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Pharmacological profile of the receptors that mediate external carotid vasoconstriction by 5-HT in vagosympathectomized dogs

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1 5-Hydroxytryptamine (5-HT) can produce vasodilatation or vasoconstriction of the canine external carotid bed depending upon the degree of carotid sympathetic tone. Hence, external carotid vasodilatation to 5-HT in dogs with intact sympathetic tone is primarily mediated by prejunctional 5-HT_1-like receptors similar to the 5-HT_{1D} subtype, which inhibit the carotid sympathetic outflow. The present investigation is devoted to the pharmacological analysis of the receptors mediating external carotid vasoconstriction by 5-HT in vagosympathectomized dogs.

2 Intracarotid (i.c.) infusions for 1 min of 5-HT (0.3, 1, 3, 10, 30 and 100 μg) resulted in dose-dependent decreases in both external carotid blood flow and the corresponding conductance; both mean arterial blood pressure and heart rate remained unchanged during the infusions of 5-HT. These responses to 5-HT were resistant to blockade by antagonists at 5-HT_2 (ritanserin) and 5-HT_3/5-HT_4 (tropisetron) receptors, but were partly blocked by the 5-HT_1-like and 5-HT_2 receptor antagonist, methiothepin (0.3 mg kg^{-1}); higher doses of methiothepin (1 and 3 mg kg^{-1}) caused little, if any, further blockade. These methiothepin (3 mg kg^{-1})-resistant responses to 5-HT were not significantly antagonized by MDL 72222 (0.3 mg kg^{-1}) or tropisetron (3 mg kg^{-1}).

3 The external carotid vasoconstrictor effects of 5-HT were mimicked by the selective 5-HT_1-like receptor agonist, sumatriptan (3, 10, 30 and 100 μg during 1 min, i.c.), which produced dose-dependent decreases in external carotid blood flow and the corresponding conductance; these effects of sumatriptan were dose-dependently antagonized by methiothepin (0.3, 1 and 3 mg kg^{-1}), but not by 5-HT_{1D}-like receptor blocking doses of metergoline (0.1 mg kg^{-1}).

4 The above vasoconstrictor effects of 5-HT remained unaltered after administration of phentolamine, propranolol, atropine, hexamethonium, brompheniramine, cimetidine and haloperidol, thus excluding the involvement of α- and β-adrenoceptors, muscarinic, nicotinic, histamine and dopamine receptors. Likewise, inhibition of either 5-HT-uptake (with fluoxetine) or cyclo-oxygenase (with indomethacin), depletion of biogenic amines (with reserpine) or blockade of calcium channels (with verapamil) did not modify the effects of 5-HT.

5 Taken together, the above results support our contention that the external carotid vasoconstrictor responses to 5-HT in vagosympathectomized dogs are mainly mediated by activation of sumatriptan-sensitive 5-HT_1-like receptors. It must be emphasized, notwithstanding, that other mechanisms of 5-HT, including an interaction with a novel 5-HT receptor (sub)type and/or an indirect action that may lead to the release of a known (or even unknown) neurotransmitter substance cannot be categorically excluded.

Keywords: Carotid blood flow; 5-hydroxytryptamine; 5-HT_1-like receptors; sumatriptan; vasoconstriction

Introduction

5-Hydroxytryptamine (5-HT) can produce vasodilatation or vasoconstriction of the canine external carotid bed depending upon the degree of carotid sympathetic tone (Saxena, 1972; Mena & Vidrio, 1979). Hence, we have shown that 5-HT-induced external carotid vasodilatation in dogs with intact sympathetic tone is primarily mediated by an inhibitory action on carotid sympathetic nerves, via the stimulation of prejunctional 5-HT_1-like receptors (Villalón *et al.*, 1993a, b; Terrón *et al.*, 1994). These prejunctional receptors are similar to the 5-HT_{1D} receptor subtype, on the basis of the rank order of agonist potency of 5-carboxamidotryptamine (5-CT) > > 5-HT > 5-methoxytryptamine ≥ sumatriptan, and the highly specific antagonist potency of metergoline (Villalón & Terrón, 1994a).

Interestingly, sumatriptan can also activate functional 5-HT_1-like receptors unrelated to the 5-HT_{1D} (and/or 5-HT_{1F}?) subtype, for instance, the increase in resistance within the canine carotid circulation (Perren *et al.*, 1991) and constriction of the porcine carotid arteriovenous anastomoses (Den Boer *et al.*, 1992). These findings have undoubtedly shed light on the mechanisms involved in the carotid vasoconstrictor effects of sumatriptan; nevertheless, the receptor mechanisms involved in the vasoconstrictor effects of 5-HT have not been completely characterized (see for example Saxena, 1972; Mena & Vidrio, 1979; Villalón & Terrón 1994a).

Hence, this study has investigated the pharmacological profile of the receptors and/or mechanisms involved in the external carotid vasoconstrictor effects of 5-HT in vagosympathectomized dogs; thus, we analysed the effects of the endogenous ligand, 5-HT, in conjunction with those of the 5-HT_1-like receptor agonist, sumatriptan (Humphrey *et al.*, 1988; Feniuk *et al.*, 1989). After use of a wide variety of antagonists and/or blockers, the results reveal the involvement of sumatriptan-sensitive 5-HT_1-like receptors; however, other mechanisms of 5-HT, including an interaction with a novel 5-HT receptor (sub)type and/or an indirect action that may lead to the release of a known (or even unknown) neurotransmitter

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substance cannot be dismissed. Preliminary results of this investigation have been communicated to the British Pharmacological Society (Villalón & Terrón, 1994b).

Methods

General

Experiments were carried out in a total of 53 dogs (18–22 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 strokes min^{-1} and a stroke volume of 13–16 ml kg^{-1}, which was adjusted to maintain arterial pH within normal limits. Catheters were placed in the inferior vena cava via a femoral vein for the administration of antagonist 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: MAP = DAP + (SAP-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 and the corresponding internal carotid and occipital arteries were ligated; then, an ultrasonic flow probe (4 mm R-Series) connected to an ultrasonic T201D flowmeter (both from Transonic Systems Inc., Ithaca, NY, U.S.A.) was placed around the right common carotid artery, and the flow through this artery was considered to represent the blood flow through the external carotid artery (for detailed considerations see Villalón *et al.*, 1993a,b). 5-HT or sumatriptan 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; the changes produced in external carotid conductance were determined from the resting value immediately prior to each dose of agonist. 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.). In 6 animals chosen at random, an additional ultrasonic flow probe was placed in the contralateral (left) common carotid artery to determine if the changes produced by 5-HT or sumatriptan on the ipsilateral external carotid blood flow were influenced by changes in the corresponding contralateral side. The body temperature of the animals was maintained between 37–38°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, external carotid blood flow and external carotid conductance were determined. At this point, consecutive i.c. infusions (for 1 min) of 5-HT (0.3, 1, 3, 10, 30 and 100 μg min^{-1}) were given; subsequently, antagonists were administered over a period of 1 min. About 10 min later, the responses to the six doses of 5-HT were elicited again.

In initial experiments it was noticed that, with the exception of methiothepin, the antagonists used (in doses sufficient for the purpose for which they were employed) did not modify the effects of 5-HT. Therefore, in order to restrict the number of animals to be used for this investigation, the responses to 5-HT were studied before and after methiothepin (0.3, 1 and 3 mg kg^{-1}) in a group of 6 animals (pretreated with 5 mg kg^{-1} i.v. of chlorisondamine) and, for the remainder antagonists, we used more than one (but no more than three) of such drugs rather then several doses of a particular drug, in any single experiment ($n=25$), varying the order of their use. In another 3 animals, the reproducibility of 5-HT-induced decreases in external carotid blood flow and conductance were checked by analyzing the responses to 5-HT before and after three consecutive doses of physiological saline (0.015, 0.05 and 0.15 ml kg^{-1}, i.v.).

Furthermore, in a group of 4 animals pretreated with chlorisondamine (5 mg kg^{-1}, i.v.) and methiothepin (3 mg kg^{-1}, i.v.), the responses to 5-HT (3–100 μg min^{-1}, i.c.) were elicited before and after MDL 72222 (0.3 mg kg^{-1}, i.v.) and tropisetron (3 mg kg^{-1}, i.v.).

In another set of experiments, the effect of consecutive i.c. infusions (for 1 min) of sumatriptan (3, 10, 30 and 100 μg min^{-1}) was analyzed before and after i.v. administration of either physiological saline (0.15 ml kg^{-1}; $n=3$), metergoline (0.1 mg kg^{-1}; $n=3$) or methiothepin (0.3, 1 and 3 mg kg^{-1}; $n=3$).

Two additional groups of animals ($n=3$ each) were pretreated intraperitoneally (i.p.) with either reserpine (1 mg kg^{-1}) or the corresponding volume of physiological saline (0.15 ml kg^{-1}); 24 h later, the animals underwent the experimental procedures previously described. 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. Subsequently, the responses produced by consecutive i.c. infusions (during 1 min) of 5-HT (0.3, 1, 3, 10, 30 and 100 μg min^{-1}) were elicited in both groups of animals.

Each dose of 5-HT or sumatriptan was in a solution which was administered at a rate of 1 ml min^{-1} for a period of 1 min. These doses of 5-HT and sumatriptan were selected on the basis of results obtained from previous experiments, in which reproducible decreases in external carotid blood flow and conductance were elicited with no changes in arterial blood pressure or heart rate (Villalón *et al.*, 1993a; Villalón & Terrón, 1994a). 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 5-HT and sumatriptan ranged between 5 and 45 min, as in each case we waited until the external carotid blood flow had returned completely to baseline values; for the antagonists, a period of 10 min was allowed to elapse before the dose-response curves to the agonists were elicited again. The dosing with all drugs used was sequential.

Data presentation and statistical analysis

All data in the text, figures and tables, unless otherwise stated, are presented as mean ± s.e.mean. The peak changes in external carotid vascular conductance produced by 5-HT and sumatriptan (as percentage change) before and after a dose of a particular antagonist were compared by: (i) use of Dunnet's t test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980); and (ii) calculation of the agonist dose-ratios, comparing the ED_{50} values (dose of agonist required to produce a 50% maximum decrease in vascular conductance) in the absence and presence of antagonist (Tallarida & Murray, 1981). Where appropriate, Student's unpaired t test was applied. Differences were considered significant when the P value was 0.05 or less (two-tailed).

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, propranolol hydrochloride, verapamil hydrochloride and cimetidine (Sigma Chemical Company, St. Louis, MO, U.S.A.); sumatriptan succinate (gift: Dr P.P.A. Humprey, Glaxo group research, Ware, U.K.); metergoline (gift: Farmitalia, Milan, Italy); ritanserin and haloperidol (gift: Dr J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium); methiothepin mal-

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 (0.15 $ml\,kg^{-1}$), methiothepin (3 $mg\,kg^{-1}$), metergoline (0.1 $mg\,kg^{-1}$), ritanserin (0.3 $mg\,kg^{-1}$) or tropisetron (3 $mg\,kg^{-1}$)

		MAP		*HR*		*ECBF*[c]	
Treatment	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Saline	3	95±6	86±6	126±22	127±21	136±23	133±27
Methiothepin[a,b]	6	95±10	97±9	125±14	124±14	85±10	94±13
Metergoline	3	117±12	122±9	161±6	146±8	158±36	148±21
Ritanserin	3	117±12	122±9	161±6	146±8	158±36	148±21
Tropisetron	3	126±6	132±8	155±9	134±7	200±35	175±28

Note that the values of MAP, HR and ECBF obtained before and after treatment with any of the antagonists used were not significantly different ($P<0.05$).
[a]Animals were pretreated with the ganglion blocker, chlorisondamine (5 $mg\,kg^{-1}$, i.v.), in order to avoid methiothepin-induced decreases in MAP, ECBF and conductance, as previously reported (Perren *et al.*, 1991; Villalón *et al.*, 1993a,b).
[b]The lower doses of methiothepin (0.3 and 1 $mg\,kg^{-1}$) were similarly without significant effects.
[c]The corresponding values of conductance before and after treatment were not significantly different ($P>0.05$), but are not shown for the sake of clarity.

eate (gift: Hoffman-La Roche Ltd., Basel, Switzerland); indomethacin, reserpine, phentolamine mesylate, atropine sulphate, MDL 72222 (1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate) and hexamethonium dichloride (Research Biomedical Inc., Natick, MA, U.S.A.); tropisetron (gift: Sandoz A.G., Basel, Switzerland); brompheniramine maleate and chlorisondamine hydrochloride (gift: Instituto Miles de Terapéutica Experimental, Mexico City, Mexico); and fluoxetine hydrochloride (gift: Eli Lilly & Company, IN, U.S.A.). All compounds were dissolved in physiological saline; when needed, either 5% propylene glycol (methiothepin), 1% ascorbic acid (ritanserin, metergoline and indomethacin) or 5% dimethyl sulphoxide (cimetidine and reserpine) was added. The vehicles had no effect on either basal mean blood pressure, heart rate, external carotid blood flow or conductance. The doses of 5-HT and sumatriptan were given as free base.

Results

Systemic haemodynamic variables

I.c. infusions of 5-HT were given in a total of 44 dogs where the baseline values of mean arterial blood pressure, heart rate, external carotid blood flow and external carotid conductance were, respectively, 142±5 mmHg, 173±9 beats min^{-1}, 123±22 $ml\,min^{-1}$ and 0.88±0.06 $ml\,min^{-1}$ mmHg. The specific values of these haemodynamic variables before and after the administration of the 5-HT receptor antagonists and other drugs (antagonists, blockers and/or inhibitors) are shown in Tables 1 and 2, respectively. At the doses used, the above haemodynamic parameters were not significantly ($P>0.05$) modified after administration of either 5-HT receptor antagonists (methiothepin, ritanserin or tropisetron; Table 1) or some other common drugs (atropine, haloperidol, brompheniramine, cimetidine, fluoxetine, verapamil or indomethacin; Table 2). In contrast, the administration of phentolamine, propranolol, chlorisondamine, hexamethonium or reserpine produced a significant decrease in one (phentolamine, propranolol and reserpine) or more (chlorisondamine and hexamethonium) of these haemodynamic variables (Table 2); irrespective of these changes, the corresponding external carotid conductance was not significantly modified before and after administration of any of the above antagonists (not shown). Moreover, blood pressure and heart rate were not modified by the i.c. infusions of 5-HT or sumatriptan before and after saline or the various antagonists (not shown, but baseline values are depicted in Tables 1 and 2).

Table 2 Mean arterial blood pressure (MAP; mmHg), heart rate (HR; beats min^{-1}) and external carotid blood flow (ECBF; $ml\,min^{-1}$) before and after i.v. administration of phentolamine (1 $mg\,kg^{-1}$), propranolol (1 $mg\,kg^{-1}$), chlorisondamine (5 $mg\,kg^{-1}$), hexamethonium (10 $mg\,kg^{-1}$), atropine (1 $mg\,kg^{-1}$), haloperidol (1 $mg\,kg^{-1}$), brompheniramine (0.5 $mg\,kg^{-1}$), cimetidine (1 $mg\,kg^{-1}$), reserpine (1 $mg\,kg^{-1}$), fluoxetine (1 $mg\,kg^{-1}$), verapamil (0.1 $mg\,kg^{-1}$) or indomethacin (5 $mg\,kg^{-1}$),

		MAP		*HR*		*ECBF*[a]	
Treatment	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Phentolamine	3	122±7	94±15*	138±26	145±33	211±77	145±43
Propranolol	3	107±17	95±13	135±7	107±1*	143±36	118±30
Chlorisondamine	6	135±11	79±11*	168±10	122±8*	183±32	94±22*
Hexamethonium	3	94±1	46±16*	99±17	78±17	176±32	84±20*
Atropine	3	94±7	87±5	127±21	128±22	135±24	134±28
Haloperidol	3	115±8	92±10	140±18	121±14	277±6	225±22
Brompheniramine	3	98±11	102±8	146±10	144±10	127±35	121±35
Cimetidine	3	105±26	113±26	120±18	119±18	179±38	180±38
Reserpine[b]	3	117±12	119±6	161±6	124±9*	158±36	113±42
Fluoxetine	3	118±4	127±7	143±12	142±12	223±26	183±22
Verapamil[c]	3	114±24	103±16	149±8	148±8	151±12	151±15
Indomethacin	3	171±3	171±4	142±25	136±25	126±21	116±19

*$P<0.05$, after vs before from the corresponding baseline value.
[a]The corresponding values of external carotid conductance before and after treatment with any of the above antagonists were not significantly different ($P>0.05$), but are not shown for the sake of clarity.
[b]The haemodynamic parameters were obtained from dogs pretreated with saline or reserpine.
[c]The dose of verapamil (0.1 $mg\,kg^{-1}$, i.v.) was followed by an infusion of 0.01 $mg\,kg^{-1}\,min^{-1}$.

Initial effects of 5-HT on the external carotid blood flow and the corresponding conductance in vagosympathectomized dogs

I.c. infusions of 5-HT elicited dose-dependent decreases in both external carotid blood flow (which had no effect in the contralateral common carotid blood flow; Figure 1) and the corresponding external carotid conductance (Figure 2); interestingly, the responses elicited by 30 and 100 μg min^{-1} of 5-HT showed a biphasic pattern (Figure 1). At the doses used, the duration of action of the responses elicited by 5-HT (to reach baseline values) was 1.3±0.2, 3±0.4, 5±0.3, 6±0.4, 8±0.8 and 10±0.8 min after 0.3, 1, 3, 10, 30 and 100 μg min^{-1} of 5-HT, respectively (n=44). In no case did 5-HT significantly change blood pressure or heart rate (not shown), implying a local vasoconstrictor effect on the external carotid bed.

Effect of physiological saline or some 5-HT receptor antagonists on the 5-HT-induced external carotid vasoconstriction

The effects of physiological saline and several 5-HT receptor antagonists on the external carotid vasoconstrictor responses to 5-HT are shown in Figure 2. The decreases in external carotid conductance (% change) produced by 5-HT, 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 2a). Similarly, the responses to all doses of 5-HT remained unaltered after ritanserin (0.3 mg kg^{-1}; Figure 2b) or tropisetron (3 mg kg^{-1}; Figure 2c) in doses that are high enough to antagonize their respective receptors (see Villalón *et al.*, 1991; 1993a). In contrast, the responses to 5-HT were partly blocked by 0.3 mg kg^{-1} of the 5-HT$_1$-like and 5-HT$_2$ receptor antagonist, methiothepin, with a dose-ratio of 2.9 (1.3–6.9, 95% confidence limits); as shown in Figure 2d, increasing the dose of methiothepin to 1 or 3 mg kg^{-1} caused, relatively little, if any, further blockade with dose-ratios of 3.1 (1.43–6.7) and 7.2 (3.2–16.5) respectively.

Interestingly, Figure 2e shows that, in dogs pretreated with methiothepin (3 mg kg^{-1}), the 5-HT-induced vasoconstrictor responses were not significantly antagonized by the subsequent i.v. administration of 0.3 mg kg^{-1} MDL 72222 or 3 mg kg^{-1} tropisetron (the corresponding dose-ratios did not significantly differ from 1; $P>0.05$).

Effect of physiological saline, metergoline or methiothepin on the sumatriptan-induced external carotid vasoconstriction in the dog

As previously observed with 5-HT, sumatriptan (3, 10, 30 and 100 μg during 1 min) also produced dose-dependent decreases in external carotid conductance (Figure 3), the pattern of which was monophasic, unlike that produced by higher doses of 5-HT (Figure 1).

The external carotid vasoconstrictor responses to sumatriptan remained unaffected after saline (Figure 3a) or metergoline (Figure 3b), but were dose-dependently antagonized

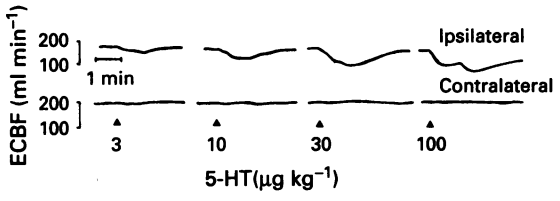

Figure 1 Experimental tracing illustrating the effects produced by i.c. infusions (during 1 min) of 5-HT on the ipsilateral (external) and contralateral (common) carotid blood flows of vagosympathectomized, anaesthetized dogs; in no case was heart rate or arterial blood pressure modified. Note that 5-HT produced transient decreases in external carotid blood flow which became biphasic at 30 and 100 μg min^{-1} of 5-HT (the effects produced by 0.3 and 1 μg min^{-1} of 5-HT are not shown for the sake of clarity).

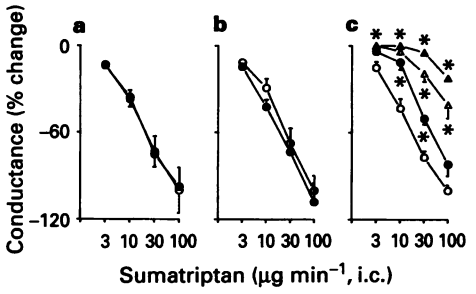

Figure 3 The effects of (a) physiological saline (○, 0 ml kg^{-1}; and ●, 0.15 ml kg^{-1}; n=3 each); (b) metergoline (○, 0 mg kg^{-1}; and ●, 0.1 mg kg^{-1}; n=3 each); and (c) methiothepin (○, 0 mg kg^{-1}; ●, 0.3 mg kg^{-1}; and ▲, 3 mg kg^{-1}; n=3 each) on the decreases in external carotid conductance (conductance: % change) induced by 1 min i.c. infusions of sumatriptan. *P<0.05 vs. control.

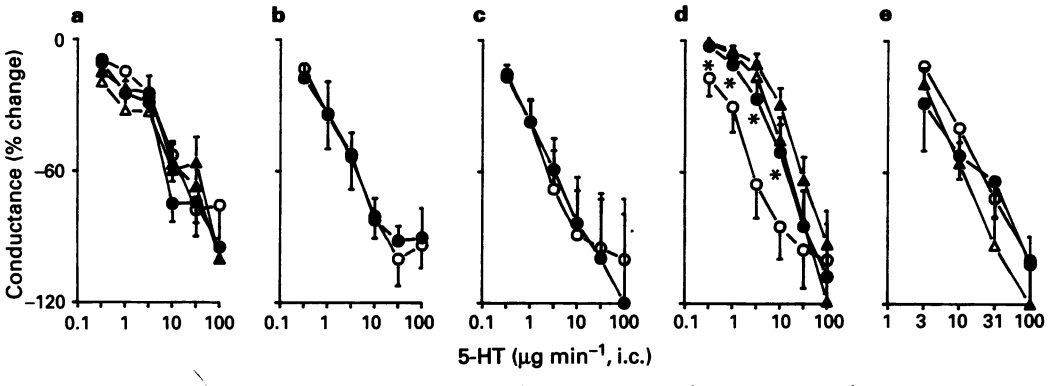

Figure 2 The effects of (a) physiological saline (○, 0 ml kg^{-1}; ●, 0.015 ml kg^{-1}; △, 0.05 ml kg^{-1}; and ▲, 0.15 ml kg^{-1}; n=3 each); (b) ritanserin (○, 0 mg kg^{-1}; ●, 0.3 mg kg^{-1}; n=3 each); (c) tropisetron (○, 0 μg kg^{-1}; ●, 3 mg kg^{-1}; n=3 each); and (d) methiothepin (○, 0 mg kg^{-1}; ●, 0.3 mg kg^{-1}; △, 1 mg kg^{-1}; and ▲, 3 mg kg^{-1}, n=6 each) on the decreases in external carotid conductance (conductance: % change) induced by 1 min i.c. infusions of 5-HT. (e) In dogs pretreated with 3 mg kg^{-1} of methiothepin (n=4), the 5-HT-induced decreases in external carotid conductance (○) were not significantly modified after MDL 72222 (●, 0.3 mg kg^{-1}) or tropisetron (△, 3 mg kg^{-1}). *P<0.05 vs. control.

by 0.3, 1 and 3 mg kg^{-1} of methiothepin (Figure 3c), with dose-ratios (95% confidence limits) of, respectively, 2.5 (1.5–4.3), 27.3 (6.9–69), and ∞ (∞-∞; as shown in Figure 3c, the slope was so low that it was impossible to calculate the corresponding dose-ratio). At the doses used, the duration of the vasoconstrictor responses to sumatriptan (7±0.7, 12±1, 23±2 and 42±7 min after 3, 10, 30 and 100 μg min^{-1}, respectively; $n=9$) was longer than that of 5-HT (see above).

Effect of some drugs (antagonists, blockers and/or inhibitors) on the 5-HT-induced external carotid vasoconstriction

In view of the differences observed in the pharmacological profile of the responses to 5-HT and sumatriptan, we decided to investigate the effect of some common drugs (antagonists, blockers and/or inhibitors) on the external carotid vasoconstrictor responses to 5-HT, in doses that are high enough to antagonize, block and/or inhibit their respective receptors and/or mechanisms (see Bom *et al.*, 1988). Thus, as shown in Table 3, none of the drugs used (phentolamine, propranolol, hexamethonium, atropine, haloperidol, brompheniramine, cimetidine, verapamil, reserpine, fluoxetine or indomethacin) significantly modified the external carotid vasoconstrictor responses to 5-HT (the respective dose-ratios did not significantly differ from 1; $P>0.05$).

In connection with the use of the above drugs, some findings deserve further comment. Thus, on the one hand, after administration of the 5-HT uptake blocker, fluoxetine, the duration of action of the responses to 5-HT (0.3, 1, 3, 10, 30 and 100 μg min^{-1}) was increased (*$P<0.05$) (1.6±0.5, 2.8±0.2, 3.8±0.2, 5.6±1.3, 6.7±0.9, 7.5±1.4 min before, and 3.7±0.2*, 4.5±0.3*, 6±0.6*, 7.2±0.2, 7.7±0.3 and 9±1 min after fluoxetine, respectively). On the other hand, although the adequacy of the dosing with reserpine to deplete monoamines (1 mg kg^{-1} 24 h before the experiment) has been verified elsewhere (Zaimis, 1964), in preliminary experiments we challenged this dosing, analyzing the effects of tyramine in control and reserpine-pretreated dogs; thus i.c. infusions of tyramine (10, 30, 100 and 300 μg min^{-1}) to control dogs resulted in dose-dependent decreases in external carotid conductance (% change) of, respectively, -5±1, -19±5, -44±5 and -82±5 ($n=3$); these responses to tyramine were markedly inhibited in reserpine-pretreated dogs (-5±2, -6±2, -8±1 and -17±1; unpublished).

Discussion

General

It is known that there are extensive communications between the cranial vascular beds in the dog (Jewell, 1952; de la Torre *et al.*, 1959); consequently, the circulatory changes ocurring in each vascular bed cannot be analyzed independently. Notwithstanding, our findings show that the effects of 5-HT, at the doses used, were localized to the ipsilateral side (Figure 1). With respect to the receptors and/or mechanisms involved in the external carotid vasoconstrictor responses to 5-HT, the present study suggests the involvement of sumatriptan-sensitive 5-HT_1-like receptors, although, as discussed below, additional mechanisms can also be involved.

Consideration of known 5-HT receptors

Since the external carotid vasoconstrictor effects of 5-HT were resistant to blockade by antagonists at 5-HT_2 (ritanserin) and 5-HT_3/5-HT_4 (tropisetron) receptors, mimicked by sumatriptan and antagonized by methiothepin, one could suggest that the 5-HT receptors involved belong to the 5-HT_1-like type, as proposed for the classification of functional 5-HT receptors (Saxena & Villalón, 1990; 1991; Hoyer *et al.*, 1994). The above suggestion gains weight when considering that the external carotid vasoconstrictor responses to both 5-HT and sumatriptan were antagonized by methiothepin (0.3 mg kg^{-1}) with similar dose-ratios (see Results section). However, it is important to note that methiothepin (1 and 3 mg kg^{-1}) was apparently weaker at blocking the effects of 5-HT (Figure 2d; the respective dose-ratios were little increased) at doses that markedly and dose-dependently blocked the effects of the more selective agonist, sumatriptan (Figure 3d; the corresponding dose-ratios were remarkably increased); this finding could suggest that, in the presence of methiothepin, 5-HT is activating a second receptor or mechanism; this implies that any indirect effect or action of 5-HT at other receptors may only become apparent after blockade of 5-HT_1-like and 5-HT_2 receptors and α_1-adrenoceptors, for which methiothepin displays high affinity (Leysen, 1985; Terrón *et al.*, 1994). Indeed, for this reason we analyzed the possible antagonist effects of MDL 72222 and tropisetron against the vasoconstriction to 5-HT in animals pretreated with methiothepin (see Figure 2e); the failure of these antagonists again confirms that 5-HT_3 and/or 5-HT_4 receptors are not involved, and thus, it is possible that a novel 5-HT receptor and/or indirect mechanism could be involved (see below). It is to be remarked that after methiothepin (3 mg kg^{-1}) the biphasic pattern of the response to 5-HT (30 and 100 μg min^{-1}) was changed into a monophasic pattern, and that even higher doses of methiothepin (10 mg kg^{-1}) failed to antagonize the vasoconstrictor responses to 30 and 100 μg min^{-1} of 5-HT (data not shown).

Nature of the 5-HT_1-like receptors mediating canine external carotid vasoconstriction

The 5-HT_1-like receptor is a group of receptors unrelated to the 5-HT_1-binding site subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}) identified in brain tissues (Humphrey *et al.*,

Table 3 Effective dose of 5-HT producing 50% of the maximum decrease in external carotid conductance (ED_{50}, μg min^{-1}), and dose-ratio (ED_{50} before and after antagonist) before and after i.v. administration of various antagonists

Antagonist	(mg kg^{-1})	n	ED_{50} *Before*	*After*	*Dose-ratio*[a]
Phentolamine	1.0	3	5.6	5.6	1.0
Propranolol	1.0	3	2.9	2.4	0.9
Hexamethonium	10.0	3	5.7	4.6	0.8
Atropine	1.0	3	3.8	3.2	0.8
Haloperidol	1.0	3	5.2	5.4	1.0
Brompheniramine	0.5	3	4.9	2.8	0.6
Cimetidine	1.0	3	5.2	3.9	0.8
Verapamil[b]	0.1	3	2.0	3.1	1.6
Reserpine[c]	1.0	3	2.9	3.6	1.3
Fluoxetine	1.0	3	4.9	2.4	0.5
Indomethacin	5.0	3	2.6	2.5	1.0

[a]All dose-ratio values did not differ significantly ($P>0.05$) from 1.
[b]The dose of verapamil (0.1 mg kg^{-1}, i.v.) was followed by an infusion of 0.01 mg kg^{-1} min^{-1}.
[c]The haemodynamic parameters were obtained from dogs pretreated with saline or reserpine.

1993; Hoyer *et al.*, 1994). Hence, the failure of metergoline to antagonize sumatriptan-induced external carotid vasoconstriction (Figure 3b) suggests the involvement of 5-HT_1-like receptors apparently unrelated to the 5-HT_{1A}, 5-HT_{1B} and/or 5-HT_{1D}-like subtypes, for which metergoline has very high affinity (Waeber *et al.*, 1988). This suggestion is reinforced when considering that the vasoconstrictor response to 5-HT was not modified by propranolol (Table 3) and (±)-pindolol (4 mg kg^{-1}; unpublished), in doses that are high enough to block functional 5-HT_{1A} and/or 5-HT_{1B} receptors (Saxena & Villalón, 1990). Furthermore, the dose of 0.1 mg kg^{-1} of metergoline is high enough to antagonize markedly the 5-HT_{1D}-like receptor-mediated external carotid vasodilatation in the dog (Villalón & Terrón, 1994a). Indeed, it could be argued that higher doses of metergoline could antagonize the sumatriptan-induced vasoconstrictor effect, but metergoline has moderate affinity ($K_i = 341 \pm 71$ nM), although lower than that of sumatriptan ($K_i = 23 \pm 11$ nM), for 5-HT_{1F} receptors (Adham *et al.*, 1993).

Thus, although the above view is in keeping with previous findings observed with sumatriptan in the canine carotid circulation (Feniuk *et al.*, 1989; Perren *et al.*, 1991), it must be stressed that the 5-HT_{1D}-like receptor is heterogeneous as at least two variants (5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$) have been described (Hoyer *et al.*, 1994) and that sumatriptan, metergoline or methiothepin cannot discriminate between 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (Kaumann *et al.*, 1993). Undoubtedly, the advent of a highly selective 5-HT_{1D} receptor antagonist will shed light on the possible involvement of 5-HT_{1D}-like receptors. Following a similar line of reasoning, since no selective 5-HT_{1F} receptor antagonist is available thus far (Hoyer *et al.*, 1994), neither can we categorically rule out the possible involvement, at least in part, of the 5-HT_{1F} subtype, particularly when the responses to 5-HT were mimicked by sumatriptan and blocked by high doses of methiothepin, a pharmacological feature of the 5-HT_{1F} receptor (see above; Adham *et al.*, 1993). It is worth noting that a dose of 0.03 mg kg^{-1} of methiothepin abolished the 5-HT_{1D} receptor-mediated external carotid vasodilatation in the dog (Villalón *et al.*, 1993a).

Consideration of other mechanisms

Apart from the possible involvement of 5-HT_{1D} and/or 5-HT_{1F} receptors, one could even suggest that a novel receptor type is involved. However, we have to admit that the mechanisms involved in the vasoconstrictor responses to 5-HT are complex and include, in addition to stimulation of 5-HT_1-like and/or 5-HT_{2A} receptors, indirect actions (see Martin, 1994). Thus, a 5-HT_{2A} receptor-stimulated release of catecholamines from the canine adrenal medulla (Feniuk *et al.*, 1981) can be excluded since ritanserin, phentolamine and propranolol failed to antagonize the effects of 5-HT. Likewise, the release of noradrenaline from sympathetic neurones either by a 5-HT_3 receptor-mediated depolarization (Fozard *et al.*, 1979) or by a tyramine-like action on these neurones (Humphrey *et al.*, 1983) is also ruled out based on the failure of MDL 72222, tropisetron, fluoxetine and reserpine (in addition to phentolamine and propranolol) to antagonize the responses to 5-HT; similarly, an endothelium-dependent vasoconstriction via the synthesis and release of pro-constrictor cyclo-oxygenase products (Rosenblum & Nelson, 1988; Seager *et al.*, 1992) seems unlikely based on the lack of effect of indomethacin.

Hence, although the possible involvement of an indirect mechanism cannot be excluded, it is significant that a range of other miscellaneous drugs failed to block the effect of 5-HT, including hexamethonium, phentolamine, propranolol, atropine, haloperidol, brompheniramine, cimetidine and verapamil at doses high enough to block their respective receptors (Bom *et al.*, 1988). Interestingly, the fact that the vasoconstrictor responses to 5-HT were slightly potentiated after hexamethonium (not shown) may suggest that additional sympathetic pathways innervating the external carotid bed are operative even after bilateral vagosympathectomy. Nonetheless, the possibility of an interaction of 5-HT with non-5 HT receptors still remains open.

Is a new type of 5-HT receptor partly involved in the external carotid vasoconstrictor responses to 5-HT?

The inability to find a specific mechanism responsible for the external carotid vasoconstrictor responses to 5-HT suggests that 5-HT may be taken up into some neurones to displace a neurotransmitter agent (other than those mentioned above: noradrenaline, acetylcholine, histamine, dopamine). If other endogenous substances (e.g. neuropeptide Y) are indeed displaced and released by 5-HT, these would involve an uptake process that is distinct from the one selectively inhibited (in the brain and blood platelets) by fluoxetine (Wong *et al.*, 1975; Lemberger *et al.*, 1978; Wright & Angus, 1989). Though such a possibility cannot be categorically dismissed, it could be similarly possible to suggest the involvement of a novel 5-HT receptor type, based on the failure of MDL 72222 and tropisetron to antagonize the vasoconstrictor responses to 5-HT in the presence of methiothepin (Figure 2e).

Lastly, it is important to emphasize that, although sumatriptan has high affinity for 5-HT_{1D} and 5-HT_{1F} receptor subtypes (Peroutka, 1990; Adham *et al.*, 1993), it is not yet clear if stimulation of these receptors is responsible for the beneficial effect of sumatriptan in migraine.

In conclusion, we suggest that sumatriptan-sensitive 5-HT_1-like receptors (5-HT_{1D}-like and/or 5-HT_{1F}?) are involved in 5-HT-induced canine external carotid vasoconstriction. Furthermore, it is plausible that (i) an interaction of 5-HT with a novel 5-HT receptor type may occur; and (ii) an indirect mechanism of 5-HT may lead to the release of a known (or even unknown) neurotransmitter substance (see Rand *et al.*, 1987).

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