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RECEPTOR MECHANISMS FOR 5-HYDROXYTRYPTAMINE IN RABBIT ARTERIES

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1 Previous investigations into the vascular actions of biogenic amines implicated in migraine have shown that the contractile effects of both 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in the rabbit ear artery are mediated by a direct sympathomimetic action at α-adrenoceptors, while in the rabbit aorta, 5-HT and NA act on pharmacologically distinct receptors. The purpose of the present investigation was to determine whether the absence of 5-HT receptors in rabbit ear arteries is characteristic of distributing arteries in general, or is confined to particular regional circulations.

2 Agonist-antagonist interactions were studied in various rabbit vascular preparations (common carotid, external carotid and femoral arterial strips, and perfused ear arteries) by determining pA_2 values for pizotifen and phentolamine against 5-HT- and NA-induced contractile responses.

3 In common carotid and femoral arteries, pizotifen was a potent competitive antagonist of 5-HT, but weak against NA. The converse applied to phentolamine. In external carotid and ear arteries, pizotifen was a weak competitive antagonist of both 5-HT and NA, whereas phentolamine was a potent competitive antagonist of both. Cocaine did not influence pA_2 values against NA.

4 5-HT and NA were of similar potency in common carotid and femoral arteries, but 5-HT was much less potent than NA in external carotid and ear arteries.

5 The results indicate that rabbit common carotid and femoral arteries contain both D-type 5-HT receptors and α-adrenoceptors, as does the aorta. However, external carotid arteries, like ear arteries, do not contain specific 5-HT receptors. The action of 5-HT in the external carotid artery is mediated by α-adrenoceptors; this is a direct sympathomimetic action since it was not inhibited by cocaine or reserpine-pretreatment.

6 The absence of 5-HT receptors in the rabbit extracranial circulation may limit the usefulness of this species as a model for research relating to migraine.

Introduction

The receptor mechanisms involved in the vasoconstrictor actions of 5-hydroxytryptamine (5-HT) have been examined by Apperley, Humphrey & Levy (1976). These authors conducted quantitative analyses of functional receptor interactions between agonists and antagonists in rabbit ear artery and aorta preparations, to characterize the receptors involved. Fozard (1976) described similar investigations in rabbit ear artery preparations. In the aorta, both D-type 5-HT receptors (Gyermek, 1966) and α-adrenoceptors were shown to be present. In the ear artery, these authors demonstrated that there are no specific 5-HT receptors, and that the constrictor action of 5-HT is mediated by a direct action at α-adrenoceptors.

A question therefore arises as to whether in the rabbit, the absence of 5-HT receptors is a peculiarity of distributing arteries in general, or is a property of particular regions of the circulation. Thus the present investigations are concerned with examining 5-HT vascular mechanisms in other rabbit vessels, namely common carotid, external carotid and femoral arteries, as well as rabbit ear arteries. The study was conducted using similar techniques to those used by Apperley *et al.* (1976) and Fozard (1976). Some of the results have been described at a meeting of the Australian Physiological & Pharmacological Society (Mylecharane, French & Black, 1979).

Findings in studies of this kind may be of particular relevance to migraine, in that 5-HT has been implicated in the cranial vascular disturbances of this disease (see Spira, Mylecharane & Lance, 1976), and many of the 5-HT antagonists used in such receptor mechanism studies (e.g. methysergide, pizotifen and cyproheptadine) are used in its therapy (see Fozard, 1975).

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Methods

Common carotid, external carotid and femoral arterial strip experiments

Preparations New Zealand white rabbits of either sex weighing 1.5 to 3 kg were killed by a blow to the back of the head. Segments of common carotid artery (15 to 20 mm in length), external carotid artery (8 to 12 mm) and femoral artery (15 to 20 mm) were excised. Close spiral strips were cut as described by Furchgott & Bhadrakom (1953). Common carotid and femoral artery segments yielded strips of approximately 15 to 25 mm in length and 1.5 to 2 mm in width, while external carotid artery strips were approximately 10 to 15 mm long and 1 to 1.5 mm wide. Two pairs of arteries (yielding 4 preparations) were usually removed from one rabbit.

The arterial strips were suspended in 10 ml organ baths containing Krebs-Henseleit solution (composition, mmol/l: Na^+ 143.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.1, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, HCO_3^- 25.0 and (+)-glucose 11.1) maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2. A load of 0.5 g was applied to each strip, as this loading was shown to be optimal in preliminary experiments. Changes in length of the strip were measured with a Harvard isotonic transducer (model 386) connected to a Heathkit potentiometric chart recorder (model IR-18M). During the initial equilibration period, contractile responses to a submaximal concentration of the agonist to be tested in that experiment were elicited at intervals of 20 to 30 min. An equilibration period of 2 to 3 h was necessary for the responses to become reproducible.

Agonist-antagonist interactions In this series of experiments, following equilibration, initial cumulative concentration-response relationships to one of the agonists, 5-HT or noradrenaline (NA), were obtained in parallel experiments on paired preparations from the same animal. When a maximal response was reached, the tissue was washed at 5 min intervals until baseline tension was re-established, usually within 45 to 90 min. In one of the paired arterial strip preparations, the concentration-response relationships for the agonist were then re-obtained in the presence of two successively increasing concentrations of an antagonist (pizotifen or phentolamine). In the other paired preparation which served as a control, the agonist concentration-response relationships were repeated at corresponding times, but in the absence of antagonist. Preliminary experiments in the absence of antagonist showed that there were small spontaneous changes in agonist sensitivity during the course of an experiment. However, the changes within paired arterial strip preparations from the same animal were highly consistent. An antagonist contact time of 30 min was allowed before re-testing the effects of agonist, since it was shown in initial experiments that the blocking effects of phentolamine and pizotifen reached equilibrium within this time.

In each preparation, all responses to agonist were expressed as percentages of the initial maximal response, and were used to plot log concentration-response curves. The concentrations of agonist producing 50% of the maximal response were determined in the initial curves and were used to calculate agonist pD_2 values. To assess the activity of the antagonists, agonist dose-ratios were determined from the agonist concentrations producing 50% of the maximal response in each curve. Correction was made for any spontaneous changes in agonist sensitivity by dividing dose-ratios obtained in the preparations exposed to antagonist by the equivalent dose-ratios obtained in the paired control preparations. In each experiment, using the corrected dose-ratio values, log (dose-ratio − 1) was plotted against log antagonist concentration (mol/l), and a pA_2 and slope were determined, as described by Arunlakshana & Schild (1959). Each agonist-antagonist interaction was studied in at least 4 experiments. In some of the experiments in which NA was the agonist, the effect of blockade of its neuronal uptake was assessed by including cocaine (10^{-5} mol/l) in the Krebs-Henseleit solution used throughout the experiment.

Assessment of indirect sympathomimetic activity In a further series of experiments, after equilibration, cumulative concentration-response curves for 5-HT and NA, and responses to a bolus dose of tyramine (10^{-4} mol/l) were obtained in each preparation. The order in which these agonists were administered varied. In some of these experiments, the responses were elicited in the presence of cocaine (10^{-5} mol/l) or in arteries removed from rabbits treated 18 to 24 h beforehand with reserpine (1 mg/kg i.p.). In each preparation, all responses were expressed as percentages of the maximal response to NA, and log concentration-response curves for 5-HT and NA were plotted.

Perfused ear artery experiments

The experimental method used was similar to that described by de la Lande & Rand (1965).

In rabbits killed as described for the arterial strip experiments, the central ear arteries were cleared of connective tissue, cannulated proximally and excised. Each singly cannulated artery (20 to 30 mm in length) was suspended in an organ bath and perfused with Krebs-Henseleit solution (maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2) by means of a Rotaline peristaltic pump at a constant

flow rate of 8 ml/min. Intraluminal pressure was recorded by means of a pressure transducer (Statham P23 Db) connected just proximal to the artery. The pressure transducer was attached to a Grass polygraph recorder (Model P79). Solutions of drugs (0.025 to 0.2 ml) were injected as bolus doses through rubber tubing into the perfusing fluid just before its passage through the artery. Any resultant vasoconstriction produced a rise in perfusion pressure which could be readily distinguished from the small, transient injection artefact caused by rapid addition of small volumes of fluid to the perfusing solution. Initially, arteries were perfused with Krebs-Henseleit solution for a period of not less than 30 min, during which time pressure stabilized to approximately 30 mmHg. Then, using a submaximal dose of one of the agonists, 5-HT or NA, contractile responses were obtained repeatedly at regular intervals of not less than 3 min. Perfusion pressure was always allowed to return to baseline values between injections. Responses to NA became reproducible within 15 min. Responses to 5-HT were more variable, reproducible responses being obtained only after 30 to 60 min. After responses to the test dose of agonist had stabilized, bolus doses of the agonist were administered so that dose-response relationships could be established.

As in the arterial strip preparation experiments, paired experiments were always used, with one artery being tested with antagonist (pizotifen or phentolamine) and the other serving as a control, so that spontaneous changes in sensitivity could be taken into account. It was found that a stable degree of blockade occurred within 30 min, thus an antagonist contact time of 30 min was always used. Agonist dose-response relationships in the absence or presence of antagonist were established in replicate two to four times. In each experiment, two or three concentrations of antagonist were tested.

Data from each preparation were plotted as log dose of agonist (mol) against mean replicate response (mmHg). To assess the activity of the antagonists, agonist dose-ratios were determined from the agonist doses producing an arbitrarily determined response for each preparation which was on the linear, parallel portion of the log dose-response curves, since maximal responses are difficult to obtain. In each experiment, dose-ratios were corrected for spontaneous changes in sensitivity, and pA_2 values and slopes of the relationship between log (dose-ratio − 1) and log antagonist concentration (mol/l) were determined, as described for the arterial strip preparation experiments.

Statistical analysis

Results were tested for significance using Student's *t* test (2-tailed) for correlated or non-correlated data as appropriate. In the latter case, when the distributions of the test samples were shown to be different using the variance ratio (F) test, the method of Cochran & Cox (1957) was used in the evaluation. Results were considered significant when $P < 0.05$.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate monohydrate, mol. wt. 405.4 (Sigma); (−)-noradrenaline hydrogen tartrate monohydrate, mol. wt. 337.3 (Sigma); tyramine hydrochloride, mol. wt. 173.6 (Sigma); cocaine hydrochloride, mol. wt. 339.8 (D.H.A.); pizotifen malate, mol. wt. 429.5 (Sandoz); phentolamine mesylate, mol. wt. 377.5 (Ciba); and reserpine hydrochloride (Sigma).

Solutions of 5-HT and NA were freshly prepared for each experiment by dissolving the salts in Krebs-Henseleit solution. Stable stock solutions of tyramine, cocaine, pizotifen and phentolamine in 0.9% w/v NaCl solution were freshly diluted for each experiment with Krebs-Henseleit solution. When NA was used, ascorbic acid (0.02 mg/ml) was always included in the Krebs-Henseleit solution and the NA solutions were stored in ice during the experiment. Concentrations are expressed as mol/l of the active moieties. Reserpine was dissolved in a vehicle comprising 10% w/v ascorbic acid in water.

Results

Agonist-antagonist interactions in arterial strip preparations

Common carotid and femoral arteries Cumulative concentration-response relationships in the absence of antagonist were obtained over the concentration ranges 2×10^{-8} to 10^{-4} mol/l of 5-HT and 10^{-7} to 10^{-4} mol/l of NA. Responses to each cumulative addition of agonist reached a plateau in 2 to 8 min in the case of 5-HT, and 2 to 5 min in the case of NA. The total times required to reach the maximal response ranged from 30 to 60 min for both 5-HT and NA. Figure 1 shows a trace of a typical cumulative concentration-response relationship for 5-HT in a common carotid artery preparation. Similar traces were obtained for 5-HT in femoral arteries. A trace of a cumulative concentration-response relationship for NA in a femoral artery preparation is illustrated in Figure 2, and is typical of those obtained in both femoral and common carotid arteries. Table 1 contains the means of the pD_2 values for 5-HT and NA determined from the initial log concentration-response curves for each agonist. The presence of

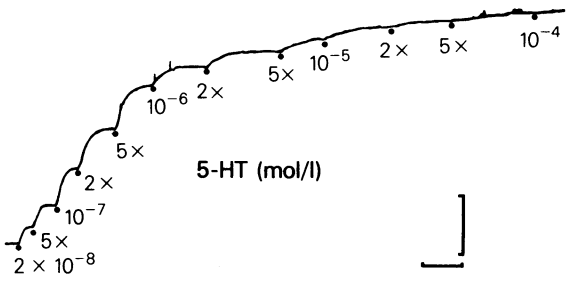

Figure 1 Trace of a cumulative concentration-response relationship for 5-hydroxytryptamine (5-HT) in a rabbit common carotid arterial strip preparation. 5-HT was added at ●; the concentrations shown represent the cumulative total concentration. The vertical scale represents an isotonic contraction equivalent to a standard change in load of 200 mg. The horizontal scale represents 5 min.

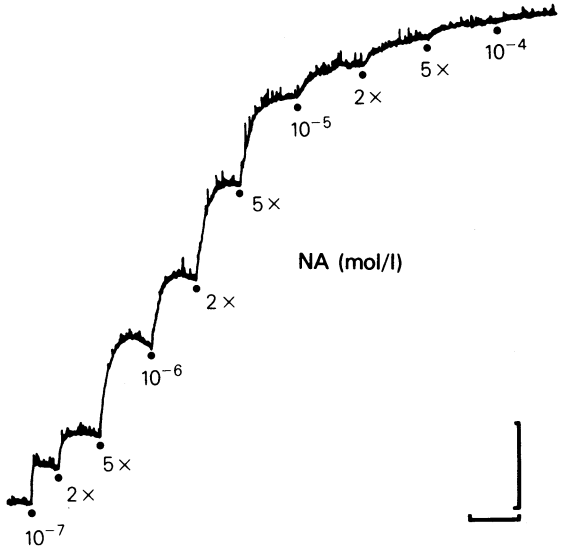

Figure 2 Trace of a cumulative concentration-response relationship for noradrenaline (NA) in a rabbit femoral arterial strip preparation, as in Figure 1.

cocaine did not produce any significant change in the NA pD_2 values in either artery. 5-HT was equipotent in the common carotid and femoral arteries, as was NA. It can also be seen that 5-HT and NA were of similar potency.

Pizotifen was used over a concentration range of 10^{-9} to 3×10^{-8} mol/l to antagonize 5-HT, and 10^{-6} to 10^{-5} mol/l against NA. Phentolamine was used at concentration ranges of 3×10^{-6} to 3×10^{-5} mol/l against 5-HT, and 10^{-7} to 10^{-6} mol/l against NA. Figure 3 illustrates log concentration-response curves obtained in a typical experiment. In the presence of antagonist, curves were shifted to the right in a parallel fashion, and there were no significant changes in maximal responses. In the paired control preparations, spontaneous changes in sensitivity occurred, resulting in shifts of curves to the left or right. The mean shifts were 0.3 and 0.2 log units to the left and right respectively in the case of 5-HT, and 0.1 and 0.3 log units to the left and right respectively in the case of NA. Slopes of the Arunlakshana & Schild (1959) plots of log (dose-ratio − 1) against log antagonist concentration did not differ significantly from unity. The means of the antagonist pA_2 values are given in Table 2. Cocaine did not produce any significant changes in the pA_2 values against NA. The pA_2 value for pizotifen against 5-HT was significantly different from those against NA, in both the common carotid and the femoral arterial strip preparations. Likewise, the pA_2 value for phentolamine against 5-HT differed significantly from those against NA in both of these arteries. Thus in the common carotid and femoral arteries, pizotifen was a potent antagonist of 5-HT, but weak as a NA antagonist. The converse was true for phentolamine.

External carotid arteries Responses to NA were similar to those obtained in common carotid and femoral arterial strip preparations. Mean pD_2 values are included in Table 1. As in the other arteries, cocaine did not alter pD_2 values for NA significantly. The external carotid artery pD_2 values for NA did not differ significantly from those in the common carotid or femoral arteries.

Table 1 pD_2 values for 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in arterial strip preparations from rabbits

Artery	*5-HT*	*NA*	*NA (with cocaine 10^{-5} mol/l)*
Common carotid	5.98 ± 0.12 (32)	5.84 ± 0.08 (31)	5.79 ± 0.07 (36)
Femoral	6.16 ± 0.14 (29)	5.90 ± 0.11 (36)	5.87 ± 0.10 (34)
External carotid	4.08 ± 0.05 (26)*	5.79 ± 0.08 (34)	5.72 ± 0.08 (34)

Each value is the mean ± s.e.mean (n) of pD_2 values determined from the initial concentration-response curves from all experiments.

*Approximate pD_2 value, since maximal response could not be elicited (see text); this value is significantly different from the 5-HT pD_2 values in the common carotid and femoral arteries ($P < 0.05$).

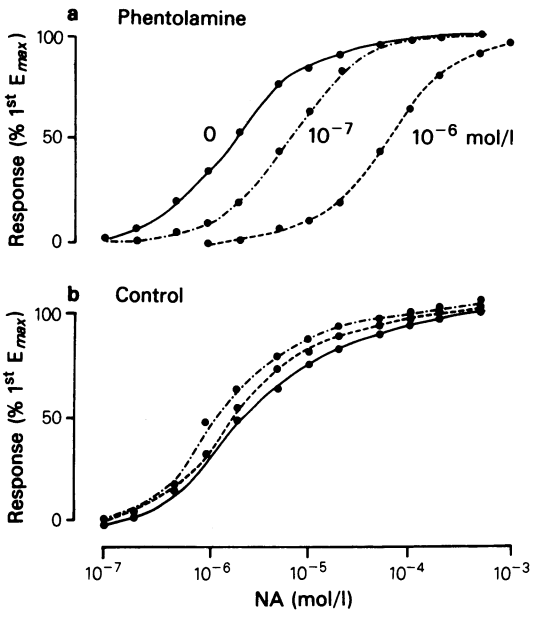

Figure 3 Log concentration-response curves for noradrenaline (NA) from an individual experiment in two rabbit femoral arterial strips. First (●——●), second (●·-·-·●), and third (●------●) curves were obtained simultaneously in both strips, in the absence (b) and presence of phentolamine (a). Responses to NA were expressed as percentages of the maximal response in the first curve (% 1st E*max*).

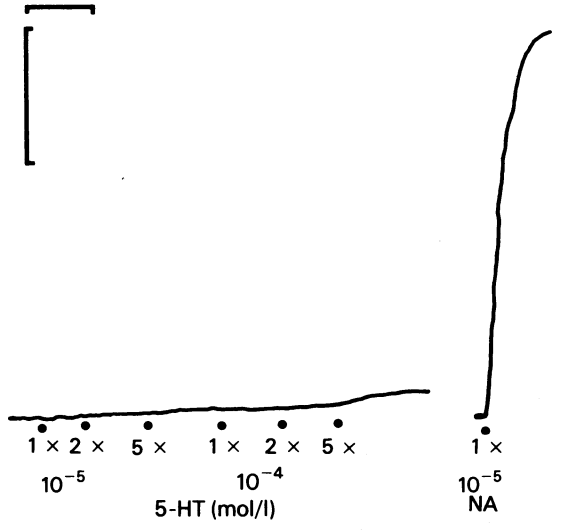

Figure 4 Trace of a cumulative concentration-response relationship for 5-hydroxytryptamine (5-HT) in a rabbit external carotid arterial strip preparation, as in Figure 1. The response to a supramaximal concentration of noradrenaline (NA) in the same strip is shown for comparison. Vertical and horizontal scale as in Figure 1.

The effect of 5-HT was markedly different from that obtained in the common carotid and femoral arteries. Practically no response was elicited at concentrations less than 10^{-5} mol/l. Concentrations of 10^{-5} to 5×10^{-4} mol/l produced small concentration-dependent contractile responses. The solubility limits of 5-HT precluded the testing of higher bath concentrations, thus maximal responses could not be obtained. A trace of a typical cumulative concentration-response relationship for 5-HT is shown in Figure 4. Because of the small magnitude of the 5-HT responses in external carotid arteries, a maximal response to NA was also obtained, and the 5-HT responses were expressed as percentages of the NA maximal response. The response to the highest concentration of 5-HT used in the initial curve, 5×10^{-4} mol/l, was 13 ± 1% of the NA maximal response (mean ± s.e.mean, $n = 27$). From the concent-

Table 2 pA_2 values for antagonism of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) by pizotifen and phentolamine in arterial strip preparations from rabbits

		Agonist		
Artery	*Antagonist*	*5-HT*	*NA*	*NA (with cocaine 10^{-5} mol/l)*
Common carotid	Pizotifen	9.20 ± 0.20 (5)*	6.35 ± 0.19 (5)	6.36 ± 0.14 (7)
	Phentolamine	5.85 ± 0.20 (5)*	7.55 ± 0.12 (5)	7.68 ± 0.17 (7)
Femoral	Pizotifen	8.84 ± 0.28 (4)*	6.63 ± 0.35 (5)	6.64 ± 0.12 (7)
	Phentolamine	5.79 ± 0.11 (4)*	7.32 ± 0.20 (5)	7.58 ± 0.18 (7)
External carotid	Pizotifen	7.3 (3)†	6.91 ± 0.17 (4)	6.61 ± 0.13 (7)
	Phentolamine	7.8 (4)†	7.96 ± 0.29 (4)	7.95 ± 0.09 (6)

Each value is the mean ± s.e.mean (n).
*Significantly different from the pA_2 values against NA ($P < 0.05$).
† Approximate pA_2 value (see text).

rations of 5-HT producing 50% of the response to 5×10^{-4} mol/l (the highest concentration used), approximate pD_2 values for 5-HT were determined. The mean value is given in Table 1; it was significantly different from the common carotid and femoral artery pD_2 values for 5-HT.

It is therefore apparent that 5-HT was far less potent an agonist in the external carotid artery than in either common carotid or femoral arteries, in which it was equipotent. NA was of similar potency in all three artery types.

Antagonism of NA was assessed using the same concentration ranges of pizotifen and phentolamine that were used in the common carotid and femoral artery experiments; findings in the external carotid arteries were similar in all respects to those in the common carotid and femoral arteries. The means of the pA_2 values are included in Table 2.

Since log concentration-response curves for 5-HT in external carotid arteries could only be obtained over the concentration range 10^{-5} to 5×10^{-4} mol/l, without yielding maximal responses, it was not possible to determine accurate pA_2 values. Measurement of antagonistic activity of phentolamine and pizotifen against 5-HT had to be restricted to determination of shifts in log concentration-response curves produced by single concentrations of each antagonist. In each experiment, an agonist dose-ratio was calculated from the agonist concentrations producing an arbitrarily determined response for each preparation which was on the linear, parallel portion of the curves. Correction was made for spontaneous changes in sensitivity using the paired control preparation. Pizotifen (10^{-6} mol/l) produced a mean shift to the right of 1.3 ± 0.3 log units (mean $\pm$ s.e.mean, $n = 3$), and phentolamine (10^{-7} mol/l) produced a mean shift to the right of 0.9 ± 0.4 log units ($n = 4$). Approximate pA_2 values were calculated from these results, using the theoretical equations employed by Arunlakshana & Schild (1959), with the assumption that blockade is competitive. These values against 5-HT are shown in Table 2; they are clearly very similar to those against NA in the same preparation. Thus in the external carotid artery, pizotifen was a weak antagonist of both 5-HT and NA, whereas phentolamine was a potent antagonist of both.

Assessment of indirect sympathomimetic activity in arterial strip preparations

Tyramine (10^{-4} mol/l) produced contractile responses which were $28 \pm 3\%$ of the NA maximal response (mean $\pm$ s.e.mean, $n = 12$) in common carotid arteries. This response was almost abolished in the presence of cocaine (10^{-5} mol/l), and in common carotid arteries removed from reserpine-pretreated rabbits. In femoral and external carotid arteries, tyramine responses were small ($4 \pm 1\%$ and $11 \pm 3\%$ of the NA maximal response respectively; means $\pm$ s.e.mean, $n = 16$), and were unaffected by cocaine or reserpine-pretreatment.

In the external carotid arteries, there was no significant reduction in the response to 5×10^{-4} mol/l of 5-HT in the presence of cocaine (10^{-5} mol/l) or in preparations obtained from reserpine-pretreated rabbits.

Agonist-antagonist interactions in perfused ear artery preparations

The results from these experiments simply confirmed the findings of Apperley *et al.* (1976) and Fozard (1976); each agonist-antagonist interaction was therefore studied in only 2 experiments. NA was 1000 times more potent than 5-HT. Pizotifen was a weak antagonist against both 5-HT and NA, whereas phentolamine was a potent antagonist of both. The pA_2 values from the individual experiments are shown in Table 3. Slopes of the Arunlakshana & Schild (1959) plots were close to unity in each experiment. All of these values were within the 95% confidence limits given by Apperley *et al.* (1976) or Fozard (1976).

Table 3 Individual pA_2 values for antagonism of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) by pizotifen and phentolamine in perfused ear artery preparations from rabbits

	Agonist	
Antagonist	*5-HT*	*NA*
Pizotifen	6.10, 6.40	6.38, 6.79
Phentolamine	7.20, 7.30	7.58, 7.71

Discussion

The results of the present study indicate that in the rabbit, specific 5-HT receptors are absent from certain extracranial arteries. Moreover, in these arteries, the effects of 5-HT are mediated by an action at α-adrenoceptors, which is direct and not indirect. In other arteries, namely the common carotid and the femoral, there are pharmacologically distinct receptors for 5-HT and NA.

In the common carotid and femoral arterial strip preparations, pizotifen and phentolamine antagonized the contractile effects of 5-HT and NA. The antagonism was competitive, according to the slopes of the Arunlakshana & Schild (1959) plots, enabling determination of pA_2 values, which can be regarded as indicative of antagonist affinity constants

(Furchgott, 1972). Functional studies aimed at characterization of receptors by this means require that pA_2 values be determined as accurately as possible. In the studies described by Apperley *et al.* (1976) and Fozard (1976), the possibility that variations in neuronal uptake of NA could influence antagonist pA_2 values (Furchgott, 1972) was not investigated. Humphrey (1978a), for example, found that in dog saphenous vein preparations, neuronal uptake influenced pA_2 values against NA by as much as 1.5 units. In the present experiments, pA_2 values against NA were therefore obtained both in the presence and absence of cocaine. The concentration of cocaine used, 10^{-5} mol/l, was in the range accepted as blocking neuronal uptake (Iversen, 1967), and accounts for our finding that cocaine blocked the action of the indirect sympathomimetic amine tyramine, in the common carotid arterial strip preparations. It was found that cocaine did not influence pD_2 or pA_2 values in the rabbit common carotid or femoral arteries. This apparent lack of influence of neuronal uptake mechanisms may be species related, as Furchgott (1967) and Brandao (1976) also found little effect with cocaine in rabbit vasculature. Thus in both common carotid and femoral arteries, the pA_2 values for each antagonist against 5-HT are different from those against NA, irrespective of any influence of neuronal uptake. Hence, 5-HT and NA are activating pharmacologically different receptors.

The pizotifen and phentolamine pA_2 values that we obtained in the common carotid and femoral arteries agree with those obtained by Apperley *et al.* (1976) in the rabbit aorta. These authors concluded that the rabbit aorta contained both D-type 5-HT receptors (Gyermek, 1966) and α-adrenoceptors, on the basis of comparisons between the pA_2 values determined in their experiments and those in the literature for various other tissues. Thus it is reasonable to conclude that the rabbit common carotid and femoral arteries, like the aorta, contain both D-type 5-HT receptors and α-adrenoceptors.

In the external carotid artery, accurate pA_2 values against NA could be determined. This was not the case with 5-HT, since 5-HT had such a weak agonist potency in this tissue. However, it proved possible to make an approximate assessment of the 5-HT blocking activity of pizotifen and phentolamine in the external carotid artery, assuming that the blockade was competitive. The pA_2 values for each antagonist against 5-HT were almost the same as those against NA. As in the common carotid and femoral arteries, cocaine did not influence pD_2 or pA_2 values. Thus the contractile effects of 5-HT and NA in the external carotid artery are mediated by an action at the same receptor, namely an α-adrenoceptor according to the actual pA_2 values. This parallels the situation in the rabbit ear artery, as shown in the investigations by Apperley *et al.* (1976) and Fozard (1976), and confirmed in our experiments.

The agonist potencies of 5-HT and NA in various rabbit arterial preparations are consistent with the above conclusions. 5-HT and NA were of similar potency in the common carotid and femoral arteries, as was the case in the rabbit aorta (Apperley *et al.*, 1976). In the external carotid and the ear arteries, 5-HT was much less potent than NA, reflecting the absence of specific receptors for 5-HT in these tissues. Although the responses to 5-HT relative to NA in the external carotid arterial strip preparations were poor in comparison with those obtained in perfused ear arteries, this apparent difference in efficacy may simply reflect inherent differences in the mechanical properties of the two types of preparation, and the mode of administration of agonist.

Although there was no indirect component to the α-adrenoceptor mediated effect of 5-HT in the rabbit ear artery (Apperley *et al.*, 1976; Fozard, 1976), an indirect sympathomimetic component has been found in other tissues such as cat spleen (Innes, 1962), dog heart (Fillion, Lluch & Uvnäs, 1971), rabbit heart (Fozard & Mwaluko, 1976), and dog saphenous vein (Humphrey, 1978b). The fact that tyramine had no indirect effect in the external carotid artery, and that the response to 5-HT was not altered by cocaine or reserpine-pretreatment, indicated that there was no indirect sympathomimetic component to the action of 5-HT in this tissue.

The results of these studies lead to speculation as to whether the distribution of 5-HT vascular receptors in rabbits is related to vessel size, function or location. It may be that the absence of 5-HT receptors in the rabbit extracranial circulation is peculiar to this species, since some recent studies suggest that in dog, cat and human extracranial vasculature, specific receptors for 5-HT are present (Edvinsson, Hardebo & Owman, 1978; Apperley, Feniuk, Humphrey & Levy, 1980; Lamar & Edvinsson, 1980). Thus in studies aimed at elucidating the mechanisms involved in the pathophysiology and treatment of cranial vascular diseases such as migraine, the use of rabbit vascular preparations as a model may be of limited value. There is an obvious need for quantitative analysis of functional receptor interactions between agonists and antagonists in human vessels.

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References

APPERLEY, E., FENIUK, W., HUMPHREY, P.P.A. & LEVY, G.P. (1980). Evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog isolated vasculature. *Br. J. Pharmac.*, **68**, 215–224.

APPERLEY, E., HUMPHREY, P.P.A. & LEVY, G.P. (1976). Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. *Br. J. Pharmac.*, **58**, 211–221.

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

BRANDAO, F. (1976). A comparative study of the role played by some inactivation pathways in the disposition of the transmitter in the rabbit aorta and the saphenous vein of the dog. *Blood Vessels*, **13**, 309–318.

COCHRAN, W.G. & COX, A.M. (1957). *Experimental Designs*, 2nd Edn. New York: Wiley.

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.

EDVINSSON, L., HARDEBO, J.E. & OWMAN, C. (1978). Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man. *Circulation Res.*, **42**, 143–151.

FILLION, G.M.B., LLUCH, S. & UVNÄS, B. (1971). Release of noradrenaline from the dog heart in situ after intravenous and intracoronary administration of 5-hydroxytryptamine. *Acta physiol. scand.*, **83**, 115–123.

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.

FOZARD, J.R. & MWALUKO, G.M.P. (1976). Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. *Br. J. Pharmac.*, **57**, 115–125.

FURCHGOTT, R.F. (1967). The pharmacological differentiation of adrenergic receptors. *Ann. N.Y. Acad. Sci.*, **139**, 553–570.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines, Handb. Exp. Pharmac.*, Vol. 33, ed. Blaschko, H. & Muscholl, E. pp. 283–335. Berlin, Heidelberg & New York: 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.

GYERMEK, L. (1966). Drugs which antagonise 5-hydroxytryptamine and related indolealkylamines. In *5-Hydroxytryptamine and Related Indolealkylamines, Handb. Exp. Pharmac.*, Vol. 19, ed. Erspamer, V. pp. 471–528. Berlin, Heidelberg & New York: Springer-Verlag.

HUMPHREY, P.P.A. (1978a). The effects of uptake$_1$ on α-adrenoceptor antagonist potency in dog saphenous vein. *Br. J. Pharmac.*, **63**, 665–669.

HUMPHREY, P.P.A. (1978b). The effects of α-adrenoceptor antagonists on contractile responses to 5-hydroxytryptamine in dog saphenous vein. *Br. J. Pharmac.*, **63**, 671–675.

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

IVERSEN, L.L. (1967). *The Uptake and Storage of Noradrenaline in Sympathetic Nerves.* pp. 151–175. London: Cambridge University Press.

LAMAR, J.C. & EDVINSSON, L. (1980). 5-Hydroxytryptamine receptors. Contractile activity and mode of inhibition by methysergide in mammalian intracranial and extracranial vessels. *Archs. Int. Pharmacodyn. Thér.*, **243**, 245–254.

MYLECHARANE, E.J., FRENCH, R.J. & BLACK, J.L. (1979). Further studies on the mechanism of action of 5-hydroxytryptamine in rabbit isolated arteries. *Proc. Aust. Physiol. Pharmac. Soc.*, **10**, 179P.

SPIRA, P.J., MYLECHARANE, E.J. & LANCE, J.W. (1976). The effects of humoral agents and antimigraine drugs on the cranial circulation of the monkey. *Res. Clin. Stud. Headache*, **4**, 37–75.

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