The 5-HT₁-like receptor mediating the increase in canine external carotid blood flow: close resemblance to the 5-HT_{1D} subtype

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1 It has recently been shown that the increase in external carotid blood flow induced by 5-hydroxytryptamine (5-HT) in the anaesthetized dog, being mimicked by 5-carboxamidotryptamine (5-CT), inhibited by methiothepin, vagosympathectomy and sympatho-inhibitory drugs, and resistant to blockade by ritanserin and MDL 72222, is mediated by stimulation of prejunctional 5-HT₁-like receptors leading to an inhibitory action on carotid sympathetic nerves; these 5-HT₁-like receptors are unrelated to either the 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1C} (now 5-HT_{2C}) receptor subtypes. Inasmuch as 5-CT, 5-methoxytryptamine, sumatriptan and metergoline display high affinity, amongst other 5-HT binding sites, for the 5-HT_{1D} subtype, in the present study we have used these drugs in an attempt to determine whether the above inhibitory prejunctional 5-HT₁-like receptors correlate with the 5-HT_{1D} subtype.

2 One-minute intracarotid (i.c.) infusions of 5-HT (0.3, 1, 3 and 10 µg), 5-CT (0.01, 0.03, 0.1 and 0.3 µg), 5-methoxytryptamine (1, 3, 10 and 30 µg) and sumatriptan (1, 3, 10, 30 and 100 µg) resulted in dose-dependent increases in external carotid blood flow (without changes in mean arterial blood pressure or heart rate) with the following rank order of agonist potency: 5-CT >> 5-HT > 5-methoxytryptamine ≥ sumatriptan. Interestingly, sumatriptan-induced vasodilatation was followed by a more pronounced vasoconstriction.

3 The external carotid vasodilator effects of 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan were dose-dependently and specifically antagonized by metergoline (10, 30 and/or 100 µg kg⁻¹, i.v.). In addition, 5-methoxytryptamine- and sumatriptan-induced vasodilator effects were, respectively, markedly inhibited or abolished after vagosympathectomy, as previously shown for 5-CT and 5-HT.

4 Sumatriptan showed tachyphylaxis in its vasodilator component and antagonized 5-HT-induced external carotid vasodilatation in a specific manner, suggesting that a common site of action may be involved.

5 Taken together, the above results support our contention that 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan produce external carotid vasodilatation in the dog by an action that might primarily involve a prejunctional inhibition on carotid sympathetic nerves; a secondary component of this vasodilator response may be postsynaptic (endothelium-dependent and/or even directly on the vasculature). Based on the rank order of agonist potency, inhibition by vagosympathectomy and blockade by metergoline, we suggest that the inhibitory prejunctional 5-HT₁-like receptors mediating external carotid vasodilatation in the dog closely resemble the 5-HT_{1D} receptor subtype. The pharmacological profile of these receptors is similar (sympathetic nerves of the rat kidney and human saphenous vein, as well as porcine coronary endothelium) to other putative 5-HT_{1D} receptors mediating vascular responses.

Keywords: Carotid blood flow; 5-hydroxytryptamine; 5-HT_{1D} receptors; metergoline; sumatriptan; vasodilatation

Introduction

Intracarotid infusion of 5-hydroxytryptamine (5-HT) increases the external carotid blood flow in the dog by an inhibitory action on carotid sympathetic nerves, via the stimulation of inhibitory prejunctional 5-HT₁-like receptors unrelated to the 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1C} (now 5-HT_{2C}) subtypes (Villalón *et al.*, 1993a,b). Admittedly, the 5-HT₁ receptor is highly heterogeneous in nature, as five 5-HT₁ binding site subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}) have been identified (Humphrey *et al.*, 1993), but even these subtypes do not seem to correspond with some 5-HT₁-like receptor-mediated functional responses (Villalón & Saxena, 1992).

Within the 5-HT₁ receptor family, sumatriptan has a relative selectivity for the 5-HT_{1D} subtype (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989). Indeed, some actions of 5-HT and/or sumatriptan, including inhibition of noradrenaline release from sympathetic nerves (Molderings *et*

al., 1990) and contraction (Bax *et al.*, 1992) of the human saphenous vein as well as endothelium-dependent coronary vasodilatation in the pig (Schoeffter & Hoyer, 1990) are mediated by the 5-HT_{1D} (or a similar) receptor. This was suggested by: (i) the blockade of these responses by methiothepin, methysergide and/or metergoline; and (ii) the rank order of agonist potency of 5-carboxamidotryptamine (5-CT) ≥ 5-HT > 5-methoxytryptamine > sumatriptan.

It should be emphasized, notwithstanding, that sumatriptan can activate functional 5-HT₁-like receptors unrelated to the 5-HT_{1D} subtype for example, the increase in resistance within the canine carotid circulation (Perren *et al.*, 1991) and constriction of porcine arteriovenous anastomoses (Den Boer *et al.*, 1992); these effects were not antagonized by metergoline, a compound displaying a very high affinity for the 5-HT_{1D} receptor as well as for the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} (5-HT_{2C}) and 5-HT₂ receptors (Waeber *et al.*, 1988).

On the basis of the above findings, the present study was carried out to characterize further the nature of the inhibitory prejunctional 5-HT₁-like receptors mediating external

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carotid vasodilatation in the dog, with particular emphasis on verifying whether these receptors belong to the 5-HT_{1D} subtype. Therefore, we analysed the external carotid vascular effects of the endogenous ligand, 5-HT, and the putative 5-HT_{1D} receptor agonists, 5-CT, 5-methoxytryptamine and sumatriptan (Waeber *et al.*, 1988; Hoyer, 1989; Peroutka & McCarthy, 1989) in the absence or the presence of metergoline. Preliminary results of this investigation have been communicated to the Western Pharmacology Society (Villalón *et al.*, 1993c).

Methods

General

Experiments were carried out in a total of 35 dogs (19.2 ± 0.8 kg) not selected for breed or sex. The animals were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} , i.v.) and additional amounts (1 mg kg^{-1} , i.v.) were provided when required. All dogs were intubated with an endotracheal tube and artificially respired with room air using a Palmer ventilation pump at a rate of $20 \text{ strokes min}^{-1}$ and a stroke volume of $13\text{--}16 \text{ ml kg}^{-1}$, which was adjusted to maintain arterial pH within normal limits. Moreover, catheters were placed in the inferior vena cava via a femoral vein for the administration of drugs and in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 ID) for the measurement of arterial blood pressure. After drug administration, the venous cannula was flushed with 3 ml of physiological saline. Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures: $\text{MAP} = \text{DAP} + (\text{SAP} - \text{DAP})/3$. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, U.S.A.) triggered from the blood pressure signal.

The right common carotid artery was dissected for approximately 10 cm proximal to its bifurcation, and the branches originating at this site were identified. In all cases, the corresponding carotid sinus was denervated in order to avoid the reflex cardiovascular effects of 5-HT. Denervation was achieved by carefully stripping the sinus of surrounding tissue, and the adequacy of the procedure was checked by verifying that occlusion of the corresponding common carotid artery did not elicit a pressor response. After ligating the right internal carotid and right occipital arteries, a pre-calibrated ultrasonic flow probe (4 mm R-Series; Transonic Systems Inc., Ithaca, N.Y., U.S.A.) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, N.Y., U.S.A.) was placed around the right common carotid artery, 1 or 2 cm proximal to the origin of the cranial thyroid artery. Under these experimental circumstances, the flow through the right common carotid artery was considered to represent the blood flow through the external carotid artery (for detailed considerations see Villalón *et al.*, 1993a,b).

To analyse the effect of agonist drugs on external carotid blood flow, the agonists were administered into the carotid artery by a Harvard model 901 pump (Harvard Apparatus Co. Inc., Millis, MA, U.S.A.) with a cannula inserted into the right cranial thyroid artery. Mean arterial blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The body temperature of the animals was maintained between $37\text{--}38^\circ\text{C}$.

Experimental protocol

After the animals had been in a stable haemodynamic condition for at least 30 min, baseline values of blood pressure, heart rate and external carotid blood flow were determined. At this point, the animals were divided into seven groups. The first group ($n = 6$) received consecutive i.c. infusions (during 1 min) of 5-HT (0.3 , 1 , 3 and $10 \mu\text{g min}^{-1}$), 5-

carboxamidotryptamine (5-CT; 0.01 , 0.03 , 0.1 and $0.3 \mu\text{g min}^{-1}$) and acetylcholine (0.003 , 0.01 , 0.03 and $0.1 \mu\text{g min}^{-1}$); subsequently, the animals were given metergoline (10 , 30 and $100 \mu\text{g kg}^{-1}$, i.v.) and the responses to 5-HT, 5-CT and acetylcholine were elicited again after each dose of the antagonist. The other three groups received 1 min i.c. infusions of 5-methoxytryptamine (1 , 3 , 10 and $30 \mu\text{g min}^{-1}$) before and after either physiological saline (0.015 , 0.05 and 0.15 ml kg^{-1} , i.v.; $n = 6$), metergoline (10 , 30 and $100 \mu\text{g kg}^{-1}$, i.v.; $n = 6$) or bilateral cervical vagosympathectomy ($n = 4$; as described by Villalón *et al.*, 1993a,b) respectively. In the saline-treated group, a dose-response curve to 5-HT was elicited before and after the four dose-response curves to 5-methoxytryptamine to investigate any possible interaction between the two agonists.

Finally, the three other groups received 1 min i.c. infusions of sumatriptan (1 , 3 , 10 , 30 and $100 \mu\text{g min}^{-1}$) before and after either saline (0.15 ml kg^{-1} , i.v.; $n = 4$), metergoline ($100 \mu\text{g kg}^{-1}$, i.v.; $n = 5$) or vagosympathectomy ($n = 4$), respectively. In the saline-treated group, the possible interaction of sumatriptan with 5-HT and acetylcholine, respectively, was analysed by eliciting dose-response curves to 5-HT and acetylcholine before and after the first dose-response curve to sumatriptan. Since sumatriptan-induced vasodilator responses showed a fading pattern after repetitive administration, only two dose-response curves to the agonist were obtained.

Each dose of agonist drug was in a solution which was administered at a rate of 1 ml min^{-1} during a period of 1 min, with intervals of $5\text{--}45$ min. The doses of agonist drugs were selected on the basis of results obtained from preliminary experiments, in which reproducible and consistent dose-dependent responses in external carotid blood flow were elicited with no changes in arterial blood pressure or heart rate (for more details see Villalón *et al.*, 1993a).

The interval between the different doses of the compounds used as agonists and/or antagonists depended on the duration of the effect produced by the preceding dose on the haemodynamic variables. Thus, the dose-intervals between the different doses of agonists (especially 5-CT and sumatriptan) ranged between 5 and 45 min, as in each case we waited until the external carotid blood flow had returned completely to the baseline values; for the antagonists, a period of 10 min was allowed to elapse before the dose-response curves to the agonists were made again. The dosing with all drugs used was sequential.

Data presentation and statistical analysis

All data in the text, figures and tables are presented as mean $\pm$ s.e. mean. The peak changes in external carotid blood flow produced by the different doses of agonists were determined. The difference between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Where appropriate, Student's paired *t* test was applied. A *P* value of 0.05 or less (two-tailed) was considered statistically significant.

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the drugs used in the present study were the following, obtained from the sources indicated: 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.); acetylcholine chloride and 5-methoxytryptamine hydrochloride (Research Biochemical Inc., Natick, MA, U.S.A.); 5-carboxamidotryptamine maleate and sumatriptan succinate (gift: Dr P.P.A. Humphrey, Glaxo group research, Ware, U.K.); and metergoline (gift: Farmitalia, Milan, Italy). All compounds were dissolved in physiological saline; when needed, 2% ascorbic acid (metergoline) was added. The

vehicles had no effect on either basal mean arterial blood pressure, heart rate or external carotid blood flow. The doses mentioned in the text refer to the free base of substances.

Results

Systemic haemodynamic variables

The values for blood pressure, heart rate and external carotid blood flow before and after saline or vagosympathectomy are shown in Table 1; similarly, the effects of metergoline are shown in Table 2. After vagosympathectomy, the external carotid blood flow was significantly increased (Table 1); in contrast, all these variables remained essentially unchanged after administration of saline (Table 1), or metergoline (Table 2). Moreover, blood pressure and heart rate were not modified by the i.c. infusions of the various agonists before and after either vagosympathectomy or metergoline (not shown, but baseline values are depicted in Table 1); however, higher doses of the 5-HT agonists tended to produce inconsistent effects on external carotid blood flow.

Initial effects of agonist drugs on external carotid blood flow

The onset of the responses induced by the 5-HT agonists was immediate; thus, Figure 1 shows that i.c. infusions of 5-HT (0.3, 1, 3 and 10 $\mu\text{g min}^{-1}$), 5-CT (0.01, 0.03, 0.1 and 0.3 $\mu\text{g min}^{-1}$), 5-methoxytryptamine (1, 3, 10 and 30 $\mu\text{g min}^{-1}$) and sumatriptan (1, 3, 10, 30 and 100 $\mu\text{g min}^{-1}$) elicited dose-dependent increases in external carotid blood flow; in no case did the 5-HT agonists significantly change blood pressure or heart rate. The rank order of agonist potency to increase external carotid blood flow was 5-CT >> 5-HT > 5-methoxytryptamine $\geq$ sumatriptan. At the doses used, the duration of action of 5-CT (2.4 ± 0.6 , $3.7 \pm$

0.6 , 5.1 ± 0.8 and 7.3 ± 1.1 min; $n = 6$) and 5-methoxytryptamine (1.3 ± 0.1 , 1.7 ± 0.1 , 2.1 ± 0.2 and 2.9 ± 0.3 min; $n = 16$) was longer than that of 5-HT, the effects of which lasted only during the period of infusion (1 min) at which time external carotid blood flow returned to the baseline value. Interestingly, 1 min i.c. infusions of sumatriptan (1, 3, 10, 30 and 100 $\mu\text{g min}^{-1}$) produced dose-dependent biphasic responses, namely, small increases (Figure 1) followed by larger decreases (-2 ± 1 , -8 ± 3 , -19 ± 4 , -46 ± 8 and -64 ± 11 ml min^{-1} after sumatriptan, respectively; $n = 6$) in external carotid blood flow; the vasodilator effects of sumatriptan lasted 1 min or less, whilst the vasoconstrictor responses were longer-lasting (2 ± 1 , 5 ± 1 , 7 ± 1 , 15 ± 2 and 24 ± 2 min after sumatriptan, respectively).

Effect of metergoline on external carotid vasodilatation to 5-HT, 5-CT and acetylcholine

We have previously reported that the external carotid vasodilatation induced by repeated administrations of 5-HT, 5-CT and acetylcholine remained unchanged in control animals receiving saline (Villalón *et al.*, 1993a). In contrast, the increases in external carotid blood flow induced by 5-HT and 5-CT were dose-dependently antagonized by metergoline (10, 30 and 100 $\mu\text{g kg}^{-1}$, i.v.), whereas those by acetylcholine remained unaffected (Figure 2). Significantly, not only were the vasodilator responses induced by 5-HT abolished by the highest dose of metergoline, but a dose-dependent vasoconstrictor effect was unmasked.

Effect of physiological saline, metergoline or vagosympathectomy on either 5-methoxytryptamine- or sumatriptan-induced increases in external carotid blood flow

The increases in external carotid blood flow produced by 5-methoxytryptamine, at the doses and time intervals (5–15 min) used in the present study, were reproducible and remained essentially unchanged in control animals receiving 3 subsequent doses (0.015, 0.05 and 0.15 ml kg^{-1} , i.v.) of saline (Figure 3a). In contrast, the responses to 5-methoxytrypt-

Table 1 Mean arterial blood pressure (MAP; mmHg), heart rate (HR; beats min^{-1}) and external carotid blood flow (ECBF; ml min^{-1} , measured by an ultrasonic flow probe) before and after i.v. administration of saline ($n = 6$) or bilateral cervical vagosympathectomy ($n = 4$)

	Treatment	Baseline value	10 min after treatment
MAP	Saline ^a	134 ± 5	141 ± 6
	Vagosympathectomy	163 ± 14	169 ± 13
HR	Saline ^a	185 ± 9	175 ± 7
	Vagosympathectomy	151 ± 17	160 ± 14
ECBF	Saline ^a	122 ± 24	115 ± 18
	Vagosympathectomy	118 ± 18	$197 \pm 25^*$

^aRefers to the effect after the third administration of physiological saline. The first and second saline administrations were without significant effects.

*Significantly different ($P < 0.05$) from the baseline value.

Table 2 Mean arterial blood pressure (MAP; mmHg), heart rate (HR; beats min^{-1}) and external carotid blood flow (ECBF; ml min^{-1} , measured by an ultrasonic flow probe) before and after i.v. administration of metergoline (10, 30 and 100 $\mu\text{g kg}^{-1}$; $n = 6$)^a

	Baseline value	10 min after metergoline 10	30	100
MAP	147 ± 9	146 ± 9	152 ± 8	153 ± 8
HR	171 ± 9	165 ± 11	165 ± 9	156 ± 9
ECBF	113 ± 18	112 ± 18	114 ± 16	118 ± 16

^aNo significant changes in either MAP, HR or ECBF were produced after metergoline (10, 30 or 100 $\mu\text{g kg}^{-1}$, i.v.).

Figure 1 Comparative effects of 1 min i.c. infusions of 5-hydroxytryptamine (○, $n = 6$), 5-carboxamidotryptamine (▲, $n = 6$), 5-methoxytryptamine (●, $n = 6$) and sumatriptan (△, $n = 6$) on the canine external carotid blood flow. Sumatriptan-induced vasodilator effects were followed by larger and longer-lasting vasoconstrictor responses (see Results section).

amine were dose-dependently antagonized by metergoline (10, 30 and 100 $\mu\text{g kg}^{-1}$, i.v.; Figure 3b) or markedly, but not totally, inhibited after vagosympathectomy (Figure 3c). Moreover, the increases in external carotid blood flow produced by repeated doses of sumatriptan in the control animals were significantly reduced (Figure 4a); nevertheless, external carotid vasodilatation due to sumatriptan was abolished after either metergoline (100 $\mu\text{g kg}^{-1}$; Figure 4b) or vagosympathectomy (Figure 4c). Although not shown, the vasoconstrictor response that followed sumatriptan-induced vasodilatation was significantly increased after metergoline or vagosympathectomy. In the saline-treated animals, where 5-HT was administered before and after the set of injections of 5-methoxytryptamine (1, 3, 10 and 30 $\mu\text{g min}^{-1}$; $n=6$) or sumatriptan (1, 3, 10, 30 and 100 $\mu\text{g min}^{-1}$; $n=4$, see Methods), the responses induced by 5-HT (0.3, 1, 3 and

10 $\mu\text{g min}^{-1}$) remained unchanged after 5-methoxytryptamine (18 ± 3 , 30 ± 4 , 43 ± 6 and 58 ± 8 ml min^{-1} before and 14 ± 2 , 22 ± 1 , 37 ± 4 and 54 ± 5 ml min^{-1} after 5-methoxytryptamine, respectively), but were significantly ($P < 0.05$) reduced by sumatriptan (25 ± 3 , 39 ± 7 , 60 ± 12 and 72 ± 14 ml min^{-1} before and 16 ± 5 , 24 ± 4 , 33 ± 4 and 40 ± 9 ml min^{-1} after sumatriptan, respectively). It has to be emphasized that the vasodilator effects of 5-HT were not 'masked' by the increase in external carotid blood flow induced by sumatriptan, as the responses to 5-HT were elicited at the time when the effect of sumatriptan had worn off. In this respect, it is important to note that the responses to acetylcholine (0.003, 0.01, 0.03 and 0.1 $\mu\text{g min}^{-1}$) remained unaltered after sumatriptan (7 ± 2 , 14 ± 5 , 24 ± 6 and 41 ± 5 ml min^{-1} before and 9 ± 4 , 14 ± 5 , 24 ± 4 and 37 ± 4 ml min^{-1} after sumatriptan, respectively).

Figure 2 The effects of i.v. bolus injections of metergoline (○, 0 $\mu\text{g kg}^{-1}$; ▲, 10 $\mu\text{g kg}^{-1}$; ■, 30 $\mu\text{g kg}^{-1}$; and ◆, 100 $\mu\text{g kg}^{-1}$; $n=6$ each) on the increases in canine external carotid blood flow induced by 1 min i.c. infusions of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and acetylcholine (ACh). * $P < 0.05$ vs. control.

Figure 3 The effects of (a) physiological saline (○, 0 ml kg^{-1} ; ●, 0.015 ml kg^{-1} ; Δ, 0.05 ml kg^{-1} and ▲, 0.15 ml kg^{-1} ; $n=6$ each), (b) metergoline (○, 0 $\mu\text{g kg}^{-1}$; ●, 10 $\mu\text{g kg}^{-1}$; Δ, 30 $\mu\text{g kg}^{-1}$; and ▲, 100 $\mu\text{g kg}^{-1}$; $n=6$ each) and (c) bilateral cervical vagosympathectomy (○, before and ▼, after; $n=4$) on the increases in canine external carotid blood flow induced by 1 min i.c. infusions of 5-methoxytryptamine. * $P < 0.05$ vs. control.

Discussion

The vasodilator effects of 5-HT on the canine external carotid bed are mediated by 5-HT₁-like receptors unrelated to the 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1C} (now 5-HT_{2C}; Humphrey *et al.*, 1993) receptor subtypes, which mediate a prejunctional inhibitory action on carotid sympathetic nerves (Villalón *et al.*, 1993a,b; Terrón *et al.*, 1994). The present investigation extends these findings and suggests that, apart from the possible involvement of secondary (postjunctional) mechanisms, the prejunctional external carotid 5-HT₁-like receptors: (i) can be stimulated by the putative 5-HT_{1D} receptor agonists, 5-methoxytryptamine and sumatriptan, the vasodilator effects of which are markedly inhibited after vagosympathectomy; and (ii) can be specifically antagonized by metergoline which, in addition to displaying a very high affinity for the 5-HT_{1D} receptors (Waeber *et al.*, 1988), is able to block either

the prejunctional (Charlton *et al.*, 1986) or postjunctional (Sumner *et al.*, 1989; Schoeffter & Hoyer, 1990; Sumner, 1991) effects of 5-HT and/or 5-CT in other vascular preparations.

Systemic and carotid haemodynamic changes

It has already been shown that the administration of three sequential i.v. bolus injections of saline to the anaesthetized dog produces no substantial changes in either blood pressure, heart rate or external carotid blood flow over a period of about 140–180 min (Villalón *et al.*, 1993a). In the present study, baseline external carotid blood flow was not significantly modified after treatment with saline or metergoline, but was markedly increased after vagosympathectomy, in accordance with previous findings (Mena & Vidrio, 1979; Villalón *et al.*, 1993a,b). That the carotid sympathetic fibres exert a tonic influence in the external carotid bed is further strengthened by the fact that a more accurate indicator of vascular tone, the external carotid conductance, did increase significantly ($P < 0.05$) from 0.62 ± 0.06 to 0.92 ± 0.11 units ($n = 8$), as previously reported (Villalón *et al.*, 1993a).

Agonist action of 5-methoxytryptamine and sumatriptan on the 5-HT receptors mediating external carotid vasodilatation

In addition to recognizing the possible interference by pharmacokinetic factors in our experimental model, we could not, admittedly, reach the maximum effects with the 5-HT agonists used, as higher doses produced inconsistent vasodilator responses (see Experimental protocol). Notwithstanding, our study shows that, like 5-HT and 5-CT (Villalón *et al.*, 1993a; present results), both 5-methoxytryptamine and sumatriptan did increase the external carotid blood flow, although there were important differences in the effects of these drugs.

Accordingly, 5-methoxytryptamine behaved as a potent agonist (being about 0.5 log units less potent than 5-HT) and failed to attenuate the responses to 5-HT; moreover, no evidence of tachyphylaxis was observed since the responses to 5-methoxytryptamine remained unaffected after three consecutive i.v. injections of saline, as shown for 5-HT and 5-CT (Villalón *et al.*, 1993a).

In contrast, sumatriptan was the weakest vasodilator agonist and attenuated the vasodilator responses to 5-HT; this attenuation by sumatriptan (and/or any possible metabolite) is specific as the vasodilatation to acetylcholine, which acts on the vascular endothelium to produce vascular smooth muscle relaxation (Furchgott & Zawadzki, 1980), remained unaffected, thereby implying a common site of action in the effects of sumatriptan and 5-HT. Moreover, the biphasic responses produced by sumatriptan (i.e. external carotid vasodilatation followed by vasoconstriction) are consistent with either the inhibitory action of sumatriptan on the sympathetic nerves of the canine (Humphrey *et al.*, 1988) and human (Molderings *et al.*, 1990) saphenous vein or the vasoconstriction produced by this agonist in the dog common carotid circulation (Feniuk *et al.*, 1989; Perren *et al.*, 1991), responses that involve the activation of 5-HT₁-like receptors. Since sumatriptan produces endothelium-dependent relaxation in the coronary artery (Schoeffter & Hoyer, 1990), but not in the vena cava (Sumner, 1991) of the pig, it is difficult to judge to what extent, if any, the vasodilator effect of sumatriptan involves an endothelium dependent (or even a direct) component in our studies.

Antagonism of the vasodilator responses to the 5-HT agonists by metergoline

The blockade of the vasodilatation to 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan by metergoline was specific and unrelated to changes in the baseline haemodynamic parameters. Notwithstanding, the profile of antagonism by

Figure 4 The effects of (a) physiological saline (○, 0 ml kg⁻¹ and ●, 0.15 ml kg⁻¹; $n = 4$); (b) metergoline (○, 0 µg kg⁻¹ and ●, 100 µg kg⁻¹; $n = 5$); and (c) bilateral cervical vagosympathectomy (○, before and ●, after; $n = 4$) on the increases in canine external carotid blood flow induced by 1 min i.c. infusions of sumatriptan. * $P < 0.05$ vs. control.

metergoline was different with each 5-HT agonist; thus, the fact that metergoline blocked the responses to 5-methoxytryptamine (Figure 3b) far more effectively than those to 5-CT (Figure 2) may reflect the higher affinity and/or efficacy of 5-CT for 5-HT₁ (including 5-HT_{1D}) binding sites and/or receptors (Hoyer, 1989; Saxena & Villalón, 1990). Indeed, the vasodilator response to 5-CT, and in some cases to 5-methoxytryptamine, remaining after metergoline may be prejunctional since it was abolished after vagosympathectomy (data not given), as shown in control animals (Villalón *et al.*, 1993a).

Role of sympathetic tone on the external carotid vasodilator effects of the 5-HT agonists

Although the vasodilator responses to 5-HT or 5-CT are specifically blocked by methiothepin, vagosympathectomy or sympatholytic drugs (Villalón *et al.*, 1993a; Terrón *et al.*, 1994), we have to recognize that the non-neurogenic vascular tone may also influence the vasodilatation produced by the 5-HT agonists, as suggested by the *inhibition* of 5-HT-induced external carotid vasodilatation by diazoxide (a direct vasodilator agent) in dogs with intact vagosympathetic trunks (Vidrio & Hong, 1976). Hence, it could be argued that the external carotid vasodilatation to 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan was inhibited after vagosympathectomy since the carotid circulation was dilated by this procedure; this seems unlikely, nevertheless, as we have reported that the subsequent restoration of the vascular tone to the prevagosympathectomy level with exogenous nor-adrenaline did not revert the effects of 5-HT and 5-CT to vasodilatation, with the vasodilator response to acetylcholine remaining unaltered (Villalón *et al.*, 1993a).

Interestingly, both vagosympathectomy and metergoline (100 µg kg⁻¹) had similar effects on the vasodilator responses to 5-HT and sumatriptan (Villalón *et al.*, 1993a; present results), namely: (i) the responses to both compounds were specifically *abolished*; (ii) a vasoconstrictor effect was unmasked in the case of 5-HT; and (iii) the vasoconstrictor component of the biphasic response to sumatriptan was magnified. Hence, it would seem that only when the vasodilator responses to 5-HT and sumatriptan have been abolished (by vagosympathectomy or metergoline), are their vasoconstrictor components made manifest and/or magnified; thus, if the vasodilator effects of 5-HT and sumatriptan have *postjunctional* (endothelium dependent and/or direct) vasodilator components, as suggested by the 5-methoxytryptamine-induced vasodilator response remaining after vagosympathectomy, they would be precluded by their vasoconstrictor response (absent in the case of 5-CT and 5-methoxytryptamine) which involves postjunctional 5-HT₁-like receptors (Villalón & Terrón, 1994). In line with this assumption, the vasodilator response to 5-methoxytryptamine may consist of a primary inhibitory prejunctional effect (sensitive to vagosympathectomy) which is apparently complemented by a small postjunctional component (resistant to vagosympathectomy), both of which seem to be blocked by metergoline. The above observations, besides being relevant to our notion that the 5-HT₁-like receptors mediating vasodilatation and vasoconstriction of the external carotid bed are heterogeneous (see below), lead us to suggest that, like 5-HT and 5-CT, both 5-methoxytryptamine and sumatriptan are acting prejunctionally, though no direct proof is provided.

Mechanisms involved in the external carotid vasodilator effects of 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan

We have previously shown that 5-HT-, 5-CT- and indorenate-induced external carotid vasodilatation involves the activation of inhibitory prejunctional 5-HT₁-like receptors (Villalón *et al.*, 1993a,b). Several experimental observations, however, have led us to consider the possible participation of

additional mechanisms in the external carotid vasodilator effects of 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan. Thus, in addition to an inhibitory action on sympathetic nerves via prejunctional 5-HT₁-like receptors (Charlton *et al.*, 1986; Humphrey *et al.*, 1988; Bond *et al.*, 1989; Molderings *et al.*, 1990; Villalón *et al.*, 1993a,b), 5-HT and some of the above agonists have been shown to induce vasodilatation in other vascular preparations by direct (vascular; Sumner *et al.*, 1989) and indirect (endothelium-dependent; Schoeffter & Hoyer, 1990; Sumner, 1991) effects.

Considering the above information, our results showing the remaining, albeit negligible, vasodilator effect of 5-methoxytryptamine as well as the abolition of 5-HT-, 5-CT- and sumatriptan-induced vasodilator responses after vagosympathectomy, suggest that the former agonist may produce *postjunctional* responses (endothelium-dependent and/or directly on the vasculature); however, these postjunctional effects seem to play a minor, if any, role in the total external carotid vasodilator effect under study (see Figure 3c). Furthermore, the fact that the external carotid vasodilatation induced by sumatriptan was totally blocked by vagosympathectomy or metergoline and that sumatriptan *specifically* attenuated the vasodilator responses to 5-HT, as reported in the perfused rat kidney (Bond *et al.*, 1988), are in keeping with the suggestion that sumatriptan and 5-HT act on a common site of action, presumably, the prejunctional 5-HT₁-like receptors producing external carotid vasodilatation, although, as previously considered, postsynaptic mechanisms cannot be completely ruled out.

Additionally, it is to be remarked that our findings with metergoline, taken individually, cannot provide any evidence on the site of action, as it is capable of blocking either the prejunctional (Charlton *et al.*, 1986) or postjunctional (Sumner *et al.*, 1989; Schoeffter & Hoyer, 1990; Sumner, 1991) effects of 5-HT and/or 5-CT in other vascular preparations.

Taken together, the pharmacological profile displayed by each agonist, as well as the inhibitory effects of vagosympathectomy and metergoline, suggest that the *primary* mechanism involved in the vasodilatation produced by the 5-HT agonists used in the present study is the stimulation of inhibitory prejunctional 5-HT₁-like receptors located on carotid sympathetic nerves, as previously described (Villalón *et al.*, 1993a; Terrón *et al.*, 1994).

Possible resemblance of the inhibitory prejunctional 5-HT₁-like receptors mediating canine external carotid vasodilatation to the 5-HT_{1D} receptor subtype

In previous publications (Villalón *et al.*, 1993a,b; Terrón *et al.*, 1994) we showed that the increases in canine external carotid blood flow produced by 5-HT, 5-CT and indorenate are *not* mimicked by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), buspirone, ipsapirone, 5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]-1-*H*-indol succinate (RU 24969), 1-(2,5-dimethoxy-4-iodophenyl)-aminopropane (DOI) or 2-methyl-5-HT. Since, in addition, the vasodilator responses to the above 5-HT receptor agonists are potently and specifically blocked by methiothepin (a 5-HT₁-like receptor antagonist) or vagosympathectomy, but not by drugs that act as antagonists at various receptors [5-HT_{1A} and/or 5-HT_{1B} (spiroxatrine, pindolol and/or cyanopindolol), 5-HT_{1C} and/or 5-HT₂ (ketanserin and/or ritanserin) and 5-HT₃ (MDL 72222)], it was concluded that 5-HT, 5-CT and indorenate increase external carotid blood flow in the dog via prejunctional 5-HT₁-like receptors unrelated to the 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1C} (now 5-HT_{2C}) receptor subtypes (Villalón *et al.*, 1993a,b; Terrón *et al.*, 1994).

The present investigation showed that 5-CT, 5-methoxytryptamine and, to a lesser extent, sumatriptan mimic 5-HT and that low doses (10, 30 and 100 µg kg⁻¹) of metergoline antagonize their respective external carotid vasodilator responses. The high affinity of the above 5-HT agonists for

5-HT_{1D} binding sites (Hoyer, 1989; Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989), coupled to the specific attenuation of the 5-HT-induced vasodilator responses by sumatriptan, raises the possibility that the 5-HT₁-like receptors involved in the increase in external carotid blood flow may correspond to the 5-HT_{1D} (or a similar) receptor subtype. Further evidence in support of this possibility is provided by the high antagonist activity of metergoline, a compound displaying the highest affinity for 5-HT_{1D} binding sites (Waeber *et al.*, 1988), against the increases in canine external carotid blood flow produced by 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan; importantly, this blockade was specific since the same doses of metergoline did not modify acetylcholine-induced external carotid vasodilatation.

Although the involvement of inhibitory *prejunctional* 5-HT_{1D} receptors cannot be suggested merely on the antagonism by metergoline of the responses to 5-HT, 5-CT, 5-methoxytryptamine and sumatriptan, we feel that this assumption is strengthened when considering the evidence in its full context (Villalón *et al.*, 1993a,b; present results): (i) complete blockade and even reversal of 5-HT-induced external carotid vasodilatation by vagosympathectomy, methiothepin and metergoline, and resistance to blockade by antagonists at other (sub)types of 5-HT receptors; (ii) the rank order of agonist potency of 5-CT, 5-methoxytryptamine and sumatriptan, which are agonists at functional 5-HT₁-like receptors (Bradley *et al.*, 1986; Humphrey *et al.*, 1988; Feniuk *et al.*, 1989; Saxena & Villalón, 1990) and display high affinity for 5-HT_{1D} binding sites (Hoyer, 1989; Peroutka & McCarthy, 1989); but no effect by agonists at other (sub)types of 5-HT receptors; (iii) inhibition of the responses to 5-CT, 5-methoxytryptamine and sumatriptan by vagosympathectomy or metergoline, which is a potent antagonist at 5-HT₁-like and 5-HT₂ receptors (Saxena & Villalón, 1990) and displays a high affinity for 5-HT_{1D} binding sites (Waeber *et al.*, 1988). Hence, our findings suggest that the *prejunctional* 5-HT₁-like receptors producing external carotid vasodilatation are similar to the 5-HT_{1D} receptors identified on: (i) sympathetic nerves (Molderings *et al.*, 1990) and vascular smooth muscle (Bax *et al.*, 1992) of the human saphenous vein; and (ii) porcine coronary endothelium (Schoeffter & Hoyer, 1990).

The physiological relevance of a *prejunctional* inhibitory 5-HT_{1D} receptor in the canine external carotid bed is, to the best of our knowledge, unknown; one possibility exists, however. 5-HT has been shown to be taken-up into and released from sympathetic nerves (see Saxena & Villalón, 1990), and may act, therefore, as a modulator of the neuroeffector transmission; thus, feed-back inhibition of noradrenaline release by 5-HT may be involved, as described for other substances (Rand *et al.*, 1987).

In conclusion, we suggest that the inhibitory *prejunctional* 5-HT₁-like receptors involved in the primary mechanism mediating external carotid vasodilatation in the dog closely resemble the 5-HT_{1D} receptor subtype. The pharmacological

profile of these receptors is similar (sympathetic nerves of the rat kidney and human saphenous vein, as well as porcine coronary endothelium) to other putative 5-HT_{1D} receptors mediating vascular responses. We also acknowledge that the external carotid vasodilatation produced by the 5-HT agonists apparently involves a secondary – though negligible – component at the postsynaptic level.

In connection with the above conclusion, it should be considered that the 5-HT_{1D} receptor represents a heterogeneous subfamily that is comprised of at least two populations, namely one that recognizes sumatriptan and has high affinity for 5-CT, and another that shows little affinity for either sumatriptan or 5-CT (Sumner & Humphrey, 1989; Humphrey *et al.*, 1993). One might further speculate that the high affinity site for sumatriptan and 5-CT might be equivalent to the inhibitory *prejunctional* 5-HT_{1D}-like receptors that mediate canine external carotid vasodilatation. Undoubtedly, the use of non-selective drugs complicates analysis of the subtypes involved, and progress will be critically dependent upon the discovery of new pharmacological probes which are endowed with high selectivity and potency.

In this regard, it is pertinent to note that methiothepin did completely antagonize the external carotid vasodilator effects of 5-HT and 5-CT at a dose of 30 µg kg⁻¹ (i.v.) (Villalón *et al.*, 1993a); this dose of methiothepin is 0.5 log units lower than the dose of metergoline (100 µg kg⁻¹; i.v.) used in the present study to produce the same effect. Therefore, the need for higher doses of metergoline to produce antagonism in the canine external carotid bed (Figures 2–4) would imply a lower affinity of metergoline for 5-HT_{1D} binding sites; an important discrepancy in this respect is that metergoline (pK_D = 9.1) displays higher affinity than methiothepin (pK_D = 6.3) for 5-HT_{1D} binding sites (Hoyer, 1989). Nevertheless, two important findings should be considered to explain the above discrepancy. Firstly, methiothepin, but not metergoline, displays very high affinity for α₁-adrenoceptor binding sites (Leysen, 1985); thus, methiothepin, but not metergoline, potentially antagonized the external carotid vasoconstrictor responses to the α₁-adrenoceptor agonist, phenylephrine, in dogs pretreated with rauwolscine (Villalón *et al.*, unpublished). Secondly, 5-HT-induced external carotid vasodilatation is inhibited by drugs that decrease the sympathetic outflow, including α₁-adrenoceptor antagonists (Terrón *et al.*, 1994). Accordingly, methiothepin is more potent than metergoline because the former produces blockade of postsynaptic α₁-adrenoceptors. Thus, the higher antagonist potency of methiothepin should not exclude the participation of 5-HT_{1D} receptors.

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