Br. J. Pharmacol. (1993), **109**, 609–611

SPECIAL REPORT

5-HT_{1B} receptors mediate potent contractile responses to 5-HT in rat caudal artery

[1]Douglas A. Craig & Graeme R. Martin

Analytical Pharmacology Group, Biochemical Sciences, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

5-Hydroxytryptamine (5-HT) evoked potent contractile responses in phenoxybenzamine-treated ring segments of rat caudal artery, partially contracted with U46619. Responses were mimicked by 5-HT_1-selective agonists with the potency order: RU24969 > 5-carboxamidotryptamine > 5-HT = CP-93,129 >> sumatriptan. 8-Hydroxy-*N*,*N*-dipropylaminotetralin was virtually inactive. Responses were unaffected by spiperone (0.1 μM) and mesulergine (1.0 μM), but were antagonized competitively by (±)-cyanopindolol affording agonist-independent pK_B estimates of 8.4 to 8.9. The pharmacological profile of this receptor is consistent with that of the 5-HT_{1B} subtype. Since the 5-HT_{1B} receptor is the rodent homologue of the 5-$HT_{1D\beta}$ subtype, it might be anticipated that 5-$HT_{1D\beta}$ receptors will be found to mediate vasoconstrictor responses in non-rodent species.

Keywords: Rat caudal artery; vasoconstriction; 5-HT_{1B} receptor; 5-HT_{1D}; 5-$HT_{1D\beta}$; CP-93,129; RU24969; sumatriptan

Introduction

In a variety of species the vasoconstrictor action of 5-hydroxytryptamine (5-HT) has been shown to involve 5-HT_1-like as well as 5-HT_2 receptors (see Feniuk & Humphrey, 1989). Classification of the 5-HT_2 receptor is unambiguous but the precise identity of the 5-HT_1-like receptor remains uncertain. Several studies have suggested that it is a 5-HT_{1D} receptor (e.g. Martin *et al.*, 1991), although this remains a contentious issue (Perren *et al.*, 1991). The problem has been confounded further by the recent disclosure that there exist pharmacologically similar 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor subtypes (Weinshank *et al.*, 1992) and that the 5-$HT_{1D\beta}$ subtype is the species homologue of the pharmacologically distinct 5-HT_{1B} receptor in rodents (Adham *et al.*, 1992). Interestingly, a 5-HT_1-like receptor mediating vascular contraction in rodents has hitherto not been reported. Here we describe such a receptor mediating contraction of rat caudal artery and show that it exhibits the pharmacology of a 5-HT_{1B} receptor.

Methods

Male Wistar rats (250–350 g) were killed by asphyxiation with CO_2. The proximal 8 cm of the caudal artery were carefully removed and cleaned of adhering tissue. Arterial rings (3 mm long) were suspended between wire hooks (150 μm diameter) for measurement of isometric tension and placed in Krebs solution (pH 7.4, 37°C), the composition of which has been detailed by Martin *et al.* (1991).

After 30 min, a force of 1 g was applied to each ring and reinstated until a resting tone of approximately 0.5 g was maintained. Tissues were treated with pargyline (500 μM, 30 min) to inhibit monoamine oxidases and, except in preliminary experiments, with phenoxybenzamine (PBZ, 3 μM for 1 h, 5 washes) to eliminate 5-HT_2- and α-adrenoceptor-mediated effects of 5-HT, as well as agonist uptake. To study the effect of agonists at the 5-HT_1-like receptor, tissues were first contracted with the stable thromboxane analogue, U46619 (10–30 nM, approximate EC_{20} concentration). When this contraction had stabilized, concentration-effect curves were constructed by the cumulative addition of agonists in 0.5 log M increments. The relative efficacies of agonists at the 5-HT_1-like receptor were estimated by the response to 10 μM 5-HT applied at the end of the concentration-effect curve. To study the effects of antagonists two concentration-effect curves were constructed in each tissue, the first serving as the control and the second constructed after 1 h incubation with antagonist. Concentration ratios (CR) for the two curves were determined for the agonist concentrations producing half-maximal effects (EC_{50}), and estimates of the apparent antagonist pK_B were calculated according to the relationship: $pK_B = \log (CR - 1/ [\text{antagonist}])$.

Drugs

5-HT creatinine sulphate, spiperone, prazosin hydrochloride, idazoxan hydrochloride, 8-OH-DPAT (8-hydroxy-N,N-dipropylaminotetralin hydrobromide) (Sigma Chemical Co., St. Louis, MO, U.S.A.); 5-carboxamidotryptamine maleate, mesulergine maleate (RBI, Natick, Mass, U.S.A.); CP-93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one) (Pfizer Inc., Groton, CT, U.S.A.); RU24969 (5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl]-1H-indole) (Roussell-Uclaf, Romaineville, France); (±)-cyanopindolol (Dr Daniel Hoyer, Sandoz Pharmaceuticals, Basel, Switzerland). Cyanopindolol was dissolved at 10 mM in ethanol and diluted in water. The remaining drugs were dissolved in distilled water.

Results

In naive arterial rings, 5-HT (0.03 to 10 μM) evoked concentration-related contractions (Figure 1a). The presence of a small sustained contraction to U46619 revealed an additional, higher potency phase of the 5-HT concentration-effect curve (not shown). The lower, but not the higher potency phase could be blocked partially by the combination of prazosin (0.1 μM) and idazoxan (1.0 μM) and totally by the further addition of spiperone (0.1 μM) or mesulergine (1.0 μM), indicating that this phase involved α-adrenoceptors as well as 5-HT_2 receptors. To isolate more effectively the receptor mediating the high potency phase, tissues were exposed to PBZ (see methods). After PBZ treatment, 5-HT failed to elicit a contraction when applied on its own (Figure 1b). In contrast, when PBZ-treated rings were first contracted with U46619, 5-HT produced a monophasic concentration-effect curve ($pEC_{50} = 7.65$; Figure 1c, Table 1). This curve

[1] Author for correspondence.

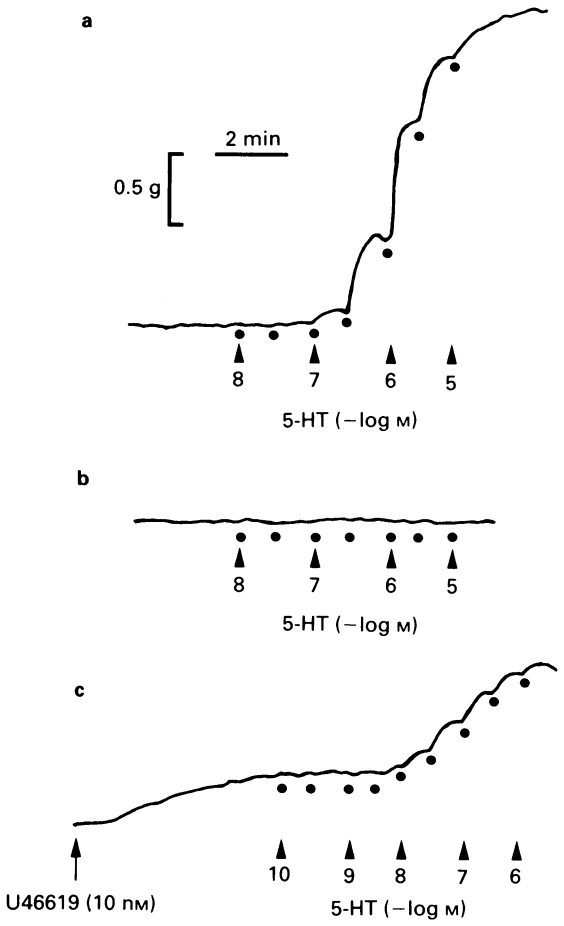

Figure 1 Contractile responses to 5-hydroxytryptamine (5-HT) in a single ring of rat caudal artery under different experimental conditions. (a) Responses to 5-HT alone, applied in 0.5 log M increments to the untreated tissue. (b) Failure of 5-HT alone to elicit contractions after phenoxybenzamine (PBZ) treatment (3 μM, 1 h). (c) High potency responses to 5-HT revealed subsequent to elevating muscle tone with U46619 after PBZ-treatment.

corresponded in location to that of the high potency 5-HT response revealed by U46619 before PBZ treatment.

In PBZ-treated rings exposed to U46619, the effect of 5-HT was mimicked by the agonists 5-CT (5-HT_1-like-selective), RU24969 and CP-93,129 (5-HT_{1B}-selective), and sumatriptan (5-HT_{1D}-selective), whereas 8-OH-DPAT (5-HT_{1A}-selective; 10 μM) evoked less than 10% of the maximum response to 5-HT (Table 1). (±)-Cyanopindolol (0.1 μM) produced parallel dextral shifts of the concentration-effect curves to 5-HT, 5-CT, RU24969, and CP-93,129 with no depression of the maximum response, affording apparent pK_B estimates of 8.4 to 8.9 (Table 1).

Discussion

In various blood vessels from the rat, including the caudal artery (Feniuk & Humphrey, 1989), 5-HT-induced contraction has been shown to be mediated by 5-HT_2 receptors. This study presents the first evidence for the presence of contractile 5-HT_1-like receptors in rodent vasculature. Although 5-HT_1-like mediated responses were not detected in tissues exposed to 5-HT alone, they could be revealed by prior contraction with a low concentration of the thromboxane analogue, U46619.

Tissues were treated with PBZ to preclude complications arising from agonist actions at 5-HT_2 receptors, α-adrenoceptors and uptake sites. Under these conditions all agonists evoked monophasic cumulative concentration-effect curves, characteristic of an interaction with a homogeneous receptor population. The pharmacology of the receptor is inconsistent with a 5-HT_2, 5-HT_3, or 5-HT_4 classification on the basis of: (1) the high potency of 5-CT relative to 5-HT (an order which is reversed for the aforementioned subtypes; (2) insensitivity to antagonism by spiperone and mesulergine at concentrations 60 to 250 fold greater than their affinities at 5-HT_2 receptors; and (3) sensitivity to antagonism by (±)-cyanopindolol, which exhibits negligible affinity at 5-HT_2, 5-HT_3 and 5-HT_4 sites.

Within the 5-HT_1-like subgroup, only 5-HT_{1A} and 5-HT_{1B} receptors exhibit affinities for (±)-cyanopindolol in the order of those obtained in the present study (pK_B range = 8.4 to 8.9). The failure of 8-OH-DPAT, a potent and selective 5-HT_{1A} agonist, to evoke significant contractions at 10 μM argues against the involvement of 5-HT_{1A} receptors. Positive identification of the present site as 5-HT_{1B} is supported by (1) potent agonism by CP-93,129 (150–650 fold selective for 5-HT_{1B} over other 5-HT_1-like receptors, Macor *et al.*, 1990). (2) the absolute and relative agonist potencies: RU24969 > 5-CT > 5-HT = CP-93,129 >> sumatriptan, and (3) the agonist-independence of antagonist pK_B estimates for (±)-cyanopindolol. Similar results have been reported elsewhere for 5-HT_{1B} receptors (Schoeffter & Hoyer, 1989; Macor *et al.*, 1990; O'Connor & Kruk, 1992).

Since 5-HT_{1D} and 5-HT_{1B} receptors evidently share similar anatomical distributions and functional roles, the demonstration that 5-HT_{1B} receptors mediate vasoconstriction is intriguing. In light of recent evidence that the 5-HT_{1B} receptor is the rodent homologue of the 5-$HT_{1D\beta}$ receptor subtype (Adham *et al.*, 1992), this novel observation suggests that 5-$HT_{1D\beta}$ receptors may subserve vasoconstriction in non-rodent species.

Table 1 Potencies and relative intrinsic activities for agonists and pK_B estimates for (±)-cyanopindolol versus various agonists in rat caudal artery

Agonist	*pEC_{50}*[a]		*i.a.*[b]	n[c]	*pK_B*[a]		n
5-HT	7.65	(0.04)	1.00	12	8.90	(0.05)	4
5-CT	8.14	(0.06)	1.00	17	8.45	(0.04)	4
RU24969	8.72	(0.21)	0.83	6	8.46	(0.15)	3
CP-93,129	7.37	(0.13)	1.00	8	8.38	(0.7)	3
Sumatriptan	5.64	(0.02)	1.00	5	ND	–	–
8-OH-DPAT	<5.0	–	0.07	3	ND	–	–

[a]Mean ± s.e.mean in parentheses. [b]Intrinsic activity relative to 5-HT. [c]Determinations made in tissues from *n* different animals. ND (not determined).

References

ADHAM, N., ROMANIENKO, P., HARTIG, P., WEINSHANK, R. & BRANCHEK, T. (1992). The rat 5-hydroxytryptamine$_{1B}$ receptor is the species homologue of the human 5-hydroxytryptamine$_{1D\beta}$ receptor. *Mol. Pharmacol.*, **41,** 1–7.

FENIUK, W. & HUMPHREY, P. (1989). Mechanisms of 5-hydroxytryptamine-induced vasoconstriction. In *The Peripheral Actions of 5-Hydroxytryptamine*. ed. Fozard, J.R. pp. 110–122. New York: Oxford University Press.

MACOR, J., BURKHART, C., HEYM, J., IVES, J., LEBEL, L., NEWMAN, M., NIELSEN, J., RYAN, K., SCHULZ, D., TORGERSEN, L. & KOE, B. (1990). 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo-3,2-b]pyrid-5-one: a potent and selective serotonin (5-HT$_{1B}$) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. *J. Med. Chem.*, **33,** 2087–2093.

MARTIN, G.R., PRENTICE, D.J. & MACLENNAN, S.J. (1991). The 5-HT receptor in rabbit saphenous vein. Pharmacological identity with the 5-HT$_{1D}$ recognition site? *Fund. Clin. Pharmacol.*, **5,** (5), 417.

O'CONNOR, J. & KRUK, Z. (1992). Pharmacological characteristics of 5-hydroxytryptamine autoreceptors in rat brain slices incorporating the dorsal raphe of the suprachiasmatic nucleus. *Br. J. Pharmacol.*, **106,** 524–532.

PERREN, M., FENIUK, W. & HUMPHREY, P. (1991). Vascular 5-HT$_1$-like receptors that mediate contraction of the dog isolated saphenous vein and carotid arterial vasoconstriction in anaesthetized dogs are not of the 5-HT$_{1A}$ or 5-HT$_{1D}$ subtype. *Br. J. Pharmacol.*, **102,** 191–197.

SCHOEFFTER, P. & HOYER, D. (1989). 5-Hydroxytryptamine 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors mediating inhibition of adenylate cyclase activity. *Naunyn Schmied. Arch. Pharmacol.*, **340,** 285–292.

WEINSHANK, R., ZGOMBICK, J., MACCHI, M., BRANCHEK, T. & HARTIG, P. (1992). Human serotonin ID receptor is encoded by a subfamily of two distinct genes: 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 3630–3634.

(Received March 30, 1993
Accepted April 26, 1993)