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The GR127935-sensitive 5-HT_1 receptors mediating canine internal carotid vasoconstriction: resemblance to the 5-HT_{1B}, but not to the 5-HT_{1D} or 5-ht_{1F}, receptor subtype

[1]David Centurión, [1]Araceli Sánchez-López, [2]Peter De Vries, [2]Pramod R. Saxena & *,[1]Carlos M. Villalón

[1]Departamento de Farmacobiologia, CINVESTAV-IPN, Calzada de los Tenorios 235, Col. Granjas Coapa, 14330 México D.F., México and [2]Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

1 This study has further investigated the pharmacological profile of the GR127935-sensitive 5-HT_1 receptors mediating vasoconstriction in the internal carotid bed of anaesthetized vagosympathectomized dogs.

2 One-minute intracarotid infusions of the agonists 5-hydroxytryptamine (5-HT; 0.1–10 μg min^{-1}; endogenous ligand) and sumatriptan (0.3–10 μg min^{-1}; 5-$HT_{1B/1D}$), but not PNU-142633 (1–1000 μg min^{-1}; 5-HT_{1D}) or LY344864 (1–1000 μg min^{-1}; 5-ht_{1F}), produced dose-dependent decreases in internal carotid blood flow without changing blood pressure or heart rate.

3 The responses to 5-HT were apparently resistant to blockade by i.v. administration of the antagonists SB224289 (300 μg kg^{-1}; 5-HT_{1B}), BRL15572 (300 μg kg^{-1}; 5-HT_{1D}) or ritanserin (100 μg kg^{-1}; 5-HT_2). In contrast, the responses to sumatriptan were antagonized by SB224289, but not by BRL15572.

4 In the animals receiving SB224289, but not those receiving BRL15572, the subsequent administration of ritanserin abolished the 5-HT-induced vasoconstriction and unmasked a vasodilator component. Similarly, in ritanserin-treated animals, the subsequent administration of SB224289, but not BRL15572, completely blocked the 5-HT-induced vasoconstriction, revealing vasodilatation. In animals receiving initially BRL15572, the subsequent administration of SB224289 did not affect (except at 10 μg min^{-1}) the vasoconstrictor responses to 5-HT.

5 Notably, in animals pretreated with 1000 μg kg^{-1} of mesulergine, a 5-$HT_{2/7}$ receptor antagonist, 5-HT produced a dose-dependent vasoconstriction, which was practically abolished by SB224289. After BRL15572, no further blockade was produced and the subsequent administration of ritanserin was similarly inactive.

6 These results suggest that the GR127935-sensitive 5-HT_1 receptors mediating canine internal carotid vasoconstriction resemble the 5-HT_{1B} but not the 5-HT_{1D} or 5-ht_{1F}, receptor subtype.
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Keywords: 5-Hydroxytryptamine (5-HT); internal carotid vasoconstriction; 5-HT_{1B} receptor; SB224289; BRL15572; ritanserin; PNU-142633; LY344864

Abbreviations: BRL15572, (S)-(−)-3,4-dihydro-1-[2-[4-(4-aminocarbonyl)phenyl]-1-piperazinyl]ethyl]-N-methyl-1H-2-benzopyran-6-carboxamide; DAP, Diastolic arterial blood pressure; LY344864, N-[3-(dimethylamino)-2,3,4,9-tetrahydro-1H-carbazol-6-yl]-4-fluorobenzamide; MAP, Mean arterial blood pressure; PNU-142633, (S)-(−)-3,4-dihydro-1-[2-[4-(4-aminocarbonyl)phenyl]-1-piperazinyl]ethyl]-N-methyl-1H-2-benzopyran-6-carboxamide; SAP, Sistolic arterial blood pressure; SB224289, 2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo[2,3f]indole-3-spiro-4′-piperidine hydrochloride

Introduction

We have previously shown that intracarotid (intra-arterial; i.a.) infusions of 5-HT result in dose-dependent decreases in canine internal carotid blood flow (Centurión *et al*., 2001). This effect, being mimicked by the 5-HT receptor agonists DOI (5-HT_2) and sumatriptan (5-$HT_{1B/1D}$), was apparently resistant to blockade by the 5-HT_2 receptor antagonist, ritanserin (100 μg kg^{-1}), but was blocked by the 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (30 μg kg^{-1}) (Centurión *et al*., 2001). In these GR127935-pretreated animals, the subsequent administration of ritanserin unmasked a vasodilator response to 5-HT, which is mainly mediated by stimulation of 5-HT_7 receptors (Centurión *et al*., 2000). The above findings led to conclude the involvement of GR127935-sensitive 5-HT_1 receptors resembling the 5-$HT_{1B/1D}$ receptor subtypes and ketanserin-sensitive receptors resembling the 5-HT_{2A} subtype (Centurión *et al*., 2001). Admittedly, the possible involvement of 5-ht_{1F} receptors could not be categorically excluded as sumatriptan and GR127935 have also moderate affinity for 5-ht_{1F} receptors (see Table 1).

With the recent advent of selective 5-HT_{1B} (SB224289; Hagan *et al*., 1997) and 5-HT_{1D} (BRL15572, Hagan *et al*.,

*Author for correspondence.

Table 1 Receptor binding affinity (pK_i) of some agonists and antagonists at 5-HT$_1$, 5-HT$_{2A}$ and 5-HT$_7$ receptors

	5-HT$_{1A}$	*5-HT$_{1B}$*	*5-HT$_{1D}$*	*5-HT$_{1E}$*	*5-HT$_{1F}$*	*5-HT$_{2A}$*	*5-HT$_7$*
Agonist							
Sumatriptan[a]	6.4	7.8	8.5	5.8	7.9	<5.5	6.2[h]
PNU-142633[b]	ND	4.8[j]	8.3[j]	ND	ND	ND	ND
LY344864[c]	6.3	6.3	6.2	5.8	8.2	5.4	5.3
Antagonist							
GR127935[d]	7.2[d]	9.0[d]	8.4[d]	5.4[d]	6.4[d]	7.8[i]	6.2[d]
SB224289[e]	5.5	8.0	6.2	<5.0	<5.0	5.8	ND
BRL15572[d]	7.7	6.1	7.9	5.2	6.0	6.6	6.3
Mesulergine	6.2[f]	4.9[f]	5.2[f]	ND	<5.0[g]	9.1[h,k]	8.2[h,k]

[a]Leysen *et al*. (1996); [b]Pregenzer *et al*. (1999); [c]Phebus *et al*. (1997); [d]Price *et al*. (1997); [e]Hagan *et al*. (1997); [f]Hoyer (1988); [g]Adham *et al*. (1993); [h]Hoyer *et al*. (1994); [i]Napier *et al*. (1999); ND, not determined. In all cases the affinity was measured in human receptors, with the exception of [j]gorilla and [k]rat.

1997; Price *et al*., 1997) receptor antagonists as well as 5-HT$_{1D}$ (PNU-142633; Prezenger *et al*., 1999) and 5-ht$_{1F}$ (LY344864; Phebus *et al*., 1997) receptor agonists, the present study was carried out to further analyse the pharmacological characteristics of the GR127935-sensitive 5-HT$_{1B/1D}$ receptors mediating vasocontriction in the canine internal carotid bed. The results show that the pharmacological profile of these receptors resemble the 5-HT$_{1B}$, but not the 5-HT$_{1D}$ or 5-ht$_{1F}$, receptor subtype. 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 33 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 reported (Villalón *et al*., 1997; 1999). 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 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 (Villalón *et al*., 1993; 1999). 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 33 dogs were divided into three groups.

The first group ($n=20$) received consecutive 1 min intracarotid (intra-arterial, i.a.) infusions of 5-HT (0.1, 0.3, 1, 3 and 10 μg min^{-1}). At this point, the dogs were subdivided into five subgroups and the effects produced by the above infusions of 5-HT were induced after i.v. treatment with each dose of either: (i) SB224289 (300 μg kg^{-1}) and, subsequently, ritanserin (100 μg kg^{-1}; $n=5$); (ii) ritanserin (100 μg kg^{-1}) followed by SB224289 (300 μg kg^{-1}; $n=4$); (iii) BRL15572 (300 μg kg^{-1}) followed by ritanserin (100 μg kg^{-1}) and, thereafter, SB224289 (300 μg kg^{-1}; $n=3$); (iv) ritanserin (100 μg kg^{-1}) and, subsequently, BRL15572 (300 μg kg^{-1}) followed by SB224289 (300 μg kg^{-1}; $n=3$); and (v) BRL15572 (300 μg kg^{-1}) and, subsequently, SB224289 (300 μg kg^{-1}) followed by ritanserin (100 μg kg^{-1}; $n=5$). In the last two subgroups (in the dogs of subgroups iv and v), the effects of i.a. infusion of PNU-142633 (1, 3, 10, 30, 100, 300 and 1000 μg min^{-1}; $n=4$) were determined before the administration of 5-HT. In some animals of each of the above subgroups that received the antagonists, the effects produced by 1 min i.a. infusions of sumatriptan (0.3, 1, 3 and 10 μg min^{-1}) were analysed after either the first administration of SB224289 (300 μg kg^{-1}; $n=5$) or BRL15572 (300 μg kg^{-1}; $n=3$).

In the second group ($n=3$), the animals were pretreated with 1000 μg kg^{-1} of the 5-HT$_{2/7}$ receptor antagonist, mesulergine. Then, the effects of i.a. infusions of 5-HT (0.1, 0.3, 1, 3 and 10 μg min^{-1}) were determined before and after the subsequent administration of SB224289 (300 μg kg^{-1}), BRL15572 (300 μg kg^{-1}) and ritanserin (100 μg kg^{-1}).

The last group ($n=10$) received consecutive i.a. infusions of LY344864 (1, 3, 10, 30, 100, 300 and 1000 μg min^{-1}; $n=4$) or sumatriptan (0.3, 1, 3 and 10 μg min^{-1}; $n=6$).

The dose-intervals between the different doses of agonists 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 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 percent 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 (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Furthermore, the peak percent changes in internal carotid blood flow induced by sumatriptan 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, from Sigma, St. Louis, MO, U.S.A.); SB224289 (2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo[2,3f]indole-3-spiro-4′-piperidine hydrochloride) and BRL15572 (1-(3-chlorophenyl)4-[3,3-diphenyl(2-(S,R) hydroxy propanyl)piperazine]hydrochloride) (both gifts from Dr A.A. Parsons, SmithKline Beecham Pharmaceuticals, Harlow, Essex, U.K.); ritanserin (gift from Dr J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium); sumatriptan succinate (gift from Dr H.E. Connor, Glaxo Group Research, Stevenage, Hertfordshire, U.K.); LY344864 (N-[3-(dimethylamino)-2,3,4,9-tetrahydro-1H-carbazol-6-yl]-4-fluorobenzamide; gift from Eli Lilly & Co., Indianapolis, IN, U.S.A.); PNU-142633 ((S)-(−)-3,4-dihydro-1-[2-[4-(4-aminocarbonyl) phenyl]-1-piperazinyl]ethyl]-N-methyl-1H-2-benzopyran-6-carboxamide; gift from Dr R.B. McCall, Pharmacia & Upjohn, Kalamazoo, MI, U.S.A.); mesulergine hydrochloride (gift from Sandoz A.G., Basel, Switzerland). All compounds were dissolved in distilled water. When needed, 4% (w v^{-1}) ascorbic acid (ritanserin), 20% (v v^{-1}) DMSO (SB224289) or 20% (v v^{-1}) propylene glycol (BRL15572) 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 and sumatriptan, where they refer to the free base.

Results

Systemic haemodynamic variables

The baseline values of mean arterial pressure, heart rate and internal carotid blood flow in the 33 dogs were 138 ± 4 mmHg, 166 ± 4 beats min^{-1} and 50 ± 3 ml min^{-1}, respectively. These haemodynamic values before and after administration of the 5-HT receptor antagonists are shown in Table 2. All haemodynamic variables remained without significant ($P>0.05$) changes after the administration of ritanserin, SB224289, BRL15572 or mesulergine.

Initial effects of agonist drugs on internal carotid blood flow

As previously demonstrated (Centurión *et al*., 2001), Figure 1 shows that i.a. infusions of 5-HT (0.1, 0.3, 1, 3 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 effects were not accompanied by changes in mean arterial blood pressure or heart rate (data not shown). The rank order of agonist potency was 5-HT > sumatriptan. At the doses used (see above), the duration of the responses elicited by sumatriptan (3.6 ± 0.7, 6.2 ± 1.8, 9.4 ± 3 and 15.7 ± 3 min) was longer than that of 5-HT (3 ± 0.4, 6 ± 0.4, 7 ± 0.4, 9 ± 0.5 and 10 ± 0.4 min). In contrast, LY344864 or PNU-142633 (both 1–1000 μg min^{-1}) failed to decrease the internal carotid blood flow *per se* (Figure 1) or to antagonize the vasoconstrictor responses to 5-HT (not shown).

Effect of 5-HT receptor antagonists on the decreases in internal carotid blood flow induced by 5-HT

The effects of several 5-HT receptor antagonists on the responses induced by 5-HT are depicted in Figures 2 and 3. We have previously demonstrated that the decreases in

Table 2 Mean arterial blood pressure, heart rate and internal carotid blood flow before and after i.v. administration of ritanserin, SB224289, BRL15572 or mesulergine in anaesthetized vagosympathectomized dogs

			Mean arterial blood pressure (mmHg)		*Heart rate* (beats min^{-1})		*Internal carotid blood flow* (ml min^{-1})	
Treatments	*Dose* (μg kg^{-1})	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Ritanserin	100	7	113 ± 13	116 ± 13	157 ± 10	150 ± 10	43 ± 10	44 ± 10
SB224289	300	5	135 ± 10	143 ± 8	158 ± 10	151 ± 10	44 ± 10	44 ± 10
BRL15572	300	8	155 ± 7	170 ± 8	164 ± 8	163 ± 6	43 ± 5	40 ± 5
Mesulergine	1000	3	155 ± 19	151 ± 19	140 ± 7	144 ± 11	48 ± 3	45 ± 3

All values are presented as mean + s.e.mean. $P<0.05$, after *vs* before from the corresponding baseline value.

internal carotid blood flow produced by 5-HT are highly reproducible as they 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 (Centurión *et al*., 2001). As shown in Figure 2a, SB224289 (300 μg kg^{-1}) did not apparently modify the vasocontrictor responses induced by 5-HT. However, in these animals, the subsequent administration of ritanserin (100 μg kg^{-1}) abolished the 5-HT-induced vasoconstriction and unmasked a dose-dependent vasodilator component (Figure 2a). When changing the order of antagonist administration, initially 100 μg kg^{-1} of ritanserin did not apparently antagonize the responses to 5-HT, but the subsequent administration of 300 μg kg^{-1} of SB224289 abolished the vasoconstrictor responses to 5-HT unmasking a vasodilator component (Figure 2b). Furthermore, Figure 2c shows that BRL15572 (300 μg kg^{-1}) did not block the responses to 5-HT; the subsequent administration of ritanserin (100 μg kg^{-1}) also failed to antagonize the responses to 5-HT (Figure 2c). In these animals, the subsequent administration of SB224289 (300 μg kg^{-1}) abolished the vasoconstrictor responses to 5-HT and, again, unmasked a vasodilator effect (Figure 2c). Moreover, as shown in Figure 3a, when this order of administration is inverted, neither the initial administration of ritanserin nor the subsequent administration of BRL15572 (300 μg kg^{-1}) modified the vasoconstrictor responses to 5-HT. In these animals, the subsequent administration of SB224289 (300 μg kg^{-1}) not only blocked the vasoconstrictor responses to 5-HT, but also unmasked a vasodilator component (Figure 3a). In addition, Figure 3b shows that the initial administration of BRL15572 (300 μg kg^{-1}) did not modify the vasoconstrictor responses to 5-HT; in these animals, the subsequent administration SB224289 (300 μg kg^{-1}) did not affect (except at 10 μg min^{-1}) the vasoconstrictor responses to 5-HT (Figure 3b). Notwithstanding, the subsequent administration of ritanserin (100 μg kg^{-1}) to these animals completely antagonized the vasoconstrictor responses to 5-HT (Figure 3b), unmasking a vasodilator component.

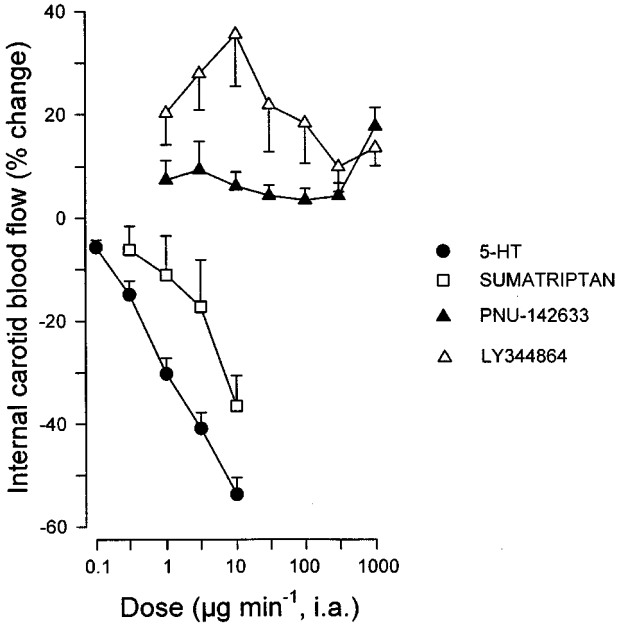

Figure 1 Comparative effects of 1 min intra-arterial (i.a.) infusions of 5-HT ($n=20$), sumatriptan ($n=6$), PNU-142633 ($n=4$) or LY344864 ($n=4$) on the internal carotid blood flow of anaesthetized vagosympathectomized dogs. All values are presented as mean$\pm$ s.e.mean.

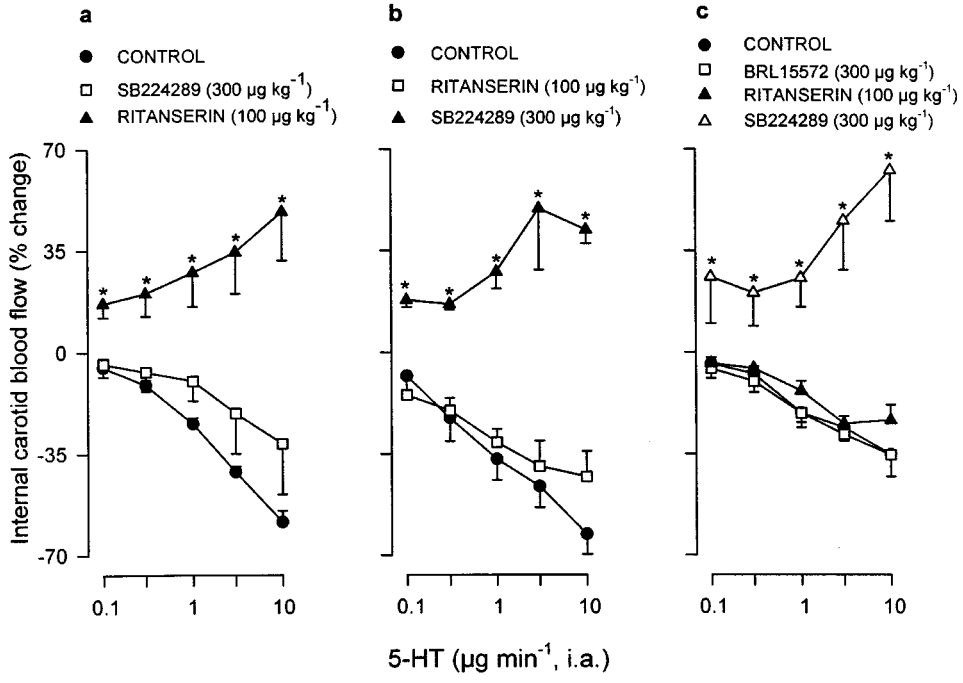

Figure 2 The effects of (a) SB224289 followed by ritanserin ($n=5$); (b) ritanserin followed by SB224289 ($n=4$) and (c) BRL15572 followed by ritanserin and, subsequently, SB224289 ($n=3$) on the decreases in internal carotid blood flow induced by 1 min intra-arterial (i.a.) infusions of 5-HT in anaesthetized vagosympathectomized dogs. *$P<0.05$ *vs* control. All values are presented as mean$\pm$s.e.mean.

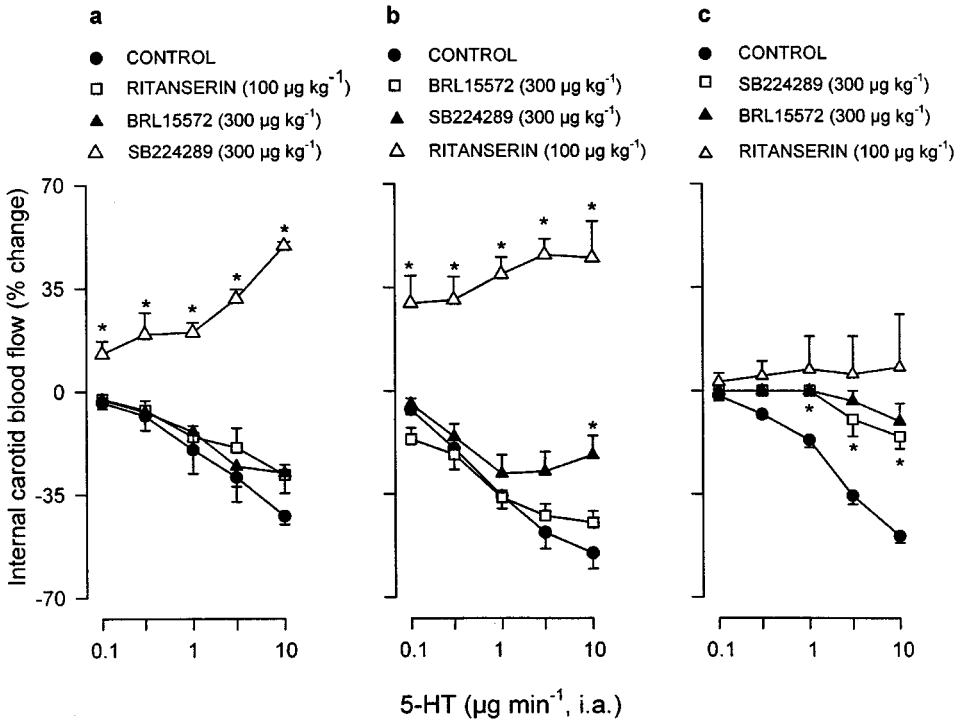

Figure 3 The effects of the subsequent administration of : (a) ritanserin, BRL15572 and SB224289 ($n=3$); (b) BRL15572, SB224289 and ritanserin ($n=5$); and (c) SB224289, BRL15572 and ritanserin ($n=3$) in animals pretreated with 1000 μg kg^{-1} of mesulergine, on the decreases in internal carotid blood 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.

With these findings, it is evident that after blockade of internal carotid vasoconstrictor receptors, 5-HT invariably produced a vasodilator effect mediated by 5-HT$_7$ receptors (Centurión *et al*., 2000), which may exert a physiological antagonism. Therefore, we decided to analyse the effects of the antagonists SB224289 (300 μg kg^{-1}), BRL15572 (300 μg kg^{-1}) and ritanserin (100 μg kg^{-1}) in animals pretreated with 1000 μg kg^{-1} of mesulergine; this ergoline is an antagonist at 5-HT$_2$ and 5-HT$_7$ receptors, which does not interact with the 5-HT$_1$ receptor family (see Table 1; Hoyer *et al*., 1994). Interestingly, under these conditions, the initial i.v. administration of SB224289 (300 μg kg^{-1}) practically abolished the 5-HT-induced vasoconstrictor responses (Figure 3c). After administration of BRL15572 (300 μg kg^{-1}) no further blockade was produced and the subsequent administration of ritanserin was similarly inactive (i.e., no vasodilator component was unmasked; Figure 3c).

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

As shown in Figure 4, sumatriptan produced dose-dependent decreases in internal carotid blood flow in animals pretreated with physiological saline. These effects were not accompanied by changes in blood pressure or heart rate (not shown). As depicted in Figure 4, the sumatriptan-induced vasoconstrictor responses were abolished in animals pretreated with SB224289 (300 μg kg^{-1}). In contrast, in animals pretreated with BRL15572 (300 μg kg^{-1}), the vasoconstrictor effects of sumatriptan were not significantly modified.

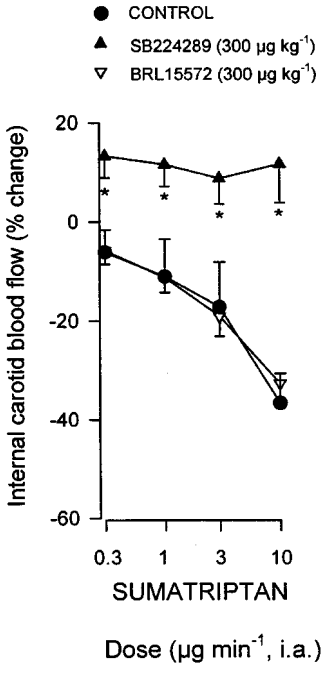

Figure 4 The decreases in internal carotid blood flow induced by 1 min intra-arterial (i.a.) infusions of sumatriptan in anaesthetized vagosympathectomized dogs pretreated with either saline ($n=6$), SB224289 ($n=5$) or BRL15572 ($n=3$). *$P<0.05$ *vs* the corresponding response in saline pretreated animals. All values are presented as mean$\pm$s.e.mean.

Discussion

General

It has been previously shown that 5-HT produces vasoconstriction in the canine internal carotid circulation by methysergide-sensitive 5-HT receptors (Vidrio & Hong, 1976). More recently, Centurión *et al*. (2001) have shown that this response to 5-HT, being mimicked by sumatriptan, 5-methoxytryptamine and DOI and blocked by GR127935 and ritanserin, mainly involves 5-HT$_{1B/1D}$ and 5-HT$_{2A}$ receptors. Admittedly, sumatriptan and GR127935 display also a moderate affinity for 5-ht$_{1F}$ receptors (see Table 1), hence the possible involvement of 5-ht$_{1F}$ receptors could not be categorically excluded (Centurión *et al*., 2001). With the recent advent of selective ligands at 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-ht$_{1F}$ receptors (see Table 1), the present study was mainly focused on investigating the possible involvement of one or more of these receptors.

Our results show that 5-HT-induced internal carotid vasoconstriction is: (1) not mimicked by the 5-HT$_{1D}$ receptor agonist PNU-142633 or the 5-ht$_{1F}$ receptor agonist, LY344864; (2) not affected by SB224289 or ritanserin alone, but potently antagonized by the combination of these two drugs; (3) essentially unaffected by BRL15572 or the combination of BRL15572 and ritanserin or BRL15572 and SB224289. Furthermore, after blockade of 5-HT$_2$ and 5-HT$_7$ receptors with mesulergine, SB224289, but not BRL15572, clearly abolished the vasoconstriction induced by 5-HT. Consistent with these findings, pretreatment with SB224289 abolished the responses to sumatriptan, whilst BRL15572 was inactive. Apart from the implications discussed below, the above data indicate that the GR127935-sensitive 5-HT$_1$ receptors mediating canine internal carotid vasoconstriction resemble the 5-HT$_{1B}$, but not the 5-HT$_{1D}$ or 5-ht$_{1F}$, receptor subtype.

Internal carotid haemodynamic changes produced by i.a. infusions of 5-HT, sumatriptan, PNU-142633 or LY344864

Intra-arterial infusions of 5-HT and sumatriptan produced dose-dependent vasoconstrictor responses in the internal carotid bed, as previously shown (Centurión *et al*., 2001). These vasoconstrictor responses to 5-HT are highly reproducible, as they remained essentially unchanged after three i.v. bolus injections of physiological saline (Centurión *et al*., 2001); 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. Furthermore, based on the vasoconstrictor activity of sumatriptan and its binding profile (see Table 1), it could be suggested that, in principle, 5-HT$_{1B/1D}$ and/or 5-ht$_{1F}$ receptors may be involved. However, as shown in Figure 1, the lack of vasoconstrictor effect of the selective 5-HT$_{1D}$ receptor agonist, PNU-142633 (Prezenger *et al*., 1999), or the selective 5-ht$_{1F}$ receptor agonist, LY344864 (Phebus *et al*., 1997), suggests that 5-HT$_{1D}$ and 5-ht$_{1F}$ receptors are not involved. This suggestion is strengthened when considering that LY344864 is also inactive in the canine external carotid circulation (De Vries *et al*., 1998) and other vascular preparations (Cohen & Schenck, 1999; Bouchelet *et al*., 2000).

Evidence that 5-HT$_{1B}$ receptors are involved on the 5-HT-induced decreases in canine internal carotid blood flow

Based on the fact that the canine external carotid as well as the porcine common carotid vasoconstrictor 5-HT$_{1B/1D}$ receptors resemble the 5-HT$_{1B}$, rather than the 5-HT$_{1D}$, receptor subtype (De Vries *et al*., 1998; 1999), we decided to investigate whether the 5-HT$_{1B}$ receptors were also producing canine internal carotid vasoconstriction. For this purpose, we employed SB224289 and BRL15572 which are selective antagonists at, respectively, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Hagan *et al*., 1997; Price *et al*., 1997).

Surprisingly, it was found that SB224289, at a dose (300 μg kg^{-1}) that completely blocked the canine external carotid vasoconstrictor responses to 5-HT and sumatriptan (De Vries *et al*., 1998), apparently failed to block the internal carotid vasoconstrictor responses to 5-HT (Figure 2a). Although this finding suggests that 5-HT$_{1B}$ receptors may not be involved, it is significant that the subsequent administration of ritanserin abolished the vasoconstrictor responses to 5-HT, unmasking a dose-dependent vasodilator component to 5-HT (Figure 2a). Similarly, when reversing the order of administration of these antagonists, the responses to 5-HT remained initially unmodified after ritanserin, but were abolished and reverted to vasodilatation after administration of SB224289 (Figure 2b). This apparent lack of antagonism by ritanserin or SB224289, when given alone, is highly likely to be explained by the fact that 5-HT can stimulate vasoconstrictor 5-HT$_{1B}$ and 5-HT$_2$ receptors (present results), as well as vasodilator 5-HT$_7$ receptors (Centurión *et al*., 2000). Thus, when blocking only one of these two vasoconstrictor receptors, the vasoconstriction produced by the unblocked receptor will overshadow the antagonism of the other receptor. Therefore, we considered it of major importance in *in vivo* studies to analyse the effect of combinations of antagonists (see Figures 2 and 3a,b). It should also be pointed out here that the doses of ritanserin and SB224289 used in the present study have previously been shown to produce selective blockade of their respective receptors (Villalón *et al*., 1993; 1996; De Vries *et al*., 1998; 1999). It is worthy of note that after BRL15572 and SB224289, a combination which seems to mimic the blocking capability of GR127935 at 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (see Table 1), the responses to 5-HT remained basically unaffected. Thus, it could be hypothesized that GR127935 blocked the responses to 5-HT (Centurión *et al*., 2001) because it also has moderate affinity for 5-HT$_{2A}$ receptors (see Table 1). Moreover, we felt that this blockade by GR127935 may also involve a physiological antagonism induced by 5-HT itself, mediated by a 5-HT$_7$ receptor-induced internal carotid vasodilation (Centurión *et al*., 2000). In order to corroborate this possibility, it would seem ideal to investigate the effects of SB224289 in animals in which vasoconstrictor 5-HT$_2$ receptors and vasodilator 5-HT$_7$ receptors have been previously blocked. In fact, this was achieved in animals pretreated with mesulergine, a 5-HT$_{2/7}$ receptor antagonist that does not interact with the 5-HT$_1$ receptor family (see Table 1). It must be emphasized that 1000 μg kg^{-1} of mesulergine is a dose high enough to specifically antagonize canine internal carotid vasodilator 5-HT$_7$ receptors (Centurión *et al*., 2000). Under these

conditions, the involvement of 5-HT$_{1B}$ receptors was definitely confirmed, as SB224289 practically abolished the vasoconstrictor responses to 5-HT (Figure 3c). Since the subsequent administration of BRL15572 followed by ritanserin did not produce further blockade (which may have been made manifest as the disclosure of a vasodilator component), the role of 5-HT$_{1B}$ receptors is clearly established. Admittedly, this conclusion is based on the assumption that species differences between the binding of sumatriptan, PNU-142633, LY344864, SB224289, BRL15572 and mesulergine to canine, human, gorilla and rat 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors do not play a major role (for references see Table 1).

Evidence against the involvement of internal carotid vasoconstrictor 5-HT$_{1D}$ receptors

The above findings, following an exclusion line of reasoning, do not seem to support the possible involvement of 5-HT$_{1D}$ receptors. In support of this view: (i) the 5-HT$_{1D}$ receptor agonist PNU-142633 (Prezenger *et al*., 1999) failed to mimic the vasoconstrictor response to 5-HT (Figure 1); (ii) SB224289, but not BRL15572, antagonized the vasoconstrictor effects of sumatriptan (Figure 4); and (iii) in one dog pretreated with 1000 μg kg^{-1} of mesulergine, the initial administration of BRL15572 did not modify the vasoconstrictor responses to 5-HT, but the subsequent administration of SB224289 abolished this response (data not shown), as demonstrated when this order was reversed (Figure 3c). Consistent with these findings, there is poor evidence that 5-HT$_{1D}$ receptors mediate vasoconstrictor effects. In this respect: (1) PNU-109291, another selective 5-HT$_{1D}$ receptor agonist (Ennis *et al*., 1998), did not elicit contraction in the human coronary artery (Nilsson *et al*., 1999) as well as in the human and bovine cerebral arteries (Bouchelet *et al*., 2000) or the feline carotid vasculature (Ennis *et al*., 1998); (2) PNU-142633 did not produce feline carotid vasoconstriction (Prezenger *et al*., 1999); and (3) no *m*RNA encoding 5-HT$_{1D}$ receptors has been shown in the human internal carotid artery (Schmuck *et al*., 1996). As previously stated, this conclusion is based on the assumption that species differences between the binding of SB224289, BRL15572 and PNU-142633 to canine, human and gorilla 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors do not play a major role (see Table 1).

Lack of resemblance of the internal carotid vasoconstrictor receptors to 5-ht$_{1F}$ receptors

The blockade of GR127935 (Centurión *et al*., 2001) and the activity of sumatriptan (Figure 4), drugs which have moderate affinity for 5-ht$_{1F}$ receptors (see Table 1), suggest that 5-ht$_{1F}$ receptors may be involved. Nevertheless, this seems unlikely because the 5-ht$_{1F}$ receptor agonist, LY344864 (see Table 1), did not produce vasoconstriction in the canine internal carotid bed (Figure 1). In line with this contention: (1) LY344864 does not produce vasoconstriction in the human and bovine cerebral arteries (Bouchelet *et al*., 2000), the canine external carotid bed (De Vries *et al*., 1998) or the rabbit saphenous vein (Cohen & Schenck, 1999); (2) no *m*RNA has been found in the human internal carotid artery (Schmuck *et al*., 1996); and (3) the 5-ht$_{1F}$ receptor agonist, LY334370, an analogue of LY344864 (Phebus *et al*., 1997), did not produce contraction of the human coronary artery (Nilsson *et al*., 1999).

Conclusion

The results of the present study in vagosympathectomized dogs show that the GR127935-sensitive 5-HT$_1$ receptors mediating internal carotid vasoconstriction resemble the 5-HT$_{1B}$ receptors, but not 5-HT$_{1D}$ or 5-ht$_{1F}$ receptor subtype. These internal carotid vasoconstrictor receptors are similar to the 5-HT$_{1B}$ receptors mediating vasoconstrictor responses in other vascular preparations, including the canine external carotid bed (De Vries *et al*., 1998), human temporal artery (Verheggen *et al*., 1998), porcine arteriovenous anastomoses (De Vries *et al*., 1999), human pulmonar arteries (Morecroft *et al*., 1999) and human and bovine cerebral arteries (Bouchelet *et al*., 2000). Moreover, when 5-HT is infused, a perceptible antagonism of 5-HT$_{1B}$ receptors with SB224289 can only be shown after simultaneous antagonism of 5-HT$_{2A}$ and 5-HT$_7$ receptors (with mesulergine; see Figure 3c and Table 1), given the coexistence of vasoconstrictor 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors as well as vasodilator 5-HT$_7$ receptors in the internal carotid bed.

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