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Short communication

5-HT_{1B} receptors and $\alpha_{2A/2C}$-adrenoceptors mediate external carotid vasoconstriction to dihydroergotamine

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Abstract

Dihydroergotamine produces external carotid vasoconstriction in vagosympathectomized dogs by 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. This study identified the specific subtypes involved in this response. One-minute intracarotid infusions of dihydroergotamine (5.6–10 μg/min) dose-dependently decreased external carotid blood flow without affecting blood pressure or heart rate. This response was: (1) partly blocked in dogs pretreated intravenously with the antagonists SB224289 (5-HT_{1B}; 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), rauwolscine (α_2), BRL44408 (α_{2A}; 2-[2H-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazole) or MK912 (α_{2C}; (2S,12bS)-13′-dimethylspiro(1,3,4,5′,6,6′,7,12b-octahydro-2Hbenzo[b]furo[2,3-a]quinazoline)-2,4′-pyrimidin-2′-one); (2) markedly blocked after SB224289 plus rauwolscine; and (3) unaffected after BRL15572 (5-HT_{1D}; 1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(S,R) hydroxypropanyl) piperazine] hydrochloride) or imiloxan (α_{2B}). Therefore, the above response involves 5-HT_{1B} receptors and $\alpha_{2A/2C}$-adrenoceptors.

Keywords: BRL44408; Dihydroergotamine; (Dog); Carotid vasoconstriction external; MK912; SB224289

1. Introduction

Several studies suggest that vasodilatation in the extracranial blood vessels, including the external carotid bed, may play a role in the pathophysiology of migraine headache (Villalón et al., 2002). Indeed, sumatriptan, ergotamine and dihydroergotamine, which are effective in the acute treatment of migraine, produce potent carotid vasoconstriction in some models predictive for antimigraine activity, including the porcine (De Vries et al., 1998b), rabbit (De Vries et al., 1997) and canine (De Vries et al., 1998a; Villalón et al., 1999) external carotid circulations.

In this respect, we have previously shown in vagosympathectomized anaesthetised dogs that the external carotid vasoconstrictor responses to intracarotid infusions of ergotamine and dihydroergotamine are partly mediated by 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors (Villalón et al., 1999), whilst those to sumatriptan are exclusively mediated by 5-HT_{1B} receptors (De Vries et al., 1998a). Moreover, activation of both α_1- and α_2-adrenoceptors produces canine external carotid vasoconstriction (Willems et al., 2001a); these receptors correlate with the α_{1A}-, α_{1D}-, α_{2A}- and α_{2C}-adrenoceptor subtypes (Willems et al., 2001c).

On this basis, the present study was designed to identify the specific subtypes of 5-$HT_{1B/1D}$ (5-HT_{1B} or 5-HT_{1D}) receptors as well as α_2-adrenoceptors (α_{2A}, α_{2B} or α_{2C}) mediating the canine external carotid vasoconstriction to dihydroergotamine.

2. Materials and methods

Experiments were carried out in a total of 32 male mongrel dogs (25–30 kg) that were anaesthetized with an intravenous (i.v.) bolus injection of sodium pentobarbitone (30 mg/kg) and additional amounts (1 mg/kg, i.v.) were provided when required throughout the experiment. All dogs were intubated with an endotracheal tube and artifi-

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cially respired with room air; for this purpose, a Palmer ventilation pump was used at a rate of 20 strokes/min and a stroke volume of 13–16 ml/kg, as previously established by Kleinman and Radford (1964). Catheters were placed in the right femoral vein for the administration of saline or antagonists and in the femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of arterial blood pressure. Heart rate was measured with a tachograph (7P4F) triggered from the arterial blood pressure signal.

Bilateral cervical vagosympathectomy was systematically performed; thereafter, 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). After i.v. administration of the respective treatments (physiological saline or antagonists), dihydroergotamine was administered (1 ml/min, for 1 min) into the right common carotid artery by a Harvard model 901 pump with a catheter inserted into the right cranial thyroid artery. The body temperature of the animals was maintained between 37–38 °C.

After a stable haemodynamic condition for at least 60 min, baseline values of mean arterial blood pressure, heart rate and external carotid blood flow were determined. Then, the animals were divided into eight groups ($n=4$ each) which received an i.v. bolus injection of, respectively: (1) physiological saline (0.1 ml/kg), (2) SB224289 (300 μg/kg), (3) BRL15572 (300 μg/kg), (4) rauwolscine (300 μg/kg), (5) the combination of SB224289 (300 μg/kg) plus rauwolscine (300 μg/kg), (6) BRL44408 (1000 μg/kg), (7) imiloxan (1000 μg/kg) or (8) MK912 (300 μg/kg). After 15 min, the effect of cumulative intracarotid infusions (1 ml/min; during 1 min) of dihydroergotamine (5.6, 10, 18, 31, 56 and 100 μg/min; given every 5 min) on the systemic and external carotid haemodynamic variables were determined in each group of animals.

Apart from the anaesthetic (sodium pentobarbitone), the compounds used in this study were: dihydroergotamine tartrate (gift: Novartis, Basel, Switzerland), SB224289 and BRL15572 (both gifts from Dr. A.A. Parsons, SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK), rauwolscine hydrochloride (Research Biochemicals, Natick, MA, USA), BRL44408 (gift from Dr. T.J. Verbeuren; Servier, 92150 Suresnes, France), imiloxan hydrochloride (gift from Dr. R. Eglen; Roche Bioscience, Palo Alto, CA 94303, USA) and MK912 (gift from Dr. W.L. Henckler; Merck, New Jersey, NJ, USA).

All compounds were dissolved in physiological saline. When needed, 20% v /v propylene glycol (dihydroergotamine, SB224289, BRL15572 and BRL44408) was added; this vehicle had no effect (when given i.v. or intracarotidly) on external carotid blood flow, blood pressure or heart rate (not shown). The doses of the antagonists and dihydroergotamine refer to their free base.

The protocol of the present investigation was approved by the Ethical Committee of the Department of Pharmacobiology, CINVESTAV-IPN (CICUAL), dealing with the use of animals in scientific experiments.

All data in the text and figures are presented as mean ± S.E.M. The peak changes in external carotid blood flow (calculated as percent change from baseline) produced by intracarotid infusions of dihydroergotamine in the antago-

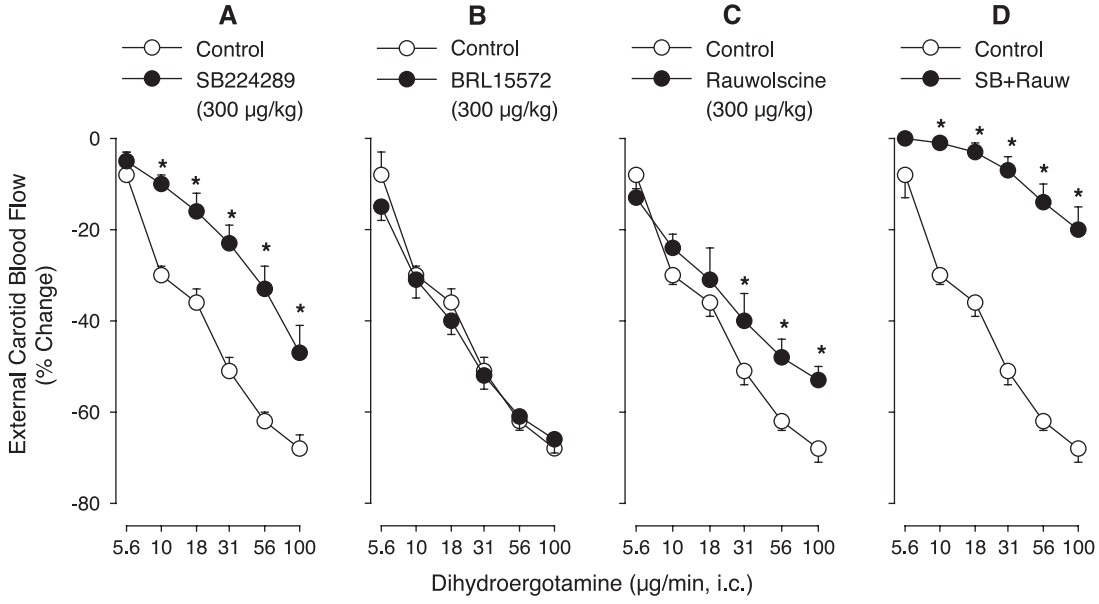

Fig. 1. Effect of i.v. SB224289 (SB; 300 μg/kg), BRL15572 (300 μg/kg), rauwolscine (Rauw; 300 μg/kg) or the combination SB224289 (300 μg/kg) plus rauwolscine (300 μg/kg) ($n=4$ each) on the decreases in external carotid blood flow induced by cumulative 1-min intracarotid (i.c.) infusions of dihydroergotamine. *$p<0.05$ vs. control.

nist-pretreated animals were determined and compared to the respective responses to dihydroergotamine in the saline-pretreated animals by Student–Newman–Keuls' test, once a two-way repeated measures analysis of variance (randomized block design) had revealed that the samples represented different populations. Statistical significance was accepted at $P < 0.05$ (two-tailed).

3. Results

As shown in Fig. 1, in the animals pretreated i.v. with saline (control), consecutive 1-min intracarotid infusions of dihydroergotamine produced dose-dependent decreases in external carotid blood flow (maximal response: 68 ± 3%); since heart rate and blood pressure remained unchanged (not shown), these decreases in external carotid blood flow were similar to those observed in the corresponding vascular conductance. These responses to dihydroergotamine were: (i) attenuated by blocking doses (300 μg/kg) of the 5-HT_{1B} receptor antagonist, SB224289 (Fig. 1A), but unaffected after blocking doses (300 μg/kg) of the 5-HT_{1D} receptor antagonist, BRL15572 (Fig. 1B); (ii) attenuated by the α_2-adrenoceptor antagonist, rauwolscine (Fig. 1C), but markedly blocked by the combination of SB224289 plus rauwolscine (Fig. 1D).

In order to identify the involvement of specific α_2-adrenoceptor subtypes (α_{2A}, α_{2B}, α_{2C}) in the dihydroergotamine-induced vasoconstrictor responses, we tested the α_2 subtype-selective antagonists, BRL44408 (α_{2A}), imiloxan (α_{2B}) and MK912 (α_{2C}). As shown in Fig. 2, the responses to dihydroergotamine remained unchanged after imiloxan (1000 μg/kg) (Fig. 2B), but were significantly attenuated after BRL44408 (1000 μg/kg, Fig. 2A) or MK912 (300 μg/kg; Fig. 2C). The doses of the antagonists used in this study were devoid of any haemodynamic effects per se (not shown).

4. Discussion

Since intracarotid infusions of dihydroergotamine elicited dose-dependent decreases in the external carotid blood flow without affecting mean arterial blood pressure or heart rate, these responses were caused by selective constriction in the external carotid circulation. The present study has further analysed the involvement of specific subtypes (5-HT_{1B}, 5-HT_{1D}, α_{2A}, α_{2B} and/or α_{2C}) in this response. Apart from the implications discussed below, our study shows that the external carotid vasoconstrictor responses to dihydroergotamine are significantly attenuated by: (i) the selective antagonist SB224289 (5-HT_{1B}), but not by BRL15572 (5-HT_{1D}) (Hagan et al., 1997), at doses high enough to block their respective receptors (De Vries et al., 1998a); thus, the 5-$HT_{1B/1D}$ receptors involved correlate with the 5-HT_{1B} (rather than the 5-HT_{1D}) subtype, as previously shown with the antimigraine agent sumatriptan (De Vries et al., 1998a); and (ii) rauwolscine, an α_2-adrenoceptor antagonist, at a dose sufficient to selectively block the canine carotid vasoconstrictor responses to the α_2-adrenoceptor agonist BHT933 (6-Ethyl-5,6,7,8-tetrahydro-4H-oxazolo[4,5-d]azepin-2-amine dihydrochloride) (Willems et al., 2001a). Consistent with the co-involvement of 5-HT_{1B} receptors and α_2-adrenoceptors in the above response, the blockade produced by the combination of SB224289 plus rauwolscine (Fig. 1D) was greater than that produced by either SB224289 (Fig. 1A) or rauwolscine (Fig. 1C) given separately. These rauwolscine-sensitive α_2-adrenoceptors, being blocked by BRL44408

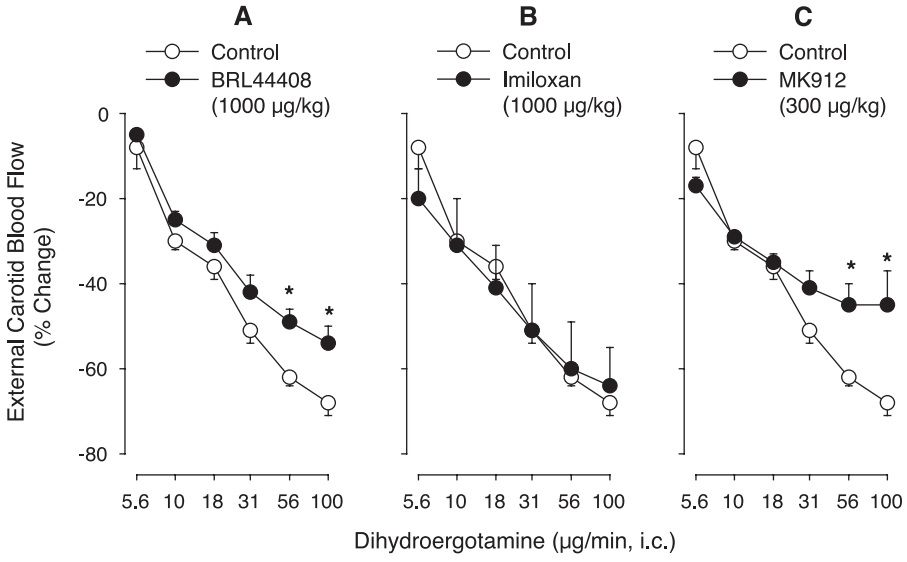

Fig. 2. Effect of i.v. BRL44408 (1000 μg/kg), imiloxan (1000 μg/kg) or MK912 (300 μg/kg) ($n = 4$ each) on the decreases in external carotid blood flow induced by cumulative 1-min intracarotid (i.c.) infusions of dihydroergotamine. *$p < 0.05$ vs. control.

(Fig. 2A) or MK912 (Fig. 2C), but not by imiloxan (Fig 2B), seem to correlate with both the α_{2A}- and α_{2C}-, but not the α_{2B}-, adrenoceptor subtypes. These results lead us to suggest that the blockade of the responses to dihydroergotamine by BRL44408 and MK912 was not marked because the 5-HT_{1B} receptors, being also stimulated by dihydroergotamine, may have overshadowed the antagonism produced on α_{2A}- and α_{2C}-adrenoceptors. The doses of BRL44408, imiloxan and MK912 used in the present study are sufficient to block their respective receptor subtypes (Willems et al., 2001c). These findings are in agreement with earlier results showing that the external carotid vasoconstrictor responses to BHT933 are also mediated by the α_{2A}- and α_{2C}-adrenoceptor subtypes (Willems et al., 2001c).

Despite our results showing the involvement of 5-HT_{1B} receptors and $\alpha_{2A/2C}$-adrenoceptors in the vasoconstrictor response to dihydroergotamine, the exact mechanism of the antimigraine activity of this ergot is elusive and, certainly, more complex (Silberstein and McCrory, 2003). However, since a common feature of all acute antimigraine drugs is their ability to produce selective vasoconstriction of extracranial blood vessels, including the external carotid bed (Villalón et al., 2002), the antimigraine activity of dihydroergotamine is, at least in part, related to its potent vasoconstrictor activity on dilated and painful blood vessels (Tfelt-Hansen et al., 2000; Silberstein and McCrory, 2003).

In contrast to the success of some 5-$HT_{1B/1D}$ receptor agonists (i.e. sumatriptan and the second-generation triptans) in acute migraine therapy (Villalón et al., 2002), other anti-migraine agents such as ergotamine (Villalón et al., 1999; Tfelt-Hansen et al., 2000) and isometheptene (Willems et al., 2001b), which interact with α_2-adrenoceptors, produce external carotid vasoconstriction in anaesthetised dogs. Hence, as previously considered (Willems et al., 2003), exploration of selective agonist activity at α_{2A}- and α_{2C}-adrenoceptor subtypes may provide further basis for the development of novel antimigraine drugs.

In conclusion, our results show that dihydroergotamine produces a cranio-selective vasoconstriction in the canine external carotid bed which is mainly (but not exclusively) mediated by 5-HT_{1B} receptors and $\alpha_{2A/2C}$-adrenoceptors.

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