Contractile 5-HT₁ receptors in human isolated pial arterioles: correlation with 5-HT_{1D} binding sites

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1 The 5-hydroxytryptamine (5-HT) receptor responsible for inducing vasoconstriction in human isolated pial arterioles has been pharmacologically characterized.

2 Of several 5-HT agonists tested, 5-carboxamidotryptamine (5-CT) was the most potent and the rank order of agonist potency can be summarized as: 5-CT > 5-HT > RU 24969 = α -methyl-5-HT = methysergide $\gg$ MDL 72832 = 2-methyl-5-HT $\gg$ 2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydro-naphthalene (8-OH-DPAT). With few exceptions, the maximal contractile responses of these agonists were comparable to that induced by 5-HT.

3 A correlation analysis performed between the agonists vascular potency (pD₂ values) and their affinities (pK_D values) published at various subtypes of 5-HT binding sites showed a positive significant correlation with rat cortical 5-HT_{1B} ($r = 0.86$; $P < 0.01$) and human caudate 5-HT_{1D} ($r = 0.98$; $P < 0.005$) subtypes.

4 Selective antagonists at 5-HT₂ (ketanserin, mianserin, MDL 11939) and 5-HT₃ (MDL 72222) sites were totally devoid of inhibitory activity on the 5-HT-induced contraction, an observation which agreed with the agonist data and further excluded activation of these receptors. In contrast, the 5-HT₁-like/5-HT₂ antagonist methiothepin and the non-selective 5-HT_{1D} compound metergoline inhibited with high affinity the contraction induced by 5-HT with respective pA₂ values of 8.55 ± 0.16 and 6.88 ± 0.05 . This contractile response was, however, insensitive to 5-HT_{1B} (propranolol) and 5-HT_{1C} (mesulergine, mianserin) antagonists.

5 It is concluded that a 5-HT₁-like receptor, which shares strong similarities with the 5-HT_{1D} binding sites identified in human caudate membranes, is mediating the vasocontractile action of 5-HT in human pial arterioles.

Introduction

The importance of 5-hydroxytryptamine (5-HT or serotonin) in the control of the brain circulation has been emphasized recently due to its probable implication in cerebrovascular disorders such as vasospasm and migraine (Anthony & Lance, 1975; MacKenzie *et al.*, 1985; Fozard, 1987). Although its precise role remains unclear and doubts have been formulated as to the origin of the vasoactive 5-HT (platelets and/or specific 5-hydroxytryptaminergic nerves) (Lee, 1989), it is beyond any doubts that various 5-HT-related compounds (primarily 5-HT₂ antagonists) are widely used in the prophylaxis of migraine (Raskin, 1986; Titus *et al.*, 1986). More recently, a 5-HT₁-like agonist (GR-43175; Sumatriptan) has reached Phase III clinical trials and was found beneficial in the treatment of acute migraine headache (Doenicke *et al.*, 1988). On consideration of the multiple subtypes of 5-HT receptors (Bradley *et al.*, 1986; Peroutka, 1988) at which sites the indoleamine can interact and the reported species-related variations in these cerebrovascular receptors (for review, see Young *et al.*, 1987; Lee, 1989), it appears important to define the nature of the contractile 5-HT receptor in human cerebral blood vessels.

Both 5-HT₁ and/or 5-HT₂ receptors have been implicated in the cerebral vasocontractile action of 5-HT depending on the species (for review, see Young *et al.*, 1987; Lee, 1989). In man, most studies were performed before the identification of the various 5-HT receptor subtypes and before the availability of selective drugs at these sites. Several non-selective 5-HT₂ antagonists were originally found to act as non-competitive inhibitors of the 5-HT-induced vasoconstriction in either human basilar (Forster & Whalley, 1982; Muller-Schweinitzer, 1983) or small pial arteries (Hardebo *et al.*, 1978; Edvinsson *et al.*, 1978). Shortly thereafter, Peroutka & Kuhar (1984) using a receptor binding radioautographic

approach reported the identification and visualization of 5-HT₁, but not 5-HT₂, receptors in human basilar artery. This contention has received further support from a recent pharmacological study (Parsons *et al.*, 1989) which suggested that the 5-HT receptor in human basilar arteries appears identical to the 5-HT₁-like receptor causing contraction of the dog isolated saphenous vein and cerebral blood vessels from the dog and primate.

In the present study, we have used selective 5-HT agonists and antagonists in an attempt to characterize the 5-HT receptor which mediates contraction of human pial arterioles, a vascular segment of cardinal importance in regulation of cerebral blood flow. The vascular potencies of these agents were compared to their published affinities at various 5-HT receptor subtypes as defined by binding studies. We conclude that a 5-HT₁ receptor, which best correlated with the 5-HT_{1D} binding site identified in human brain, is responsible for the contraction elicited by the indoleamine.

Methods

In vitro study of human pial arterioles

Pial arterioles from eleven humans of either sex were obtained post-mortem (delay of 4 to 12 h) from the Montreal Brain Bank at the Douglas Hospital. Patients had died from neurological or non-neurological causes but, in all cases, 5-HT-related medication was not required. The specimens corresponded to a proximal ramification of the middle cerebral artery and were approximately 0.8 mm outside diameter; all vessels were used within 1 h following removal from the cortical surface. The small arteries were cleaned of surrounding tissue under a dissecting microscope and placed in a cold Krebs-Ringer solution of the following composition (in mM): NaCl 118, KCl 4.5, MgSO₄·7H₂O 1.0, KH₂PO₄ 1.0,

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NaHCO₃ 25, CaCl₂·2H₂O 2.5 and glucose 6.0. Circular segments (3–4 mm in length) were used for recording of the isometric tension developed by the smooth muscle as described previously (Högestätt *et al.*, 1983); changes in tension were measured by a force displacement transducer (GRASS FT 103D) and recorded on a Grass Polygraph Model 7E. All vessel segments were mounted between two L-shaped metal prongs in temperature-controlled (37°C) tissue baths containing the Krebs solution gassed continuously with 5% CO₂ in 95% O₂, in order to maintain the pH at 7.4. Arteriolar segments were then given a mechanical tension of 0.4 g and allowed to stabilize at this level for 60 min. During this period the bathing fluid was replaced regularly by fresh buffer (every 15 min).

Response curves to agonists

Log concentration-response curves were generated for each agonist by cumulative addition of the drug (0.1 nM–1 mM) to vessels equilibrated at the level of the resting tension (mechanical tone of 0.4 g). In every experiment the maximal contractile capacity of the arterial segments was assessed by exposure to 124 mM K⁺. For this purpose, NaCl was replaced by KCl in equimolar concentration in the Krebs buffer solution. Relative potencies of the agonists were determined according to their respective pD₂ values (the negative logarithm of the molar concentration of the agonist which produced 50% of the maximal contractile effect; –log EC₅₀) calculated from individual curves with the following equation:

$$pD_2 = -\log[A] - \log\left(\frac{E_{Amax}}{E_A} - 1\right) \quad (1)$$

where E_{Amax} is the maximal contraction induced by agonist A and E_A the contractile effect in response to a given concentration [A] of agonist (Van den Brink, 1977). The E_{Amax} (in g) of the vessel segments to the various agonists were compared with the maximal vasoconstriction elicited by 5-HT in the same preparations, and expressed as percentage of the maximal 5-HT response. For reference, the maximal constriction elicited by 5-HT (10 μM) corresponded to 71 ± 5% ($n = 33$) of the maximal contractile capacity of the cerebrovascular smooth muscle (1.64 ± 0.09 g), as determined by 124 mM K⁺.

Determination of antagonist potency

A concentration-response curve to 5-HT was first obtained and when the maximal contraction was reached, the agonist was washed out from the bath and the potency of antagonist determined on the same vessels. Cumulative addition of the agonist was again performed but in the presence of graded concentrations of a given antagonist in the range of 10 nM to 1 μM. In each case, the antagonist was in contact with the vessels for a period of 20 min before reconstruction of the agonist concentration-effect curve. When appropriate the potency of the antagonist was evaluated by the use of pA₂ value for dual antagonist (competitive and metatoid) or the negative logarithm of the molar antagonist concentration, in the presence of which twice the original agonist concentration is needed to cause an effect that is 'relatively' equal to the original effect (Van Den Brink, 1977). The pA₂ was calculated as:

$$pA_2 = -\log[B] + \log\left(\frac{[A]_2}{[A]_1} - 1\right) \quad (2)$$

in which [B] is the antagonist concentration; [A]₂ the concentration of agonist needed to reach a vasoconstriction relatively equivalent to the control half-maximal response, in the presence of [B]; [A]₁ the concentration of agonist needed to elicit the half-maximal response in the absence of [B] (Van Den Brink, 1977). In addition, the pA₂ values were estimated from the regression lines obtained in the Schild plot analysis

(Arunlakshana & Schild, 1959). The competitive nature of the antagonism was also assessed by Schild plot analysis of the agonist dose-ratios obtained for two (metergoline) to three (methiothepin) different antagonist concentrations. The slope of the regression line was determined for each individual arteriolar segment.

Statistical analysis

All results are presented as means ± s.e.mean. Differences in agonists potency (pD₂ values and maximal responses) were evaluated according to one-way analysis of variance (ANOVA) for unequal sample size and Student-Newman-Keuls multiple comparison test. Student's *t* test was used to evaluate significant antagonist activity. Linear regression lines and correlation coefficients were calculated in order to detect and quantify correlations between vascular and binding potencies of the various agonists. In all cases, statistical significance was assumed when $P \leq 0.05$.

Drugs

The following drugs were purchased: 5-HT creatinine sulphate, (±)-propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO), ketanserin tartrate, mianserin hydrochloride, and 8-OH-DPAT ((±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydro-naphthalene) hydrobromide (RBI, Natick, MA). All other compounds were kindly provided by the following: (±)-α-methyl-5-HT (α-CH₃-5-HT), 2-methyl-5-HT (2-CH₃-5-HT), methysergide hydrogen maleinate and mesulergine (Sandoz, Basel, Switzerland); 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl) 1H indole succinate (RU 24969; Roussel UCLAF, Paris, France); α-phenyl-1-(2-phenylethyl)-4-piperidine methanol (MDL 11939); 1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222) and 8-[4-(1,4-benzodioxan-2-ylmethylamino)butyl]-8-azaspiro[4,5]decane-7,9-dione (MDL 72832) were from the Centre de Recherche Merrell Dow International, Strasbourg, France and methiothepin maleate from Hoffman-Laroche, Basel, Switzerland. Metergoline was a generous gift from Dr Rémi Quirion, Douglas Hospital Research Centre, Verdun, Québec, Canada.

Results

Agonist potency

All 5-HT agonists tested were found to induce concentration-dependent constrictions of human pial arterioles under resting tension (Figure 1, Table 1). Except for methysergide which elicited a contractile response corresponding to only 53% ($P < 0.05$) of that induced by 5-HT itself, all other agonists tested elicited full constriction over the concentration range studied (Figure 1). 5-HT was four fold more potent than 5-HT itself followed by RU 24969, methysergide and α-CH₃-5-HT which were approximately 5 to 12 times less potent than 5-HT in inducing vasoconstriction, as evaluated from their EC₅₀ ratios (Table 1). Agonists at 5-HT_{1A} (8-OH-DPAT and MDL 72832) and 5-HT₃ (2-CH₃-5-HT) sites were the least potent compounds and were one hundred to one thousand fold less active than 5-HT on a concentration basis. However, these agents produced maximal responses which either compared well (MDL 72832), were significantly higher (8-OH-DPAT, $P < 0.05$) or slightly less (2-CH₃-5-HT) than that elicited by 5-HT itself (Table 1).

A correlation analysis was performed between the vascular potencies (expressed as pD₂ values taken from Table 1) of the agonists in human pial arterioles and the affinities of these compounds at the various subtypes of 5-HT binding sites as described in the literature (taken as pK_D, pK_i or pIC₅₀ values as detailed in legend to Figure 2). No significant correlation could be detected between the affinities of these agents at 5-HT_{1A}, 5-HT_{1C} or 5-HT₂ binding sites characterized in rat

Figure 1 Concentration-response curves to 5-hydroxytryptamine (5-HT) and 5-HT agonists on human pial arterioles under resting tension. The curve to 5-HT (○) represents data obtained with all tissues. Shown are the responses to 5-carboxamidotryptamine (●); RU 24969 (□); α-methyl-5-HT (■); methysergide (△); MDL 72832 (▲); 2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydro-naphthalene (▽) and 2-methyl-5-HT (▼). The complete information for individual potency, maximal response and number of vascular segments is given in Table 1. Vertical bars show s.e. means of $n = 4$ to 33 (see Table 1).

brain or pig choroid plexus membranes and their potencies in inducing vasoconstriction of human pial arterioles (Figure 2). However, significant correlations were found between their affinities at 5-HT_{1B} sites in rat cortex ($r = 0.86$; $P < 0.01$; Figure 2) as well as 5-HT_{1D} sites characterized in human caudate membranes ($r = 0.98$; $P < 0.005$; Figure 2).

Antagonists

Consecutive administration of 5-HT alone to arteriolar segments resulted in a good reproducibility of the vasoconstriction induced by the amine, provided that a 45 to 60 min resting period was allowed before reconstruction of the concentration-response curves. In any instance, a trend towards an increase in maximal response and potency was noted rather than a decrease in vasomotor effects induced by 5-HT.

Antagonists with affinities at 5-HT_{1B} (propranolol), 5-HT_{1C} (mesulergine, mianserin), 5-HT₂ (ketanserin, mianserin and MDL 11939) and 5-HT₃ (MDL 72222) binding sites were tested as inhibitors of the 5-HT-induced constriction of human pial arterioles. In concentrations which varied between 10 nM to 1 μ M, all these compounds were devoid of antagonist activity as neither 5-HT potency (EC_{50}) nor maximal response (E_{Amax}) were significantly modified in the presence of the antagonist (Figure 3 and Table 2). In some cases, the 5-HT response was enhanced rather than depressed as was observed with the 5-HT₂ antagonists ketanserin and mianserin (for illustration, see ketanserin Figure 3e). However, such an effect was not noted with the more recently described 5-HT₂ antagonist MDL 11939 (Dudley *et al.*, 1988).

In contrast, in the presence of various concentrations of methiothepin, a compound with a broad spectrum of activity which includes all 5-HT₁ subtypes as well as the 5-HT₂ sites, an apparent parallel rightward shift in the concentration-response curves to 5-HT was observed (Figure 3a; Table 2) with a slight decrease in the maximal effect at higher antagonist concentrations. Similarly, the compound metergoline which exhibits high affinity for various 5-HT receptor subtypes, including 5-HT_{1D} recognition sites in human brain

Table 2 Effects of antagonists on 5-hydroxytryptamine (5-HT)-induced contraction of human pial arterioles

Antagonist	n	Concentration used	pA_2 value* (Schild slope)
Methiothepin	6	10 nM–1 μ M	8.55 ± 0.16 (1.41 ± 0.09)
Metergoline	4	10 nM–1 μ M	6.88 ± 0.05 (1.72 ± 0.23)
Mesulergine	3	0.1–1 μ M	No effect
Propranolol	3	0.1–1 μ M	No effect
Ketanserin	5	0.1–1 μ M	No effect
Mianserin	5	1 μ M	No effect
MDL 11939	3	10 nM–1 μ M	No effect
MDL 72222	3	10 nM–1 μ M	No effect

The concentrations of antagonist used are listed and given pA_2 values were obtained according to Arunlakshana & Schild as described in Methods. Results are the mean $\pm$ s.e. mean from the number n of arteriolar segments indicated. The slopes of the Schild plots are given within parentheses and were significantly different from unity with a $P < 0.05$.

* In the Van Den Brink calculation, the pA_2 values were 8.46 ± 0.24 and 7.24 ± 0.09 , respectively, for methiothepin and metergoline.

Table 1 Potencies of various 5-hydroxytryptamine (5-HT) agonists for inducing contraction on human pial arterioles

Agonist	n	E_{Amax} (% of 5-HT E_{Amax})	pD_2 ($-\log EC_{50}$)	$\frac{EC_{50} \text{ agonist}}{EC_{50} \text{ 5-HT}}$
5-HT	33	100	7.61 ± 0.08^b	1
5-CT	21	90 ± 5	8.18 ± 0.09^b	0.25
RU 24969	8	86 ± 7	6.85 ± 0.15^c	5
α -CH ₃ -5-HT	10	97 ± 9	6.57 ± 0.16^c	12
Methysergide	4	53 ± 14^a	6.30 ± 0.06^c	13
MDL 72832	5	103 ± 19	5.45 ± 0.08^d	96
2-CH ₃ -5-HT	18	68 ± 5	5.20 ± 0.13^d	450
8-OH-DPAT	10	137 ± 19^b	4.67 ± 0.21^b	1160

All values are mean $\pm$ s.e. mean from n individual segments as indicated. Those used for 5-HT represent pooled data obtained with all agonist experiments. An EC_{50} of 40.0 ± 6.7 nM was calculated for 5-HT and was used for determination of the agonist relative potency defined as $EC_{50} \text{ agonist}/EC_{50} \text{ 5-HT}$.

^a $P < 0.05$ when compared with all agonists except 2-CH₃-5-HT; ^b $P < 0.05$ with respect to all other agonists; ^c $P < 0.05$ with all agonists except α -CH₃-5-HT, methysergide or RU 24969, respectively; ^d $P < 0.05$ with all compounds but 2-CH₃-5-HT and MDL 72832, respectively, by one way analysis of variance and Student Newman-Keuls multiple range test. For key to abbreviations used see legend of Figure 1.

Figure 2 Correlation between the 5-hydroxytryptamine (5-HT) agonists vascular potencies (pD_2 values in human arterioles from Table 1) and their binding affinities (pK_D or pK_i) at various 5-HT receptor subtypes. The agonists used for the correlation analysis are 5-HT ($\circ$); 5-CT ($\bullet$); RU 24969 ($\square$); α -CH₃-5-HT ($\blacksquare$); methysergide (Δ); MDL 72832 ($\blacktriangle$); 8-OH-DPAT (∇) and 2-CH₃-5-HT ($\blacktriangledown$). We have used pK_D values published by Engel *et al.* (1986) in rat brain cortex membranes at 5-HT_{1A}, 5-HT_{1B} and 5-HT₂ binding sites for all agonists except RU 24969 (pK_i values from Glennon, 1987 in rat brain cortex), MDL 72832 (pIC_{50} values obtained in rat brain membranes; Mir *et al.*, 1988) and methysergide (pK_D values from Hoyer, 1988; at 5-HT_{1A} site, pig frontal cortex was used in this study). Binding affinities at 5-HT_{1C} were determined in pig choroid plexus and, as above, pK_D values were used except for RU 24969 and MDL 72832 (Engel *et al.*, 1986; Glennon, 1987; Hoyer, 1988; Mir *et al.*, 1988). At the 5-HT_{1D} subtype, affinities (pK_D or pK_i values) were taken from those obtained in human caudate membranes (Waeber *et al.*, 1988; Herrick-Davis *et al.*, 1988). Correlation coefficients (r) are given in the figure. For the 5-HT_{1B} subtype: $r = 0.86$ ($P < 0.01$) and for the 5-HT_{1D} site, $r = 0.98$ ($P < 0.005$). For key to abbreviations used see legend of Figure 1.

membranes (Waeber *et al.*, 1988), potently inhibited the 5-HT-induced vasoconstriction in human pial arterioles (Figure 3b and Table 2). The pA_2 values estimated in the Schild plot analysis were 8.55 ± 0.16 and 6.88 ± 0.05 for methiothepin and metergoline, respectively. When calculated according to Van Den Brink, pA_2 values of 8.46 ± 0.24 and 7.24 ± 0.09 were obtained for methiothepin and metergoline, respectively. For both antagonists, the slopes of the regression lines in the Schild plots analysis differed significantly from unity (Table 2).

Discussion

The present study on the pharmacological identification of human 5-HT receptors in pial arterioles suggests that 5-HT_{1A},

Figure 3 Concentration-response curves for 5-hydroxytryptamine (5-HT) on human pial arterioles in the absence ($\circ$, control) and presence of 10 nM ($\bullet$), 100 nM ($\square$) or 1000 nM ($\blacksquare$) methiothepin (a, $n = 6$); metergoline (b, $n = 4$); propranolol (c, $n = 3$); mesulergine (d, $n = 3$); ketanserin (e, $n = 5$); and MDL 72222 (f, $n = 3$). Vertical bars represent s.e. means of $n = 3-6$.

5-HT_{1C}, 5-HT₂ and 5-HT₃ receptor subtypes are not involved in the vasoconstriction induced by the indoleamine in this cerebrovascular tissue. More likely, a 5-HT₁-like receptor, which correlated best with the 5-HT_{1D} binding sites identified in human caudate membranes, is responsible for the 5-HT-induced contractile response. This contention is based on both our agonist and antagonist data as well as on the pharmacological properties of these various subtypes of 5-HT receptors (Bradley *et al.*, 1986; Richardson & Engel, 1986; Peroutka, 1988).

We found that two selective 5-HT_{1A} agonists, 8-OH-DPAT (Gozlan *et al.*, 1983; Middlemiss & Fozard, 1983) and MDL 72832 (Mir *et al.*, 1988) had a very low potency in inducing vasoconstriction of human isolated pial arterioles. In fact, their vascular potencies were more than 1000 fold less than their reported affinities at the 5-HT_{1A} subtype (Engel *et al.*, 1986; Glennon, 1987; Hoyer, 1988; Mir *et al.*, 1988), an observation which virtually rules out the stimulation of 5-HT_{1A}

receptors in the 5-HT-induced contraction of pial arterioles. Most probably, at the concentration range at which they were able to elicit potent vasoconstriction (10 μ M–1 mM), these agents interacted with other 5-HT receptor subtypes (Engel *et al.*, 1986; Mir *et al.*, 1988) and/or with α -adrenoceptors as suggested previously in other tissues (Timmermans *et al.*, 1984) including cerebrovascular beds (Hamel *et al.*, 1989; Parsons *et al.*, 1989). The exclusion of the 5-HT_{1A} subtype pinpoints to apparent species-related variations, as in the dog basilar artery this subtype of 5-HT receptor appears to mediate contraction and 8-OH-DPAT was found to be as potent as (Taylor *et al.*, 1986) or more potent than (Peroutka *et al.*, 1986) 5-HT. However, more recent studies have suggested that a 5-HT₁-like receptor, different from the 5-HT_{1A}, mediates contraction in this cerebral artery (Connor *et al.*, 1989).

Our experimental data also suggest that 5-HT_{1C} receptors cannot be involved in the 5-HT-mediated vasoconstriction of human pial arterioles. This hypothesis is based primarily on the observation that the potent 5-HT_{1C} antagonists, mesulergine (tested at 0.1 and 1 μ M) and mianserin (tested at 1 μ M), showed no inhibitory effect in this vascular response. Furthermore, the rank order of agonist potency at this subtype: 5-HT = α -CH₃-5-HT > RU 24969 = 5-CT = 2-CH₃-5-HT > 8-OH-DPAT (Pazos *et al.*, 1984; Engel *et al.*, 1986; Glennon, 1987; Hoyer, 1988) is different from the vascular potency observed in the present study, as clearly evidenced by the correlation analysis. The weak agonist activity of 2-CH₃-5-HT and the lack of antagonism of MDL 72222, two compounds with selective affinity at the 5-HT₃ receptor subtype (Fozard, 1984; Richardson & Engel, 1986), on the 5-HT-induced contraction of human pial arterioles further suggest that this vasomotor effect is not mediated by a 5-HT₃ receptor. Such a conclusion would support the finding that the beneficial effect of MDL 72222 in acute migraine attack (Loisy *et al.*, 1985) is related to nonvascular interactions (Richardson *et al.*, 1985; Orwin & Fozard, 1986).

Earlier studies have demonstrated the non-competitive nature of the weak antagonism exerted by several selective and non-selective 5-HT₂ antagonists on the 5-HT-induced contraction of human cerebral blood vessels (Hardebo *et al.*, 1978; Edvinsson *et al.*, 1978; Foster & Whalley, 1982; Muller-Schweinitzer, 1983). Recently, Parsons and colleagues (1989) showed the absence of antagonist effects of 5-HT-induced constriction of human basilar artery by the 5-HT₂ blocking drug, ketanserin. This observation supports our present findings in human pial arterioles which showed that selective 5-HT₂ compounds including ketanserin, mianserin and MDL 11939 (Engel *et al.*, 1986; Glennon, 1987; Hoyer, 1988; Dudley *et al.*, 1988) failed to exhibit any inhibitory effect on the 5-HT-mediated contraction. In some instances, a slight (non-significant) enhancement in both 5-HT maximal response and potency was noted with 5-HT₂ antagonists such as ketanserin and mianserin. This observation is difficult to explain but it is interesting that such a phenomenon had previously been noted in human cerebral vasculature (Hardebo *et al.*, 1978; Edvinsson *et al.*, 1978) and in other tissues with 5-HT₁-like mediated effects (Newberry & Gilbert, 1989). The exclusion of the 5-HT₂ receptor is further supported by the complete absence of correlation noted between the vascular and binding potencies of the various agonists studied. These results contrast with our previous study in feline cerebral arteries in which we found that the 5-HT₂ antagonists were the most potent inhibitors of the 5-HT vasocontractile response, albeit in an apparently non-competitive manner (Hamel *et al.*, 1989). These discrepancies further emphasize the species-related variations in the identity of contractile 5-HT cerebrovascular receptors.

Several aspects from both our agonist and antagonist data suggest that a 5-HT₁-like receptor is being stimulated by 5-HT to induce contraction of human pial arterioles. On one hand, this hypothesis is based on the higher potency of 5-CT as compared to 5-HT itself (Bradley *et al.*, 1986) and on the overall rank order of agonist potency, in which the agonist

property of methysergide is clearly evidenced and which resembles quite closely the order of potency described for 5-HT₁-like-mediated vascular function in the dog saphenous vein (Engel *et al.*, 1983). On the other hand, the participation of a 5-HT₁-like receptor is further adduced by the fact that the selective 5-HT₂ and 5-HT₃ antagonists were completely inactive whereas methiothepin (which has affinity at 5-HT_{1A}, 1B, 1C, 1D and 5-HT₂ receptors) was able to block potentially the 5-HT-induced contraction of human pial arterioles.

Based on our agonist correlation data, two subtypes of 5-HT₁-like receptors, namely 5-HT_{1B} and 5-HT_{1D}, are possible candidates for the receptor mediating constriction of human pial arterioles. However, experiments carried out with propranolol, a potent antagonist at 5-HT_{1B} sites in brain membranes (Middlemiss, 1984; Engel *et al.*, 1986; Glennon, 1987; Hoyer, 1988), showed that this compound was completely devoid of inhibitory activity (up to 1 μ M) on the contractile response of human pial arterioles to 5-HT. This observation is supported by the work of Parsons *et al.* (1989) who similarly found that the 5-HT_{1B} antagonist cyanopindolol was inactive in blocking 5-HT vasomotor effects in human basilar artery. Moreover, species differences in the distribution of 5-HT_{1B} and 5-HT_{1D} receptors (Hoyer *et al.*, 1986; Waeber *et al.*, 1988; Herrick-Davis *et al.*, 1988) indicate that the former are absent from human brain, an observation which would argue in favour of the activation of contractile 5-HT_{1D} receptors in human pial arterioles. The strong correlation obtained with the agonist's potency at the 5-HT_{1D} site ($r = 0.98$) would also support such a contention. However, the most convincing evidence to attest for the involvement of a receptor similar to the 5-HT_{1D} subtype was provided by the antagonist activity of metergoline on the 5-HT-induced cerebrovascular response. Though the correlation analysis illustrated in Figure 2 included only the agonists, addition of the metergoline vascular potency (pA_2 of 6.88 or 7.24, see results) and the pK_D value found in human caudate membranes (7.93 ± 0.23 ; Waeber *et al.*, 1988) still yielded a highly significant correlation ($r = 0.98$, $P < 0.01$ for either one of the pA_2 values). Methiothepin, on the other hand, was much more potent in inhibiting the 5-HT-induced constriction than predicted from its affinity at human 5-HT_{1D} binding sites (pK_D of 6.77, Waeber *et al.*, 1988). The reason for a higher affinity at cerebrovascular 5-HT receptors is unclear, but it might suggest that the cerebrovascular 5-HT₁ receptor is somewhat different from the caudate 5-HT_{1D} binding sites. Interestingly, such a high potency was also reported for methiothepin at 5-HT₁-like receptors in human basilar artery (Parsons *et al.*, 1989). The apparent lack of antagonism of mianserin, which expectedly should have exerted some blockade effect due to its relative affinity at 5-HT_{1D} sites (Waeber *et al.*, 1988; Hoyer, 1988), most probably results from our experimental conditions (tested at a single dose of 1 μ M) which did not allow for any forthcoming effect to be observed. Such an explanation seems likely when considering that 1 μ M metergoline potentially antagonized the 5-HT response while being only slightly active at 0.1 μ M. Had this compound been tested at a unique concentration of 0.1 μ M, we would have classified it as being virtually inactive. Such might actually have been the case in the dog saphenous vein where, on the basis of no antagonism of 0.1 μ M metergoline, it was concluded that the contractile response to 5-HT was not mediated by 5-HT_{1D} receptors (Humphrey *et al.*, 1988). Indeed, the overall pharmacology of this arterial bed resembles quite closely that described previously in human basilar artery (Parsons *et al.*, 1989) and here in human pial arterioles. Whether or not the 5-HT₁-like contractile cerebrovascular receptor identified in dog, primate and human basilar artery (Connor *et al.*, 1989; Parsons *et al.*, 1989) corresponds to the 5-HT_{1D} subtype remains to be established, but this possibility has not been ruled out (Parsons *et al.*, 1989).

In conclusion, our study suggests that a 5-HT₁-like receptor, which best correlated with the 5-HT_{1D} site identified in

human brain caudate membranes, mediates the vasoconstriction elicited by 5-HT in human pial arterioles. That we have identified a receptor slightly different from the brain caudate nucleus 5-HT_{1D} binding site cannot be excluded; however, our results and the current understanding of 5-HT receptor pharmacology and species distribution strongly emphasize its similarity to the 5-HT_{1D} subtype. When comparing the results of the present study in human pial arterioles with our recent data obtained in cat cerebral arteries, there is a major difference which resides in the participation of 5-HT₂ receptors exclusively in the latter species which, nevertheless, also exhibited the presence of a 5-HT₁-like contractile receptor (Hamel *et al.*, 1989). In the search for anti-migraine drugs, the 5-HT_{1D} site would appear as a good target for controlling cerebrovascular function since agonists and antagonists at this site would, respectively, induce constriction and potentially block the contractile effect of endogenous 5-HT thus resulting in vasodilatation, two vasomotor effects which have been associated with the manifestation of migraine (See Toole,

1984; Fozard, 1987). However, a better understanding of the pharmacology of peripheral 5-HT receptors would appear essential in order to insure the selectivity of 5-HT_{1D}-related drugs for use in cerebrovascular treatment without considerable interaction with non-cerebral vessels (see Angus, 1989).

This work was supported by a grant from the "Fondation des Maladies du Cœur du Québec", a Summer Medical Fellowship (D.B.) from the "Fonds de Recherche en Santé du Québec", as well as a grant and a scholarship (E.H.) from the Medical Research Council of Canada. The following individuals or drug companies which provided us with compounds that are not available commercially are greatly acknowledged: Dr Rémi Quirion, Douglas Hospital Research Centre; Merrell Dow International, Hoffman-Laroche, Roussel UCLAF and Sandoz. The authors are most grateful to the Douglas Hospital Brain Bank and especially Mrs P. Lemoine for providing us with human specimens. We want to thank Ms. L. Grégoire, S. Kaupp and Mr C. Hodge for expert technical, artistic and photographic work, respectively. We also thank Carolyn Elliot for the preparation of the manuscript.

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(Received June 13, 1990

Revised July 30, 1990

Accepted September 6, 1990)