5-HT₁-like receptors mediate 5-hydroxytryptamine-induced contraction of guinea-pig isolated iliac artery

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1 The effects of 5-hydroxytryptamine (5-HT) and of the 5-HT₁-like receptor agonists, 5-carboxamidotryptamine (5-CT) and sumatriptan (GR43175) were investigated in isolated ring preparations of guinea-pig common iliac artery.

2 The three agonists induced very weak, if any, contractions of unstimulated preparations, whereas they elicited concentration-dependent contractions in preparations given a moderate tone by a threshold concentration of prostaglandin F_{2α} (PGF_{2α}).

3 Under the latter conditions, *E*_{max} values for 5-HT and 5-CT reached about 45% of PGF_{2α} maximal effect, whereas the *E*_{max} value of sumatriptan was significantly lower (about 35%). The rank order of potency (mean EC₅₀ value, nM) was 5-CT (6.6) > 5-HT (22.9) > sumatriptan (155). Pargyline, cocaine or deoxycorticosterone were without significant effect on the contractions induced by 5-HT.

4 The 5-HT₃ receptor antagonist, (1αH,3α,5αH-tropan-3-yl) 1-H-indole-3-carboxylic acid ester (ICS 205-930; 1 μM), had no effect on 5-HT-, 5-CT- and sumatriptan-induced contractions.

5 The 5-HT₂ receptor antagonist, ketanserin (1 μM) caused only small rightward shifts (concentration-ratios, about 2) in the concentration-response curves to 5-HT, 5-CT and sumatriptan without significantly depressing the maximum effects.

6 In the presence of ketanserin (1 μM), the non-selective 5-HT receptor antagonist, methiothepin (0.1 μM), shifted the concentration-response curves to 5-HT and 5-CT to the right in a parallel manner and to a similar extent for both agonists (respective mean pK_B values, 8.07 and 8.27). The effect of sumatriptan was also antagonized by methiothepin, but solvent effects precluded quantitative analysis of this antagonism.

7 It is concluded that 5-HT₁-like receptors mediate the contractions induced by 5-HT, 5-CT and sumatriptan in guinea-pig isolated iliac artery. For reasons not yet understood, these receptors are detected only when the tissues are moderately pre-contracted by PGF_{2α}.

Introduction

Contractile responses of blood vessels to 5-hydroxytryptamine (5-HT) are mostly mediated by 5-HT₂ receptors (for a review see Saxena, 1989). Notable exceptions are the dog isolated saphenous vein (Apperley *et al.*, 1980; Feniuk *et al.*, 1985; Humphrey *et al.*, 1988), isolated basilar arteries from various species including man (Bradley *et al.*, 1986b; Chang & Owman, 1989; Connor *et al.*, 1989; Parsons & Whalley, 1989; Parsons *et al.*, 1989), cranial arteriovenous anastomoses of pig and cat (Saxena *et al.*, 1986; Feniuk *et al.*, 1987) and dog carotid arterial bed (Feniuk *et al.*, 1989). In all these vessels, 5-HT₁-like receptors have been implicated in the vasoconstrictor response to 5-HT.

5-HT₁-like receptors have been defined as those 5-HT receptors satisfying the following three criteria: 5-carboxamidotryptamine (5-CT) should behave as an agonist and be at least as potent as 5-HT, itself; 5-HT₂ and 5-HT₃ receptor antagonists should be inactive; methiothepin should be a potent antagonist (Bradley *et al.*, 1986a). In addition, sumatriptan (or GR43175) has recently emerged as a selective 5-HT₁-like receptor agonist and as such is potentially a useful tool in the search for 5-HT₁-like receptors (Humphrey *et al.*, 1988).

In the present study, evidence is provided that 5-HT receptors fulfilling these criteria are present in guinea-pig isolated iliac artery. Contraction mediated by these 5-HT₁-like receptors is unmasked when the vessels are moderately precontracted by prostaglandin F_{2α} (PGF_{2α}). A preliminary account of this work was presented at the 1990 Spring Meeting of the

German Pharmacological Society (Schoeffter & Sahin-Erdemli, 1990).

Methods

Experimental protocol

Male albino guinea-pigs (250–300 g) were killed by a blow to the neck. The abdominal aorta, iliac and femoral arteries were exposed, removed and placed in a Krebs bicarbonate solution (composition in mM: NaCl 112, KCl 5, NaHCO₃ 25, KH₂PO₄ 1, MgSO₄ 1.2, CaCl₂ 1.25 and glucose 11.5). The right and left common iliac arteries were carefully cleaned of loosely adherent tissue under a microscope. Two ring segments (1.5–2 mm long) were cut in each iliac artery. No attempt was made to remove the endothelium. Rings were mounted between L-shaped stainless steel wires in 10 ml organ baths filled with Krebs bicarbonate solution bubbled with 95% O₂:5% CO₂ at 37°C. An initial tension of 0.5 g was applied and repeatedly readjusted during a 2 h period of washing and equilibration. Isometric changes in tension were recorded. Rings were first contracted by a maximally effective concentration of PGF_{2α} (30 μM). When the contraction had plateaued, carbachol (10 μM) was added and tissues were allowed to relax. PGF_{2α}-induced contraction and carbachol-elicited relaxation amounted to 1.00 ± 0.02 g and 54 ± 2% reduction in tone, respectively (*n* = 167). After a further 60–90 min period of washout and resting, during which the tension was readjusted to 0.5 g, tissues were challenged with 5-HT, 5-CT or sumatriptan, either at rest or after the tone had been moderately increased by a threshold concentration of PGF_{2α} (an EC₁₀ to EC₂₀, i.e. 0.3 to 1 μM; see Figure 1). 5-HT, 5-CT and sumatriptan were added cumulatively in concentrations increasing by about 3 fold steps. When studied, 5-HT receptor

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antagonists, pargyline and uptake inhibitors were present 30 min before PGF_{2α}. Agonists and antagonists were tested on only one arterial ring from a given animal; hence *n* numbers refer to animal numbers. The effects of antagonists and inhibitors were investigated in rings adjacent to those used as controls.

Analysis of data

Concentration-response curves for 5-HT, 5-CT and sumatriptan were analysed using SCTFIT, a non linear regression computerized programme (De Lean *et al.*, 1980). EC₅₀ and *E*_{max} values were derived from this analysis. Antagonist *K*_B values were calculated according to the formula: $K_B = [B]/(CR - 1)$, where [B] is the concentration of antagonist used and CR (concentration-ratio) is the ratio of agonist EC₅₀ measured in the presence of antagonist over that measured in the absence of antagonist (Furchgott, 1972). *E*_{max} values are expressed as percentages of the amplitude of PGF_{2α} (30 μM)-induced contraction and given as arithmetic means ± s.e.mean. EC₅₀ values and concentration-ratios are expressed as geometric means with 95% confidence limits in parentheses. *K*_B values were given as negative logarithms (p*K*_B). Statistical comparisons were made by the use of Student's *t* test. A *P* level less than 0.05 was considered significant.

Materials

5-HT creatinine sulphate, pargyline hydrochloride, cocaine hydrochloride and carbachol (carbamylcholine chloride) were obtained from Sigma, Saint Louis, MO, U.S.A. PGF_{2α} was purchased from Upjohn in the form of Dinolytic (PGF_{2α} tromethamine salt). 11-Deoxycorticosterone acetate was from Schuchard, Munich, F.R.G. 5-CT hydrogenomaleate, sumatriptan and (1αH,3α,5αH-tropan-3-yl)1-H-indole-3-carboxylic acid ester (ICS 205-930) hydrochloride were synthesized at Sandoz, Basle, Switzerland. Ketanserine tartrate and methiothepin were gifts from Janssen, Beerse, Belgium and from Hoffmann-La Roche, Basle, Switzerland, respectively.

Stock solutions (10 mM) of sumatriptan, ketanserine and methiothepin were prepared daily in a mixture of ethanol:1-methyl-2-pyrrolidone:water (1:1:2) containing ascorbic acid 10 mg ml⁻¹. Subsequent dilutions were made in distilled water. Deoxycorticosterone (10 mM) was dissolved in ethanol. Other substances were dissolved in distilled water.

Results

Effects of 5-hydroxytryptamine, 5-carboxamidotryptamine and sumatriptan

When added to resting rings of guinea-pig iliac arteries, 5-HT (1 nM–0.1 mM), 5-CT (1 nM–0.1 mM) and sumatriptan (10 nM–3 μM) were generally without effect (Figure 1). Occasionally, the highest concentrations of 5-HT and 5-CT (≥ 10 μM) elicited small contractile responses. By contrast, when tissues had been moderately pre-contracted by a threshold concentration of PGF_{2α} (see Methods), they were further contracted by 5-HT, 5-CT and sumatriptan in a concentration-dependent manner (Figure 1).

The concentration-response curves for 5-HT, 5-CT and sumatriptan obtained under these conditions are shown in Figure 2. 5-HT and 5-CT induced contractions at a concentration as low as 1 nM. With 0.1 μM 5-CT and 0.3 μM 5-HT, the curves reached similar *E*_{max} values amounting to about 45% the contraction induced by PGF_{2α} (30 μM). At concentrations higher than 1 μM, 5-CT did not induce further contraction, whereas responses to 5-HT were inconsistent: relaxation was frequently observed at 3 μM 5-HT, sometimes followed by re-contraction at higher concentrations; in the other cases, no further increases in tension were produced by 3 μM 5-HT, but 10 μM and higher concentrations produced additional contrac-

Figure 1 Experimental recordings illustrating the effects of 5-hydroxytryptamine (5-HT) in guinea-pig isolated iliac artery rings, with (a) and without (b) moderate pre-contraction induced by prostaglandin F_{2α} (PGF_{2α}). The figure also shows the first stimulation by PGF_{2α} (30 μM) followed by the relaxation to carbachol (CCh, 10 μM). Negative logarithms of molar concentrations are indicated. Intermediate concentrations of 5-HT correspond to 3 fold increases.

tions. Sumatriptan (10 nM–3 μM) induced concentration-dependent increases in tension. The *E*_{max} value was not fully reached at 3 μM, but higher concentrations could not be used because the vehicle began to display relaxing effects. Nevertheless, the computer fitting of the sumatriptan curve yielded an *E*_{max} of about 35% of that of PGF_{2α} (see Table 1), which is in

Figure 2 Concentration-effect curves to 5-hydroxytryptamine (○), 5-carboxamidotryptamine (□) and sumatriptan (◇) in guinea-pig isolated iliac artery. Experimental conditions were those described in Figure 1 (a). Points are means with s.e.mean (shown by vertical bars) of 28, 11 and 21 values, respectively.

Table 1 Parameters of 5-hydroxytryptamine, 5-carboxamidotryptamine and sumatriptan agonist activities in guinea-pig isolated iliac arteries

Agonist	EC ₅₀ (nM)	E _{max} (%)	n
5-Hydroxytryptamine	22.9 (18.2–28.8)	46.3 ± 2.6	28
5-Carboxamidotryptamine	6.6 (4.4–10.0)	44.1 ± 5.1	11
Sumatriptan	155 (100–240)	36.2 ± 3.4	21

EC₅₀ values are expressed as geometric means with 95% confidence limits in parentheses; E_{max} values are arithmetic means ± s.e.mean of contractions expressed in percentages of the maximal contraction induced by prostaglandin F_{2α} (30 μM). Experimental conditions were those described in Figure 1a.

line with the overall sigmoidal aspect of the curve (Figure 2). The E_{max} value of sumatriptan was found to be significantly ($P < 0.02$) smaller than that of 5-HT (Table 1). The rank order of potency of the three agonists was as follows (mean EC₅₀ values, nM): 5-CT (6.6) > 5-HT (22.9) > sumatriptan (155). Table 1 gives the 95% confidence limits of the EC₅₀ values, which do not overlap.

The concentration-response curve to 5-HT was not significantly altered in the presence of either pargyline (50 μM),

Figure 3 Concentration-effect curves to 5-hydroxytryptamine in guinea-pig isolated iliac artery in the absence (○) and in the presence (●) of (a) pargyline (50 μM); (b) cocaine (30 μM); (c) deoxycorticosterone (3 μM). Experimental conditions were those described in Figure 1 (a), the inhibitors being added 30 min before the second administration of prostaglandin F_{2α}. Points are means with s.e.mean (shown by vertical bars) of 5 values. **Figure 4** Concentration-effect curves to 5-hydroxytryptamine (circles), 5-carboxamidotryptamine (squares) and sumatriptan (diamonds) in guinea-pig isolated iliac artery in the absence (open symbols) and in the presence (closed symbols) of ICS 205-930 (1 μM). Experimental conditions were those described in Figure 1a, the antagonist being added 30 min before the second administration of prostaglandin F_{2α}. Points are means with s.e.mean (shown by vertical bars) of 4 values.

cocaine (30 μM) or deoxycorticosterone (3 μM), conditions for inhibition of monoamine oxidase, neuronal uptake and extra-neuronal uptake, respectively (Figure 3).

Effects of 5-hydroxytryptamine receptor antagonists

The 5-HT₃ receptor antagonist, ICS 205-930 (1 μM), did not significantly affect concentration-response curves to 5-HT, 5-CT and sumatriptan (Figure 4).

In the presence of the 5-HT₂ receptor antagonist, ketanserin (1 μM) the concentration-response curves to 5-HT, 5-CT and sumatriptan were slightly shifted to the right, without significant depression of the E_{max} values (Figure 5). Concentration-ratio shifts induced by ketanserin (1 μM), as measured at the EC₅₀ level, were 2.6 (1.6–4.2), 2.3 (1.5–3.5) and 1.8 (1.1–2.8) against 5-HT ($n = 11$), 5-CT ($n = 9$) and sumatriptan ($n = 13$),

Figure 5 Concentration-effect curves to 5-hydroxytryptamine (circles), 5-carboxamidotryptamine (squares) and sumatriptan (diamonds) in guinea-pig isolated iliac artery in the absence (open symbols) and in the presence (closed symbols) of ketanserin (1 μM). Experimental conditions were those described in Figure 1a, the antagonist being added 30 min before the second administration of prostaglandin F_{2α}. Points are means with s.e.mean (shown by vertical bars) of 11 (5-HT), 9 (5-CT) and 13 (sumatriptan) values.

Figure 6 Concentration-effect curves to 5-hydroxytryptamine (circles), 5-carboxamidotryptamine (squares) and sumatriptan (diamonds) in guinea-pig isolated iliac artery in the absence (open symbols) and in the presence (closed symbols) of methiothepin ($0.1 \mu\text{M}$). Experiments were performed in the presence of ketanserin ($1 \mu\text{M}$). Experimental conditions were those described in Figure 1a, the antagonists being added 30 min before the second administration of prostaglandin $\text{F}_{2\alpha}$. Points are means with s.e.mean (shown by vertical bars) of 5 (5-HT), 6 (5-CT) and 8 (sumatriptan) values.

respectively (geometric means with 95% confidence intervals). That is, the effect of ketanserin ($1 \mu\text{M}$) was at the borderline of significance.

The effect of the 5-HT receptor antagonist, methiothepin ($0.1 \mu\text{M}$) was investigated in the presence of ketanserin ($1 \mu\text{M}$). Control concentration-response curves to 5-HT and to 5-CT (that is, in the presence of ketanserin $1 \mu\text{M}$) were displaced to the right by methiothepin $0.1 \mu\text{M}$, without loss of E_{max} values (Figure 6). Concentration-ratios were 16 (3–100) and 20 (9–44) for 5-HT ($n = 5$) and 5-CT ($n = 6$), corresponding to pK_{B} values of 8.07 ± 0.41 and 8.27 ± 0.15 , respectively. Methiothepin ($0.1 \mu\text{M}$) also exerted an inhibitory effect on sumatriptan-induced contractions (Figure 6); in this particular case the surmountable nature of the antagonism could not be established, owing to vehicle effects, as mentioned above. In the presence of methiothepin ($0.1 \mu\text{M}$), nevertheless, a concentration of $3 \mu\text{M}$ sumatriptan was necessary to reach about the same level of contraction as that produced by $0.3 \mu\text{M}$ under control conditions (Figure 6). This indicates an approximate concentration-ratio and pK_{B} value of 10 and 8 respectively.

Discussion

The present study shows that 5-HT and the 5-HT₁-like receptor agonists, 5-CT and sumatriptan can elicit concentration-dependent contractions of guinea-pig isolated iliac artery, provided that a minimal tone is previously induced by $\text{PGF}_{2\alpha}$. The 5-HT receptor mediating these responses has the characteristics of the 5-HT₁-like receptor as proposed by Bradley *et al.* (1986a). Thus, the contractions induced by 5-HT, 5-CT and sumatriptan were relatively resistant to, and unaffected by, the 5-HT₂ and 5-HT₃ receptor antagonists, ketanserin and ICS 205-930, respectively. The weak antagonist effect displayed by ketanserin in guinea-pig iliac artery is indeed hard to reconcile with its potency in the nanomolar range at 5-HT₂ receptors (Van Nueten *et al.*, 1981; Feniuk *et al.*, 1985). In fact, the deduced pK_{B} value of ketanserin (about 6) fits well with its reported affinity for the various 5-HT₁ binding sites (Hoyer, 1988). Second, methiothepin was found to be a potent antagonist of 5-HT, 5-CT and sumatriptan in guinea-pig iliac artery. It was about equiactive and apparently

competitive against 5-HT and 5-CT; it also inhibited sumatriptan-induced contractions with an apparently similar potency, although in this case the analysis was hampered by vehicle effects. Third, 5-CT was more potent than 5-HT. Sumatriptan, introduced more recently, was less potent than 5-HT by 6 to 7 fold, an observation in line with its activity at the prototype 5-HT₁-like receptor mediating contraction of dog saphenous vein (Humphrey *et al.*, 1988).

As a whole, these results strongly support the view that 5-HT and the 5-HT₁-like receptor agonists, 5-CT and sumatriptan evoke contraction of guinea-pig iliac artery through stimulation of a 5-HT₁-like receptor. Thus, guinea-pig iliac artery appears to represent another vessel endowed with 5-HT₁-like receptors mediating vascular contraction, besides the saphenous vein of the dog and basilar arteries of various species (see Introduction). Removal of agonists by monoamine oxidase, neuronal or extraneuronal uptake are unlikely to bias this interpretation, since pargyline, cocaine and deoxycorticosterone did not significantly affect 5-HT-induced contraction. It is interesting to compare the intrinsic activities of 5-CT and sumatriptan, with respect to that of 5-HT, in the present model and in other models of 5-HT₁-like receptor-mediated contraction. In guinea-pig iliac artery, the rank order of intrinsic activities was $5\text{-CT} = 5\text{-HT} > \text{sumatriptan}$. In other models, 5-CT usually displays less efficacy than 5-HT (Feniuk *et al.*, 1985; Connor *et al.*, 1989; Parsons & Whalley, 1989; Parsons *et al.*, 1989). In some of these cases, a minor population of 5-HT₂ receptors may explain the greater response to 5-HT. This seems not to be the case in guinea-pig iliac artery since 5-CT and 5-HT displayed about equal efficacy. On the other hand, sumatriptan behaved as a full agonist in dog saphenous vein (Humphrey *et al.*, 1988), whereas it behaved as a partial agonist (with about 80% of the intrinsic activity of 5-HT) in guinea-pig iliac artery. Partial agonist activity of sumatriptan has been reported elsewhere (Bond *et al.*, 1989; Parsons *et al.*, 1989; Schoeffter & Hoyer, 1989a; 1990). Such variations in intrinsic activities do not necessarily imply that the receptors involved are heterogeneous, since they may be interpreted in terms of different receptor densities or receptor-coupling efficiencies (see Kenakin, 1984). It is thus quite possible that the 5-HT₁-like receptor mediating contraction of guinea-pig iliac artery is the same as that reported in dog saphenous vein (Feniuk *et al.*, 1985; Humphrey *et al.*, 1988).

As regards the identity of the present receptor with one or other of the 5-HT₁ receptor subtypes defined by binding studies, it appears difficult to draw any conclusion on the basis of agonist potency ratio and without the use of discriminating 5-HT₁ receptor antagonists. It is noteworthy, nevertheless, that sumatriptan is preferentially recognized by 5-HT_{1D} binding sites (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989b; Sumner & Humphrey, 1989; Waeber *et al.*, 1989). In addition, the compound behaves as a full agonist in a proposed 5-HT_{1D} receptor model, inhibition of adenylate cyclase in calf substantia nigra (Schoeffter & Hoyer, 1989b). However, the agonist effect of sumatriptan does not necessarily imply that 5-HT_{1D} binding sites and the 5-HT₁-like receptor mediating contraction of guinea-pig iliac artery are identical. Sumatriptan also behaves as a full agonist in 5-HT_{1A} and 5-HT_{1B} receptor models based on second messengers, although with less potency than in the 5-HT_{1D} receptor model (Schoeffter & Hoyer, 1989b). On the other hand, sumatriptan-induced contraction of dog saphenous vein is reportedly unaffected by metergoline ($0.1 \mu\text{M}$; Humphrey *et al.*, 1988), a drug showing nanomolar affinity to 5-HT_{1D} recognition sites (Heuring & Peroutka, 1987; Waeber *et al.*, 1988).

It is not clear at present why the 5-HT₁-like receptor present in guinea-pig iliac artery requires a state of moderate pre-contraction, induced by $\text{PGF}_{2\alpha}$, to be functionally 'expressed'. Interestingly, this situation is reminiscent of that described by MacLennan *et al.* (1989) in the human isolated umbilical artery. In that vessel, 5-HT₁-like receptors mediating contraction were 'unmasked' under conditions of high

oxygen tension, a mechanism which involved cyclo-oxygenase products. Thus, although 5-HT₂ receptors appear to mediate contraction of most vascular preparations, 5-HT₁-like receptors might be of physiological importance under conditions of increased levels of eicosanoids.

In conclusion, 5-HT and the 5-HT₁-like receptor agonists, 5-CT and sumatriptan induced contraction of isolated rings from guinea-pig iliac arteries, moderately pre-contracted by

PGF_{2α}. This effect is most probably mediated by a 5-HT₁-like receptor, which could be similar to the 5-HT_{1D} type but further experiments are required to test this possibility.

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