA_2	unsurmountable antagonism**	unsurmountable antagonism***	7.23 (7.02–7.44)
	slope	—	—	1.02 (0.81–1.23)
Phentolamine	pA_2	6.11† (5.92–6.30)	6.10 (5.73–6.47)	7.90† (7.66–8.14)
	slope	0.95 (0.54–1.36)	—	1.00 (0.80–1.20)

Each value is the mean (95% confidence limits) of 4 to 6 estimates except for phentolamine against methysergide, where $n = 9$ (see text).
* See Figure 3; **See Figure 4; ***See Figure 5a.
† Results from Humphrey (1978).

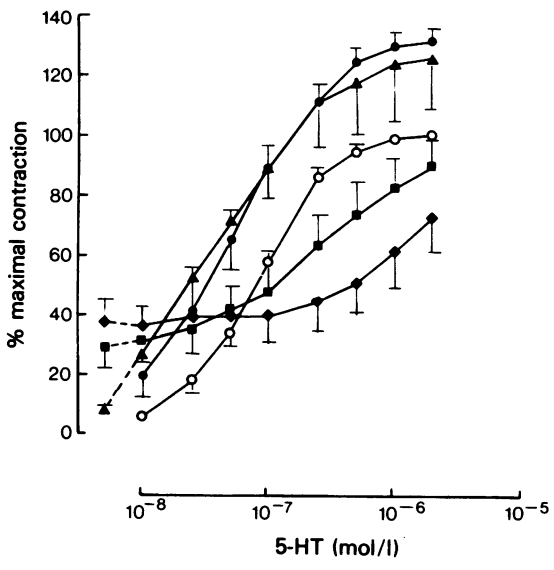

Figure 3 Dog isolated saphenous vein strip. Effect of methysergide on contractile responses to 5-hydroxytryptamine (5-HT). The interval between curves was 120 min and both the first (○) and second (●) control concentration-effect curves to 5-HT were obtained in the absence of antagonist. Methysergide concentrations examined were 1.0×10^{-7} mol/l (▲), 1.0×10^{-6} mol/l (■) and 1.0×10^{-5} mol/l (◆). Ordinate represents the response as a percentage of the maximum 5-HT response obtained in the first concentration-effect curve. Ordinate values to the left of the abscissa scale represent contractile responses to methysergide alone; subsequent responses to 5-HT have been expressed relative to the resting tension immediately prior to dosing with methysergide. Each value is the mean of 7 estimates; vertical lines show s.e. mean.

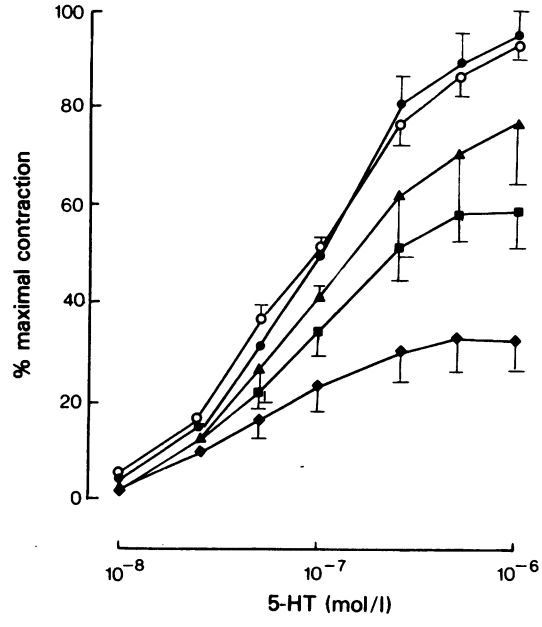

Figure 4 Dog isolated saphenous vein strip. Effect of cyproheptadine on contractile responses to 5-hydroxytryptamine (5-HT). Both the first (○) and second (●) control concentration-effect curves to 5-HT were obtained in the absence of antagonist. Cyproheptadine concentrations examined were 1.0×10^{-8} mol/l (▲), 1.0×10^{-7} mol/l (■) and 1.0×10^{-6} mol/l (◆). Ordinate scale represents the response as a percentage of the maximum response obtained in the first concentration-effect curve. Each value is the mean of 4 estimates; vertical lines show s.e. mean.

had no effect on contractile responses to 5-HT or methysergide in dog saphenous vein ($n = 4$ for each compound).

Dog external carotid, lingual and intermediate auricular artery preparations

Agonists The agonistic activities of 5-HT and methysergide were compared in a number of dog isolated vascular preparations, including the femoral artery and saphenous vein (Table 3). 5-HT was an agonist in all the preparations examined. Methysergide was an agonist in the saphenous vein and intermediate auricular arteries, but was without agonist activity in the femoral, external carotid and lingual arteries. The maximum contractile response obtained with methysergide in the saphenous vein and auricular artery varied between 30 and 60% of the 5-HT maximum and methysergide was about 10 times less potent than 5-HT (Table 3).

Antagonists The antagonistic activity of a single concentration (1.0×10^{-7} mol/l) of cyproheptadine against 5-HT was examined in all the vascular preparations, including the femoral artery and saphenous vein (Table 4). In the femoral artery, external carotid artery and lingual artery, cyproheptadine was a potent antagonist of 5-HT. The 5-HT concentration-effect curves were displaced to the right in a parallel manner, suggesting competitive antagonism. The pA_2 values estimated from these experiments were similar to each other and the value for the femoral artery is close to that obtained in the more detailed study (see Table 1). In contrast, in the saphenous vein and auricular artery, cyproheptadine was a weak and unsurmountable antagonist of 5-HT. Cyproheptadine was

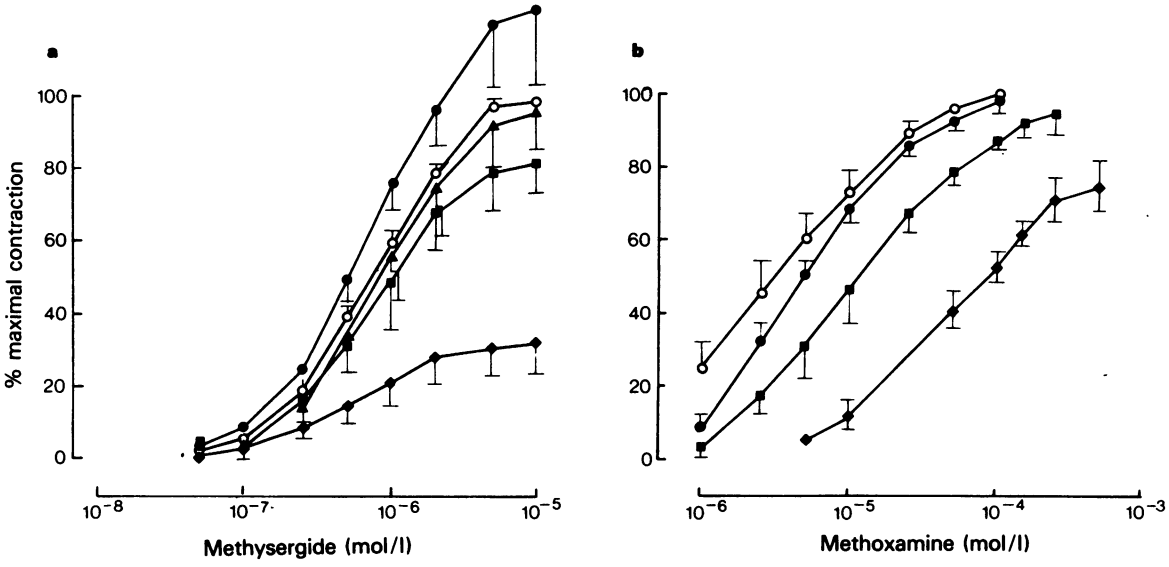

Figure 5 Dog isolated saphenous vein strip. Effects of cyproheptadine on contractile responses produced by methysergide (a) and methoxamine (b). Both the first (○) and second (●) control agonist concentration-effect curves were obtained in the absence of cyproheptadine. Cyproheptadine concentrations examined were 1.0×10^{-8} mol/l (▲), 1.0×10^{-7} mol/l (■) and 1.0×10^{-6} mol/l (◆). Ordinate scale represents the responses as a percentage of the maximum response obtained in the first concentration-effect curve. Each value is the mean of 4 and 5 estimates respectively; vertical lines show s.e. mean. In (b) the curve for methoxamine in the presence of 1.0×10^{-8} mol/l cyproheptadine was omitted for clarity.

also a weak and unsurmountable antagonist of the contractile responses to methysergide in the two preparations.

Discussion

The results in this study demonstrate that 5-HT is a potent contractile agonist in both the dog femoral artery and saphenous vein, but suggest that different types of 5-HT receptor are involved. In the femoral artery both methysergide and cyproheptadine were potent and competitive antagonists of 5-HT. However, in the saphenous vein methysergide was only a weak antagonist of 5-HT and had contractile activity of its own, while cyproheptadine was a weak, unsurmountable antagonist of 5-HT.

The results in the femoral artery show that 5-HT

Table 3 Contractile effects of 5-hydroxytryptamine (5-HT) and methysergide in various dog isolated vessels

Vessel	*Agonist*	*Maximum response*	EC_{25} (*mol/l*)	n
Femoral artery	5-HT	1.12 ± 0.19 g	$1.0 \pm 0.2 \times 10^{-7}$	10
	Methysergide	*	—	10
External carotid artery	5-HT	0.43 ± 0.10 g	$1.4 \pm 0.2 \times 10^{-7}$	7
	Methysergide	*	—	9
Lingual artery	5-HT	0.68 ± 0.15 g	$1.7 \pm 0.2 \times 10^{-7}$	8
	Methysergide	*	—	9
Saphenous vein	5-HT	1.41 ± 0.16 g	$5.1 \pm 0.5 \times 10^{-8}$	19
	Methysergide	0.60 ± 0.08 g	$4.0 \pm 0.6 \times 10^{-7}$	19
Intermediate auricular artery	5-HT	199 ± 31 mmHg	$5.5 \pm 1.3 \times 10^{-8}$	9
	Methysergide	63 ± 13 mmHg	$1.6 \pm 0.6 \times 10^{-7}$	7

Each value is the mean ± s.e. mean; *n* is the number of estimates.
* no agonistic activity in concentrations up to 1.0×10^{-5} mol/l.
EC_{25} = concentration required to produce 25% of the maximum response to the individual agonist.

mediates its contractile action through a specific receptor and that this receptor can be clearly differentiated from the α-adrenoceptor that is also present in the preparation. As judged by the high antagonistic potencies of methysergide and cyproheptadine, the 5-HT receptor in the femoral artery has the same characteristics as the 5-HT receptors in smooth muscle from the gastro-intestinal tract (Bartlet & Hassan, 1968; Gorlitz & Frey, 1973), uterus (Fanchamps *et al.*, 1960) and rabbit aorta (Apperley *et al.*, 1976), that is, the characteristics of the so-called 'D-receptor' (Gaddum & Picarelli, 1957; Day & Vane, 1963; Drakontides & Gershon, 1968).

On the other hand, in the saphenous vein a D-receptor mechanism does not appear to be involved. Methysergide was a much weaker antagonist of responses to 5-HT in the saphenous vein than in the femoral artery (compare Figures 1a and 3). It cannot be argued that the weak antagonism was due to the additional contractile action of methysergide in the saphenous vein because the lowest concentrations examined had no agonistic or antagonistic effects. With cyproheptadine the possibility that the marked difference in antagonistic activity in the two preparations could be attributed to an agonistic action does not even arise because cyproheptadine was devoid of agonistic activity in the concentrations tested. Because contractile responses to 5-HT in the saphenous vein were not potently antagonized by two classical 5-HT antagonists, methysergide and cyproheptadine, other possible mechanisms were investigated. The low antagonistic potency of phentolamine against 5-HT rules out the possibility that 5-HT mediates its contractile effect through α-adrenoceptors, either directly, as has been demonstrated in other tissues, for example the rabbit ear artery (Apperley *et al.*, 1976; Fozard, 1976), or indirectly through release of noradrenaline from noradrenergic nerves. This latter possibility deserves particularly careful consideration because the noradrenaline-releasing effect of 5-HT has been well documented and also because the dog saphenous vein possesses a dense noradrenergic innervation (Brandao, 1976; Feniuk, Humphrey & Watts, 1978). Further evidence on this point has been obtained in a separate study by one of us in which it was shown that the contractile response to 5-HT was unaffected by cocaine and was still present in saphenous veins from dogs pretreated with syrosingopine (Humphrey, 1978). 5-HT is also known to be capable of releasing a number of mediators other than noradrenaline, but our results show that the contractile response of the saphenous vein to 5-HT was not caused by release of a prostaglandin, acetylcholine or histamine.

In the absence of any obvious alternative we suggest that 5-HT exerts its contractile effect on the saphenous vein directly through a specific 5-HT receptor and that this receptor differs from the 'D-receptor' found in the femoral artery and elsewhere. The high potency of 5-HT in the saphenous vein, higher than that in the femoral artery, is consistent with the idea of an action on a specific 5-HT receptor. We suggest, furthermore, that methysergide is a partial agonist at the 5-HT receptor in the saphenous vein. The finding that methysergide antagonized responses to 5-HT in the saphenous vein only at concentrations that produced contractile effects, is consistent with this hypothesis (see Ariens, Simonis & Van Rossum, 1964). Verification of this hypothesis requires the identification of a specific antagonist of 5-HT and methysergide in the saphenous vein. In the search for such a compound we have

Table 4 Effects of cyproheptadine (1.0×10^{-7} mol/l) on contractile responses to 5-hydroxytryptamine (5-HT) in various dog isolated vessels

Vessel	*5-HT concentration-ratio*†	*pA_2 estimate against 5-HT*	n
Femoral artery	54 (24–119)	8.72 (8.36–9.07)	10
External carotid artery	81 (24–267)	8.90 (8.36–9.43)	7
Lingual artery	52 (21–131)	8.71 (8.30–9.11)	8
Saphenous vein	3.2 (1.4–7.4)	*	19
Intermediate auricular artery	2.3 (1.5–3.6)	*	9

Each value is the mean (95% confidence limits); n = number of estimates.
† calculated from EC_{25} values (see methods).
* 5-HT concentration-effect curves not displaced in a parallel manner (see text).

examined other 5-HT antagonists including mianserin (Saxena, van Houwelingen & Bonta, 1971), BW 501C67 (Mawson & Whittington, 1970) and bromolysergic acid diethylamide (Kohli, 1968), but they were without significant effect (unpublished observations). A morphine-sensitive mechanism like that in the guinea-pig lung (Bakhle & Smith, 1974) can also be excluded. In the absence of a specific antagonist we are attempting to characterize the putative 5-HT receptor in the saphenous vein by determining the agonistic potencies of a series of tryptamine analogues for comparison with those obtained in other preparations containing 5-HT receptors (see Fozard & Mobarok Ali, 1978a). If the saphenous vein does contain a specific 5-HT receptor and if this receptor is distinct from the 'D-receptor', then the question arises of whether it has the characteristics of the 'M-receptor' (Gaddum & Picarelli, 1957; Drakontides & Gershon, 1968; Fozard & Mobarok Ali, 1978a, b). The answer appears to be no, because responses to 5-HT in saphenous vein were not blocked by the M-receptor antagonists, phenylbiguanide and metoclopramide (unpublished observations). In addition, the saphenous vein was contracted by low concentrations of tryptamine which is a very weak M-receptor agonist (unpublished observation).

The pattern of activity obtained with methysergide and cyproheptadine in dog femoral artery was also found in the external carotid and lingual arteries; likewise, the pattern of activity obtained in the saphenous vein was also found in the auricular artery. The results in the external carotid and lingual arteries are of interest because they extend the range of preparations known to contain 'D-receptors'. The results in the auricular artery are of more fundamental importance, however, because they show that the novel pattern of activity observed in the saphenous vein is not attributable to some pecularity of the preparation itself but that it has a more general validity. If our interpretation given above is correct, then it follows that the auricular artery and saphenous vein contain the same type of 5-HT receptor. Other responses to 5-HT that are not blocked but instead are mimicked by methysergide have been demonstrated in the common carotid bed of the dog (Saxena, 1972; Vargaftig & Lefort, 1974) and in the cat brain (Jalfre, Monachon & Haefely, 1974).

The present results may provide a simple explanation of the selective vasoconstrictor action of methysergide in the carotid arterial bed of the dog and thereby of the basis of its efficacy in the treatment of migraine. The hypothesis is that the type of 5-HT receptor identified in the saphenous vein and auricular artery predominates in the resistance vessels of the carotid vasculature. This is consistent with the finding that cyproheptadine was surprisingly ineffective as a 5-HT antagonist in this bed (Saxena, 1972). We are currently investigating the distribution of the different types of 5-HT receptor throughout the vasculature of the anaesthetized dog in order to test our hypothesis.

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