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Short communication

The canine external carotid vasoconstrictor 5-HT_1 receptor: blockade by 5-HT_{1B} (SB224289), but not by 5-HT_{1D} (BRL15572) receptor antagonists

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Abstract

In vagosympathectomised dogs pre-treated intravenously (i.v.) with mesulergine (300 μg/kg), 1-min intracarotid (i.c.) infusions of 5-hydroxytryptamine (5-HT; 0.3–30 μg/min) and sumatriptan (1–30 μg/min) dose-dependently decreased external carotid blood flow, without affecting mean blood pressure or heart rate. Treatment with the selective 5-HT_{1B} receptor antagonist SB224289 (2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl]furo[2,3*f*]indole-3-spiro-4′-piperidine hydrochloride; 30–300 μg/kg, i.v.) produced a potent, specific and dose-dependent blockade of this response, whereas the selective 5-HT_{1D} receptor antagonist BRL15572 (1-(3-chlorophenyl)-4-[3,3-diphenyl(2-(*S*,*R*) hydroxypropanyl)piperazine]hydrochloride; 30–300 μg/kg, i.v.) was ineffective. It is concluded that mainly 5-HT_{1B}, but not 5-HT_{1D} receptors mediate the canine external carotid vasoconstriction by 5-HT and sumatriptan. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: 5-HT_{1B} receptor; BRL15572; Carotid vasoconstriction, canine, external; SB224289; Sumatriptan

1. Introduction

We have previously reported that 1-min intracarotid (i.c.) infusions of 5-hydroxytryptamine (5-HT) or sumatriptan produce selective vasoconstriction in the external carotid vascular bed of vagosympathectomized dogs via 5-HT_1-like receptors (Villalón et al., 1995). Subsequently, it was shown that these 5-HT_1-like receptors closely resemble the 5-$HT_{1B/1D}$ receptor subtypes (Villalón et al., 1996), as they are highly sensitive to the antagonist action of GR127935, a potent and selective 5-$HT_{1B/1D}$ receptor ligand (Skingle et al., 1996). Based on the presence of 5-HT_{1B}, but not of 5-HT_{1D} receptor mRNA in vascular smooth muscle (Ullmer et al., 1995; Bouchelet et al., 1996), it was suggested that the receptor mediating this response appears to be of the 5-HT_{1B} subtype (Villalón et al., 1996). Notwithstanding, it remained virtually impossible to pharmacologically attribute the external carotid vasoconstrictor responses to either the 5-HT_{1B} or 5-HT_{1D} receptor due to the lack of potent and selective ligands at these subtypes. In this context it should be emphasised that, in contrast to other species, ketanserin cannot discriminate between the canine 5-HT_{1B} and 5-HT_{1D} receptor subtypes (Branchek et al., 1995). Considering the recent availability of potent and selective antagonists at either 5-HT_{1B} (SB224289) or 5-HT_{1D} (BRL15572) receptors (Hagan et al., 1997), in the present study we decided to further analyse the 5-$HT_{1B/1D}$ receptors involved in canine external carotid vasoconstrictor responses to 5-HT and sumatriptan.

2. Materials and methods

Experiments were carried out in a total of 12 dogs (16–26 kg) not selected for breed or sex. The animals were anaesthetized with intravenously (i.v.) administered sodium pentobarbitone (30 mg/kg) and additional amounts (1 mg/kg) were provided when required. All dogs were

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intubated with an endotracheal tube and artificially respired with room air using a Palmer ventilation pump at a rate of 20 strokes/min and a stroke volume of 13–16 ml/kg. Bilateral cervical vagosympathectomy was systematically performed and mean arterial blood pressure and heart rate were recorded. The right femoral vein was cannulated for drug injection. The right common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. The blood flow through the right common carotid artery, measured with ultrasonic flowmetry, was considered as the external carotid blood flow (for further details see Villalón et al., 1993). The agonists were administered into the right common carotid artery with a cannula inserted into the right cranial thyroid artery. The body temperature of the animals was maintained between 37 and 38°C.

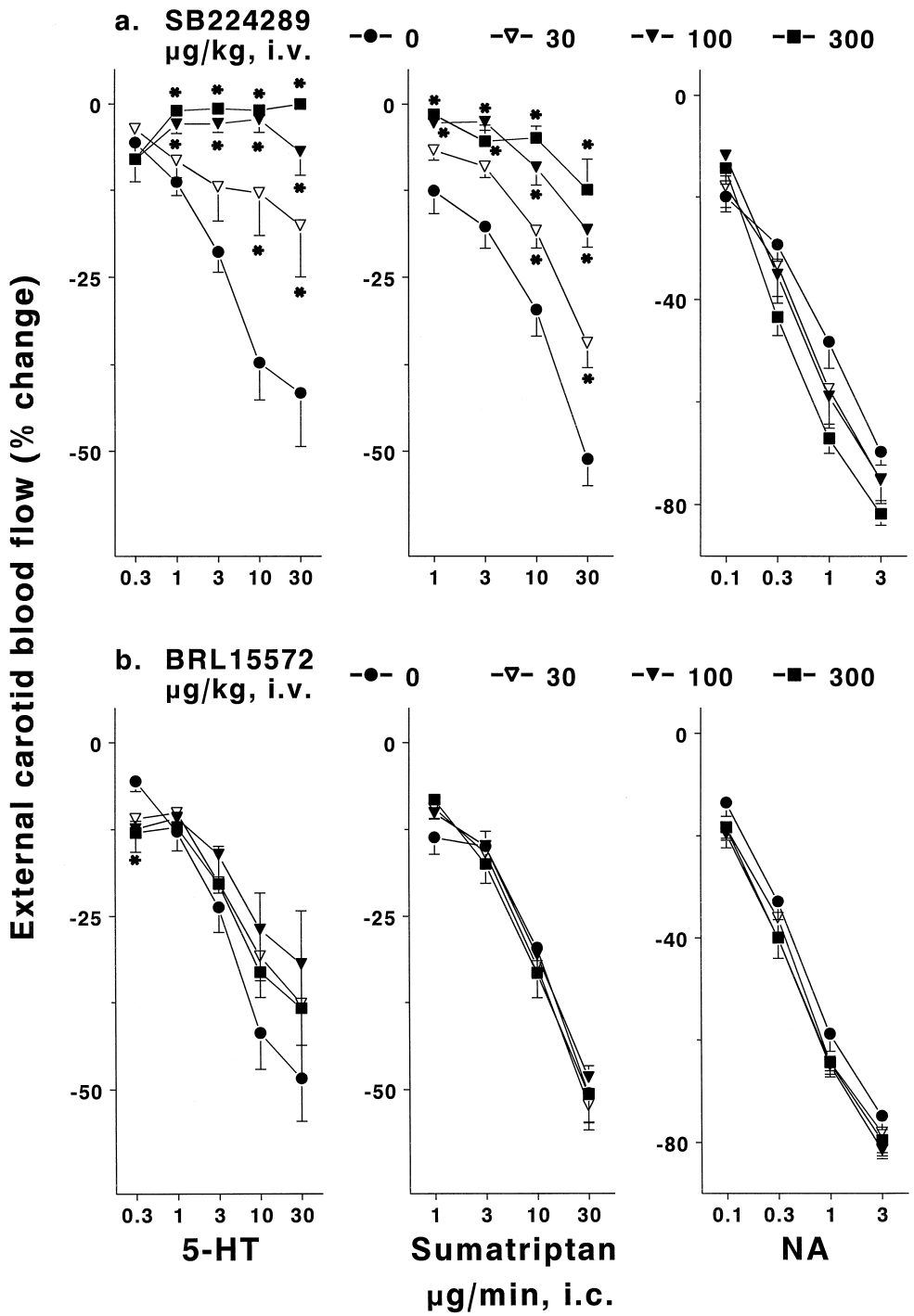

Fig. 1. The effects of SB224289 (a) and BRL15572 (b) on the decreases in canine external carotid blood flow observed with 1-min intracarotid (i.c.) infusions of 5-HT, sumatriptan or noradrenaline (NA). All values are presented as mean ± S.E.M. $* P < 0.05$ vs. control.

After a stable haemodynamic condition for at least 30 min, baseline values of blood pressure, heart rate and external carotid blood flow were determined. After i.v. pre-treatment with 300 μg/kg of mesulergine, in order to block 5-HT_7 receptor-mediated external carotid vasodilatation (see Villalón et al., 1997), sequential 1-min i.c. infusions of 5-HT (0.3, 1, 3, 10 and 30 μg/min), sumatriptan (1, 3, 10 and 30 μg/min) and noradrenaline (0.1, 0.3, 1 and 3 μg/min) were given. The animals were then divided into 2 groups ($n = 6$ each), where the responses to the above agonists were reanalysed after i.v. treatment with 30, 100 and 300 μg/kg of either SB224289 or BRL15572. The dose-intervals between the different doses of agonists ranged between 5 and 20 min, as in each case we waited until the blood flow had returned completely to baseline values. After the administration of an antagonist or saline a period of about 10 min was allowed to elapse before the responses to the respective agonists were elicited.

The drugs used in this study were: 5-HT creatinine sulphate and noradrenaline bitartrate (Sigma, St. Louis, MO, USA), sumatriptan succinate (gift: Dr. M. Skingle, Glaxo Group Research, Ware, UK), mesulergine hydrochloride (gift: Sandoz, Basel, Switzerland) and SB224289 and BRL15572 (both gifts from Dr. A.A. Parsons, SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK). All compounds were dissolved in distilled water; when needed 20% (v/v) propylene glycol (SB224289 and BRL15572) was added. This vehicle did not affect any haemodynamic variable. The peak changes in external carotid blood flow by the agonists, expressed as percent changes from baseline, before and after the different doses of antagonists were compared by Student's paired *t*-test. A *P*-value of 0.05 or less (two-tailed) was considered statistically significant. All data are reported as mean ± S.E.M.

3. Results

3.1. Initial effects of 5-HT, sumatriptan and noradrenaline on external carotid blood flow

Baseline values ($n = 12$) of mean arterial blood pressure, heart rate and external carotid blood flow were 125 ± 2 mm Hg, 175 ± 3 beats/min and 184 ± 5 ml/min, respectively. 5-HT (0.3, 1, 3, 10 and 30 μg/min), sumatriptan (1, 3, 10 and 30 μg/min) and noradrenaline (0.1, 0.3, 1 and 3 μg/min) produced dose-dependent decreases in external carotid blood flow in mesulergine-pre-treated vagosympathectomized dogs (Fig. 1). The apparent rank order of agonist potency was noradrenaline > 5-HT ≥ sumatriptan. One-minute i.c. infusions of corresponding volumes of saline did not affect any haemodynamic parameter for the duration of the experiments (data not shown). Since the agonists were devoid of effects on mean arterial blood pressure and heart rate (data not shown), a local vasoconstrictor effect in the external carotid vasculature is implied, as previously discussed (Villalón et al., 1993). The 5-HT-induced decreases in external carotid blood flow were preceded by small, non-dose-dependent increases in external carotid blood flow (see below).

3.2. Effects of SB224289 and BRl15572 on the external carotid vascular responses to 5-HT, sumatriptan and noradrenaline

As shown in Fig. 1a, SB224289 dose-dependently antagonised the external carotid vasoconstriction induced by 5-HT and sumatriptan, without affecting that to noradrenaline. Additionally, after SB224289 the non-dose-dependent 5-HT-induced increases in external carotid blood flow were potentiated and a dose-dependency was unmasked. The increases after 0.3, 1, 3, 10 and 30 μg/min of 5-HT were 6.6 ± 2.7, 15.5 ± 6.5, 17.2 ± 10.3, 14.0 ± 6.6 and 13.1 ± 9.9%, respectively before SB224289 and 0.5 ± 0.1, 7.2 ± 3.5, 21.8 ± 9.9, 43.6 ± 14.8 and 63.2 ± 17.4%, respectively after the highest dose of SB224289 (300 μg/kg). In contrast, BRL15572 did not inhibit the decreases in external carotid blood flow by either 5-HT, sumatriptan or noradrenaline (Fig. 1b); at 300 μg/kg, BRL15572 even slightly enhanced the decrease in external carotid blood flow by 0.3 μg/min of 5-HT. The small increases in the external carotid blood flow by 5-HT remained unchanged after BRL15572 (data not given).

The doses of the antagonists used in this study were devoid of any haemodynamic effects per se.

4. Discussion

We have previously shown that 5-HT and sumatriptan constrict the canine external carotid vasculature via GR127935-sensitive 5-$HT_{1B/1D}$ receptors (Villalón et al., 1996). The recent availability of silent and selective antagonists for the 5-HT_{1B} and 5-HT_{1D} receptor subtypes led us to further analyse the receptors mediating these responses.

5-HT, sumatriptan and noradrenaline produced external carotid vasoconstriction with an apparent rank order of agonist potency (noradrenaline > 5-HT ≥ sumatriptan), similar to that reported earlier (Villalón et al., 1993, 1995, 1996). These effects are highly reproducible as they remain essentially unchanged after three subsequent infusions of saline (Villalón et al., 1995, 1996). The 5-HT_{1B} receptor ligand SB224289 (pK_i values of 8.0 and 6.2 at human 5-HT_{1B} and 5-HT_{1D} receptors, respectively; Hagan et al., 1997) dose-dependently antagonised these responses and the highest dose of SB224289 virtually abolished both the 5-HT- as well as sumatriptan-induced carotid vasoconstriction. This antagonism by SB224289 was specific, as the noradrenaline-induced effects remained unaffected. In contrast, the 5-HT_{1D} receptor ligand BRL15572 (pK_i values of 6.1 and 7.9 at human 5-HT_{1B} and 5-HT_{1D} receptors, respectively; Hagan et al., 1997) did not affect the carotid

vascular effects of 5-HT, sumatriptan or noradrenaline in any way. As SB224289 and BRL15572 display similar affinities at their respective receptors (see above), the lack of inhibitory effects by BRL15572, combined with the potent blockade by SB224289 at similar doses, clearly indicates that 5-HT_{1B}, but not 5-HT_{1D} receptors, are involved in the canine external carotid vasoconstriction by 5-HT and sumatriptan. Admittedly, this conclusion is based on the assumption that species differences between the binding of SB224289 and BRL15572 to canine and human 5-HT_{1B} and 5-HT_{1D} receptors do not play a major role. In any case, the involvement of 5-HT_{1B}, but not 5-HT_{1D} receptors is supported by the following observations: (i) the 5-HT-induced contraction of the human isolated temporal artery is similarly antagonized by SB224289 with a potency of 1 nM, but not by BRL15572 in doses up to 500 nM (Verheggen et al., 1998); (ii) mRNA (Bouchelet et al., 1996; Verheggen et al., 1998) and even the corresponding receptor protein (Longmore et al., 1997) of the 5-HT_{1B}, but not of the 5-HT_{1D} receptor, has been detected in cranial blood vessels; and (iii) high doses of ketanserin or ritanserin (potential 5-HT_{1D} receptor antagonists) do not display antagonism against sumatriptan-induced vasoconstriction (for reviews see Kaumann et al., 1993; Saxena et al., 1998). Additionally, in view that SB224289 produced a complete blockade of the 5-HT- and sumatriptan-induced carotid vascular effects, it seems highly unlikely that additional receptors/mechanisms play a role, if any.

The 5-HT-induced decreases in external carotid blood flow, but not those to sumatriptan and noradrenaline, were preceded by a small vasodilator response, which was enhanced after blockade of the carotid vasoconstriction by SB224289, as also previously observed with GR127935 (Villalón et al., 1996). This carotid vasodilator response was recently shown to be mediated by the 5-HT_7 receptor (Villalón et al., 1997). Indeed, due to the systematic pre-treatment in the present experiments with the 5-HT_7 receptor ligand, mesulergine, the magnitude of the external carotid dilatory responses was much less marked than that observed previously (Villalón et al., 1997).

In conclusion, using selective antagonists the present results obtained in the canine external carotid vasculature represent the first in vivo evidence that vascular constriction induced by 5-HT and sumatriptan is mediated primarily via 5-HT_{1B}, but not 5-HT_{1D} receptors. Thus, SB224289 and BRL15572 seem to be excellent tools for further investigating the pharmacology of the 5-$HT_{1B/1D}$ receptors.

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