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No contractile effect for 5-HT_{1D} and 5-HT_{1F} receptor agonists in human and bovine cerebral arteries: similarity with human coronary artery

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1 Using subtype-selective 5-HT_1 receptor agonists and/or the 5-HT_1 receptor antagonist GR127935, we characterized *in vitro* the 5-HT receptor that mediates the contraction of human and bovine cerebral arteries. Further, we investigated which sumatriptan-sensitive receptors are present in human coronary artery by reverse-transcriptase polymerase chain reaction (RT–PCR).

2 Agonists with affinity at the 5-HT_{1B} receptor, such as sumatriptan, alniditan and/or IS-159, elicited dose-dependent contraction in both human and bovine cerebral arteries. They behaved as full agonists at the sumatriptan-sensitive 5-HT_1 receptors in both species. In contrast, PNU-109291 and LY344864, selective agonists at 5-HT_{1D} and 5-HT_{1F} receptors, respectively, were devoid of any significant vasocontractile activity in cerebral arteries, or did not affect the sumatriptan-induced vasocontraction. The rank order of agonist potency was similar in both species and could be summarized as 5-HT = alniditan > sumatriptan = IS-159 > > > PNU-109291 = LY344864.

3 In bovine cerebral arteries, the 5-HT_1 receptor antagonist GR127935 dose-dependently inhibited the vasoconstrictions elicited by both 5-HT and sumatriptan, with respective pA_2 values of 8.0 and 8.6.

4 RT–PCR studies in human coronary arteries showed a strong signal for the 5-HT_{1B} receptor while message for the 5-HT_{1F} receptor was weak and less frequently detected. Expression of 5-HT_{1D} receptor mRNA was not detected in any sample.

5 The present results demonstrate that the triptan-induced contraction in brain vessels is mediated exclusively by the 5-HT_{1B} receptor, which is also present in a majority of human coronary arteries. These results suggest that selective 5-HT_{1D} and 5-HT_{1F} receptor agonists might represent new antimigraine drugs devoid of cerebro- and cardiovascular effects.

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Abbreviations: CGRP, calcitonin gene-related peptide; GR127935, (N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide hydrochloride; 5-HT, 5-hydroxytryptamine, serotonin; IS-159, serotonin-*O*-carboxymethylglycyltyrosinamide; LY344864, (R)-(+)-N-(3-dimethylamino-1,2,3,4-tetrahydro-9H-carbazol-6-yl)-4-fluorobenzamide; PNU-109291, (S)-(-)-1[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-N-methylisochroman-6-carboxamide; RT, reverse transcriptase; RT–PCR, reverse transcriptase-polymerase chain reaction

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter which exerts a wide spectrum of modulatory effects in the peripheral and central nervous systems. It has been implicated in various disorders including the pathogenesis of migraine (Martin, 1997). 5-HT interacts with multiple receptors and numerous studies have emphasized the role of 5-HT_1 receptors in the acute treatment of migraine headache (for reviews, see Martin, 1997; Schoenen, 1997; Goadsby, 1998). In this respect, the 5-HT_1 receptor agonist sumatriptan (Peroutka & McCarthy, 1989; Humphrey & Feniuk, 1991; Adham *et al*., 1993) has proven to be a highly effective antimigraine compound (Ferrari, 1993; Gross *et al*., 1993; Tansey *et al*., 1993). Two mechanisms have been proposed to explain its clinical efficacy, namely a vasoconstriction of meningeal blood vessels and an inhibition of the pro-inflammatory response that results from the release of substance P and calcitonin gene-related peptide (CGRP) from activated trigeminovascular afferents (Humphrey & Feniuk, 1991; Moskowitz, 1992; Buzzi *et al*., 1995). However, it is still unclear whether or not the vascular and neuronal sites of action are both necessary for clinical efficacy.

Recent findings of distinct populations of 5-HT_1 receptors in the trigeminovascular system and cerebral blood vessels (Hamel *et al*., 1993a,b; Rebeck *et al*., 1995; Bouchelet *et al*., 1996b; Longmore *et al*., 1997; Bonaventure *et al*., 1998) may offer an opportunity to highlight which of these neuronal or vascular effects, if any, confers anti-migraine properties. Indeed, pharmacological (Hamel & Bouchard, 1991), molecular (Hamel *et al*., 1993b; Bouchelet *et al*., 1996b) and immunocytochemical investigations (Longmore *et al*., 1997) suggest that a 5-HT_{1B} receptor is involved in the sumatriptan-induced cerebral vaosoconstriction, while 5-HT_{1D} (Rebeck *et al*., 1995; Bouchelet *et al*., 1996b; Longmore *et al*., 1997; Bonaventure *et al*., 1998) and/or 5-HT_{1F} (Bouchelet *et al*., 1996b; Johnson *et al*., 1997; Phebus *et al*., 1997) receptors would mediate the pre-synaptic inhibition of the trigeminovascular inflammatory response. Although this represents the

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predominant pattern of receptor distribution, expression of 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor mRNAs has also been detected, respectively, in a subset and in a majority of human brain vessels (Bouchelet *et al*., 1996b). Such findings suggest that these receptors could possibly participate in the 5-HT$_1$ receptor-mediated vasocontractile response to sumatriptan in a subpopulation of human cerebral vessels. In addition, sumatriptan and many of its new derivatives have been shown *in vitro* to induce contraction of human coronary arteries (Kaumann *et al*., 1994; Ferro *et al*., 1995; Maassen VanDenBrink *et al*., 1998), an undesirable side-effect of this therapy which has limited its use in individuals at risk for cardiovascular problems (Otterwanger *et al*., 1997; Dahlöf & Mathew, 1998). Hence, it appears of primary importance to clearly identify the 5-HT receptor(s) responsible for the vasomotor properties of sumatriptan and to understand their distribution not only in human brain vessels but also in coronary arteries. The present study was thus undertaken to assess the vasocontractile effect of selective 5-HT$_{1D}$, 5-HT$_{1B/1D}$ and 5-HT$_{1F}$ receptor agonists in human and bovine brain vessels, two species in which sumatriptan has been shown to mediate vasoconstriction through a similar 5-HT$_1$ receptor, suggested to correspond primarily to the 5-HT$_{1B}$ receptor subtype (Hamel *et al*., 1993a,b; Kaumann *et al*., 1993). Further, the expression of mRNAs for the three sumatriptan-sensitive 5-HT$_1$ receptor was studied in human coronary arteries. Altogether, the results clearly indicate that 5-HT$_{1B}$ receptors are the only mediators of the vasocontractile response to sumatriptan and non-selective 5-HT$_1$ receptor agonists in human and bovine brain vessels, and that this receptor is also expressed in a majority of human coronary arteries. Part of these results have appeared in an abstract form (Chauveau *et al*., 1994; Bouchelet *et al*., 1996a).

Methods

Tissue preparation

Vasomotor reactivity was measured in isolated segments of a temporal ramification of the middle cerebral artery obtained from calf or human (surgical biopsies of epileptic patients of either sex, obtained with permission from the Institutional research ethics committee) brain. Vessels were carefully isolated from the pia-arachnoid membrane, cleaned of blood and surrounding tissue under a dissecting microscope. They were then cut in 2–3 mm long segments and kept in an ice-cold Krebs-Ringer buffer solution (pH 7.4) (in mM): NaCl 118, KCl 4.5, $MgSO_4$-$7H_2O$ 1.0, KH_2PO_4 1.0, $NaHCO_3$ 25, $CaCl_2$-$2H_2O$ 2.5 and glucose 6.0. Coronary arteries used for the identification of 5-HT$_1$ receptor mRNA expression were obtained with approval from the research ethics committee from human subjects of either sex who died from diseases not related to cardiovascular complications ($n=6-12$; post-mortem delay of 13.3 ± 1.4 h). Distal portions of the coronary arteries were dissected from the hearts, cleaned of surrounding fat and adherent tissues, and were kept frozen at -80°C until use. They were then powdered with a pestle in liquid nitrogen and homogenized in TRIzol reagent (Gibco-BRL, Gaitherburg, MD, U.S.A.) with a polytron and by passage through a 18½ needle gauge. Total RNA was extracted according to Chomczynski (1993), treated with RQ1-DNAase (Promega, Madison, WI, U.S.A.), precipitated with ethanol and used in reverse transcriptase-polymerase chain reaction (RT–PCR) experiments, taking the human trigeminal ganglion as control tissue (for details, see Bouchelet *et al*., 1996b).

Functional assays

The vasocontractile responses to 5-HT and 5-HT$_1$ receptor agonists were determined in intact human and bovine brain vessels as routinely performed in our laboratory (Hamel & Bouchard, 1991; Hamel *et al*., 1993a,b). In brief, vessel segments were mounted between two L-shaped metal prongs in temperature-controlled (37°C) tissue baths (volume of 5 ml) containing the Krebs-Ringer solution (see above) bubbled with a mixture of 95% O_2 and 5% CO_2, and replaced every 15 min. Changes in muscle tension were measured by a force displacement transducer (Grass FT 103 D) and recorded on a Grass Polygraph Model 7E. The vessels were allowed to stabilize (45 min, 0.4 g for human and 0.4–0.6 g for bovine) and then the maximal contractile capacity was evaluated with a Krebs solution supplemented with K^+ (124 mM). The vessels were washed and allowed to recover for an additional 30–45 min period.

Agonists Log-concentration response curves were generated by cumulative addition (1 nM–10 μM) of either 5-HT, sumatriptan or selective 5-HT$_{1B/1D}$, 5-HT$_{1D}$ or 5-HT$_{1F}$ receptor agonists. The order of the compounds was randomized from one experiment to another. For comparison, the maximal contractile response (E_{Amax}) and relative potency (pD_2 values or $-\log EC_{50}$, Van den Brink, 1977) were determined for each agonist, taking 5-HT as the reference compound. In a different series of experiments in bovine cerebral arteries, dose-response curves to sumatriptan were generated in the absence (control) and presence (plus a 30 min pre-incubation) of 10^{-6} M of the selective 5-HT$_{1D}$ receptor agonist (see below). In this case, the dose-response curves to sumatriptan in the presence of the agonist were expressed as a per cent of the control curves obtained for sumatriptan in the same vascular segments. The 5-HT$_1$ receptor agonists used were: the non-selective 5-HT$_{1B/1D/1F}$ receptor agonist, sumatriptan (Peroutka & McCarthy, 1989; Humphrey & Feniuk, 1991; Adham *et al*., 1993; GlaxoWellcome, Greenford, U.K.), the 5-HT$_{1B/1D}$ receptor agonists alniditan (Leysen *et al*., 1996; Janssen Research Foundation, Beerse, Belgium) and/or serotonin-*O*-carboxymethylglycyltyrosinamide (IS-159, Boulenguez *et al*., 1991; Chauveau *et al*., 1994; Immunotech, Marseille, France), the selective 5-HT$_{1D}$ receptor agonist (S)-(−)-1[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-N-methylisochroman-6-carboxamide (PNU-109291, Ennis *et al*., 1998; Pharmacia and Upjohn, Kalamazoo, MI, U.S.A.) and the selective 5-HT$_{1F}$ receptor agonist (R)-(+)-N-(3-dimethylamino-1,2,3,4,-tetrahydro-9H-carbazol-6-yl)-4-fluorobenzamide (LY344864, Phebus *et al*., 1997; Eli Lilly, Indianapolis, IN, U.S.A.). All compounds were graciously provided by the above respective companies. They were first diluted in water (10^{-2} or 10^{-3} M), with following dilutions being made directly in buffer.

Antagonist The participation of 5-HT$_1$ receptors in the vasocontractile response to 5-HT in bovine cerebral arteries was further assessed with the 5-HT$_1$ receptor antagonist (N-[methoxy-3- (4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide hydrochloride (GR127935, a gift from GlaxoWellcome, Greenford, U.K.) (Skingle *et al*., 1996). Dose-response curves to 5-HT and sumatriptan (1 nM to 10–100 μM) were performed in the absence and then in the presence (30 min pre-incubation) of different concentrations (1 or 10 nM to 0.1 or 1 μM) of GR127935, with the vessels being exposed only once (on rare occasions twice) to the antagonist. The potency of GR127935 was expressed as a pA_2 value, and was calculated according to

Van den Brink (1977) (for more details, see Hamel & Bouchard, 1991, Hamel *et al*., 1993b). The nature of the antagonism was further determined by Schild plot analysis (Arunlakshana & Schild, 1959) and the pA_2 obtained from the Schild analysis compared to that calculated as described above. The pA_2 values obtained from both methods were averaged and data are presented as overall pA_2 values for GR127935 against either 5-HT or sumatriptan.

RT–PCR experiments

Following synthesis of cDNAs from the total RNA using random primers and avian myeloblastosis virus reverse transcriptase (RT) (for details, Bouchelet *et al*., 1996b), cDNA was heat denaturated (95°C, 3–5 min) and then subjected (2–4 μl) to PCR amplification with Taq polymerase using selective primers for the three sumatriptan-sensitive 5-HT$_1$ receptors, namely the 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ receptors, (for more details see Bouchelet *et al*., 1996b). The timing for amplification was 85°C 3 min, 56°C 1 min, 72°C 5 min followed by 39 cycles of amplification (94°C 40 s, 55°C 40 s, 72°C 40 s) and a final elongation 5 min at 72°C. The PCR products (fragment size of 340 bp, 595 bp and 482 bp for the 5-HT$_{1D}$, 5-HT$_{1B}$ and 5-HT$_{1F}$ receptors, respectively) were analysed by gel electrophoresis. Control reactions lacking reverse transcriptase were used to monitor for DNA contamination (−RT lane in Figure 5).

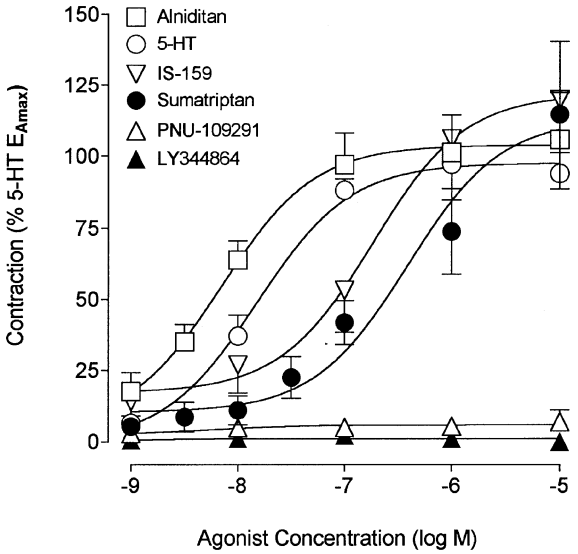

Figure 1 Concentration-response curves for 5-HT, sumatriptan, alniditan, IS-159, PNU-109291 and LY344864 in human cerebral arteries under resting tension. The E_{Amax} for each agonist is expressed as a percentage of 5-HT$_{EAmax}$ measured in the same vascular segments. Complete information on potency, maximal response and number of vascular segments is given in Table 1. Vertical bars show s.e.mean of $n=5-8$.

Statistical analysis

All results are mean ± s.e.mean. Statistical significance was assumed when $P<0.05$, as determined by ANOVA followed by a Newman-Keuls comparison test, or Student *t*-test in the case of sumatriptan-induced contraction with and without PNU-109291.

Results

Vasomotor responses in cerebral arteries

In human cerebral arteries at basal tone, 5-HT, sumatriptan, alniditan and IS-159 consistently elicited a strong and dose-dependent vasoconstriction (Figure 1). The 5-HT$_1$ receptor agonists sumatriptan, alniditan and IS159 behaved as full agonists, inducing maximal contractions which were not significantly different from that elicited by 5-HT (Table 1). However, while alniditan was as potent as 5-HT in inducing contraction of human cerebral arteries, sumatriptan and IS-159 both exhibited significantly lower pD_2 values (Table 1). In contrast, the selective 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor agonists PNU-109291 and LY344864, respectively, were both devoid of any significant vasocontractile activity in human brain vessels (Figure 1, Table 1).

5-HT, sumatriptan and alniditan also elicited potent contraction of bovine cerebral arteries at basal tone (Figure 2). In this species, alniditan and sumatriptan both induced a significantly weaker maximal contraction than that mediated by 5-HT (55 ± 3% and 36 ± 5%, respectively of 5-HT E_{Amax}, $P<0.001$, Table 2). As in human brain vessels, alniditan was as potent as 5-HT and sumatriptan exhibited a significantly lower pD_2 values than these two agonists (Table 2). The selective 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor agonists PNU-109291 and LY344864, respectively, did not elicit any significant contraction of bovine cerebral arteries (Figure 2, Table 2). In vessels incubated with 10^{-6} M PNU-109291, sumatriptan induced concentration-dependent contractions with a similar potency and a slightly lower but not significantly different maximal response ($E_{Amax}=88\pm8\%$, $n=7$) than those obtained in the same vessels tested before application of the selective 5-HT$_{1D}$ receptor agonist. The rank order of agonists potency in human and bovine cerebral arteries was thus comparable and could be summarized as: 5-HT = alniditan > sumatriptan = IS-159 >>> PNU-109291 = LY344864.

The contractions elicited by 5-HT and sumatriptan in bovine vessels were antagonized by GR127935, which induced a rightward shift in the dose-response curve to both agonists,

Table 1 Potencies of 5-HT$_1$ receptor agonists in inducing contraction of human cerebral arteries

Agonist	*n*	*E_{Amax} (% 5-HT E_{Amax})*	*PD_2* ($-\log EC_{50}$)	*EC_{50} agonist / EC_{50} 5-HT*
5-HT[a]	8	100	7.82 ± 0.1	1
Sumatriptan	7	114 ± 26	6.41 ± 0.21*	25.6
Alniditan	7	106 ± 16	8.16 ± 0.04	0.5
IS-159	8	120 ± 19	6.83 ± 0.25*	9.9
PNU-109291	6	8 ± 4*	–	–
LY344864	5	0	–	–

Values are the means ± s.e.means of the number of determinations indicated. [a]For reference, the E_{Amax} value for 5-HT was 0.41 ± 0.06 g. *($P<0.001$) indicates a statistically significant difference from 5-HT (ANOVA).

together with a small but significant decrease in maximal responses for 5-HT (28%, $P<0.001$ at 10^{-6} M) and sumatriptan (21%, $P<0.05$) at the highest antagonist concentration (Figures 3 and 4). The mean pA_2 value calculated for all GR127935 concentrations against 5-HT was 8.06 ± 0.18. Schild plot analysis ($r=0.99$) of the inhibition yielded a pA_2 value evaluated at the intercept of 7.89 (Figure 3B). An overall pA_2 value of 7.98 ± 0.12 (s.d.) was thus obtained for GR127935 against 5-HT. When sumatriptan was used as the agonist, GR127935 was a slightly more potent antagonist in inhibiting the contractile response with a calculated pA_2 value of 8.50 ± 0.39 (Figure 4A). In the Schild plot analysis ($r=0.96$), the pA_2 value at intercept was 8.73 (Figure 4B), for an overall pA_2 value of 8.61 ± 0.12 (s.d.).

5-HT1 receptor mRNAs expression in human coronary arteries

Upon gel electrophoresis, PCR products of the expected size for the 5-HT$_{1D}$ receptor were never detected in human coronary arteries despite successful amplification in control tissue (Figure 5). In contrast, the 5-HT$_{1B}$ receptor message was strongly expressed in a majority (>60%) of human coronary arteries and messages for the 5-HT$_{1F}$ receptor were detected in about 40% of human coronary arteries. The intensity of the

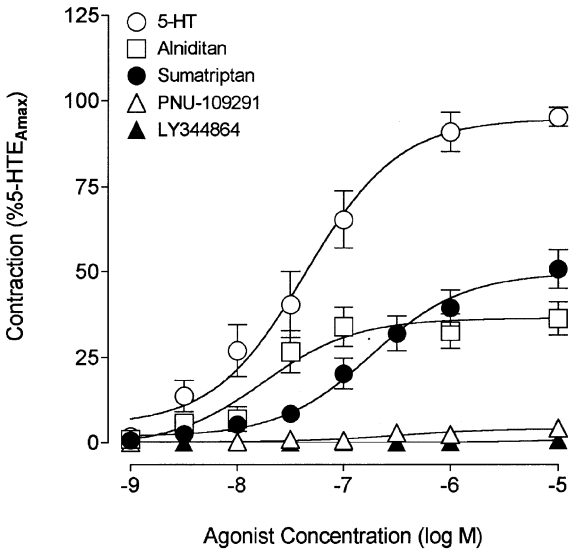

Figure 2 Concentration-response curves for 5-HT, sumatriptan, alniditan, PNU-109291 and LY344864 in bovine cerebral arteries under resting tension. The E_{Amax} for each agonist is expressed as a percentage of 5-HT E_{Amax} obtained in the same vascular segments. See Table 2 for detailed information on individual potency, maximal response and number of vascular segments. Vertical bars indicate s.e.mean of $n=10-12$.

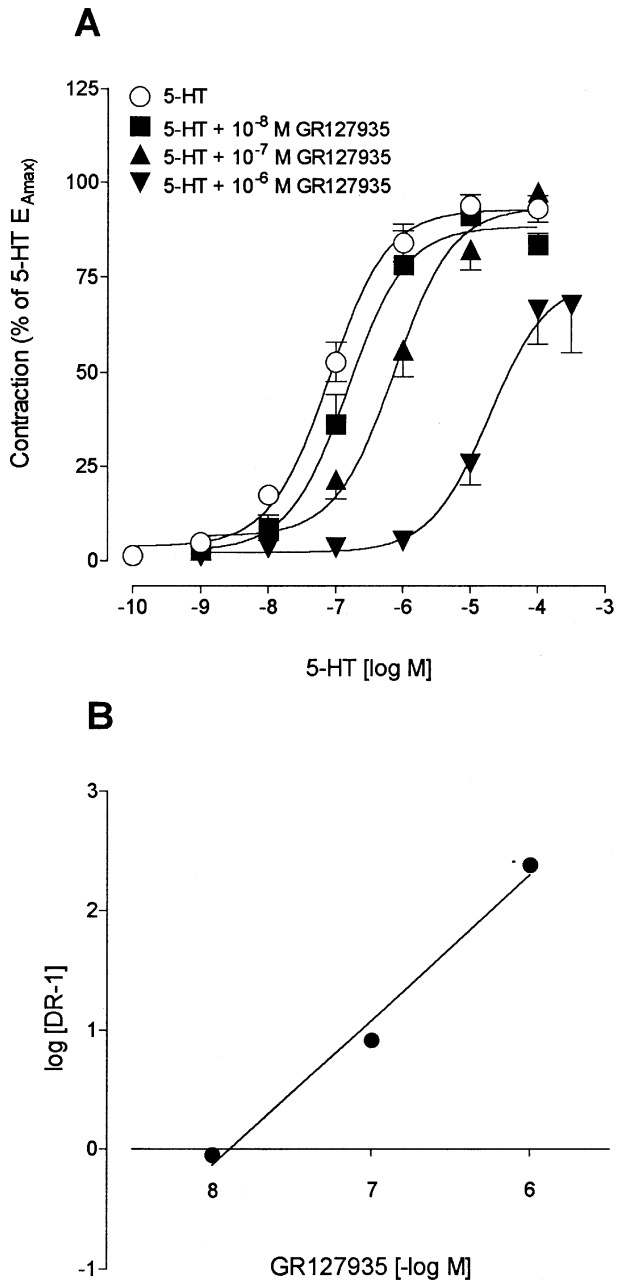

Figure 3 (A) Concentration-response curves for 5-HT in bovine cerebral arteries in the absence (control) and in presence of various concentrations ($10^{-8}-10^{-6}$ M) of GR127935. Values are expressed as percentage of the 5-HT E_{Amax} measured in the same arterial segments and are means±s.e.means of $n=20$, 7, 11 and 9, respectively. (B) Schild plot analysis of the effect of GR127935 on 5-HT-induced contraction of bovine cerebral arteries ($r=0.99$, slope of 1.20 ± 0.02).

Table 2 Potencies of 5-HT$_1$ receptor agonists in inducing contraction of bovine cerebral arteries

Agonist	*n*	*E_A max (% 5-HT E_{Amax})*	*PD_2 ($-logEC_{50}$)*	*EC_{50} agonist / EC_{50} 5-HT*
5-HT[a]	12	100	7.35±0.09	1
Sumatriptan	10	55±3**	6.74±0.07*	4.1
Alniditan	11	36±5**	7.76±0.17	0.4
PNU-109291	10	4±2**	–	–
LY344864	10	0.6±0.4**	–	–

Values are the means±s.e.means of the number of determinations indicated. [a]For reference, the E_{Amax} value for 5-HT was 3.12±1.6g. *($P<0.05$) and **($P<0.001$) indicate a statistically significant difference from 5-HT (ANOVA).

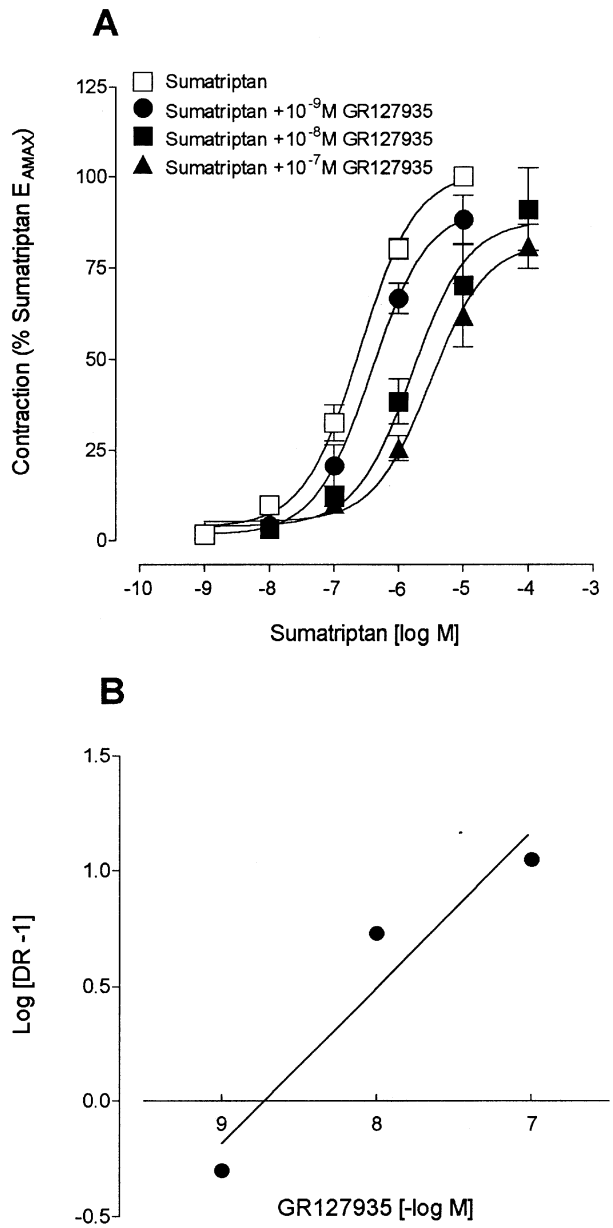

Figure 4 (A) Concentration-response curve for sumatriptan in bovine arteries in the absence (control) and in the presence of various concentrations (10^{-9}–10^{-7} M) of GR127935. Values are expressed as percentage of the sumatriptan E_{Amax} measured in the same arterial segments and are means ± s.e.means of $n = 18$, 5, 7, and 6, respectively. (B) Schild plot analysis of the effect of GR127935 on sumatriptan-induced contraction of bovine cerebral arteries ($r = 0.96$, slope of 0.68 ± 0.20).

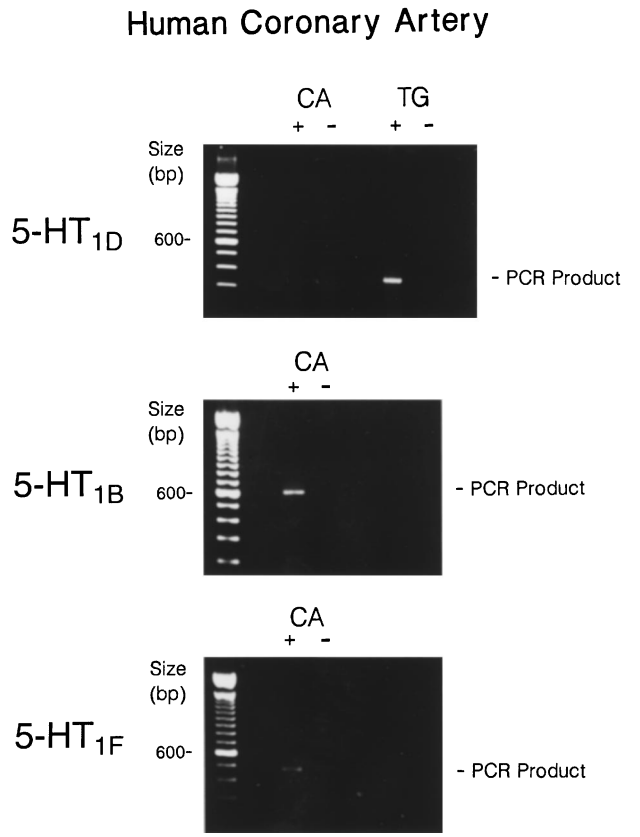

Figure 5 Identification of sumatriptan-sensitive 5-HT$_1$ receptors in human coronary artery (CA) by RT–PCR. Representative agarose gel electrophoresis of PCR products showing the absence of 5-HT$_{1D}$ receptors in human coronary artery despite consistent amplification in the human trigeminal ganglion (TG). High intensity PCR products were obtained for 5-HT$_{1B}$ receptors in 60% of human coronary arteries while a weak signal was obtained for the 5-HT$_{1F}$ receptor in 40% of cases. Samples without reverse transcriptase (−) were included to monitor for possible contamination.

latter PCR products, however, was systematically very faint on the ethidium bromide-stained agarose gels (Figure 5).

Discussion

The present data with selective 5-HT$_{1D}$, 5-HT$_{1B/1D}$ and 5-HT$_{1F}$ receptor agonists allow to conclude that the 5-HT$_{1B}$ receptor is the exclusive mediator of the cerebral constriction elicited by sumatriptan and pharmacologically related compounds in both bovine and human brain arteries at basal tone. Furthermore, the results indicate that this receptor subtype is expressed in a majority of human coronary arteries, in which it is possibly responsible for the reported arterial vasoconstriction elicited by sumatriptan and other non-selective 5-HT$_1$ receptor agonists (Kaumann *et al*., 1994; Ferro *et al*., 1995; Maassen VanDenBrink *et al*., 1998). Altogether these results suggest that selective 5-HT$_{1D}$ and/or 5-HT$_{1F}$ receptor agonists, if proven clinically effective, could represent a new generation of antimigraine compounds with a neuronal site of action and an increased cardiovascular safety.

The 5-HT$_1$ receptor agonist sumatriptan was found to elicit a contraction of both human and bovine cerebral arteries, with a potency similar to that reported in previous studies in human (Parsons, 1991; Hamel *et al*., 1993b), bovine (Hamel *et al*., 1993b) and dog (Beattie & Connor, 1995) cerebral arteries. In bovine cerebral arteries, the maximal response elicited by sumatriptan and other 5-HT$_1$ receptor agonists was predictably less than that of 5-HT, but was also slightly smaller than that reported previously by us for sumatriptan in the same preparation (Hamel *et al*., 1993b), probably a consequence of the seasonal variations in contractile 5-HT receptors (Vinall *et al*., 1991). The difference between 5-HT and sumatriptan maximal response, however, is most likely due to the participation of 5-HT$_{2A}$ receptors in the 5-HT-mediated vasoconstriction in this species (Frenken & Kaumann, 1984; De Wever *et al*., 1990; Foy *et al*., 1992), a population of receptors which is either not present or not functional in human brain vessels (Hamel & Bouchard, 1991; Kaumann *et al*., 1993). The vasocontractile response to 5-HT in bovine cerebral arteries was inhibited by the 5-HT$_1$ receptor

antagonist GR127935 with an overall potency ($pA_2 \approx 8$) which was slightly less than that observed when sumatriptan was used as the agonist ($pA_2 \approx 8.6$). The fact that, in bovine vessels, 5-HT also interacts with 5-HT$_{2A}$ receptors for which GR127935 has a lower affinity (pK_i of 7.4) as compared to 5-HT$_{1B/1D}$ receptors (Skingle *et al*., 1996), most likely account for this weaker potency of GR127935 against 5-HT. Interestingly, GR127935 potency against sumatriptan in bovine vessels was identical to that observed for this antagonist against sumatriptan in ovine main branch (pKb = 8.5) and second branch (pKb = 8.7) middle cerebral arteries (Teng *et al*., 1998), and compared very well with that reported at rodent 5-HT$_{1B}$ receptors ($pK_i = 8.5$) (Skingle *et al*., 1996). However, it was slightly less than expected from a previous study in the dog basilar artery where it behaved as a very potent insurmountable antagonist (Skingle *et al*., 1996). These apparent discrepancies in potency and type of antagonism in dog cerebrovascular tissues may be partly related to species differences but could also be due to the fact that, in the present study, most vessels were exposed only once to this highly lipophilic antagonist in order to avoid tachyphylaxis, a phenomenon which would artificially increase the antagonist potency. The cerebrovascular potency of GR127935 in bovine vessels is thus fully compatible with an interaction with functional 5-HT$_{1B/1D}$ receptors, and not with 5-HT$_{1F}$ receptors for which it exhibits much lower affinity (pK_i of 7.1, personal communication Dr H Connor).

The vasocontractile response obtained with alniditan and/or IS-159, two compounds not structurally related to sumatriptan and with low or no affinity at the 5-HT$_{1F}$ receptor subtype (respective pK_i values of 6 and <5; Leysen *et al*.,1996; Hamel, 1996), further indicates that 5-HT$_{1F}$ receptors are not involved in the 5-HT$_1$ receptor-mediated cerebral vasoconstriction in both man and bovine. The potent contraction elicited by the benzopyran derivative alniditan in human and bovine cerebral arteries also agrees with previous reports in the dog carotid artery (Van de Water *et al*., 1995) and the pig carotid arteriovenous anastomoses (De Vries *et al*., 1997), in which alniditan was found to be significantly more potent than sumatriptan. The high efficacy of alniditan in the present study fully agrees with its reported higher affinity at 5-HT$_{1B/1D}$ receptors than sumatriptan (Hamel *et al*., 1996; Leysen *et al*., 1996; Lesage *et al*., 1998). Moreover, alniditan is reportedly about ten times more potent than sumatriptan at the human 5-HT$_{1B}$ receptor and only twice as potent as sumatriptan at the human 5-HT$_{1D}$ receptor in mediating inhibition of adenylyl cyclase (Lesage *et al*., 1998). Based on this observation and the significantly greater potency for alniditan as compared to sumatriptan in eliciting cerebral vasoconstriction in the present study (at least 10 fold), we can suggest that these compounds act at a 5-HT$_{1B}$ receptor to elicit cerebral vasoconstriction.

This assumption is unequivocally supported by the lack of vasocontractile effect of the selective 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor agonists PNU-109291 and LY344864, respectively. Indeed, PNU-109291 which has a 5000 times higher affinity at the 5-HT$_{1D}$ than the 5-HT$_{1B}$ receptor (K_i of 0.9 and 5775 nM, respectively; Ennis *et al*., 1998), did not elicit any significant contraction at concentrations up to 10^{-5} M. Further, the fact that 10^{-6} M PNU-109291, a concentration which is well in excess to its affinity at 5-HT$_{1D}$ receptors, failed to significantly affect the sumatriptan-induced vasocontractile response provided additional arguments for the absence of functional 5-HT$_{1D}$ receptors in cerebral vessels. Such a statement also agrees with recent studies which showed this compound to be devoid of any effect on carotid resistance in the cat (Ennis *et al*., 1998), and the sumatriptan-induced contraction of the porcine carotid arteriovenous anastomoses to be blocked by selective 5-HT$_{1B}$, but not 5-HT$_{1D}$ receptor antagonists (DeVries *et al*., 1999). Similarly, a lack of vasomotor effect for the selective 5-HT$_{1F}$ receptor agonist LY344864 (Ki of 6 nM at 5-HT$_{1F}$ as compared to 549 and 575 nM at 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors, respectively) (Phebus *et al*., 1997) has also been reported in peripheral blood vessels (Johnson *et al*., 1997; Phebus *et al*., 1997). Together with our present findings, these results suggest that the 5-HT$_{1F}$ receptor does not exert vasomotor effects in either brain or peripheral blood vessels. This statement is in line with our previous pharmacological correlation analyses (Hamel *et al*., 1993b) in human brain vessels that excluded the 5-HT$_{1F}$ receptor in the vasocontractile response to 5-HT and sumatriptan, this despite the presence of 5-HT$_{1F}$ mRNA associated with human brain vessels (Bouchelet *et al*., 1996b). However, a vascular localization for the receptor appears most unlikely. Indeed, smooth muscle cells from human pulmonary artery and aorta (Ullmer *et al*., 1995) and human brain microvessels (Cohen *et al*., 1999) were found not to express any, or very low levels of mRNA for either the 5-HT$_{1D}$ or the 5-HT$_{1F}$ receptor. Interestingly, in the human brain microcirculation, 5-HT$_{1F}$ receptors were expressed in astroglial cells which are closely associated with microvessels. It is thus possible that the 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor messages detected in some human pial vessels (Bouchelet *et al*., 1996b) are localized in fibroblasts and cells of the pia-arachnoid membrane which may be closely attached to the vessel wall.

Expression of mRNA for 5-HT$_1$ receptors in human coronary arteries indicated an absence of 5-HT$_{1D}$, a predominance of 5-HT$_{1B}$ with a less frequent and overall weaker expression of 5-HT$_{1F}$ receptor subtype, an overall statement which is well compatible with the results from two recent studies (Ishida *et al*., 1999; Nilsson *et al*., 1999) which found barely detectable 5-HT$_{1D}$ receptors expression and no (Ishida *et al*., 1999) or relatively high (Nilsson *et al*., 1999) expression levels of 5-HT$_{1F}$ receptors.

In a certain proportion of human coronary arteries, however, we were unable to detect mRNA for either 5-HT$_{1B}$ or 5-HT$_{1F}$ receptor subtypes. Although we believe that this is not due to the selected oligonucleotide primers since they yielded highly reproducible results in human trigeminal ganglia (this study and Bouchelet *et al*., 1996b), we cannot exclude that the post mortem delay, RNAase activity and difference in the caliber of the arteries may play a role in this variability and small discrepancies between studies. In this regards, it is interesting to note that in a previous functional study (Ferro *et al*., 1995), about 50% of the human coronary artery segments were unable to constrict in response to either 5-HT or sumatriptan. As well, a large interpatient variability in coronary artery responses to 5-HT was previously reported by Kaumann *et al*. (1994). Taking this variability into account, the relatively low level of expression of the 5-HT$_{1F}$ receptor mRNA and the non-vascular cellular localization of this receptor in another isolated human cerebrovascular preparation (Cohen *et al*., 1999), the results of the present study strongly argues that the 5-HT$_{1B}$ is the most likely receptor to be activated by sumatriptan in human coronary arteries. Interestingly, many functional studies in human coronary arteries (Kaumann *et al*., 1994; Ferro *et al*., 1995; Maassen VanDenBrink *et al*., 1998) have previously attributed the sumatriptan-induced vasoconstriction to a pharmacologically defined 5-HT$_{1D}$-like receptor, best characterized as 5-HT$_{1B}$ receptor (Kaumann *et al*., 1994). Together with a recent study in human temporal arteries (Verheggen *et al*., 1998), and the lack of association of 5-HT$_{1F}$ receptors with vascular cells of

human brain (Cohen *et al*., 1999) and peripheral (Ullmer *et al*., 1995) vessels, our results in human coronary and cerebral arteries would support the statement that 5-HT$_{1B}$ receptors may be the general mediators of regional arterial vasoconstriction elicited by both 5-HT and sumatriptan in man (Verheggen *et al*., 1998).

These findings are important as they imply that antimigraine drugs with affinity at the 5-HT$_{1B}$ receptor would be endowed with intrinsic potential cardiovascular activity (see Maassen VanDenBrink *et al*., 1998), a side-effect that has been seriously considered in susceptible patients (Dahlöf & Mathew, 1998).Whether or not a contractile effect at the level of brain vessels is necessary for a drug to be effective in migraine treatment still remains to be demonstrated. However, it is clear that effective antimigraine compounds such as sumatriptan, alniditan (Goldstein *et al*., 1995) and IS-159 (Hamel *et al*., 1996) share a common characteristic of high affinity at the 5-HT$_{1B/1D}$ receptors. As both 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor agonists were found to be devoid of cerebrovascular activity (this study) while being active inhibitors of the trigeminovascular-mediated neurogenic inflammation response and/or c-*fos* expression in trigeminal nucleus caudalis in animal models (Cutrer *et al*., 1999; Ennis *et al*., 1998; Phebus *et al*., 1997), their clinical efficacy in migraine treatment, if proven, might offer new means to selectively target the putative neuronal locus of action of sumatriptan and derivatives. Clinical studies with subtype-selective compounds are mandatory and likely to provide new insights into the pathophysiology of migraine headache.

In conclusion, our results indicate that the 5-HT$_{1B}$ receptor is the exclusive mediator of the constriction elicited by 5-HT$_1$ receptor agonists such as sumatriptan in human and bovine cerebral vessels, and they further show that this receptor is present in human coronary arteries. These findings raise the interesting possibility that new antimigraine drugs targeting selectively the neuronal 5-HT$_{1D}$ and/or 5-HT$_{1F}$ receptors may provide a safer cardiovascular profile. Furthermore, would the clinical efficacy of such compounds be demonstrated, the present findings would suggest that the vascular site of action of the triptans and other 5-HT$_1$ receptor agonists is not required for clinical efficacy.

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