Presence of vasoconstrictor 5HT₁-like receptors revealed by precontraction of rabbit isolated mesenteric artery

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1 A series of 5-hydroxytryptamine (5-HT) receptor agonists including 5-HT, 5-carboxamidotryptamine (5-CT) and sumatriptan produced little or no contraction of rabbit isolated mesenteric arteries under resting tone conditions, even at concentrations up to 10^{-4} M.

2 When the same agonists were retested in mesenteric artery preparations pre-contracted with the thromboxane-mimetic, U46619, each demonstrated concentration-related vasoconstrictor activity. 5-CT and 5-HT were especially potent and effective in this model giving EC_{50} values of 4.3×10^{-9} M and 1.6×10^{-8} M respectively and maximum effects equivalent to those of KCl 80 mM. In preparations precontracted by U46619 (conditions retained throughout the rest of the study) the order of agonist potency was 5-CT > 5-HT > RU 24969 = sumatriptan > 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) > cisapride.

3 The vasoconstrictor effects of 5-CT were competitively antagonized by methiothepin (pA_2 8.20) but resistant to antagonism by a range of other 5-HT receptor antagonists, i.e. pindolol (5-HT_{1A}/5-HT_{1B}), propranolol (5-HT_{1B}), spiperone (5-HT_{2A}), ondansetron (5-HT₃), ICS 205930 (5-HT₃/5-HT₄) and SDZ 205557 (5-HT₄). 5-CT responses were slightly antagonized by a high concentration of ritanserin (5-HT_{2A}/5-HT_{2C}). Responses to 5-HT and sumatriptan were also antagonized by methiothepin with similar affinity (pA_2/pK_B values ≈ 8.0).

4 Metergoline and rauwolscine (10^{-7} – 10^{-6} M) antagonized the effects of 5-CT in a non-competitive fashion giving pK_{Bapp} values of 7.13 (metergoline) and 6.86 (rauwolscine).

5 Vasoconstrictor responses to 5-HT were not modified in the presence of ritanserin (3×10^{-7} M) or spiperone (3×10^{-7} M) and only modestly antagonized by ketanserin (10^{-6} M) suggesting that 5-HT_{2A} receptors do not make a significant contribution in this model.

6 Hence, precontraction of rabbit mesenteric arteries reveals potent vasoconstrictor effects of 5-HT and related agonists. Based on the agonist potency order and the antagonist studies performed, the receptor subtype responsible has the characteristics of a 5-HT₁-like (probably 5-HT_{1D}) receptor. This study therefore demonstrates a particularly striking example of vasoconstrictor synergy involving 5-HT₁-like receptors.

Keywords: 5-HT₁-like receptors; 5-HT_{1D} receptors; vasoconstriction; rabbit isolated mesenteric arteries; 5-hydroxytryptamine (5-HT); 5-carboxamidotryptamine (5-CT); sumatriptan; vasoconstrictor synergy

Introduction

5-Hydroxytryptamine (5-HT) demonstrates potent vasoconstrictor effects *in vitro* or *in vivo*. Although in most vascular beds this effect is mediated by activation of the 5-HT_{2A} subtype, an additional or exclusive role for 5-HT₁-like receptors has also been determined in certain vessels. These 5-HT₁-like receptors have been identified on the basis of their sensitivity to antagonism by methiothepin, relative insensitivity to ketanserin and potent agonist effects of 5-carboxamidotryptamine (5-CT) and, to a lesser extent, sumatriptan, according to criteria originally established by Bradley *et al.* (1986). 5-HT₁-like receptor-mediated vasoconstriction is most closely associated with specific types of blood vessel, notably cerebral arteries from a variety of species, including man (Parsons *et al.*, 1989; Connor *et al.*, 1989; Hamel & Bouchard, 1991), canine or human coronary arteries (Parsons *et al.*, 1992) and canine saphenous vein (Humphrey *et al.*, 1988).

However, recent *in vitro* observations suggest a wider presence of vasoconstrictor 5-HT₁-like receptors than would be indicated by the above analysis. Furthermore, in certain vascular tissues, the 5-HT₁-like receptor is virtually 'silent' and becomes functional only if the tissue is maintained at or above a threshold level of vasoconstriction. To date, such observations have been made in guinea-pig iliac artery

(Sahin-Erdemli *et al.*, 1991), rabbit femoral artery (MacLennan & Martin, 1992), human coronary artery (Cocks *et al.*, 1993) and rabbit renal artery (Choppin & O'Connor, 1993). In the rabbit renal artery, we have demonstrated that agonists such as 5-HT, 5-CT and sumatriptan are virtually without activity under normal conditions of resting tone but show prominent methiothepin-sensitive spasmogenic effects in the presence of threshold vasoconstriction produced by histamine, U46619 (Choppin & O'Connor, 1993) or potassium chloride (Choppin & O'Connor, 1994).

In the present study, we have investigated the responsiveness of rabbit isolated mesenteric arteries to the vasoconstrictor effects of 5-HT and other 5-HT receptor agonists. We report that in this preparation, as in the examples cited above, 5-HT₁-like receptors (probably of the 5-HT_{1D}-subtype), mediate a vasoconstrictor effect which manifests itself only in pre-contracted vessels.

Methods

Male New Zealand white rabbits (2.0–2.2 kg) were killed by rapid exsanguination after being rendered unconscious by a blow to the neck. The main mesenteric arteries were cleared of adherent tissue or fat and placed in Krebs solution (composition in mM: NaCl 118, KCl 4.7, MgCl₂ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.6, NaHCO₃ 25.0 and glucose 11.7) containing

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prazosin 10^{-6} M and idazoxan 10^{-6} M to eliminate the possibility that 5-HT receptor agonists could directly or indirectly activate contractile α_1 - and α_2 -adrenoceptors (Black *et al.*, 1981; McGrath, 1977). The vascular endothelium was removed by passing a scored catheter through the arterial lumen. Ring segments (1.5–2 mm long) were suspended between two stainless steel wire hooks in 20 ml organ baths containing Krebs solution bubbled with 95% O₂:5% CO₂ at 37°C. Changes in isometric tension were recorded on a Grass polygraph model 7D.

Preparations were maintained at an initial resting tension of 1 g, repeatedly re-adjusted over a 2 h equilibration period during which the tissues were washed regularly. Following this, the tissues were first contracted by a maximal effective concentration of KCl (80 mM) with which all subsequent responses were compared. In a representative series of experiments the tension generated in response to 80 mM KCl was 3.23 ± 0.19 g ($n = 41$). A series of different 5-HT receptor agonists were tested for spasmogenic activity. A single cumulative agonist concentration-response curve was performed in each tissue, either in normal Krebs solution, or in the presence of a threshold vasoconstriction produced by the thromboxane-mimetic, U46619 (10^{-8} M). U46619 10^{-8} M caused a modest and sustained contraction (approximately 20–30% of the KCl maximum). With the exception of the preliminary studies which involved the comparison of resting tone conditions and pre-contracted tissues, all experiments were performed in tissues pre-contracted with U46619, 10^{-8} M. To evaluate the mechanism responsible for agonist-induced contraction, in certain experiments, receptor antagonists were added to the buffer solution 30 min before commencing the agonist concentration-response curve. Since a single agonist curve design was retained for the antagonist experiments, antagonist effects were analysed by comparing the antagonist-treated groups of tissues with the control group.

Contractions produced by agonists were measured as changes in tension from baseline (i.e. the tension immediately before agonist administration) and expressed as a percentage of KCl maximum as means $\pm$ s.e. Agonist potency and maximum response ($E_{\max}$) were calculated by computer-assisted regression analysis of the full (sigmoid) curves ('ALLFIT'). Agonist potency was expressed as EC_{50} values, i.e. the molar concentration necessary to produce 50% of the maximum agonist response. EC_{50} values obtained in the presence of a receptor antagonist were compared with control EC_{50} values and the significance of the difference between the two means was determined by a Fisher's test. Differences with $P < 0.05$ were considered significant. n values refer to the number of tissue rings tested, with the number of animals used shown in parenthesis afterwards. The antagonist potency of methiothepin was evaluated by linear regression analysis according to the Schild equation using mean agonist EC_{50} values obtained in the presence and absence of antagonist to give a pA_2 value. Where full agonist concentration-response curves could only be achieved in the presence of a single antagonist concentration, a pK_B value was estimated with the following formula:

$$pK_B = -\log [\text{antagonist}] + \log \left(\frac{\text{mean } EC_{50} \text{ in the presence of antagonist}}{\text{mean } EC_{50} \text{ control}} - 1 \right)$$

Where the effects of certain antagonists were insurmountable in nature, the apparent pK_B (pK_{Bapp}) was derived from a double-reciprocal regression as described by Kenakin (1993).

Drugs

Sources of compounds used were as follows: 5-HT creatinine sulphate, prazosin hydrochloride, (–)-propranolol hydrochloride, spiperone, U46619 (9,11-dideoxy-11 α , 9 α -epoxy-methano-prostaglandin F_{2a}) (Sigma); methiothepin maleate

(Roche); rauwolscine hydrochloride (Research Biochemicals Inc.); 5-CT sulphate and maleate, 8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT), 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H indole succinate (RU 24969), sumatriptan succinate, cisapride, idazoxan, (3 α -tropanyl)1H-indole-3-carboxylate hydrochloride (ICS 205-930), ondansetron hydrochloride, ketanserin tartrate, pindolol, cyanopindolol, metergoline, ritanserin and 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester (SDZ 205557) were all synthesized by the Department of Chemistry, Synthelabo Recherche.

Results

In mesenteric arteries bathed in normal Krebs solution under conditions of resting tone, the agonists used in this study exhibited little or no spasmogenic activity. Specifically, sumatriptan, RU 24969, 8-OH DPAT and cisapride were inactive at concentrations up to 10^{-4} M. 5-HT and 5-CT produced weak increases in tension at concentrations $> 10^{-7}$ M attaining $67 \pm 13\%$ and $31 \pm 12\%$ of the KCl maximum respectively at 10^{-4} M.

However, in tissues pre-contracted with U46619 (10^{-8} M), all the above agonists produced vasoconstrictor responses which were markedly more prominent than those observed in standard preparations. Figure 1 illustrates this comparison by showing concentration-response curves for 5-HT and sumatriptan in the presence and absence of U46619. In pre-

Figure 1 Comparison of the contractile effects of (a) 5-HT, (b) sumatriptan in rabbit mesenteric arteries either in normal Krebs buffer (○), or in Krebs buffer containing U46619 10^{-8} M (●) to cause a modest precontraction. All experiments were performed in the presence of prazosin 10^{-6} M and idazoxan 10^{-6} M. Contractile effects were expressed as percentages of the response to 80 mM KCl. Values shown are means $\pm$ s.e. $n = 6-7$ (3) (●) and $n = 5-7$ (2-3) (○). A single concentration-response curve was obtained in each tissue.

contracted tissues, the 5-HT concentration-response curve was displaced leftwards approximately 3000-fold giving a $E_{\max}$ of $114 \pm 5\%$ and a EC_{50} of $1.6 \pm 0.4 \times 10^{-8}$ M, $n = 6$ (3). The level of potentiation of 5-CT, sumatriptan and RU 24969 in the presence of U46619 was equally dramatic. The vasoconstrictor effects of each of the agonists in pre-contracted tissues is shown in Figure 2, $n = 6-9$ (3-5). 5-CT was the most potent compound tested with an EC_{50} of $4.3 \pm 0.8 \times 10^{-9}$ M and an $E_{\max}$ of $125 \pm 6\%$, $n = 8$ (5). RU 24969 exhibited a complex concentration-response curve which reached a plateau between 10^{-6} and 3×10^{-5} M but then showed a further response at 10^{-4} M, probably representing a second component. For this compound, calculation of the EC_{50} was based on concentrations up to 3×10^{-5} M. The relative order of agonist potencies (mean EC_{50} values $\pm$ S.E.) was: 5-CT ($4.3 \pm 0.8 \times 10^{-9}$ M) > 5-HT ($1.6 \pm 0.4 \times 10^{-8}$ M) > RU 24969 ($1.6 \pm 0.5 \times 10^{-7}$ M) = sumatriptan ($1.9 \pm 0.3 \times 10^{-7}$ M) > 8-OH DPAT ($8.0 \pm 1.8 \times 10^{-7}$ M) > cisapride ($> 10^{-5}$ M).

The sensitivity of the three key agonists, 5-CT, 5-HT and sumatriptan to antagonism by methiothepin was evaluated using antagonist concentrations in the range 1×10^{-8} – 3×10^{-7} M. Figure 3 shows the effects of methiothepin against 5-CT. Agonist-response curves were displaced rightwards in a concentration-related fashion. Schild analysis of these data gave a pA_2 value of 8.20 (95% confidence limits 7.92–8.48) with a slope of 0.98 which was not significantly different from 1, suggesting that the antagonism was competitive in nature. 5-HT and sumatriptan were similarly antagonized by methiothepin. In the case of 5-HT, the pA_2 value was 8.04 (95% confidence limits 7.66–8.42) (slope 1.17, not different from 1). The vasoconstrictor effects of sumatriptan were also antagonised concentration-dependently by methiothepin. Since sumatriptan is less potent than 5-CT and 5-HT, maximum agonist responses could not be clearly established after the higher concentrations of methiothepin. Therefore, antagonist affinity has been expressed as a pK_B estimate (8.20) calculated for the lowest dose of methiothepin (3×10^{-8} M) where a maximum response was achieved.

Metergoline and rauwolscine, over the concentration range 10^{-7} – 10^{-6} M, also antagonized the vasoconstrictor responses to 5-CT and 5-HT (Figures 4 and 5). These antagonists generally demonstrated non-competitive characteristics, i.e. depression of agonist maximum response accompanied by rightwards displacement of the agonist concentration-response curve which sometimes showed a reduced slope. The

pK_{Bapp} values calculated for metergoline 10^{-6} M were 7.13 against 5-CT and 7.86 against 5-HT. $E_{\max}$ values following 10^{-6} M metergoline were $80 \pm 4\%$ ($P < 0.01$) for 5-CT and $68 \pm 4\%$ ($P < 0.01$) for 5-HT. Rauwolscine antagonised 5-CT non-competitively ($pK_{Bapp} = 6.86$) with a $E_{\max}$ of $67 \pm 2\%$ ($P < 0.01$) in the presence of rauwolscine 10^{-6} M. However, rauwolscine (10^{-6} M) did not significantly reduce the maximum response to 5-HT. Therefore a pK_B value (6.87) was calculated for the highest antagonist concentration.

The effects of a range of other 5-HT receptor antagonists were tested against 5-CT-induced vasoconstriction and these results are summarised in Table 1. For antagonists that have potent 5-HT_{2A} blocking properties (spiperone, ketanserin, ritanserin), these experiments were also performed with 5-HT as agonist (Table 1). With the exception of ketanserin 10^{-6} M (pK_B 6.43) and ritanserin 3×10^{-7} M which demonstrated a weak effect (pK_{Bapp} 6.08), none of these antagonists modified

Figure 3 Antagonism by methiothepin of the vasoconstrictor effects of 5-carboxamidotryptamine (5-CT) in rabbit mesenteric arteries pre-contracted with U46619 (10^{-8} M). Cumulative concentration-response curves to the agonist were established under control conditions (●), or in the presence of 3×10^{-8} M (○), 10^{-7} M (△) or 3×10^{-7} M (□) methiothepin. The buffer also contained prazosin 10^{-6} M and idazoxan 10^{-6} M throughout. Contractile effects were expressed as a percentage of the response to 80 mM KCl. Values shown are means $\pm$ s.e. $n = 8$ (4–5). A single concentration-response curve was obtained in each tissue.

Figure 2 Comparison of the contractile effects of cumulative administration of various 5-hydroxytryptamine (5-HT) receptor agonists in rabbit mesenteric arteries pre-contracted with U46619 (10^{-8} M). Compounds shown at 5-CT (●), 5-HT (△), sumatriptan (○), RU 24969 (■), 8-OH-DPAT (▲) and cisapride (□). All experiments were performed in the presence of prazosin 10^{-6} M and idazoxan 10^{-6} M. Contractile effects were expressed as percentages of the response to 80 mM KCl. Values shown are means $\pm$ s.e. $n = 6-9$ (3–5). A single concentration-response curve was obtained in each tissue.

Figure 4 Antagonism by rauwolscine of the vasoconstrictor effects of 5-carboxamidotryptamine (5-CT) in rabbit mesenteric arteries pre-contracted with U46619 (10^{-8} M). Cumulative concentration-response curves to 5-CT were established under control conditions (●), or in the presence of 10^{-7} M (□), 3×10^{-7} M (○) or 10^{-6} M (△) rauwolscine. The buffer also contained prazosin 10^{-6} M and idazoxan 10^{-6} M throughout. Contractile effects were expressed as a percentage of the response to 80 mM KCl. Values shown are means $\pm$ s.e. $n = 6-7$ (4–5). A single concentration-response curve was obtained in each tissue.

the effects of 5-CT. 5-HT responses were also weakly antagonized by ketanserin ($pK_B = 6.45$) but unaffected by spiperone 3×10^{-7} M or ritanserin 3×10^{-7} M. The 5-HT₃ antagonist ondansetron, the 5-HT₄ antagonist SDZ 205557 and the mixed 5-HT₃/5-HT₄ antagonist ICS 205930 had no effect on responses to 5-CT (Table 1).

Figure 5 Antagonism by metergoline of the vasoconstrictor effects of (a) 5-carboxamidotryptamine (5-CT) and (b) 5-hydroxytryptamine (5-HT) in rabbit mesenteric arteries pre-contracted with U46619 (10^{-8} M). Cumulative concentration-response curves to each agonist were established under control conditions (●), or in the presence of 10^{-7} M (□), 3×10^{-7} M (○) or 10^{-6} M (△) metergoline. The buffer also contained prazosin 10^{-6} M and idazoxan 10^{-6} M throughout. Contractile effects were expressed as a percentage of the response to 80 mM KCl. Values shown are means $\pm$ s.e. $n = 5-8$ (2-5). A single concentration-response curve was obtained in each tissue.

Discussion

In this study, we report a striking amplification of the vasoconstrictor effects of a series of 5-HT receptor agonists in modestly pre-contracted (20–30% of KCl maximum) rabbit mesenteric arteries when compared with the same preparation under conditions of resting tone. For those agonists for which concentration-related responses could be detected using resting tone conditions (5-HT and 5-CT) their response curves were displaced rightwards by 3–4 orders of magnitude. Similarly, sumatriptan and RU 24969 which were inactive up to 10^{-4} M in normal buffer showed vasoconstrictor activity at concentrations as low as 10^{-8} M in the presence of U46619. In precontracted tissues, 5-HT receptor agonists were capable of producing contractions of a magnitude at least equivalent to the response to 80 mM KCl. Therefore, this study provides an excellent example of synergy between vasoconstrictors acting by different mechanisms. The subsequent discussion will characterize the receptor involved in the contractile effects of 5-CT and the other agonists as the 5-HT₁-like subtype. Our study therefore extends the range of blood vessels in which vasoconstrictor 5-HT₁-like receptors have been identified to include the rabbit mesenteric artery, and emphasizes the importance of the choice of experimental conditions. The application of this practice to other vascular tissues could reveal additional examples of this receptor type. Conversely, evaluation of 5-HT-receptor agonists under conditions of resting tone only, may lead to erroneous conclusions regarding the possible existence of 5-HT₁-like receptors.

We have already observed an apparently similar phenomenon in rabbit renal arteries (Choppin & O'Connor, 1993; 1994). The level of agonist activity noted in the present study indicates that the mesenteric artery is more sensitive than the renal artery in this respect. 5-CT (EC_{50} 4×10^{-9} M in rabbit mesenteric artery, E_{max} 125%) was approximately 10 times less potent with a lower E_{max} in rabbit renal arteries precontracted with either 22 mM KCl, histamine or U46619. Indeed, the vasoconstrictor potency of 5-CT in precontracted mesenteric arteries equals or exceeds that reported in tissues which contain functionally active 5-HT₁-like receptors under resting tone conditions, e.g. human pial arterioles (Hamel & Bouchard, 1991), rabbit basilar arteries (Parsons & Whalley, 1989).

We have studied a range of receptor agonists and antagonists in U46619-precontracted preparations with the objective of identifying the 5-HT receptor subtype responsible for agonist-induced vasoconstriction. The potent agonist activity of 5-CT (>5 -HT) clearly implicates a 5-HT₁-receptor. A role of 5-HT₃ and 5-HT₄ receptors was not anticipated given their non-vascular locations and can be rapidly eliminated based on the relatively low affinity of

Table 1 The potencies of 5-carboxamidotryptamine (5-CT) and 5-hydroxytryptamine (5-HT) in rabbit mesenteric arteries in the absence and presence of various receptor antagonists

Antagonist		EC_{50} 5-CT	EC_{50} 5-HT
Control		$2.9 \pm 0.7 \times 10^{-9}$ M	$1.6 \pm 0.4 \times 10^{-8}$ M
SDZ 205557	3×10^{-6} M	$1.7 \pm 0.7 \times 10^{-9}$ M	—
Pindolol	3×10^{-6} M	$3.2 \pm 0.1 \times 10^{-9}$ M	—
Propranolol	10^{-7} M	$4.3 \pm 1.4 \times 10^{-9}$ M	—
Ondansetron	10^{-6} M	$2.6 \pm 0.6 \times 10^{-9}$ M	—
ICS 205930	3×10^{-6} M	$2.6 \pm 0.6 \times 10^{-9}$ M	—
Ritanserin	3×10^{-7} M	$1.1 \pm 0.5 \times 10^{-8}$ M*	$1.3 \pm 0.3 \times 10^{-8}$ M
Ketanserin	10^{-6} M	$9.3 \pm 0.7 \times 10^{-9}$ M**	$6.4 \pm 2.1 \times 10^{-8}$ M**
Spiperone	3×10^{-7} M	$5.2 \pm 2.8 \times 10^{-9}$ M	$1.3 \pm 0.3 \times 10^{-8}$ M
Methiothepin	3×10^{-7} M	$1.8 \pm 0.5 \times 10^{-7}$ M**	$2.1 \pm 0.8 \times 10^{-6}$ M**

All studies were performed in tissue pre-contracted by U46619 and in the presence of prazosin 10^{-6} M and idazoxan 10^{-6} M. Studies of ritanserin and ketanserin against 5-CT were performed in a separate series of experiments in which the control EC_{50} value for 5-CT was $4.3 \pm 0.8 \times 10^{-9}$ M. Data for each antagonist represents a separate group of tissues. * $P < 0.05$, ** $P < 0.01$ versus control EC_{50} (Fisher's test). $n = 4-8$ (3-5).

5-CT for these subtypes (Eglen *et al.*, 1992), the inability of 5-HT₃ antagonists (ondansetron, ICS 205-930) and 5-HT₄-antagonists (SDZ 205557, ICS 205-930) to block 5-CT-induced vasoconstriction, and the weak spasmogenic effects of the 5-HT₄ agonist, cisapride. At least five 5-HT₁-subtypes have now been recognised on the basis of ligand binding studies and receptor cloning (Humphrey *et al.*, 1993), of which the recently-identified 5-HT_{1E} subtype is unlikely to be involved because it shows low affinity for 5-CT (McAllister *et al.*, 1992). Considering the remaining 5-HT₁-subtypes characterized by radioligand binding techniques, the relative order of agonist potencies observed in the present study (5-CT > 5-HT > RU 24969 = sumatriptan > 8-OH-DPAT) most closely resembles the 5-HT_{1D} recognition site (Hoyer, 1989; Hoyer & Schoeffter, 1991). Comparisons of relative agonist potencies obtained from a functional study (such as the present one) with relative binding site affinities ignore the possible dependence of the former on factors such as agonist efficacy and tissue receptor reserve. Nevertheless, the antagonist characterization performed also supports the involvement of a receptor similar to the 5-HT_{1D} subtype. The ineffectiveness of the 5-HT_{1A}/5-HT_{1B} antagonist pindolol and weak effects of the 5-HT_{2A}/5-HT_{2C} antagonist, ritanserin, confirm the exclusion of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} subtypes. Furthermore, methiothepin, a potent but non-selective 5-HT₁-antagonist, blocked the vasoconstrictor effects of 5-CT, 5-HT and sumatriptan with similar affinity giving pA₂/pK_B values within the range 8.0–8.5. The antagonist effects of rauwolscine are also consistent with this hypothesis since this potent α_2 -antagonist also shows significant affinity for 5-HT_{1D} binding sites (Hoyer & Schoeffter, 1991).

The lack of antagonist properties of the potent 5-HT_{2A} antagonists, spiperone and ritanserin and the weak inhibitory effects of ketanserin when tested against 5-HT induced contractions excludes a significant role for 5-HT_{2A} receptors since these antagonists were tested at concentrations 200–1000 times greater than their published affinity for 5-HT_{2A} receptors (Hoyer & Schoeffter, 1991). Hence, the vasoconstrictor properties of 5-HT receptor agonists in pre-contracted rabbit mesenteric arteries appear to be largely if not solely due to stimulation of a 5-HT₁-receptor with similarities to the 5-HT_{1D} binding site. The ability to eliminate a contribution from 5-HT_{2A} receptors is also important for the interpretation of the methiothepin data since, in addition to being a potent 5-HT₁-antagonist, methiothepin also blocks 5-HT_{2A} receptors at low concentrations (Hoyer & Schoeffter, 1991). The lack of a significant 5-HT_{2A} component distinguishes this tissue by comparison with rabbit femoral artery (MacLennan & Martin, 1992) and rabbit renal artery (Choppin & O'Connor, 1994), both of which demonstrate vasoconstriction mediated through dual 5-HT₁-like/5-HT_{2A} receptor populations.

The 5-HT₁-receptor implicated in the present study fulfills the criteria established by Bradley *et al.* (1986) for a func-

tional 5-HT₁-like receptor. Unfortunately, no direct radioligand binding site equivalent to these functional responses in vascular tissues has been identified and a recent revision of 5-HT receptor nomenclature concluded that such sites are still best described as 'vascular 5-HT₁ receptors' (Humphrey *et al.*, 1993). Analysis of agonist and antagonist characteristics indicates that the receptor present in the pre-contracted rabbit mesenteric artery is very similar to other 5-HT receptors mediating vasoconstriction. Notably, those in guinea-pig iliac artery (Schoeffter & Sahin-Erdemli, 1992), rabbit renal artery (Choppin & O'Connor, 1994) and human pial artery (Hamel & Bouchard, 1991) which have been variously classified as 5-HT₁-like, 5-HT_{1D}-like or 5-HT_{1D} in nature. We have already underlined the similarity of the receptor found in the rabbit mesenteric artery to the 5-HT_{1D} binding site, however, the relatively modest antagonist potency of metergoline in our study appears somewhat inconsistent with a 5-HT_{1D} classification. The possibility that metergoline sensitivity could provide a means of differentiating between the functional 5-HT₁-like vascular receptor and the 5-HT_{1D} recognition site has already been proposed (Parsons, 1991). Metergoline shows very high 5-HT_{1D} binding affinity (pIC₅₀ 9.1, Hoyer & Schoeffter, 1991), but is notably less active as an antagonist of 5-HT₁-like receptor-mediated vasoconstriction (Humphrey *et al.*, 1988; Hamel & Bouchard, 1991). Our own data tend to confirm this distinction; however, whether or not this difference alone is sufficient to disallow the 5-HT_{1D} classification is debatable.

Ketanserin was a weak antagonist of both 5-CT and 5-HT induced responses in our study (pK_B $\approx$ 6.4). At these relatively high concentrations such antagonist activity is clearly unrelated to 5-HT_{2A} receptors. These results are consistent with reports from other rabbit vascular preparations that ketanserin shows a low affinity for 5-HT₁-like receptors (Martin & MacLennan, 1990; Tadipatri *et al.*, 1991). In this context it is interesting to note that ketanserin is one of the few compounds capable of distinguishing between the cloned human 5-HT_{1DA}- and 5-HT_{1DB}-subtypes, showing moderate affinity for the former (pK_D 7.0–7.5) while being inactive at the latter (pK_D < 5) (Kaumann *et al.*, 1993). Although this could suggest that rabbit vascular 5-HT₁-like receptors are similar to the cloned 5-HT_{1DA}-subtype, the available data is insufficient allow a definitive conclusion. An additional factor which may complicate interpretation of low affinity antagonist properties of ketanserin in functional studies such as our own, is its weak calcium antagonist effects (Razzaque *et al.*, 1994).

In summary, this study demonstrates the existence in the rabbit mesenteric artery of 5-HT₁-like receptors mediating vasoconstriction. These receptors are functionally silent unless tissues are subjected to a modest pre-contraction, applied, in the case of this study, by the thromboxane-mimetic, U46619.

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