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Pharmacological evidence for the 5-HT$_7$ receptor mediating smooth muscle relaxation in canine cerebral arteries

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1 We investigated in the present study whether 5-HT is able to exert direct relaxant responses in canine basilar and middle cerebral arteries *via* the 5-HT$_7$ receptor.

2 In arterial rings deprived of endothelium and pre-contracted with prostaglandin $F_{2\alpha}$ (2 μM), 5-HT, 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine, sumatriptan or α-methyl-5-HT produced further increase in tone and/or slight relaxation. Blockade of 5-HT$_{1B/1D}$ and 5-HT$_{2A}$ receptors with GR127935 (1 μM) and ketanserin (0.1 μM), respectively, antagonized the vasoconstrictor component of the response and unmasked a concentration-dependent relaxation to 5-HT, 5-CT and 5-methoxytryptamine; sumatriptan and α-methyl-5-HT remained inactive as relaxant agonists. The rank order of agonist potency in both arteries was 5-CT > 5-HT > 5-methoxytryptamine >> sumatriptan $\geqslant$ α-methyl-5-HT.

3 In dog basilar artery, pre-incubated with GR127935 (1 μM) and ketanserin (0.1 μM) and pre-contracted with prostaglandin $F_{2\alpha}$ (2 μM), the 5-HT$_7$ ligands, clozapine (1 μM), mesulergine (0.3 μM), methiothepin (3 nM), risperidone (3 nM), spiperone (1 μM) and LY215840 (10–100 nM), produced significant rightward shifts of the concentration-response curves for 5-HT and 5-CT. Only methiothepin and risperidone reduced significantly the maximum relaxant response (E_{max}), whilst the other drugs behaved as competitive antagonists with affinity values (pK_B) that significantly correlated with their binding affinity (pK_i) at recombinant 5-HT$_7$ receptors.

4 These data disclosing the involvement of the 5-HT$_7$ receptor in cerebrovascular relaxation may be strongly relevant in the light of : (1) the involvement of 5-HT in migraine; (2) the putative linkage between cephalovascular vasodilatation and migraine headache; and (3) the relatively high 5-HT$_7$ receptor affinity of migraine prophylactic 5-HT antagonists.

Keywords: Basilar artery; 5-hydroxytryptamine; middle cerebral artery; migraine; 5-HT$_7$ receptor; relaxation; vascular smooth muscle

Abbreviations: C-R, concentration-response; IUPHAR, International Union of Pharmacology; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; PKA, cyclic AMP-dependent protein kinase; Rp-cAMPS, Rp diastereoisomer of adenosine cyclic 3′,5′-monophosphothioate

Introduction

Serotonin (5-hydroxytryptamine; 5-HT) has long been implicated in the pathophysiology of migraine (Fozard, 1982; Kimball *et al*., 1960; Sicuteri *et al*., 1961; Sjaastad, 1975; Silberstein, 1994) though the precise mechanisms involved are still a matter of debate. From the standpoint of the 'vascular' hypothesis of migraine, very early data suggested that vasodilatation of the extra-cerebral branches of the external carotid artery (Ostfeld & Wolff, 1958; Saxena, 1972; Saxena & De Vlaam-Schluter, 1974; Tunis & Wolff, 1952) and to some extent also in the cerebral vessels (O'Brien, 1971; Skinhöj & Paulson, 1969) could be associated with the headache phase. Although new concepts have emerged from extensive research in this field (Fozard & Kalkman, 1994; Moskowitz, 1992), in the context of this vascular link (Humphrey & Feniuk, 1991; Saxena & Ferrari, 1989) the mechanisms underlying cerebrovascular vasodilatation in migraine and the possible role of 5-HT in its aetiology, remain to be determined.

More recently, given the evidence that nitric oxide (NO) may be involved in migraine headaches (Olesen *et al*., 1994), it was suggested that endogenous 5-HT, released perhaps from platelets, but most likely from perivascular 5-HT containing neurons in response to stress, stimulates endothelial 5-HT$_{2B}$ receptors in cerebral blood vessels to release NO (Fozard, 1995; Fozard & Kalkman, 1994; Schmuck *et al*., 1996). This latter, in addition to causing vasodilatation, would produce the 'sterile inflammatory response' in the cerebral vasculature which, according to the 'neurogenic' hypothesis of migraine, is believed to be the key step in the development of this disorder (Fozard, 1995; Fozard & Kalkman, 1994; Moskowitz, 1992). These observations notwithstanding, it should be noted that no convincing evidence for an endothelium-dependent NO-mediated relaxant effect by 5-HT in cerebral blood vessels has yet been provided (Schmuck *et al*., 1996; Connor & Feniuk, 1989). If cerebrovascular vasodilatation does underlie the development of headache, as suggested by the 'vascular' hypothesis of migraine (Humphrey & Feniuk, 1991; Saxena & Ferrari, 1989), and 5-HT is involved in this process, one could expect from the above observations that another mechanism e.g. 5-HT$_7$ receptor, may be involved in a putative vasodilator effect induced by 5-HT in the cerebral vasculature. In fact, it is very interesting to note that most of the migraine prophylactic drugs, including amitriptyline, chlorpromazine, cyproheptadine, lisuride, LY215840, metergoline, methysergide, mianserin, and sergolexole, display moderate to high affinity for the recombinant 5-HT$_7$ receptor (see Terrón, 1998a for review). On this basis, the present study was aimed to investigate whether, as previously reported in the dog coronary artery (Terrón, 1996), treatment with the potent and selective 5-HT$_{1B/1D}$ receptor antagonist, GR127935 (Skingle *et al*., 1996), could unmask a direct relaxing mechanism probably related to the 5-

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HT_7 receptor in canine cerebral arteries. Since large intracranial arterial dilatation has been associated with migraine headache in patients (Friberg *et al*., 1991), we selected the basilar and middle cerebral arteries of a model system, i.e. the dog, for the study. The results reveal that 5-HT does produce direct relaxation in the above vessels through a receptor that displays close pharmacological alignment to the 5-HT_7 type. Preliminary accounts of this investigation have been published previously in abstract form (Terrón, 1997a; 1998b).

Methods

Tissue preparation

A total of 28 mongrel dogs (15–25 kg) of either sex were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) and killed by rapid exsanguination from the carotid arteries. Brains were quickly removed and basilar and middle cerebral arteries were dissected and placed in Krebs bicarbonate solution and stored overnight at 4°C. The vessels were cleaned of adherent tissue and cut into ring segments 3–4 mm in length. Up to eight (basilar) or four (middle cerebral) adjacent rings from the same vessel were used as experimental and control rings. The middle cerebral artery of both sides was employed. In order to remove the endothelium, the vessels were perfused intraluminally with Triton X-100 (0.1%, 0.5 ml min^{-1} for 1 min), as previously reported (Verrechia *et al*., 1986).

Organ chamber studies

Rings were suspended horizontally in an organ bath by two L-shaped stainless-nikrom wire hooks (0.2 mm diameter). The lower hook was attached by a clamp to a tissue holder, whilst

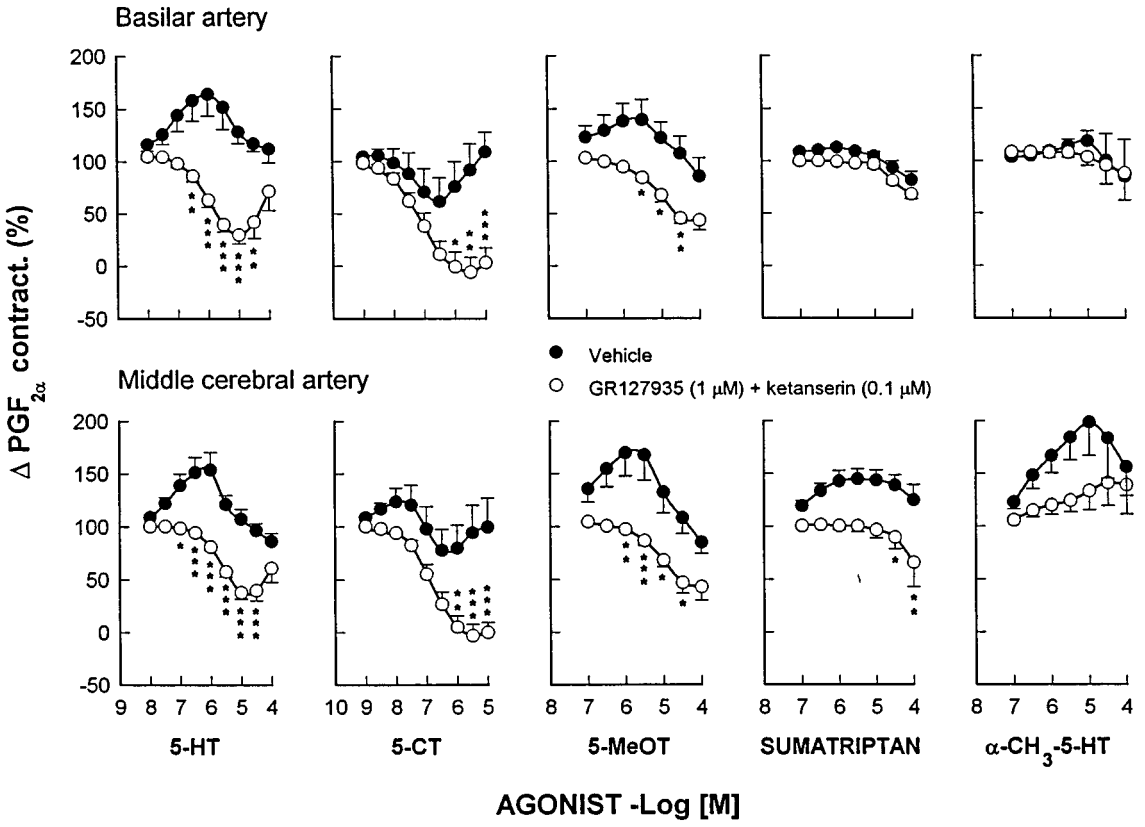

Figure 1 Effect of ketanserin and GR127935, as compared to that of vehicle, on cumulative concentration-response curves for 5-HT, 5-CT, 5-methoxytryptamine (5-MeOT), sumatriptan and α-methyl-5-HT (α-CH_3-5-HT) in endothelium-denuded canine basilar and middle cerebral artery rings taken from the same animal. Changes in tension are expressed as percentage of the contraction to prostaglandin $F_{2\alpha}$ (2 μM). Points are the mean, and vertical bars denote the s.e.mean of 3–6 observations. Contraction to prostaglandin $F_{2\alpha}$ in the basilar artery was 2.06±0.13 and 1.83±0.1 g while in the middle cerebral artery was 1.49±0.17 and 1.33±0.11 g in vehicle- and antagonist-treated rings, respectively. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 1 Relaxing potency (−log EC_{50}; pD_2) and efficacy (E_{max}) of 5-HT receptor agonists in endothelium-denuded canine basilar and middle cerebral arteries

	Basilar artery			*Middle cerebral artery*		
Agonist	n	pD_2	E_{max} (%)[a]	n	pD_2	E_{max} (%)[a]
5-HT	5	6.00±0.13	75.4±8.5	5	5.76±0.04	64.4±7.8
5-CT	5	7.24±0.14	105.6±14	6	7.11±0.14	103.3±11
5-MeOT	4	5.03±0.1	65.3±4.9	5	5.16±0.15	59.3±11.9
Sumatriptan	4	<5	nd	4	<5	nd
α-Methyl-5-HT	3	<5	nd	4	<5	nd

[a]Expressed as percentage of contraction to prostaglandin F2α (2 μM). Preparations had been pre-incubated (45 min) with GR127935 (1 μM) and ketanserin (0.1 μM). 5-MeOT, 5-methoxytryptamine; nd, not determined.

the upper hook was connected directly to an isometric force displacement transducer (Grass FT03C) so that isometric changes in force could be recorded on a Grass model 7D Polygraph. The rings were mounted in organ chambers containing 10 ml of Krebs bicarbonate solution of the following composition (mM concentrations): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.15; $MgSO_4$, 1.2; $NaHCO_3$, 24; KH_2PO_4, 1.2; dextrose, 11; and Ca_2EDTA, 0.026. This solution was maintained at 37°C and aerated continuously with 95% O_2 and 5% CO_2 to give pH 7.4. Tissues were gradually stretched over a period of 30 min to a basal tension of 3 g; this amount of resting tension has been shown to maximize the contractile response to 5-HT and other spasmogens, including prostaglandin $F_{2\alpha}$ (Allen *et al*., 1974). The preparations were allowed to equilibrate for 2 h with elicitation of contractile responses every 30 min by depolarization with 30 mM potassium chloride. Then, two or three contractile responses to prostaglandin $F_{2\alpha}$ (2 μM) were obtained. This procedure ensured strong and sustained contractile responses to prostaglandin $F_{2\alpha}$ for analysis of agonist-induced relaxation. The absence of functional endothelium was verified by exposure to oxytocin (1 μM) under pre-contraction with prostaglandin $F_{2\alpha}$ (2 μM). Those rings that relaxed to oxytocin were not used for the study.

Experimental protocols

After completion of the above procedures, two protocols were followed. The first protocol was designed to evaluate the vasomotor activity of 5-HT (10 nM – 100 μM), 5-carboxamidotryptamine (5-CT; 1 nM – 10 μM), 5-methoxytryptamine (100 nM – 100 μM), sumatriptan (100 nM – 100 μM) and α-methyl-5-HT (100 nM – 100 μM) in basilar and middle cerebral artery rings pre-incubated with either vehicle or GR127935 (1 μM) to block 5-HT$_{1B/1D}$ receptors. Since 5-HT$_{2A}$ receptors have been suggested to partly mediate the contractile response to 5-HT in the dog basilar artery (Connor *et al*., 1989), the experiments were conducted in the presence of ketanserin (0.1 μM) as well. Thus, after 30 min of equilibration with either vehicle or GR127935 and ketanserin, preparations were contracted with prostaglandin $F_{2\alpha}$ (2 μM). In the plateau phase of the contraction, which became stable in about 15 min, the agonists were cumulatively added to obtain full concentration-response (C-R) curves. Therefore, the incubation time with vehicle or GR127935 and ketanserin was approximately 45 min.

The second protocol explored the effects of the high-affinity 5-HT$_7$ receptor ligands, clozapine (1 μM), mesulergine (0.3 μM), methiothepin (3 nM), risperidone (3 nM), spiperone (1 μM) and LY215840 (10 – 100 nM), on the relaxation induced by 5-HT and 5-CT in the canine basilar artery. These experiments were conducted in the presence of GR127935 (1 μM) and ketanserin (0.1 μM). Then, cumulative C-R curves for 5-HT and 5-CT were generated in tissues incubated with either vehicle (controls) or antagonist for 1 h. Each concentration of agonist (spaced by a factor of 10½) was added only after the maximum response to the previous concentration had been attained. Responses to 5-HT and 5-CT in vehicle- and

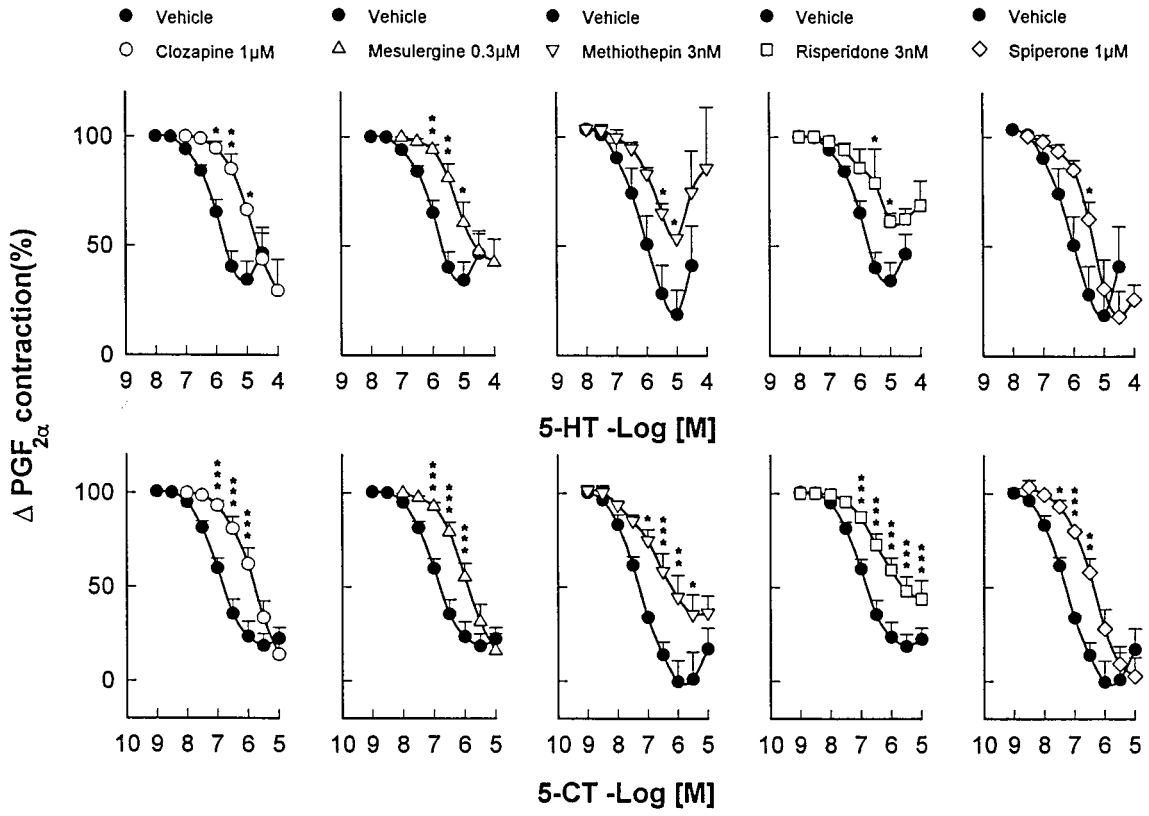

Figure 2 Effect of clozapine, mesulergine, methiothepin, risperidone or spiperone, as compared to that of vehicle, on cumulative concentration-response curves for 5-HT and 5-CT in endothelium-denuded canine basilar artery rings taken from the same animal. The vessels had been pre-incubated with GR127935 (1 μM) and ketanserin (0.1 μM). Changes in tension are expressed as percentage of the contraction to prostaglandin $F_{2\alpha}$ (2 μM). Points are the mean and vertical bars denote the s.e.mean of 3–8 observations. Contraction to prostaglandin $F_{2\alpha}$ was 2.45 ± 0.31 and 2.75 ± 0.25 g (5-HT set) and 2.45 ± 0.27 and 2.24 ± 0.2 g (5-CT set) in vehicle- and antagonist-treated rings, respectively. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

antagonist-treated tissues were elicited in separate rings so that only one C-R curve was obtained in each tissue.

Data presentation and statistical evaluation

All data in the text and figures are expressed as the mean ± s.e.mean, where *n* represents the number of dogs from which the vessels were taken. In order to restrict the number of dogs used in the present study, no more than one tissue was used from each animal for any given treatment. Changes in tension are expressed as percentage of the contraction to prostaglandin $F_{2\alpha}$ (2 μM). Comparisons between vehicle- and antagonist-treated rings obtained from the same animal were performed in separate tissues and no tissue was used to generate more than one agonist C-R curve. The pD_2 values (negative logarithm of EC_{50}, the agonist concentration producing 50% of the maximum relaxant response, calculated by nonlinear regression analysis) and the maximum relaxant response (E_{max}) were determined from individual C-R curves. Apparent antagonist dissociation constants (K_B), expressed as the negative logarithm ($-\log K_B = pK_B$), were determined according to the equation $K_B = B/(\text{dose ratio-1})$ where B is the concentration of the antagonist and dose ratio is the EC_{50} of the agonist in the presence of the antagonist divided by the EC_{50} of the agonist in vehicle-treated preparations.

Comparisons of the relaxant responses obtained in vehicle- and antagonist-treated rings excised from the same animal were performed in separate tissues using one-way analysis of variance, followed by a Newman-Keuls' test. For comparison of the agonist potency (pD_2) and efficacy (E_{max}) in the basilar and middle cerebral arteries, and the antagonist affinity estimates (pK_B) obtained against 5-HT and 5-CT in the basilar artery, *t*-test was applied. Statistical significance was defined at $P<0.05$. The data for LY215840 were also analysed in accordance with the procedure of Arunlakshana & Schild (1959). The dose-ratios were determined at various concentrations of LY215840. If blockade is competitive under equilibrium conditions, then a plot of the logarithm of (dose ratio − 1) against the negative logarithm of the molar concentration of the antagonist should yield a straight line whose slope is not different from unity and whose intercept on the abscissa is the pA_2 which is generally considered to be equivalent to $-\log K_B$.

Drugs

The drugs used in the present study (obtained from the sources indicated) were the following: 5-hydroxytryptamine creatinine sulphate, oxytocin, prostaglandin $F_{2\alpha}$ (Sigma Chemical Company, St. Louis, MO, U.S.A.); 5-methoxytryptamine hydrochloride and α-methyl-5-HT (Research Biochemicals Int., Natick, MA, U.S.A.); 5-carboxamidotryptamine maleate, GR127935 and sumatriptan succinate (gift: Glaxo Group Research, Ware, U.K.); clozapine and mesulergine (gift: Sandoz A.G., Basel, Switzerland); ketanserin tartrate, risperidone and spiperone (gift: Janssen Pharmaceutica, Beerse, Belgium); LY215840 (gift: Eli Lilly, Indianapolis, IN, U.S.A.); and methiothepin maleate (gift: Hoffman-La Roche Ltd., Basel, Switzerland).

All compounds were dissolved in distilled water. When needed, 4% ascorbic acid (clozapine, LY215840 and spiperone) or 5% (v v^{-1}) DMSO (mesulergine, methiothepin and risperidone) was employed to prepare stock solutions from which aqueous dilutions were made. Fresh solutions were prepared for each experiment and vehicles had no effect on baseline tension or agonist-induced responses.

Results

As shown in Figure 1, 5-HT, 5-CT, 5-methoxytryptamine, sumatriptan and α-methyl-5-HT had no effects or produced further increase in tone and/or slight relaxation in control pre-contracted vessels. However, in the presence of GR127935 (1 μM) and ketanserin (0.1 μM), 5-HT, 5-CT and 5-methoxytryptamine, but not sumatriptan or α-methyl-5-HT, produced concentration-dependent relaxant responses in both the basilar and middle cerebral artery (Figure 1). Based upon the corresponding pD_2 values, the rank order of agonist potency in both arteries was 5-CT > 5-HT > 5-methoxytryptamine >> sumatriptan ≥ α-methyl-5-HT. Agonist affinity estimates were not significantly different when comparing the basilar and the middle cerebral artery and only small differences in agonist efficacy were observed (Table 1; Figure 1). For this reason, we decided to perform the interaction experiments in the basilar artery only.

Thus, incubation of basilar artery ring segments with clozapine (1 μM), mesulergine (0.3 μM), methiothepin (3 nM), risperidone (3 nM) or spiperone (1 μM), produced significant rightward shifts of the C-R curves for 5-HT and 5-CT (Figure 2). As previously noticed in other vascular preparations

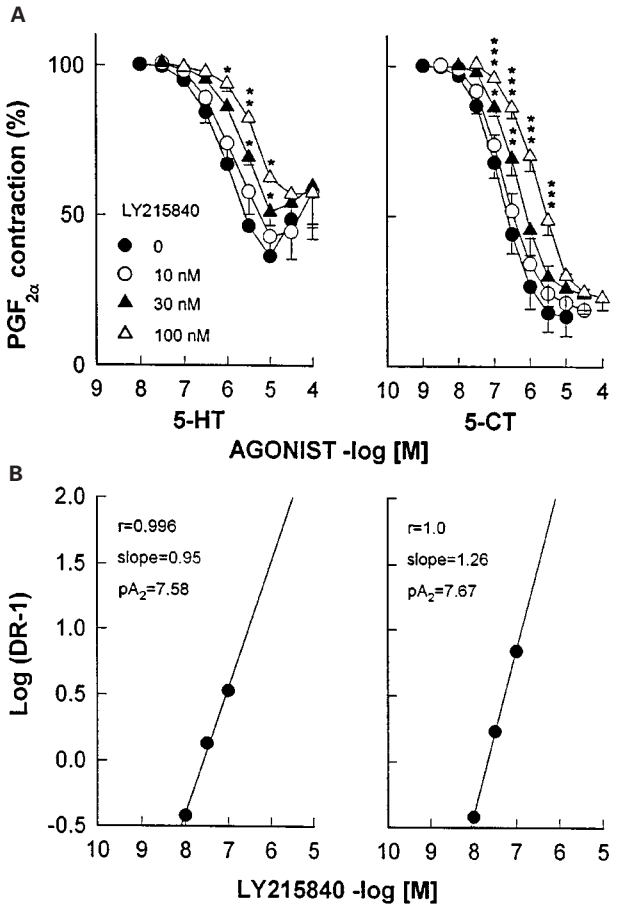

Figure 3 (A) Effect of increasing concentrations of LY215840 on cumulative concentration-response curves for 5-HT and 5-CT in endothelium-denuded canine basilar artery ring segments taken from the same animal. The vessels had been pre-incubated with GR127935 (1 μM) and ketanserin (0.1 μM). Changes in tension are expressed as percentage of the contraction to prostaglandin $F_{2\alpha}$ (2 μM). Points are the mean and vertical bars denote the s.e.mean of 4–5 observations. Contraction to prostaglandin $F_{2\alpha}$ was 3.46 ± 0.35 and 2.95 ± 0.23 g (5-HT set) and 3.36 ± 0.25 and 3.19 ± 0.21 g (5-CT set) in vehicle- and antagonist-treated rings, respectively. (B) Schild plots for the antagonist effects of LY215840. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

(Martin & Wilson, 1995; Terrón, 1996; 1997b), methiothepin and risperidone behaved as potent unsurmountable antagonists as both caused a significant reduction in the E_{max} of 5-HT and 5-CT, while clozapine, mesulergine and spiperone were simple competitive antagonists (Figure 2). In addition, LY215840 produced a parallel concentration-dependent rightward shift of the C-R curves for 5-HT and 5-CT (Figure 3A). Schild analysis yielded straight lines with pA_2 values (7.58 and 7.67 against 5-HT and 5-CT, respectively) that matched the affinity of LY215840 for the human 5-HT$_7$ receptor ($pK_i = 7.83$; Cushing *et al*., 1996); the slope of the Schild plot (0.95 and 1.26 against 5-HT and 5-CT, respectively) was not significantly different from unity (Figure 3B).

Discussion

The present study demonstrates, for the first time, that 5-HT does produce endothelium-independent relaxant responses in major conduit cerebral arteries. On the basis of operational criteria, the 5-HT receptor involved can be classified within the 5-HT$_7$ type. Despite the role of the 5-HT$_7$ receptor in peripheral vascular and nonvascular smooth muscle relaxation has been elucidated (Eglen *et al*., 1997; Terrón, 1998a), its potential implication in the regulation of cerebroarterial tone may be strongly relevant in the light of: (1) the involvement of 5-HT in the pathogenesis of migraine; (2) the putative linkage between cephalovascular vasodilatation and migraine headache; and (3) the relatively high affinity of several migraine prophylactic drugs for the 5-HT$_7$ receptor (see Terrón, 1998c for review). Apart from the implications discussed below, these findings may provide new insights into the regulatory role of 5-HT in cerebrovascular tone and perhaps also in the pathophysiological mechanisms of migraine.

Direct relaxant effects of 5-HT receptor agonists in dog basilar and middle cerebral arteries

It seems clear, from the use of GR127935 in the present experiments, that blockade of 5-HT$_{1B/1D}$ receptors is a requisite for the direct relaxant activity of 5-HT and some 5-HT receptor agonists to be manifest. Thus, the previously reported inability of 5-HT and 5-CT to cause relaxation in canine endothelium-intact basilar artery rings pre-contracted with prostaglandin $F_{2\alpha}$, U46619, uridine triphosphate or potassium chloride, even in the presence of a high concentration of ketanserin (1 μM) and phenoxybenzamine (30 μM) (Connor & Feniuk, 1989), most likely reflects a predominant role of vasoconstrictor mechanisms over those mediating relaxation due to the inability of these blockers to abolish 5-HT$_{1B/1D}$ receptor-mediated vasoconstriction. Since endothelium-denuded preparations were employed in the present study, the relaxant effect of 5-HT is most likely due to an action on smooth muscle cells. In fact, the relaxant response induced by 5-HT and 5-CT in endothelium-denuded GR127935- and ketanserin-treated preparations of basilar and middle cerebral artery could not be inhibited by pre-treatment with the NO synthase and cyclo-oxygenase inhibitors, N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 μM) and indomethacin (10 μM), respectively (unpublished). On the other hand, the rather selective 5-HT$_{2B}$ receptor agonist, α-methyl-5-HT (Baxter *et al*., 1995), which is well-documented to produce strong and potent endothelium-dependent 5-HT$_{2B}$ receptor-mediated relaxant responses in peripheral vessels (Bodelson *et al*., 1993; Glusa & Richter, 1993; Leff *et al*., 1987; Sumner, 1991), failed to relax both endothelium-intact (not shown) and endothelium-free dog basilar and middle cerebral artery ring segments (Figure 1) pre-incubated with GR127935 (1 μM) and ketanserin (0.1 μM). These observations in dog cerebral arteries may therefore argue against the mechanistic concept in migraine involving a 5-HT-induced release of NO, *via* activation of endothelial 5-HT$_{2B}$ receptors, in cerebral blood vessels (Fozard & Kalkman, 1994; Fozard, 1995; Schmuck *et al*., 1996).

Pharmacological profile at the relaxant 5-HT receptor in canine cerebral arteries

As observed in other vascular preparations in which 5-HT mediates smooth muscle relaxation through the 5-HT$_7$ receptor (Leung *et al*., 1996; Martin & Wilson, 1995; Terrón, 1996; 1997b), the rank order of agonist potency obtained in the canine basilar and middle cerebral arteries i.e. 5-CT > 5-HT > 5-methoxytryptamine > > sumatriptan ≥ α-methyl-5-HT, is consistent with the binding profile reported at recombinant 5-HT$_7$ receptors (Bard *et al*., 1993; Lovenberg *et al*., 1993; Plassat *et al*., 1993; Ruat *et al*., 1993; Shen *et al*., 1993). Since agonist pD_2 values obtained in the basilar and middle cerebral arteries were not significantly different (Table 1), it is likely that a closely related or even an identical receptor mediates the relaxant response to 5-HT in both tissues.

On the other hand, the 5-HT$_7$ receptor ligands, clozapine, mesulergine, methiothepin, risperidone and spiperone, which are well-documented antagonists at vascular 5-HT$_7$ receptors (Leung *et al*., 1996; Martin & Wilson, 1995; Sumner *et al*., 1989; Terrón, 1996; 1997a,b; Villalón *et al*., 1997), significantly blocked the relaxant response to 5-HT and 5-CT in the basilar artery (Figure 2). Importantly, these effects were achieved at concentrations consistent with the affinity of the antagonists at the 5-HT$_7$ receptor (Table 2; see Bard *et al*., 1993; Ruat *et al*., 1993; Shen *et al*., 1993). In order to further characterize the relaxant cerebrovascular 5-HT receptor, we decided to evaluate the effects of LY215840 (Cushing *et al*., 1996), on 5-HT and 5-CT-induced relaxation in the basilar artery. In addition to having high affinity for 5-HT$_2$ receptor subtypes, this ergoline was recently demonstrated to display high affinity for a transiently expressed human 5-HT$_7$ receptor and to behave as potent competitive antagonist at the relaxant 5-HT$_7$ receptor in the canine coronary artery smooth muscle (Cushing *et al*., 1996). As expected, LY215840 exerted a concentration-dependent rightward displacement of the C-R curves for 5-HT and 5-CT with no significant reduction in the maximum relaxant response to the agonists (Figure 3A). Both pK_B values calculated from a single concentration of LY215840 (Table 2) and pA_2 values obtained from the Schild plots depicted in Figure 3B are in close agreement with the binding affinity of LY215840 at the human 5-HT$_7$ receptor ($pK_i = 7.83$; Cushing

Table 2 Affinity estimates for antagonists against 5-HT- and 5-CT-induced relaxation in endothelium-denuded canine basilar artery

Antagonist	*Concentration* (μM)	*Affinity estimates* (pK_B) *5-HT*	*5-CT*
Clozapine	1	7.1 ± 0.1(4)	6.9 ± 0.1(6)
Mesulergine	0.3	7.3 ± 0.1(4)	7.3 ± 0.2(6)
Methiothepin	0.003	8.7 ± 0.2(3)	9.0 ± 0.3(3)
Risperidone	0.003	9.2 ± 0.2(3)	8.9 ± 0.1(6)
Spiperone	1	6.6 ± 0.2(3)	6.9 ± 0.3(3)
LY215840	0.1	7.7 ± 0.13(4)	7.7 ± 0.2(5)

pK_B values (mean ± s.e.mean for *n* experiments in parenthesis) were determined as described in Methods.

et al., 1996). In view that the antagonist dissociation constants (pK_B values) for all the above drugs were similar regardless of whether 5-HT or 5-CT was used as relaxant agonist (Table 2), the involvement of a common receptor site can be suggested.

Although the antagonist drugs used in the present study display moderate to high affinity for other 5-HT receptors (see Terrón, 1998a for review), their affinity estimates at the relaxant 5-HT receptor significantly ($P<0.01$) correlated with their binding affinity at the recombinant 5-HT$_7$ receptor ($r=0.972$ and $r=0.981$ against 5-HT and 5-CT, respectively, Figure 4). Correlation with other 5-HT receptors, including the 5-HT$_{1A}$ subtype (Figure 4), which is targeted by some agonists (e.g, 8-OH-DPAT and 5-CT) that also stimulate the 5-HT$_7$ receptor (Eglen *et al.*, 1997; Terrón, 1998a), and the 5-HT$_{2B}$ receptor (Figure 4), which has been associated with migraine pathogenesis (see below), were not only much lower than those obtained with the 5-HT$_7$ type, but also a theoretical straight line connecting the correlation points could be rejected ($P>0.05$). Similarly, non-significant correlations were obtained with 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-ht$_6$ receptors (not shown).

Finally, it is worth mentioning that a high concentration (100 μM) of the Rp diastereoisomer of adenosine cyclic 3′,5′-monophosphothioate (Rp-cAMPS), an antagonist of the cyclic AMP-dependent protein kinase (PKA; Van Haastert *et al.*, 1984), partially – but significantly – inhibited the relaxant response induced by 5-CT in the dog basilar artery pre-incubated with GR127935 (1 μM) and ketanserin (0.1 μM) (Terrón, unpublished observation). Although other transduction mechanisms could be involved, this finding suggests that, like native (Trevethick *et al.*, 1986; Sumner *et al.*, 1989) and cloned (Bard *et al.*, 1993; Lovenberg *et al.*, 1993; Plassat *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993) 5-HT$_7$ receptors, the cerebrovascular 5-HT$_7$ receptor may be positively coupled to the adenylyl cyclase system. This possibility could be further supported by an earlier study showing that 5-HT-induced inhibition of spontaneous rhythmic contraction of porcine pial veins, an effect displaying a 5-HT$_7$-like receptor pharmacology, was (1) enhanced by a cyclic AMP phosphodiesterase inhibitor; (2) diminished by a PKA inhibitor; and (3) accompanied by an increase in cyclic AMP, but not cyclic GMP synthesis (Ueno *et al.*, 1995). Although additional experiments are required to further elucidate the transductional pathway(s) linked to the cerebrovascular 5-HT$_7$ receptor, the above observations are in agreement with the transductional criterion under which the 5-HT$_7$ receptor was classified by the International Union of Pharmacology (IUPHAR) serotonin receptor classification committee (Hoyer *et al.*, 1994).

Potential impact of the 5-HT$_7$ receptor in migraine

An interesting observation prompting us to search for a relaxant 5-HT$_7$-like receptor mechanism in cerebral vessels was the fact that, with the exception of pizotifen and propranolol for which no binding data at the 5-HT$_7$ receptor have been reported thus far, most of the migraine prophylactic drugs such as amitriptyline, cyproheptadine, lisuride, methysergide and mianserin, display relatively high affinity (pK_i values between 9 and 7.1) for the recombinant 5-HT$_7$ receptor (Bard *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993). The same applies to the antimigraine drugs, sergolexole and LY215840, two ergoline-derivatives which are in phases II and III of clinical development, respectively, as well as to the recently launched antimigraine compound, metergoline (see Terrón, 1998a for review). In fact, some of the above drugs i.e. lisuride, LY215840, metergoline, methysergide, mianserin and sergolexole (the others have not been tested thus far), have been shown to antagonize functional 5-HT$_7$ receptors mediating vasorelaxation in several vascular smooth muscle preparations

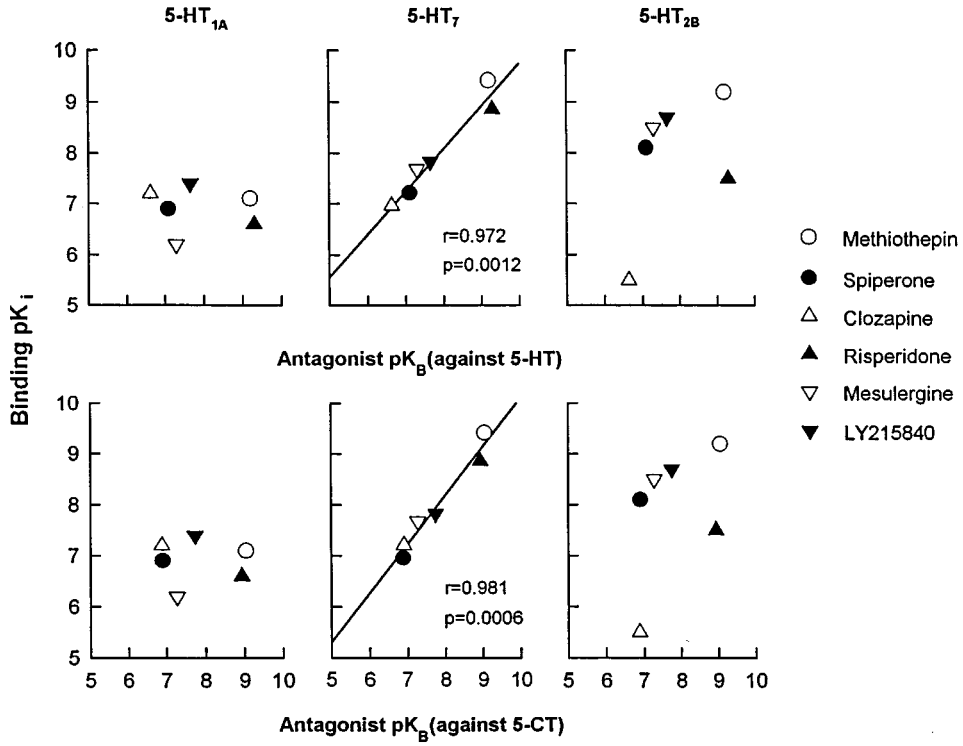

Figure 4 Correlation of antagonist affinity estimates (pK_B values) against 5-HT and 5-CT at the relaxant 5-HT receptor in the canine basilar artery smooth muscle and binding affinity (pK_i values) at 5-HT receptor subtypes.

(Cushing *et al*., 1996; Leung *et al*., 1996; Martin & Wilson, 1995; Sumner *et al*., 1989; Terrón, 1996; 1997a,b; Ueno *et al*., 1995), including the external carotid circulation (Villalón *et al*., 1997) which was long suggested to be involved in the pathophysiology of migraine (Ostfeld & Wolf, 1958; Saxena, 1972; Saxena & de Vlaam-Schluter, 1974; Tunis & Wolff, 1952). Most interesting is the observation that the average – pharmaceutically-active – doses of several migraine prophylactic drugs, including amitriptyline, chlorpromazine, cyproheptadine, lisuride, methysergide and mianserin, significantly correlate ($r = 0.989$; $P < 0.001$) with their reported affinity at the recombinant 5-HT$_7$ receptor (Terrón, unpublished observation). On these bases, it seems reasonable to hypothesize that the migraine prophylactic efficacy of 5-HT$_{2B}$/5-HT$_{2C}$ (and 5-HT$_7$) receptor antagonists is due, at least in part, to blockade of craniovascular 5-HT$_7$ receptors. In the case of propranolol, i.e. another major migraine prophylactic drug, it should be recalled its early-reported ability to potently antagonize 5-HT-induced relaxant responses in human pial vessels and temporal artery ($pA_2 = 8.29$ and 8.50, respectively; Edvinsson *et al*., 1978). If such an interaction is confirmed to involve the 5-HT$_7$ receptor, a similar mechanism of anti-migraine action for this drug could be speculated.

The potential involvement of the relaxant 5-HT$_7$ receptor in the regulation of cerebroarterial tone and perhaps in migraine is further supported by recent molecular biological studies showing high expression of 5-HT$_7$ receptor transcripts in both pig cerebral blood vessels (Ullmer *et al*., 1995) and several human meningeal tissues, including the internal carotid and middle meningeal artery (Schmuck *et al*., 1996). Although a consistent expression of the 5-HT$_{2B}$ message in these human tissues was observed, no convincing evidence for a functional role of the 5-HT$_{2B}$ receptor in the pig cerebral artery was provided i.e. the nonselective 5-HT$_2$ receptor agonist, DOI, hardly relaxed the vessel by about 15% of the spasmogen-induced contraction (Schmuck *et al*., 1996). This is in marked contrast to the profound and potent 5-HT$_{2B}$ receptor-mediated endothelium-dependent relaxant responses produced by 5-HT and α-methyl-5-HT in various peripheral blood vessels, such as the rabbit jugular vein (Leff *et al*., 1987; Martin *et al*., 1987), pig vena cava (Sumner, 1991), pig pulmonary artery (Glusa & Richter, 1993) and rat jugular vein (Bodelsson *et al*., 1993). It must be recalled that the hypothesis suggesting a link between 5-HT and endothelial NO in the pathogenesis of migraine was primarily based on the unsubstantiated assumption that an endothelial 5-HT$_{2B}$ receptor-mediated mechanism, similar to that observed in peripheral vessels, would promote release of NO in the cerebral vasculature (Fozard, 1995; Fozard & Kalkman, 1994). However, the weak endothelium-dependent cerebroarterial relaxation referred to above may argue against this hypothesis. It follows that other mechanisms, such as the one mediating cerebrovascular dilatation through the 5-HT$_7$ receptor (present results), could be involved in the pathophysiological events that occur in migraine.

Finally, another implication arising from the present study is concerned with the controversy as to whether it is depletion or mobilization of 5-HT that predisposes to migraine. If the relaxant mechanism shown here and elsewhere (Ueno *et al*., 1995; Villalón *et al*., 1997) is actually involved in cephalovascular vasodilatation and migraine, the proposed excess of 5-HT as a key event in the initiation of migraine (Fozard, 1992; 1995; Fozard & Kalkman, 1994) is supported. Interestingly, the 5-HT$_7$ receptor, being located in craniovascular smooth muscle, would be best targeted by neuronal 5-HT released from perivascular 5-HT-containing neurons so that smooth muscle relaxation is produced without a need of interacting with the endothelial compartment. In this context, and relevant to the present observations in canine basilar and middle cerebral arteries, previous transcranial Doppler sonography studies showed a significant decrease in middle cerebral artery blood velocity, and its reversal by sumatriptan, on the headache side in migraine patients; in contrast, blood flow velocity was unchanged in the non-headache side (Friberg *et al*., 1991).

In conclusion, the present study demonstrates that 5-HT elicits direct relaxation in canine basilar and middle cerebral arteries through a receptor highly resembling the 5-HT$_7$ type. These findings, along with those reported in the canine external carotid circulation (Villalón *et al*., 1997) and the porcine pial vein (Ueno *et al*., 1995), may be strongly relevant in the context of the role of 5-HT in cerebrovascular vasodilatation and migraine. This contention does gain weight when considering the relatively high affinity of several migraine prophylactic 5-HT receptor antagonists for the 5-HT$_7$ receptor and its highly significant correlation with their orally-active clinical doses. Provided that this mechanism operates in the human cerebral vasculature, the potential implication of the 5-HT$_7$ receptor in migraine and other vascular headaches deserves a serious consideration.

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