Analysis of the 5-HT receptors mediating contractions in the rabbit isolated renal artery

Sreekanth Tadipatri, Dicky van Heuven-Nolsen, *Wasył Feniuk & ¹Pramod R. Saxena

Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, Post Box 1738, 3000 DR Rotterdam, The Netherlands and *Glaxo Group Research, Ware, Herts

1 Using a number of agonist and antagonist compounds, we have attempted to characterize the responses and receptors involved in the effects of 5-hydroxytryptamine (5-HT) in the rabbit isolated renal artery.

2 In vessel segments precontracted with the thromboxane-mimetic agent, U46619 (100 nM), neither 5-HT (10^{-8} to 10^{-4} M) nor 5-carboxamidotryptamine (5-CT; 10^{-8} to 3×10^{-4} M) caused relaxations like those observed with methacholine. Both 5-HT and 5-CT further increased the tone of the vessels, with pD_2 values of 7.1 and 7.9, respectively.

3 In the absence of U46619, both 5-HT (10^{-7} to 3×10^{-3} M) and 5-CT (10^{-7} to 10^{-3} M) contracted the rabbit renal artery, but with reduced potencies. The contractions to 5-HT were reproducible and the rank order of potency (pD_2) of the agonists was: α -methyl-5-HT (5.7), sumatriptan (5.3), 5-HT (5.1), 8-hydroxy-2(di-n-propylamino)tetralin (5.0), 5-CT (4.7) and 5-methoxytryptamine (4.3). 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane, fleroxan and RU 24969 elicited either only small contractions or none at all.

4 The contractile effect of 5-HT was unaffected by MDL 72222 (10^{-6} M) and metergoline (10^{-8} and 10^{-7} M), was weakly antagonized by ketanserin and phentolamine (pK_B : 6.6 and 6.8, respectively), but was effectively antagonized by methiothepin (pK_B : 8.6). Responses to 5-CT and sumatriptan were affected by ketanserin, phentolamine and methiothepin similarly to 5-HT-induced responses.

5 Ketanserin was ineffective against noradrenaline-induced contractions, which were antagonized by phentolamine with a pK_B of 7.3. The pK_B values of phentolamine against 5-HT, 5-CT or sumatriptan were about half a log unit lower than against noradrenaline.

6 In vascular preparations treated with cocaine (3×10^{-5} M), the potency (pK_B) of phentolamine as an antagonist of the responses to noradrenaline (7.6) and 5-HT (6.7) did not differ significantly from the values in untreated preparations. However, the difference between the pK_B values of phentolamine against the two agonists was now about one log unit.

7 Pretreatment of the vascular strips with 6-hydroxydopamine (1.5×10^{-3} M) did not significantly affect responses to 5-HT or 5-CT, but almost eliminated those to tyramine. Cocaine (3×10^{-5} M) slightly potentiated noradrenaline-induced contractions, but did not significantly affect those induced by 5-HT.

8 These data suggest that: (i) 5-HT receptors mediating vasodilatation are not present in the rabbit renal artery smooth muscle or endothelium; (ii) the contractile effect of 5-HT does not involve the release of noradrenaline from sympathetic nerve stores; (iii) the 5-HT receptor in the rabbit renal artery is not of the 5-HT₂, 5-HT₃ or 5-HT₄ type. The pharmacological properties of this receptor most closely resemble those described for the heterogeneous 5-HT₁-like category.

Keywords: 5-Hydroxytryptamine, 5-hydroxytryptamine receptors; rabbit, renal artery; sumatriptan; 5-carboxamidotryptamine; 8-hydroxy-2(di-n-propylamino)tetralin; U46619 interactions; ketanserin; phentolamine

Introduction

There is a wide variation in the renal vascular response to 5-hydroxytryptamine (5-HT; serotonin) with investigators reporting either no change (Caldicott *et al.*, 1970), increases (McCubbin *et al.*, 1962), decreases (Vyden *et al.*, 1974; Saxena *et al.*, 1978; Wright & Angus, 1987) or even bi- or multiphasic changes (Blackshear *et al.*, 1986; Wright & Angus, 1989) in renal blood flow. Moreover, there are conflicting claims about the 5-HT receptors which mediate the effects of 5-HT in the renal vasculature. In the canine renal vasculature, Blackshear *et al.* (1986) reported 5-HT₁-like receptor-mediated vasodilator and 5-HT₂ receptor-mediated vasoconstrictor components, while Shoji *et al.* (1989) concluded that 5-HT₂ receptors mediate vasodilatation and 5-HT₁-like receptors are involved in vasoconstriction. In the rabbit, notwithstanding that 5-carboxamidotryptamine (5-CT), a selective agonist at 5-HT₁-like receptors (Bradley *et al.*, 1986; Saxena & Villalón, 1990), decreases renal blood flow, Wright & Angus (1987) have suggested that renal vasodilatation and vasoconstriction in this species is mediated by 5-HT₁-like and 5-HT₂ receptors, respectively. The vasoconstrictor effect of 5-CT has been

explained by it causing release of 5-HT from blood platelets (Wright & Angus, 1989). In view of this and other complicating factors operating in intact animals, such as prostaglandin release (Blackshear *et al.*, 1986) and sympathetic neuro-inhibition (Charlton *et al.*, 1986), the present study was performed in the isolated renal artery of the rabbit. Using a number of agonist and antagonist compounds, we have attempted to characterize the nature of 5-HT receptors in this tissue.

Methods

Tissue preparation

New Zealand white rabbits (ENKI, Helmond, The Netherlands), weighing 2.0–2.8 kg, were killed by injections of pentobarbitone sodium (Nembutal; 60 mg kg⁻¹) into a marginal ear vein. The right and left renal arteries were cleaned of fat and connective tissue *in vitro*, and placed in Krebs solution (composition in mM: NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 8.3; pH 7.4). Arteries were either used on the same day or after overnight storage at 4°C in the Krebs solution. The maximum effect and pD_2 value of 5-HT were unaffected by storage.

¹ Author for correspondence.

Ring segments (3–5 mm in length) of rabbit renal artery were placed in organ bath chambers filled with 10 ml of Krebs solution maintained at 37°C and bubbled constantly with a mixture of 95% O₂ and 5% CO₂. The segments were carefully suspended between two stainless steel wire hooks (0.2 mm diameter) inserted into the lumen to record changes in isometric tension. No attempt was made to remove the endothelium. Tension was measured with a Harvard isometric transducer. Preparations were maintained at an initial resting tension of 2 g. During the equilibration period, the Krebs solution was changed at 15 min intervals and the vascular segments were re-stretched to the resting tension. This procedure was repeated three times. After equilibration, contractile responses to 30 mM KCl were ascertained and the preparations washed; the responses to KCl and subsequent washings were repeated once again. Concentration-effect curves were then established for 5-HT and other agonists.

The relaxant effects of 5-HT (in the presence of 10⁻⁵ M ketanserin to avoid 5-HT₂ receptor-mediated responses) and 5-CT were studied in vascular segments precontracted to a near maximal level with 100 nM U46619. The contractile effects of 5-HT and other agonists were investigated in quiescent vessels (i.e. without precontraction with U46619). In all vessel segments precontracted with U46619, and in about one third of the experiments on quiescent vessels, 10⁻⁷ M methacholine was added in the bath at the end of the experiment to verify endothelial function. In all cases, a relaxant response to methacholine was observed thus showing the viability of the endothelium.

Determination of agonist potency

5-HT was added to the fluid bathing the renal artery preparations by a cumulative concentration schedule, allowing sufficient time for the effects of each concentration to become fully established before adding the next concentration, until a maximum response ($E_{\max}$) was obtained or relaxation started to occur. After frequent washing, tissues were left for at least 30 min before re-challenging with either 5-HT or another agonist. On each preparation, one or two concentration-effect curves to 5-HT were obtained; preliminary experiments showed that at least three consecutive concentration-effect curves to 5-HT were reproducible. One preparation was used as a control in which the concentration-effect curve to 5-HT was repeated, while concentration-effect curves to various other agonists were constructed on other preparations from the same animal. Not more than two agonists were tested on one preparation.

Determination of antagonist potency

Concentration-effect curves to 5-HT were established in the rabbit renal artery as described. One preparation was used as a control and a single concentration of antagonist was added to the other organ baths. An antagonist equilibration time of 30 min was allowed before agonist concentration-effect curves were re-determined.

Acute sympathetic denervation and uptake blockade

In vitro sympathetic denervation of the renal artery segments was achieved by the method described by Aprigliano & Hermesmeyer (1976) with certain modifications. Briefly, a solution (composition in mM: NaCl 112, KCl 5, CaCl₂ 1.25, MgSO₄ 1.2, glucose 11.5 and glutathione 0.1; pH 4.9) was supplemented with 1.5 × 10⁻³ M 6-hydroxydopamine. After incubation in this solution for 15 min at 37°C, the renal artery segments were washed several times and equilibrated in Krebs solution for a further 60 min. Paired experiments were performed on preparations treated either with 6-hydroxydopamine or the vehicle alone. The effectiveness of the sympathetic denervation was checked by measuring contractions elicited by 10⁻⁴ M tyramine. After washing,

concentration-effect curves were constructed for 5-HT and 5-CT in both control and 6-hydroxydopamine-treated segments.

The effect of sympathetic neuronal uptake blockade on the responses to 5-HT and noradrenaline were studied in experiments conducted in the continuous presence of cocaine (3 × 10⁻⁵ M).

Analysis of the data

The concentration-effect curves for the agonists were analysed by means of a computerized curve fitting technique described by De Lean *et al.* (1978). The parameters thus obtained (i.e. pD₂ and $E_{\max}$) were averaged for the respective agonists. In the case of antagonists, the shifts in the agonist-induced concentration-effect curves were calculated from the pD₂ values in each individual preparation before and after addition of an antagonist. pK_B values were calculated from equations described by Kenakin (1987), with the assumption that the antagonism was competitive. All data are presented as mean ± s.e.mean. Student's *t* test was used to evaluate the significance of the differences in pD₂ and $E_{\max}$ values.

Materials

The compounds used in this study were kindly supplied by, or purchased from, the companies mentioned: 5-carboxamidotryptamine maleate (gift: Glaxo, Ware, U.K.), cocaine (Sigma Chemical Co., St Louis, U.S.A.), 1-(2,5-dimethoxy-4-iodo-phenyl)-2-aminopropane hydrochloride (DOI) (Research Biochemicals Inc., Natic, U.S.A.), fleroxan hydrochloride (gift: Duphar B.V., Weesp, The Netherlands), glutathione (E. Merck, Amsterdam, The Netherlands), 6-hydroxydopamine hydrochloride (Sigma Chemical Co., St Louis, U.S.A.), 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Co., St Louis, U.S.A.), ketanserin tartrate (gift: Janssen Pharmaceutica, Beerse, Belgium), metergoline (gift: Farmitalia, Milan, Italy), methacholine bromide (Janssen Chimica, Beerse, Belgium), methiothepin methane sulphate (gift: Hoffman La Roche, Mijdrecht, The Netherlands), 5-methoxytryptamine hydrochloride (Research Biochemicals Inc., Natic, U.S.A.), α-methyl-5-hydroxytryptamine maleate (gift: Glaxo, Ware, U.K.), MDL 72222 ([1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate]; gift: Marion Merrell Dow Research Institute, Strasbourg, France), noradrenaline hydrochloride (gift: Fluka AG, Switzerland), 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT); gift: Marion Merrell Dow Research Institute, Strasbourg, France), phentolamine hydrochloride (gift: Ciba-Geigy, Basel, Switzerland), RU 24969 ([5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate]; gift: Roussel Laboratories, Hoevelaken, The Netherlands), sumatriptan (GR 43175; gift: Glaxo, Ware, U.K.), tyramine hydrochloride (gift: Fluka AG, Switzerland) and U46619 ([9,11-dideoxy-11α,9α-epoxymethano- prostaglandin F_{2α}]; The Upjohn Chemical Company, Kalamazoo, U.S.A.).

Results

Investigation of vasodilator responses

The possibility that 5-HT and 5-CT may elicit vasodilatation in the rabbit isolated renal artery was investigated in vessels precontracted with 100 nM of the thromboxane receptor agonist, U46619, which increased the vessel tone submaximally by 2.4 ± 0.3 g (*n* = 11). As illustrated in Figure 1, neither 5-HT (10⁻⁸ to 10⁻⁴ M; in vessels treated with ketanserin to avoid 5-HT₂ receptor-mediated responses) nor 5-CT (10⁻⁸ to 3 × 10⁻⁴ M) relaxed precontracted renal artery segments, which, as expected, responded to 10⁻⁷ M methacholine with a decrease in tone. Instead, both 5-HT and 5-CT caused further contractions. In these vessel segments precontracted with U46619, the pD₂ and $E_{\max}$ values were 7.1 ± 0.3 and

Figure 1 Effects of different concentrations of 5-hydroxytryptamine (5-HT) (a) and 5-carboxamidotryptamine (5-CT) (b) on rabbit isolated renal artery segments precontracted with U46619 (10^{-7} M). Ketanserin (10^{-5} M) was added to the segment exposed to 5-HT to antagonize 5-HT_2 receptors. At each dot (•) increasing (half log molar sequence) concentrations of 5-HT (10^{-8} to 10^{-4} M) or 5-CT (10^{-8} to 3×10^{-4} M) were added. Neither compound elicited vasorelaxation; instead contractile responses were observed. Administration of methacholine (10^{-7} M) resulted in relaxation of the tissues.

1.7 ± 0.5 g ($n = 6$), respectively for 5-HT and 7.9 ± 0.2 and 1.0 ± 0.2 g ($n = 5$), respectively, for 5-CT.

Investigation of contractile responses

Agonist studies In the absence of U46619, 5-HT caused contractions of the rabbit renal artery, but with a reduced potency (pD_2 ; 5.1 ± 0.1 ; $E_{\max}$ 3.6 ± 0.2 g; $n = 20$). As illustrated in Figure 2, the 5-HT-induced concentration-effect curves were reproducible; the $E_{\max}$ ($109.5 \pm 2.5\%$ of the first curve) and pD_2 (5.2 ± 0.1) values of the second concentration-

Table 1 The pD_2 and $E_{\max}$ values of a number of 5-hydroxytryptamine (5-HT) receptor agonists calculated from concentration-effect curves obtained on the rabbit isolated renal artery

Agonist	pD_2	$E_{\max}$
α -Methyl-5-HT	5.7 ± 0.2	$109 \pm 4^*$
Sumatriptan	5.3 ± 0.2	$52 \pm 13^*$
5-HT 1st curve	5.1 ± 0.1	100
5-HT 2nd curve	5.2 ± 0.2	$109 \pm 3^*$
8-OH-DPAT	5.0 ± 0.3	$87 \pm 6^*$
5-CT	4.7 ± 0.2	$90 \pm 2^*$
5-Methoxytryptamine	4.3 ± 0.2	117 ± 14
DOI	Inactive	
Flesinoxan	Inactive	
RU 24969	Inactive	

For abbreviations, see Materials.

The pD_2 value and maximal response ($E_{\max}$) were calculated by a computerized analysis of the individual curves according to De Lean *et al.* (1978). Values are mean $\pm$ s.e.mean ($n = 4-6$). The maximal response is expressed as percentage of the initial $E_{\max}$ obtained for 5-HT. * $P < 0.05$ vs. 5-HT 1st curve.

effect curves were not significantly different from those of the first curves. The 5-HT receptor agonists DOI, flesinoxan and RU 24969 elicited either small or no contractions but α -methyl-5-HT, 5-CT, 8-OH-DPAT, sumatriptan and 5-methoxytryptamine all caused concentration-dependent contractions (Figure 2). The pD_2 and $E_{\max}$ values for each agonist are shown in Table 1. The rank order of potency was α -methyl-5-HT > sumatriptan = 5-HT = 8-OH-DPAT > 5-CT > 5-methoxytryptamine. The $E_{\max}$ developed by sumatriptan was significantly ($P < 0.05$) less than that of the other agonists.

It should be noted that, in the absence of U46619, the pD_2 values were lower and the $E_{\max}$ values were higher than those obtained for 5-HT and 5-CT in the presence of U46619. While the differences in the pD_2 values may represent an increase in the affinity of 5-HT and 5-CT in the presence of U46619 (see Discussion), the decrease in the $E_{\max}$ may just be due to the fact that the tissues probably cannot contract further.

Effects of antagonists and of cocaine on contractile responses to 5-HT, 5-CT, sumatriptan and noradrenaline

The 5-HT receptor antagonists methiothepin, ketanserin, metergoline, MDL 72222 and the α -adrenoceptor antagonist,

Figure 2 Rabbit isolated renal artery: concentration-effect curves to: 5-hydroxytryptamine (5-HT) 1st curve (○); 5-HT, 2nd curve (●); 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (▽); α -methyl-5-HT (□); and 5-methoxytryptamine (▼) (a) and to: 8-hydroxy-2-(di-n-propylamino)tetralin (Δ); 5-carboxamidotryptamine (▲); sumatriptan (■); flesinoxan (◆) and RU 24969 (+) (b). Points represent mean (with s.e.mean shown by vertical bars) ($n = 4-6$) of the increase in force expressed as a percentage of the initial maximal responses to 5-HT.

Figure 3 Rabbit isolated renal artery: concentration-effect curves to 5-hydroxytryptamine (5-HT) in the absence (●) and presence (open symbols) of ketanserin, MDL 72222, metergoline or methiothepin. The concentrations of the antagonists used were 10^{-8} (○), 10^{-7} (△) and 10^{-6} (□) M. Points represent mean (with s.e.mean shown by vertical bars) ($n = 4-6$) of the increase in force expressed as a percentage of the initial maximal responses to 5-HT.

Figure 4 Rabbit isolated renal artery: concentration-effect curves to 5-carboxamidotryptamine (5-CT, a) and sumatriptan (b) in the absence (●) and presence (open symbols) of ketanserin, metergoline or methiothepin. The concentrations of the antagonists used were 10^{-7} (△) and 10^{-6} (□) M. Points represent mean (s.e.mean shown by vertical bars) ($n = 4-6$) of the increase in force expressed as a percentage of the initial maximal responses to 5-HT.

phentolamine, were investigated as antagonists of the contractile responses elicited by 5-HT, 5-CT and sumatriptan as well as by noradrenaline in the rabbit isolated renal artery. MDL 72222 (10^{-6} M) and metergoline (10^{-8} and 10^{-7} M) did not antagonize the effects of 5-HT, but metergoline had some contractile activity at 10^{-7} M. Both ketanserin (10^{-6} M) and methiothepin (10^{-8} and 10^{-7} M) antagonized 5-HT-induced contractile responses (Figure 3).

The responses to 5-CT and sumatriptan were not significantly affected by the presence of 10^{-7} M metergoline, but in the presence of 10^{-6} M ketanserin they were shifted significantly to the right. Responses to the agonists were non-competitively antagonized by 10^{-7} M methiothepin (Figure 4).

Phentolamine (10^{-6} M) produced small rightward shifts of the concentration-effect curves of 5-HT, 5-CT and sumatriptan (Figure 5).

The effects of methiothepin (10^{-7} M) and ketanserin (10^{-6} M) on noradrenaline-induced contractions are shown in Figure 6. Ketanserin did not significantly affect the responses to noradrenaline, but methiothepin antagonized the responses, producing a parallel shift to the right of the concentration-effect curve.

Since the concentration-effect curves to 5-HT, 5-CT and sumatriptan were obtained over the range 10^{-6} to 10^{-4} M, the antagonistic activity of most compounds had to be restricted to determination of shifts in concentration-effect curves produced by a single concentration of the antagonist. Therefore, where applicable, only antagonist affinities (pK_B) were calculated (Kenakin, 1987) from the results and these are listed in Table 2. Ketanserin, being equiactive against 5-HT, 5-CT and sumatriptan, was less potent than methiothepin. The pK_B

Table 2 The pK_B values for the antagonism of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), sumatriptan and noradrenaline by ketanserin, methiothepin and phentolamine in the rabbit isolated renal artery

Agonist	Ketanserin	Antagonist Methiothepin	Phentolamine
<i>In the absence of cocaine</i>			
5-HT	6.6 ± 0.1	8.6 ± 0.4	$6.8 \pm 0.1^*$
5-CT	6.7 ± 0.4	NC	6.9 ± 0.1
Sumatriptan	6.6 ± 0.2	NC	$6.8 \pm 0.1^*$
Noradrenaline	Inactive	8.1 ± 0.2	7.3 ± 0.1
<i>In the presence of cocaine (3×10^{-5} M)</i>			
5-HT	—	—	$6.7 \pm 0.1^*$
Noradrenaline	—	—	7.6 ± 0.4

The pK_B values were calculated by an analysis of the individual curves according to Kenakin (1987). Values are mean $\pm$ s.e.mean ($n = 4-6$). NC; Not calculated as there was a near complete suppression of responses in the presence of 10^{-7} M of the antagonist.

* $P < 0.05$ vs. corresponding pK_B values against noradrenaline.

values for phentolamine against 5-HT, 5-CT and sumatriptan were also similar, but they were slightly less (half a log unit) than that found when noradrenaline was the agonist. The effects of phentolamine on the responses to 5-HT and noradrenaline were also studied in the presence of cocaine (3×10^{-5} M). Whether cocaine was used or not, the pK_B

Figure 5 Rabbit isolated renal artery: concentration-effect curves to 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and sumatriptan in the absence (●) and presence of phentolamine (10^{-6} M; □). Points represent mean (s.e.mean shown by vertical bars) ($n = 4-6$) of the increase in force expressed as a percentage of the initial maximal responses to 5-HT.

Figure 6 Rabbit isolated renal artery: concentration-effect curves to noradrenaline (NA) in the absence (●) and presence (open symbols) of ketanserin and methiothepin. The concentrations of the antagonists used were 10^{-7} (Δ) and 10^{-6} M (□). Points represent mean (s.e.mean shown by vertical bars) ($n = 4-6$) of the increase in force expressed as a percentage of the initial maximal responses to noradrenaline.

Table 3 The responses to 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), tyramine and noradrenaline observed in rabbit isolated renal artery segments, with or without treatment with 6-hydroxydopamine (1.5×10^{-3} M) and cocaine (3.0×10^{-5} M)

		pD_2	E_{max} (g)
<i>6-Hydroxydopamine</i>			
5-HT	Untreated ($n = 8$)	5.8 ± 0.3	5.1 ± 0.4
	Treated ($n = 4$)	5.8 ± 0.3	4.4 ± 0.3
5-CT	Untreated ($n = 6$)	4.9 ± 0.2	4.6 ± 0.7
	Treated ($n = 4$)	4.7 ± 0.4	4.2 ± 0.6
Tyramine	Untreated ($n = 12$)	—	$4.4 \pm 0.4\ddagger$
	Treated ($n = 8$)	—	$0.6 \pm 0.1\ddagger$
<i>Cocaine</i>			
5-HT	Before ($n = 6$)	5.1 ± 0.1	3.5 ± 0.6
	After ($n = 4$)	5.0 ± 0.1	3.7 ± 0.8
Noradrenaline	Before ($n = 7$)	6.0 ± 0.1	4.7 ± 0.1
	After ($n = 4$)	$6.8 \pm 0.1^*$	5.6 ± 0.5

The pD_2 values and maximal responses (E_{max}) were calculated by a computerized analysis of the individual curves according to De Lean *et al.* (1978). Values are mean $\pm$ s.e.mean. Number of observations in parentheses.

* Significantly different ($P < 0.05$) from the corresponding values before treatment.

‡ Response to a single concentration (10^{-4} M) of tyramine.

values of phentolamine against 5-HT (6.7 ± 0.1 and 6.8 ± 0.1 , respectively) and noradrenaline (7.6 ± 0.4 and 7.3 ± 0.1 , respectively) were not statistically different. However, the difference between the pK_B values of phentolamine against the two agonists was more marked (about one long unit) in the presence of cocaine than in its absence (Table 2).

Effects of 6-hydroxydopamine and cocaine on the contractile responses to 5-HT, 5-CT, tyramine and noradrenaline

Table 3 presents the pD_2 and E_{max} values of agonists acting on the rabbit isolated renal artery before and after treatment with either 6-hydroxydopamine or cocaine. Treatment with 6-hydroxydopamine markedly attenuated the effects of tyramine, but responses to 5-HT and 5-CT were not significantly affected. The potency of noradrenaline but not that of 5-HT, was significantly increased by cocaine.

Discussion

The aim of the present study was to examine the effects of 5-HT, and the receptors involved in mediating them, in the isolated renal artery of the rabbit. Unlike the results obtained in whole animals (McCubbin *et al.*, 1962; Blackshear *et al.*, 1986; Wright & Angus, 1989; Shoji *et al.*, 1989) or in the perfused isolated kidney (Verbeuren *et al.*, 1990), no vasodilatation was observed with either 5-HT or 5-CT when the renal artery segments were precontracted with U46619. This suggests that the receptors mediating vasodilator responses to 5-HT are predominantly located on arterioles and not on the large conduit renal artery. On the other hand, as observed in many investigations *in vivo* (Vyden *et al.*, 1974; Saxena *et al.*, 1978; Blackshear *et al.*, 1986; Wright & Angus, 1987; Shoji *et al.*, 1989), 5-HT did elicit a contractile response. This response was further analysed by comparing the effects of 5-HT with those of a series of potent 5-HT receptor agonists (5-CT, 8-OH-DPAT, RU 24969, sumatriptan, flesinoxan, α -methyl-5-HT, 5-methoxytryptamine and DOI) and of noradrenaline, and by examining the antagonist properties of ketanserin, methiothepin, MDL 72222, metergoline and phentolamine.

The nature of the 5-HT receptor mediating renal artery contractions

The scheme proposed by Bradley *et al.* (1986) has served as a framework for the classification of 5-HT receptors. MDL 72222 (Fozard, 1984) and ketanserin (Feniuk *et al.*, 1985) are highly potent antagonists at 5-HT₃ and 5-HT₂ receptors, respectively. In the present experiments we found that MDL 72222 was inactive and that ketanserin was only a weak antagonist of responses to 5-HT. The resistance to blockade by MDL 72222 suggests that 5-HT₃ receptors are not involved. The weak antagonism by ketanserin (pK_B : 6.6 against about 8.5 at 5-HT₂ receptors) and the inactivity of the selective 5-HT₂ receptor agonist DOI (Richardson & Hoyer, 1990; Saxena & Villalón, 1990) suggests that 5-HT₂ receptors are also not involved. Though another 5-HT₂ receptor agonist, α -methyl-5-HT (Hoyer, 1989; Saxena & Villalón, 1990), contracted renal artery, this compound is also a potent 5-HT₁-like receptor agonist (Feniuk *et al.*, 1985; Ismaiel *et al.*, 1990). Indeed, the mimicking of the contractile effect of 5-HT by other 5-HT₁-like receptor agonists (5-CT, 8-OH-DPAT, 5-methoxytryptamine and sumatriptan) and the potent antagonist action of methiothepin not only rule out the involvement of putative 5-HT₄ receptors (see Saxena & Villalón, 1990), but also suggest that 5-HT₁-like receptors may mediate the 5-HT-induced contractions of rabbit renal artery.

However, it must be pointed out that the effects of the 5-HT receptor agonists are not entirely in keeping with the participation of 5-HT₁-like receptors in 5-HT-induced contractions of the rabbit isolated renal artery. The pD_2 values of the different 5-HT₁-like receptor agonists in the quiescent vessels (i.e. without precontraction with U46619) were substantially lower (4.3 to 5.7; Table 1) than might be expected from their affinity at 5-HT₁ binding sites (see Hoyer *et al.*, 1985; Hoyer, 1989; Saxena & Villalón, 1990). Moreover, in contrast to the accepted criteria (Bradley *et al.*, 1986), 5-CT (pD_2 : 4.7) proved to be less potent than 5-HT (pD_2 : 5.1). Surprisingly however, in the vessels precontracted with U46619 (used primarily for the purpose of detecting vasodilatation), the pD_2 values of 5-CT (7.9) and 5-HT (7.1) were nearer to those that are usually reported for 5-HT₁-like receptor-mediated responses (Bradley *et al.*, 1986; Hoyer, 1989; Saxena & Villalón, 1990). The reasons for the changes in pD_2 values of 5-CT and 5-HT are not known, but a very similar phenomenon has recently been described for the 5-HT₁-like receptors in the guinea-pig isolated iliac artery where 5-HT, 5-CT and sumatriptan were virtually ineffective in the absence of tone, but generated appreciable force when tissues were precontracted with threshold concentrations of prostaglandin F_{2 α} (Sahin-Erdemli *et al.*, 1991).

The antagonism by ketanserin of the responses to 5-HT, 5-CT and sumatriptan could reflect a low affinity of this drug for the 5-HT₁-like receptors present in the rabbit renal artery. Ketanserin does attenuate 5-HT₁-like receptor-mediated contractions in some other vascular tissues, such as the rabbit saphenous vein (Martin & MacLennan, 1990; Van Heuven-Nolsen *et al.*, 1990) and porcine cranial arteriovenous anastomoses (Saxena *et al.*, 1989; Den Boer *et al.*, 1991). These vessels are contracted by 5-HT, 5-CT and sumatriptan and the effects of these agonists are effectively antagonized by methiothepin, but only weakly by ketanserin.

Does the putative 5-HT₁-like receptor in the renal artery resemble any of the known 5-HT₁ receptor subtypes?

Bradley *et al.* (1986) have pointed out that 5-HT₁-like receptors are heterogeneous and several subtypes, some having a corresponding binding site (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D}), have been described (see Saxena & Villalón, 1990). In order to characterize further the 5-HT₁-like receptor in the rabbit renal artery, experiments were carried out with compounds that are considered relatively selective for the putative

subtypes: 8-OH-DPAT and flesinoxan (5-HT_{1A}; Middlemiss & Fozard, 1983; Richardson & Hoyer, 1989), RU 24969 (5-HT_{1A} and 5-HT_{1B}; Hoyer *et al.*, 1985), sumatriptan (5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}; Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989; Sumner & Humphrey, 1989), DOI (5-HT_{1C} and 5-HT₂; Hoyer, 1989), α -methyl-5-HT (low affinity for all four subtypes; Hoyer, 1989; Ismaiel *et al.*, 1990) and metergoline (high affinity for all four subtypes; Hoyer, 1989). A contribution by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} receptors to responses to 5-HT can be excluded since RU 24969, flesinoxan and DOI were inactive and several of the agonists effective in this study (5-CT, 8-OH-DPAT and sumatriptan) have very low affinities for 5-HT_{1C} binding sites. The ineffectiveness of metergoline against the renal vascular contractions elicited by 5-HT, 5-CT and sumatriptan tends to rule out the involvement of the 5-HT_{1D} subtype as well. There are several examples of vascular locations of 5-HT₁-like receptors not entirely resembling any of the above subtypes: the dog and rabbit saphenous vein (Feniuk *et al.*, 1985; Humphrey *et al.*, 1988; Van Heuven-Nolsen *et al.*, 1990), human basilar artery (Parsons *et al.*, 1989) or porcine cranial arteriovenous anastomoses (Saxena *et al.*, 1989; Den Boer *et al.*, 1991). Though the rank order of potency (5-CT > 5-HT > α -methyl-5-HT > sumatriptan > 8-OH-DPAT = RU 24969) found at the dog saphenous vein 5-HT₁-like receptor differs from that (α -methyl-5-HT > sumatriptan = 5-HT = 8-OH-DPAT > 5-CT > 5-methoxytryptamine; RU 24969 inactive) found at the 5-HT₁-like receptor in the rabbit renal artery in the absence of tone, the relative potencies of 5-HT and 5-CT were similar in precontracted vessels.

Does the renal artery 5-HT receptor differ from α -adrenoceptors?

In view of the fact that the contractile effects of 5-HT in some blood vessels of the rabbit may be mediated by a direct action on α -adrenoceptors (Apperley *et al.*, 1976; Black *et al.*, 1981; Feniuk, 1984), we have attempted to distinguish between the effects of noradrenaline and 5-HT receptor agonists. Several observations indicate that noradrenaline and 5-HT contract the rabbit isolated renal artery by activating different receptors: firstly the pK_B values of phentolamine against 5-HT, 5-CT and sumatriptan (6.8–6.9) were somewhat lower than that against noradrenaline (7.3); secondly, though the potency of phentolamine against noradrenaline and 5-HT did not differ significantly in vascular preparations in the absence or presence of cocaine, the difference between the pK_B values against the two agonists became more marked after cocaine treatment (Table 2); thirdly, ketanserin (10⁻⁶ M) did not significantly affect noradrenaline-induced renal artery contractions, but significantly affected responses to 5-HT, 5-CT and sumatriptan with pK_B values around 6.6; however, noradrena-

line may still act via α_2 -adrenoceptors in the presence of ketanserin; and, fourthly, not only 5-HT, but several other 5-HT₁-like receptor agonists, which are not known to activate α -adrenoceptors, contracted the rabbit renal artery in the present study.

The antagonism by phentolamine of 5-HT-, 5-CT- and sumatriptan-induced responses (pK_B values of 6.8–6.9) suggests that phentolamine may also antagonize the 5-HT₁-like receptor in the rabbit isolated renal artery. Indeed, it is known that phentolamine is an antagonist with low potency at 5-HT receptors (Hoffman & Lefkowitz, 1990), including the 5-HT₁-like receptors in the human isolated basilar artery (Parsons *et al.*, 1989).

Is the 5-HT-induced contraction of the renal artery mediated by noradrenaline release?

It has been reported that high concentrations of 5-HT can have indirect sympathomimetic actions by releasing noradrenaline (McGrath, 1977; Saxena & Villalón, 1990). Since relatively high concentrations of 5-HT, 5-CT and other agonists were needed to elicit renal artery contractions, it is possible that, like tyramine, these agents may release noradrenaline from sympathetic nerve endings. However, this is unlikely since the contractile responses to 5-HT and 5-CT were unaltered upon treatment of tissues with 6-hydroxydopamine, which clearly inhibited tyramine-induced contractions (see Table 3). *In vitro* treatment with 6-hydroxydopamine has been shown to produce noradrenergic denervation of vascular tissues (Aprigliano & Hermesmeyer, 1976). Furthermore, the response to 5-HT was not altered by cocaine, which moderately potentiated contractile responses to noradrenaline.

In conclusion, the present study shows that 5-HT contracts the rabbit isolated renal artery by activation of a 5-HT₁-like receptor. This effect was mimicked by a number of agonists having affinity for 5-HT₁-like receptors, but DOI, flesinoxan and RU 24969 were inactive. Methiothepin was a potent antagonist, while metergoline, ketanserin and MDL 72222 showed little or no antagonist activity. These results suggest that the renal vasoconstrictor effects of 5-CT in the conscious rabbit may not be entirely due to 5-HT₂ receptor activation following the release of 5-HT from blood platelets by 5-CT (Wright & Angus, 1989), but could be mediated, partly or entirely, by a 5-HT₁-like receptor on renal vessels. Such a 5-HT₁-like receptor mediating a short-lasting renal vasoconstriction has also recently been described in the anaesthetized dog (Cambridge *et al.*, 1991).

This work was partly supported by a grant from Glaxo Group Research, Ware, Hertfordshire.

References

- 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. Pharmacol.*, **58**, 211–221.
- APRIGLIANO, O. & HERMSMEYER, K. (1976). *In vitro* denervation of the portal vein and caudal artery of the rat. *J. Pharmacol. Exp. Ther.*, **198**, 568–577.
- BLACK, J.L., FRENCH, R.J. & MYLECHARANE, E.J. (1981). Receptors mechanism for 5-hydroxytryptamine in rabbit arteries. *Br. J. Pharmacol.*, **74**, 619–626.
- BLACKSHEAR, J.L., ORLANDI, C. & HOLLENBERG, N.K. (1986). Serotonin and the renal blood supply: Role of prostaglandins and the 5-HT₂ receptor. *Kidney Int.*, **30**, 304–310.
- BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563–576.
- CALDICOTT, W.H.H., HOLLENBERG, N.K. & ABRAMS, H.L. (1970). Characteristics of response of renal vascular bed to contrast media. Evidence for vasoconstriction induced by renin-angiotensin system. *Invest. Radiol.*, **5**, 539–547.
- CAMBRIDGE, D., WHITING, M.V. & BUTTERFIELD, L.J. (1991). 5-Carboxamidotryptamine induced renal vasoconstriction in the dog. In *Serotonin, Molecular Biology, Receptors and Function*. ed. Fozard, J.R. & Saxena, P.R. Basel: Birkhauser, (in press).
- CHARLTON, K.G., BOND, R.A. & CLARKE, D.E. (1986). An inhibitory prejunctional 5-HT₁-like receptor in the isolated perfused rat kidney. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **332**, 8–15.
- DE LEAN, A., MUNSON, P.J. & ROBBARD, R. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am. J. Physiol.*, **235**, 97–102.
- DEN BOER, M.O., VILLALÓN, C.M., HEILIGERS, J.P.C., HUMPHREY, P.P.A. & SAXENA, P.R. (1991). Role of 5-HT₁-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. *Br. J. Pharmacol.*, **102**, 323–330.

- FENIUK, W. (1984). An analysis of 5-hydroxytryptamine receptors mediating contraction of isolated smooth muscle. *Neuropharmacology*, **23**, 1467–1472.
- FENIUK, W., HUMPHREY, P.P.A. & WATTS, A.D. (1985). A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by use of selective agonists and antagonists. *Br. J. Pharmacol.*, **86**, 697–704.
- FOZARD, J.R. (1984). MDL 72222, a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **326**, 36–44.
- HOFFMAN, B.B. & LEFKOWITZ, R.J. (1990). Adrenergic receptor antagonists. In *The Pharmacological Basis of Therapeutics*, ed. Goodman Gilman, A., Rall, T.W., Nies, A.S. & Taylor, P. pp. 221–243. New York: Pergamon Press.
- HOYER, D. (1989). 5-Hydroxytryptamine receptors and effector coupling mechanisms in peripheral tissues. In *The Peripheral Actions of 5-hydroxytryptamine*, ed. Fozard, J.R. pp. 73–99. Oxford: Oxford University Press.
- HOYER, D., ENGEL, G. & KALKMAN, H.O. (1985). Molecular Pharmacology of 5-HT₁ and 5-HT₂ recognition sites in rat and pig brain membranes: radioligand binding studies with [³H]5-HT, [³H]8-OH-DPAT, (–)[¹²⁵I]iodocyanopindolol, [³H]mesulergine and [³H]ketanserin. *Eur. J. Pharmacol.*, **118**, 13–23.
- HUMPHREY, P.P.A., FENIUK, W., PERREN, M.J., CONNOR, H.E., OXFORD, A.W., COATES, I.H. & BUTINA, D. (1988). GR 43175, a selective agonist for 5-HT₁-like receptor in dog isolated saphenous vein. *Br. J. Pharmacol.*, **94**, 1123–1132.
- ISMAIEL, A.M., TITELER, M., MILLER, K.J., SMITHS, T.S. & GLENNON, R.A. (1990). 5-HT₁ and 5-HT₂ binding profiles of the serotonergic agents α -methyl-serotonin and 2-methyl-serotonin. *J. Med. Chem.*, **33**, 755–758.
- KENAKIN, T.P. (1987). *Pharmacologic Analysis of Drug Receptor Interaction*, p. 213. New York: Raven Press.
- MARTIN, G.R. & MACLENNAN, S.J. (1990). Analysis of the 5-HT receptor in rabbit saphenous vein exemplifies the problems of using exclusion criteria for receptor classification. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **342**, 111–119.
- MCCUBBIN, J.W., KANEKO, Y. & PAGE, I.H. (1962). Inhibition of neurogenic vasoconstriction by serotonin. Vasodilator action of serotonin. *Circ. Res.*, **11**, 74–83.
- McGRATH, M.A. (1977). 5-Hydroxytryptamine and neurotransmitter release in canine blood vessels, inhibition by low and augmentation by high concentrations. *Circ. Res.*, **41**, 428–435.
- MIDDLEMISS, D.N. & FOZARD, J.R. (1983). 8-Hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT₁ recognition site. *Eur. J. Pharmacol.*, **90**, 151–153.
- PARSONS, A.A., WHALLEY, E.T., FENIUK, W., CONNOR, H.E. & HUMPHREY, P.P.A. (1989). 5-HT₁-like receptors mediate 5-hydroxytryptamine-induced contraction of human isolated basilar artery. *Br. J. Pharmacol.*, **96**, 434–440.
- PEROUTKA, S.J. & MCCARTHY, B.G. (1989). Sumatriptan (GR 43175) interacts selectively with 5-HT_{1B} and 5-HT_{1D} binding sites. *Eur. J. Pharmacol.*, **163**, 133–136.
- RICHARDSON, B.P. & HOYER, D. (1990). Selective agonists and antagonists at 5-hydroxytryptamine receptor subtypes. In *Serotonin: from Cell Biology to Pharmacology and Therapeutics*, ed. Paolietti, R., Vanhoutte, P.M., Brunello, N. & Maggi, F.M. pp. 265–276. Dordrecht: Kluwer Academic Publishers.
- SAHIN-ERDEMLI, I., HOYER, D., STOLL, A., SEILER, M.P. & SCHOEFFTER, P. (1991). 5-HT₁-like receptors mediate 5-hydroxytryptamine-induced contraction in guinea-pig isolated iliac artery. *Br. J. Pharmacol.*, **102**, 386–390.
- SAXENA, P.R., BOM, A.H. & VERDOUW, P.D. (1989). Characterization of 5-hydroxytryptamine receptors in the cranial vasculature. *Cephalalgia*, **9** (Suppl. 9), 15–22.
- SAXENA, P.R., FORSYTH, R.P., JOHNSTON, B.M. & DE WERK, A. (1978). Regional and systemic haemodynamic changes evoked by 5-hydroxytryptamine in awake and anaesthetized rabbits. *Eur. J. Pharmacol.*, **50**, 61–68.
- SAXENA, P.R. & VILLALÓN, C.M. (1990). Cardiovascular effects of serotonin agonists and antagonists. *J. Cardiovasc. Pharmacol.*, **15** (Suppl. 7), S17–S34.
- SCHOEFFTER, P. & HOYER, D. (1989). How selective is GR43175? Interactions with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**, 135–138.
- SHOJI, T., TAMAKI, T., FUKUI, K., IWAO, H. & ABE, Y. (1989). Renal haemodynamic responses to 5-hydroxytryptamine (5-HT): involvement of the 5-HT receptor subtypes in the canine kidney. *Eur. J. Pharmacol.*, **171**, 219–228.
- SUMNER, M.J. & HUMPHREY, P.P.A. (1989). 5-HT_{1D} binding sites in porcine brain can be subdivided by GR43175. *Br. J. Pharmacol.*, **98**, 29–31.
- VAN HEUVEN-NOLSEN, D., TYSSE KLASSEN, T.H.M., LUO, Q. & SAXENA, P.R. (1990). 5-HT₁-like receptors mediate contractions in the rabbit saphenous vein. *Eur. J. Pharmacol.*, **191**, 375–382.
- VERBEUREN, T.J., LAUBIE, M., MENNECIER, P., PROST, J.-F. & ROCHAT, C. (1990). Does 5-HT_{1A} receptor activation cause renal vasodilation. *Eur. J. Pharmacol.*, **183**, 666.
- VYDEN, J.K., LENT, D., NAGASAWA, K., CARVAHLO, M., SERRUYA, A. & CORDAY, E. (1974). The effects of serotonin on regional haemodynamics in the vascular system. *J. Clin. Pharmacol.*, **14**, 434–441.
- WRIGHT, C.E. & ANGUS, J.A. (1987). Diverse vascular responses to serotonin in the conscious rabbit: effects of serotonin antagonists on renal artery spasm. *J. Cardiovasc. Pharmacol.*, **10**, 415–423.
- WRIGHT, C.E. & ANGUS, J.A. (1989). 5-Carboxamidotryptamine elicits 5-HT₂ and 5-HT₃ receptor-mediated cardiovascular responses in the conscious rabbit: Evidence for 5-HT release from platelets. *J. Cardiovasc. Pharmacol.*, **13**, 557–564.

(Received April 29, 1991)

Revised August 14, 1991

Accepted August 22, 1991)