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Evidence for 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors mediating constriction of the canine internal carotid circulation

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1 The present study has investigated the preliminary pharmacological profile of the receptors mediating vasoconstriction to 5-hydroxytryptamine (5-HT) in the internal carotid bed of vagosympathectomised dogs.

2 One minute intracarotid infusions of the agonists 5-HT (0.1–10 μg min^{-1}), sumatriptan (0.3–10 μg min^{-1}; 5-$HT_{1B/1D}$), 5-methoxytryptamine (1–100 μg min^{-1}; 5-HT_1, 5-HT_2, 5-HT_4, 5-ht_6 and 5-HT_7) or DOI (0.31–10 μg min^{-1}; 5-HT_2), but not 5-carboxamidotryptamine (0.01–0.3 μg min^{-1}; 5-HT_1, 5-ht_{5A} and 5-HT_7), 1-(m-chlorophenyl)-biguanide (*m*CPBG; 1–1000 μg min^{-1}; 5-HT_3) or cisapride (1–1000 μg min^{-1}; 5-HT_4), resulted in dose-dependent decreases in internal carotid blood flow, without changing blood pressure or heart rate.

3 The vasoconstrictor responses to 5-HT, which remained unaffected after saline, were resistant to blockade by i.v. administration of the antagonists ritanserin (100 μg kg^{-1}; 5-$HT_{2A/2B/2C}$) in combination with tropisetron (3000 μg kg^{-1}; 5-$HT_{3/4}$) or the cyclo-oxygenase inhibitor, indomethacin (5000 μg kg^{-1}), but were abolished by the 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (30 μg kg^{-1}). Interestingly, after administration of GR127935, the subsequent administration of ritanserin unmasked a dose-dependent vasodilator component. GR127935 or saline did not practically modify the vasoconstrictor effects of 5-MeO-T. In animals receiving GR127935, the subsequent administration of ritanserin abolished the vasoconstrictor responses to 5-MeO-T unmasking a dose-dependent vasodilator component.

4 The vasoconstriction induced by sumatriptan was antagonized by GR127935, but not by ritanserin. Furthermore, ritanserin (100 μg kg^{-1}) or ketanserin (100 μg kg^{-1}; 5-HT_{2A}), but not GR127935, abolished DOI-induced vasoconstrictor responses.

5 The above results suggest that 5-HT-induced internal carotid vasoconstriction is predominantly mediated by 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors.

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Abbreviations: 5-CT, 5-carboxamidotryptamine; DAP, Diastolic arterial blood pressure; DOI, (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; GR127935, 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; MAP, Mean arterial blood pressure; *m*CPBG, 1-(m-chlorophenyl)-biguanide; 5-MeO-T, 5-methoxytryptamine; NC-IUPHAR, Committee for Receptor Nomenclature and Drug Classification of the International Union of Pharmacology; SAP, Systolic arterial blood pressure

Introduction

It has been demonstrated that intracarotid (intra-arterial; i.a.) infusions of 5-HT produce vasodilatation in the external carotid circulation of dogs with intact vagosympathetic trunks (Villalón *et al*., 1993) *via* the activation of both prejunctional sympatho-inhibitory 5-HT_{1B} receptors and musculotropic 5-HT_7 receptors (Villalón *et al*., 2001). Interestingly, after vagosympathectomy, 5-HT produces vasoconstriction in this vascular bed (Villalón *et al*., 1995), *via* the stimulation of vascular smooth muscle 5-HT_{1B} receptors (De Vries *et al*., 1998), without the involvement of 5-HT_2 receptors (Villalón *et al*., 1993). In contrast, 5-HT produces vasoconstriction in the internal carotid circulation of dogs irrespective of the presence or absence of sympathetic tone (Mena & Vidrio, 1979). This effect was: (i) resistant to ganglion blockade, α-adrenoceptor blockade and changes in non-neurogenic vascular tone (Vidrio & Hong, 1976); and (ii) potently antagonized by methysergide, a finding that led Vidrio & Hong (1976) to conclude the involvement of so called 'D' 5-HT receptors. However, it is nowadays clear that methysergide can interact with a wide array of 5-HT receptors, including 5-HT_1, 5-HT_2, 5-ht_5, 5-ht_6 and 5-HT_7 receptors (Hoyer *et al*., 1994). Therefore, the present study set out to investigate the receptor mechanisms involved in 5-HT-induced vasoconstriction of the canine internal carotid bed with regard to the classification schemes recently proposed by

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the NC-IUPHAR subcommittee on 5-HT receptors (see Hoyer *et al*., 1994; Villalón *et al*., 1997b; Saxena *et al*., 1998). Hence, the drugs employed included: (i) the 5-HT receptor agonists 5-carboxamidotryptamine (5-CT; 5-HT_1, 5-ht_{5A} and 5-HT_7); 5-methoxytryptamine (5-MeO-T; 5-HT_1, 5-HT_2, 5-HT_4, 5-ht_6 and 5-HT_7); sumatriptan (5-HT_{1B}, 5-HT_{1D} and 5-ht_{1F}); (±)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; 5-$HT_{2A/2B/2C}$); 1-(m-chlorophenyl)-biguanide (*m*CPBG; 5-HT_3) and cisapride (5-HT_4); and (ii) the 5-HT receptor antagonists GR127935 (5-$HT_{1B/1D}$), ritanserin (5-$HT_{2A/2B/2C}$), ketanserin (5-HT_{2A}) and tropisetron (5-HT_3 and 5-HT_4). In addition, indomethacin was employed in order to evaluate the possible involvement of indirect mechanisms mediated by the release of vasoconstrictor prostanoids, as previously shown by Rosenblum and Nelson (1988).

The characterization of the 5-HT receptors involved in the above vasoconstrictor response may offer further information about the mechanisms involved in the 'aura' phase of migraine, characterized by high plasma levels of 5-HT (Deshmukh & Meyer, 1977; Ferrari *et al*., 1989).

A preliminary account of this study has been communicated to the British Pharmacological Society (Centurión *et al*., 1999).

Methods

General

Experiments were carried out in a total of 57 mongrel dogs (15–30 kg) not selected for breed or sex. The animals were anaesthetized with an intravenous (i.v.) bolus injection of sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}, i.v.) were provided every 45 min to maintain anaesthesia, as previously established (Villalón *et al*., 1997a; 1999b).

After intubation of the trachea, the dogs were artificially respired with room air using a Palmer positive pressure pump at a rate of 20 strokes min^{-1} and a stroke volume of 13–16 ml kg^{-1}, as previously established by Kleinman & Radford (1964). Stroke volume was adjusted to maintain arterial pH within normal limits (7.35–7.45). Moreover, catheters were placed in the femoral vein for the administration of drugs and in the 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 saline. Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial blood 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 free and the corresponding external carotid and occipital arteries were ligated. Following bilateral cervical vagosympathectomy a precalibrated ultrasonic flow probe (2 mm R-Series; Transonic Systems Inc., Ithaca, NY, U.S.A.) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, NY, U.S.A.) was placed around the right common carotid artery and the flow through this artery was considered as the internal carotid blood flow (for further considerations, see Villalón *et al*., 1993; 1999b). To analyse the effect of agonist drugs on internal carotid blood flow, the agonists were administered into the carotid artery by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.) with a cannula inserted into the right cranial thyroid artery. Blood pressure, heart rate and internal carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., MA, U.S.A.).

Experimental protocol

After a stable haemodynamic condition for at least 30 min, baseline of values of blood pressure, heart rate and internal carotid blood flow were determined. Then, the 57 dogs were divided into three groups.

The first group ($n=24$) received consecutive 1 min intracarotid (intra-arterial; i.a.) infusions of 5-HT (0.1, 0.3, 1, 3 and 10 $\mu g\ min^{-1}$). At this point, the dogs were subdivided into five subgroups and the effects produced by the above i.a. infusions of 5-HT were elicited again after i.v. treatment with each dose of either: (i) physiological saline (0.01, 0.05 and 0.1 ml kg^{-1}; $n=5$); (ii) ritanserin (100 $\mu g\ kg^{-1}$; $n=3$); (iii) the combination of ritanserin (100 $\mu g\ kg^{-1}$) and tropisetron (3000 $\mu g\ kg^{-1}$) given simultaneously ($n=4$); (iv) GR127935 (30 $\mu g\ kg^{-1}$) and, subsequently, ritanserin (100 $\mu g\ kg^{-1}$; $n=7$) or (v) indomethacin (5000 $\mu g\ kg^{-1}$; $n=5$). In most animals of each of the above subgroups (except in the subgroups treated with the combination of ritanserin and tropisetron, or with indomethacin), the effects produced by 1 min i.a. infusions of sumatriptan (0.3, 1, 3 and 10 $\mu g\ min^{-1}$) were analysed after either the first administration of saline (0.01 ml kg^{-1}; $n=4$) or the first administered antagonists, namely, GR127935 (30 $\mu g\ kg^{-1}$; $n=5$) or ritanserin (100 $\mu g\ kg^{-1}$; $n=4$), respectively. It should be noted that only these animals ($n=13$) received simultaneously 5-HT (0.1, 0.3, 1, 3 and 10 $\mu g\ min^{-1}$) and sumatriptan (0.3, 1, 3 and 10 $\mu g\ min^{-1}$).

In the second group ($n=18$), the effects of *cumulative* 1 min i.a. infusions of DOI (0.31, 0.56, 1, 1.8, 3.1, 5.6 and 10 $\mu g\ min^{-1}$) were analysed in animals pretreated i.v. with either saline (0.1 ml kg^{-1}; $n=5$), GR127935 (30 $\mu g\ kg^{-1}$; $n=4$), ritanserin (100 $\mu g\ kg^{-1}$; $n=5$) or ketanserin (100 $\mu g\ kg^{-1}$; $n=4$).

Finally, the third group ($n=15$) was subdivided into two subgroups. In the first subgroup ($n=4$), the effects produced by consecutive 1 min i.a. infusions of *m*CPBG and, subsequently, of cisapride (both given at 1, 3, 10, 30, 100, 300, and 1000 $\mu g\ min^{-1}$) were analysed. Moreover, the second subgroup ($n=11$) received consecutive 1 min i.a. infusions of 5-methoxytryptamine (5-MeO-T; 1, 3, 10, 30 and 100 $\mu g\ min^{-1}$). Then, the effects of 5-MeO-T were elicited again after i.v. administration of: (i) saline (0.01 and 0.05 ml kg^{-1}; $n=6$) or (ii) GR127935 (30 $\mu g\ kg^{-1}$) followed by ritanserin (100 $\mu g\ kg^{-1}$; $n=5$). In four of these saline-treated animals, the effects produced by 1 min i.a. infusions of 5-CT (0.01, 0.03, 0.1 and 0.3 $\mu g\ min^{-1}$) were determined before the administration of 5-MeO-T.

The dose-intervals between the different doses of agonists, except for DOI (given cumulatively), ranged between 5 and 20 min, as in each case we waited until the internal carotid blood flow had returned completely to baseline values. The dosing with all drugs used, except for DOI, was sequential. The doses of the above agonists were infused in a volume of

1 ml during a period of 1 min. Moreover, after the administration of a specific dose of an antagonist or saline a period of about 10 min was allowed to elapse before the responses to the respective agonists were elicited again.

Data presentation and statistical analysis

All data in the text and figures are represented as the mean ± s.e.mean. The peak changes in internal carotid blood flow were expressed as per cent change from baseline. The difference between the variables within one group of animals was evaluated with Student–Newman–Keuls test once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Furthermore, the peak per cent changes in internal carotid blood flow induced by sumatriptan and DOI in the different groups of animals were compared by using unpaired Student's *t*-test. A *P*-value of 0.05 or less (two-tailed) was considered statistically significant.

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the drugs used in the present study were the following, obtained from the sources indicated: 5-hydroxytryptamine creatinine sulphate (5-HT) and 5-methoxytryptamine hydrochloride (5-MeO-T) (both from Sigma, St. Louis, MO, U.S.A.); indomethacin, *m*CPBG (1-[m-chlorophenyl]-biguanide hydrochloride) and DOI ((±)1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane) (Research Biochemicals Int., Natick, MA, U.S.A.); ritanserin and cisapride (gifts from Dr J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium); tropisetron (gift: Sandoz A.G., Basel, Switzerland); 5-carboxamidotryptamine maleate (5-CT), sumatriptan succinate and GR127935 (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) (gifts from Dr M. Skingle, Glaxo Group Research, Ware, Herts, U.K. and Dr H.E. Connor, Glaxo Group Research, Stevenage, Hertfordshire, U.K.). All compounds were dissolved in distilled water. When needed, 4% (w v^{-1}) ascorbic acid (ritanserin) was added. These vehicles had no effect on the haemodynamic variables. The doses mentioned in the text refer to the salts of substances except in the case of 5-HT, 5-CT, 5-MeO-T, sumatriptan, DOI, cisapride and *m*CPBG, where they refer to the free base.

Results

Systemic haemodynamic variables

The baseline values of mean arterial blood pressure, heart rate and internal carotid blood flow in the 57 dogs were 133 ± 3 mmHg, 173 ± 4 beats min^{-1} and 45 ± 2 ml min^{-1}, respectively. These haemodynamic values before and after administration of physiological saline, indomethacin or the 5-HT receptor antagonists are shown in Table 1. All haemodynamic variables remained essentially unchanged after the administration of saline, ritanserin, ketanserin, tropisetron or GR127935. Only indomethacin produced a slight, though significant, decrease in internal carotid blood flow (Table 1).

Initial effects of agonist drugs on internal carotid blood flow

Figure 1 shows that i.a. infusions of 5-HT (0.1, 0.3, 1, 3 and 10 μg min^{-1}), 5-MeO-T (1, 3, 10, 30 and 100 μg min^{-1}), DOI (0.31, 0.56, 1, 1.8, 3.1, 5.6 and 10 μg min^{-1}) and sumatriptan (0.3, 1, 3 and 10 μg min^{-1}) elicited dose-dependent decreases in internal carotid blood flow. These responses were not accompanied by changes in mean blood pressure or heart rate (not shown), implying a local vasoconstrictor effect. The above responses, which were immediate in onset, are drug-induced as 1 min i.a. infusions of the corresponding volumes of saline did not affect any haemodynamic parameter for the duration of the experiments (data not shown). The rank order of agonist potency was: 5-HT = DOI > sumatriptan = 5-MeO-T. At the doses used (see above), the duration of the responses elicited by sumatriptan (3.8 ± 1, 7.4 ± 2.4, 12 ± 3.7, 12.7 ± 3.3 min) was longer than that of 5-HT (2.6 ± 0.5, 5.0 ± 0.4, 7.1 ± 0.4, 8.7 ± 0.4, 9.6 ± 0.2 min) and 5-MeO-T (2.0 ± 0.7, 3.7 ± 0.5, 3.6 ± 0.3, 4.2 ± 0.3, 5.0 ± 0.3 min). The duration of action of DOI could not be established, as its effect on the internal carotid blood flow did not return to baseline values. In any case, this finding led us to employ a cumulative administration schedule for DOI. In addition,

Table 1 Mean arterial blood pressure, heart rate and internal carotid blood flow before and after i.v. administration of the different compounds in anaesthetized vagosympathectomised dogs

			Mean arterial blood pressure (mmHg)		*Heart rate* (beats min^{-1})		*Internal carotid blood flow* (ml min^{-1})	
Compound	*Dose* (μg kg^{-1})	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Saline[a]	0.01[b]	16	133 ± 9	133 ± 9	140 ± 14	140 ± 14	39 ± 4	39 ± 4
Ritanserin	100	12	139 ± 5	141 ± 5	173 ± 8	168 ± 9	39 ± 5	40 ± 5
Tropisetron[c]	3000	4	145 ± 5	154 ± 5	184 ± 15	180 ± 17	46 ± 7	45 ± 7
GR127935	30	16	137 ± 7	146 ± 6	157 ± 8	152 ± 8	41 ± 4	36 ± 3
Ketanserin	100	4	133 ± 7	128 ± 7	176 ± 9	169 ± 10	37 ± 4	35 ± 3
Indomethacin	5000	5	133 ± 6	139 ± 9	163 ± 12	162 ± 12	40 ± 6	31 ± 6*

*$P < 0.05$, after *vs* before from the corresponding baseline value. [a]Refers to the effect after the first administration of physiological saline. The second and third administrations of saline were similarly without significant effects (not shown). [b]ml kg^{-1}. [c]This was subsequently followed by ritanserin (100 μg kg^{-1}; i.v.) which did not produce any significant effects (not shown). All values are presented as mean ± s.e.mean.

*m*CPBG and cisapride (1–1000 μg min^{-1}) failed to decrease the internal carotid blood flow *per se* (Figure 1) or to antagonise the vasoconstrictor responses to 5-HT (not shown). It should be noted that cisapride produced small increases in internal carotid blood flow at the highest doses.

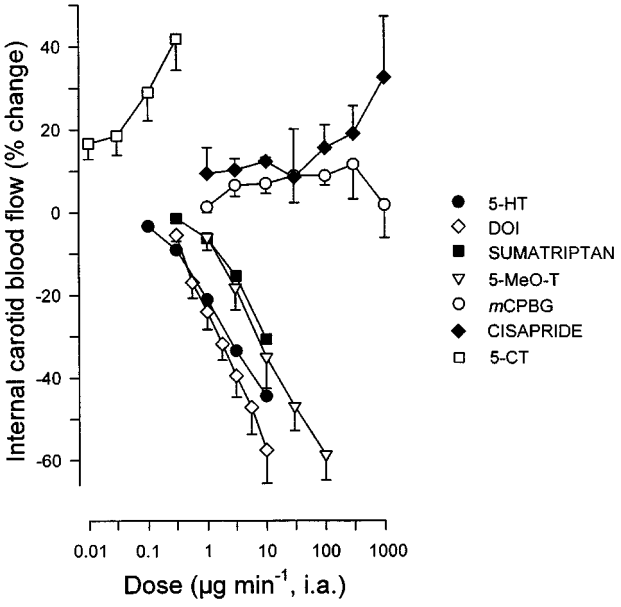

Figure 1 Comparative effects of 1 min intra-arterial (i.a.) infusions of 5-HT ($n=24$), DOI ($n=5$), sumatriptan ($n=4$), 5-MeO-T ($n=11$), *m*CPBG ($n=4$), cisapride ($n=4$) and 5-CT ($n=4$) on the internal carotid blood flow of anaesthetized vagosympathectomized dogs. All values are presented as mean$\pm$s.e.mean.

Interestingly, 5-CT (0.01, 0.03, 0.1 and 0.3 μg min^{-1}) elicited dose-dependent increases (rather than decreases) in internal carotid blood flow.

Effect of physiological saline or 5-HT receptor antagonists on the decreases in internal carotid blood flow induced by 5-HT or 5-MeO-T

The effects of physiological saline or several 5-HT receptors antagonists on the responses induced by 5-HT and 5-MeO-T are depicted in Figures 2 and 3, respectively. The decreases in internal carotid blood flow 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 three subsequent i.v. bolus injections (0.01, 0.05 and 0.1 ml kg^{-1}) of saline (Figure 2a). Similarly, the responses to 5-HT remained unaltered after administration of 100 μg kg^{-1} ritanserin (Figure 2b) or the combination of 100 μg kg^{-1} of ritanserin and 3000 μg kg^{-1} of tropisetron (Figure 2c). As shown in Figure 2d, the vasoconstrictor responses to 5-HT were abolished by GR127935; interestingly, the subsequent administration of ritanserin unmasked a dose-dependent vasodilator component to 5-HT. The effects of saline or the 5-HT receptor antagonists on the decreases in internal carotid blood flow induced by 5-MeO-T are shown in Figure 3. The vasoconstrictor effects of 5-MeO-T were not modified after two i.v. bolus injections of saline (Figure 3a). Interestingly, only the vasoconstrictor response to 30 μg min^{-1} of 5-MeO-T was slightly, though significantly ($P<0.05$), blocked by GR127935 (30 μg kg^{-1}). Notably, the subsequent administration of ritanserin (100 μg kg^{-1}) not only abolished the 5-MeO-T-induced vasoconstriction, but

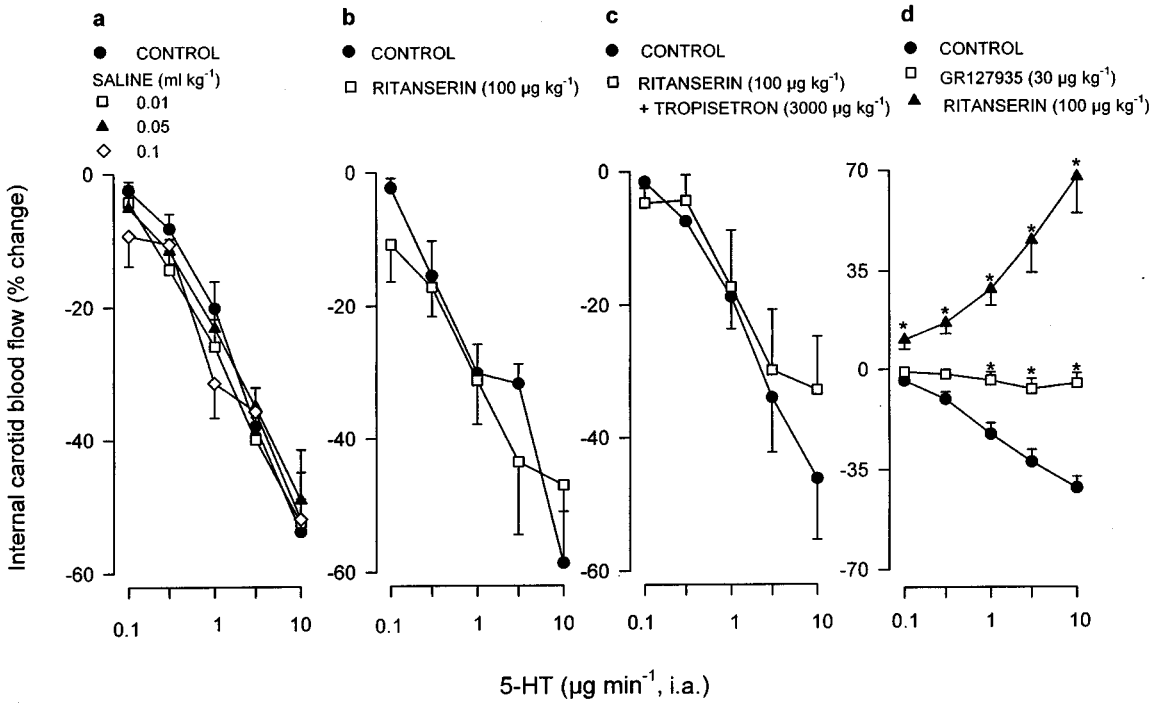

Figure 2 The effects of (a) saline ($n=5$); (b) ritanserin ($n=3$); (c) the combination of ritanserin and tropisetron ($n=4$); and (d) GR127935 followed by ritanserin ($n=7$) on the decreases in internal carotid blood flow induced by 1 min intra-arterial (i.a.) infusions of 5-HT in anaesthetized vagosympathectomized dogs. *$P<0.05$ *vs* control. All values are presented as mean$\pm$s.e.mean.

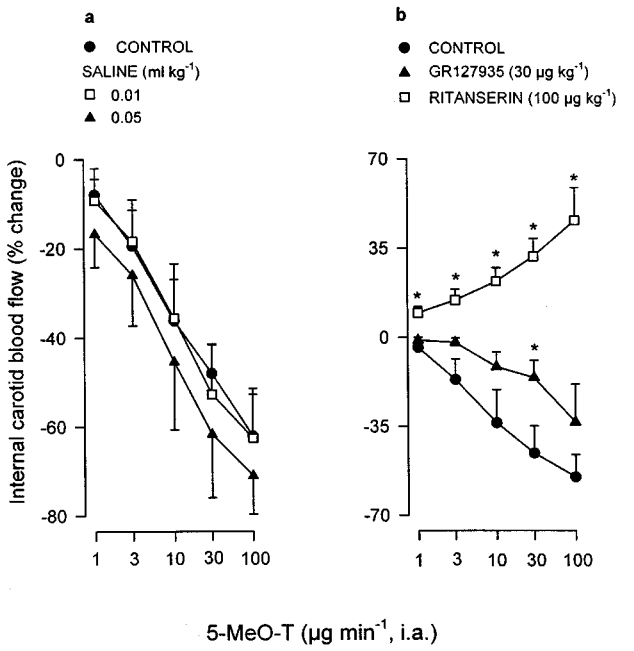

Figure 3 The effects of (a) saline ($n=6$); or (b) GR127935 followed by ritanserin ($n=5$) on the decreases in internal carotid blood flow induced by 1 min intra-arterial (i.a.) infusions of 5-MeO-T in anaesthetized vagosympathectomized dogs. $^*P<0.05$ *vs* control. All values are presented as mean ± s.e.mean.

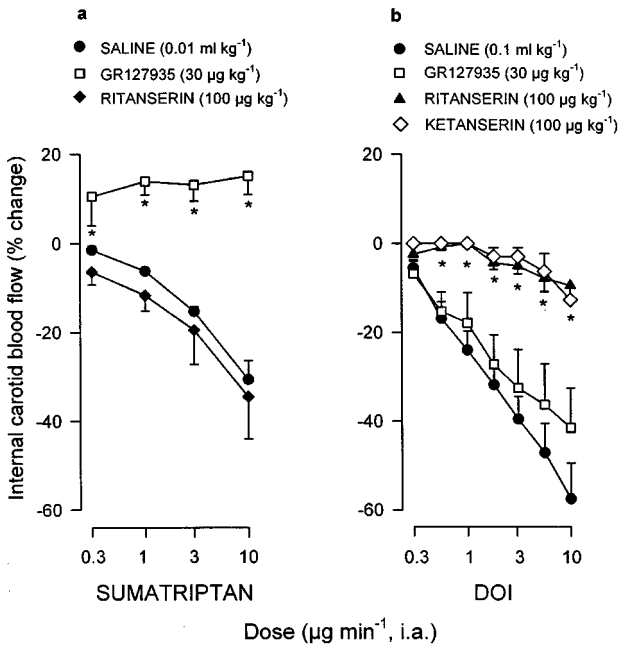

Figure 4 The decreases in canine internal carotid blood flow induced by 1 min intra-arterial (i.a.) infusions of (a) sumatriptan in animals pretreated with either saline ($n=4$), GR127935 ($n=5$), or ritanserin ($n=4$); or (b) DOI in animals pretreated with either saline ($n=5$), GR127935 ($n=4$), ritanserin ($n=5$), ketanserin ($n=4$). $^*P<0.05$ *vs* the corresponding response in saline pretreated animals. All values are presented as mean ± s.e.mean.

also unmasked a dose-dependent vasodilator component (Figure 3b), as previously observed with 5-HT (Figure 2d).

Effect of indomethacin on the decreases in internal carotid blood flow by 5-HT

Indomethacin (5000 μg kg^{-1}) failed to block the decreases in internal carotid blood flow induced by 5-HT; even a slight, but significant, potentiation of the response to 1 μg min^{-1} of 5-HT was observed. Thus, the per cent changes induced by 0.1, 0.3, 1, 3 and 10 μg min^{-1} of 5-HT on the internal carotid blood flow were, respectively, -2 ± 1, -9 ± 1, -25 ± 4, -49 ± 8 and -64 ± 8% before indomethacin and -7 ± 2, -20 ± 8, -46 ± 8*, -56 ± 8 and -65 ± 8% after indomethacin ($^*P<0.05$ *vs* before indomethacin).

Effect of physiological saline or 5-HT receptor antagonists on either sumatriptan- or DOI-induced decreases in internal carotid blood flow

In animals pretreated with physiological saline, sumatriptan (Figure 4a) or DOI (Figure 4b) produced dose-dependent decreases in internal carotid blood flow, without modifying blood pressure or heart rate (not shown). As depicted in Figure 4a, the sumatriptan-induced vasoconstrictor responses remained unaffected in animals pretreated i.v. with ritanserin (100 μg kg^{-1}), but were abolished in those pretreated with GR127935 (30 μg kg^{-1}). Moreover, as shown in Figure 4b, the DOI-induced vasoconstrictor responses were abolished in animals pretreated with either ritanserin (100 μg kg^{-1}) or ketanserin (100 μg kg^{-1}), but not with GR127935 (30 μg kg^{-1}).

Discussion

General

The pharmacology of 5-HT receptors in the canine external carotid vascular bed has been extensively studied (see Villalón *et al.*, 1999a; 2001). In contrast, only limited data are available concerning the type/s of 5-HT receptors mediating vasoconstriction in the internal carotid circulation, apart from being independent of the sympathetic tone and amenable to blockade by methysergide (Vidrio & Hong, 1976; Mena & Vidrio, 1979). Therefore, in the present study we decided to analyse the preliminary pharmacological profile of the receptors involved in the 5-HT-induced vasoconstrictor effects in the canine internal carotid circulation, using selective agonists and antagonists.

The major findings of the present study are that the 5-HT receptors producing canine internal carotid vasoconstriction are: (i) stimulated, with a rank order of agonist potency, by 5-HT = DOI > sumatriptan = 5-MeO-T but not by *m*CPBG, cisapride or 5-CT; (ii) not affected by ritanserin and tropisetron; and (iii) antagonized by GR127935. Consistent with these findings, pretreatment with GR127935, but not with ritanserin, abolished the responses to sumatriptan, whilst ritanserin and ketanserin, but not GR127935, antagonized the responses to DOI. Apart from the implications discussed below, the above data indicate that the canine internal carotid vasoconstriction induced by 5-HT is predominantly mediated by 5-HT$_{1B/1D}$ and 5-HT$_{2A}$ receptors. A detailed analysis of the exact 5-HT$_{1B/1D}$ receptor subtype involved in

this vasoconstrictor response has been carried out in the following paper (Centurión *et al*., 2001).

Internal carotid haemodynamic changes produced by i.a. infusions of 5-HT receptor agonists

The present study confirms and extends previous observations (Vidrio & Hong, 1976) showing that 5-HT induces a dose-dependent decrease in the canine internal carotid blood flow. Since no changes in blood pressure or heart rate were produced, a selective vasoconstriction in the internal carotid circulation is implied. The above responses to 5-HT were highly reproducible as they remained essentially unchanged in control animals receiving three consecutive i.v. bolus injections of physiological saline. Therefore, we can conclude that no time-dependent changes occurred in the above haemodynamic variables during the experimental period (180–240 min) in the animal model used here. Moreover, it should be noted that 5-CT produced internal carotid vasodilatation (rather than vasoconstriction). This effect of 5-CT is consistent with the presence of 5-HT_7 receptors which mediate vasodilatation, a mechanism recently shown in this vascular bed (Centurión *et al*., 2000). On the other hand, we do not have a clear-cut explanation for the long-lasting effect of DOI; it may be due to a slow dissociation from its receptors and/or a sequestration and subsequent diffusion out of a local nonsaturable compartment, as hypothesized for the long-lasting effect of ergotamine and dihydroergotamine (Müller-Schweinitzer & Weidmann, 1978; Martin *et al*., 1995). It should be emphasized that, at the doses used, the selective agonists DOI, sumatriptan, *m*CPBG and cisapride used in this study retained their selectivity (see Villalón *et al*., 1996).

Consideration of indirect mechanisms on the 5-HT-induced canine internal carotid vasoconstriction

The mechanisms involved in the vasoconstrictor responses to 5-HT are complex and include, in addition to direct stimulation of vascular smooth muscle receptors, indirect actions (Martin, 1994). Thus, a 5-HT_2 receptor-stimulated release of catecholamines from the canine adrenal medulla (Feniuk *et al*., 1981), as well as the release of noradrenaline from sympathetic neurons by a 5-HT_3 receptor-mediated depolarisation (Fozard *et al*., 1979) can be excluded since zolertine, in doses high enough to block α-adrenoceptors, failed to antagonize the 5-HT-induced internal carotid vasoconstriction (Vidrio & Hong, 1976). In agreement with the latter, the responses to 5-HT were not blocked by tropisetron (present results), in doses high enough to antagonise both 5-HT_3 and 5-HT_4 receptors (Villalón *et al*., 1991). Similarly, an endothelium-dependent vasoconstriction *via* the synthesis and release of pro-constrictor cyclo-oxygenase products (Rosenblum & Nelson, 1988) seems unlikely based on the failure of indomethacin to block 5-HT-induced vasoconstriction (present results). In fact, our findings showing a significant decrease in blood flow after indomethacin (Table 1), suggest that pro-dilator cyclo-oxygenase products may exert a tonic influence on the internal carotid bed. This line of reasoning is consistent with the slight potentiation to a 5-HT-induced vasoconstrictor response after indomethacin (see Results section).

Lack of resemblance of the 5-HT receptors mediating internal carotid vasoconstriction with either 5-HT_3, 5-HT_4, 5-ht_5, 5-ht_6 or 5-HT_7 receptors

Considering that *m*CPBG and cisapride, which are agonists at 5-HT_3 and 5-HT_4 receptors, respectively (see Villalón *et al*., 1991; Hoyer *et al*., 1994), failed to decrease internal carotid blood flow (Figure 1), it is unlikely that these receptors are involved. Accordingly, the effects of 5-HT were resistant to blockade by tropisetron (Figure 2c), at doses high enough to block 5-HT_3 and 5-HT_4 receptors (Villalón *et al*., 1991); moreover, the agonist activity of 5-MeO-T (Figure 1) is an additional criterion to exclude the participation of 5-HT_3 receptors (Hoyer *et al*., 1994). Furthermore, it should be noted that the 5-HT_4 receptor is, by definition, positively coupled to adenylyl cyclase (Hoyer *et al*., 1994), a signal transduction system usually associated with relaxation, not constriction (Rand *et al*., 1987; Lincoln & Cornwell, 1991).

The 5-HT receptors mediating canine internal carotid vasoconstriction also seem to differ from the 5-$ht_{5A/5B}$, 5-ht_6 and 5-HT_7 types on the basis that: (i) sumatriptan, which has low affinity for 5-ht_{5A}, 5-ht_{5B} and 5-HT_7 (pK_D: 6.8, 5.1 and 6.2, respectively) receptors and DOI, with low affinity for these receptors (pK_D: <6, <6 and 4.6, respectively; Hoyer *et al*., 1994) mimicked the vasoconstrictor responses to 5-HT (Figure 1); (ii) the 5-ht_6 and 5-HT_7 receptors are positively coupled to adenylyl cyclase, a transductional system generally associated with vasodilator rather than vasoconstrictor responses; and (iii) mRNA encoding 5-$ht_{5A/5B}$ and 5-ht_6 receptors has not been detected in blood vessels (Ullmer *et al*., 1995).

Contribution of 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors to canine internal carotid vasoconstriction

The 5-HT-induced internal vasoconstrictor responses were potently mimicked by 5-MeO-T, sumatriptan and DOI (Figure 1) implying, in principle, the possible involvement of 5-$HT_{1B/1D}$ and 5-$HT_{2A/2B/2C}$ receptors. In agreement with this suggestion, 30 $\mu g\ kg^{-1}$ of the 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (Skingle *et al*., 1996), markedly blocked the 5-HT-induced vasoconstriction, whereas the subsequent administration of 100 $\mu g\ kg^{-1}$ of the 5-$HT_{2A/2B/2C}$ receptor antagonist, ritanserin (Hoyer *et al*., 1994), even unmasked a vasodilator component in response to 5-HT (Figure 2d); the above doses of GR127935 and ritanserin have previously been shown to completely antagonise cardiovascular 5-$HT_{1B/1D}$ (Villalón *et al*., 1996; 1999b) and 5-HT_2 (Villalón *et al*., 1993; 1996) receptors, respectively, in the dog. This finding indicates that after blockade of GR127935-sensitive 5-$HT_{1B/1D}$ receptors, a vasoconstrictor mechanism mediated by ritanserin-sensitive 5-HT_2 receptors remains operative together with a vasodilator component; the latter has been recently shown to involve 5-HT_7 receptors (Centurión *et al*., 2000).

The fact that, unlike ritanserin (Figure 2b), GR127935 blocked 5-HT-induced vasoconstrictor effects (Figure 2d) may be explained by: (i) the moderate 5-HT_{2A} receptor blocking properties of GR127935, as previously shown in rats and cats (De Vries *et al*., 1997) although, admittedly, GR127935 failed to produce a marked blockade of DOI-induced responses (Figure 4b); and/or (ii) physiological

antagonism by stimulation of internal carotid vasodilator 5-HT_7 receptors, which are potently activated by 5-HT (Centurión *et al*., 2000). Following a similar line of reasoning, the lack of antagonism of ritanserin on the 5-HT-induced vasoconstrictor responses (Figure 2b) may be explained by the additional capability of this monoamine to stimulate 5-$HT_{1B/1D}$ receptors; this may have overshadowed the antagonism of 5-$HT_{2A/2B/2C}$ receptors with ritanserin. In contrast, the apparent lack of antagonism of GR127935 on the 5-MeO-T-induced vasoconstriction (Figure 3b) may be explained by the lower activity of this monoamine (relative to 5-HT) at vasodilator 5-HT_7 receptors on the canine internal carotid bed (Centurión *et al*., 2000). In line with the above contention, GR127935 and ritanserin selectively abolished the sumatriptan- and DOI-induced effects, respectively (Figure 4a,b). Although 5-ht_{1F} receptors cannot be categorically excluded since sumatriptan and GR127935 also display moderate affinity for these receptors (Hoyer *et al*., 1994), our results, taken together, suggest a predominant involvement of 5-$HT_{1B/1D}$ and 5-$HT_{2A/2B/2C}$ receptors. With respect to the latter, it is well known that 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors mediate vasoconstriction in many blood vessels (see Hoyer *et al*., 1994; Martin, 1994; Bouchelet *et al*., 2000). Consistent with this view, ketanserin, a quinazoline-dione derivative with high affinity and selectivity for 5-HT_{2A} receptors (Hoyer *et al*., 1994), abolished DOI-induced internal carotid vasoconstriction (Figure 4b). The 5-$HT_{2B/2C}$ receptors do not seem to be involved on the basis that: (i) ketanserin displays lower affinity for 5-HT_{2B} and 5-HT_{2C} receptors ($pK_i = 5.5$ and 6.5, respectively; Hoyer *et al*., 1994); and (ii) no mRNA encoding 5-HT_{2c} receptors has been detected in blood vessels (Ullmer *et al*., 1995), including the human internal carotid artery (Schmuck *et al*., 1996).

In conclusion, the results of the present study show that 5-HT constricts the internal carotid vasculature predominantly via 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors. After simultaneous blockade of these receptors a vasodilator response, mediated by 5-HT_7 receptors (Centurión *et al*., 2000), is unmasked. Admittedly, further experiments with selective ligands at 5-HT_{1B}, 5-HT_{1D} and 5-ht_{1F} receptors will be required to identify the definitive pharmacological profile of the vasoconstrictor 5-HT_1 receptors involved. These experiments should be ideally performed in the absence and the presence of 5-HT_2 and 5-HT_7 receptor antagonists in order to exclude a possible interference by physiological antagonism. Obviously, these studies fall beyond the scope of the present investigation, but have been described in the following paper (Centurión *et al*., 2001).

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