Mediation by 5-HT_{1D} receptors of 5-hydroxytryptamine-induced contractions of rabbit middle and posterior cerebral arteries

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1 5-Hydroxytryptamine (5-HT) receptor-mediated contraction of endothelium denuded rabbit middle (MCA) and posterior (PCA) cerebral arteries was characterized by use of selective agonists and antagonists for different 5-HT receptor subtypes.

2 5-HT and various 5-HT receptor agonists contracted the arteries with the following rank order of potency in MCA: 5-carboxamidotryptamine (5-CT) > 5-HT > 5-methoxytryptamine (5-MeOT) > sumatriptan > α -methyl-5-HT (α -Me-5-HT) >> 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and in PCA: 5-CT > 5-HT > sumatriptan > 5-MeOT > α -Me-5-HT >> 8-OH-DPAT. With few exceptions, the maximal contractile responses of these agonists were similar to that induced by 5-HT.

3 The selective antagonists of 5-HT_{2A/2C} (ketanserin), 5-HT₄ (SDZ 205-557) and 5-HT_{1A/1B} (S(-)-propranolol) sites were devoid of inhibitory effect on 5-HT-mediated contraction in both MCA and PCA, thus excluding activation of the corresponding receptors.

4 In both arteries, the contraction-response curve to 5-HT was unaffected by the 5-HT₃ receptor antagonist, ICS 205-930 (0.01 and 0.1 μ M) whilst a small (3 and 6 fold displacement) was seen with MDL 72222 (0.1 and 1 μ M).

5 The mixed 5-HT₁-like/5-HT_{2A} receptor antagonist, methiothepin (0.001–0.1 μ M), was a potent antagonist of 5-HT-induced contractions in both arteries, giving pA₂ values of 9.4 $\pm$ 0.7 and 9.6 $\pm$ 0.8 in MCA and PCA, respectively.

6 Rauwolscine (0.1–10 μ M) and yohimbine (0.3, 3 μ M) inhibited contractions to 5-HT in a competitive manner, pA₂ values of 7.1 $\pm$ 0.6 and 6.7 $\pm$ 0.6 were determined for rauwolscine in MCA and PCA, respectively. An apparent pA₂ value of 6.9 $\pm$ 0.2 was calculated for yohimbine (3 μ M) in both MCA and PCA.

7 In conclusion, these results suggest that the contractile response to 5-HT in rabbit isolated MCA and PCA is predominantly mediated by the 5-HT_{1D} receptor subtype, although a small contribution by 5-HT₃ receptors cannot be excluded.

Keywords: Rabbit cerebral arteries; 5-hydroxytryptamine (5-HT); 5-HT receptors; 5-HT_{1D} receptor subtype; sumatriptan

Introduction

5-Hydroxytryptamine (5-HT) is a potent constrictor of cerebral arteries in mammals. 5-HT has been localized in platelets (Tranzer *et al.*, 1972), and mast cells (Aubineau & Dimitriadou, 1989). In addition, nerves containing 5-HT have been identified in animal and human brain vessels (Griffith *et al.*, 1982; Edvinsson *et al.*, 1983; Griffith & Burnstock, 1983; Scatton *et al.*, 1985; Saito & Lee, 1987). 5-HT may therefore contribute to the control of cerebral blood flow and has been implicated in the aetiology of several cerebrovascular disorders, such as migraine.

Four main types of 5-HT receptors have been defined, the 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ receptors and a new nomenclature has been proposed to introduce additional subtypes (see Humphrey *et al.*, 1993). Attempts have been made to characterize the type of receptor mediating the contraction to 5-HT in a number of cerebral arteries including basilar, pial, meningeal and middle cerebral arteries and it appears that the distribution of 5-HT receptor subtypes varies according to the species. Thus, in the rat (Deckert & Angus, 1992) and the sheep (Gaw *et al.*, 1990) 5-HT_{2A} (Humphrey *et al.*, 1993) receptors predominantly mediate the contraction of cerebral arteries whilst in other species, including man (Parsons *et al.*, 1989; Hamel & Bouchard, 1991), rabbit (Parsons

& Whalley, 1989), guinea-pig (Chang & Owman, 1989), dog and primate (Connor *et al.*, 1989), 5-HT₁-like receptors are primarily involved. 5-HT₁-like receptors which underly the contraction of the rabbit basilar artery appear to share some similarities with 5-HT₁-like receptors that mediate contractions to 5-HT in cerebral blood vessels from man, primate and dog (Parsons *et al.*, 1989). While the 5-HT_{1D} receptor subtype mediates contraction to 5-HT in human cerebral arteries (Hamel & Bouchard, 1991; Jansen *et al.*, 1993), the subtype of 5-HT₁ receptors implicated in the contraction of rabbit cerebral arteries remains to be fully characterized.

Therefore, this study was undertaken to determine the type of 5-HT receptors involved in the contraction of rabbit isolated middle (MCA) and posterior (PCA) cerebral arteries. By using selective agonists and antagonists for 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ receptor subtypes, we have shown that 5-HT-induced contractions of MCA and PCA are dependent on the 5-HT_{1D} receptor subtype.

Methods

Preparation of vessels

Vessels were prepared according to a method previously described (Deckert *et al.*, 1994). Briefly, New Zealand white rabbits, weighing 3–3.5 kg, were killed by an overdose of pentobarbitone sodium via the marginal ear vein. The brain

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was rapidly removed and placed in a Krebs solution bubbled with 95% O₂:5% CO₂. The Krebs solution had the following composition (in mM): NaCl 119, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.17, CaCl₂ 2.5, ethylenediaminetetracetic acid (EDTA) 0.027, glucose 5.5 and NaHCO₃ 15, pH 7.3. The arteries were carefully dissected out under a binocular microscope (Nachet Z45P, Dijon, France). Two millimeter long segments of the arteries were threaded onto 40 µm stainless steel wires and mounted as ring preparations in a dual channel isometric myograph (J-P Trading, Aarhus, Denmark). The endothelium was mechanically removed by gentle rubbing of the luminal surface of the artery with a thin cotton thread previously hardened by coating with cyanoacrylate glue. Artery segments were left unstretched on the wires for 30 min at 37°C in Krebs solution containing 10 µM indomethacin. The vessels were then submitted to a resting tension of 100 mg and left to equilibrate for 30 min before concentration-effect curves to 5-HT or to agonists were constructed. 5-HT or agonists were added to the organ bath cumulatively in 0.5 log unit increments. In all experiments, a 1 h delay was allowed between two consecutive concentration-effect curves while three washings with fresh (drug-free apart from indomethacin) solution was performed after obtaining the maximal effect for each curve. Previous experiments showed that at least three consecutive concentration-effect curves to 5-HT were reproducible in both arterial preparations (Deckert *et al.*, 1994).

Determination of agonist potency

After completion of the control concentration-effect curve to 5-HT, cumulative concentration-effect curves to either 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeOT), α -methyl-5-HT (α -Me-5-HT), sumatriptan and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) were constructed. Only one agonist other than 5-HT was tested per artery.

Determination of antagonist potency

Concentration-effect curves to 5-HT were established first in the absence and then repeated in the presence of methiothepin (0.001–0.1 µM), ketanserin (0.003–0.3 µM), MDL 72222 (0.01–1 µM), S(-)-propranolol (0.1, 1 µM), yohimbine (0.3, 3 µM), rauwolscine (0.1–10 µM), ICS 205-930 (0.01, 0.1 µM) or SDZ 205-557 (0.1, 1 µM). In each case, the antagonist was present for 45 min before 5-HT was added. No more than two concentrations of antagonist were tested on the same artery.

Test for functional endothelium

The integrity of the endothelium was assessed functionally, at the end of the experiment, by application of acetylcholine (ACh, 10 µM) on a sustained tension to 30 mM KCl. Vessels relaxing to ACh were discarded.

Analysis of data

The concentration-effect curves to agonists or to 5-HT (in the absence or in the presence of an antagonist) were expressed as percentage of the maximum contraction induced by 5-HT (% 5-HT max. contraction) in a control concentration-response curve constructed within the same artery. The mean maximal contraction ($E_{\max}$) $\pm$ s.e.mean was determined from experimental data whereas the mean $pD_2 \pm$ s.e.mean (the negative logarithm of the concentration required to produce a half maximum contractile effect (EC_{50})) was calculated after fitting each curve according to a sigmoidal equation of the form:

$$Y = P_1 + P_2/[1 + e^{P_3(\log X - P_4)}]$$

in which, X = agonist concentration, P_1 = lower plateau response, P_2 = range between the lower and the maximal

plateau of the concentration-effect curve, P_3 = a negative curvature index indicating the slope independently of the range and $P_4 = \log EC_{50}$ (Elghozi & Head, 1990). The relative potency of the agonist was determined by calculating the concentration ratio: EC_{50} for agonist divided by EC_{50} for 5-HT obtained from the corresponding control curve.

Antagonist potency was assessed by calculating the pA_2 value giving a concentration-ratio of two, according to the relation $pA_2 = \log (CR - 1) - \log (B)$, where (B) is the antagonist concentration, (CR) the concentration ratio of 5-HT EC_{50} values in the absence and in the presence of the antagonist (Van Den Brink, 1977). When concentration-effect curves to 5-HT were shifted in parallel in the presence of three concentrations of antagonist, the pA_2 was determined from a Schild plot analysis and linear regression (Arunlakshana & Schild, 1959). When concentration-effect curves to 5-HT were shifted in parallel in the presence of one or two concentrations of antagonist, we calculated an apparent pA_2 from the formula given above, by assuming that the slope of the Schild plot did not differ from unity.

A one-way analysis of variance followed by a Student's *t* test was used to establish significant differences between mean values of pD_2 or $E_{\max}$. A *P* value less than 0.05 was considered to be statistically significant.

Drugs

The following drugs were used, 5-hydroxytryptamine creatinine sulphate, indomethacin, acetylcholine chloride, yohimbine hydrochloride; 5-methoxytryptamine hydrochloride, S(-)-propranolol hydrochloride (Sigma Chemical Co., St. Louis, U.S.A.), 5-carboxamidotryptamine maleate, α -methyl-5-hydroxytryptamine maleate, 8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide, ketanserin tartrate, rauwolscine hydrochloride (Research Biochemicals Inc., Natick, U.S.A.), methiothepin maleate (gift from Drs E. Kyburz and P. Weber, Hoffman La Roche, Basel, Switzerland), sumatriptan (3-[2-dimethylamino]-ethyl-N-methyl-1H-indole-5-methane sulphonamide, Glaxo, Greenford, U.K.), MDL 72222 (1 α H,3 α ,5 α H-tropan-3-yl 3,5-dichlorobenzoate methanesulphonate, gift from Drs R.C. Miller and C.D. Houldsworth, Marion Merrel Dow Research Institute, Strasbourg, France). ICS 205-930 ([3 α -tropanyl]-1-H-indole-3-carboxylic acid ester hydrochloride) and SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino)-ethyl ester hydrochloride) (gifts from Drs J.R. Fozard, D. Römer and E. Rissi, Sandoz Pharma Ltd. Basel, Switzerland).

All drugs were freshly dissolved and diluted in distilled water and kept on ice for the duration of the experiment. Indomethacin was freshly dissolved in dimethylsulphoxide (DMSO) and kept at room temperature.

Results

Agonist studies

No significant differences occurred between the resting force of the vessels prior to concentration-effect curves to 5-HT and to the 5-HT agonists. 5-HT was a potent agonist causing contraction of rabbit MCA and PCA with pD_2 values of 7.32 ± 0.12 and 7.16 ± 0.13 , respectively (Table 1). 5-CT, sumatriptan, 5-MeOT, α -Me-5-HT and 8-OH-DPAT also produced concentration-dependent contractions of rabbit MCA and PCA. The corresponding concentration-effect curves are presented in Figure 1 and the pD_2 and $E_{\max}$ values are given in Table 1. A comparison of the relative potency of each agonist in respect to 5-HT gave the following rank order of potency in MCA: 5-CT > 5-HT > 5-MeOT > sumatriptan > α -Me-5-HT >> 8-OH-DPAT; and in PCA: 5-CT > 5-HT > sumatriptan > 5-MeOT > α -Me-5-HT >> 8-OH-DPAT. In MCA and PCA, all the agonists, except 8-OH-DPAT, produced maximal contractions of similar magnitude

Table 1 Potencies of various 5-hydroxytryptamine (5-HT) receptor agonists for inducing contraction of rabbit middle and posterior cerebral arteries

Agonist	n	Middle cerebral artery			n	Posterior cerebral artery		
		pD ₂ (-log EC ₅₀)	Relative potency	E _{max} (%5-HT E _{max})		pD ₂ (-log EC ₅₀)	Relative potency	E _{max} (%5-HT E _{max})
5-HT	32	7.32 ± 0.12	1	100	31	7.16 ± 0.13	1	100
5-CT	7	8.06 ± 0.13*	0.07 (0.02–0.22)	107 ± 28	6	7.41 ± 0.22	0.31 (0.06–1.64)	76 ± 16
Sumatriptan	7	7.01 ± 0.32* (7.76 ± 0.17)	5.6 (1.3–24.3)	116 ± 10	6	6.98 ± 0.12 (7.35 ± 0.08)	2.3 (0.1–5.7)	135 ± 14
5-MeOT	5	5.93 ± 0.36 (6.49 ± 0.14)	3.6 (0.5–24.7)	108 ± 6	6	5.84 ± 0.22* (6.45 ± 0.15)	4.1 (1.9–8.8)	90 ± 11
α-Me-5-HT	6	5.79 ± 0.32* (7.67 ± 0.22)	74.1 (15.9 ± 345.5)	130 ± 17	6	6.16 ± 0.12* (7.69 ± 0.05)	33.9 (16.6–68.9)	132 ± 17
8-OH-DPAT	7	— (7.59 ± 0.29)	—	32 ± 10*	7	— (7.52 ± 0.33)	—	24 ± 10*

Values are mean ± s.e.mean for pD₂, E_{max} (maximal contraction expressed as % of E_{max} obtained for 5-HT) and geometric mean (95% confidence limits) for relative potency (EC₅₀ agonist/EC₅₀ 5-HT). pD₂ values obtained from respective control response curve to 5-HT constructed before each agonist response curve are given in brackets. The EC₅₀ for 8-OH-DPAT could not be determined (see Results section for details). *P < 0.05 compared with respective 5-HT data. For abbreviations, see text.

Figure 1 Average concentration-effect curves to 5-hydroxytryptamine (5-HT) and to 5-HT receptor agonists in rabbit middle (a,b) and posterior (c,d) cerebral arteries. 5-HT control curve (●); 5-carboxamidotryptamine (■); sumatriptan (□); 5-methoxytryptamine (Δ); α-methyl-5-HT (▲); 8-hydroxy-2-(di-n-propylamino) tetralin (×). The average control curve to 5-HT, which is the same curve in left and right panels, represents the pooled data obtained with all tissues. Mean ± s.e.mean is shown of *n* = 5 to 32 experiments.

to that of 5-HT (Table 1). Although the maximum response to α-Me-5-HT in MCA appeared larger than that to 5-HT, this was not statistically significant (Table 1). 8-OH-DPAT was the weakest contractile agent in both rabbit MCA and PCA (*P* < 0.05). Because of the non-sigmoid form of its concentration-effect curve and the poor contraction it elicited, EC₅₀ values for this agonist could not be determined.

Antagonist studies

The effects of various antagonists with known selectivity towards 5-HT receptor subtypes were assessed against 5-HT-induced contraction of rabbit isolated MCA and PCA. The contractile response to 5-HT in both arteries was resistant to ketanserin, a selective 5-HT_{2A/2C} receptor antagonist (Figure 2), to SDZ 205-557 an antagonist of 5-HT₄ receptor and to

Figure 2 Average concentration-effect curves to 5-hydroxytryptamine (5-HT) in rabbit middle (a) and posterior (b) cerebral arteries in the absence (○) and in the presence of ketanserin 3 nM (▲) and 300 nM (■). Mean ± s.e.mean of *n* = 6 to 9 experiments.

S-(–)-propranolol, a β-adrenoceptor blocker with a relatively high affinity for 5-HT_{1A} and 5-HT_{1B} receptors (Table 2).

MDL 72222, an antagonist of 5-HT₃ receptors, produced a significant 3 and 6 fold rightward shift of the 5-HT concentration-effect curve at 0.1 and 1 μM, respectively (Figure 3). The maximum contractile response was un-

Table 2 Effects of antagonists on 5-hydroxytryptamine (5-HT)-induced contraction of rabbit middle and posterior cerebral arteries

Antagonist	μM	<i>n</i>	Middle cerebral artery			Posterior cerebral artery				
			5-HT conc.ratio	Apparent pA_2	E_{max}	<i>n</i>	5-HT conc.ratio	Apparent pA_2	E_{max}	
Propranolol	0.1	4	1.2 (0.1–20.4)	–	88 ± 6	4	1.5 (0.4–5.6)	–	99 ± 9	
	1	4	1.1 (0.5–2.5)	–	84 ± 17	4	2.0 (0.91–4.6)	–	85 ± 15	
Yohimbine	0.3	6	1.9 (0.8–4.7)	6.77 ± 0.22	80 ± 9	6	3.6 (2.4–5.5)	6.93 ± 0.09	74 ± 15	
	3	5	23.4 (7.9–69.5)	6.86 ± 0.18	68 ± 14	6	28.5 (7.0–116.0)	6.95 ± 0.24	91 ± 21	
SDZ 205-557	0.1	4	1.6 (0.15–16.5)	–	74 ± 10	4	0.7 (0.1–5.3)	–	98 ± 8	
	1	4	2.2 (0.5–9.4)	–	72 ± 12	4	2.7 (0.3–20.7)	–	87 ± 16	
ICS 205-930	0.01	6	0.8 (0.2–2.7)	–	78 ± 9	4	0.7 (0.33–1.25)	–	85 ± 10	
	0.1	6	1.9 (0.2–20.2)	–	88 ± 24	4	1.5 (0.4–5.4)	–	76 ± 13	

5-HT concentration-ratio in the presence of indicated antagonist concentration are given as geometric mean (95% confidence limits), apparent pA_2 and 5-HT E_{max} are given as mean value $\pm$ s.e.mean of *n* experiments. * $P < 0.05$ compared with respective 5-HT data.

Figure 3 Average concentration-effect curves to 5-hydroxytryptamine (5-HT) in rabbit middle (a) and posterior (b) cerebral artery in the absence (○) and in the presence of MDL 72222 0.01 μM (▲), 0.1 μM (■) and 1 μM (◆). Mean $\pm$ s.e.mean of *n* = 4 to 9 experiments.

changed in PCA but was significantly reduced in MCA by MDL 72222 ($P < 0.05$). ICS 205-930 at low concentrations (0.1–10 nM) has a known antagonist action on 5-HT₃ receptors whilst at higher concentrations (1 μM) it antagonizes 5-HT₄ receptors (Clarke *et al.*, 1989). ICS 205-930, at 0.01 and 0.1 μM , did not affect in terms of maximal contraction and sensitivity the 5-HT-induced contraction in either MCA or PCA (Table 2).

Methiothepin (0.001–0.1 μM), a mixed 5-HT₁-like/5-HT_{2A} receptor antagonist, behaved as a potent antagonist causing a concentration-dependent parallel rightward shift of the concentration-response curve to 5-HT with no significant depres-

Figure 4 Average concentration-effect curves to 5-hydroxytryptamine (5-HT) in rabbit middle (a,b) and posterior (c,d) cerebral arteries in the absence (○) and in the presence of methiothepin 0.001 μM (▲), 0.01 μM (■) and 0.1 μM (◆). The respective Schild regression plots are given on the right part of the figure. Mean $\pm$ s.e.mean of *n* = 5 to 10 experiments.

sion of the maximum response (Figure 4). The Schild plot analysis of methiothepin against 5-HT gave a pA_2 value of 9.4 ± 0.7 for MCA and a pA_2 value of 9.6 ± 0.8 for PCA. The regression line had a slope of 0.9 ± 0.15 for MCA and of 0.75 ± 0.15 for PCA. The slopes were not significantly different from one indicating competitive antagonism.

Concentration-dependent shifts of the 5-HT concentration effect curve were observed with both yohimbine and its enantiomer, rauwolscine (Table 2 and Figure 5). 5-HT concentration-ratios and corresponding apparent pA_2 values of yohimbine are given in Table 2. Schild plot analysis of rauwolscine effects against 5-HT (Figure 5), gave a pA_2 value of 7.1 ± 0.6 and a slope of 1.05 ± 0.2 for MCA. For PCA, a pA_2 value of 6.7 ± 0.6 and a slope of 1.13 ± 0.26 were determined. In each case, the slopes were not significantly different from 1, indicating competitive antagonism.

Discussion

These results demonstrate that contractions of rabbit isolated MCA and PCA to 5-HT are mediated by 5-HT_{1D} receptors with no contribution by 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT₄

Figure 5 Average concentration-effect curves to 5-hydroxytryptamine (5-HT) in rabbit middle (a,b) and posterior (c,d) cerebral arteries in the absence (○) and in the presence of rauwolscline 0.1 μM (▲), 1 μM (■) and 10 μM (◆). The respective Schild regression plots are given on the right side of the figure. Mean $\pm$ s.e.mean of $n = 4$ to 8 experiments.

receptors. Nevertheless, a participation of 5-HT₃ receptors could not be ruled out.

In some cerebrovascular preparations such as the rabbit (Trezise *et al.*, 1992) and the dog basilar artery (Connor & Feniuk, 1989), the reactivity of smooth muscle has been shown to be influenced by the presence of the endothelium (see Cocks & Angus, 1983). In particular, endothelial removal potentiated the response to 5-HT and increased the maximum of the contraction. Therefore, in order to assay the full constrictor effect of 5-HT and 5-HT agonists, we conducted all the experiments on arteries without endothelium. Under these conditions, the involvement of 5-HT_{2A} receptors was first ruled out in the 5-HT-mediated contraction of rabbit MCA and PCA. This was based on the observation that the potent 5-HT_{2A} antagonist, ketanserin was devoid of antagonist activity. 5-HT_{2A} receptors, however, have been shown to mediate 5-HT contractions of rabbit vertebral artery (Griffith & Burnstock, 1982) and rabbit aorta (Feniuk *et al.*, 1985). In the latter preparation, ketanserin was a potent antagonist with a pA_2 value of 8.7 (Feniuk *et al.*, 1985). In addition, Parsons & Whalley (1989) showed that in the rabbit basilar artery, 5-HT activated a small population of 5-HT_{2A} receptors. Thus, marked differences in the contribution of 5-HT_{2A} receptors to 5-HT-induced contractions exist between cerebral arteries from the same species. It is also unlikely that activation of vascular 5-HT₄ receptors (Cocks & Arnold, 1993) mediated contractions to 5-HT in rabbit MCA and PCA since the 5-HT₄ receptor antagonist, SDZ 205-557, a compound previously shown to be a potent selective antagonist of 5-HT₄ receptors in the guinea-pig isolated ileum had no effect (Buchheit *et al.*, 1992). MDL 72222, an antagonist of 5-HT₃ receptors (Fozard, 1984) produced a 6 fold shift at 1 μM of the concentration-effect curve to 5-HT in both arteries which was associated with a decrease in the maximum contraction in MCA only. ICS 205-930, an antagonist of 5-HT₃ receptors at low concentrations (0.1–10 nM; Clarke *et al.*, 1989) had no effect at 0.01 and 0.1 μM against the contraction to 5-HT in either artery; ICS 205-930 has been shown to have a greater affinity for 5-HT₃ receptors than MDL 72222 (Hoyer, 1990) and thus it

is possible that MDL 72222 produced some non specific effect in our experiments. Although we cannot reach a definite conclusion on the role of 5-HT₃ receptors in the 5-HT-mediated contraction, their participation is likely to be minor. In support of this, previous reports showed that the contractions to 5-HT of the rabbit, dog and primate basilar artery (Parsons & Whalley, 1989; Connor *et al.*, 1989), the cat middle cerebral artery (Hamel *et al.*, 1989), the human basilar artery (Parsons *et al.*, 1989) and the human pial arterioles (Hamel & Bouchard, 1991) were not mediated by 5-HT₃ receptors. So far, 5-HT₃ receptors have been found only on neurones where they are responsible for eliciting the depolarizing action of 5-HT (Bradley *et al.*, 1986) and further studies would be needed to investigate such a mechanism in our vessel preparations.

The mixed 5-HT₁-like/5-HT_{2A} receptor antagonist, methiothepin, was a potent competitive antagonist of 5-HT-induced contractions in rabbit MCA and PCA. Respective pA_2 values of 9.4 (MCA) and 9.6 (PCA) were determined suggesting that 5-HT was acting at the same receptor type in the two vessels to produce contraction. These observations are consistent with the presence of 5-HT₁-like receptors in both preparations, with similar pA_2 values to those found in vessels where 5-HT₁-like receptors mediate vasoconstriction; 8.8 in human basilar artery (Parsons *et al.*, 1989), 8.6 in rabbit renal artery (Tadipatri *et al.*, 1992) and 8.5 in human pial arterioles (Hamel & Bouchard, 1991). Interestingly, our pA_2 values are also close to the pA_2 value of 10.3 reported by Parsons & Whalley (1989) in the rabbit basilar artery.

When comparing the contractile potency of various agonists, 5-CT, a selective agonist at 5-HT₁-like receptors, was found to be the most potent in both types of arteries. The EC_{50} values for 5-CT were 7.1 fold and 1.3 fold higher than that for 5-HT in MCA and PCA, respectively. In addition, sumatriptan, another selective 5-HT₁-like agonist effective in the acute treatment of migraine (Doenicke *et al.*, 1988), was found to be a potent vasoconstrictor of rabbit MCA and PCA.

Taken together, the ineffectiveness of ketanserin, ICS 205-930 and SDZ 205-557 in preventing the contractile effect of 5-HT and the potent antagonism by methiothepin as well as the higher potency of 5-CT as compared with 5-HT and the agonist effect of sumatriptan, strongly suggest, according to the criteria of classification proposed by Bradley *et al.* (1986), that 5-HT₁-like receptors are predominantly involved in the contraction of rabbit MCA and PCA.

Both α -Me-5-HT and 5-MeOT contracted the rabbit MCA and PCA. These compounds are usually considered as selective for 5-HT_{2A} and 5-HT₄ receptors, respectively (Bradley *et al.*, 1986; Saxena & Villalon, 1990). Nevertheless, their ability to contract rabbit MCA and PCA could result from a stimulation of 5-HT₁-like receptors rather than 5-HT_{2A} or 5-HT₄ receptors since it has been demonstrated by Tadipatri *et al.* (1992) that these agonists contracted the rabbit isolated renal artery via 5-HT₁-like receptors. Thus, the selectivity of these tryptamine derivatives is questionable, particularly since α -Me-5-HT and 5-MeOT have identical binding affinity at 5-HT₁, 5-HT_{2A} and 5-HT₄ sites (Hoyer & Fozard, 1991).

The β -adrenoceptor blocker, S-(–)-propranolol, did not alter the 5-HT concentration-effect curve, ruling out an involvement of 5-HT_{1A} and 5-HT_{1B} receptor subtypes. Furthermore, the weak effect of 8-OH-DPAT a highly selective agonist at 5-HT_{1A} receptors argues against the presence of 5-HT_{1A} receptors. Involvement of 5-HT_{2C} receptors was also unlikely since ketanserin which in addition to its high affinity for 5-HT_{2A} sites, exhibits an affinity for 5-HT_{2C} sites (pK_D values of 8.9 and 7, respectively, Hoyer & Fozard, 1991) was without effect. However, we found that yohimbine (0.3, 3 μM) and rauwolscline (0.1–10 μM), two drugs with affinity for 5-HT_{1D} sites (Hoyer & Fozard, 1991) antagonized the contractile effect of 5-HT. The apparent pA_2 value for yohimbine and the pA_2 value for rauwolscline were close to their respective affinity constants reported at 5-HT_{1D} binding

sites in mammalian brain membranes (7.1 and 7.7, respectively, Hoyer & Fozard, 1991), an observation which would favour the activation of 5-HT_{1D} receptors in rabbit MCA and PCA. In addition, agonists displaying high affinity towards 5-HT_{1D} binding site such as, 5-CT (pK_D , 9), 5-MeOT (pK_D , 8.4) and sumatriptan (pK_D , 7.5) matched the effect of 5-HT (pK_D , 8.4) better than α -Me-5-HT (pK_D = 6.2, Hoyer & Fozard, 1991). Furthermore, the apparent pA_2 value for methiothepin and yohimbine as well as the pD_2 values for 5-HT and 5-CT found in this study are in accordance with the corresponding values observed for these compounds at the presynaptic 5-HT autoreceptor in the rabbit brain cortex (Limberger *et al.*, 1991). Taken together our results support the view that the receptor underlying 5-HT-mediated contraction of rabbit middle and posterior cerebral arteries is of the 5-HT_{1D} subtype. Interestingly, such a subtype of 5-HT receptor was not found in the rabbit basilar artery, where yohimbine failed to antagonize the contractile effect of 5-HT and of sumatriptan (Parsons & Whalley, 1989). Such a difference seems to reflect a differential distribution of 5-HT receptors along the cerebral vasculature of the rabbit.

This study provides the first demonstration of 5-HT_{1D}

receptor-mediated contractile response in rabbit cerebral arteries. Since this type of 5-HT receptor has been shown to be involved in 5-HT-induced contraction of human cerebral arterioles (Hamel & Bouchard, 1991) and human cerebral arteries (Jansen *et al.*, 1993), the rabbit isolated MCA and PCA might constitute useful pharmacological preparations for the development of new compounds acting at the 5-HT_{1D} receptor. Recently, two genes encoding the human 5-HT_{1D} receptor subtypes have been cloned and named 5-HT_{1D α} and 5-HT_{1D β} (Weinshank *et al.*, 1992). A weak homology of sequence (63%) between 5-HT_{1D β} and 5-HT_{1D α} receptors has been reported but their pharmacological profiles appeared almost indistinguishable (Hartig *et al.*, 1992). While it appears that the human 5-HT_{1D β} is the species homologous of the rat 5-HT_{1B} receptor (Hartig *et al.*, 1992), further studies will be needed to determine the subtype of 5-HT_{1D} receptor present in rabbit MCA and PCA.

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