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Mediation of 5-HT-induced external carotid vasodilatation in GR 127935-pretreated vagosympathectomized dogs by the putative 5-HT_7 receptor

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1 The vasodilator effects of 5-hydroxytryptamine (5-HT) in the external carotid bed of anaesthetized dogs with intact sympathetic tone are mediated by prejunctional sympatho-inhibitory 5-$HT_{1B/1D}$ receptors and postjunctional 5-HT receptors. The prejunctional vasodilator mechanism is abolished after vagosympathectomy which results in the reversal of the vasodilator effect to vasoconstriction. The blockade of this vasoconstrictor effect of 5-HT with the 5-$HT_{1B/1D}$ receptor antagonist, GR 127935, unmasks a dose-dependent vasodilator effect of 5-HT, but not of sumatriptan. Therefore, the present study set out to analyse the pharmacological profile of this postjunctional vasodilator 5-HT receptor in the external carotid bed of vagosympathectomized dogs pretreated with GR 127935 (20 μg kg^{-1}, i.v.).

2 One-minute intracarotid (i.c.) infusions of 5-HT (0.3–30 μg min^{-1}), 5-carboxamidotryptamine (5-CT; 0.01–0.3 μg min^{-1}), 5-methoxytryptamine (1–100 μg min^{-1}) and lisuride (3–1000 μg min^{-1}) resulted in dose-dependent increases in external carotid blood flow (without changes in blood pressure or heart rate) with a rank order of agonist potency of 5-CT > > 5-HT $\geqslant$ 5-methoxytryptamine > lisuride, whereas cisapride (100–1000 μg min^{-1}, i.c.) was practically inactive. Interestingly, lisuride (mean dose of 85 ± 7 μg kg^{-1}, i.c.), but not cisapride (mean dose of 67 ± 7 μg kg^{-1}, i.c.), specifically abolished the responses induced by 5-HT, 5-CT and 5-methoxytryptamine, suggesting that a common site of action may be involved. In contrast, 1 min i.c. infusions of 8-OH-DPAT (3–3000 μg min^{-1}) produced dose-dependent decreases, not increases, in external carotid blood flow and failed to antagonize (mean dose of 200 ± 33 μg kg^{-1}, i.c.) the agonist-induced vasodilator responses.

3 The external carotid vasodilator responses to 5-HT, 5-CT and 5-methoxytryptamine were not modified by intravenous (i.v.) pretreatment with either saline, (±)-pindolol (4 mg kg^{-1}) or ritanserin (100 μg kg^{-1}) plus granisetron (300 μg kg^{-1}), but were dose-dependently blocked by i.v. administration of methiothepin (10 and 30 μg kg^{-1}, given after ritanserin plus granisetron), mesulergine (10 and 30 μg kg^{-1}), metergoline (1 and 3 mg kg^{-1}), methysergide (1 and 3 mg kg^{-1}) or clozapine (0.3 and 1 mg kg^{-1}). Nevertheless, the blockade of the above responses, not significant after treatment with the lower of the two doses of metergoline and mesulergine, was nonspecific after administration of the higher of the two doses of methysergide and clozapine.

4 Based upon the above rank order of agonist potencies and the antagonism produced by a series of drugs showing high affinity for the cloned 5-ht_7 receptor, our results indicate that the 5-HT receptor mediating external carotid vasodilatation in GR 127935-pretreated vagosympathectomized dogs is operationally similar to the putative 5-HT_7 receptor mediating relaxation of vascular and non-vascular smooth muscles (e.g. rabbit femoral vein, canine coronary artery, rat systemic vasculature and guinea-pig ileum) as well as tachycardia in the cat.

Keywords: Carotid blood flow; GR 127935; 5-hydroxytryptamine; 5-HT_7 receptor; vagosympathectomized dog; vasodilatation

Introduction

5-Hydroxytryptamine (5-HT) can produce vasodilatation or vasoconstriction of the canine external carotid bed depending on the degree of carotid sympathetic tone (Terrón *et al*., 1994). Thus, the external carotid vasodilator effect of 5-HT in dogs with intact sympathetic tone is primarily mediated by prejunctional sympatho-inhibitory 5-$HT_{1B/1D}$ receptors (previously described as, respectively, the 5-$HT_{1D\beta/1D\alpha}$ subtypes of the 5-HT_{1D} receptor; Hartig *et al*., 1996), but a postjunctional vasodilator mechanism is also involved (Villalón & Terrón, 1994). Indeed, the existence of the latter mechanism has been recently confirmed (Villalón *et al*., 1996a) by the observation that blockade of the 5-HT receptors mediating vasoconstriction in vagosympathectomized dogs with the potent and selective 5-$HT_{1B/1D}$ receptor antagonist, GR 127935 (Clitherow *et al*., 1994; Skingle *et al*., 1996), not only abolished the vasoconstrictor responses induced by 5-HT and sumatriptan, but also unmasked a dose-dependent vasodilator effect in the case of 5-HT, but not sumatriptan (Villalón *et al*., 1996a).

In the light of these observations, the present study was designed to investigate the pharmacological properties of this postjunctional vasodilator mechanism. Hence the agonist and/or antagonist effects of several drugs acting at the different 5-HT receptor (sub)types were analysed in the external carotid bed of vagosympathectomized dogs pretreated with GR 127935. The drugs employed included antagonists at 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors, as well as some compounds with high affinity for 5-ht_6 and/or 5-HT_7 receptors (see Hoyer *et al*., 1994). The results show that the postjunctional 5-HT receptor mediating canine external carotid vasodilatation is operationally similar to the 5-HT_7 re-

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ceptor. A preliminary account of this study has been communicated to the British Pharmacological Society (Villalón *et al*., 1996b).

Methods

General

Experiments were carried out in a total of 36 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.) provided every 45 min to maintain the anaesthesia. After intubation of the trachea, the dogs were artificially respired with room air with 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 the normal limits (7.35–7.45). Catheters were placed in the femoral vein for the administration of antagonists and in the femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of blood pressure. After drug administration, the venous cannula was flushed with 3 ml of saline. Mean blood pressure (MAP) was calculated for 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. Following bilateral cervical vagosympathectomy an ultrasonic flow probe (4 mm R-Series) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, N.Y.) was placed around the right common carotid artery, and the flow through this artery was considered as the external carotid blood flow (see Villalón *et al*., 1993a,b). 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. Blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, U.S.A.).

Experimental protocol

After a stable haemodynamic condition for at least 30 min, baseline values of blood pressure, heart rate and external carotid blood flow were determined. Then, the dogs were given 20 μg kg^{-1} (i.v.) of GR 127935, which is two fold the dose sufficient to antagonize completely vasoconstrictor 5-HT$_{1B/1D}$ receptors in the canine external carotid bed (Villalón *et al*,. 1996a). Subsequently, the animals received consecutive i.c. infusions (1 min duration) of 5-HT (0.3, 1, 3, 10 and 30 μg), 5-CT (0.01, 0.03, 0.1 and 0.3 μg), 5-methoxytryptamine (1, 3, 10, 30 and 100 μg) and acetylcholine (0.01, 0.03, 0.1, 0.3 and, in some cases, 1 μg when the lowest doses were inactive); at this point, the dogs were divided into ten groups. In seven groups, the effects of the above agonists were induced after i.v. treatment with either: physiological saline (0.03 and 0.1 ml kg^{-1}; $n=4$), (±)-pindolol (4 mg kg^{-1}, $n=3$), ritanserin (100 μg kg^{-1}) plus granisetron (300 μg kg^{-1}) given together and then methiothepin (10 and 30 μg kg^{-1}, $n=3$), methysergide (1 and 3 mg kg^{-1}, $n=4$), metergoline (1 and 3 mg kg^{-1}, $n=4$), mesulergine (10 and 30 μg kg^{-1}, $n=4$) or clozapine (0.3 and 1 mg kg^{-1}, $n=4$). A period of 10 min was allowed to elapse before the dose-response curves for the agonists were elicited again. In the other three groups, the effects of 5-HT, 5-CT, 5-methoxytryptamine and acetylcholine were evaluated before and after consecutive i.c. infusions (1 min duration) of lisuride (3, 10, 30, 100, 300 and 1000 μg; mean cumulative dose of 85±7 μg kg^{-1}; $n=3$), cisapride (100, 300 and 1000 μg; mean cumulative dose of 67±7 μg kg^{-1}; $n=4$) or 8-OH-DPAT (3, 10, 100, 300, 1000 and 3000 μg; mean cumulative dose of 200±33 μg kg^{-1}; $n=3$). The dose-intervals between the different doses of agonists ranged between 5 and 20 min, as in each case we waited until the external carotid blood flow had returned completely to baseline values. The dosing with all drugs used was sequential.

Data presentation and statistical analysis

All data in the text and figures are presented as the mean± s.e.mean. The difference between the variables within one group of animals 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). 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 (obtained from the sources indicated) were the following: 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.); 5-methoxytryptamine hydrocholoride, (±)-8-hydroxy-dipropylaminotetraline hydrobromide (8-OH-DPAT), lisuride hydrogen maleate and acetylcholine chloride (Research Biochemicals Int., Natick, MA, U.S.A.); granisetron hydrochloride (gift: SmithKline Beecham Pharmaceuticals, Harlow, U.K.); methiothepin maleate (gift: Hoffman-La Roche Ltd., Basel, Switzerland); 5-carboxamidotryptamine maleate and 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 hydrochloride monohydrate (GR 127935) (gift: Glaxo Group Research, Ware, U.K.); metergoline (gift: Farmitalia, Milan, Italy); ritanserin and cisapride (gift: Janssen Pharmaceutica, Beerse, Belgium); and clozapine, (±)-pindolol, mesulergine hydrochloride and methysergide hydrogen maleate (gift: Sandoz A.G., Basel, Switzerland). All compounds were dissolved in distilled water. When needed, 4% (w/v) ascorbic acid (clozapine and metergoline) or 5% (v/v) DMSO (lisuride, (±)-pindolol and methiothepin) was added; these vehicles had no effect on the haemodynamic variables or the agonist-induced responses.

Results

Systemic haemodynamic variables

Baseline values of mean arterial blood pressure, heart rate and external carotid blood flow in the 36 vagosympathectomized dogs were 135±5 mmHg, 173±6 beats min^{-1} and 166±14 ml min^{-1}, respectively. These haemodynamic variables remained essentially unchanged after two i.v. bolus injections of physiological saline (Table 1), indicating that no time-dependent changes occurred during the experimental period (360–390 min) in the animal model used here. Table 1 also shows that all the above variables were not significantly changed ($P<0.05$) after administration of GR 127935, (±)-pindolol, methysergide, mesulergine, metergoline, clozapine or ritanserin plus granisetron followed by methiothepin, at all doses tested.

In contrast to previous results showing a vasoconstrictor effect of methysergide in the canine carotid circulation (Saxena, 1972; 1974; Villalón *et al*., 1996a), in the present experiments with GR 127935-pretreated dogs, methysergide produced no haemodynamic changes.

Initial effects of agonist drugs on external carotid blood flow

The onset of the responses induced by the agonists under study was immediate. Figure 1 shows that i.c. infusions of 5-CT, 5-HT, 5-methoxytryptamine and lisuride elicited dose-dependent

Table 1 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 various 5-HT receptor antagonists

		MAP		*HR*		*ECBF*	
Treatment	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
GR 127935	36	135±5	137±5	173±6	178±5	166±14	154±11
Saline	4	151±10	153±11	156±7	163±7	181±29	181±23
(±)-Pindolol	3	134±1	99±3	173±6	178±5	166±14	154±11
Rit.+Gran.	3	183±12	181±10	167±6	167±9	165±28	147±23
Methiothepin	3	155±5	142±4	175±3	170±8	138±9	132±15
Methysergide	4	152±19	122±27	154±11	130±14	143±23	98±24
Meterogoline	4	155±9	147±15	143±9	124±7	125±18	85±13
Mesulergine	4	125±14	123±14	146±16	140±18	190±30	188±28
Clozapine	4	110±5	110±15	113±3	103±1	163±17	138±16

The drugs used were: GR 127935 (20 μg kg^{-1}), saline (0.1 ml kg^{-1}), (±)-pindolol (4 mg kg^{-1}), ritanserin (Rit; 100 μg kg^{-1}) plus granisteron (Gran; 300 μg kg^{-1}), methiothepin (30 μg kg^{-1}), methysergide (3 mg kg^{-1}), metergoline (3 mg kg^{-1}), mesulergine (30 μg kg^{-1}) or clozapine (1 mg kg^{-1})
None of the above compounds produced significant haemodynamic changes ($P<0.05$). The lower doses of saline (0.03 ml kg^{-1}), methiothepin (10 μg kg^{-1}), methysergide (1 mg kg^{-1}), metergoline (1 mg kg^{-1}), mesulergine (10 μg kg^{-1}) or clozapine (0.3 mg kg^{-1}) were similarly without significant effects on MAP, HR or ECBF.

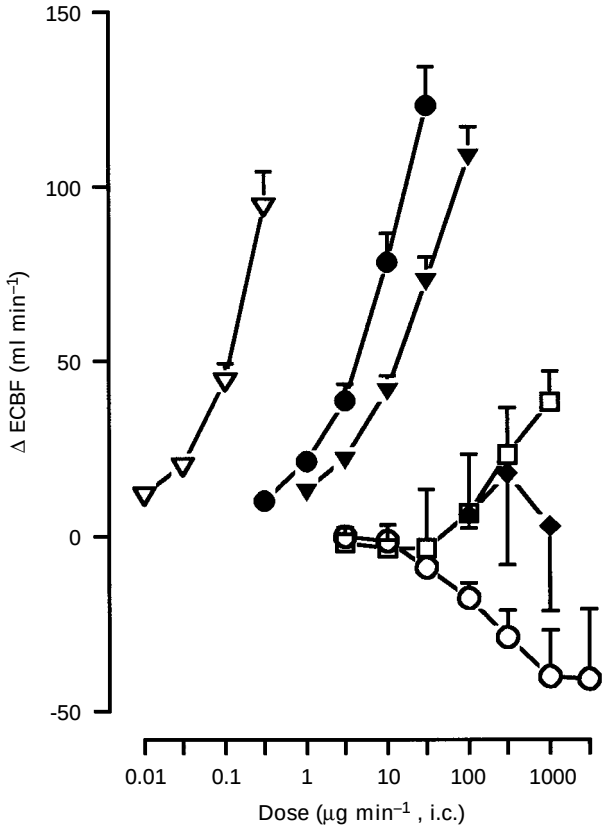

Figure 1 Comparative effects of 1 min intracarotid (i.c.) infusions of 5-CT (▽, $n=36$), 5-HT (●, $n=36$), 5-methoxytryptamine (▼, $n=36$), lisuride (□, $n=3$), cisapride (◆, $n=4$) and 8-OH-DPAT (○, $n=3$) on the external carotid blood flow (ECBF) in vagosympathectomized dogs pretreated with GR 127935 (20 μg kg^{-1}, i.v.). Vertical lines show s.e.mean.

increases in external carotid blood flow whereas cisapride produced no consistent effects; in constrast, i.c. infusions of 8-OH-DPAT produced dose-dependent decreases, not increases, in this variable. In no case did these agonists significantly change blood pressure or heart rate (not shown).

At the doses used, the vasodilator responses to acetylcholine lasted only during the period of infusion (1 min). In contrast, the duration of action of the vasodilator responses to 5-CT (1.2±0.1, 2.2±0.2, 3.9±0.4 and 6.2±0.5 min) was longer than that of 5-HT (0.9±0.08, 1.3±0.08, 1.7±0.1, 2.5±0.2 and 3.8±0.3 min), 5-methoxytryptamine (1.2±0.06, 1.3±0.06, 1.5±0.07, 1.9±0.09 and 3±1.4 min) or lisuride (0.6±0.2, 1.1±0.07, 1.3±0.2, 1.6±0.2, 1.8±0.2 and 2.3±0.2 min) with an apparent rank order of agonist potency of 5-CT>>5-HT⩾5-methoxytryptamine>lisuride. With the exception of lisuride, which produced a significantly lower maximum vasodilator effect than that of 5-HT, the above agonists displayed similar maximum effects (Figure 1); these latter effects, nevertheless, were dose-limited rather than effect-limited. In this respect, it should be noted that higher doses of these compounds produced marked changes in blood pressure and/or heart rate (not shown).

Effect of physiological saline and 5-HT receptor antagonists on the external carotid vasodilator responses induced by 5-HT, 5-CT, 5-methoxytryptamine and acetylcholine

The effects of saline on the responses induced by 5-HT, 5-CT, 5-methoxytryptamine and acetylcholine are depicted in Figure 2 (a); no evidence of tachyphylaxis was observed, with the responses to these agonists, at the doses and time intervals used here, were reproducible and remained essentially unchanged in control animals receiving 2 subsequent doses of saline (0.03 and 0.1 ml kg^{-1}, i.v.). Similar results were obtained after blockade of 5-HT$_{1A}$ receptors with (±)-pindolol (4 mg kg^{-1}, i.v.; Figure 2b) or 5-HT$_2$ and 5-HT$_3$ receptors with ritanserin (100 μg kg^{-1}, i.v.) plus granisetron (300 μg kg^{-1}, i.v.), respectively (Figure 3a). The subsequent administration of methiothepin (10 and 30 μg kg^{-1}, i.v.) to those animals previously given ritanserin plus granisetron potently and dose-dependently blocked the responses to 5-HT, 5-CT and 5-methoxytryptamine; this blockade was specific as the responses to acetylcholine were not significantly modified (Figure 3a). A similar pattern of blockade was observed after administration of methysergide (1 and 3 mg kg^{-1}, i.v.; Figure 3b); in this case, notwithstanding, 3 mg kg^{-1} of methysergide induced a significant attenuation of the vasodilator response to 0.3 μg min^{-1} acetylcholine (Figure 3b).

The responses to 5-HT, 5-CT and 5-methoxytryptamine could also be significantly antagonized by the higher of the two doses of metergoline (1 and 3 mg kg^{-1}; Figure 4a) and mesulergine (10 and 30 μg kg^{-1}; Figure 4b); the blocking effects of both antagonists, in contrast to methysergide, were specific as they did not modify the responses to acetylcholine (Figure 4, right panels). Interestingly, the atypical antipsychotic drug, clozapine (0.3 and 1 mg kg^{-1}, i.v.), which also has a relatively high affinity for 5-HT$_7$ receptors (Hoyer *et al*., 1994), produced, in general, a dose-dependent blockade of the responses

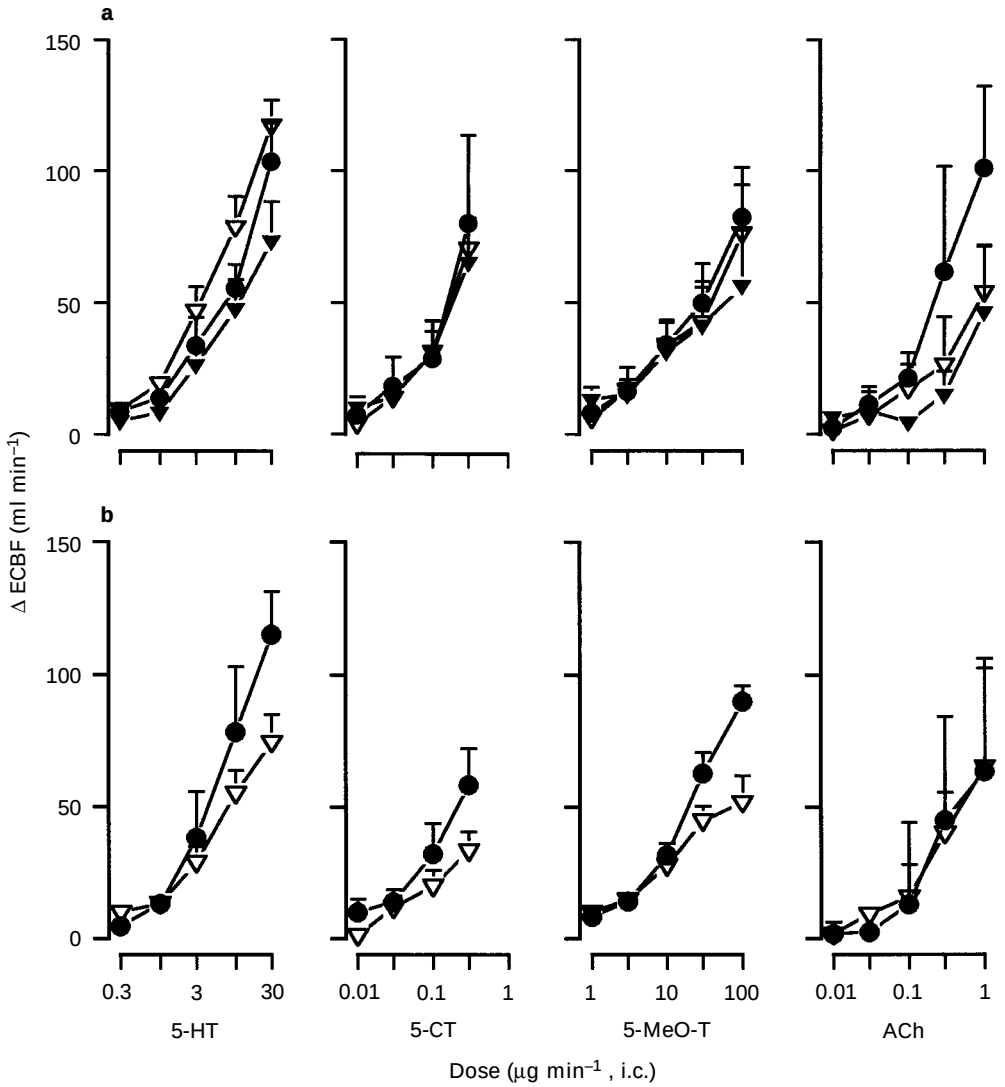

Figure 2 The effects of i.v. bolus injection of (a) physiological saline (●, 0 ml kg^{-1}; ▽, 0.03 ml kg^{-1} and ▼, 0.1 ml kg^{-1}; $n=4$) or (b) (±)-pindolol (●, 0 μg kg^{-1} and (▽) 4 mg kg^{-1}, $n=3$) on the increases in external carotid blood flow (ΔECBF) induced by 1 min i.c. infusions of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeO-T) and acetylcholine (ACh) in vagosympathectomized dogs pretreated with GR 127935 (20 μg kg^{-1}, i.v.). Vertical lines show s.e.mean.

to 5-HT, 5-CT and 5-methoxytryptamine (although 0.3 mg kg^{-1} clozapine did not significantly block the responses to 5-CT; Figure 5a). The blockade produced by 1 mg kg^{-1} clozapine was clearly nonspecific as the drug also blocked the responses to acetylcholine (Figure 5a).

In those experiments designed to search for either a partial agonist activity (lisuride and cisapride) or antagonism (8-OH-DPAT) of agonist-induced vasodilator effects, only lisuride (mean dose of 85±7 μg kg^{-1}, i.c.) abolished the external carotid vasodilator responses to 5-HT, 5-CT and 5-methoxytryptamine, but not those to acetylcholine (Figure 5b). These agonist-induced responses, in contrast, were not significantly affected by cisapride (mean dose of 67±7 μg kg^{-1}, i.c.) or 8-OH-DPAT (mean dose of 200±33 μg kg^{-1}, i.c.) (not shown).

It should be pointed out that after i.v. treatment of the animals with GR 127935, the duration of the experiments with saline (360–390 min) was: (i) similar to that of the experiments with ritanserin plus granisetron and subsequently with methiothepin (350–400 min); or (ii) longer than those with methysergide, metergoline, mesulergine or clozapine (310–340 min each) or with (±)-pindolol (230–250 min). The reason for this difference in the experimental periods is that after administration of most antagonists the duration of action of the responses to the 5-HT receptor agonists (to reach baseline values) was shorter, except after administration of (±)-pindolol or ritanserin plus granisetron given together, which did not block the responses to the agonists (see above).

Discussion

The major finding of the present study was that the 5-HT receptors producing external carotid vasodilatation in the presence of the 5-HT$_{1B/1D}$ receptor antagonist, GR 127935, were: (i) stimulated, in order of potency, by 5-CT, 5-HT, 5-methoxytryptamine and lisuride (Figure 1), but not by sumatriptan (Villalón *et al.*, 1996a); (ii) potently blocked by a series of drugs showing high affinity at cloned 5-ht$_7$ receptors, i.e. methiothepin (pK_D: 8.99), lisuride (pK_D: 9.28), mesulergine (pK_D: 8.15), clozapine (pK_D: 7.87) and methysergide (pK_D: 7.9), but less potently by metergoline (pK_D: 8.69); and (iii) resistant to blockade by compounds at doses that selectively interact with 5-HT$_{1A}$ [(±)-pindolol], 5-HT$_2$ (ritanserin), 5-HT$_3$ (granisetron) or 5-HT$_4$ (cisapride) receptors. Apart from the implications discussed below, these data indicate that the pharmacological profile of the canine external carotid vasodilator 5-HT receptors is similar to that obtained for the 5-HT$_7$ receptor (e.g. Hoyer *et al.*, 1994; Martin, 1994; Terrón, 1996).

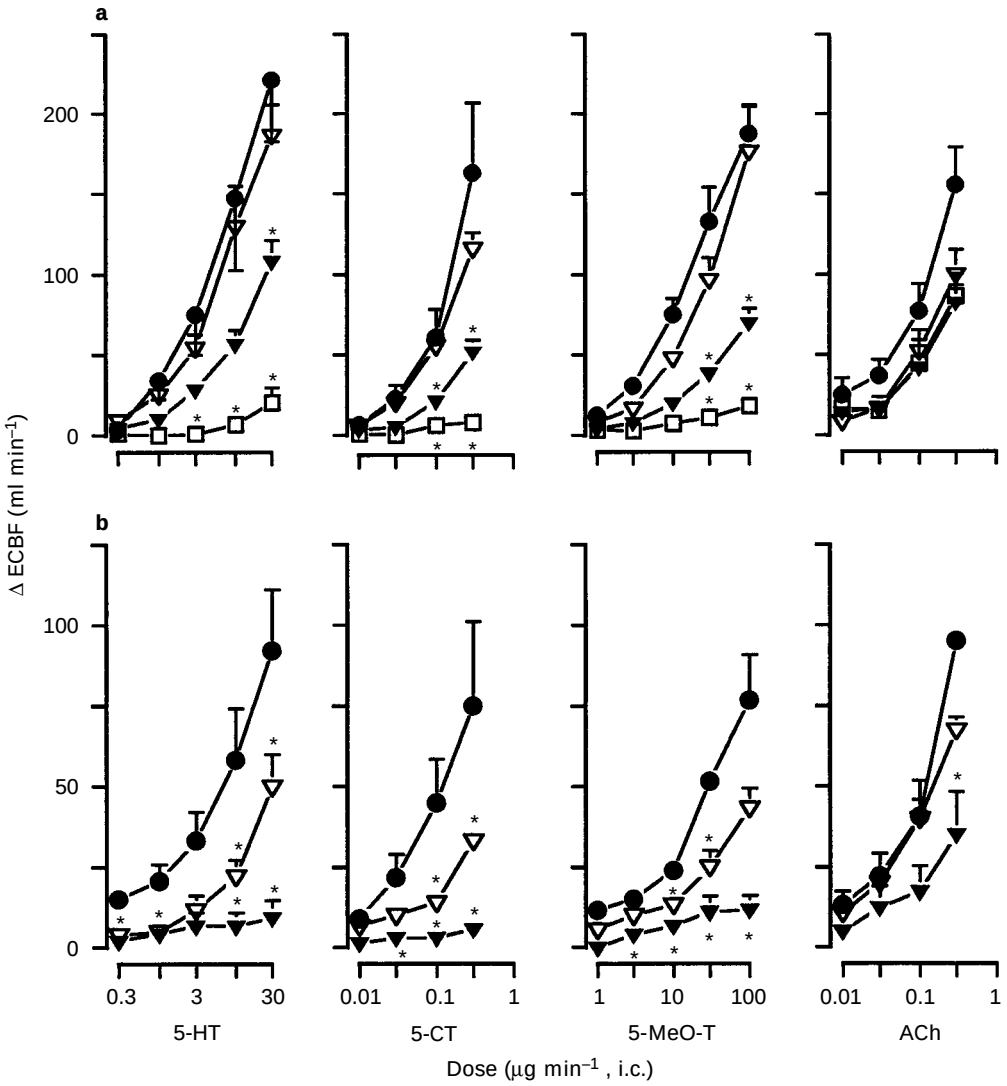

Figure 3 The effects of i.v. bolus injections of (a) ritanserin plus granisetron (●, 0 μg kg^{-1} of each; ▽, 100 μg kg^{-1} of ritanserin plus 300 μg kg^{-1} of granisetron; $n=3$) and the effect of subsequent i.v. bolus injections of methiothepin (▼, 10 μg kg^{-1}; and □, 30 μg kg^{-1}; $n=3$) or (b) methysergide (●, 0 mg kg^{-1}; ▽, 1 mg kg^{-1}; and ▼, 3 mg kg^{-1}; $n=4$) on the increases in external carotid blood flow (ΔECBF) induced by 1 min i.c. infusions of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeO-T) and acetylcholine (ACh) in vagosympathectomized dogs pretreated with GR 127935 (20 μg kg^{-1}, i.v.). Vertical lines show s.e.mean. *$P<0.05$ vs control.

Blockade of vasoconstrictor 5-HT$_{1B/1D}$ receptors: an experimental condition required for the study of the external carotid vasodilator 5-HT receptor in vagosympathectomized dogs

5-HT-induced external carotid vasoconstriction is primarily mediated by sumatriptan-sensitive 5-HT$_1$ receptors (Villalón *et al*., 1995) which are antagonized by GR 127935 (Villalón *et al*., 1996a); notably, not only did GR 127935 abolish the vasoconstrictor effects of 5-HT and sumatriptan, but also unmasked a dose-dependent vasodilator effect in the case of 5-HT, but not sumatriptan (Villalón *et al*., 1996a). Thus, our experimental design using vagosympathectomized dogs pretreated with GR 127935 was intended to exclude the stimulation of: (i) prejunctional sympatho-inhibitory 5-HT$_{1B/1D}$ receptors which produce external carotid vasodilatation (Villalón & Terrón, 1994); and (ii) postjunctional 5-HT$_{1B/1D}$ receptors which produce external carotid vasoconstriction (Villalón *et al*., 1996a). These conditions, consequently, 'unmask' vasodilator mechanisms suggested here to be mediated by 5-HT$_7$-like receptors. Whether these receptors are located in the vascular smooth muscle and/or the endothelium remains to be determined.

Rank order of potency of 5-HT receptor agonists on the canine external carotid blood flow

It should be stressed that the rank order of potency of 5-CT > > 5-HT ⩾ 5-methoxytryptamine observed here resembles that obtained for the sympatho-inhibitory 5-HT$_{1B/1D}$ receptors mediating external carotid vasodilatation in dogs with intact vagosympathetic trunks (Villalón & Terrón, 1994). However, in this case, sumatriptan behaved as an active agonist. Hence, the possible involvement of vasodilator 5-HT$_7$-like receptors in our study stems from two additional findings: (i) sumatriptan, an agonist at 5-HT$_{1B/1D/1\text{-like}}$ receptors (Hoyer *et al*., 1994), does not produce external carotid vasodilatation in GR 127935-pretreated vagosympathectomized dogs (Villalón *et al*., 1996a); and (ii) lisuride, which has a very high affinity for the cloned 5-ht$_7$ receptor (pK_i = 8.2 – 9.05; Ruat *et al*., 1993; Shen *et al*., 1993), mimicked 5-HT producing external carotid vasodilatation and specifically abolished the responses to 5-CT, 5-HT and 5-methoxytryptamine (Figure 5b), suggesting that a common site of action may be involved. Accordingly, the above rank order of agonist potency parallels that found at both the cloned 5-ht$_7$ receptor (e.g. Lovenberg *et al*., 1993) and the putative 5-HT$_7$ receptors mediating direct vasorelaxation

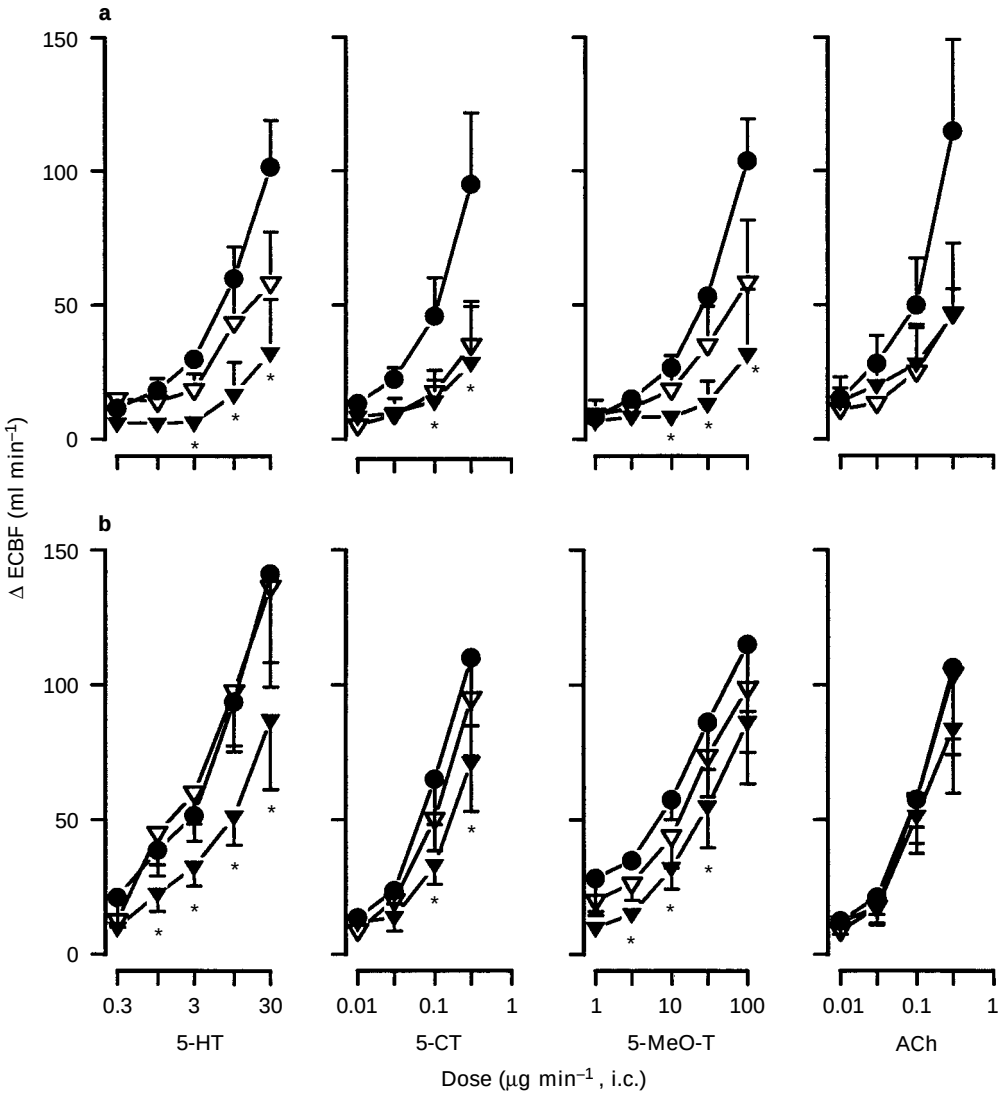

Figure 4 The effects of i.v. bolus injections of (a) metergoline (●, 0 μg kg^{-1}; ▽, 1 mg kg^{-1}; and ▼, 3 mg kg^{-1}; $n=4$) or (b) mesulergine (●, 0 μg kg^{-1}; ▽, 10 μg kg^{-1}; and ▼, 30 μg kg^{-1}; $n=4$) on the increases in external carotid blood flow (ΔECBF) induced by 1 min i.c. infusions of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeO-T) and acetylcholine (ACh) in vagosympathectomized dogs pretreated with GR 127935 (20 μg kg^{-1}, i.v.). Vertical lines show s.e.mean. *$P<0.05$ vs control.

(e.g. Martin & Wilson, 1995; Leung *et al*., 1996; Terrón, 1996), but differs from that obtained at cloned 5-ht$_6$ receptors (5-methoxytryptamine > 5-HT > 5-CT; Monsma *et al*., 1993).

Interestingly, 8-OH-DPAT, which is a partial agonist at cloned 5-ht$_7$ receptors (Lovenberg *et al*., 1993), produced vasoconstriction, not vasodilatation, in the dog external carotid bed. Conceivably, a vasodilator effect of this drug could have been masked by its vasoconstrictor activity mediated by α_1-adrenoceptors (Castillo *et al*., 1994; Terrón *et al*., 1996). Whatever the case, the lack of apparent agonist or antagonist activity of 8-OH-DPAT in our experiments is not an argument against the possible participation of a 5-HT$_7$-like receptor, as this drug exhibits moderate affinity for the mouse and rat (pK_i = 7.3 – 7.5; Plassat *et al*., 1993; Ruat *et al*., 1993; Shen *et al*., 1993), but not for the human (pK_i = 6.3; Bard *et al*., 1993) 5-ht$_7$ receptor clone.

Effects of 5-HT receptor antagonists on the agonist-induced vasodilator responses

The above contention supporting the role of 5-HT$_7$ receptors would be strengthened by the capability of some putative 5-HT$_7$ receptor antagonists to block the vasodilator responses to 5-HT, 5-CT and 5-methoxytryptamine, provided that other 5-HT receptors have been excluded. Thus, in principle, support for excluding the involvement of 5-HT$_1$ receptors, in addition to the inactivity of sumatriptan (Villalón *et al*., 1996a), comes from our findings showing that the above vasodilator responses are elicited in the presence of the 5-HT$_{1B/1D}$ receptor antagonist, GR 127935 (Clitherow *et al*., 1994; Skingle *et al*., 1996). Furthermore, the failure of (±)-pindolol, ritanserin and granisetron to antagonize the above responses at doses that block, respectively, 5-HT$_{1A}$, 5-HT$_2$ and 5-HT$_3$ receptors (Bom *et al*., 1989; Villalón *et al*., 1991; 1993a,b), excludes their involvement; similarly, the failure of cisapride to block (or to mimic) the vasodilator responses to the above agonists at doses that antagonize/stimulate the cardiac 5-HT$_4$ receptor in the pig (Villalón *et al*., 1991), precludes its participation.

On the basis of the above, the high blocking potency of methiothepin in our experiments suggests the involvement of a putative 5-HT$_7$ receptor as this drug: (i) displays a very high affinity for cloned 5-ht$_7$ receptors (pK_i = 9.42; Shen *et al*., 1993); and (ii) is a potent antagonist of other putative 5-HT$_7$ receptors mediating smooth muscle relaxation (e.g. Terrón, 1996). It should be noted that our experiments with methiothepin were carried out in animals previously given ritanserin

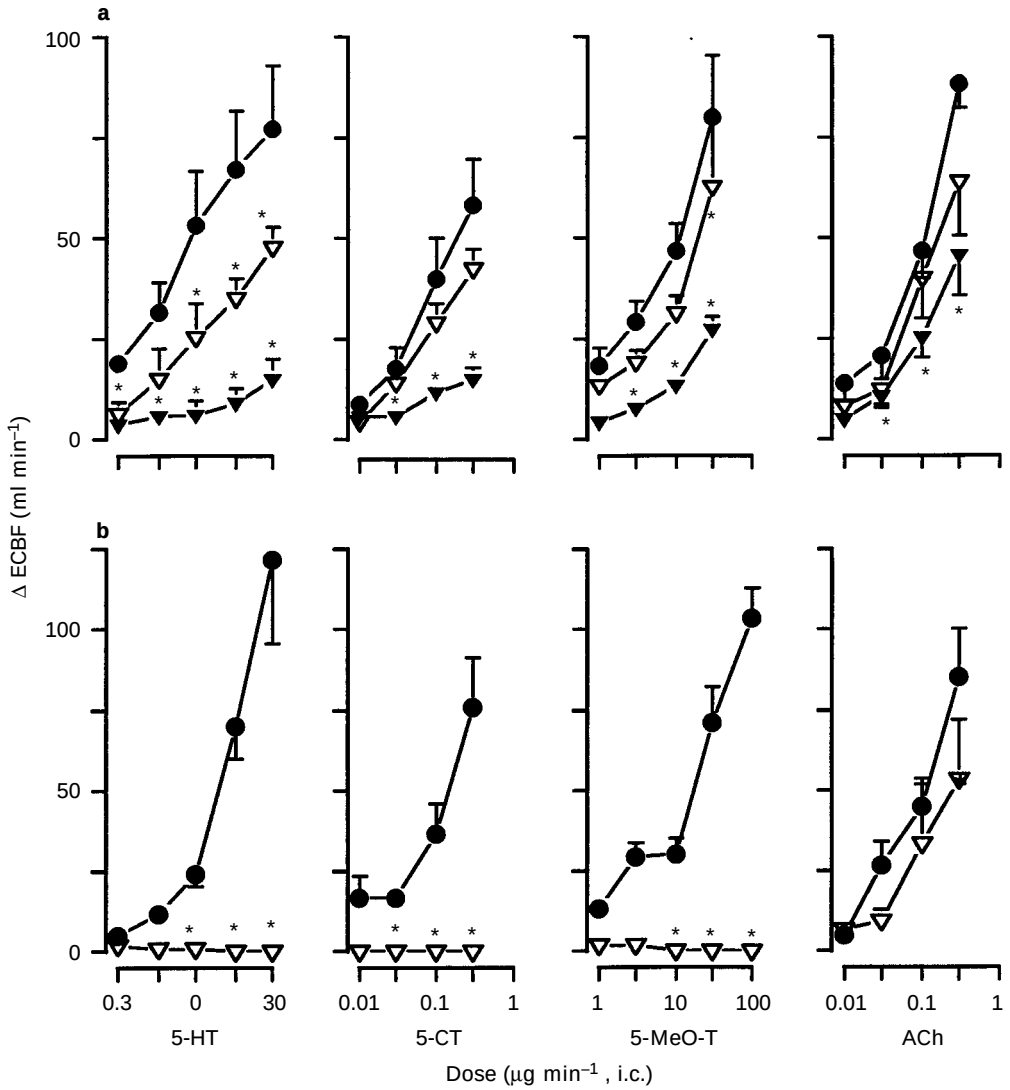

Figure 5 The effects of (a) clozapine (●, 0 mg kg^{-1}; △, 0.3 mg kg^{-1}; and ▼, 1 mg kg^{-1}, i.v.; $n=4$) or (b) lisuride (●, 0 μg kg^{-1}; and ▽, 85±7 μg kg^{-1}, i.c.; $n=3$) on the increases in external carotid blood flow (ΔECBF) induced by 1 min i.c. infusions of 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeO-T) and acetylcholine (ACh) in vagosympathectomized dogs pretreated with GR 127935 (20 μg kg^{-1}, i.v.). Vertical lines show s.e.mean. *$P<0.05$ vs control.

and granisetron (see Methods) in order to exclude: the potential role of 5-HT$_2$ and 5-HT$_3$ receptors in the same animals; and the potent blocking component of methiothepin at vascular 5-HT$_2$ receptors (Saxena & Villalón, 1990).

In order to reinforce the above suggestion, we deliberately selected methysergide, metergoline, mesulergine, lisuride and clozapine as potential antagonists in our study because they show either high affinity (methysergide, metergoline, lisuride and clozapine) or relative selectivity (mesulergine) for the cloned 5-ht$_7$ receptor (Hoyer *et al*., 1994). Of particular significance is the fact that a low dose (30 μg kg^{-1}, i.v.) of mesulergine specifically antagonized the 5-CT, 5-HT and 5-methoxytryptamine-induced vasodilator response, because this ergoline displays an almost 300 fold selectivity for the cloned 5-ht$_7$ receptor (pK_D=8.15) over the cloned 5-ht$_6$ receptor (pK_D=5.76) and does not interact with the 5-HT$_1$ receptor family (Hoyer *et al*., 1994). In accordance with the above observations, methysergide, metergoline and clozapine blocked, whereas lisuride abolished, the above agonist-induced vasodilator responses, though these blocking effects were specific only for metergoline and lisuride. Since these drugs have also been shown to antagonize smooth muscle relaxant responses mediated by 5-HT$_7$ receptors (e.g. Carter *et al*., 1995; Martin & Wilson, 1995; Terrón, 1996), the involvement of a vasodilator 5-HT$_7$ receptor in the present study is reinforced.

Lastly, the apparent rank order of antagonist potency observed in our study, i.e. methiothepin (8.99)>mesulergine (8.15)>>clozapine (7.87)≥methysergide (7.9)>metergoline (8.69), shows that the blocking potency of metergoline does not correlate with its pK_D for 5-HT$_7$ receptors (Hoyer *et al*., 1994). Although the reason for this discrepancy is not clear, this ergoline has similarly shown a poor antagonist potency at other putative 5-HT$_7$ receptors mediating cardiovascular responses, including feline tachycardia (Saxena, 1988) and porcine arteriolar dilatation (Den Boer *et al*., 1992).

Resemblance of the external carotid vasodilator 5-HT receptors to other 5-HT$_7$ receptors

The most prominent haemodynamic response to i.v. 5-HT in anaesthetized animals is vasodepression, first described by Page & McCubbin (1953) as the tertiary component of the triphasic response to 5-HT; the pharmacological profile of this vasodepressor response, mediated by '5-HT$_1$-like' receptors (Saxena & Lawang, 1985; Connor *et al*., 1986; Martin *et al*., 1987), insensitive to the agonist action of sumatriptan (Feniuk *et al*., 1989; Perren *et al*., 1989), closely resembles that of previously called pro-relaxant '5-HT$_1$-like' receptors (e.g. Feniuk *et al*., 1983; Sumner *et al*., 1989; Saxena & Villalón, 1990; Martin, 1994). These receptors, characterized by the high

agonist potency of 5-CT (relative to 5-HT and other tryptamines), the inactivity of sumatriptan, blockade by methiothepin, methysergide and/or spiperone and insensitivity to antagonists at 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors (see Martin, 1994), mediate relaxation of endothelium-denuded blood vessels, including the cat saphenous vein (Feniuk *et al*., 1983), rabbit jugular vein (Martin *et al*., 1987), beagle coronary artery (Martin, 1994) and neonatal pig vena cava (Trevethick *et al*., 1984; 1986). In this latter case, significantly, 5-HT and 5-CT produced relaxation and a concomitant elevation of tissue adenosine 3′:5′-cyclic AMP (cyclic AMP) levels (a transductional system associated to activation of 5-HT$_7$ receptors; Hoyer *et al*., 1994), and both responses were potently and specifically antagonized, to a similar extent, by methiothepin, methysergide and spiperone (Sumner *et al*., 1989); interestingly, metergoline and mesulergine also block the above relaxant response, as observed in our study.

In spite of the absence of selective agonists or antagonists, the above lines of evidence, taken together, strongly suggest that the operational characteristics of 5-HT receptors mediating hypotension and vasorelaxation are very similar to the 5-HT$_7$ receptors mediating canine external carotid vasodilatation (present study) as well as direct relaxation of the rabbit femoral vein (Martin & Wilson, 1995), canine coronary artery (Terrón, 1996), *Cynomolgus* monkey isolated jugular vein (Leung *et al*., 1996) and guinea-pig ileum (Feniuk *et al*., 1983; Carter *et al*., 1995).

The 5-HT$_7$ receptor may also be involved in the tachycardia induced by 5-HT in spinal cats. This response, being mimicked with higher potency by 5-CT and insensitive to ketanserin or MDL 72222, is blocked by methiothepin, methysergide or mesulergine (Saxena *et al*., 1985; Saxena, 1988). Tryptamines produce dose-dependent tachycardic responses with a potency order of 5-CT > > 5-HT > 5-methoxytryptamine (with sumatriptan, indorenate and cisapride inactive as agonists or antagonists) and such effects are blocked (in order of potency) by lisuride, mesulergine, ergotamine and clozapine, but not by GR 127935 (unpublished observations).

In conclusion, it is apparent from the foregoing that the effects of 5-HT on the carotid vascular bed are complex. When sympathetic tone is removed by vagosympathectomy, the predominant effect is vasoconstriction mediated by postjunctional 5-HT$_{1B/1D}$ receptors. Interestingly, blockade of these receptors unmasks a vasodilator response to 5-HT that is also postjunctional. Based upon its operational characteristics, the receptor mediating this response bears a close resemblance to the receptor previously described as '5-HT$_1$-like' in the cat heart and a variety of vascular and non-vascular smooth muscles; it is now evident that this receptor corresponds to the 5-HT$_7$ receptor (see Hoyer *et al*., 1994; Martin, 1994). The precise role of this receptor type in the carotid arterial circulation is presently unclear; although, admittedly, we cannot categorically exclude the possible involvement of additional mechanisms (e.g. endothelial nitric oxide release), the data described in this paper indicate that modulation of local blood flow is likely only when sympathetic tone is low and the reactivity of 5-HT$_{1B/1D}$ receptors is somehow depressed. Albeit highly speculative, such a situation might pertain in migraine headache, a condition that is accompanied, in some cases, by increases in extracerebral blood flow and which is effectively relieved by 5-HT$_{1B/1D}$ receptor agonists like sumatriptan. However, a more precise understanding of the physiological and pathophysiological roles of the 5-HT$_7$ receptors waits the development of selective agonist and antagonist probes.

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