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RESEARCH PAPER

Donitriptan, but not sumatriptan, inhibits capsaicin-induced canine external carotid vasodilatation via 5-HT_{1B} rather than 5-HT_{1D} receptors

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Background and purpose: It has been suggested that during a migraine attack capsaicin-sensitive trigeminal sensory nerves release calcitonin gene-related peptide (CGRP), resulting in cranial vasodilatation and central nociception; hence, trigeminal inhibition may prevent this vasodilatation and abort migraine headache. This study investigated the effects of the agonists sumatriptan (5-$HT_{1B/1D}$ water-soluble), donitriptan (5-$HT_{1B/1D}$ lipid-soluble), PNU-142633 (5-HT_{1D} water-soluble) and PNU-109291 (5-HT_{1D} lipid-soluble) on vasodilator responses to capsaicin, α-CGRP and acetylcholine in dog external carotid artery.
Experimental approach: 59 vagosympathectomized dogs were anaesthetized with sodium pentobarbitone. Blood pressure and heart rate were recorded with a pressure transducer, connected to a cannula inserted into a femoral artery. A precalibrated flow probe was placed around the common carotid artery, with ligation of the internal carotid and occipital branches, and connected to an ultrasonic flowmeter. The thyroid artery was cannulated for infusion of agonists.
Key results: Intracarotid infusions of capsaicin, α-CGRP and acetylcholine dose-dependently increased blood flow through the carotid artery. These responses remained unaffected after intravenous (i.v.) infusions of sumatriptan, PNU-142633, PNU-109291 or physiological saline; in contrast, donitriptan significantly attenuated the vasodilator responses to capsaicin, but not those to α-CGRP or acetylcholine. Only sumatriptan and donitriptan dose-dependently decreased the carotid blood flow. Interestingly, i.v. administration of the antagonist, SB224289 (5-HT_{1B}), but not of BRL15572 (5-HT_{1D}), abolished the inhibition by donitriptan.
Conclusions and implications: Our results suggest that the inhibition produced by donitriptan of capsaicin-induced external carotid vasodilatation is mainly mediated by 5-HT_{1B}, rather than 5-HT_{1D}, receptors, probably by a central mechanism.
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Keywords: capsaicin; α-CGRP; donitriptan; external carotid artery; 5-HT_{1B} receptors; migraine; sumatriptan; vasodilatation

Abbreviations: BRL15572, (1-(3-chlorophenyl)-4-[3,3-diphenyl(2-(S,R)hydroxypropanyl) piperazine]) hydrochloride; CGRP, calcitonin gene-related peptide; CNS, central nervous system; PNU-142633, [(S)-(−)-3,4-dihydro-1-[2-[4-aminocarbonyl)phenyl]-1-piperazinyl]ethyl]-*N*-methyl-1H-2-benzopyran-6-carboxamide; PNU-109291, [(S)-(−)-1-[2-[4-(4-methoxy-phenyl)-piperazin-1-yl]-ethyl]isochroman-6-carboxylic acid methylamide]; 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

Introduction

Migraine, a syndrome with an elusive pathophysiology (see Villalón *et al*., 2002), involves vasodilatation of cranial blood vessels and activation of perivascular trigeminal sensory nerves that results in the release of several neuropeptides, particularly calcitonin gene-related peptide (CGRP) (see Edvinsson, 2003). Indeed, plasma concentrations of CGRP, but not of other neuropeptides, are elevated during migraine headache and these are normalized by the triptans (such as sumatriptan) in parallel with alleviation of headache (see Goadsby *et al*., 2002).

CGRP is predominantly located on sensory neurons and perivascular nerves surrounding blood vessels, where it is co-localized with other vasoactive neuropeptides, such as substance P and neurokinin A (Van Rossum *et al*., 1997). In

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addition, it dilates cranial blood vessels and transmits vascular nociception (see Arulmani *et al*., 2004b). The release of endogenous CGRP can be experimentally induced by either trigeminal electrical stimulation (Buzzi *et al*., 1991; Goadsby and Edvinsson, 1993) or chemical stimulation with capsaicin (Potenza *et al*., 1994; Hou *et al*., 2002; Dux *et al*., 2003). Interestingly, the water-soluble antimigraine drug, sumatriptan (see Humphrey *et al*., 1991), attenuated the increased release of CGRP evoked by trigeminal electrical stimulation in rats and cats (Buzzi *et al*., 1991; Goadsby and Edvinsson, 1993), but failed to modify the carotid vasodilatation and the increased release of CGRP induced by capsaicin in pigs (Arulmani *et al*., 2004a). In this context, it is noteworthy that sumatriptan does not easily cross an intact blood–brain barrier (see Humphrey *et al*., 1991). However, during a migraine attack, the blood–brain barrier is transiently disrupted (Harper *et al*., 1977) and this phenomenon may facilitate the penetration of sumatriptan into the central nervous system (CNS), as previously shown with a hyperosmolar mannitol solution (Kaube *et al*., 1993), resulting in trigeminal inhibition. With the advent of more lipid-soluble compounds, including second-generation triptans (see Villalón *et al*., 2002) and selective 5-hydroxytriptamine (5-HT)$_{1D}$ receptor agonists (Ennis *et al*., 1998), the question remains open as to whether lipid-soluble compounds given i.v. could inhibit the carotid vasodilatation induced by capsaicin and α-CGRP *in vivo*. On this basis, and considering that central and peripheral trigeminal inhibition involves activation of 5-HT$_{1B/1D}$ receptors (see Goadsby *et al*., 2002; Villalón *et al*., 2002), the present study set out to investigate in vagosympathectomized dogs: (a) whether the agonists sumatriptan (5-HT$_{1B/1D}$, water-soluble), donitriptan (5-HT$_{1B/1D}$, lipid-soluble), [(S)-(−)-3,4-dihydro-1-[2-[4-aminocarbonyl)phenyl]-1-piperazinyl]ethyl]-*N*-methyl-1H-2-benzopyran-6-carboxamide (PNU-142633) (5-HT$_{1D}$, water-soluble) and [(S)-(−)-1-[2-[4-(4-methoxy-phenyl)-piperazin-1-yl]-ethyl]isochroman-6-carboxylic acid methylamide] (PNU-109291) (5-HT$_{1D}$, lipid-soluble) (see Table 1) are capable of inhibiting the carotid vasodilator responses to capsaicin, α-CGRP and acetylcholine; and (b) whether the 5-HT$_{1B}$ and/or 5-HT$_{1D}$ receptor subtypes are involved in these effects of the agonists by means of using the selective antagonists 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 (SB224289) (5-HT$_{1B}$) and (1-(3-chlorophenyl)-4-[3,3-diphenyl(2-(S,R)hydroxypropanyl) piperazine]) hydrochloride (BRL15572) (5-HT$_{1D}$) (see Table 1). Our results showed that only donitriptan inhibited capsaicin-induced external carotid vasodilatation, a response that seems to be mainly mediated by central 5-HT$_{1B}$, rather than 5-HT$_{1D}$, receptors.

Materials and methods

General methods

Experiments were carried out in a total of 59 dogs (body weight: 15–25 kg) not selected for breed or sex. As previously reported (Villalón *et al*., 1999), the animals were anaesthetized with an i.v. bolus injection of sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}, i.v.) were provided when required throughout the experiment. All dogs were intubated with an endotracheal tube and artificially respired with room air, using a Palmer ventilation pump (rate: 20 strokes min^{-1}; stroke volume: 13–16 ml kg^{-1}) as previously established by Kleinman and Radford (1964). Catheters were placed in: (i) a femoral vein for the administration of vehicle, agonists and antagonists at 5-HT$_{1B/1D}$ receptors; and (ii) a femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of arterial blood pressure. After administration of vehicle, agonists or antagonists, the venous catheter was flushed with 3 ml of physiological saline. Mean arterial blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures as follows: MAP = DAP + (SAP−DAP)/3. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, USA) triggered from the blood pressure signal.

The right common carotid artery was dissected free and the corresponding internal carotid and occipital branches were ligated; under these experimental conditions, the blood flow through the right common carotid artery was considered to represent that of the external carotid artery (for further considerations see Villalón *et al*., 1993). Thereafter, an ultrasonic flow probe (4 mm R-Series) connected to an ultrasonic T206 flow meter (Transonic Systems Inc., Ithaca, NY, USA) was placed around the right common carotid artery. Bilateral cervical vagosympathectomy was systematically performed in order to prevent possible baroreceptor reflexes produced by the intracarotid infusions of capsaicin, α-CGRP and acetylcholine. Subsequently, a catheter was introduced into the right cranial thyroid artery for the administration of intracarotid infusions of capsaicin, α-CGRP and acetylcholine. It is to be noted that the carotid arterioles are dilated under our experimental conditions; as the main objective of our study is to investigate the responses to vasodilator agents (such as capsaicin, α-CGRP and acetylcho-

Table 1 Binding affinity constants (p*Ki*) of several 5-HT receptor agonists and antagonists for cloned human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors

	5-HT$_{1B}$	*5-HT$_{1D}$*
Sumatriptan	7.8[a]	8.5[a]
Donitriptan	9.4[b]	9.3[b]
PNU-142633	4.8[c]	8.3[c]
PNU-109291	5.23[d]	9.04[d]
SB224289	8.0[e]	6.2[e]
BRL15572	6.1[f]	7.9[f]

BRL15572, (1-(3-chlorophenyl)-4-[3,3-diphenyl(2-(S,R)hydroxypropanyl) piperazine]) hydrochloride; PNU-142633, [(S)-(−)-3,4-dihydro-1-[2-[4-aminocarbonyl)phenyl]-1-piperazinyl]ethyl]-*N*-methyl-1H-2-benzopyran-6-carboxamide; PNU-109291, [(S)-(−)-1-[2-[4-(4-methoxy-phenyl)-piperazin-1-yl]-ethyl] isochroman-6-carboxylic acid methylamide]; 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; 5-HT, 5-hydroxytriptamine.
Data taken from
[a]Leysen *et al*. (1996).
[b]John *et al*. (1999).
[c]Pregenzer *et al*. (1999).
[d]Ennis *et al*. (1998).
[e]Hagan *et al*. (1997).
[f]Price *et al*. (1997).

line) on the external carotid circulation, we had to produce a selective carotid preconstriction with an intracarotid continuous infusion of phenylephrine (an α_1-adrenoceptor agonist). For this purpose, a 0.5 mm (external diameter) needle, connected to a suitable catheter, was inserted into the right common carotid artery for the continuous infusion of phenylephrine by another motor-driven syringe. This phenylephrine-induced carotid vasoconstriction, which allowed us to obtain greater vasodilator responses (Arulmani *et al*., 2004a), was compared with the effects produced after 10-min i.v. infusions of the highest doses of sumatriptan, donitriptan, PNU-142633, PNU-109291 or equivalent volumes of physiological saline. Thus, by using WPI model sp100i pumps (World Precision Instruments Inc., Sarasota, FL, USA): (i) capsaicin, α-CGRP and acetylcholine as well as phenylephrine were infused into the carotid artery; and (ii) sumatriptan, donitriptan, PNU-142633, PNU-109291 or equivalent volumes of physiological saline were infused into the femoral vein (for further details, see the Experimental protocol described below).

Arterial blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, USA). The body temperature of the animals was maintained between 37 and 38°C.

Experimental protocol

After the animals ($n=59$) had been in a stable haemodynamic condition for at least 60 min, baseline values of mean blood pressure, heart rate and external carotid blood flow were determined. Subsequently, the animals were divided into four groups ($n=20$, 8, 20 and 11).

The first group ($n=20$) was subdivided into five subgroups ($n=4$ each) that received consecutive 10-min i.v. infusions of, respectively: (i) sumatriptan (1, 3, 10, 30, 100 and 300 $\mu g\,kg^{-1}$); (ii) donitriptan (0.1, 0.3, 1, 3, 10 and 30 $\mu g\,kg^{-1}$); (iii) PNU-142633 (1, 3, 10, 30, 100 and 300 $\mu g\,kg^{-1}$); (iv) PNU-109291 (0.3, 1, 3, 10, 30 and 100 $\mu g\,kg^{-1}$); and (v) equivalent volumes of physiological saline (0.5 ml min^{-1} during 10 min; given six times). The above compounds were given consecutively following a cumulative dose-schedule as i.v. infusions (at a rate of 0.5 ml min^{-1} during 10 min for each dose).

The second group ($n=8$) received consecutive intracarotid infusions (1 ml min^{-1}, for 1 min) of capsaicin (10, 18, 30 and 56 $\mu g\,min^{-1}$), α-CGRP (0.1, 0.3, 1 and 3 $\mu g\,min^{-1}$) and acetylcholine (0.01, 0.03 and 0.1 $\mu g\,min^{-1}$). Then, this group was subdivided into two subgroups ($n=4$ each) that received an intracarotid continuous infusion throughout the experiment of, respectively: (i) vehicle (0.3 ml min^{-1} of physiological saline); and (ii) phenylephrine (1.5 $\mu g\,min^{-1}$, given at a rate of 0.3 ml min^{-1}), which produced a carotid vasoconstriction similar to that elicited by the highest dose of sumatriptan (300 $\mu g\,kg^{-1}$, i.v.) or donitriptan (30 $\mu g\,kg^{-1}$, i.v.) (see first group for details). Twenty minutes after the start of the infusion of physiological saline or phenylephrine, the responses to the above doses of capsaicin, α-CGRP and acetylcholine (in this order) were elicited again as described above *during* the intracarotid continuous infusion of each compound.

The third group ($n=20$) received a continuous intracarotid infusion of phenylephrine (1.5 $\mu g\,min^{-1}$) as described previously and, 20 min later, the responses to the above doses of capsaicin, α-CGRP and acetylcholine (in this order) were elicited *during* the infusion of phenylephrine. Then, this group was subdivided into five subgroups ($n=4$ each) so that *the infusion of phenylephrine was stopped in the first two subgroups (waiting about 60 min for the recovery of baseline external carotid blood flow), whereas it remained continuously infusing throughout the experiment in the remaining three subgroups*. Subsequently, by the use of another motor-driven syringe inserted into the femoral vein and infusing at a rate of 0.5 ml min^{-1} during 10 min (following the procedures described for the first group): (i) the first two subgroups (60 min after stopping the phenylephrine infusion) received, consecutively, cumulative 10 min i.v. infusions of, respectively, sumatriptan (1–300 $\mu g\,kg^{-1}$) and donitriptan (0.1–30 $\mu g\,kg^{-1}$); and (ii) the remaining three subgroups (during phenylephrine infusion) received, consecutively, cumulative 10-min i.v. infusions of, respectively, PNU-142633 (1–300 $\mu g\,kg^{-1}$), PNU-109291 (0.3–100 $\mu g\,kg^{-1}$) and equivalent volumes of physiological saline (0.5 ml min^{-1} during 10 min; given 6 times). Then, the responses to the above doses of capsaicin, α-CGRP and acetylcholine were reanalysed. It is important to note that, with these procedures, the carotid vasoconstriction was similar in all subgroups before the 1 min intracarotid infusions of capsaicin, α-CGRP and acetylcholine.

Finally, the fourth group ($n=11$) received an intracarotid continuous infusion of phenylephrine (1.5 $\mu g\,min^{-1}$) and, 20 min later, the responses to the above doses of capsaicin were elicited as described above *during* the infusion of phenylephrine. At this point, this group was subdivided into three subgroups that received i.v. bolus injections of, respectively, SB224289 (300 $\mu g\,kg^{-1}$; $n=4$), BRL15572 (300 $\mu g\,kg^{-1}$; $n=4$) and an equivalent volume of physiological saline (0.15 ml kg^{-1}; $n=3$). After 10 min, each subgroup received, consecutively, cumulative 10-min i.v. infusions of donitriptan (0.1–30 $\mu g\,kg^{-1}$) as described previously. It is important to note that after the administration of SB224289 the donitriptan-induced vasoconstriction was completely blocked; therefore, in order to maintain the carotid circulation under a vasoconstriction state similar to that observed previously with the administration of this antagonist, the infusion of phenylephrine was maintained at a constant rate (1.5 $\mu g\,min^{-1}$) throughout the experiments in this subgroup. In contrast, as BRL15572 or physiological saline did not modify the donitriptan-induced carotid vasoconstriction, the infusion of phenylephrine was interrupted just before the administration of these compounds (results obtained from preliminary experiments; not shown). Ten minutes after the last i.v. dose of donitriptan (30 $\mu g\,kg^{-1}$) had been given, the responses to the above 1-min intracarotid infusions of capsaicin were elicited again.

Each dose of capsaicin, α-CGRP and acetylcholine was in a solution that was administered at a rate of 1 ml min^{-1} during a period of 1 min. The dose intervals between the different doses of capsaicin, α-CGRP and acetylcholine (given sequentially as they produced transient responses) ranged between 5 (acetylcholine) and 20 (capsaicin and α-CGRP) min,

as in each case we waited until the carotid blood flow had returned completely to baseline values. The doses of these compounds were selected on the basis of results obtained from preliminary experiments, in which reproducible and consistent dose-dependent increases in carotid blood flow were elicited with no changes in blood pressure or heart rate.

The Ethical Committee of our institution (CICUAL), dealing with the use of animals in scientific experiments, approved the protocols of the present investigation.

Data presentation and statistical evaluation

All data are presented as mean ± s.e.m. The peak changes in external carotid blood flow were expressed as percent change from baseline. The difference between the variables within one group of animals was compared by using a two-way repeated measures analysis of variance or between different groups), a two-way analysis of variance (both randomized block design) followed by the Student–Newman-Keuls' test (Steel and Torrie, 1980). Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the compounds used in the present study were obtained from the sources indicated: L-phenylephrine hydrochloride, capsaicin, rat α-CGRP, acetylcholine chloride and SB224289 (2,3,6,7-tetrahydro-1′-methyl-5-[2′-mathyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl] furo [2,3f] indole-3-spiro-4′-piperidine hydrochloride) (Sigma Chemical Co., St Louis, MO, USA); sumatriptan succinate (gift from Dr Paul J Strijbos, GlaxoSmithKline, Harlow, Essex, UK); donitriptan (gift from Dr Gareth John, Institut de Recherche Pierre Fabre, Castres, France); PNU-142633 [(S)-(−)-3,4-dihydro-1-[2-[4-aminocarbonyl)phenyl]-1-piperazinyl]ethyl]-*N*-methyl-1H-2-benzopyran-6-carboxamide] (gift from Dr Robert B McCall, Pharmacia & Upjohn, Kalamazoo, MI, USA); PNU-109291 [(S)-(−)-1-[2-[4-(4-methoxy-phenyl)-piperazin-1-yl]-ethyl]isochroman-6-carboxylic acid methylamide] (gift from Dr Robert B McCall, Pfizer Inc., Kalamazoo, MI, USA); and BRL15572 (1-(3chorophenyl)4-[3,3-diphenyl (2-(S,R)hydroxy propanyl) piperazine] hydrochoride) (Tocris Cookson Inc. Ellisville, MO, USA). All compounds were dissolved in physiological saline. When needed, some drops of 20% ($v\,v^{-1}$) propylene glycol (SB224289 and BRL15572) or 20% ($v\,v^{-1}$) ethanol (capsaicin) were added and, then, the resulting solution was finally diluted with physiological saline; fresh solutions were prepared for each experiment. These vehicles had no effect (when given i.v. or intracarotidly) on external carotid blood flow, blood pressure or heart rate (data not shown). The doses of the antagonists refer to their respective salts, whereas those of the agonists refer to their free base.

Results

Systemic and carotid haemodynamic effects of the different treatments

Baseline values of mean blood pressure, heart rate and external carotid blood flow in the 59 anaesthetized dogs were 175 ± 23 mm Hg, 177 ± 3 beats min^{-1} and 213 ± 8 ml min^{-1}, respectively. These variables were not significantly modified in the subgroups that were about to receive an i.v. bolus injection of either physiological saline (182 ± 23 mm Hg, 157 ± 7 beats min^{-1} and 240 ± 36 ml min^{-1}; respectively), SB224289 (165 ± 7 mm Hg; 205 ± 6 beats min^{-1} and 209 ± 40 ml min^{-1}; respectively) or BRL15572 (164 ± 29 mm Hg; 174 ± 7 beats min^{-1} and 189 ± 13 ml min^{-1}; respectively) after the administration of these compounds (not shown); likewise, no significant changes occurred during the consecutive administration of the cumulative 10-min i.v. infusions of physiological saline (0.5 ml min^{-1}; given six times). Therefore, no time-dependent changes occurred in the haemodynamic variables during the experimental period in the animal model used here.

In contrast, the continuous intracarotid infusion of phenylephrine (1.5 μg min^{-1}; 20 min after starting the infusion; $n=35$) significantly decreased the external carotid blood flow (211 ± 12 ml min^{-1} before and 123 ± 10 ml min^{-1} during treatment) without significant changes in mean blood pressure (136 ± 7 mm Hg before and 146 ± 8 mm Hg during treatment) or heart rate (179 ± 3 beats min^{-1} before and 177 ± 3 beats min^{-1} during treatment). It is to be noted that the decreased external carotid blood flow during the phenylephrine infusion (123 ± 10 ml min^{-1}, equivalent to an approximate decrease of 41%) did not significantly differ from that produced after the 10-min i.v. infusion of the highest dose of sumatriptan or donitriptan (see below and Figure 1). That is why the enhanced vasodilator responses to capsaicin, α-CGRP and acetylcholine during the intracarotid continuous infusion of phenylephrine were considered as the control responses when compared to those produced after the 10-min i.v. infusion of the highest dose of sumatriptan or donitriptan (see below).

Effects of sumatriptan, donitriptan, PNU-142633 or PNU-109291 on systemic and carotid haemodynamic variables

At the doses used, the cumulative 10-min i.v. infusions of sumatriptan or donitriptan, but not of PNU-142633, PNU-109291 or physiological saline, induced dose-dependent decreases in carotid blood flow, with maximal percent changes of 43 ± 6% (sumatriptan) and 40 ± 6% (donitriptan) (see Figure 1). These effects were not accompanied by changes in arterial blood pressure or heart rate and lasted unchanged for a long time, as reported previously (De Vries *et al*., 1998; John *et al*., 1999).

Effect of a continuous intracarotid infusion of physiological saline or phenylephrine on the external carotid vasodilator responses to capsaicin, α-CGRP and acetylcholine

One-minute intracarotid infusions of capsaicin, α-CGRP and acetylcholine induced dose-dependent increases in the carotid blood flow (see Figure 2; control responses); these effects were not accompanied by changes in heart rate or mean blood pressure (not shown). These carotid vasodilator responses: (i) remained without significant changes during a continuous intracarotid infusion of vehicle (0.3 ml min^{-1} of physiological saline throughout the experiment, 20 min after

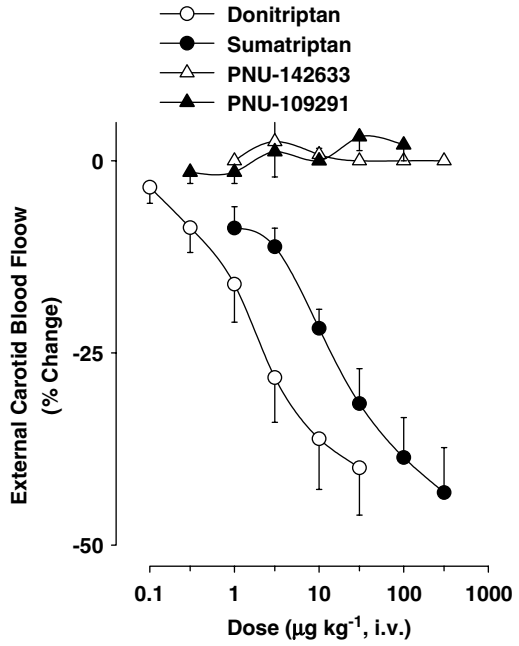

Figure 1 Effect of consecutive administration of cumulative 10 min i.v. infusions of donitriptan, sumatriptan, PNU-142633 or PNU-109291 ($n=4$ for each group) on blood flow through the external carotid artery in dogs. The corresponding i.v. infusions of physiological saline (0.5 ml min^{-1} during 10 min; given 6 times) ($n=4$) were devoid of any effect, but are not shown for the sake of clarity.

the infusion had been started; see Figures 2a, b and c) and (ii) were significantly enhanced (particularly the highest doses) during a continuous intracarotid infusion of phenylephrine (1.5 μg min^{-1} throughout the experiment, 20 min after the infusion had been started; see Figures 2d, e and f). The duration of action of the vasodilator responses to capsaicin and α-CGRP (between 5 and 20 min) was longer-lasting than those to acetylcholine (between 1 and 5 min).

Moreover, during the infusion of phenylephrine (1.5 μg min^{-1}), the enhanced vasodilator responses to capsaicin, α-CGRP and acetylcholine (control responses) remained unchanged after consecutive administration of the 10-min i.v. infusions of physiological saline (data not shown). Hence, the enhanced vasodilator responses to capsaicin, α-CGRP and acetylcholine during phenylephrine infusion were considered as the control responses when compared to those elicited after sumatriptan (300 μg kg^{-1}) or donitriptan (30 μg kg^{-1}).

Effects of i.v. administration of sumatriptan, donitriptan, PNU-142633 or PNU-109291 on the carotid vasodilator responses to capsaicin, α-CGRP and acetylcholine

The carotid vasodilator responses to capsaicin: (i) were significantly inhibited after administration of donitriptan (0.1–30 μg kg^{-1}; Figure 3b), particularly at 30 and 56 μg min^{-1}; and (ii) remained unchanged after administration of sumatriptan (1–300 μg kg^{-1}; Figure 3a), PNU-142633 (1–300 μg kg^{-1}; Figure 3c) or PNU-109291 (0.3–100 μg kg^{-1}; Figure 3d). In

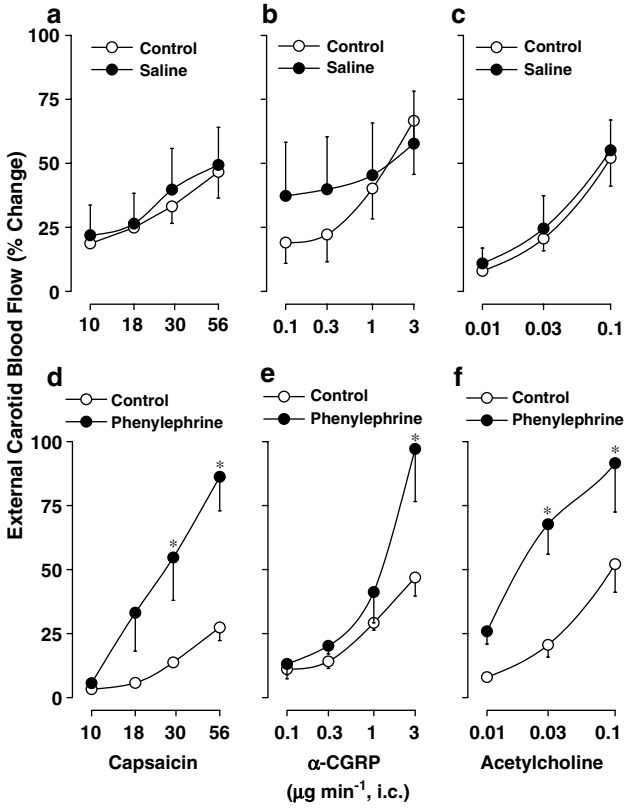

Figure 2 Effect of 1 min intracarotid (i.c.) infusions of capsaicin, α-CGRP or acetylcholine on carotid blood flow before (control responses) and during an i.c. continuous infusion throughout the experiment of either ($n=4$): (i) vehicle (0.3 ml min^{-1} of physiological saline, 20 min later; **a, b, c**) or (ii) phenylephrine (1.5 μg min^{-1}, 20 min later; **d, e, f**). *$P<0.05$ vs the corresponding control response.

contrast, the carotid vasodilator responses to α-CGRP (see Figure 4) and acetylcholine (see Figure 5) were not significantly modified after i.v. administration of these doses of sumatriptan, donitriptan, PNU-142633 or PNU-109291.

Effect of i.v. bolus injections of physiological saline, SB224289 or BRL15572 on the donitriptan-induced inhibition of the carotid vasodilator responses to capsaicin

As the inhibition produced by the 5-$HT_{1B/1D}$ receptor agonist donitriptan on capsaicin-induced carotid vasodilator responses was specific (see above), we decided to investigate the potential involvement of 5-HT_{1B} and/or 5-HT_{1D} receptors by administering i.v. bolus injections of the selective antagonists, SB224289 (5-HT_{1B}) and BRL15572 (5-HT_{1D}) (see Table 1). Hence, Figure 6 shows that the inhibition produced by donitriptan on capsaicin-induced carotid vasodilator responses was: (i) completely antagonized in the animals receiving 300 $\mu g\,kg^{-1}$ of SB224289 (Figure 6b) and (ii) unaffected in the animals receiving 300 $\mu g\,kg^{-1}$ of BRL15572 (Figure 6c) or equivalent volumes of physiological saline (Figure 6a). In fact, when comparing the inhibitory effects of donitriptan after BRL15572 (Figure 6c) or saline (Figure 6a), there was no significant difference ($P>0.05$). It must be emphasized that the above doses of SB224289 and BRL15572 were high enough to completely and selectively block their respective receptors in the external carotid circulation of dogs (De Vries *et al*., 1998).

Discussion and conclusions

General

Apart from the implications discussed below, our results show that capsaicin and α-CGRP produced dose-dependent vasodilator responses, as reported previously in the carotid circulation of anaesthetized pigs (Kapoor *et al*., 2003a, b). Indeed, capsaicin-induced carotid vasodilatation in pigs, which is associated with increased plasma levels of CGRP, can be antagonized by the $CGRP_1$ receptor antagonist,

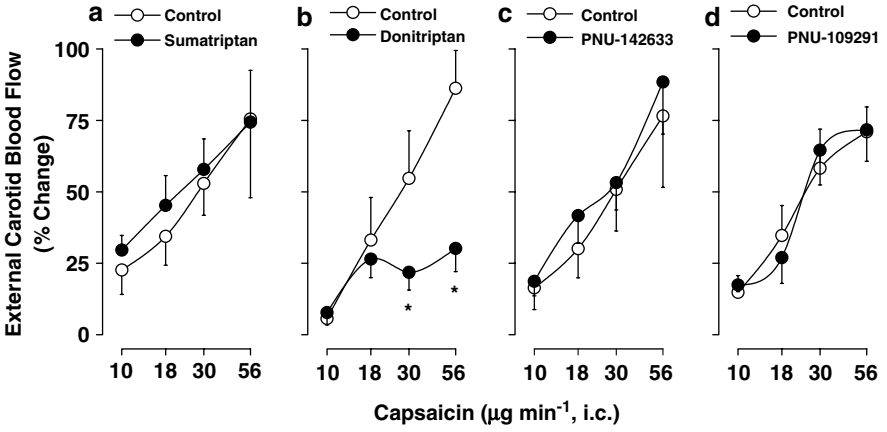

Figure 3 Carotid vasodilator responses induced by 1 min intracarotid (i.c.) infusions of capsaicin before (control responses; elicited during an i.c. continuous infusion of phenylephrine 1.5 $\mu g\,min^{-1}$, 20 min later) and after consecutive administration of cumulative 10 min i.v. infusions of either: (**a**) sumatriptan (1–300 $\mu g\,kg^{-1}$); (**b**) donitriptan (0.1–30 $\mu g\,kg^{-1}$); (**c**) PNU-142633 (1–300 $\mu g\,kg^{-1}$); or (**d**) PNU-109291 (0.3–100 $\mu g\,kg^{-1}$) ($n=4$ for each group). *$P<0.05$ vs the corresponding control response.

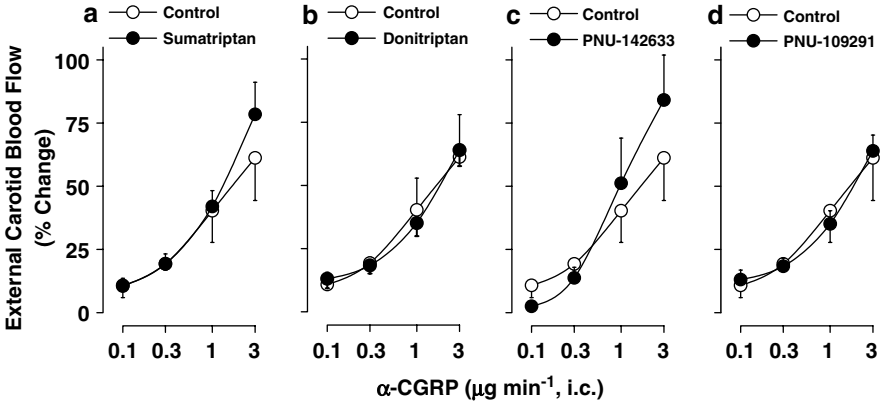

Figure 4 Carotid vasodilator responses induced by 1 min intracarotid (i.c.) infusions of α-CGRP before (control responses; elicited during an i.c. continuous infusion of phenylephrine 1.5 $\mu g\,min^{-1}$, 20 min later) and after consecutive administration of cumulative 10 min i.v. infusions of either: (**a**) sumatriptan (1–300 $\mu g\,kg^{-1}$); (**b**) donitriptan (0.1–30 $\mu g\,kg^{-1}$); (**c**) PNU-142633 (1–300 $\mu g\,kg^{-1}$); or (**d**) PNU-109291 (0.3–100 $\mu g\,kg^{-1}$) ($n=4$ for each group).

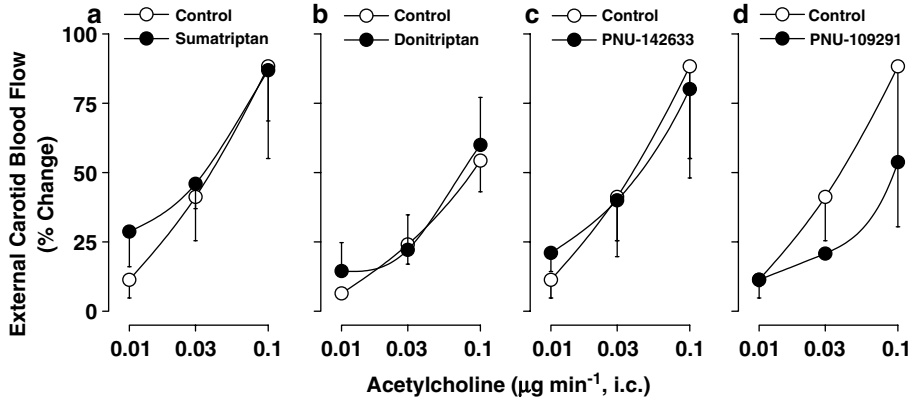

Figure 5 Carotid vasodilator responses induced by 1 min intracarotid (i.c.) infusions of acetylcholine before (control responses; elicited during an i.c. continuous infusion of phenylephrine 1.5 μg min^{-1}, 20 min later) and after consecutive administration of cumulative 10 min i.v. infusions of either: (**a**) sumatriptan (1–300 μg kg^{-1}); (**b**) donitriptan (0.1–30 μg kg^{-1}); (**c**) PNU-142633 (1–300 μg kg^{-1}); or (**d**) PNU-109291 (0.3–100 μg kg^{-1}) ($n=4$ for each group).

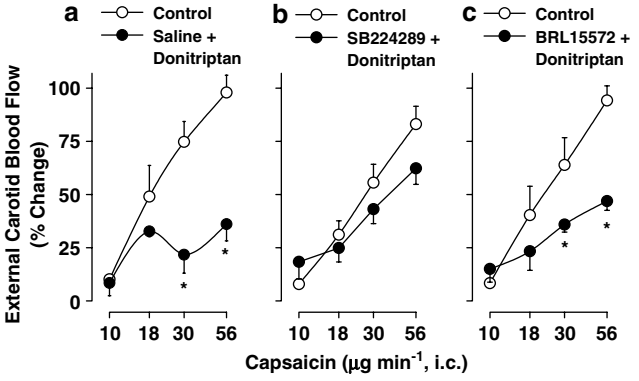

Figure 6 Carotid vasodilator responses induced by 1 min intracarotid (i.c.) infusions of capsaicin before (control responses; elicited during an i.c. continuous infusion of phenylephrine 1.5 μg min^{-1}, 20 min later) and after i.v. bolus injections of either: (**a**) vehicle (physiological saline: 0.15 ml kg^{-1}; $n=3$); (**b**) SB224289 (300 μg kg^{-1}; $n=4$); or (**c**) BRL15572 (300 μg kg^{-1}; $n=4$) followed by i.v. administration of donitriptan (30 μg kg^{-1}). *$P<0.05$ vs the corresponding control response.

BIBN4096BS (Kapoor *et al*., 2003b). Although it is tempting to speculate from our results that capsaicin-induced carotid vasodilatation is also mediated by CGRP release, this hypothesis remains unproven. Notwithstanding, our study clearly demonstrates that, unlike sumatriptan, PNU-142633 and PNU-109291, donitriptan specifically inhibited the carotid vasodilatation to capsaicin, but not that to α-CGRP or acetylcholine. Considering the lipid solubility and brain penetration of donitriptan (John *et al*., 1999), its inhibitory action may involve a central mechanism. This inhibition, being blocked by the antagonist SB224289 (5-HT$_{1B}$), but not by BRL15572 (5-HT$_{1D}$) (Table 1), is mainly mediated by the 5-HT$_{1B}$ receptor subtype.

Systemic and carotid haemodynamic changes produced by the different treatments

The fact that mean blood pressure, heart rate and external carotid blood flow remained unchanged in the animals receiving i.v. saline, SB224289 or BRL15572, as well as continuous intracarotid infusions of saline indicates that no time-dependent changes occurred in the haemodynamic variables during our experimental protocol. Moreover, as the phenylephrine infusion decreased the external carotid blood flow (about 41%) without changing mean blood pressure or heart rate, a local vasoconstrictor effect (mediated by α_1-adrenoceptors; Willems *et al*., 2001) is implied. A similar carotid vasoconstrictor effect was induced after the i.v. infusion of the highest dose of donitriptan (40$\pm$6%) or sumatriptan (43$\pm$6%), with the former being more potent than the latter (Figure 1) as previously reported (John *et al*., 1999). Our results also confirm that the receptors involved in these vasoconstrictor effects resemble the 5-HT$_{1B}$, rather than the 5-HT$_{1D}$ subtype as, at the doses tested: (i) both PNU-142633 and PNU-109291 were inactive on external carotid blood flow (Figure 1); and (ii) the antagonist SB224289, but not BRL15572, abolished the vasoconstriction to donitriptan (see experimental protocol section), as previously shown for sumatriptan (De Vries *et al*., 1998). These findings are in keeping with the clinical antimigraine efficacy exhibited by sumatriptan and donitriptan, but not by PNU-142633 (Gómez-Mancilla *et al*., 2001). It remains to be investigated whether PNU-109291 has antimigraine properties.

It is noteworthy that phenylephrine, given into the carotid artery, and the 5-HT$_1$ receptor agonists mentioned above, given i.v., were administered by different routes. This was due to the fact that in preliminary experiments we observed that, unlike PNU-142633 and PNU-109291, sumatriptan and donitriptan given intracarotidly produced a decrease of 50% in external carotid blood flow at very low doses (up to 1 μg kg^{-1} min^{-1} during 1 min). This low dose of sumatriptan or donitriptan did not inhibit capsaicin-induced external carotid vasodilatation (not shown) probably because the plasma levels of these drugs (particularly donitriptan) were not high enough to reach (and have discernible effects in) the CNS. Consequently, we decided to administer these compounds as i.v. cumulative infusions, so that after the last infusion of each compound had been given, they reached much higher levels just before starting the administration of capsaicin.

Mechanisms involved in the external carotid vasodilator responses to capsaicin, α-CGRP and acetylcholine

The fact that capsaicin, α-CGRP and acetylcholine produced dose-dependent increases in external carotid blood flow without modifying blood pressure or heart rate suggests a local vasodilator response. Regarding the mechanisms involved in these responses, other lines of evidence in the carotid circulation have previously shown the involvement of: (i) CGRP release and activation of BIBN4096BS-sensitive $CGRP_1$ receptors for capsaicin (Kapoor *et al*., 2003b); (ii) BIBN4096BS-sensitive $CGRP_1$ receptors for α-CGRP (Kapoor *et al*., 2003a); and (iii) atropine-sensitive muscarinic receptors located on the vascular endothelium for acetylcholine (Villalón *et al*., 1993).

Potentiation of the vasodilator responses to capsaicin, α-CGRP and acetylcholine during the intracarotid infusion of phenylephrine, but not of saline

The external carotid vasodilator responses to capsaicin, α-CGRP and acetylcholine were highly reproducible as they did not significantly differ before and during the intracarotid infusion of saline in control dogs (Figures 2a, b and c). In contrast, these responses were enhanced during the intracarotid infusion of phenylephrine (Figure 2d, e and f) and were reproducible during the intracarotid infusion of saline (see Results section). This finding is attributed to the decrease in external carotid conductance resulting from an increase in the non-neurogenic vascular tone via the activation of phenylephrine-sensitive α_1-adrenoceptors (Willems *et al*., 2001). Thus, the enhanced vasodilator responses to capsaicin, α-CGRP and acetylcholine during the phenylephrine infusion were considered as the control responses when compared to those elicited after i.v. donitriptan ($30\,\mu g\,kg^{-1}$) or sumatriptan ($300\,\mu g\,kg^{-1}$) (see below).

Specific inhibition by donitriptan, but not by sumatriptan, PNU-142633 or PNU-109291 on the vasodilator responses to capsaicin

Because i.v. donitriptan or sumatriptan produced a carotid vasoconstriction similar to that produced by phenylephrine (see above), any effect of donitriptan or sumatriptan on capsaicin-, α-CGRP- or acetylcholine-induced carotid vasodilatation should be attributed to a direct interaction of these triptans with their respective receptors, rather than to decreases in baseline carotid blood flow. Moreover, it should be kept in mind that the effects of PNU-142633 and PNU-109291 on the vasodilator responses to capsaicin, α-CGRP and acetylcholine were analysed *during* an intracarotid infusion of phenylephrine, as these drugs failed to produce vasoconstriction *per se*. Hence, the fact that donitriptan (but not sumatriptan, PNU-142633 or PNU-109291) significantly inhibited the vasodilator responses to capsaicin (Figure 3) has several possible explanations, including:

(i) Sumatriptan and PNU-142633, being water-soluble, do not easily cross the blood–brain barrier (see Humphrey *et al*., 1991; McCall *et al*., 2002) and, thus, sumatriptan-sensitive $5\text{-}HT_{1B/1D}$ receptors or PNU-142633-sensitive $5\text{-}HT_{1D}$ receptors located peripherally do not seem to be involved; and

(ii) Donitriptan and PNU-109291, being lipid soluble, cross the blood–brain barrier and have access to the CNS (Ennis *et al*., 1998; John *et al*., 1999); nevertheless, as only donitriptan inhibited capsaicin-induced vasodilatation, donitriptan-sensitive $5\text{-}HT_{1B/1D}$ (rather than PNU-109291-sensitive $5\text{-}HT_{1D}$) receptors, probably located in the CNS, seem to be involved.

In keeping with the above suggestions, i.v. sumatriptan failed to inhibit the trigeminal activity induced by electrical stimulation unless the blood–brain barrier had been disrupted (Kaube *et al*., 1993). In addition, i.v. zolmitriptan (another lipid soluble triptan), but not sumatriptan, inhibited the expression of *c*-Fos produced by electrical stimulation of trigeminal neurons (Hoskin and Goadsby, 1998); these differential effects can be explained by central effects produced by zolmitriptan.

On the other hand, our findings showing that donitriptan, sumatriptan, PNU-142633 or PNU-109291 failed to modify the carotid vasodilator responses to α-CGRP (Figure 4) or acetylcholine (Figure 5) demonstrates that the inhibitory effect of donitriptan on the capsaicin-induced vasodilatation: (i) is specific and (ii) does not involve a postjunctional interaction with vascular CGRP or muscarinic receptors. Accordingly, these findings reinforce our assertion that the inhibition by donitriptan involves a central, rather than a peripheral, mechanism.

Involvement of 5-HT_{1B} rather than 5-HT_{1D}, receptors in the inhibition by donitriptan

It has previously been shown that activation of $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$ and $5\text{-}HT_{1F}$ receptors (in decreasing order of potency) inhibits the trigeminovascular nociceptive transmission in cats (Goadsby and Classey, 2003). Our study in dogs is in keeping with the above finding only regarding the role of $5\text{-}HT_{1B}$ receptors (probably owing to differences in species and/or experimental conditions), as the inhibition by donitriptan on capsaicin-induced vasodilatation was: (i) antagonized by SB224289, but not by BRL15572 or vehicle (Figure 6); and (ii) not mimicked by the agonists PNU-142633 and PNU-109291 (Figure 3). Likewise, the role of $5\text{-}HT_{1F}$ receptors in our study is unlikely as donitriptan displays a rather low affinity for these receptors (p*K*i: 5.47; John *et al*., 1999).

Admittedly, the above interpretation is based on the assumption that species differences between the binding of sumatriptan, donitriptan, PNU-142633, PNU-109291, SB224289 and BRL15572 to canine and human $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors do not play a major role (see Table 1).

Possible locus of the 5-HT_{1B} receptors involved in the specific inhibitory action of donitriptan

Our suggestion that donitriptan inhibits capsaicin-induced external vasodilatation mainly by central mechanisms is consistent with previous studies. In this context, electrical stimulation of the sagital sinus (Kaube *et al*., 1993), dural blood vessels (Davis and Dostrovsky, 1986) or the middle meningeal artery (Hoskin *et al*., 1999) has been shown to activate a group of cells in the trigeminocervical complex. It

has been suggested that the trigeminocervical complex is a potential target for 5-$HT_{1B/1D}$ receptor agonists that cross the blood–brain barrier, as a specific binding site for [^{3}H]-sumatriptan has been demonstrated in cats, guinea-pigs and humans (Mills and Martin, 1995; Waeber and Moskowitz, 1995; Pascual *et al*., 1996), as well as for [^{3}H]-zolmitriptan in cats (Goadsby and Knight, 1997). Thus, our results imply that the 5-HT_{1B} receptors involved in the inhibitory action of donitriptan are most likely located in the trigeminocervical complex.

In conclusion, the above results, taken together, suggest that the inhibition produced by donitriptan on capsaicin-induced vasodilatation of the external carotid artery is mainly mediated by 5-HT_{1B} receptors, probably by a central mechanism.

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Conflict of interest

The authors state no conflict of interest.

References

Arulmani U, Heiligers JPC, Garrelds IM, Sánchez-López A, Willems EW, Villalón CM *et al*. (2004a). Effects of sumatriptan on capsaicin-induced carotid haemodynamic changes and CGRP release in anaesthetised pigs. *Cephalalgia* **24:** 717–727.

Arulmani U, Massenvandenbrink A, Villalón CM, Saxena PR (2004b). Calcitonin gene-related peptide and its role in migraine pathophysiology. *Eur J Pharmacol* **500:** 315–330.

Buzzi MG, Carter WB, Shimizu T, Heath H, Moskowitz MA (1991). Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. *Neuropharmacology* **30:** 1193–1200.

Davis KD, Dostrovsky JO (1986). Activation of trigeminal brain-stem nociceptive neurons by dural artery stimulation. *Pain* **25:** 395–401.

De Vries P, Sánchez-López A, Centurión D, Heiligers JP, Saxena PR, Villalón CM (1998). The canine external carotid vasoconstrictor 5-HT_1 receptor: blockade by 5-HT_{1B} (SB224289), but not by 5-HT_{1D} (BRL15572) receptor antagonists. *Eur J Pharmacol* **362:** 69–72.

Dux M, Sántha P, Jancsó G (2003). Capsaicin-sensitive neurogenic sensory vasodilatation in the dura mater of the rat. *J Physiol* **552:** 859–867.

Edvinsson L (2003). New therapeutic target in primary headaches – blocking the CGRP receptor. *Expert Opin Ther Targets* **7:** 377–383.

Ennis MD, Ghazal NB, Hoffman RL, Smith MW, Schlachter SK, Lawson CF *et al*. (1998). Isochroman-6-carboxamides as highly selective 5-HT_{1D} agonists: potential new treatment for migraine without cardiovascular side effects. *J Med Chem* **41:** 2180–2183.

Goadsby PJ, Classey JD (2003). Evidence for serotonin (5-$HT)_{1B}$, 5-HT_{1D} and 5-HT_{1F} receptor inhibitory effects on trigeminal neurons with craniovascular input. *Neuroscience* **122:** 491–498.

Goadsby PJ, Edvinsson L (1993). The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in man and cat. *Ann Neurol* **33:** 48–56.

Goadsby PJ, Knight YE (1997). Direct evidence for central sites of action of zolmitriptan (311C90): an autoradiographic study in cat. *Cephalalgia* **17:** 153–158.

Goadsby PJ, Lipton RB, Ferrarri MD (2002). Migraine–current understanding and treatment. *N Engl J Med* **346:** 257–270.

Gómez-Mancilla B, Cutler NR, Leibowitz MT, Spierings ELH, Klapper JA, Diamond S *et al*. (2001). Safety and efficacy of PNU-142633, a selective 5-HT_{1D} agonist, in patients with acute migraine. *Cephalalgia* **21:** 727–732.

Hagan JJ, Slade PD, Gaster L, Jeffrey P, Hatcher JP, Middlemiss DN (1997). Stimulation of 5-HT_{1B} receptors causes hypothermia in the guinea pig. *Eur J Pharmacol* **331:** 169–174.

Harper AM, Mackenzie ET, Mcculloch J, Pickard JD (1977). Migraine and the blood–brain barrier. *Lancet* **1:** 1034–1036.

Hoskin KL, Goadsby PJ (1998). Comparison of more and less lipophilic serotonin (5.$HT_{1B/1D}$) agonists in a model of trigeminovascular nociception in cat. *Exp Neurol* **150:** 45–51.

Hoskin KL, Zagami A, Goadsby PJ (1999). Stimulation of the middle meningeal artery leads to Fos expression in the trigeminocervical nucleus: a comparative study of monkey and cat. *J Anat* **194:** 579–588.

Hou M, Uddman R, Tajti J, Kanje M, Edvinsson L (2002). Capsaicin receptor immunoreactivity in the human trigeminal ganglion. *Neurosci Lett* **330:** 223–226.

Humphrey PPA, Feniuk W, Marriott AS, Tanner RJ, Jackson MR, Tucker ML (1991). Preclinical studies on the anti-migraine drug, sumatriptan. *Eur Neurol* **31:** 282–290.

John GW, Pauwels PJ, Perez M, Halazy S, Le Grand B, Verscheure Y (1999). F11356, a novel 5-hydroxytriptamine (5-HT) derivative with potent, selective, and unique high intrinsic activity at 5-$HT_{1B/1D}$ receptors in models relevant to migraine. *J Pharmacol Exp Ther* **290:** 83–95.

Kapoor K, Arulmani U, Heiligers JP, Willems EW, Doods H, Villalón CM *et al*. (2003a). Effects of BIBN4096BS on cardiac output distribution and on CGRP-induced carotid haemodynamic responses in the pig. *Eur J Pharmacol* **475:** 69–77.

Kapoor K, Arulmani U, Heiligers JPC, Garrelds IM, Willems EW, Doods H *et al*. (2003b). Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetized pigs. *Br J Pharmacol* **140:** 329–338.

Kaube H, Hoskin KL, Goadsby PJ (1993). Inhibition by sumatriptan of central trigeminal neurons only after blood–brain barrier disruption. *Br J Pharmacol* **109:** 788–792.

Kleinman LI, Radford EP (1964). Ventilation standards for small mammals. *J Appl Physiol* **19:** 360–362.

Leysen JE, Gommeren W, Heylen L, Luyten WH, Van De Weyer I, Vanhoenacker P *et al*. (1996). Alniditan, a new 5-hydroxytryptamine$_{1D}$ agonist and migraine-abortive agent: ligand-binding properties of human 5-hydroxytryptamine$_{1D\alpha}$, human 5-hydroxytryptamine$_{1D\beta}$, and calf 5-hydroxytryptamine$_{1D}$ receptors investigated with [^{3}H]5-hydroxytryptamine and [^{3}H]alniditan. *Mol Pharmacol* **50:** 1567–1580.

McCall RB, Huff R, Chio CL, Tenbrink R, Bergh CL, Ennis MD *et al*. (2002). Preclinical studies characterizing the anti-migraine and cardiovascular effects of the selective 5-HT_{1D} receptor agonist PNU-142633. *Cephalalgia* **22:** 799–806.

Mills A, Martin GR (1995). Autoradiographic mapping of [^{3}H]sumatriptan binding in cat brain stem and spinal cord. *Eur J Pharmacol* **280:** 175–178.

Pascual J, Arco CD, Romon T, Olmo C, Pazos A (1996). [^{3}H]sumatriptan binding sites in human brain regional-dependent labeling of 5HT_{1D} and 5-HT_{1F} receptors. *Eur J Pharmacol* **295:** 271–274.

Potenza MA, De Salvatore G, Montagnani M, Serio M, Mitolo-Chieppa D (1994). Vasodilatation induced by capsaicin in rat mesenteric vessels is probably independent of nitric oxide synthesis. *Pharmacol Res* **30:** 253–261.

Pregenzer JF, Alberts GL, Bin Im W, Slightom JL, Ennis MD, Hoffman RL *et al*. (1999). Differential pharmacology between the guinea-pig and the gorilla 5-HT_{1D} receptor as probed with isocromans (5-HT_{1D}-selective ligands). *Br J Pharmacol* **127:** 468–472.

Price GW, Burton MJ, Collin LJ, Duckworth M, Gaster L, Gothert M *et al*. (1997). SB-216641 and BRL-15572-compounds to pharmacologically discriminate h5-HT_{1B} and h5-HT_{1D} receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **356:** 312–320.

Steel RGD, Torrie JH (1980). *Principles and Procedures of Statistics. A Biomedical Approach*. McGraw Hill Kogakusha Ltd: Tokyo.

Van Rossum D, Hanisch UK, Quirion R (1997). Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors. *Neurosci Biobehav Rev* **21:** 649–678.

Villalón CM, Centurión D, Valdivia LF, De Vries P, Saxena PR (2002). An introduction to migraine: from ancient treatment to functional pharmacology and antimigraine therapy. *Proc West Pharmacol Soc* **45:** 199–210.

Villalón CM, De Vries P, Rabelo G, Centurión D, Sánchez-López A, Saxena PR (1999). Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. *Br J Pharmacol* **126:** 585–594.

Villalón CM, Terrón JA, Hong E (1993). Role of 5-HT_1-like receptors in the increase in external carotid blood flow induced by 5-hydroxytryptamine in the dog. *Eur J Pharmacol* **24:** 9–20.

Waeber C, Moskowitz MA (1995). [^{3}H]sumatriptan labels both 5-HT_{1D} and 5-HT_{1F} receptor binding sites in the guinea pig brain: an autoradiographic study. *Naunyn-Schmiedeberg's Arch Pharmacol* **352:** 263–275.

Willems EW, Valdivia LF, Saxena PR, Villalón CM (2001). The role of several α_1- and α_2-adrenoceptor subtypes mediating vasoconstriction in the canine external carotid circulation. *Br J Pharmacol* **132:** 1292–1298.