Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: role of 5-HT_{1B/1D} receptors and α_2 -adrenoceptors

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1 The antimigraine drugs methysergide, ergotamine and dihydroergotamine (DHE) produce selective vasoconstriction in the external carotid bed of vagosympathectomized dogs anaesthetized with pentobarbital and artificially respired, but the receptors involved have not yet been completely characterized. Since the above drugs display affinity for several binding sites, including α -adrenoceptors and several 5-HT₁ and 5-HT₂ receptor subtypes, this study has analysed the mechanisms involved in the above responses.

2 Intracarotid (i.c.) infusions during 1 min of methysergide (31–310 $\mu\text{g min}^{-1}$), ergotamine (0.56–5.6 $\mu\text{g min}^{-1}$) or DHE (5.6–31 $\mu\text{g min}^{-1}$) dose-dependently reduced external carotid blood flow (ECBF) by up to 46 ± 4 , 37 ± 4 and $49 \pm 5\%$, respectively. Blood pressure and heart rate remained unchanged.

3 The reductions in ECBF by methysergide were abolished and even reversed to increases in animals pre-treated with GR127935 (10 $\mu\text{g kg}^{-1}$, i.v.).

4 The reductions in ECBF by ergotamine and DHE remained unchanged in animals pre-treated (i.v.) with prazosin (300 $\mu\text{g kg}^{-1}$), but were partly antagonized in animals pre-treated with either GR127935 (10 or 30 $\mu\text{g kg}^{-1}$) or yohimbine (1000 $\mu\text{g kg}^{-1}$). Pre-treatment with a combination of GR127935 (30 $\mu\text{g kg}^{-1}$) and yohimbine (1000 $\mu\text{g kg}^{-1}$) abolished the responses to both ergotamine and DHE. The above doses of antagonists were shown to produce selective antagonism at their respective receptors.

5 These results suggest that the external carotid vasoconstrictor responses to methysergide primarily involve 5-HT_{1B/1D} receptors, whereas those to ergotamine and DHE are mediated by 5-HT_{1B/1D} receptors as well as α_2 -adrenoceptors.

Keywords: Carotid artery; dihydroergotamine; dog; ergotamine; GR127935; 5-hydroxytryptamine (5-HT); 5-HT_{1B/1D} receptors; methysergide; prazosin; yohimbine

Abbreviations: DHE, dihydroergotamine; ECBF, external carotid blood flow; HR, heart rate; 5-HT, 5-hydroxytryptamine; i.c., intracarotid; L-NAME, L-N^G-nitroarginine methyl ester; MAP, mean arterial blood pressure

Introduction

Several lines of pharmacological evidence show that serotonin (5-hydroxytryptamine; 5-HT) and the antimigraine drug sumatriptan constrict the carotid vascular bed in vagosympathectomized animals *via* 5-HT₁-like receptors (Den Boer *et al.*, 1991b; Villalón *et al.*, 1995). Using the potent and selective 5-HT_{1B/1D} receptor antagonist, 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) (see Pauwels, 1996; Skingle *et al.*, 1996), it was subsequently demonstrated that these vasoconstrictor 5-HT₁-like receptors correspond to 5-HT_{1B/1D} subtypes (De Vries *et al.*, 1996; Villalón *et al.*, 1996). Additionally, it has been previously demonstrated that the classical antimigraine agents methysergide, ergotamine and dihydroergotamine (DHE) exert a selective vasoconstrictor action in the carotid vascular bed of several species, including dogs (Saxena, 1974b; Saxena *et al.*, 1983), pigs (Saxena & Verdouw, 1984; Den Boer *et al.*, 1991a),

cats (Spierings & Saxena, 1980) and monkeys (Mylecharane *et al.*, 1978).

Surprisingly, very little is known about the receptor mechanisms involved in the external carotid vasoconstriction induced by methysergide, ergotamine and DHE in the dog. In this respect, we have shown that vasoconstriction in the canine external carotid bed may be mediated by activation of 5-HT_{1B/1D} receptors (Villalón *et al.*, 1996), α_1 - and α_2 -adrenoceptors (Villalón & Terrón, 1994; Terrón *et al.*, 1996), but not by 5-HT₂ (Villalón *et al.*, 1995) or D₂ receptors (Villalón & Terrón, 1994). Indeed, as shown in Table 1, ergotamine and DHE are able to interact with a wide variety of receptors mediating vasoconstriction, including 5-HT_{1/2} receptors and $\alpha_{1/2}$ -adrenoceptors, whereas methysergide seems to interact mainly with 5-HT_{1/2} receptors. In the light of these findings, the present study set out to characterize the pharmacological profile of the receptors involved in the vasoconstriction of the canine external carotid vascular bed in response to methysergide, ergotamine and DHE, with particular emphasis on verifying the possible involvement of the 5-HT_{1B/1D} receptor subtypes and α_1 - and α_2 -adrenoceptors. For this purpose, we made use of antagonists at 5-HT_{1B/1D} receptor subtypes (GR127935;

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Skingle *et al.*, 1996), α_1 - (prazosin) and α_2 - (yohimbine) adrenoceptors (Hoffman & Lefkowitz, 1996), after determining the doses of these antagonists that produce selective blockade at their respective receptors. Preliminary results of this investigation have been communicated to the XIIIth International Congress of Pharmacology (Villalón *et al.*, 1998).

Methods

General

Experiments were carried out in a total of 72 dogs (15–31 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 when required. All dogs were intubated with an endotracheal tube and artificially respired with room air; for this purpose, a Palmer ventilation pump was used 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). Catheters were placed in the inferior vena cava *via* a femoral vein for the administration of antagonist drugs and in the aortic arch *via* a femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of blood pressure. After drug administration, the venous catheter was flushed with 3 ml of saline. Mean 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 internal carotid and occipital arteries were ligated. Thereafter, an ultrasonic flow probe (4 mm R-Series) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, N.Y., U.S.A.) was placed around the right common carotid artery, and the flow through this artery was considered as the external carotid blood flow (for further details, see Villalón *et al.*, 1993). Bilateral cervical vagosympathectomy was systematically performed in order to produce one of the main features of migraine, i.e. external carotid

vasodilatation (Saxena & De Vlaam-Schluter, 1974). The agonists were administered into the carotid artery by a Harvard model 901 pump (Harvard Apparatus Co. Inc., Millis, MA, U.S.A.) with a catheter inserted into the right cranial thyroid artery. Blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The body temperature of the animals was maintained between 37–38°C.

Experimental protocol

After a stable haemodynamic condition for at least 30 min, baseline values of blood pressure, heart rate and external carotid blood flow were determined. At this point, the dogs were divided into two groups ($n = 56$ and 16 , respectively). The first group ($n = 56$) was subdivided into three subgroups. In the first subgroup ($n = 8$), the effects of sequential 1 min intracarotid (i.c.) infusions of methysergide (31, 100 and 310 $\mu\text{g min}^{-1}$) were analysed in animals pre-treated i.v. with either saline (0.1 ml kg⁻¹, $n = 4$) or GR127935 (10 $\mu\text{g kg}^{-1}$; $n = 4$). In the second and third subgroups ($n = 24$ each), the effects of cumulative 1 min i.c. infusions of ergotamine (0.56, 1, 1.8, 3.1 and 5.6 $\mu\text{g min}^{-1}$) or DHE (5.6, 10, 18 and 31 $\mu\text{g min}^{-1}$), respectively, were analysed in animals pre-treated with i.v. infusions of either ($n = 4$ each) saline (0.1 ml kg⁻¹), GR127935 (10 $\mu\text{g kg}^{-1}$), GR127935 (30 $\mu\text{g kg}^{-1}$), prazosin (300 $\mu\text{g kg}^{-1}$), yohimbine (1000 $\mu\text{g kg}^{-1}$) or the combination of GR127935 (30 $\mu\text{g kg}^{-1}$) and yohimbine (1000 $\mu\text{g kg}^{-1}$). In the second group ($n = 16$), all animals received subsequent 1 min i.c. infusions of clonidine (1 $\mu\text{g min}^{-1}$), phenylephrine (10 $\mu\text{g min}^{-1}$) and sumatriptan (30 $\mu\text{g min}^{-1}$). Then, this group of animals was subdivided into four subgroups ($n = 4$ each). In these subgroups, the responses to clonidine, phenylephrine and sumatriptan were reanalysed after i.v. administration of either prazosin (300 $\mu\text{g kg}^{-1}$), yohimbine (1000 $\mu\text{g kg}^{-1}$), GR127935 (30 $\mu\text{g kg}^{-1}$) or the combination of GR127935 (30 $\mu\text{g kg}^{-1}$) and yohimbine (1000 $\mu\text{g kg}^{-1}$).

The dose-intervals between the different doses of methysergide ranged between 5 and 10 min, as in each case we waited

Table 1 Pharmacological profile of several agonists and antagonists used in the present study

Receptor	Methysergide	Ergotamine	DHE	GR127935	Prazosin	Yohimbine
5-HT _{1A}	7.63 ^{a,*}	8.37 ^{a,*}	9.30 ^b	7.20 ^c	5.00 ^{a,*}	6.86 ^{a,*}
r5-HT _{1B}	5.82 ^{a,*}	8.69 ^{a,*}	7.85 ^b	8.50 ^d	5.10 ^{a,*}	5.46 ^{a,*}
h5-HT _{1B}	n.d.	n.d.	9.22 ^b	9.90 ^c /9.00 ^d	n.d.	n.d.
h5-HT _{1D}	n.d.	n.d.	8.60 ^b	8.90 ^c /8.60 ^d	n.d.	n.d.
5-HT _{1B/1D}	8.42 ^{a,*}	7.60 ^{a,*}	7.65 ^b	n.d.	7.12 ^{a,*}	7.12 ^{a,*}
5-HT _{1E}	6.64 ^c	6.22 ^c	6.22 ^b	6.20 ^c /5.40 ^d	n.d.	5.90 ^c
5-HT _{1F}	7.47 ^c	6.77 ^c	6.96 ^b	7.30 ^c /6.40 ^d	n.d.	7.04 ^c
5-HT _{2A}	8.57 ^{a,*}	7.69 ^{a,*}	8.54 ^b	7.20 ^c /7.80 ^d	4.95 ^{a,*}	5.95 ^{a,*}
5-HT _{2B}	u.a. ^f	8.17 ^{a,*}	7.70 ^{a,*}	6.20 ^d	n.d.	7.90 ^{f,*}
5-HT _{2C}	8.90 ^{b,*}	7.25 ^{a,*}	7.48 ^{a,*} /7.43 ^b	6.20 ^c /7.00 ^d	4.70 ^{a,*}	4.37 ^{a,*}
5-HT _{5A}	7.20 ^{b,*}	8.40 ^{b,*}	n.d.	5.20 ^c	n.d.	6.00 ^{b,*}
5-HT _{5B}	6.90 ^{b,*}	8.50 ^{b,*}	n.d.	n.d.	n.d.	6.00 ^{b,*}
5-HT ₆	6.43 ^{b,*}	n.d.	7.88 ^{b,*} /8.00 ^b	5.80 ^d	n.d.	n.d.
5-HT ₇	7.90 ^{b,*}	7.49 ^{b,*}	6.82 ^{b,*} /7.82 ^b	5.50 ^c /6.20 ^d	n.d.	5.55 ⁱ
α_1	5.64 ^j	8.00 ^k	8.00 ^b	<6.00 ^c	9.24 ^j	6.39 ^j
α_2	5.59 ^j	8.20 ^k	8.00 ^b	<6.00 ^c	5.33 ^j	6.82 ^j
D ₂	6.70 ^j	8.50 ^k	7.00 ^k	<5.00 ^c	<6.00 ^j	6.22 ^j

^aHoyer (1988); ^bLeysen *et al.*, (1996); ^cPauwels (1996); ^dPrice *et al.* (1997); ^eAdham *et al.*, (1993); ^fBaxter *et al.* (1994); ^gGlusa & Roos (1996); ^hHoyer *et al.* (1994); ⁱBard *et al.* (1993); ^jLeysen (1985); ^kLeysen & Gommeren (1984). All values have been presented as pK_i, except for: $\$$, pA₂; $\dagger$, pEC₅₀; *, pK_D. Abbreviations: u.a., unsurmountable antagonist; n.d., not determined. r5-HT_{1B} refer to the rodent 5-HT_{1B} and receptor; h5-HT_{1B} and h5-HT_{1D} refer to the previously called 5-HT_{1D β} and 5-HT_{1D α} human receptor binding sites, respectively (Hartig *et al.* (1996); 5-HT_{1B/1D} refers to the, then called, 5-HT_{1D} receptor binding site in calf caudate membrane, at which time the experimental conditions allowed the inclusion of the 5-HT_{1B} (5-HT_{1D β}) and 5-HT_{1D} (5-HT_{1D α}) receptor sites.

until the blood flow had returned completely to baseline values. Moreover, 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.

Data presentation and statistical analysis

All data have been expressed as the means $\pm$ s.e.mean. The peak changes in external carotid blood flow (calculated as percent change from baseline) by methysergide, ergotamine and DHE after pre-treatment with a dose of a particular antagonist were compared to the respective responses by these agonists in saline-pre-treated animals by Student's unpaired *t*-test. Furthermore, the peak percent changes in external carotid blood flow by clonidine, phenylephrine and sumatriptan before and after antagonist treatment were compared by using Student-Newman-Keuls test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the drugs used in the present study (obtained from the sources indicated) were the following: phenylephrine hydrochloride, clonidine hydrochloride and prazosin hydrochloride (Sigma Chemical Company, St. Louis, MO, U.S.A.); GR127935 and sumatriptan succinate (gifts from Dr M. Skingle, Glaxo Group Research, Ware, Herts, U.K. and Dr H.E. Connor, Glaxo Group Research, Stevenage, Hertfordshire, U.K. respectively); methysergide maleate, DHE mesylate and ergotamine tartrate (gift: Sandoz A.G., Basel, Switzerland). All compounds were dissolved in physiological saline. When needed, 5% (v/v⁻¹) propylene glycol (prazosin) was added; this vehicle had no effect on the haemodynamic variables. GR127935 was solubilized according to the instructions of the supplier by heating the dispersion in bidistilled water to about 70°C for 10 s and then allowing to cool down to room temperature. All doses of antagonists refer to the respective salts, whereas those of the agonists refer to the free base.

Results

Systemic haemodynamic variables

Baseline values of mean arterial blood pressure, heart rate and external carotid blood flow in the 72 vagosympathectomized dogs were, respectively, 142 ± 5 mmHg, 178 ± 8 beats min⁻¹ and 163 ± 21 ml min⁻¹. No significant differences were observed in the initial values between the 18 subgroups. As

depicted in Table 2, these haemodynamic variables remained essentially unchanged after i.v. administrations of saline, GR127935, yohimbine, or the combination of GR127935 and yohimbine, at all doses tested. Only prazosin produced a significant decrease in both mean blood pressure and external carotid blood flow by 29 ± 15 and $29 \pm 16\%$, respectively, without affecting the corresponding carotid vascular conductance (not shown). This prazosin-induced hypotension and the resulting decrease in external carotid blood flow has previously been reported to be attributable to its α_1 -adrenoceptor blocking properties (Terrón *et al.*, 1996). In any case, these effects were not accompanied by changes in heart rate (Table 2), as previously shown by Massingham & Hayden (1975).

Initial effects of methysergide, ergotamine and DHE on external carotid blood flow

As shown in Figure 1, i.c. infusions (during 1 min) of methysergide (31, 100 and 310 $\mu\text{g min}^{-1}$), ergotamine (0.56, 1, 1.8, 3.1 and 5.6 $\mu\text{g min}^{-1}$) and DHE (5.6, 10, 18 and 31 $\mu\text{g min}^{-1}$) produced dose-dependent decreases in external carotid blood flow, without changes in mean blood pressure or heart rate (not shown). The above responses, which were immediate in onset, are drug-induced as 1 min i.c. infusions of the corresponding volumes of saline did not affect any haemodynamic parameter for the duration of the experiments (data not shown). The duration of action of ergotamine and DHE could not be established, as external carotid blood flow did not return to baseline values, as previously reported (Saxena, 1974a; Villalón *et al.*, 1992). In contrast, the duration of action of methysergide was 2.0 ± 0.7 , 7.0 ± 1.2 and 9.0 ± 1.9 min after 31, 100 and 310 $\mu\text{g min}^{-1}$, respectively. The apparent rank order of potency obtained in the present experiments was ergotamine > DHE > methysergide (Figure 1).

Effects of GR127935 on the methysergide-induced external carotid vasoconstriction

As depicted in Figure 2, in animals pre-treated with 10 $\mu\text{g kg}^{-1}$ of GR127935, the decreases in external carotid blood flow by methysergide were abolished and even reversed to an external carotid vasodilatation.

Effects of GR127935, prazosin, yohimbine or the combination of GR127935 and yohimbine on the ergotamine- and DHE-induced external carotid vasoconstriction

As shown in Figures 3 (ergotamine) and 4 (DHE), 10 $\mu\text{g kg}^{-1}$ of GR127935 only partly blocked the external carotid vasoconstrictor responses to ergotamine and DHE. Interest-

Table 2 Mean arterial blood pressure (MAP), heart rate (HR) and external carotid blood flow (ECBF) values before and after different i.v. treatments

Treatment	Dose ($\mu\text{g kg}^{-1}$)	n	MAP (mmHg)		HR (beats min ⁻¹)		ECBF (ml min ⁻¹)	
			Before	After	Before	After	Before	After
Saline	0.1†	12	152 $\pm$ 11	152 $\pm$ 12	176 $\pm$ 12	173 $\pm$ 11	151 $\pm$ 8	153 $\pm$ 6
GR127935	10	12	141 $\pm$ 6	148 $\pm$ 5	183 $\pm$ 7	182 $\pm$ 8	163 $\pm$ 13	155 $\pm$ 13
GR127935	30	8	137 $\pm$ 5	145 $\pm$ 6	181 $\pm$ 5	181 $\pm$ 7	134 $\pm$ 12	127 $\pm$ 9
Prazosin	300	8	143 $\pm$ 8	101 $\pm$ 14*	184 $\pm$ 8	188 $\pm$ 10	171 $\pm$ 21	122 $\pm$ 15*
Yohimbine	1000	8	143 $\pm$ 6	138 $\pm$ 10	171 $\pm$ 8	186 $\pm$ 14	177 $\pm$ 32	139 $\pm$ 22
GR127935 + Yohimbine	30 + 1000	8	138 $\pm$ 5	128 $\pm$ 7	176 $\pm$ 9	199 $\pm$ 18	170 $\pm$ 19	143 $\pm$ 18

All values have been presented as means $\pm$ s.e.mean. †, ml kg⁻¹; * $P < 0.05$ vs before values.

Figure 1 Comparative effects (expressed as per cent change from baseline values) of 1 min i.c. infusions ($n=4$ each) of ergotamine (ERG), dihydroergotamine (DHE) and methysergide (METHY) on external carotid blood flow in vagosympathectomized dogs. All values have been presented as means $\pm$ s.e.mean.

Figure 2 Percentage changes from baseline values by 1 min i.c. infusions of methysergide (METHY; 31–310 $\mu\text{g min}^{-1}$) in external carotid blood flow in animals pre-treated (i.v.; $n=4$ each) with either saline (SAL; 0.1 ml kg^{-1}) or GR127935 (GR; 10 $\mu\text{g kg}^{-1}$). All values have been presented as means $\pm$ s.e.mean. * $P<0.05$ vs response in saline-pre-treated animals.

ingly, a higher dose of GR127935 (30 $\mu\text{g kg}^{-1}$) did not produce a further blockade. Moreover, whereas prazosin (300 $\mu\text{g kg}^{-1}$) did not affect the ergotamine- and DHE-induced decreases in external carotid blood flow, yohimbine (1000 $\mu\text{g kg}^{-1}$) brought about a partial blockade. Significantly, the combination of GR127935 (30 $\mu\text{g kg}^{-1}$) and yohimbine (1000 $\mu\text{g kg}^{-1}$) completely blocked the external carotid vasoconstriction induced by ergotamine (Figure 3) and DHE (Figure 4). The above doses of antagonists, as explained below, were shown to produce selective antagonism at their respective receptors.

Effects of GR127935, prazosin, yohimbine and the combination of GR127935 and yohimbine on the external carotid vasoconstriction produced by clonidine, phenylephrine and sumatriptan

As depicted in Figure 5, GR127935 (30 $\mu\text{g kg}^{-1}$) selectively antagonized the external carotid vasoconstriction to sumatriptan, as the responses to clonidine and phenylephrine remained unaffected. Similarly, prazosin (300 $\mu\text{g kg}^{-1}$) exclusively abolished the phenylephrine-induced decreases in external carotid blood flow, leaving the clonidine- and sumatriptan-induced changes intact. Lastly, while yohimbine (1000 $\mu\text{g kg}^{-1}$) abolished only the clonidine-induced carotid vascular effects, the combination of GR127935 (30 $\mu\text{g kg}^{-1}$) and yohimbine (1000 $\mu\text{g kg}^{-1}$) completely blocked the clonidine-, as well as the sumatriptan-induced constriction of the canine carotid vascular bed. It should be pointed out that the external carotid vasoconstrictor responses to phenylephrine, clonidine and sumatriptan are highly reproducible, as

they remained essentially unchanged in control animals receiving two subsequent bolus injections of physiological saline (Villalón & Terrón, 1994; Villalón *et al*, 1996).

Discussion

General

The major findings of the present study in vagosympathectomized dogs were that: (i) the methysergide-induced external carotid vasoconstriction was abolished, and even reversed to carotid vasodilatation, by the selective 5-HT_{1B/1D} receptor antagonist, GR127935 and (ii) the external carotid vasoconstrictor responses by ergotamine and DHE were only partly antagonized by GR127935 or yohimbine, but were completely blocked by the combination of GR127935 and yohimbine. Apart from the implications discussed below, these data indicate that the vasoconstrictor responses to ergotamine and DHE in the external carotid vascular bed involve both 5-HT_{1B/1D} receptors and α_2 -adrenoceptors, whereas methysergide seems to exert external carotid vasoconstriction primarily via 5-HT_{1B/1D} receptors.

Figure 3 Percentage changes from baseline values of external carotid blood flow by 1 min i.c. infusions of ergotamine ($0.56\text{--}5.6\text{ }\mu\text{g min}^{-1}$) in animals pre-treated (i.v.; $n=4$ each) with either saline (0.1 ml kg^{-1}), GR127935 (10 or $30\text{ }\mu\text{g kg}^{-1}$), prazosin ($300\text{ }\mu\text{g kg}^{-1}$), yohimbine ($1000\text{ }\mu\text{g kg}^{-1}$) or the combination of GR127935 (GR; $30\text{ }\mu\text{g kg}^{-1}$) and yohimbine (YHB; $1000\text{ }\mu\text{g kg}^{-1}$). All values have been presented as means $\pm$ s.e.mean. For the sake of clarity, the responses to ergotamine in saline-pre-treated animals are shown in all panels. * $P<0.05$ vs response in saline-pre-treated animals.

Receptors involved in the methysergide-induced carotid vasoconstrictor responses

As previously observed using i.v. administrations (Villalón *et al.*, 1996), 1 min i.c. infusions of methysergide dose-dependently decreased external carotid blood flow, an effect which has been suggested to be due to an agonist action of the ergot derivative at smooth muscle 5-HT_1 -like receptors (Saxena & Verdouw, 1984; Martin, 1994). As methysergide did not affect heart rate or blood pressure, these data suggest that under our experimental conditions methysergide induced a selective vasoconstriction in the external carotid circulation. A complete blockade of the methysergide-induced carotid vasoconstriction was observed after treatment with $10\text{ }\mu\text{g kg}^{-1}$ of GR127935; this dose of GR127935 has previously been shown to abolish sumatriptan-induced external carotid vasoconstriction without affecting that to noradrenaline or oxymetazoline (Villalón *et al.*, 1996). In the present study, our results further demonstrate that doses of GR127935 up to $30\text{ }\mu\text{g kg}^{-1}$ selectively abolish sumatriptan-induced canine external carotid vasoconstriction, since the corresponding responses to phenylephrine and clonidine remained unaltered (Figure 5), as expected from its binding profile (see Table 1). Thus, these data show that methysergide constricts the external

carotid vasculature predominantly *via* the $5\text{-HT}_{1B/1D}$ receptor subtypes, at which methysergide displays high affinities (see Table 1). In view of the complete blockade by GR127935, as well as the low affinities exhibited by methysergide at α_1 - and α_2 -adrenoceptors (see Table 1), we decided not to further analyse the effects of prazosin or yohimbine on the methysergide-induced external carotid vasoconstriction.

Contribution of $5\text{-HT}_{1B/1D}$ receptors in the carotid vasoconstrictor responses to ergotamine and DHE

One minute i.c. infusions of ergotamine and DHE induced dose-dependent decreases in the external carotid blood flow, without affecting heart rate or mean arterial blood pressure. Thus, these findings suggest that such responses were caused by a selective constriction in the external carotid vascular bed. Notwithstanding, unlike methysergide, the responses to ergotamine and DHE were of long duration, as previously observed *in vivo* (Saxena, 1974a; Den Boer *et al.*, 1991a; Villalón *et al.*, 1992; De Vries *et al.*, 1998b) and *in vitro* (Müller-Schweinitzer & Weidmann, 1978; MaassenVanDenBrink *et al.*, 1998). Although there is no clear-cut explanation for this difference, it has been suggested, albeit not categorically proven, that it may be due to a slow dissociation

Figure 4 Percentage changes from baseline values of external carotid blood flow by 1 min i.c. infusions of dihydroergotamine ($5.6\text{--}31\text{ }\mu\text{g min}^{-1}$) in animals pre-treated (i.v.; $n=4$ each) with either saline (0.1 ml kg^{-1}), GR127935 (10 or $30\text{ }\mu\text{g kg}^{-1}$), prazosin ($300\text{ }\mu\text{g kg}^{-1}$), yohimbine ($1000\text{ }\mu\text{g kg}^{-1}$) or the combination of GR127935 (GR; $30\text{ }\mu\text{g kg}^{-1}$) and yohimbine (YHB; $1000\text{ }\mu\text{g kg}^{-1}$). All values have been presented as means $\pm$ s.e.mean. For the sake of clarity, the responses to dihydroergotamine in saline-pre-treated animals are shown in all panels. * $P < 0.05$ vs response in saline-pre-treated animals.

from its receptors or a sequestration and subsequent diffusion out of a local nonsaturable compartment (Müller-Schweinitzer & Weidmann, 1978; Martin *et al.*, 1995).

Regarding the possible receptor mechanisms involved in the vasoconstrictor responses to ergotamine and DHE, previous results in anaesthetized dogs have shown that the external carotid vasoconstrictor responses to ergotamine and DHE are not mediated by 5-HT_2 receptors, whereas only part of the effects seem to be mediated by phentolamine-sensitive α -adrenoceptors (Saxena *et al.*, 1983). Moreover, 5-HT_1 -like receptors are only partly involved in the ergotamine- and DHE-induced constriction of the carotid vasculature in pigs (Den Boer *et al.*, 1991a). Since 5-HT_1 -like receptors mediating porcine as well as canine carotid vasoconstriction are similar to the $5\text{-HT}_{1B/1D}$ receptor subtypes (De Vries *et al.*, 1996; Villalón *et al.*, 1996; Saxena *et al.*, 1998), we decided to explore the possible involvement of $5\text{-HT}_{1B/1D}$ receptors in the present study. Indeed, the responses to ergotamine and DHE in dogs were, in contrast to the methysergide- and sumatriptan-induced effects, only partly antagonized by $10\text{ }\mu\text{g kg}^{-1}$ of GR127935; the fact that pre-treatment with a higher dose of GR127935 did not produce any further blockade, implies that the ergotamine- and DHE-induced external carotid vasoconstriction involves a mixed population of receptors. Thus, we

suggest that the responses to ergotamine and DHE are partly mediated *via* $5\text{-HT}_{1B/1D}$ receptors, but a considerable part is mediated *via* GR127935-resistant receptors. Consistent with these findings, we have recently shown that ergotamine and DHE act, at least partly, *via* GR127935-sensitive $5\text{-HT}_{1B/1D}$ receptors in the porcine carotid vascular bed, but additional receptors/mechanisms seem to be involved (De Vries *et al.*, 1998b).

Contribution of α_1 - and/or α_2 -adrenoceptors in the carotid vasoconstrictor responses to ergotamine and DHE

As discussed above, a considerable part of the external carotid vasoconstriction by both ergotamine and DHE is mediated by non- $5\text{-HT}_{1B/1D}$ receptors. These GR127935-resistant receptors may be of the α -adrenoceptor class, as (i) agonists at α_1 - and α_2 -adrenoceptors can produce potent vasoconstrictor responses in the carotid circulation of dogs (Villalón & Terrón, 1994; Terrón *et al.*, 1996) and pigs (Willems, E.W., personal communication); and (ii) ergotamine and DHE display high affinities at α_1 - and α_2 -adrenoceptors (Table 1).

Nevertheless, $300\text{ }\mu\text{g kg}^{-1}$ of prazosin, a dose which is 3 fold higher than that required to abolish methoxamine-

Figure 5 Percentage changes from baseline values of external carotid blood flow by 1 min i.c. infusions of clonidine ($1 \mu\text{g min}^{-1}$), phenylephrine ($10 \mu\text{g min}^{-1}$) or sumatriptan ($30 \mu\text{g min}^{-1}$) before and after i.v. administration ($n=4$ each) of either GR127935 ($30 \mu\text{g kg}^{-1}$), prazosin ($300 \mu\text{g kg}^{-1}$), yohimbine ($1000 \mu\text{g kg}^{-1}$) or the combination of GR127935 (GR; $30 \mu\text{g kg}^{-1}$) and yohimbine (YHB; $1000 \mu\text{g kg}^{-1}$) in vagosympathectomized dogs. All values have been presented as means $\pm$ s.e.mean. * $P < 0.05$ after vs before.

induced canine external carotid vasoconstriction (Terrón *et al.*, 1996), did not affect the vasoconstrictor responses to either ergotamine (Figure 3) or DHE (Figure 4). These results suggest that ergotamine and DHE do not display α_1 -adrenoceptor agonist properties under our experimental conditions. Indeed, this suggestion is strengthened by our results showing that the above dose of prazosin produced a selective and complete blockade of the α_1 -adrenoceptor-mediated external carotid vasoconstriction induced by phenylephrine (Figure 5). It might still be argued that ergotamine and DHE could have behaved as α -adrenoceptor antagonists. Nonetheless, at such low total doses (5.6 and $31 \mu\text{g}$ for ergotamine and DHE, respectively) these ergot derivatives did not modify the external carotid vasoconstrictor responses to phenylephrine and clonidine (data not shown).

Contrasting with prazosin, yohimbine was capable of producing a partial blockade of the vasoconstrictor responses to ergotamine and DHE (see Figures 3 and 4) at a dose ($1000 \mu\text{g kg}^{-1}$) that selectively abolished clonidine-induced canine external carotid vasoconstriction (Figure 5). Taken together, the above results imply that α_2 -, but not α_1 -adrenoceptors, are involved, as previously suggested by other studies (Müller-Schweinitzer & Weidmann, 1978; Kalkman, 1983; Müller-Schweinitzer, 1984).

Nevertheless, it must be recognized that yohimbine also displays moderate affinity for other receptors, including α_1 -adrenoceptors and $5\text{-HT}_{1B/1D}$ receptors (see Table 1); thus, it is reasonable to assume that the above dose of the drug could have been high enough to block these, and other, receptors. Yet, lower doses of yohimbine (100 and $300 \mu\text{g kg}^{-1}$, i.v.) did not completely block clonidine-induced external carotid vasoconstriction and higher doses ($3000 \mu\text{g kg}^{-1}$, i.v.) were not selective in our model as they also blocked phenylephrine-induced external carotid vasoconstriction (data not shown). Indeed, other *in vivo* studies have shown that yohimbine displays moderate selectivity for α_2 - over the α_1 -adrenoceptor in anaesthetized cats (Ramage & Tomlinson, 1985) and dogs (Shepperson *et al.*, 1981).

However, as shown in Figure 5, the antagonism produced by $1000 \mu\text{g kg}^{-1}$ of yohimbine at external carotid α_2 -receptors was selective, since this dose completely blocked the clonidine-induced carotid vascular effects without affecting those to sumatriptan (mediated by $5\text{-HT}_{1B/1D}$ receptors; Villalón *et al.*, 1996) and phenylephrine (mediated by α_1 -adrenoceptors; Villalón & Terrón, 1994). In any case, the latter suggestion is validated by the fact that prazosin, at doses producing selective α_1 -adrenoceptor antagonism did not affect the responses to ergotamine and DHE. Although beyond the scope of the

present investigation, further studies using highly selective agonists and antagonists will be required to ascertain which specific subtype(s) of the α_2 -adrenoceptor is (are) producing vasoconstriction.

Possible involvement of other receptors in the carotid vasoconstrictor responses to ergotamine and DHE

As discussed above, the constriction of the canine carotid vascular bed by ergotamine and DHE is likely to be mediated by 5-HT_{1B/1D} receptors, as well as α_2 -adrenoceptors. The question remains open, however, whether additional receptors and/or mechanisms play a role in their effects. For this reason we studied the effects of a combination of GR127935 and yohimbine on the carotid vascular responses by the ergot alkaloids. Indeed, after simultaneous, selective blockade of both 5-HT_{1B/1D} receptors and α_2 -adrenoceptors, the carotid vascular effects of ergotamine and DHE are abolished. Therefore, the above results, taken collectively, suggest that the vasoconstriction of the carotid vascular bed induced by ergotamine and DHE in vagosympathectomized dogs is primarily mediated by both 5-HT_{1B/1D} receptors and α_2 -adrenoceptors.

On the other hand, since methysergide, ergotamine and DHE (as well as GR127935 and yohimbine) display also moderate affinity for 5-HT_{1F} receptors (see Table 1), its potential role cannot be categorically excluded in the present study, particularly when considering that mRNA for 5-HT_{1F} receptors has been shown in cranial blood vessels (Bouchelet *et al.*, 1996). Indeed, sumatriptan, which displays reasonable affinity (pK_i : 7.64) for the recombinant 5-HT_{1F} receptor (Adham *et al.*, 1993), also produced a GR127935-sensitive vasoconstriction in the canine external carotid bed (Villalón *et al.*, 1996). Notwithstanding, two recent findings from our laboratory apparently argue against the role of 5-HT_{1F} receptors (and simultaneously reinforce the role of 5-HT_{1B} receptors): (i) 1 min i.c. infusions of LY344864 ($1-3100 \mu\text{g min}^{-1}$), a selective 5-HT_{1F} receptor agonist (Phebus *et al.*, 1997), did not produce canine external carotid vasoconstriction (unpublished); and (ii) SB224289 ($300 \mu\text{g kg}^{-1}$), a selective 5-HT_{1B} ligand with very low affinity (pK_i : <5.0) for 5-HT_{1F} receptors (Hagan *et al.*, 1997), completely blocked 5-HT- as well as sumatriptan-induced canine external carotid vasoconstriction (De Vries *et al.*, 1998a).

Possible involvement of external carotid vasodilator mechanisms by ergot derivatives

Interestingly, after treatment with GR127935, not only was the methysergide-induced carotid constriction abolished, but a clear vasodilator response was unmasked. We have previously demonstrated that the 5-HT-induced external carotid vasodilatation in GR127935-pretreated vagosympathectomized dogs is mediated *via* 5-HT₇ receptors (Villalón *et al.*, 1997a), as shown in other vascular preparations (Saxena *et al.*, 1998). Therefore, it is tempting to suggest that methysergide could have acted *via* smooth muscle relaxant 5-HT₇ receptors, for which the compound displays high affinity (see Table 1).

Nevertheless, this seems less likely, as methysergide behaves as a silent antagonist at other cardiovascular 5-HT₇ receptors, including those mediating feline tachycardia (Villalón *et al.*, 1997b) and rat hypotension (Saxena & Lawang, 1985). Alternatively, methysergide may have stimulated an endothelial receptor resulting in the release of nitric oxide, possibly of the 5-HT_{2B} receptor subtype, as shown previously for other ergot derivatives in the porcine pulmonary circulation (Glusa & Roos, 1996). In support of this contention, the methysergide-induced carotid dilatory response was inhibited in animals treated with the nitric oxide synthetase inhibitor L-N^G-nitroarginine methyl ester (L-NAME; unpublished observations).

As opposed to methysergide, ergotamine and DHE did not induce any vasodilator effects in the external carotid circulation, even after complete blockade of the vasoconstrictor effects by GR127935 and yohimbine. This seems to argue the possible involvement of endothelial dilatory (5-HT) receptors, as these ergot derivatives have been shown to be potent agonists at the (5-HT_{2B}) receptors mediating endothelium-dependent vasorelaxation of porcine isolated pulmonary arteries (Glusa & Roos, 1996). It should be noted, however, that yohimbine has been shown to possess high antagonist affinity at 5-HT_{2B} receptors (Table 1) and, therefore, may have inhibited the potential ergot-induced vasorelaxant effects at these receptors. In this context, in view of the low affinity displayed by yohimbine at 5-HT₇ receptors (Table 1), it is highly unlikely that 5-HT₇ receptors mediate the potential ergotamine- and DHE-induced external carotid vasodilatation.

In conclusion, our results show that in vagosympathectomized, anaesthetized dogs, methysergide constricts the external carotid vascular bed predominantly *via* GR127935-sensitive 5-HT_{1B/1D} receptors. After administration of GR127935, an external carotid vasodilator (possibly endothelial) component was unmasked. Additionally, the present results show the predominant involvement of both 5-HT_{1B/1D} receptors and α_2 -adrenoceptors, but not α_1 -adrenoceptors, in the external carotid constriction by ergotamine and DHE. The therapeutic efficacy of these classical antimigraine drugs is likely to be mediated by these receptors, as constriction of the carotid vascular bed is highly predictive of antimigraine potential (Saxena *et al.*, 1997). In this context, we have clearly demonstrated that the decrease in carotid blood flow by antimigraine agents is exclusively due to vasoconstriction of carotid arteriovenous anastomoses (for references see Saxena, 1995; Saxena *et al.*, 1997), which have been suggested to be in a dilated state during migraine headaches (e.g. Heyck, 1969; Saxena, 1995). Lastly, although we cannot categorically exclude other, possibly novel receptors/mechanisms, as shown recently in the porcine carotid vasculature (De Vries *et al.*, 1998b), it seems that, if present at all, these are of minor importance in the dog external carotid vascular bed.

The technical assistance of Mr Arturo Contreras is gratefully acknowledged. The authors also thank CONACyT (Mexico City, Mexico) and the pharmaceutical companies (see Drugs Section) for their support.

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(Received August 17, 1998

Revised September 28, 1998

Accepted October 28, 1998)