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RECEPTORS FOR 5-HYDROXYTRYPTAMINE AND NORADRENALINE IN RABBIT ISOLATED EAR ARTERY AND AORTA

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1 5-Hydroxytryptamine (5-HT) is thought to be implicated in the vascular disturbances of the external carotid artery bed associated with migraine. As part of a study of the pharmacology of some 5-HT antagonists used in the treatment of migraine we have examined the interactions of these drugs with 5-HT and noradrenaline in rabbit isolated ear artery and aortic strip. The results provide new information on the distribution of 5-HT-receptors and α-receptors in these preparations.

2 In the aorta, 5-HT and noradrenaline were of similar potency in producing contractions. Methysergide produced very small contractions and was about 1000 times less potent than the other two agonists.

3 In the ear artery noradrenaline produced monophasic vasoconstrictor responses, whereas 5-HT and methysergide produced prolonged biphasic responses. 5-HT was about 700 times less potent and methysergide about 4500 times less potent than noradrenaline. Methysergide was a better agonist in the ear artery than in the aorta. Biphasic responses to 5-HT and methysergide were also obtained in ear arteries from reserpine-treated rabbits indicating that neither agonist was acting by releasing endogenous noradrenaline.

4 Pizotifen, cyproheptadine and phentolamine had no agonistic actions in either the aorta or ear artery.

5 In the aorta methysergide, pizotifen and cyproheptadine were potent antagonists of 5-HT and much weaker antagonists of noradrenaline. Phentolamine possessed the opposite profile of selectivity. These results show that there are distinct receptors for 5-HT and noradrenaline in rabbit aorta.

6 In the ear artery the pA_2 values for each of the four antagonists were virtually identical against 5-HT and noradrenaline and similar to those obtained on α-adrenoceptors in the aorta. We conclude that 5-HT and noradrenaline act directly at α-receptors to produce vasoconstriction in the ear artery and that this preparation does not contain specific 5-HT-receptors.

7 This insight into the distribution of 5-HT-receptors and α-receptors allows interpretation of the various actions of methysergide. In the aorta, methysergide was a potent antagonist at 5-HT-receptors and a weak partial agonist at α-receptors. In the ear artery, methysergide was a partial agonist at α-receptors; it was only a weak antagonist of 5-HT because this preparation does not contain specific 5-HT-receptors.

8 The cross-reactivity demonstrated throughout these experiments indicates that 5-HT-receptors and α-receptors, although distinct entities, have features in common.

9 These results are discussed in relation to the mode of action of methysergide, pizotifen and cyproheptadine in the treatment of migraine.

Introduction

Although the aetiology of migraine is not clear there is considerable evidence implicating 5-hydroxytryptamine (5-HT) in the vascular disturbances of the external carotid artery bed associated with the syndrome (see Lance, Anthony & Hinterberger, 1970). Methysergide, a proven migraine prophylactic drug, is well known as a 5-HT antagonist (see Fozard, 1975 for references). However, recent studies reveal that methysergide also has an agonistic vasoconstrictor action in the carotid vasculature (Fozard, 1973; Saxena, 1974; Apperley, Humphrey & Levy, 1974). As part of a wider study into the mechanisms of action of drugs used in the treatment of migraine we have analysed the agonistic and antagonistic actions

of methysergide at the receptor level. Two other drugs used in the treatment of migraine and known to interact with 5-HT, namely pizotifen and cyproheptadine, have also been examined. It was hoped that a better understanding of the actions of these drugs at the receptor level would help to explain their therapeutic effects and also provide clues about the aetiology of the syndrome. Experiments have been carried out with the rabbit central ear artery, a branch of the external carotid artery, and for comparison, the aorta. The results show that methysergide, pizotifen and cyproheptadine can act on both 5-HT and noradrenaline receptors and provide new insight into the distribution of these receptors in the two preparations.

Some of these results were presented to a meeting of the British Pharmacological Society (Apperley, Humphrey & Levy, 1974).

Methods

New Zealand white rabbits of either sex, body weight 2–3 kg, were used.

Rabbit aortic strip

Thoracic aortic arteries were removed from animals killed with pentobarbitone (400 mg i.v.). The aortae were cut spirally into strips as described by Furchgott & Bhadrakom (1953), four preparations being obtained from each animal. Each strip was suspended in a 20 ml organ bath containing a modified Krebs solution. Tension was measured with a Statham Microscale Accessory (Model UL5) attached to a Statham Universal Transducing Cell (Model UC3). Strips were approximately 2 cm in length at an applied resting tension of 400–500 mg.

Preparations were left for 3 h before the start of the experiment. During this period three doses of potassium chloride (3.0×10^{-2} mol/l) were administered at intervals of 45–60 minutes. The resting tension decreased slightly and the size of the contractile response to potassium chloride increased to an almost constant level.

A single agonist (noradrenaline, 5-HT, methysergide or potassium chloride) was administered cumulatively to each strip. Increase in tension, expressed as % maximum response, was plotted against agonist concentration on a log scale. The potency of each agonist was calculated from its concentration-effect curve. The agonist was then washed from the bath and the potency of an antagonist (phentolamine, methysergide, pizotifen or cyproheptadine) determined in the same preparations. Graded concentrations of antagonist were added to three of the strips whilst the fourth strip acted as a control. After 30 min, concentration-effect curves to the agonist were re-determined in all four strips. The degree of antagonism was quantified in terms of the dose-ratio, which was determined by the ratio of the EC_{50} values before and after the addition of antagonist. Correction was made for any spontaneous change in sensitivity by dividing the dose-ratios obtained in the strips exposed to antagonist by the dose-ratio obtained in the control strip. The sensitivity of the control strips to the various agonists decreased during the experiments by about 2-fold. The results were plotted graphically in the form of a Schild plot as log (agonist dose-ratio −1) on log antagonist concentration (mol/l) (Arunlakshana & Schild, 1959). The slope of the regression and the pA_2 value were estimated.

Rabbit isolated ear artery

Right and left auricular arteries were removed from animals anaesthetized with pentobarbitone (36 mg/kg i.v.). The vessels were carefully cleared of all connective tissue and perfused intraluminally with modified Krebs solution at a constant flow rate of 7 ml/min by means of a peristaltic pump (H.R. Flow Inducer, Watson-Marlow Ltd.). The distal end of the artery was not cannulated, so that the perfusion fluid, after contact with the intraluminal surface, bathed the extraluminal surface before being removed by overflow. The bath volume was thereby kept at a constant volume of 15 ml. Changes in intraluminal pressure were recorded from a pressure transducer (Bell & Howell Ltd., type 4–422–0001) attached to a side arm of the perfusion cannula. Bolus injections of the agonists were made into the perfusion fluid immediately before its passage through the peristaltic pump. A constant injection volume of 100 µl which produced no injection artefact was used. This procedure is similar to that described by de la Lande & Rand (1965).

Paired preparations from the same animal were used in parallel experiments. The arteries were perfused for 60 min before the experiment started. A single agonist was then administered at 5 or 6 dose-levels to the two arteries, with a 5–10 min interval between doses. Increase in perfusion pressure was plotted against dose on a log scale. The potency of each agonist was estimated from the dose-effect curves. The potency of an antagonist was then determined in the same two preparations. One artery acted as a control and was perfused with Krebs solution alone. The second artery was perfused for 30 min with Krebs solution containing the test antagonist. Agonist dose-effect curves were then re-determined in test and control arteries. This procedure was repeated twice more using successively higher concentrations of antagonist. Since maximum responses were rarely obtained and individual dose-response curves varied slightly in shape, dose-ratios were estimated at arbitrarily determined response levels on the linear portion of the curves. Dose-ratios

were corrected for spontaneous changes in sensitivity. The sensitivity to vasopressin decreased during the experiment by about 2-fold; sensitivity to noradrenaline, 5-HT or methysergide usually increased, the maximum increase being 5-fold. The results were analysed by the Schild plot as described under rabbit aortic strip.

Pretreatment with reserpine

Reserpine was dissolved in distilled water containing 20% w/v ascorbic acid. Rabbits were injected with reserpine (1 mg/kg i.p.) 18 h before removal of the arteries. This procedure has been shown to eliminate the indirect action of tyramine, which results from release of endogenous noradrenaline (Farmer, 1966).

Physiological saline

A modified Krebs solution with the following composition (mmol/l) was used: Na^+ 143.4, K^+ 5.9, Mg^{++} 0.6, Ca^{++} 1.3, Cl^- 124.5, $H_2PO_4^-$ 1.2, SO_4^{++} 0.6, HCO_3^- 25.0, glucose 11.1, gassed with 95% O_2 and 5% CO_2 at 37°C.

Drugs

The following drugs were used: (−)-noradrenaline bitartrate, mol. wt. 337.3 (Koch-Light), 5-hydroxytryptamine creatinine sulphate, mol. wt. 405.4 (Koch-Light), methysergide bimaleate, mol. wt. 469.5 (Sandoz), pizotifen hydrogen maleate, mol. wt. 295.4 (Sandoz), phentolamine methane sulphonate, mol. wt. 337.5 (Ciba), cyproheptadine hydrochloride, mol. wt. 350.9 (Merck Sharp & Dohme) and lysine-vasopressin mol. wt. 1056.3 (Sigma). 5-HT, methysergide, pizotifen and cyproheptadine were dissolved in distilled water, and phentolamine and vasopressin in 0.9% w/v NaCl solution (saline). Noradrenaline was dissolved in saline containing ascorbic acid (0.2 mg/ml).

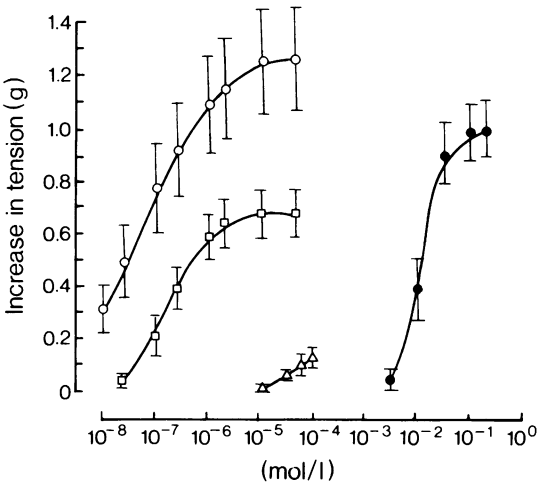

Figure 1 Rabbit aortic strip. Concentration-effect curves for noradrenaline (○), 5-hydroxytryptamine (□), methysergide (△) and potassium chloride (●). Each point is the mean response from 8 strips, each taken from a different animal. Vertical lines show s.e. mean. Note the very weak agonistic action of methysergide.

Results

Rabbit aortic strip

Agonists Potassium chloride produced contractions of the aorta in the concentration-range 3.0×10^{-3}–2.0×10^{-1} mol/l with a maximum tension of 1.0 ± 0.1 g (mean ± s.e. mean, $n=8$) (Figure 1). Noradrenaline and 5-HT produced contractions over similar concentration ranges, 1.0×10^{-8}–5.0×10^{-5} mol/l, with a maximum tension of 1.3 ± 0.2 g ($n=8$) and 0.7 ± 0.1($n=8$) respectively. 5-HT (EC_{50} 1.7×10^{-7} mol/l) was about 4 times less active than noradrenaline (EC_{50} 4.3×10^{-8} mol/l) (Figure 1).

Table 1 Effect of pretreatment with high concentrations of noradrenaline or 5-hydroxytryptamine on contractile responses to methysergide or histamine in rabbit aortic strip

Pretreatment	*Agonist*	*Maximal response as % of the maximal response in strips pretreated with potassium chloride (3.0×10^{-2} mol/l)*
Noradrenaline (5.0×10^{-5} mol/l)	Methysergide	19 (0–34)
5-Hydroxytryptamine (5.0×10^{-6} mol/l)	Methysergide	101 (49–198)
Noradrenaline (5.0×10^{-5} mol/l)	Histamine	87 (60–121)

Each value is the mean of 4–8 determinations (range).

Methysergide was a weak agonist over the concentration-range 1.0×10^{-5}–1.0×10^{-4} mol/l (Figure 1), the response at the highest concentration being 0.14 ± 0.04 g ($n = 8$), which is only 11% of the maximum contraction produced by noradrenaline. Comparison of potencies at this level shows that methysergide is over 1000 times less potent than noradrenaline or 5-HT. Concentrations of methysergide higher than 1.0×10^{-4} mol/l were not tested because this was the practical limit of its solubility.

During the course of these experiments we noticed that methysergide was even less active in preparations

Table 2 Interactions between agonists and antagonists in rabbit aortic strip

		Agonist	
Antagonist		*Noradrenaline*	*5-hydroxytryptamine*
Phentolamine	pA_2	7.96 (7.76–8.16)	6.21 (5.52–6.90)
	Slope	0.91 (0.79–1.03)	0.93 (0.60–1.26)
Pizotifen	pA_2	6.69 (6.23–7.15)	9.42 (8.18–10.66)
	Slope	0.78 (0.53–1.03)	1.01 (0.29–1.73)
Cyproheptadine	pA_2	6.69 (6.05–7.33)	8.73 (8.36–9.10)
	Slope	1.02 (0.69–1.35)	0.90 (0.71–1.09)
Methysergide	pA_2	5.29 (4.95–5.63)	8.49 (7.85–9.14)
	Slope	1.20 (0.74–1.66)	0.78 (0.51–1.05)

Each value is the mean (95% confidence limits) of 4–8 separate estimates. Tissues exposed to antagonists for 30 min before agonist concentration-effect curves re-determined.

Table 3 Comparison of the specific and non-specific blocking actions of phentolamine, pizotifen, cyproheptadine and methysergide in rabbit aortic strip

		Agonist dose-ratio at antagonist concentration (mol/l) of			
Antagonist	*Agonist*	1.0×10^{-6}	5.0×10^{-6}	1.0×10^{-5}	1.0×10^{-4}
Phentolamine	5-Hydroxytryptamine	—	—	11 (6–22)	92 (56–151)
	Potassium chloride	—	—	1.2 (0.8–1.8)	3.7 (2.6–5.5)
Pizotifen	Noradrenaline	4.0 (3.0–5.3)	25 (20–31)	50 (32–79)	—
	Potassium chloride	0.9 (0.8–1.1)	2.9 (2.5–3.3)	4.9 (3.5–6.6)	—
Cyproheptadine	Noradrenaline	7.4 (6.7–8.0)	42 (25–71)	96 (64–145)	—
	Potassium chloride	2.4 (1.8–3.1)	5.2 (3.7–7.3)	5.0 (3.4–7.2)	—
Methysergide	Noradrenaline	—	—	2.6 (1.7–3.9)	22 (14–33)
	Potassium chloride	—	—	0.7 (0.5–1.0)	0.9 (0.5–1.6)

Each value is the mean of 4–8 determinations (95% confidence limits). Tissues exposed to antagonists for 30 min before agonist concentration-effect curves re-determined. For each antagonist the potassium chloride blocking action is compared with the weaker of the two amine blocking actions.

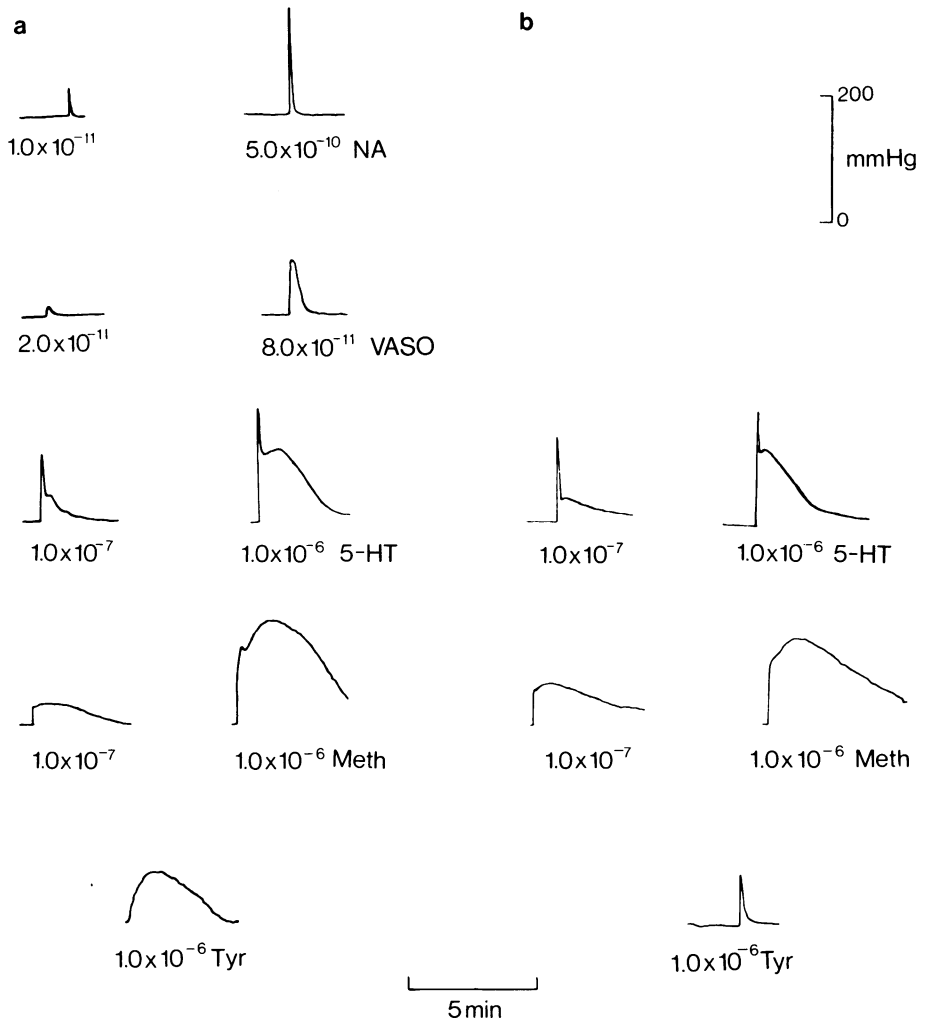

Figure 2 Rabbit isolated ear artery. Vasoconstrictor responses to bolus injections of noradrenaline (NA), vasopressin (VASO), 5-hydroxytryptamine (5-HT), methysergide (Meth) and tyramine (Tyr) (a) in normal arteries and (b) in arteries removed from reserpine-treated rabbits. Doses are expressed in mol. Note that reserpine-treatment converts the prolonged response to tyramine to a transient response but has no effect on the prolonged responses to 5-HT and methysergide.

previously exposed to noradrenaline and the phenomenon was further investigated. In each experiment two aortic strips were exposed to either noradrenaline (5.0×10^{-5} mol/l) or 5-HT (5.0×10^{-6} mol/l) and the other two strips to potassium chloride (3.0×10^{-2} mol/l), the concentrations of each agonist being selected to produce a virtually maximal response. The agonists were washed out after 10 min and the strips left for 90 min before concentration-effect curves to either methysergide (1.0×10^{-5}–1.0×10^{-4} mol/l) or histamine (1.0×10^{-8}–1.0×10^{-4} mol/l) were determined. The results are summarized in Table 1. Prior exposure to noradrenaline, but not to 5-HT, greatly reduced the maximal response to methysergide. In contrast, the maximal response to histamine was unaffected by prior exposure to noradrenaline.

Phentolamine (1.0×10^{-8}–1.0×10^{-4} mol/l), pizotifen (1.0×10^{-8}–1.0×10^{-4} mol/l), cyproheptadine (1.0×10^{-8}–1.0×10^{-4} mol/l) and vasopressin (0.8×10^{-10}–0.8×10^{-7} mol/l) were not agonists.

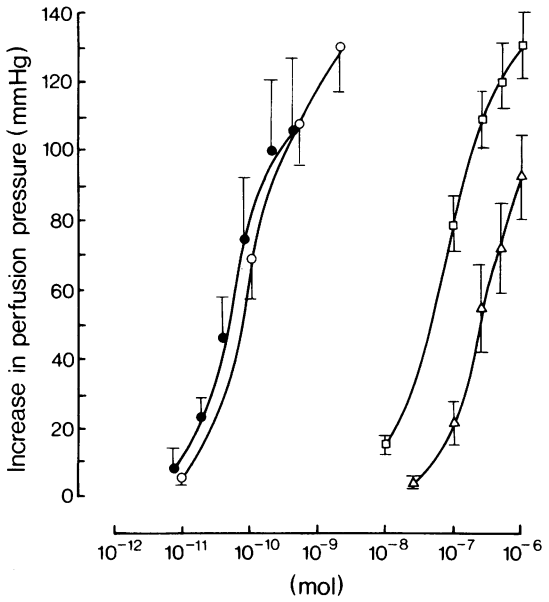

Figure 3 Rabbit isolated ear artery. Dose-effect curves for vasopressin (●), noradrenaline (○), 5-hydroxytryptamine (□) and methysergide (△). Each point is the mean response from 16 arteries obtained from eight animals. Vertical lines show s.e. mean. Note that 5-HT and methysergide are considerably less potent than noradrenaline.

Antagonists Methysergide, pizotifen, cyproheptadine and phentolamine were investigated as antagonists of 5-HT and noradrenaline. The results are summarized in Table 2. Each caused parallel concentration-dependent shifts to the right in the 5-HT and noradrenaline concentration-effect curves. The Schild plots were linear with slopes not significantly different from unity. These findings suggest competitive antagonism in each case.

For each antagonist there was a clear difference in potency against 5-HT and noradrenaline (Table 2). Methysergide, pizotifen and cyproheptadine were potent 5-HT antagonists and much weaker noradrenaline antagonists. Phentolamine had the opposite profile of selectivity.

The effect of these antagonists on contractile responses to potassium chloride was also examined in order to distinguish between specific and any non-specific blocking activity. The results, summarized in Table 3, show that over the concentration ranges tested these antagonists possess little non-specific blocking activity.

The effects of antagonists on the small contractile responses to methysergide (1.0×10^{-5}–1.0×10^{-4} mol/l) were also investigated. These responses were abolished by phentolamine at a concentration (1.0×10^{-6} mol/l) which antagonized noradrenaline with little or no effect on 5-HT, but were only slightly antagonized (methysergide dose-ratio of 2 to 3, $n=4$) by pizotifen at a concentration (1.0×10^{-7} mol/l) which produced a dose-ratio of about 400 against 5-HT.

Rabbit isolated ear artery

Agonists Individual vasoconstrictor responses are shown in Figure 2 and dose-response curves in Figure 3.

Vasopressin (8.0×10^{-12}–4.0×10^{-10} mol) produced prolonged responses, usually monophasic but occasionally biphasic, with a maximal response of 106 ± 21 mmHg (mean ± s.e. mean, $n=6$).

Noradrenaline (1.0×10^{-11}–2.0×10^{-9} mol) produced transient monophasic responses with a maximal or near-maximal response of 130 ± 13 mmHg ($n=16$).

Responses to 5-HT (1.0×10^{-8}–1.0×10^{-6} mol) were prolonged and biphasic, with the initial phase consistently larger but of shorter duration than the second phase. Results have been presented only for the initial phase. 5-HT produced a near-maximal response of 131 ± 9 mmHg ($n=16$) at 1.0×10^{-6} mol; it was about 700 times less potent than noradrenaline, in contrast to the finding in the aorta where these two agonists were similar in potency.

Methysergide was a better agonist in the ear artery than in the aorta. The responses were prolonged and often two phases could be distinguished, an initial phase of short duration and a prolonged and slightly larger second phase. Results have been presented only for the second phase. At 1.0×10^{-6} mol, the highest dose that could be tested, methysergide produced a response of 93 ± 12 mmHg ($n=16$). Methysergide was about 6 times less potent than 5-HT and about 4500 times less potent than noradrenaline. The sensitivity of the ear artery to methysergide was not reduced by prior exposure to noradrenaline, in contrast to our findings in the aorta.

In order to determine the concentration-range over which methysergide was an agonist, a second series of experiments was carried out in which the drug was perfused intraluminally in graded concentrations instead of being given by bolus injection. In four arteries, threshold vasoconstriction (5% of the maximum response to noradrenaline) occurred at 1.0×10^{-6} mol/l and maximum vasoconstriction (76% of the maximum response to noradrenaline) at 1.0×10^{-4} mol/l.

Experiments were carried out in arteries from reserpine-treated rabbits to determine whether part of the response to 5-HT or methysergide was mediated through release of endogenous noradrenaline. Responses to 5-HT and methysergide were unchanged

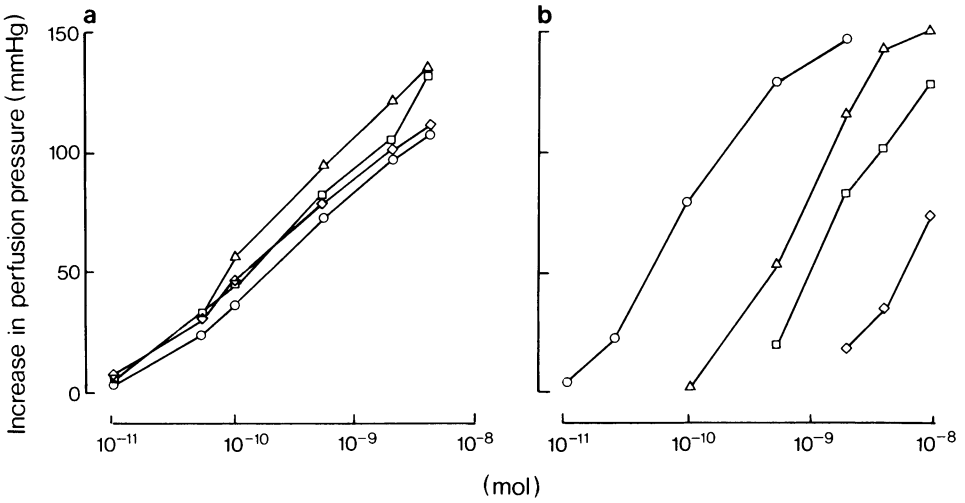

Figure 4 Rabbit isolated ear artery. Dose-effect curves to noradrenaline (a) in a control artery perfused with Krebs alone and (b) in a test artery perfused with Krebs plus pizotifen. In each artery the first (○) second (△), third (□) and fourth (◇) dose-effect curves were determined at intervals of about 30 minutes. Pizotifen was perfused at 2.0×10^{-6}, 4.0×10^{-6} and 1.6×10^{-5} mol/l for 30 min prior to the second, third and fourth dose-effect curves respectively. The dose-ratios calculated for successive dose-effect curves in the control artery were 0.4, 0.7 and 0.7 and in the test artery 7.7, 18.9 and 112. When corrections were made for changes in sensitivity in the control artery the dose-ratios in the test artery became 19.9, 29.0 and 153. These values gave a Schild plot with a pA_2 of 6.88 and a slope of 1.03.

in these preparations (Figure 2). In contrast, the prolonged response to tyramine (1.0×10^{-6} mol) obtained in arteries from untreated rabbits was converted to a transient response in arteries from reserpinized rabbits (Figure 2).

Phentolamine (1.0×10^{-6} mol) and cyproheptadine (1.0×10^{-6} mol) had no vasoconstrictor activity. Pizotifen (1.0×10^{-6} mol) produced a transient vasoconstriction (30–45 mmHg) after the first but not after subsequent doses.

Antagonists Phentolamine, pizotifen and cyproheptadine antagonized vasoconstrictor responses to 5-HT, noradrenaline and methysergide. In every case, agonist dose-response curves were displaced to the right by the antagonist in a parallel, concentration-dependent manner (for example see Figure 4). Schild plots were linear with slopes not significantly different from unity. As in the aorta, these findings indicate competitive antagonism. The pA_2 values for each antagonist against 5-HT, noradrenaline and methysergide were virtually identical, which contrasts with the results in the aorta. These results are summarized in Table 4.

We also noted that the two phases of the response to 5-HT were antagonized equally by phentolamine or pizotifen. The pA_2 and slope values for phentolamine were 8.41 (7.99–8.83) and 1.02 (0.54–1.50) against the initial phase (see Table 4) and 8.34 (8.10–8.58) and 0.86 (0.62–1.11) against the second phase; the corresponding values for pizotifen were 6.79 (6.39–7.18) and 1.08 (0.77–1.39) against the initial phase (see Table 4) and 6.89 (5.70–8.08) and 1.25 (0.65–1.84) against the second phase. Similarly, the two phases of the response to methysergide, when distinguishable, were antagonized equally by phentolamine or pizotifen.

Experiments were carried out in the ear artery, as in the aorta, to determine whether the antagonists possessed any non-specific blocking activity. The results, summarized in Table 5, show that phentolamine, pizotifen and cyproheptadine had little or no effect against vasopressin-induced vasoconstriction in concentrations that strongly antagonized noradrenaline. We conclude that these antagonists possess little or no non-specific blocking action over the concentration ranges tested.

The ability of methysergide to antagonize constrictor responses to noradrenaline, 5-HT and vasopressin was also investigated. At 1.0×10^{-6} mol/l, methysergide sensitized the ear artery to noradrenaline and vasopressin but not to 5-HT (Table 6). Higher concentrations of methysergide (1.0×10^{-5}–1.0×10^{-4} mol/l) antagonized responses to noradrenaline and 5-HT but had little or no effect on those to vasopressin. The combination of sensitizing, vaso-

Table 4 Interactions between agonists and antagonists in rabbit isolated ear artery

Antagonist		*Agonist*		
		Noradrenaline	*5-Hydroxytryptamine*	*Methysergide*
Phentolamine	pA_2	8.09 (7.54–8.64)	8.41 (7.99–8.83)	8.25 (7.96–8.54)
	Slope	1.12 (0.57–1.67)	1.02 (0.54–1.50)	0.88 (0.75–1.02)
Pizotifen	pA_2	6.57 (6.15–6.98)	6.79 (6.39–7.18)	6.95 (6.48–7.42)
	Slope	1.00 (0.76–1.24)	1.08 (0.77–1.39)	1.07 (0.45–1.69)
Cyproheptadine	pA_2	6.80 (6.41–7.20)	7.02 (6.58–7.66)	7.04 (6.59–7.49)
	Slope	1.10 (0.51–1.70)	0.87 (0.71–1.03)	1.25 (0.57–1.52)

Each value is the mean (95% confidence limits) of 4–8 separate determinations. Tissues exposed to antagonists for 30 min before agonist dose-effect curves re-determined.

Table 5 Comparison of the specific and non-specific blocking actions of phentolamine, pizotifen and cyproheptadine in rabbit isolated ear artery

Antagonist	*Agonist*	*Agonist dose-ratio at antagonist concentration (mol/l) of*			
		1.0×10^{-8}	1.0×10^{-7}	1.0×10^{-6}	4.0×10^{-6}
Phentolamine	Noradrenaline	2.7 (1.8–3.9)	9.0 (4.3–19.2)	—	—
	Vasopressin	1.2 (0.8–1.7)	1.9 (1.0–3.3)	—	—
Pizotifen	Noradrenaline	—	—	4.4 (2.1–9.3)	19 (9–42)
	Vasopressin	—	—	0.9 (0.6–1.2)	1.1 (0.8–1.7)
Cyproheptadine	Noradrenaline	—	—	7.7 (5.4–10.9)	34 (13–85)
	Vasopressin	—	—	2.5 (1.3–4.5)	3.7 (2.2–6.3)

Each value is the mean of 4–8 determinations (95% confidence limits). Tissues exposed to antagonists for 30 min before agonist dose-effect curves re-determined.

Table 6 Effects of methysergide on responses to noradrenaline, 5-hydroxytryptamine and vasopressin in rabbit isolated ear artery

	Agonist dose-ratio at methysergide concentration (mol/l) of		
	1.0×10^{-6}	1.0×10^{-5}	1.0×10^{-4}
Noradrenaline	0.24 (0.15–0.36)	3.7 (1.6–8.2)	89 (29–279)
5-Hydroxytryptamine	1.2 (0.62–2.3)	29 (10–83)	343 (67–1758)
Vasopressin	0.36 (0.22–0.60)	0.86 (0.23–3.4)	3.6 (1.3–10.1)

Each value is the mean of 4 determinations (95% confidence limits). Dose-ratios greater than unity indicate antagonism, those less than unity indicate sensitization. Tissues exposed to methysergide for 30 min before agonist dose-effect curves re-determined.

constrictor and blocking properties present in methysergide makes it difficult to quantify its antagonist potency. However, it is worth noting that methysergide was a much weaker antagonist of 5-HT in the ear artery than in aorta, but was similar in potency against noradrenaline in these two preparations.

Discussion

Monophasic and biphasic responses to agonists in the ear artery

Noradrenaline produced transient monophasic responses in the ear artery, whereas 5-HT and methysergide produced prolonged biphasic responses. Because these prolonged responses resembled those obtained with tyramine and because 5-HT has been shown to act by releasing endogenous noradrenaline in some tissues (Innes, 1962), we sought evidence of such an action in the ear artery. Responses to both 5-HT and methysergide were unchanged in arteries from reserpine-treated rabbits, as found by Fozard (1973), indicating that neither drug was acting by releasing noradrenaline. The biphasic responses to 5-HT and methysergide probably occur because of the method of administration of the agonists (Apperley & Humphrey, 1976), the initial phase being caused by diffusion of drug through the intraluminal surface to the smooth muscle cells immediately after injection, and the second phase by diffusion through the extraluminal surface after the drug bolus has traversed the length of the artery and entered the fluid in which the artery is immersed. The response to noradrenaline is monophasic because noradrenaline is rapidly inactivated, mainly by $Uptake_1$, during diffusion through the extraluminal surface (de la Lande, Frewin & Waterson, 1967).

Receptors for noradrenaline and 5-hydroxytryptamine in aorta and ear artery

The data obtained from this study provide new information on the distribution of (noradrenaline) α-receptors and 5-HT-receptors in the rabbit aorta and ear artery. We used antagonists to classify the receptors involved and assumed that the pA_2 value corresponds to the affinity constant (Furchgott, 1972). This seems to be a reasonable assumption since, invariably, the noradrenaline and 5-HT dose-response curves were displaced to the right in a parallel manner by the antagonists, the Schild plots had slopes not significantly different from unity, and the possibility of non-specific antagonism had been eliminated.

The results show that noradrenaline and 5-HT act on different receptors in rabbit aorta. Phentolamine was a potent antagonist at the (noradrenaline) α-receptors, and methysergide, pizotifen and cyproheptadine were potent antagonists at the 5-HT-receptors. The pA_2 values obtained with these antagonists agree well with those obtained in other tissues containing α-receptors (Gulati, Parikh & Umar, 1968; Clineschmidt, Geller, Govier & Sjoerdsma, 1970; Patil, Fudge & Jacobwitz, 1972; Kennedy & Levy, 1974) and 5-HT-receptors (Gorlitz & Frey, 1973). Phentolamine was also a weak antagonist at 5-HT-receptors, and methysergide, pizotifen and cyproheptadine weak antagonists at α-receptors.

The results in the ear artery were strikingly different from those in the aorta. The pA_2 values for each of the four antagonists in the ear artery were virtually identical against noradrenaline and 5-HT and this indicates that the agonists act on a common receptor to produce vasoconstriction. Furthermore, since the pA_2 values for the four antagonists in the ear artery were close to those obtained on α-receptors in the aorta, we conclude that the common receptor is an α-receptor and that the ear artery does not contain specific 5-HT-receptors. These conclusions are consistent with the finding that noradrenaline and 5-HT had similar potencies at their specific receptors in the aorta but that 5-HT was about 700 times less potent than noradrenaline in the ear artery.

Analysis of the actions of methysergide

This insight into the distribution of α-receptors and 5-HT-receptors in rabbit aorta and ear artery helps in the interpretation of the various actions of methysergide. As described above, methysergide was a potent antagonist at 5-HT-receptors and a weaker antagonist at α-receptors in rabbit aorta. Methysergide also possesses very weak agonistic action in this preparation and this appears to be mediated through α-receptors since it was antagonized by phentolamine at concentrations which did not affect 5-HT.

The finding that the constrictor response to methysergide was even weaker than normal in strips previously incubated with noradrenaline, but was unchanged in strips previously incubated with 5-HT, is consistent with an action at α-receptors. It is possible that the prior exposure to noradrenaline causes desensitization of α-receptors which reduces the response to methysergide, a weak partial agonist. Thus, methysergide acts as a potent antagonist at 5-HT-receptors and as a weak partial agonist at α-receptors in rabbit aorta.

Methysergide was also a partial agonist compared to noradrenaline in the ear artery. Its vasoconstrictor action was antagonized by phentolamine, pizotifen and cyproheptadine, with pA_2 values virtually identical to those obtained against noradrenaline and 5-HT. This shows that methysergide activates α-

receptors in this tissue. Fozard (1973) originally reported that the action of methysergide was unaffected by phentolamine, in direct contrast to our results, but this major discrepancy has now been resolved since Fozard (1976) recently reported that phentolamine does antagonize methysergide. Methysergide antagonized the actions of the more potent α-receptor agonists noradrenaline and 5-HT, as would be expected with a partial agonist. Thus, methysergide acts as a partial agonist at α-receptors in the ear artery; it is only a weak antagonist of 5-HT because this preparation does not contain specific 5-HT-receptors.

Methysergide was a better agonist in the ear artery than in the aorta. A possible explanation is that the relationship between stimulus and response is different in the two tissues. The potency of a partial agonist would be particularly susceptible to a difference of this sort (Furchgott, 1972; Jenkinson, 1973). Hence, if the stimulus required for the same fractional response was higher in the aorta than in the ear artery then the activity of a partial agonist such as methysergide would be less pronounced in the former preparation.

Our results confirm previous reports (de la Lande, Cannell & Waterson, 1966; Fozard, 1973; Carrol, Ebeling & Glover, 1974) that methysergide also sensitizes the rabbit ear artery to the vasoconstrictor action of noradrenaline. Fozard (1973) has suggested that sensitization results from sub-threshold vasoconstriction achieving threshold or greater levels in the presence of other vasoconstrictor agents. However, this explanation is unlikely to apply to the sensitization of the ear artery to noradrenaline by methysergide if, as our results show, these two agonists act at the same receptor. From classical drug receptor theory it can be predicted that a partial agonist such as methysergide would antagonize the effect of a full agonist such as noradrenaline (see Barlow, 1964; Ariens & Simonis, 1964). An alternative explanation is that methysergide blocks noradrenaline uptake; an amine-uptake blocking action has been reported in cat spleen (Owen, Herd, Kalberer, Pacha & Salzmann, 1971). On the other hand, there is no theoretical objection to the idea that potentiation of vasopressin responses by methysergide results from sub-threshold vasoconstriction, since these two agents act at different sites.

Similarity between α-receptors and 5-hydroxytryptamine-receptors

Our results demonstrate that 5-HT and 5-HT-receptor blocking drugs can act at α-receptors and, conversely, that an α-receptor blocking drug, phentolamine, can act at 5-HT-receptors. It was not possible in the two preparations examined to determine whether noradrenaline can act at 5-HT-receptors but Vane (1960) has shown that certain α-receptor agonists contract rat stomach strip by an action at 5-HT-receptors. This cross reactivity is in accord with a large body of evidence (see Fozard, 1973 for references) and indicates that the two types of receptor, although distinct entities, have features in common. This can give rise to problems of interpretation unless detailed quantitative data are obtained. For example, Carroll *et al.* (1974), in a semi-quantitative study, showed that the vasoconstrictor action of methysergide in rabbit ear artery was abolished by pizotifen and concluded that methysergide acts at 5-HT-receptors. Our analysis shows that this interaction actually takes place at α-receptors. Indeed, it is ironic that the rabbit ear artery, which is frequently used for the study of 5-HT agonist-antagonist interactions, does not contain specific 5-HT-receptors.

Relevance to migraine therapy

Since three of the drugs examined in this study, namely methysergide, pizotifen and cyproheptadine, are used in the treatment of migraine, it is appropriate to consider whether our results have any bearing on their mode of action. Pizotifen and cyproheptadine were more potent than methysergide as 5-HT-receptor blocking drugs in rabbit aorta yet are less effective in the treatment of migraine (Lance, Anthony & Somerville, 1970). These findings are in accord with a growing concensus of opinion that antagonism of the vascular actions of 5-HT may not be relevant to the effectiveness of anti-migraine drugs. Fozard (1975) suggested that it might well be the sedative properties of cyproheptadine and the combined sedative and anti-depressant properties of pizotifen, rather than their anti-5-HT properties, that benefit the migraine sufferer because they alleviate the stress symptoms which may trigger an attack (Lance, 1973). The ability to mimic the vasoconstrictor action of 5-HT is now considered to be an important property of specific anti-migraine drugs (Saxena, 1972). Methysergide effectively mimics the vasoconstrictor action of 5-HT in the rabbit ear artery and initially we thought that this might be the basis of its therapeutic effect. However, we subsequently rejected this hypothesis because this action of methysergide, in the ear artery at least, occurs at higher concentrations than could be achieved after administration of therapeutic doses of the drug (estimated maximum blood concentration of about 10^{-7} mol/litre). Thus, it does not seem that the α-adrenoceptor stimulation produced by methysergide in the ear artery can explain its therapeutic action in migraine. However, it may be that the ear artery is unrepresentative of the external carotid vasculature in general or that the rabbit differs from other species. For example, we have recently shown that very low concentrations of methysergide (threshold 10^{-8} mol/l) constrict the dog isolated ear artery (Apperley, Humphrey & Levy, unpublished observations), and Saxena (1974) has shown that low doses of methy-

sergide selectively increase the resistance of the dog carotid vascular bed *in vivo*. We are currently investigating the mechanisms involved in these actions of methysergide.

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References

APPERLEY, EIRA & HUMPHREY, P.P.A. (1976). A proposed mechanism for the biphasic vasoconstrictor response to serotonin and methysergide in the rabbit ear artery. *J. Pharm. Pharmac.* In press.

APPERLEY, EIRA, HUMPHREY, P.P.A. & LEVY, G.P. (1974). Receptors for 5-hydroxytryptamine and noradrenaline in rabbit aorta and central ear artery. *Br. J. Pharmac.*, **52**, 131–132P.

ARIENS, E.J. & SIMONIS, A.N. (1964). Drug receptor interaction: interaction of one or more drugs with one receptor system. In *Molecular Pharmacology*, vol. 1, pp. 119–286, ed. Ariens, E.J., New York: Academic Press.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BARLOW, R.B. (1964). *Introduction to Chemical Pharmacology*, pp. 1–26, 2nd edition. London: Methuen.

CARROLL, P.R., EBELING, P.W. & GLOVER, W.E. (1974). The responses of the human temporal and rabbit ear artery to 5-hydroxytryptamine and some of its antagonists. *Aust. J. exp. Biol. med. Sci.*, **52**, 813–823.

CLINESCHMIDT, B.V., GELLER, R.G., GOVIER, W.C. & SJOERDSMA, A. (1970). Reactivity to norepinephrine and nature of the alpha adrenergic receptor in vascular smooth muscle of a genetically hypertensive rat. *Eur. J. Pharmac.*, **10**, 45–50.

FARMER, J.B. (1966). The biphasic response of an isolated artery to tyramine. *Br. J. Pharmac. Chemother.*, **28**, 340–347.

FOZARD, J.R. (1973). Drug interactions on an isolated artery. In *Background to Migraine*, **5**, pp. 150–169. ed. Cumings, J.N., London: Heinemann.

FOZARD, J.R. (1975). The animal pharmacology of drugs used in the treatment of migraine. *J. Pharm. Pharmac.*, **27**, 297–321.

FOZARD, J.R. (1976). Comparative effects of four migraine prophylactic drugs on an isolated extracranial artery. *Eur. J. Pharmac.*, **36**, 127–139.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines, Handb. exp. Pharmac.* N.S. Vol. 33, pp. 288–289. ed. Blaschko, H. & Muscholl, E. Berlin and Heidelberg: Springer-Verlag.

FURCHGOTT, R.F. & BHADRAKOM, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmac. exp. Ther.*, **108**, 129–143.

GORLITZ, B.D. & FREY, H.H. (1973). Comparison of the blocking effects of antagonists of adrenaline and 5-hydroxytryptamine on their mutual receptors. *J. Pharm. Pharmac.*, **25**, 651–653.

GULATI, O.D., PARIKH, H.M. & UMAR, M.S. (1968). Receptors for noradrenaline and histamine in the rabbit's posterior vena cava. *Br. J. Pharmac. Chemother.*, **32**, 87–95.

INNES, I.R. (1962). An action of 5-hydroxytryptamine on adrenaline receptors. *Br. J. Pharmac. Chemother.*, **19**, 427–441.

JENKINSON, D.H. (1973). Classification and properties of peripheral adrenergic receptors. *Br. med. Bull.*, **29**, 142–147.

KENNEDY, I. & LEVY, G.P. (1974). Combined α and β-adrenoceptor blocking drug, AH 5158: further studies on specificity and α-adrenoceptor blocking action. *Proc. Aust. physiol. pharmac. Soc.*, **5**, No. 2, 216–217.

LANCE, J.W. (1973). The pathogenesis of migraine. In *Mechanism and Management of Headache*, pp. 113–133, 2nd edition. London: Butterworths.

LANCE, J.W., ANTHONY, M. & HINTERBERGER, H. (1970). Migraine and 5-hydroxytryptamine. In *Background to Migraine*, pp. 155–161. London: William Heinemann Medical Books.

LANCE, J.W., ANTHONY, M. & SOMERVILLE, B. (1970). Comparative trial of serotonin antagonists in the management of migraine. *Br. med. J.*, **2**, 327–330.

DE LA LANDE, I.S., CANNELL, V.A. & WATERSON, J.G. (1966). The interaction of serotonin and noradrenaline on the perfused artery. *Br. J. Pharmac. Chemother.*, **28**, 255–272.

DE LA LANDE, I.S., FREWIN, D. & WATERSON, J.G. (1967). The influence of sympathetic innervation on vascular sensitivity to noradrenaline. *Br. J. Pharmac. Chemother.*, **31**, 82–93.

DE LA LANDE, I.S. & RAND, M.J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.

OWEN, D.A.A., HERD, J.K., KALBERER, F., PACHA, W. & SALZMANN, R. (1971). The influence of ergotamine and methysergide on the storage of biogenic amines. *Proc. Int. Headache Symposium*, Elsinore, Denmark. pp. 153–161.

PATIL, P.N., FUDGE, K. & JACOBWITZ, D. (1972). Steric aspects of adrenergic drugs. XVIII α-adrenergic receptors of mammalian aorta. *Eur. J. Pharmac.*, **19**, 79–87.

SAXENA, P.R. (1972). The effects of antimigraine drugs on the vascular responses by 5-hydroxytryptamine and related biogenic substances on the external carotid bed of dogs: possible pharmacological implications to their antimigraine action. *Headache*, **12**, 44–54.

SAXENA, P.R. (1974). Selective vasoconstriction in carotid vascular bed by methysergide: possible relevance to its antimigraine effect. *Eur. J. Pharmac.*, **27**, 99–105.

VANE, J.R. (1960). The actions of sympathomimetic amines on tryptamine receptors. In *Adrenergic Mechanisms*, pp. 356–372. ed. Vane, J.R., Wolstenholme, A.E.W. & O'Connor, M. London: Churchill.

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