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External carotid effects of 2-(2-aminoethyl)-quinoline (D-1997) in vagosympathectomized dogs

Carlos M. Villalón [a], Francisco J. López-Muñoz [a], Mercedes Perusquía [b], José A. Terrón [a,*]

[a] *Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apdo. Postal 22026, 14000 México, D.F., Mexico*

[b] *Instituto de Investigaciones Biomédicas, UNAM, Apdo. Postal 70-492, México, D.F., Mexico*

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Abstract

Serotonin (5-hydroxytryptamine; 5-HT) elicits external carotid vasoconstriction in vagosympathectomized dogs via 5-$HT_{1B/1D}$ receptors and a mechanism unrelated to the 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 types. In order to further explore the nature of this novel mechanism, the canine external carotid effects of 2-(2-aminoethyl)-quinoline (D-1997), a novel 5-HT_1 receptor agonist, were analyzed and compared with those of 5-HT and sumatriptan. Intracarotid (i.c.) infusions of 5-HT, D-1997 and sumatriptan to vagosympathectomized dogs dose-dependently decreased external carotid conductance, the rank order of agonist potency being 5-HT > sumatriptan > D-1997. The effects to D-1997 were resistant to intravenous (i.v.) pretreatment with 5-HT_2 and 5-HT_3/5-HT_4 receptor antagonists. Remarkably, the effects induced by lower (10–100 μg/min), but not higher (300–1000 μg/min), doses of D-1997 were blocked by high doses of methiothepin (1 and 3 mg/kg, i.v.), as previously shown with 5-HT. In addition, GR-127935 (1–10 μg/kg, i.v.), partially and dose-dependently antagonized D-1997-induced responses. However, the effects of D-1997 remained unaltered after blockade of α- and ß-adrenoceptors, muscarinic, nicotinic, histamine and dopamine receptors, or inhibition of 5-HT-uptake or cyclo-oxygenase, depletion of biogenic amines or blockade of Ca^{2+} channels. These results may support our previous contention that lower doses of 5-HT elicit external carotid vasoconstriction in vagosympathectomized dogs by activation of 5-$HT_{1B/1D}$ receptors, whilst higher doses of 5-HT stimulate a novel vasoconstrictor mechanism.

Keywords: Carotid blood flow; D-1997; 5-HT (5-hydroxytryptamine, serotonin); 5-$HT_{1B/1D}$ receptors; Sumatriptan; Vasoconstriction

1. Introduction

It has previously been reported that intracarotid (i.c.) infusions of 5-hydroxytryptamine (5-HT) to vagosympathectomized dogs induce dose-dependent external carotid vasoconstrictor responses involving activation of sumatriptan-sensitive 5-$HT_{1B/1D}$ receptors (Villalón et al., 1996) and a receptor unrelated to the 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 types (Villalón et al., 1995). Since the vasoconstrictor effects induced by the highest doses (30 and 100 μg/min, i.c.) of 5-HT were resistant to blockade by antagonists at the above 5-HT receptor types, the hypothesis alluding to the possible involvement of a novel 5-HT receptor (sub)type was put forward (Villalón et al., 1995). In addition, the 5-HT-induced vasoconstrictor effects remained unaltered after administration of multiple drugs inhibiting and/or blocking other common receptors and/or mechanisms (Villalón et al., 1995).

The possibility of identifying a drug tool that selectively stimulates the novel vasoconstrictor mechanism referred to above would undoubtedly shed light on the nature of the receptor involved. Therefore, we decided to evaluate the effects of the quinoline derivative, 2-(2-aminoethyl)-quinoline (D-1997), a novel agonist at the '5-HT_1-like' receptor mediating contraction in the canine basilar artery (Terrón et al., 1994a), in the external carotid bed of vagosympathectomized dogs. This latter '5-HT_1-like' receptor has indeed been suggested to be similar to that mediating vasoconstriction in the canine carotid circulation (Feniuk et al., 1989; Perren et al., 1991).

The results of the present study indicate that D-1997, like 5-HT, produces external carotid vasoconstriction via stimulation of 5-$HT_{1B/1D}$ receptors and a mechanism unre-

* Corresponding author. Tel.: +52-5-675 9341; Fax: +52-5-675 9168. Present address: Laboratory of Cerebovascular Research, Montréal Neurological Institute, Room 751, 3801 University Street, Montréal, Québec, Canada H3A 2B4.

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lated to the 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-ht_5, 5-ht_6 and 5-HT_7 receptors. These data apparently support our previous contention that a novel mechanism may be partly involved in the external carotid vasoconstrictor effects of 5-HT in vagosympathectomized dogs.

A preliminary account of this investigation was communicated to the 1994 meeting of the International Union of Pharmacology (Terrón et al., 1994b).

2. Methods

2.1. General

Experiments were carried out in a total of 33 dogs (body weight: 18–22 kg) not selected for breed or sex. The animals were anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.) and additional amounts (1 mg/kg, 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 and a stroke volume of 13–16 ml/kg, 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 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 each 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, Quincy, MA, USA) 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, Ithaca, NY) was placed around the right common carotid artery, and the flow through this artery was considered to represent the external carotid blood flow (for detailed considerations see Villalón et al., 1993a,b). Arterial blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument, Quincy, MA, USA). The vascular conductance in the external carotid bed was calculated by dividing the values of external carotid blood flow (ml/min) by the mean arterial blood pressure (mm Hg), assuming the venous pressure to be zero.

To analyze the effects of 5-HT, sumatriptan or D-1997 on external carotid blood flow, the agonists were administered into the carotid artery by a Harvard model 901 pump (Harvard Apparatus, Millis, MA, USA) with a cannula inserted into the right cranial thyroid artery. The body temperature of the animals was maintained between 37–38°C.

2.2. Experimental protocol

After the animals had been in a stable hemodynamic condition for at least 30 min, baseline values of blood pressure, heart rate, external carotid blood flow and conductance were determined. At this point, in order to compare the effects of D-1997 with those of 5-HT and sumatriptan, consecutive i.c. infusions (during 1 min) of 5-HT (0.3, 1, 3, 10, 30 and 100 μg/min), sumatriptan (1, 3, 10, 30 and 100 μg/min) and D-1997 (10, 30, 100, 300 and 1000 μg/min) were given to one group of animals in order to determine that D-1997, like 5-HT and sumatriptan, elicits dose-dependent vasoconstrictor responses. In other groups of dogs, the dose–response curves for D-1997 were obtained before and after the i.v. administration of one of the several antagonist drugs (for names, doses and number of experiments, see Sections 2.4 and 3) employed to analyze the effects of D-1997.

In initial experiments it was noticed that, with the exception of methiothepin and GR-127935, the other antagonists used (in doses sufficient for the purpose for which they were employed) did not modify the effects of D-1997. Therefore, in order to restrict the number of animals to be used, the responses to D-1997 were studied before and after methiothepin (0.3, 1 and 3 mg/kg) or GR-127935 (1, 3 and 10 μg/kg, i.v.) in two groups of 6 and 3 animals, respectively; for the remainder antagonists, we used more than one (but no more than three) of such drugs rather than several doses of a particular drug, in any single experiment, varying the order of their use. Furthermore, in 3 animals, the reproducibility of D-1997-induced decreases in external carotid conductance was checked by analyzing the responses to D-1997 before and after three consecutive doses of physiological saline (0.015, 0.05 and 0.15 ml/kg, i.v.).

Two additional groups of animals ($n = 3$ each) were pretreated intraperitoneally (i.p.) with either reserpine (1 mg/kg) or the corresponding volume of physiological saline (0.15 ml/kg); 24 h later, the animals underwent the experimental procedures previously described. After a stabilization period of at least 30 min, baseline values of blood pressure, heart rate and external carotid blood flow and conductance were determined. Subsequently, the responses produced by consecutive i.c. infusions (during 1 min) of D-1997 (10–1000 μg/min) were elicited in both groups of animals.

Each dose of 5-HT, sumatriptan or D-1997 was in a solution which was administered at a rate of 1 ml/min during a period of 1 min. The doses of D-1997, 5-HT and sumatriptan were selected on the basis of results obtained from previous experiments, in which reproducible and dose-dependent decreases in external carotid blood flow were elicited with no changes in arterial blood pressure or heart rate.

The interval between the different doses of agonist and/or antagonist drugs depended on the duration of the hemodynamic effect produced by the preceding dose. Thus, the dose-intervals of 5-HT, sumatriptan and D-1997 ranged between 5 and 45 min, as in each case we waited until the external carotid blood flow had returned to baseline values; for the antagonists, a period of 10 min was allowed to elapse before the dose–response curves for D-1997 were elicited again. The dosing with all drugs used was sequential.

2.3. Data presentation and statistical analysis

All data in the text, figures and tables, unless otherwise stated, are presented as mean ± S.E.M. The peak changes in external carotid conductance produced by D-1997 (as percentage change) before and after a dose of a particular antagonist were compared by: (i) Dunnet's *t*-test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980); and (ii) calculation of the agonist dose-ratios, comparing the ED_{50} values (dose of D-1997 required to produce 50% of the response obtained with the highest dose of D-1997 used) in the absence and the presence of antagonist (Tallarida and Murray, 1981). Where appropriate, paired or unpaired Student's *t*-test was applied. In all cases a P value of 0.05 or less (two-tailed) was considered statistically significant.

2.4. Drugs

The drugs used in the present study (obtained from the sources indicated) were the following: 5-hydroxytryptamine creatinine sulphate, propranolol hydrochloride, verapamil hydrochloride and cimetidine (Sigma, St. Louis, MO, USA); brompheniramine maleate and 2-(2-aminoethyl)-quinoline hydrochloride (D-1997) (gift: Instituto Miles de Terapéutica Experimental, Mexico City, Mexico); *N*-[4-Methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1, 2, 4-oxadiazol-3-yl)[1,1, -biphenyl]-4-carboxamide (GR-127935) and sumatriptan succinate (gift: Glaxo group research, Ware, UK); ritanserin and haloperidol (gift: Janssen Pharmaceutica, Beerse, Belgium); methiothepin maleate (gift: Hoffman-La Roche, Basel, Switzerland); 1α *H*,3α,5α *H*-tropan-3yl-3,5-dichlorobenzoate (MDL-72222), indomethacin, reserpine, phentolamine mesylate, atropine sulphate and hexamethonium dichloride (Research Biochemical International, Natick, MA, USA); tropisetron (gift: Sandoz, Basel, Switzerland); and fluoxetine hydrochloride (gift: Elli Lilly, Indianapolis, IN, USA).

All compounds were dissolved in physiological saline; when needed, either 5% propylene glycol (methiothepin), 1% ascorbic acid (ritanserin, metergoline) or sodium carbonate (indomethacin), or 5% (v/v) dimethyl sulfoxide (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, sumatriptan and D-1997 were given as the free base.

3. Results

3.1. Systemic hemodynamic variables

The baseline values of mean arterial blood pressure, heart rate, external carotid blood flow and conductance in the 33 dogs used in the present study were, respectively, 152 ± 6 mm Hg, 186 ± 5 beats/min, 128 ± 19 ml/min and 0.89 ± 0.05 ml/min mm Hg. The values for these variables before and after the administration of either the 5-HT receptor antagonists or some other commonly used drugs (antagonists, blockers and/or inhibitors) are depicted in Tables 1 and 2, respectively. At the doses used, the above hemodynamic variables were not significantly

Table 1
Mean arterial blood pressure (MAP; mm Hg), heart rate (HR; beats/min) and external carotid blood flow (ECBF; ml/min) before and after i.v. administration of saline (0.15 ml/kg), methiothepin (3 mg/kg), GR-127935 (10 μg/kg), metergoline (0.1 mg/kg), ritanserin (0.3 mg/kg) or tropisetron (3 mg/kg)

Treatment	*n*	MAP		HR		ECBF[d]	
		Before	After	Before	After	Before	After
Saline	3	143 ± 15	145 ± 16	165 ± 33	163 ± 34	170 ± 25	175 ± 28
Methiothepin[a,b]	6	94 ± 9	93 ± 8	115 ± 15	118 ± 14	91 ± 10	98 ± 12
GR-127935[c]	3	155 ± 1	156 ± 2	144 ± 13	145 ± 14	139 ± 38	137 ± 37
Metergoline	3	127 ± 10	132 ± 10	148 ± 7	139 ± 9	180 ± 32	166 ± 30
Ritanserin	3	156 ± 14	162 ± 10	162 ± 9	149 ± 11	145 ± 22	138 ± 21
Tropisetron	3	156 ± 9	165 ± 10	185 ± 9	164 ± 9	190 ± 28	167 ± 25

The values of MAP, HR and ECBF 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, 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) were similarly without significant effects.
[c]The lower doses of GR-127935 (1 and 3 μg/kg) were similarly without significant effects.
[d]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.

Table 2
Mean arterial blood pressure (MAP; mm Hg), heart rate (HR; beats/min) and external carotid blood flow (ECBF; ml/min) before and after i.v. administration of phentolamine (1 mg/kg), propranolol (1 mg/kg), hexamethonium (hexam; 10 mg/kg) plus atropine (atrop; 1 mg/kg), haloperidol (1 mg/kg), brompheniramine (Bromphen.; 0.5 mg/kg), cimetidine (1 mg/kg), reserpine (1 mg/kg), fluoxetine (1 mg/kg), verapamil (0.1 mg/kg) or indomethacin (5 mg/kg)

Treatment	*n*	MAP		HR		ECBF[a]	
		Before	After	Before	After	Before	After
Phentolamine	3	145 ± 7	110 ± 12[d]	148 ± 24	155 ± 24	198 ± 45	152 ± 29
Propranolol	3	115 ± 12	100 ± 13	138 ± 8	102 ± 2[d]	186 ± 31	165 ± 28
Hexam + atrop	3	98 ± 3	51 ± 9[d]	120 ± 15	90 ± 14	178 ± 28	91 ± 18[d]
Haloperidol	3	112 ± 8	95 ± 10	160 ± 17	142 ± 13	187 ± 9	145 ± 20
Bromphen.	3	112 ± 10	109 ± 8	156 ± 10	159 ± 10	168 ± 31	170 ± 22
Cimetidine	3	115 ± 18	123 ± 19	180 ± 12	178 ± 15	120 ± 21	126 ± 24
Reserpine[b]	3	126 ± 1	111 ± 9	155 ± 9	114 ± 11[d]	150 ± 36	79 ± 41
Fluoxetine	3	128 ± 6	135 ± 9	118 ± 12	124 ± 12	210 ± 25	218 ± 22
Verapamil[c]	3	125 ± 19	112 ± 9	161 ± 8	158 ± 10	132 ± 12	135 ± 12
Indomethacin	3	135 ± 4	136 ± 5	175 ± 22	176 ± 19	145 ± 20	138 ± 19

[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 variables were obtained from dogs pretreated with saline or reserpine.
[c] The dose of verapamil (0.1 mg/kg, i.v.) was followed by an infusion of 0.01 mg/kg min.
[d] $P < 0.05$, after vs. before from the corresponding baseline value.

($P > 0.05$) modified after administration of either 5-HT receptor antagonists (methiothepin, GR-127935, metergoline, ritanserin or tropisetron; Table 1) or some other common drugs (haloperidol, brompheniramine, cimetidine, fluoxetine, verapamil or indomethacin; Table 2). In contrast, the administration of phentolamine, propranolol, hexamethonium plus atropine or reserpine produced a significant decrease in one (phentolamine, propranolol and reserpine) or more (hexamethonium plus atropine) of these hemodynamic variables (Table 2); irrespective of these changes, the corresponding external carotid conductance remained unmodified (not shown). Moreover, neither was systemic blood pressure nor heart rate modified by i.c. infusions of D-1997 either before or after saline or the various other drugs (not shown, but baseline values are depicted in Tables 1 and 2).

3.2. Initial effects of D-1997 on the external carotid blood flow

As shown in Fig. 1, i.c. infusions of 5-HT, sumatriptan and D-1997 elicited dose-dependent decreases in external carotid blood flow, the rank order of agonist potency being 5-HT > sumatriptan > D-1997. Sumatriptan and D-1997 displayed higher efficacy with respect to 5-HT. Interestingly, unlike the biphasic effects elicited by high doses of 5-HT (30 and 100 μg/min, i.c.; Villalón et al., 1995), D-1997 and sumatriptan exhibited monophasic effects at all doses tested (not shown). Furthermore, the duration of action of the responses elicited by D-1997 (1.2 ± 0.1, 2.4 ± 0.1, 3 ± 0.2, 4.6 ± 0.2 and 5.8 ± 0.2 min after 10, 30, 100, 300 and 1000 μg/min, respectively; $n = 33$) was similar to that of 5-HT (1.6 ± 0.1, 3.5 ± 0.6, 5 ± 0.4, 6.2 ± 0.4, 8.3 ± 0.6 and 10.6 ± 0.9 min after 0.3, 1, 3, 10, 30 and 100 μg/min, respectively; $n = 6$), but shorter than that of sumatriptan (3.2 ± 0.2, 7 ± 0.5, 13.4 ± 2, 20 ± 3 and 45 ± 8 min after 1, 3, 10, 30 and 100 μg/min, respectively; $n = 6$). In no case did D-1997 significantly change blood pressure or heart rate implying a local vasoconstrictor effect.

3.3. Effect of saline or 5-HT receptor antagonists on D-1997-induced responses

The effects of physiological saline and several 5-HT receptor antagonists on the external carotid vasoconstrictor responses induced by D-1997 are depicted in Fig. 2. The decreases in external carotid conductance (% change) pro-

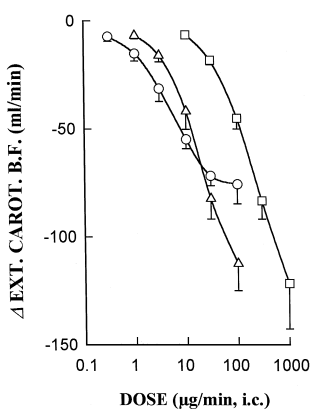

Fig. 1. Comparative effects of 1 min i.c. infusions of 5-HT (-○-, $n = 6$), sumatriptan (-△-, $n = 6$) and D-1997 (-□-, $n = 6$) on external carotid blood flow (EXT. CAROT. B.F.) in vagosympathectomized dogs. D-1997 and sumatriptan produced more pronounced vasoconstrictor effects than 5-HT. None of these agonists produced concomitant changes in mean arterial blood pressure or heart rate (see Section 3).

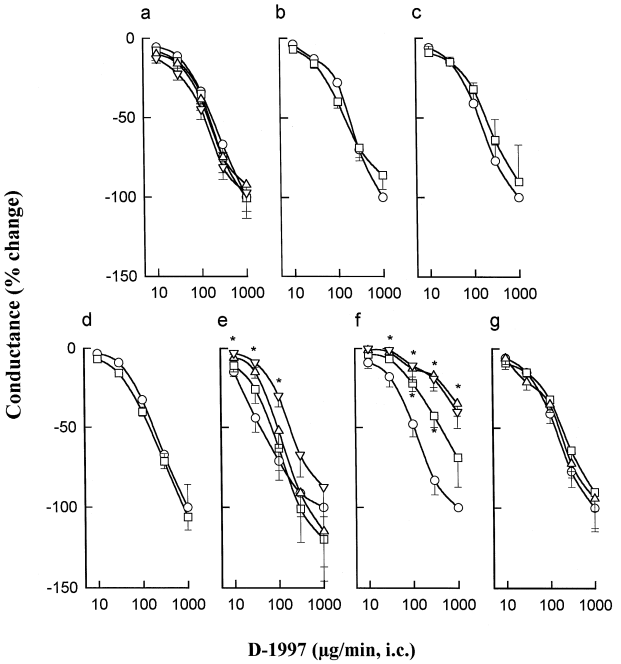

Fig. 2. The effects of (a) physiological saline (-○-, 0 ml/kg; -□-, 0.015 ml/kg; -△-, 0.05 ml/kg; and -▽-, 0.15 ml/kg; $n=3$ each); (b) ritanserin (-○-, 0 mg/kg; -□-, 0.3 mg/kg; $n=3$ each); (c) tropisetron (-○-, 0 mg/kg; -□-, 3 mg/kg; $n=3$ each); (d) metergoline (-○-, 0 mg/kg; -□-, 0.1 mg/kg; $n=3$ each); (e) methiothepin (-○-, 0 mg/kg; -□-, 0.3 mg/kg; -△-, 1 mg/kg; and -▽-, 3 mg/kg; $n=6$ each); and (f) GR-127935 (-○-, 0 μg/kg; -□-, 1 μg/kg; -△-, 3 μg/kg; and -▽-, 10 μg/kg; $n=3$ each) on the decreases in external carotid conductance (conductance; % change) induced by 1 min i.c. infusions of D-1997. (g) In dogs pretreated with 3 mg/kg of methiothepin ($n=4$), the D-1997-induced decreases in external carotid conductance (-○-) were not significantly modified after MDL-72222 (-□-, 0.3 mg/kg) or tropisetron (-△-, 3 mg/kg). *, $P<0.05$ vs. control.

duced by D-1997, 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; i.v.) of saline (Fig. 2a). Similar results were obtained in those animals treated with ritanserin (0.3 mg/kg; Fig. 2b), tropisetron (3 mg/kg; Fig. 2c) or metergoline (0.1 mg/kg; Fig. 2d), in doses that are high enough to antagonize functional responses mediated by their respective receptors (see Villalón et al., 1993a, 1995; Villalón and Terrón, 1994). In contrast, the responses to D-1997 were partly blocked by 0.3 mg/kg of methiothepin, with a dose-ratio of 2.1 (1.3–2.9, 95% confidence limits) which was significantly different ($P<0.05$) from that obtained in 0.015 ml/kg saline-treated dogs (0.9 [0.7–1.1]); as depicted in Fig. 2e, increasing the dose of methiothepin to 1 and even 3 mg/kg caused relatively little, if any, further blockade with dose-ratios of 3.5 (0.7–6.3) and 3.8 (1–6.6), respectively, as previously found with 5-HT (Villalón et al., 1995); these dose-ratios, however, did not differ significantly from the corresponding dose-ratios obtained in 0.05 (1.1 [0.7–1.5]) and 0.15 (0.9 [0.7–1.0]) ml/kg saline-treated animals. In support of the involvement of 5-$HT_{1B/1D}$ receptors in the vasoconstrictor responses to 5-HT and sumatriptan (Villalón et al., 1996), and D-1997 (present study), the potent and selective 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (1–10 μg/kg, i.v.), antagonized D-1997-induced responses in a dose-dependent manner, the maximum blockade being achieved with the dose of 3 μg/kg; increasing the dose of the antagonist up to 10 μg/kg caused no further rightward displacement of the dose–response curve to D-1997 (Fig. 2f). Unlike 5-HT (Villalón et al., 1996), D-1997 did not produce clear vasodilator responses after GR-127935 treatment.

Finally, Fig. 2f shows that the above pattern of responses was unchanged after i.v. administration of 0.3 mg/kg MDL 72222 and 3 mg/kg tropisetron in animals pretreated with 3 mg/kg methiothepin (the dose-ratios did not significantly differ from unity; $P>0.05$).

3.4. Effect of some common drugs (antagonists, blockers and/or inhibitors) on D-1997-induced responses

Since responses to D-1997, like those to 5-HT (Villalón et al., 1995), could not be completely antagonized by drugs blocking known 5-HT receptor (sub)types, the effects of some common drugs (antagonists, blockers and/or inhibitors) on D-1997-induced vasoconstrictor responses, in doses that are high enough to antagonize, block and/or inhibit their respective receptors and/or mechanisms (Bom et al., 1988) were investigated. Thus, as shown in Table 3, none of the drugs used (phentolamine, propranolol, hexamethonium plus atropine, haloperidol, brompheniramine, cimetidine, reserpine, fluoxetine, verapamil or indomethacin) significantly modified the effects to D-1997 (the

Table 3
Effective dose of D-1997 producing 50% of the decrease in external carotid conductance obtained with the highest dose of D-1997 used (ED_{50}, μg/min), and dose-ratio (ED_{50} before and after antagonist) before and after i.v. administration of various antagonists and inhibitors

Antagonist	(mg/kg)	n	ED_{50}		Dose-ratio[a]
			Before	After	
Phentolamine	1.0	3	187±45	168±55	0.8±0.1
Propranolol	1.0	3	131±30	76±30	0.6±0.2
Hexamethonium	10.0				
+ atropine	1.0	3	173±21	123±24	0.7±0.1
Haloperidol	1.0	3	256±16	217±21	0.9±0.1
brompheniramine	0.5	3	163±60	168±33	1.4±0.4
Cimetidine	1.0	3	179±15	154±14	0.9±0.1
Reserpine[b]	1.0	3	131±30	72±10	0.6±0.1
Fluoxetine	1.0	3	200±21	202±5	1.1±0.1
Verapamil[c]	0.1	3	148±38	182±23	1.3±0.2
Indomethacin	5.0	3	188±15	160±41	0.9±0.3

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

respective dose ratios did not significantly differ from unity; $P > 0.05$).

4. Discussion

4.1. General

The complex effects of 5-HT in the canine external carotid bed comprise both vasodilatation (Villalón et al., 1993a,b, 1997a; Villalón and Terrón, 1994) and vasoconstriction (Villalón et al., 1993a, 1995, 1996). The former effect can be observed either in dogs with intact vagosympathetic trunks or in vagosympathectomized animals pretreated with the potent and selective 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (Skingle et al., 1996), involving, respectively, sympatho-inhibitory prejunctional 5-$HT_{1B/1D}$ receptors (Villalón and Terrón, 1994) and vasodilator 5-HT_7 receptors (Villalón et al., 1997a). The vasoconstrictor effect, which is less well defined, involves, at least partly, activation of '5-HT_1-like' receptors (Villalón et al., 1995), similar to the 5-$HT_{1B/1D}$ subtype (Villalón et al., 1996), and a mechanism unrelated to 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors (Villalón et al., 1995). The possibility that a novel mechanism mediates canine external carotid vasoconstriction may be reinforced in the present study as the quinoline derivative with '5-HT_1-like' receptor agonist properties, D-1997 (Terrón et al., 1994a), closely matched the pharmacological effects of 5-HT.

4.2. Role of 5-HT_1 receptors in the external carotid effects of D-1997

D-1997 exhibits some pharmacological properties similar to those of sumatriptan. In this context, D-1997, like sumatriptan (Connor et al., 1989; Humphrey et al., 1988), behaved as an agonist at '5-HT_1-like' receptors mediating contraction in the canine isolated basilar artery (Terrón et al., 1994a) and saphenous vein (Terrón et al., unpublished). Accordingly, the responses to both agonists in the dog basilar artery were potently blocked by methiothepin, but were resistant to blockade by antagonists at 5-$HT_{1A/1B}$, 5-HT_2, 5-HT_3 and/or 5-HT_4 receptors (Connor et al., 1989; Terrón et al., 1994a). Given the previous suggestion that the canine carotid '5-HT_1-like' receptors are similar to those mediating contraction in the canine basilar artery and saphenous vein (Perren et al., 1991), it may be expected that the effects of D-1997 are mediated by '5-HT_1-like' receptors. In accordance with this hypothesis, the external carotid vasoconstrictor responses to D-1997 were resistant to blockade by antagonists at 5-HT_2 (ritanserin) and 5-HT_3/5-HT_4 (tropisetron) receptors, and partially antagonized by GR-127935 or methiothepin thereby suggesting that 5-$HT_{1B/1D}$ receptors are involved. In this connection, it is worth noting that D-1997-induced responses were antagonized by methiothepin (0.3 mg/kg) with a dose-ratio (2.1) that is similar to that obtained against 5-HT (2.9) and sumatriptan (2.5) (Villalón et al., 1995). Nevertheless, the responses to D-1997, like those of 5-HT (Villalón et al., 1995), were not further antagonized by 1 and 3 mg/kg of methiothepin (Fig. 2e; the corresponding dose-ratios were little increased and they were not significantly different from the corresponding dose-ratios obtained in saline-treated dogs; see Section 3). In marked contrast, these higher doses of methiothepin strongly and dose-dependently antagonized the external carotid vasoconstrictor responses induced by sumatriptan (Villalón et al., 1995), thus implying that methiothepin is more potent in blocking the effects of sumatriptan in comparison to those of D-1997 and 5-HT. This may support the possibility that an additional mechanism is involved in the effects of D-1997 and 5-HT. A similar profile was observed when comparing the effects of GR127935 against D-1997- and sumatriptan-induced responses; thus, GR127935 (1, 3 and 10 μg/kg) partially antagonized D-1997-induced effects but completely antagonized sumatriptan-induced responses (Villalón et al., 1996). This observation implicating a lower antagonist potency of GR127935 against D-1997 in comparison to sumatriptan, may also support the involvement of an additional mechanism in the vasoconstrictor effects of D-1997. These results also suggest that in the presence of methiothepin, 5-HT and D-1997 may activate a second receptor or mechanism; it is indeed for this reason that we evaluated the effects of MDL-72222 and tropisetron against the responses induced by D-1997 in animals pretreated with 3 mg/kg of methiothepin (Fig. 2g).

Taken in concert, these observations may support our previous contention that a novel 5-HT receptor may be involved in the external carotid vasoconstrictor responses induced by high doses of 5-HT in the vagosympathectomized dog (Villalón et al., 1995). If this were indeed the case, it would be tentative to speculate that D-1997, being less sensitive than sumatriptan to the blocking effects of GR-127935 and methiothepin, is a more selective agonist at such a novel vasoconstrictor mechanism. In this framework, we were prompted to think that D-1997, like 5-HT (Villalón et al., 1995), decreases the external carotid blood flow and conductance in vagosympathectomized dogs primarily by two mechanisms: i) a GR-127935- and methiothepin-sensitive component which is operative at lower doses of D-1997 and involves sumatriptan-sensitive 5-$HT_{1B/1D}$ receptors (Villalón et al., 1995, 1996); and ii) a methiothepin- and GR-127935-resistant component, which may involve a mechanism unrelated to 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-ht_5, 5-ht_6 and 5-HT_7 receptors. This latter component is predominantly stimulated by high doses (300 and 1000 μg/min) of D-1997. Interestingly, the ergot alkaloids, ergotamine and dihydroergotamine, have also been shown to reduce arteriovenous anastomotic blood

flow in anaesthetized pigs via stimulation of '5-HT_1-like' receptors and a novel methiothepin-resistant mechanism (Den Boer et al., 1991). Whether or not this latter mechanism is the same as that mediating canine external carotid vasoconstriction (Villalón et al., 1995; present study) remains to be determined.

4.3. Nature of the 5-HT_1 receptors mediating D-1997-induced responses

The functional '5-HT_1-like' receptors (Hoyer et al., 1994) can, at present, be reclassified into 5-$HT_{1B/1D}$ receptors (stimulated by 5-CT and sumatriptan; blocked by GR-127935, but not by mesulergine) and 5-HT_7 receptors (potently stimulated by 5-CT, but not by sumatriptan; blocked by mesulergine, but not by GR-127935) (see Saxena et al., 1998; Terrón, 1998; Villalón et al., 1997b). Although the failure of propranolol and metergoline to block D-1997-induced responses could suggest the involvement of '5-HT_1-like' receptors unrelated to the 5-HT_{1A} and 5-$HT_{1B/1D}$ subtypes, the potent antagonist effect by GR127935, at doses that antagonized the external carotid vasoconstrictor responses to 5-HT and sumatriptan (Villalón et al., 1996), clearly suggests that the '5-HT_1-like' receptor involved in the effects to D-1997 (and also to 5-HT and sumatriptan) belongs to the 5-$HT_{1B/1D}$ subtype.

4.4. Consideration of other mechanisms

Based upon the failure of 5-HT receptor antagonists, including methiothepin and GR-127935, to completely block the vasoconstrictor effects of D-1997, which is suggestive of involvement of a 5-HT receptor unrelated to the 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-ht_5, 5-ht_6 and 5-HT_7 types, we considered the potential participation of indirect mechanisms. In this context, a 5-HT_2 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 D-1997; this finding is consistent with previous results showing no activity of D-1997 at vascular 5-HT_2 receptors mediating contraction in the rat aorta (Terrón et al., 1994a). In addition, the release of noradrenaline from sympathetic neurons either by a 5-HT_3 receptor-mediated depolarization (Fozard et al., 1979) or by a tyramine-like action (Humphrey et al., 1983; Saxena and Villalón, 1990) can also be ruled out based on the failure of tropisetron, fluoxetine and reserpine (in addition to phentolamine and propranolol, see below) to antagonize the responses induced by D-1997. Similarly, an endothelium-dependent vasoconstriction via the synthesis and release of pro-constrictor cyclo-oxygenase products (Rosenblum and Nelson, 1988; Seager et al., 1992) seems unlikely since the responses produced by D-1997 remained unaltered after a high dose of indomethacin. In fact, the possible involvement of an indirect mechanism seems unlikely when considering the failure of several drugs to block the effect of D-1997, including: i) fluoxetine, a highly selective inhibitor of 5-HT uptake (Lemberger et al., 1978; Wong et al., 1975); ii) indomethacin, a cyclo-oxygenase inhibitor (Filep et al., 1991; Takahashi et al., 1991); and iii) reserpine, an agent producing depletion of monoamines, i.e., catecholamines and 5-HT, not only peripherally, but also in the central nervous system (Zaimis, 1964).

Despite the above findings, the possibility of an interaction of D-1997 (and 5-HT) with non-5-HT receptors cannot be categorically excluded. Nonetheless, the external carotid vasoconstrictor effects of D-1997 remained unaffected after complete blockade of either ganglia (with hexamethonium) or sympathetic (with phentolamine and propranolol) and parasympathetic (with atropine) tone; the use of these drugs precluded the involvement of nicotinic and muscarinic receptors, and α- and ß-adrenoceptors. The fact that the vasoconstrictor effects of D-1997 were unaffected by hexamethonium excludes the involvement of any prejunctional mechanism in the effects of D-1997; in agreement with this, it should be mentioned that D-1997, unlike 5-HT and sumatriptan (Villalón and Terrón, 1994), produced no vasodilator responses in dogs with intact sympathetic tone (Terrón et al., unpublished). Finally, D-1997-induced responses were not mediated by dopamine D_2, histamine H_1 and H_2 or Ca^{2+} channels, because the respective blockers—haloperidol, brompheniramine, cimetidine and verapamil—did not block such effects at doses high enough to block their respective mechanisms (Bom et al., 1988).

4.5. Possible support of a novel 5-HT receptor-mediated vasoconstrictor mechanism by D-1997

Taking into consideration our previous study in which we were unable to find a specific mechanism responsible for the external carotid vasoconstrictor responses induced by high doses of 5-HT in vagosympathectomized dogs (Villalón et al., 1995), the present results using the novel '5-HT_1-like' receptor agonist, D-1997 (Terrón et al., 1994a), may support our contention that a novel 5-HT receptor mediates external carotid vasoconstriction. In this connection, it may be highly significant that the pharmacology of 5-HT in the canine external carotid bed (Villalón et al., 1995) was closely mimicked by D-1997 (present study), i.e., their vasoconstrictor responses were resistant to blockade by a range of drugs blocking and/or inhibiting multiple receptor and/or mechanisms. In addition, it may be relevant that the responses induced by D-1997 were apparently less sensitive to methiothepin than those to 5-HT, i.e., the dose-ratios for D-1997 (present study) appeared to be lower than those obtained for 5-HT (see Villalón et al., 1995). Further, the responses to D-1997, unlike those to sumatriptan, were not completely antagonized by GR-127935, which implies that D-1997—but not

sumatriptan—activates a second mechanism unrelated to the 5-$HT_{1B/1D}$ subtype. Since no noticeable vasodilator responses to D-1997 were unmasked after GR-127935 (present results), which contrasts to the potent vasodilator responses to 5-HT under GR-127935 treatment (Villalón et al., 1996, 1997a), D-1997 may have weak or no agonist effects at carotid 5-HT_7 receptors mediating vasodilatation (Villalón et al., 1997b).

In conclusion, we suggest that D-1997, in addition to stimulating 5-$HT_{1B/1D}$ receptors, causes vasoconstriction by activating a novel receptor mechanism. It is to be recognized, however, that we suggest the involvement of a novel 5-HT receptor in the present study based upon the similar pharmacological effects of D-1997 and 5-HT, and that further experiments will be required to provide a direct evidence that high doses of D-1997 activate a 5-HT receptor unrelated to the currently known (sub)types. This will be possible when selective antagonists for recently cloned 5-HT receptors (e.g., 5-ht_{1E} and 5-ht_{1F}) become available. Since the effects of D-1997 closely paralleled those previously described for 5-HT in the dog external carotid bed (Villalón et al., 1995) and ergotamine and dihydroergotamine in the pig (Den Boer et al., 1991), it is likely that all these agonists produce carotid vasoconstriction via the same mechanisms and/or receptors. On this basis, it would be tentative to speculate that the novel mechanism mediating reduction in external carotid blood flow represents another target for novel antimigraine drugs.

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