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Evidence for a 5-HT_1-like receptor mediating the amplifying action of 5-HT in the rabbit ear artery

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1 The nature of the 5-hydroxytryptamine (5-HT) receptors which amplify the vasoconstrictor effect of methoxamine was examined in the rabbit isolated perfused ear artery with intact endothelium. Indices of amplification were leftward shifts of methoxamine dose-response (DR) curves produced by 5-HT (0.3 μM) (Method I), and the appearance of vasoconstrictor responses to 5-HT receptor agonists when methoxamine was present in a near-threshold concentration (Method II).

2 The amplifying effect of 5-HT (Method I) was unaffected by prazosin (0.08 μM), was partly depressed by 5-HT_2-receptor antagonists in high concentrations (ketanserin 0.5 μM, LY53857, 1.0 μM), and was abolished by a non-selective antagonist of 5-HT_1 and 5-HT_2 receptors (methiothepin, 0.01 μM).

3 Amplifying potencies of agonists assessed by both Methods I and II were in the order 5-carboxamidotryptamine (5-CT) > 5-HT > α-methyl 5-HT. The potency of sumatriptan (assessed by Method II only) was intermediate between those of 5-HT and α-methyl 5-HT.

4 Ketanserin and LY53857 inhibited the amplifying action of 5-CT to about the same extent as that of 5-HT.

5 The amplifying potencies of the agonists are in marked contrast to the reported contractile potencies in the rabbit aorta where the receptor is 5-HT_2, but are almost identical with reported contractile potencies in the dog saphenous vein where the receptor is 5-HT_1-like.

6 It is concluded that a 5-HT_1-like receptor mediates the amplifying interaction between 5-HT and methoxamine in the rabbit ear artery which can be weakly blocked by ketanserin and LY53857.

7 Since 5-CT was equipotent when applied separately to the intimal and adventitial surfaces of the artery, it is suggested that the 5-HT_1-like receptors are distributed uniformly across the artery wall.

Keywords: Rabbit ear artery; amplifying interactions; 5-hydroxytryptamine$_1$-like receptors; 5-carboxamidotryptamine; sumatriptan

Introduction

In concentrations that are sub-threshold for contraction, 5-hydroxytryptamine (5-HT) exerts an excitatory effect in arteries *in vitro* which is manifested either by an increased response to a second contractile agent or by the appearance of a contractile response to 5-HT in the presence of the second agent. This synergistic interaction, which is commonly termed the amplifying action of 5-HT, is prominent in the rabbit ear artery where it was first documented (de la Lande *et al.*, 1966). The nature of the receptor mediating this amplifying action of 5-HT in the rabbit ear artery has been explored in the present study.

Previous attempts to identify the receptor have focussed on the possibility that amplification is simply a sub-threshold manifestation of excitability changes associated with the contractile response to 5-HT. The latter response, in the absence of other treatment, appears to be mediated only by the α-adrenoceptor (Apperley *et al.*, 1976); sensitivity to prazosin (Purdy *et al.*, 1981) implies that the receptor is of the α_1-subtype. However a 5-HT_2-receptor appears to play a contributory role in artery strips from reserpinized rabbits (de la Lande & Kennedy, 1985) and in artery rings exposed to ouabain (Xu *et al.*, 1990). So far, attempts to establish the role of these receptors in amplification have yielded results which are not easy to interpret. In reserpinized-strip preparations, both prazosin and ketanserin depressed amplification in the interaction between 5-HT and noradrenaline (NA) (see de la Lande, 1989), but had much less effect on the interaction between 5-HT and methoxamine (de la Lande & Kennedy, 1985). Nevertheless, Meehan *et al.* (1986), using the perfused segment preparation from untreated rabbits, noted that the 5-HT interaction with NA was unaffected by prazosin but was depressed by ketanserin in a concentration (0.03 μM) that was considered to be indicative of a role for the 5-HT_2 receptor.

The present study was confined to experiments on perfused segments from untreated rabbits, with methoxamine as the reference contractile agent. In order to characterize the amplifying receptor, two approaches were adopted. In one, the effects on the 5-HT-methoxamine interaction of α_1-adrenoceptor blockade by prazosin, of 5-HT_2 receptor blockade by ketanserin and LY53857 (for references see Mylecharane, 1990), and of non-selective 5-HT_1 and 5-HT_2 receptor blockade by methiotepin, were examined. In the other, the amplifying actions of 5-HT were compared with those of a 5-HT_2 selective agonist (α-methyl 5-HT) and two agonists with selectivity for receptors within the 5-HT_1 group, namely, 5-carboxamidotryptamine (5-CT) and sumatriptan. The agonists were selected in view of evidence that they are of value in distinguishing between effects mediated by 5-HT_1-like and 5-HT_2 receptors (e.g. Feniuk & Humphrey, 1989; Summer *et al.*, 1989).

A preliminary account of the present findings was presented to the Australasian Society for Clinical and Experimental Pharmacologists (de la Lande, 1990).

Methods

Preparation

Semi-lop eared rabbits, bred at the Central Animal House, University of Adelaide from the Belgian Lop-Eared strain,

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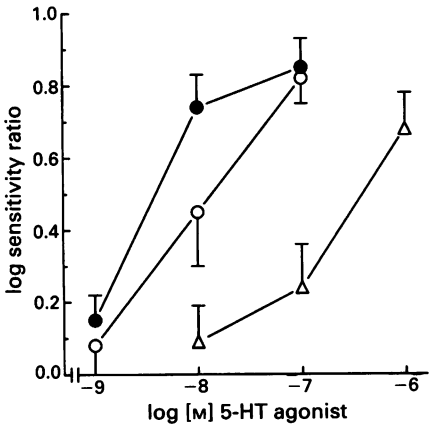

Figure 2 Amplifying potencies of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and α-methyl 5-HT: (●) 5-CT; (○) 5-HT; (△) α-methyl 5-HT. The graphs show leftward shifts of methoxamine dose-response curves (expressed as log sensitivity ratios) at three concentrations of each agonist ($n = 4$). Values shown are means with s.e.mean indicated by vertical bars.

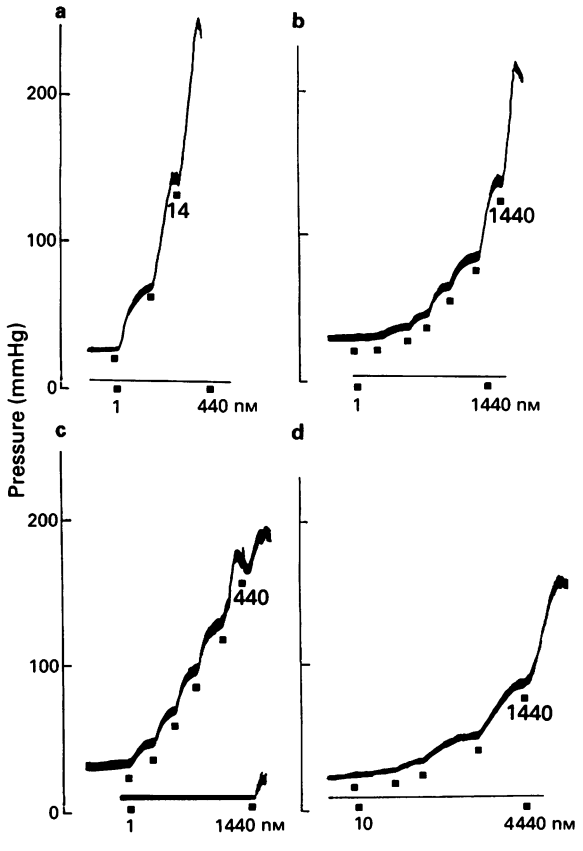

Figure 3 Effect of methoxamine on the contractile activities of 5-hydroxytryptamine (5-HT) receptor agonists: (a) 5-carboxamidotryptamine (5-CT); (b) sumatriptan; (c) 5-HT; (d) α-methyl 5-HT. The agonist concentration was cumulated by factors of 3.2–4.0 between the lowest and highest concentrations (shown in μM). The lower (horizontal) trace is the perfusion pressure in the absence of methoxamine and shows that the agonists were without constrictor effect, except for 5-HT at the highest concentration. The superimposed trace shows that, in the same arteries, the agonists became potent constrictor agents when methoxamine was present in a concentration which elevated the perfusion pressure by 15–30 mmHg.

Responses below 100–150 mmHg increase in pressure were well sustained, but above this range they displayed a pronounced tendency to fade. Comparison of concentration-constrictor response curves (Figure 4) indicated an order of amplifying potency: 5-CT > 5-HT > sumatriptan > α-methyl 5-HT. Concentrations which elicited a response of 60 mmHg (in nM, with 95% confidence limits in parentheses, $n = 4$–6) were: 5-CT (2.3, 0.8–6.6), 5-HT (6.2, 3.5–11), sumatriptan (76, 35–257), and α-methyl 5-HT (186, 62–562). Ratios of concentrations (with 95% confidence limits in parentheses) which were equipotent with 5-HT were: 5-CT, 0.4 (0.3–0.7, $n = 6$), sumatriptan, 11 (5–25, $n = 5$) and α-methyl 5-HT, 28 (13–58, $n = 4$).)

In the preceding experiments, the 5-HT receptor agonists were present only in the extraluminal solution. However experiments in which the effects of intraluminal and extraluminal 5-CT were compared, failed to reveal any difference in amplifying potencies. Thus concentrations of intraluminal and of extraluminal 5-CT which elicited a response of 60 mmHg in six arteries (in nM, with 95% confidence limits) were 1.6 (0.52–4.8) and 2.2 (0.74–6.8) respectively.

Vasodilator effect of acetylcholine

To assess the functional state of the endothelium, the vasodilator response to ACh (0.5 μM, applied intraluminally) was measured before terminating each experiment; the artery was constricted to between 100 and 200 mmHg by methoxamine. The ACh caused a decrease in perfusion pressure of 50 mmHg or greater in most (82 of 97) artery segments. In six pairs of arteries, responses to extraluminally-applied ACh (0.5 μM) were compared with those to intraluminal ACh (0.5 μM); physostigmine (3.0 μM) was present to minimize hydrolysis of the ACh. The response to the extraluminal ACh (a decrease in pressure of 48 ± 5%) was considerably less than to the intraluminal ACh (a decrease of 93 ± 4%).

Discussion

Effects of the 5-HT receptor agonists are discussed first since they provide a clearer insight into the nature of the receptors mediating amplification than do the effects of the agonists.

When assessed in terms of increases in the contractile activity of methoxamine, the amplifying potency of 5-HT was

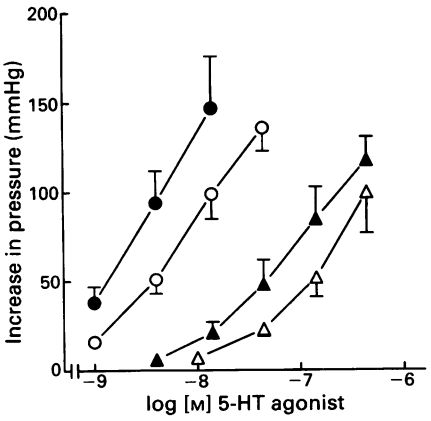

Figure 4 Concentration-response (CR) curves to 5-hydroxytryptamine (5-HT) receptor agonists in the presence of methoxamine. The increased steady-state levels of perfusion pressure due to methoxamine (0.2–0.4 μM) at the time the agonists were added were (in mmHg): 5-HT (27 ± 3, $n = 6$), 5-carboxamidotryptamine (5-CT, 31 ± 5, $n = 6$), sumatriptan (28 ± 5, $n = 5$), α-methyl 5-HT (20 ± 7, $n = 4$). Symbols: (●) 5-CT; (○) 5-HT, (▲) sumatriptan; (△) α-methyl 5-HT. Values shown are means with s.e.mean (vertical bars) from n experiments.

greater than that of α-methyl 5-HT but less than that of 5-CT. A similar rank order of potencies prevailed when the constrictor response to the 5-HT agonist in the presence of methoxamine was used as the index of amplification. The latter method indicated also an amplifying potency of sumatriptan between that of α-methyl 5-HT and 5-CT. As indicated in Table 2, the relative potencies agree closely with those for contractile activity in the dog saphenous vein, and are not too dissimilar from those for endothelium-dependent relaxation in the pig coronary artery; in both these vessels the effects of 5-HT are believed to be mediated by 5-HT_1-like receptors (Feniuk & Humphrey, 1989; Schoeffter & Hoyer, 1990). On the other hand, the relative amplifying potencies contrast markedly with those for 5-HT_2 receptor-mediated contraction in the rabbit aorta, where the rank order is 5-HT > α-methyl 5-HT > 5-CT > sumatriptan (inactive) (Feniuk *et al.*, 1985; Humphrey *et al.*, 1988).

In view of evidence that the 5-HT_1-like receptor in the pig coronary artery is very similar to the 5-HT_{1D} receptor (Shoeffter & Hoyer, 1990) it is noteworthy that the absolute amplifying potencies when assessed in terms of the concentrations eliciting a response of 60 mmHg in the methoxamine-exposed artery, are in the same rank order as IC_{50}s for binding to 5-HT_{1D} recognition sites in brain. The respective values (amplifying potency followed by binding affinity, in nM) on which the comparison of rank order is based are: 5-CT (2.3, 2.5), 5-HT (6.2, 4.0), sumatriptan (76, 17–68) and α-methyl 5-HT (186, 158). The data on binding are from the studies of Engel *et al.* (1986), Hoyer (1989), Shoeffter & Hoyer (1990), Peroutka & McCarthy (1989) and Van Wijngaarden *et al.* (1990).

It follows from the above considerations that there are reasonable grounds for placing the amplifying receptor in the rabbit ear artery in the 5-HT_1-like category.

Effects of antagonists

Criteria for the presence of a 5-HT_1-like receptor include insensitivity to 5-HT_2 selective antagonists (e.g. ketanserin) and sensitivity to antagonists which do not discriminate beween 5-HT_1 and 5-HT_2 receptors e.g. methysergide, methiothepin (Bradley *et al.*, 1986).

At first sight, the ability of the 5-HT_2 receptor antagonists, LY53857 and ketanserin, to inhibit amplification implies a departure from the above criteria. However, the following factors must be taken into account. Firstly, the effect of ketanserin was not impressive in the sense that 50 nM appeared to be close to the inhibitory threshold, yet this concentration is considerably greater than the pA_2 for the 5-HT_2 receptor, namely 0.4–8 nM (Mylecharane, 1990). Similarly, the inhibitory threshold for LY53857, which was in the vicinity of 100 nM, also seems high when compared with its pA_2 for the 5-HT_2 receptor namely 0.4–40 nM (Fenuik & Humphrey, 1989; Mylecharane, 1990). Secondly, the finding that both ketanserin (0.5 μM) and LY53857 (1.0 μM) inhibited the amplifying action of 5-CT to about the same extent as that of 5-HT suggests that these antagonists possess sufficient affinity for the 5-HT_1-like receptor to account for their effects on the action of 5-HT.

Methysergide was not used in the present study, since it is known to behave like an agonist of the amplifying receptor in the rabbit ear artery (de la Lande *et al.*, 1966; Fozard, 1976). However methiothepin proved to be a potent inhibitor of the amplifying action of 5-HT, abolishing the action in a concentration of 0.01 μM. The effects of these antagonists again emphasise the similarity between the actions of 5-HT in the rabbit ear artery and dog saphenous vein, since in the latter vessel methysergide also is a partial agonist and methiothepin a potent antagonist of the 5-HT_1-like receptor mediating the contractile response (Fenuik *et al.*, 1985; Fenuik & Humphrey, 1989).

Distribution of the amplifying receptor

In view of evidence that a 5-HT_1-like receptor is associated with the endothelium in pig coronary artery (see Table 2), an indication of the distribution of the amplifying receptor in the wall of the rabbit ear artery was sought by comparing the potencies of intraluminal and extraluminal 5-CT. The finding that the potencies did not differ is interpreted as evidence that the receptors are fairly evenly distributed between the inner and outer regions of the artery wall, and hence are probably associated with the smooth muscle cells. That this interpretation is valid is supported by the finding that the vasodilator effect of intraluminal ACh is considerably greater than that of extraluminal ACh. This finding accords with the substantial evidence that the cholinoceptors mediating vasodilatation are located on endothelial cells (Furchgott & Zawadski, 1980).

Relationship between amplifying and contractile actions of 5-hydroxytryptamine

In accord with evidence that the amplifying effect of 5-HT is manifested in concentrations below those eliciting constriction (de la Lande *et al.*, 1966), there was no indication of constrictor activity on the part of any of the 5-HT receptor agonists in concentrations of up to 100 fold greater than those eliciting detectable amplification. Earlier studies on the perfused artery from the untreated rabbit are in agreement that the constrictor activity is due to α-adrenoceptor activation (Apperley *et al.*, 1976; Fozard, 1976; see also Introduction). 5-HT_2 receptor-mediated contraction has also been demonstrated although only under certain conditions e.g. in reserpine-treated artery strips (de la Lande & Kennedy, 1985) and in oubain-exposed artery rings (Xu *et al.*, 1990). Nevertheless, the present results argue against a role for either of these receptors in the amplifying interaction between 5-HT and methoxamine. It is suggested therefore that the 5-HT_1-like receptor functions primarily as an amplifying receptor. Whether it simply facilitates constrictor activity of other agonists, or whether it requires the presence of a second excitatory agonist for expression of its own constrictor function, are complex questions which remain to be addressed.

Table 2 Relative potencies† of 5-hydroxytryptamine (5-HT) receptor agonists

			Agonist			
Receptor	*Tissue*	*Response*	*5-CT*	*5-HT*	*Sumatr*	*α-Me 5-HT*
?	Rabbit ear art	amplifn.	2.5	1.0	0.09	0.04
5-HT_1-like	Dog saph. vein	contrn.	3.3	1.0	0.20	0.08
5-HT_1-like	Pig cor. art.	relaxn*	1.0	1.0	0.03	0.06
5-HT_2	Rabbit aorta	contrn.	0.04	1.0	nil	0.5

†Concentration of 5-HT divided by equipotent concentrations of the agonist. *Endothelium-dependent. Data on dog saphenous vein from Humphrey *et al.* (1988), on pig coronary artery from Schoeffter & Hoyer (1989) and on rabbit aorta from Feniuk *et al.* (1985).

were used in all experiments. Ear artery segments (1–2 cm long) were removed from rabbits which had been killed under pentobarbitone anaesthesia. The segments were perfused by the method of de la Lande *et al.* (1966). The segment was cannulated at both ends, placed in an organ bath containing Krebs solution (vol. 15 ml), and perfused intraluminally with Krebs solution (3.0 ml min^{-1}). After passing through the lumen, the perfusate escaped without mixing with the solution in the organ bath (termed 'extraluminal'). Intraluminal perfusion pressure was measured by a Statham Pressure Transducer; vasoconstriction was manifested by an increase in perfusion pressure. The perfusion pressure in the absence of vasoactive agents was usually between 5 and 10 mmHg and did not vary within an experiment. Note: Higher base-line pressures (10–20 mmHg) in the earlier experiments of de la Lande *et al.* (1966) reflected higher flow rates (6–8 ml min^{-1}).

Effects of 5-hydroxytryptamine on responses to methoxamine

Vasoconstrictor responses to methoxamine (administered as an intraluminal bolus in 0.1 ml) were measured in the absence and presence of 5-HT (0.03 μM). The 5-HT was present in both the intraluminal and extraluminal solutions. Responses to methoxamine were elicited in duplicate at three dose levels (most commonly 0.03, 0.10, and 0.30 μmol). The 5-HT was added 5–10 min before starting a second dose-response (DR) curve. Its amplifying effect was quantitated in terms of the ratio (termed sensitivity ratio) of doses of methoxamine which were equipotent in eliciting a response of 60 mmHg in the absence (numerator) and presence (denominator) of 5-HT.

In experiments where 5-HT was applied for a second time to the artery, a period of approximately 60 min after the preceding wash-out of 5-HT was allowed to enable sensitivity to methoxamine to return to its pre-5-HT level. Although recovery from the amplifying effect of 5-HT was extremely rapid (less than 5 min), it was followed by a period of depressed sensitivity to methoxamine which lasted about 30 min.

In all experiments, two or more arteries from the same animal were used. When effects of antagonists were studied, one of the arteries served as the control, the other (test artery) being exposed to the antagonist 60 min prior to commencing the first DR curve to methoxamine. The same time interval was allowed in the control artery. 5-HT sensitivity ratios were compared in the control and test arteries. Antagonists tested comprised prazosin, ketanserin, LY53857 and methiothepin.

The only departure from the above procedure was that in some experiments 5-HT was replaced by 5-CT (0.03 μM).

Interactions between 5-hydroxytryptamine receptor agonists and methoxamine

The agonists comprised 5-HT, α-methyl 5-HT, 5-CT and sumatriptan. With the exception of sumatriptan their effects were measured in two ways. In one, the agonist was present in the infusion and bathing media and its effects on the vasoconstrictor responses to intraluminally-injected methoxamine were measured. The concentration of the agonist was progressively increased in multiples of ten between 0.001 and 1.0 μM. The sensitivity ratio was measured at each concentration.

The second method used the constrictor response to the 5-HT-receptor agonist in the presence of methoxamine, as the index of amplification. Methoxamine was added to the extraluminal solution in a concentration (usually 0.3 μM) which resulted in a steady-state constrictor response between 15 and 40 mmHg. The agonist was then added cumulatively to the same solution in concentrations which, in the absence of methoxamine, did not constrict the artery. The concentration of agonist which elicited a constrictor response of 60 mmHg in the presence of methoxamine was estimated from the agonist concentration-response (CR) curve. When the second method was used, cocaine (3 μM) was present in both the intraluminal and extraluminal solutions to minimize any loss of the 5-HT agonists resulting from uptake into the sympathetic nerves.

Comparison of intraluminal and extraluminal amplifying potencies of 5-carboxamidotryptamine

Methoxamine was added to the extraluminal solution in a concentration that elicited a steady-state constrictor response of between 15 and 40 mmHg. Constrictor responses to 5-CT applied extraluminally were then compared with those to 5-CT perfused intraluminally. Concentrations which were equipotent in eliciting a response of 60 mmHg were estimated from the 5-CT CR curves. Cocaine (3 μM) was present in both the intraluminal and extraluminal solutions.

Endothelial function

Towards the end of each experiment, the vasodilator response to intraluminally-infused acetylcholine (ACh, 0.5 μM) was measured after the perfusion pressure had been elevated to between 100 and 200 mmHg by extraluminal methoxamine.

Maximum constriction

Before terminating an experiment, an indication of the maximum response to methoxamine was obtained. Depending on the nature of the experiment, methoxamine was either injected intraluminally in a large dose (3 or 10 μmol) or was applied extra-luminally in a high concentration (30 μM). The resultant response was usually in the 300 to 400 mmHg range but was often difficult to quantify due to a tendency for the perfusion pressure to fluctuate erratically as it reached this range. In view of this tendency, which was noted earlier (de la Lande, 1975), the response in mmHg rather than the response as a percentage of maximum was used in plotting dose-response relationships.

Statistics

Effects of drugs on constrictor responses and on sensitivity ratios, the latter converted to log units, were assessed for significance by the paired t test. Significance refers to $P<0.05$; except where otherwise stated n refers to the number of rabbits.

Drugs

The following compounds were purchased: cocaine hydrochloride (MacFarlane-Smith); 5-hydroxytryptamine creatinine sulphate (Sigma), methoxamine hydrochloride (Sigma); α-methyl 5-hydroxytryptamine maleate (Research Biochemicals); physostigmine salicylate (Sigma), prazosin hydrochloride (Sigma).

The following were gifts: methiothepin maleate (Roche); ketanserin hydrochloride (Janssen Pharmaceuticals); 5-carboxamidotryptamine maleate, sumatriptan (Glaxo); LY53857 (Lilly Research Laboratories).

LY53857 is 4-isopropyl-7-methyl-9-(2-hydroxy-1-methyl-propoxy-carbonyl) -4, 6, 6A, 7, 8, 9, 10, 10A-octahydro-indolol [4.3-FG] quinoline.

Results

Effects of antagonists on the amplifying action of 5-hydroxytryptamine

5-HT (0.03 μM) did not affect the perfusion pressure but caused an approximately four fold leftward shift of the

methoxamine DR curve (Figure 1a). The action of 5-HT was unchanged when sensitivity to methoxamine was depressed by prazosin (0.08 μM); the depression was manifested as a twenty fold rightward shift of the methoxamine DR curve (Figure 1a).

Ketanserin (0.05 μM and 0.5 μM) also shifted the methoxamine DR curve to the right by factors of 3 and 21 respectively. However, unlike prazosin, it also decreased the amplifying action of 5-HT (Table 1). Although not significant at the lower concentration of ketanserin, the effect of amplification was unequivocal at the higher concentration and is illustrated in Figure 1b.

LY53857 decreased the amplifying action of 5-HT but did not decrease the sensitivity to methoxamine. In the two concentrations tested, namely 0.1 and 0.5 μM, the inhibitory effects of LY53857 on amplification were comparable with those of ketanserin 0.05 and 0.5 μM respectively (Table 1, Figure 1b).

Methiothepin (0.01 μM) caused an approximately forty fold rightward shift of the methoxamine DR curve and abolished the action of 5-HT (Table 1).

Amplifying actions of 5-hydroxytryptamine receptor agonists

The agonists were 5-HT, 5-CT (5-HT_1 selective) and α-methyl 5-HT (5-HT_2 selective). Each increased the vasoconstrictor activity of methoxamine. Leftward shifts of methoxamine DR curves, measured at three subconstrictor concentrations of each agonist, indicated that 5-HT was less potent than 5-CT, but considerably more potent than α-methyl 5-HT (Figure 2). From the concentration-amplifying effect curves, it was estimated that concentrations eliciting a leftward shft of 0.5 log units (in nM with 95% confidence limits, $n = 4$) were 5-CT (3.4, 1.3–8.7), 5-HT (15, 3.5–64) and α-methyl 5-HT (300, 87–1040).

Since the above experiments revealed that 5-CT was a highly potent amplifying agent, the effects of ketanserin (0.5 μM) and of LY53857 (1.0 μM) on the 5-CT-methoxamine interaction were examined. As shown in Table 1, both agents inhibited the amplifying action of 5-CT to about the same extent as that of 5-HT.

Effect of methoxamine on vasoconstrictor activities of 5-hydroxytryptamine receptor agonists

In these experiments the vasoconstrictor response to the 5-HT receptor agonist in the presence of methoxamine (both applied extraluminally), was used as the index of amplification. The agonists included sumatriptan.

In the absence of methoxamine, none of the four agonists displayed vasoconstrictor activity when tested in concentrations ranging up to 0.4 μM (for 5-HT and 5-CT) and 1.4 μM (for α-methyl 5-HT and sumatriptan). However, when applied in much lower concentrations during a steady-state constriction of between 10 and 50 mmHg induced by methoxamine, the agonists displayed marked constrictor activity (Figure 3).

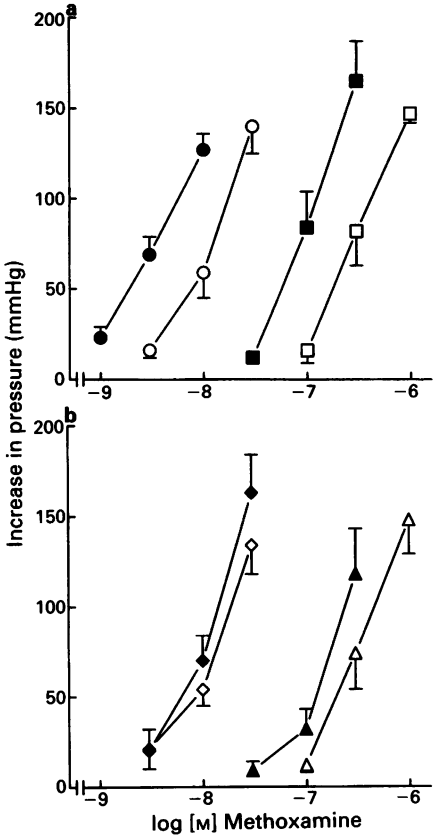

Figure 1 Effects of antagonists on the amplifying action of 5-hydroxytryptamine (5-HT). Shown are effects of prazosin (a), LY53857 and ketanserin (b) on dose-response (DR) curves to methoxamine in the absence and presence of 5-HT (0.03 μM): (○) Control ($n = 17$); (□) prazosin (0.08 μM, $n = 5$); (◇) LY53857 (1.0 μM, $n = 6$); (△) ketanserin (0.5 μM, $n = 6$). DR curves elicited in the presence of 5-HT are indicated by the closed symbols. Values shown are means with s.e.mean (vertical bars) from n experiments.

Table 1 Effects of 5-hydroxytryptamine (5-HT) receptor antagonists on amplifying actions of 5-HT and 5-carboxytryptamine (5-CT) on responses to methoxamine

Antagonist (μM)	*5-HT-receptor agonist*	n	*Sensitivity ratio geometric mean (with 95% confidence limits)* *Control*	*Antagonist present*
Ketanserin (0.05)	5-HT (0.03 μM)	5	4.6 (3.8–5.5)	2.8 (2.0–3.9)[a]
Ketanserin (0.5)	5-HT (0.03 μM)	6	3.6 (3.0–4.3)	1.8 (1.3–2.4)*
LY53857 (0.1)	5-HT (0.03 μM)	5	4.6 (3.7–5.6)	2.8 (1.7–4.6)[a]
LY53857 (1.0)	5-HT (0.03 μM)	6	3.7 (2.9–4.8)	1.3 (0.9–1.9)*
Methiothepin (0.01)	5-HT (0.03 μM)	4	4.5 (2.3–8.7)	1.1 (0.7–1.7)*
Ketanserin (0.5)	5-CT (0.03 μM)	5	4.8 (3.5–6.6)	2.3 (1.2–3.4)*
LY53857 (1.0)	5-CT (0.03 μM)	5	5.2 (3.2–8.7)	1.8 (1.1–3.0)*

* $P < 0.05$. [a] $0.1 > P > 0.05$.

In conclusion, it is emphasised that the present evidence refers only to the interaction between 5-HT and methoxamine. It remains to be established whether amplifying interactions between 5-HT and other agonists such as NA and histamine also involve the 5-HT$_1$-like receptor. That these interactions may be more complex than the one between 5-HT and methoxamine is suggested by findings that amplification in the 5-HT interaction with NA is inhibited by prazosin and ketanserin (see de la Lande, 1989), while amplification in the 5-HT interaction with histamine is potentiated by histamine H_2-receptor activation (de la Lande *et al.*, 1989).

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