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Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetised pigs

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1 Calcitonin gene-related peptide (CGRP), a potent vasodilator released from capsaicin-sensitive trigeminal sensory nerves, seems to be involved in the pathogenesis of migraine. Hence, CGRP receptor antagonists may serve as a novel treatment for migraine. This study was therefore designed to investigate the effects of BIBN4096BS (100, 300 and 1000 $\mu g\,kg^{-1}$, i.v.), a potent and selective CGRP receptor antagonist, on capsaicin-induced carotid haemodynamic changes in anaesthetised pigs. Both vagosympathetic trunks were cut and phenylephrine was infused into the carotid artery (i.c.) to support carotid vascular tone.

2 Infusions of capsaicin (0.3, 1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$, i.c.) did not alter the heart rate, but dose-dependently increased the mean arterial blood pressure. This moderate hypertensive effect was not modified by BIBN4096BS.

3 Capsaicin infusion (10 $\mu g\,kg^{-1}\,min^{-1}$, i.c.) increased total carotid, arteriovenous anastomotic and tissue blood flows and conductances as well as carotid pulsations, but decreased the difference between arterial and jugular venous oxygen saturations. These responses to capsaicin were dose-dependently blocked by BIBN4096BS.

4 Capsaicin infusion (10 $\mu g\,kg^{-1}\,min^{-1}$, i.c.) more than doubled the jugular venous plasma concentration of CGRP. This effect was not blocked, but rather increased, by BIBN4096BS.

5 The above results show that BIBN4096BS behaves as a potent antagonist of capsaicin-induced carotid haemodynamic changes that are mediated *via* the release of CGRP. Therefore, this compound may prove effective in the treatment of migraine.
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Keywords: Arteriovenous anastomoses; BIBN4096BS; capsaicin; carotid circulation; CGRP; CGRP receptor antagonists; microspheres; migraine and pig

Abbreviations: A−V SO_2 difference, difference between arterial and jugular venous oxygen saturations; BIBN4096BS, 1-piperidinecarboxamide, *N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2 H)-quinazolinyl)-, [*R*-(*R**,*S**)]-; CGRP, calcitonin gene-related peptide; i.c., intracarotid arterial route of administration

Introduction

Although a complete understanding of the pathogenesis of migraine remains elusive thus far, there seems little doubt that dilatation of cranial blood vessels, including carotid arteriovenous anastomoses, is involved in the headache phase (De Vries *et al*., 1999). Moreover, evidence is accumulating that a release of vasoactive neuropeptides from the trigeminal sensory nerves may be an important factor in the genesis of migraine (Goadsby *et al*., 2002). In this respect, a high-circulating plasma concentration of calcitonin gene-related peptide (CGRP) has been demonstrated during migraine headache (Goadsby *et al*., 1990), and these concentrations can be normalised by tryptans in parallel with alleviation of headache (Goadsby *et al*., 1990; Ashina *et al*., 2000). Indeed, CGRP is widely distributed in the body, including the central and peripheral parts of the trigeminovascular system (Brain *et al*., 1985; Van Rossum *et al*., 1997; Juaneda *et al*., 2000; Poyner & Marshall, 2001), where it is colocalised with substance P, neurokinin A and/or 5-HT_{1D} receptors (Gulbenkian *et al*., 1995; 2001; Smith *et al*., 2002). CGRP can mediate neurogenic dilatation of cranial blood vessels, as well as sensory nerve transmission between the first- and second-order afferent input from these vessels during migraine headache (Gulbenkian *et al*., 2001; Williamson & Hargreaves, 2001; Goadsby *et al*., 2002; Smith *et al*., 2002). Thus, it follows that inhibition of CGRP-mediated cranial vasodilatation and sensory nerve transmission with a potent and selective CGRP receptor antagonist may prove a novel strategy in treating migraine.

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The recent discovery of a dipeptide CGRP receptor antagonist 1-piperidinecarboxamide, *N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl] amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]- 4-(1,4-dihydro-2-oxo-3(2 H)-quinazolinyl)-, [*R*-(*R**,*S**)]-(BIBN4096BS) (Doods *et al*., 2000; Doods, 2001) represents a significant advance in exploring the pathophysiological role of CGRP in migraine. BIBN4096BS displays a very high affinity for human CGRP receptors (Doods *et al*., 2000; Wu *et al*., 2000; Edvinsson *et al*., 2002; Moreno *et al*., 2002; Verheggen *et al*., 2002). This compound is undergoing clinical trials for aborting migraine headache, and the clinical results are awaited with great interest.

Using an animal model that seems to be predictive of antimigraine activity (Spierings & Saxena, 1980; Villalón & Terrón, 1994; Saxena, 1995; Saxena *et al*., 1998; De Vries *et al*., 1999; Tfelt-Hansen *et al*., 2000), the present study in anaesthetised pigs was designed (i) to investigate the effects of capsaicin (pungent substance in red chilli pepper), which releases neuropeptides, including CGRP (Alving *et al*., 1991; Jansen-Olesen *et al*., 1996; Szallasi & Blumberg, 1999; Eltorp *et al*., 2000), on systemic and carotid haemodynamics, and (ii) to establish if BIBN4096BS is able to attenuate the responses induced by capsaicin. A preliminary account of this investigation was presented at the XIVth World Congress of Pharmacology (Kapoor *et al*., 2002).

Methods

General

After an overnight fast, a total of 22 pigs (Yorkshire × Landrace, females, 10–14 kg; $n=11$ each for vehicle and BIBN4096BS) were sedated with azaperone (120 mg, i. m.) and midazolam hydrochloride (10 mg, i.m.), and then anaesthetised with sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35–7.48; pCO_2: 35–48 mmHg; pO_2: 100–120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (12–20 mg kg^{-1} h^{-1}). This anaesthetic regimen, together with bilateral vagosympathectomy (see below), increases the heart rate and markedly dilates carotid arterioles and arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Consequently, carotid blood flow, particularly its arteriovenous anastomotic fraction, is considerably higher in these pigs than in conscious or thiopental-anaesthetised pigs (Den Boer *et al*., 1993).

Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Both common carotid arteries were dissected free, and the accompanying vagosympathetic trunks were cut between two ligatures to prevent any possible influence *via* baroreceptor reflexes on the carotid vascular responses produced by capsaicin. Pulsatile and mean carotid blood flows were measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). The amplitude of carotid blood flow signals provided an *index* of carotid flow pulse. Subsequently, three hubless needles, connected to a polyethylene tube, were inserted into the right common carotid artery for the administration of capsaicin, radioactive microspheres and the α_1-adrenoceptor agonist phenylephrine. The use of phenylephrine is necessitated by the fact that the carotid arterioles and arteriovenous anastomoses are already in a dilated state under the present anaesthetic regime (Den Boer *et al*., 1993), and, therefore, to study the effects of vasodilator agents (in the present case capsaicin), one has to constrict them first. As described earlier (Willems *et al*., 1999), phenylephrine decreases total carotid conductance exclusively by constricting carotid arteriovenous anastomoses, which results in an increase in the difference between arterial and jugular venous oxygen saturations (A−V SO_2 difference) (Saxena, 1987).

Lastly, catheters were placed in the right external jugular vein for the withdrawal of venous blood samples to measure blood gases (ABL-510; Radiometer, Copenhagen, Denmark) and plasma concentrations of CGRP (see below), inferior vena cava (*via* the left femoral vein) for the administration of the vehicle or BIBN4096BS and aortic arch (*via* the left femoral artery) for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) as well as withdrawal of arterial blood samples to measure blood gases.

Heart rate and systolic, diastolic and mean arterial blood pressures as well as mean and pulsatile carotid artery blood flows were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). Vascular conductances were calculated by dividing respective blood flows (ml min^{-1}) by mean arterial blood pressure (mmHg), multiplied by 100 and expressed as 10^{-2} ml min^{-1} mmHg^{-1}. During the experiment, body temperature was maintained at 37 ± 1°C by a heating pad, and the animal was infused with physiological saline to compensate for fluid losses.

Distribution of carotid blood flow

The distribution of common carotid blood flow into tissue (capillary) and arteriovenous anastomotic fractions was determined in 13 pigs (later receiving vehicle, $n=7$ or BIBN4096BS, $n=6$) with radioactive microspheres (diameter: 15.5 ± 0.1 μm; s.d.), and labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, U.S.A.). For each measurement, a suspension of about 200,000 microspheres, labelled with one of the isotopes, was mixed and injected into the carotid artery. At the end of the experiment, the animal was killed using an overdose of pentobarbital, and the heart, kidneys, lungs and different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows for discriminating the different isotopes (^{141}Ce: 120–167 keV, ^{113}Sn: 355–435 keV, ^{103}Ru: 450–548 keV, ^{95}Nb: 706–829 keV and ^{46}Sc: 830–965 keV). All data were processed by a set of specially designed computer programs (Saxena *et al*., 1980).

The distribution of total carotid blood flow to different tissues (Q_{tis}) was calculated by the formula: $Q_{tis}=(I_{tis}/I_{total})\times Q_{carotid}$, where I_{tis} is the tissue radioactivity, I_{total} is the

sum of radioactivity counted in tissues and $Q_{carotid}$ is the total common carotid blood flow at the time of microsphere injection. Since little or no radioactivity was detected in the heart or kidneys, it can be assumed that all microspheres trapped in lungs reach the lungs from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs can be used as an *index* of the arteriovenous anastomotic fraction of carotid blood flow (Saxena *et al*., 1980; Saxena & Verdouw, 1982).

Determination of plasma concentration of CGRP

Jugular venous blood samples were obtained from 12 pigs, receiving vehicle or BIBN4096BS ($n=6$ each). Four of these animals (two each for vehicle and BIBN4096BS) had been used for carotid haemodynamic experiments, while the other eight were separate experiments using the same protocol, except that the radioactive microspheres were not used. Blood was transferred immediately into a polypropylene tube containing ethylene dinitrotetraacetic acid (1 mg ml^{-1} of blood) and aprotinin (500 KIU ml^{-1} of blood). Aprotinin was used to inhibit endogenous plasma proteases, since we observed that CGRP is not detectable in biological samples without aprotinin (unpublished). After centrifugation at $1600\times g$ for 15 min, plasma samples were coded and stored at -80°C until CGRP measurements were performed. The person measuring CGRP concentrations remained blind to the treatments, until all data had been collated.

CGRP was extracted from plasma using a C_{18}SEP-COLUMN, dried by lypholisation, and measured by radioimmunoassay (Dwenger, 1984), as per the protocol of the Peninsula Laboratories, Inc (Belmont, CA, U.S.A.). The recovery of CGRP from the extraction procedure was ascertained by assaying control samples paired with a duplicate sample spiked with known quantities of CGRP. The column recovery values were 85, 79, 81, 89 and 92% (mean = 85.2; standard deviation = 5.4; coefficient of variation = 6.3%). The CGRP concentrations measured in the actual samples were, however, not corrected for the loss in the extraction procedure.

Experimental protocol

Following surgery and after haemodynamic condition of the animals ($n=22$) had been stable for 15–20 min (heart rate: 107 ± 4 beats min^{-1}, mean arterial blood pressure: 95 ± 2 mmHg, mean carotid blood flow: 120 ± 12 ml min^{-1} and A−V SO_2 difference: 7.6 ± 1.1%), phenylephrine was infused into the right common carotid artery at a rate of 10 μg kg^{-1} min^{-1} for 10 min, followed by 3–6 μg kg^{-1} min^{-1} throughout the rest of the experiment. The latter dose of phenylephrine was chosen so that the external jugular venous oxygen saturation was between 60 and 70%, and the mean carotid blood flow was about 40% of the original value. After a period during which haemodynamic variables remained constant for at least 60 min (heart rate: 130 ± 4 beats min^{-1}, mean arterial blood pressure: 105 ± 2 mmHg, mean carotid blood flow: 48 ± 5 ml min^{-1} and A−V SO_2 difference: 31 ± 2.3%; $n=22$), the animals received consecutive infusions (0.15, 0.45, 1.5 and 4.5 ml, i.c. during 3 min each) of capsaicin vehicle (see Compounds and kits section). It is important to mention that the vehicle of capsaicin was devoid of any systemic and carotid haemodynamic responses (data not shown).

At 5–10 min after the last infusion of capsaicin vehicle, blood samples were obtained for the measurements of blood gases and CGRP concentration, and values of heart rate, arterial blood pressure and total carotid blood flow and conductance were collated (baseline values; 11 pigs each for vehicle and BIBN4096BS). In 13 of the 22 pigs (7 and 6 for vehicle and BIBN4096BS, respectively), the first batch of radioactive microspheres was injected for determining the baseline distribution of carotid blood flow. The animals then received consecutive infusions of capsaicin (0.3, 1, 3 and 10 μg kg^{-1} min^{-1}, i.c. for 3 min each) and heart rate, arterial blood pressure and total carotid blood flow were determined at the end of each infusion. In addition, after the last infusion of capsaicin (10 μg kg^{-1} min^{-1}), blood gases, plasma CGRP concentration and carotid blood flow distribution were measured as described above (control values). Subsequently, a recovery period of 20 min was allowed until all haemodynamic parameters had returned to baseline levels. At this point, the animals were divided into two groups, which were treated with i.v. infusions (rate: 0.5 ml min^{-1} for 10 min) of either vehicle (three times 5 ml of acidified distilled water) or BIBN4096BS (100, 300 and 1000 μg kg^{-1}). At 10 min after each infusion, capsaicin was given, and haemodynamic and biochemical variables were measured again, as described above.

Data presentation and statistical analysis

All data are presented as mean ± s.e.m., unless stated otherwise. The statistical analysis was performed using the SPSS package for windows (version 10.0; SPSS Inc., Chicago, IL, U.S.A.). The significance of changes within one group (vehicle or BIBN4096BS) was analysed with repeated-measures ANOVA, followed by Greenhouse–Geisser correction for serial autocorrelation (Ludbrook, 1994) and Bonferroni correction for multiple comparisons (Overall & Doyle, 1996). The significance of the between-group changes (vehicle vs BIBN4096BS treatments) was first analysed with repeated-measures ANOVA, including baseline measurements as a covariate (Overall & Doyle, 1994) and the Greenhouse–Geisser correction. If the two groups differed significantly, pairwise comparisons of corresponding values in the vehicle- and BIBN4096BS-treated groups were performed using univariate analysis (Overall & Atlas, 1999), followed by Bonferroni correction. Statistical significance was accepted at $P<0.05$ (two-tailed).

Ethical approval

The Ethics Committee of the Erasmus MC, Rotterdam, dealing with the use of animals in scientific experiments, approved the protocols for this investigation.

Compounds and kits

The following compounds were used: aprotinin (5850 KIU mg^{-1}; Roth, Karlsruhe, Germany), azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), BIBN4096BS (gift from Boehringer Ingelheim Pharma KG, Biberach, Germany), capsaicin, Tween 80, ethanol and

phenylephrine hydrochloride (all from Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands), ethylene dinitrotetraacetic acid (Merck, Darmstadt, Germany), heparin sodium (to prevent blood clotting in catheters; Leo Pharmaceutical Products, Weesp, The Netherlands), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands), phenylephrine hydrochloride (Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands) and sodium pentobarbital (Sanofi Sante b.v., Maasluis, The Netherlands). The radioimmunoassay kit for CGRP was purchased from Peninsula Laboratories, Inc. (Belmont, CA, U.S.A.).

Capsaicin was initially dissolved in tween 80, ethanol and physiological saline in the ratio of 0.5 : 1 : 8.5 ml, respectively. Phenylephrine was dissolved in distilled water, while BIBN4096BS was initially dissolved in 0.5 ml of 1N HCl, then diluted with 4 ml of distilled water and adjusted to pH 6.5 by 1N NaOH.

Results

Baseline values

Baseline values in the 22 pigs used were: heart rate, 126 ± 3 beats min^{-1}; mean arterial blood pressure, 105 ± 3 mmHg; total carotid blood flow, 40 ± 5 ml min^{-1}; total carotid vascular conductance, 39 ± 5 10^{-2} ml min^{-1} $mmHg^{-1}$ and A−V SO_2 difference, 38 ± 2%. No significant differences in baseline values were found between the two groups of animals ($n = 11$ each) that later received vehicle or BIBN4096BS.

Effect of different doses of capsaicin on heart rate, blood pressure and carotid blood flow

Figure 1 depicts heart rate, mean arterial blood pressure and total carotid blood flow and conductance changes produced by the infusions of capsaicin (0.3, 1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$, i.c.) before (control response) and after treatments with BIBN4096BS (100, 300 and 1000 $\mu g\,kg^{-1}\,min^{-1}$, i.v.) or the corresponding volumes of vehicle. In both groups of animals, capsaicin elicited dose-dependent increases in mean arterial blood pressure as well as total carotid blood flow and conductance, without significantly affecting heart rate. These effects of capsaicin remained essentially unchanged after the administration of vehicle (0.5 ml), except that a slight attenuation was noticed in the increases in carotid blood flow and conductance after the third dose of vehicle. In contrast, BIBN4096BS produced a dose-dependent attenuation of capsaicin-induced increases in total carotid blood flow and conductance, but not in blood pressure (Figure 1).

Carotid haemodynamic changes following capsaicin infusion

The carotid haemodynamic effects observed after the highest infusion (10 $\mu g\,kg^{-1}\,min^{-1}$) of capsaicin were examined in more detail in animals receiving vehicle or BIBN4096BS.

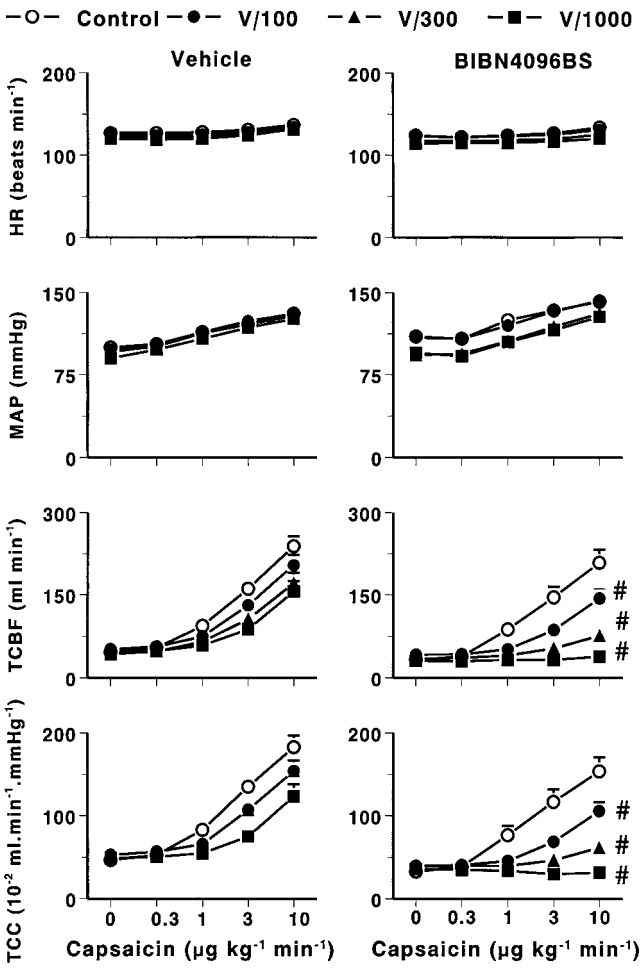

Figure 1 Heart rate (HR), mean arterial blood pressure (MAP) and total carotid blood flow (TCBF), and vascular conductance (TCC) values at baseline (capsaicin 0 $\mu g\,kg^{-1}\,min^{-1}$), and following infusions of capsaicin (0.3, 1,3, 10 $\mu g\,kg^{-1}\,min^{-1}$, i.c.) in anaesthetised pigs before (control) and after i.v. administrations of vehicle (V, 5 ml three times; $n = 11$) or BIBN4096BS (100, 300 and 1000 $\mu g\,kg^{-1}$, $n = 11$). All values are expressed as mean ± s.e.m. While HR was not affected, capsaicin increased MAP as well as TCBF and conductance (significance not shown for the sake of clarity). BIBN4096BS dose-dependently antagonised capsaicin-induced carotid haemodynamic changes, but not the increase in arterial blood pressure. $^{\#}P < 0.05$ vs response after the corresponding volume of vehicle.

Effect on carotid blood flow and pulsations

As shown in Figure 2, i.c. infusions of capsaicin (10 $\mu g\,kg^{-1}\,min^{-1}$) clearly increased carotid blood flow and conductance (both depicted as maximum absolute changes) as well as pulsations. In animals treated with vehicle, there was some decrease in the responses to capsaicin, but these responses were significantly more attenuated in animals treated with BIBN4096BS, particularly the two highest doses.

Fractionation of carotid blood flow and vascular conductance

In both vehicle and BIBN4096BS groups, capsaicin (10 $\mu g\,kg^{-1}\,min^{-1}$, i.c.) significantly increased total carotid blood flow and conductance as well as those distributed to

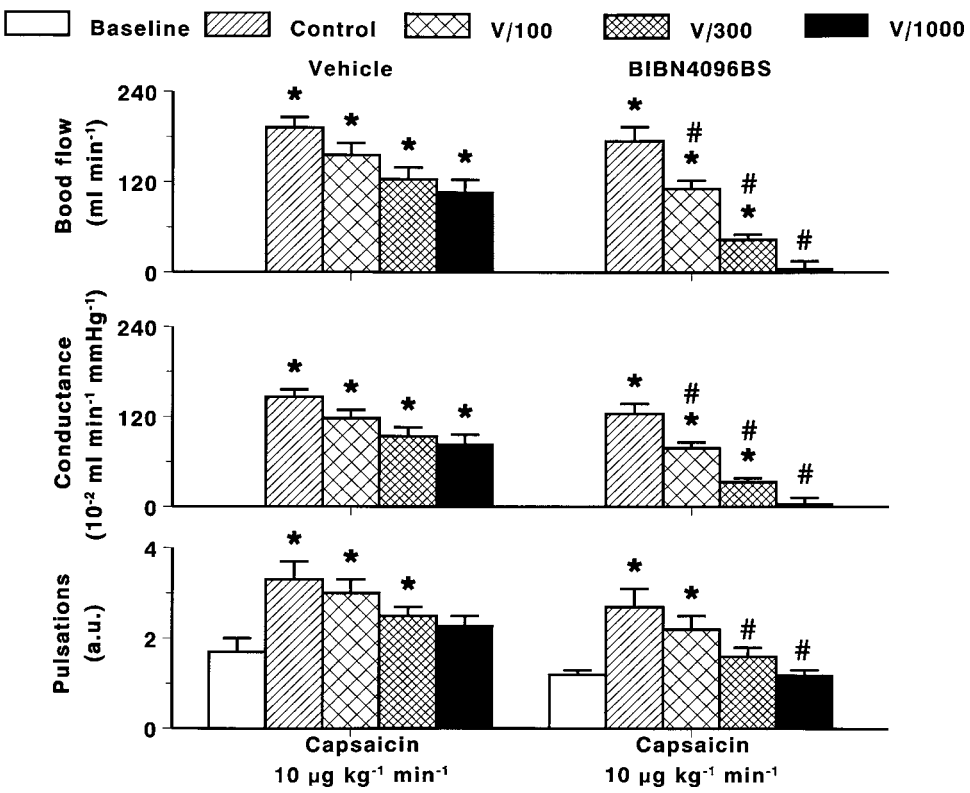

Figure 2 Maximum changes in carotid blood flow, vascular conductance and pulsations measured at baseline and following infusions of capsaicin ($10\,\mu g\,kg^{-1}\,min^{-1}$, i.c.) given in anaesthetised pigs before (control) and after i.v. administrations of vehicle (V, 5 ml three times; $n=11$) or BIBN4096BS (100, 300 and $1000\,\mu g\,kg^{-1}$, $n=11$). All values are expressed as mean ± s.e.m. a.u., arbitrary units. $^{*}P<0.05$ vs baseline values; $^{\#}P<0.05$ vs response after the corresponding volume of vehicle.

arteriovenous anastomoses and capillaries. The increases from baseline values in blood flows and vascular conductances were, respectively: total carotid, 494±59 and 362±40%; arteriovenous anastomotic fraction, 726±282 and 505±188% and capillary fraction, 526±48 and 389±32% ($n=13$ in each case).

The effects of BIBN4096 as well as of its vehicle on the carotid haemodynamic responses to capsaicin are illustrated in Figure 3. Compared to the corresponding volumes of vehicle, the increases in total, arteriovenous anastomotic as well as capillary blood flows and vascular conductances were clearly antagonised after the two highest infusions (300 and $1000\,\mu g\,kg^{-1}\,min^{-1}$) of BIBN4096BS.

Figure 4 shows that capsaicin ($10\,\mu g\,kg^{-1}\,min^{-1}$, i.c.) increased vascular conductances to the different cranial tissues, including the skin, ear, skeletal muscles, fat, bone, eye, tongue and dura mater, but not in the brain or salivary glands. As has been described with 5-hydroxytryptamine (Saxena & Verdouw, 1982), the increase in skin blood flow was most likely responsible for the redness of skin on the side of capsaicin infusion (not shown in the figure). These effects of capsaicin were significantly and dose-dependently antagonised by BIBN4096BS (100, 300 and $1000\,\mu g\,kg^{-1}\,min^{-1}$, i.v.), but not by the corresponding volumes of vehicle.

Difference between arterial and jugular venous oxygen saturations (A−V SO_2 difference)

Consistent with the increase in arteriovenous anastomotic blood flow, capsaicin ($10\,\mu g\,kg^{-1}\,min^{-1}$, i.c.) significantly decreased A−V SO_2 difference from baseline values of 38±2 to 4.5±0.4% ($n=22$). This response remained unaffected in animals treated with vehicle, but was dose-dependently antagonised by BIBN4096BS (Figure 5).

Jugular venous plasma concentrations of CGRP

In the 12 pigs used for this purpose, the baseline value of CGRP concentration in jugular venous plasma was 27±2 pg ml^{-1} and following capsaicin infusion ($10\,\mu g\,kg^{-1}\,min^{-1}$, i.c.) it increased by 119±17% to 58±5 pg ml^{-1}.

Figure 6 shows the effects of capsaicin ($10\,\mu g\,kg^{-1}\,min^{-1}$, i.c.) on jugular venous plasma concentration of CGRP in pigs receiving either three i.v. infusions of vehicle (5 ml each) or BIBN4096BS (100, 300 and $1000\,\mu g\,kg^{-1}$). Capsaicin increased plasma CGRP concentration in both animal groups by a similar magnitude, and this increase was not attenuated in either vehicle- or BIBN4096BS-treated group of animals. Interestingly, following the two highest doses of BIBN4096BS (300 and $1000\,\mu g\,kg^{-1}$, i.v.) there was even a potentiation of capsaicin-induced increases in plasma CGRP concentrations (control response: 138±29%; response after BIBN4096BS: 211±30 and 211±38%, respectively; $n=6$).

Discussion

General

Although there is much debate about the pathogenesis of migraine, there seems to be a general agreement regarding its

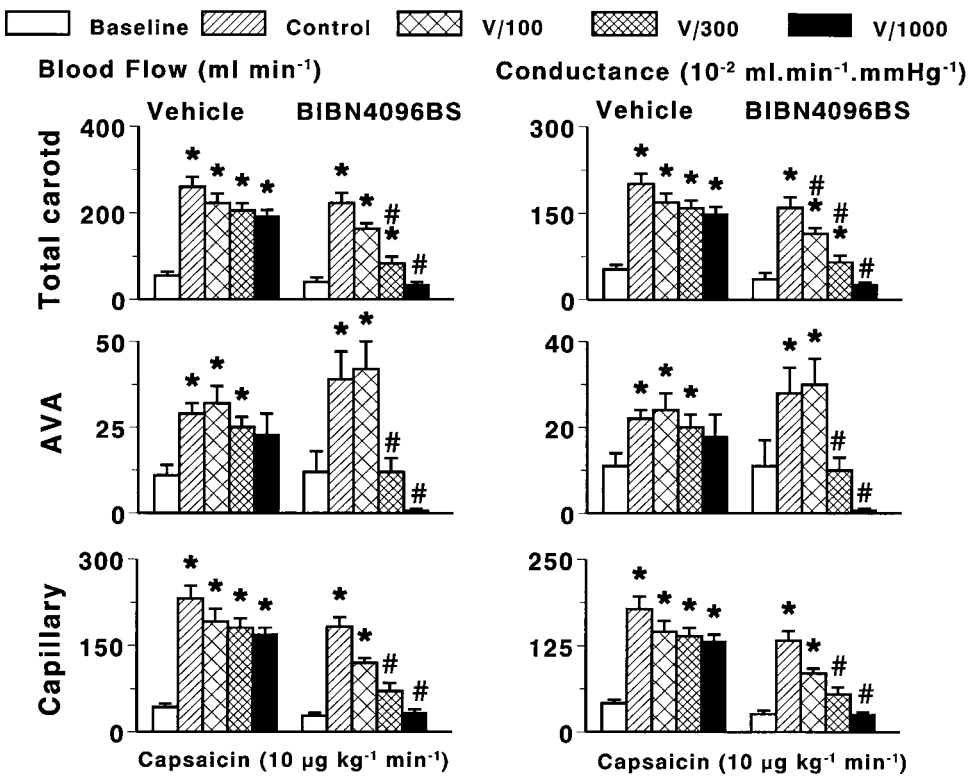

Figure 3 Total carotid, arteriovenous anastomotic (AVA) and capillary blood flows (left panel) and vascular conductances (right panel) measured at baseline and following infusions of capsaicin (10 μg kg^{-1} min^{-1}, i.c.) given in anaesthetised pigs before (control) and after i.v. administrations of vehicle (V, 5 ml three times; $n=7$) or BIBN4096BS (100, 300 and 1000 μg kg^{-1}, $n=6$). All values are expressed as mean $\pm$ s.e.m. $^{*}P<0.05$ vs baseline values; $^{\#}P<0.05$ vs response after the corresponding volume of vehicle.

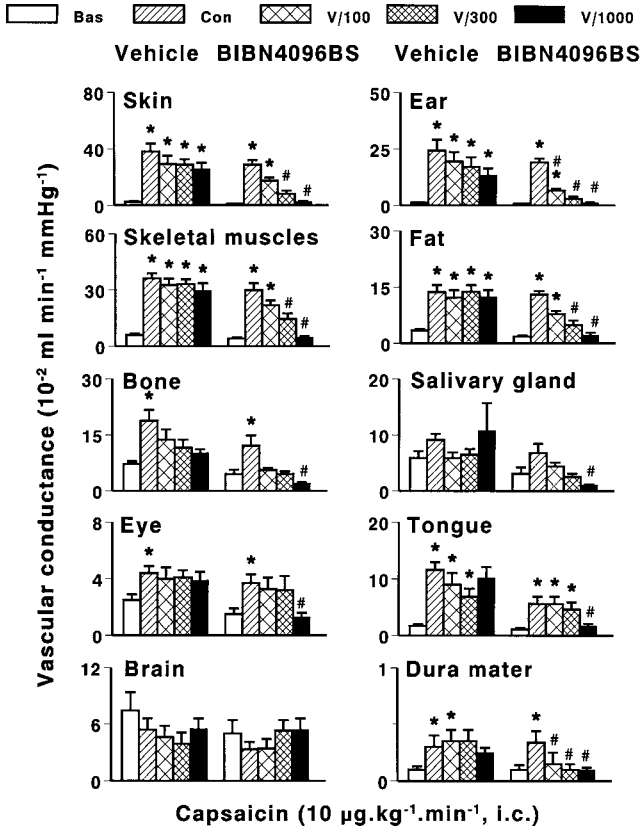

Figure 4 Distribution of carotid vascular conductances to head tissues measured at baseline (Bas) and following infusions of capsaicin (10 μg kg^{-1} min^{-1}, i.c.) given in anaesthetised pigs before (Con) and after i.v. administrations of vehicle (V, 5 ml three times; $n=7$) or BIBN4096BS (100, 300 and 1000 μg kg^{-1}, $n=6$). All values are expressed as mean $\pm$ s.e.m. $^{*}P<0.05$ vs baseline values; $^{\#}P<0.05$ vs response after the corresponding volume of vehicle.

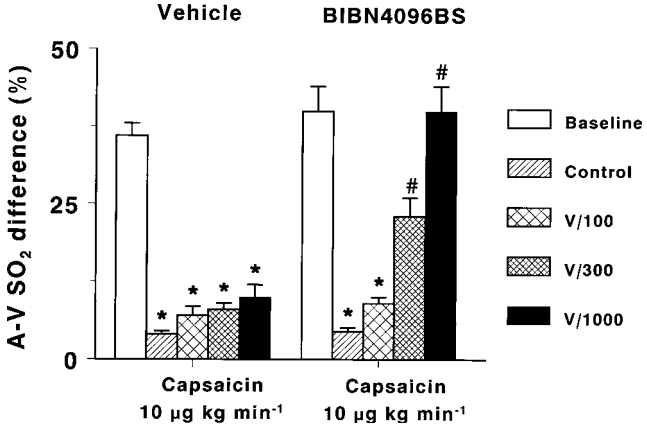

Figure 5 Differences between arterial and jugular venous oxygen saturations (A–V SO_2 difference) measured at baseline and following infusions of capsaicin (10 μg kg^{-1} min^{-1}, i.c.) given in anaesthetised pigs before (control) and after i.v. administrations of vehicle (V, 5 ml three times; $n=11$) or BIBN4096BS (100, 300 and 1000 μg kg^{-1}, $n=11$). All values are expressed as mean $\pm$ s.e.m. $^{*}P<0.05$ vs baseline values; $^{\#}P<0.05$ vs response after the corresponding volume of vehicle.

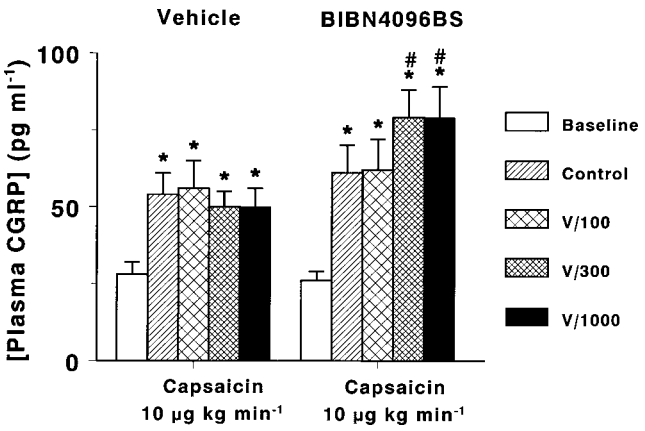

Figure 6 Jugular venous plasma CGRP concentrations measured at baseline and after infusions of capsaicin ($10\,\mu g\,kg^{-1}\,min^{-1}$, i.c.) given in anaesthetised pigs before (control) and after i.v. administrations of vehicle (V, 5 ml three times; $n=6$) or BIBN4096BS (100, 300 and $1000\,\mu g\,kg^{-1}$, $n=6$). All values are expressed as mean + s.e.m. $^*P<0.05$ vs baseline values; $P<0.05$ vs response after the corresponding volume of vehicle.

neurovascular nature (Goadsby & Edvinsson, 1993; De Vries *et al*., 1999; Goadsby *et al*., 2002; Villalón *et al*., 2002). Thus, there is a release of vasoactive peptides producing intense cranial vasodilatation, increased arterial pulsations and a sterile inflammatory reaction with pain (Moskowitz *et al*., 1989; De Vries *et al*., 1999). Among these neuropeptides, CGRP is considered as a biological marker in migraine pathogenesis (Van Rossum *et al*., 1997; Goadsby *et al*., 2002; Hagner *et al*., 2002). Moreover, stimulation of trigeminal sensory neurones with electrical procedures or chemical substances, like capsaicin, releases endogenously stored CGRP (Buzzi *et al*., 1995; Eltorp *et al*., 2000) that, in turn, dilates cranial vessels (Williamson & Hargreaves, 2001), including carotid arteriovenous anastomoses (Van Gelderen *et al*., 1995). In addition, CGRP may also facilitate sensory nerve transmission between the first- and second-order afferent input from these vessels during migraine headache (Gulbenkian *et al*., 2001; Goadsby *et al*., 2002; Smith *et al*., 2002). On this basis, it is reasonable to assume that CGRP receptor antagonists can be a novel approach to antimigraine therapy. In this respect, recent *in vitro* studies have shown that BIBN4096BS, a potent and 'silent' CGRP receptor antagonist (Doods *et al*., 2000), inhibits CGRP-induced dilatation of isolated cranial blood vessels (Edvinsson *et al*., 2002; Verheggen *et al*., 2002). BIBN4096BS can also effectively antagonise CGRP-induced carotid vasodilatation in anaesthetised pigs (Kapoor *et al*., 2003). Therefore, it seems important to investigate the effects of BIBN4096BS on the carotid haemodynamic responses produced by endogenous CGRP released by capsaicin, in a porcine model predictive of antimigraine activity (Saxena, 1995; De Vries *et al*., 1999; Tfelt-Hansen *et al*., 2000). Our results show that: (i) i.c. administration of capsaicin increased blood pressure, but dilated carotid arteriovenous anastomoses and arterioles, together with an increase in carotid pulsations and a narrowing of A−V SO_2 difference as well as an elevation of jugular venous plasma CGRP concentration; and (ii) BIBN4096BS dose-dependently antagonised the changes in carotid haemodynamics and A−V SO_2 difference caused by capsaicin, but it enhanced the capsaicin-induced increase in jugular venous plasma CGRP concentration.

Systemic haemodynamic responses to capsaicin

The widespread distribution of CGRP immunoreactivity in cardiovascular tissues suggests that CGRP may play a role in the regulation of systemic and regional haemodynamics (Bell & McDermott, 1996; Hagner *et al*., 2002). In fact, several *in vivo* studies have evidenced a hypotensive response to CGRP due to its potent vasodilator action (Bell & McDermott, 1996; Shen *et al*., 2001). In contrast, our study shows a significant increase in mean blood pressure following i.c. capsaicin, and this increase was not abolished by BIBN4096BS. Despite the absence of clear tachycardic responses to i.c. capsaicin, the simplest interpretation of these findings may be that the vasopressor response to capsaicin is not mediated *via* CGRP receptors, but is rather due to an interaction with vasoconstrictor mechanisms. Indeed, not only do high subcutaneous doses ($50\,mg\,kg^{-1}$) of capsaicin increase plasma CGRP concentrations, but also plasma catecholamines, neurokinin A and neuropeptide Y concentrations (Alving *et al*., 1991).

Carotid haemodynamics

Stimulation of the trigeminal ganglion increases cerebral blood flow and releases endogenous vasoactive neuropeptides, including CGRP (Goadsby *et al*., 1988). Vasoactive neuropeptides are also released from sensory afferent nerves by capsaicin, but its relaxant effect on isolated cerebral blood vessels is mediated by CGRP, rather than by substance P or neurokinin A (Jansen *et al*., 1990; O'Shaughnessy *et al*., 1993; Jansen-Olesen *et al*., 1996). These findings are in full agreement with our results in anaesthetised pigs showing dose-dependent vasodilator responses to capsaicin in the carotid circulation, including arteriovenous anastomoses and arterioles. Admittedly, as reported earlier (Szallasi & Blumberg, 1999), vasodilator responses to capsaicin tended to wear off in vehicle-treated animals, suggestive of tachyphylaxis. This tachyphylaxis was rather limited, possibly due to a neuronal reuptake of released CGRP into capsaicin-sensitive perivascular nerves (Sams-Nielsen *et al*., 2001). However, compared to the vehicle-treated animals, the carotid haemodynamic effects of capsaicin were clearly much more attenuated by the potent and selective CGRP receptor antagonist BIBN4096BS (Doods *et al*., 2000; Wu *et al*., 2000; 2002; Doods, 2001). BIBN4096BS has also been demonstrated to effectively block the relaxation of blood vessels by CGRP, both *in vitro* (Doods *et al*., 2000; Edvinsson *et al*., 2002; Moreno *et al*., 2002; Verheggen *et al*., 2002; Wu *et al*., 2002) and *in vivo* (Doods *et al*., 2000), including the porcine carotid vascular bed (Kapoor *et al*., 2003). Therefore, it is clear that carotid vasodilatation by capsaicin in the present investigation is mediated by the release of CGRP.

A−V *SO_2 difference*

During the headache phase of migraine, the A−V SO_2 difference is abnormally low, presumably due to an opening of arteriovenous shunts (Heyck, 1969). Thus, a reduction of carotid arteriovenous anastomotic blood flow, with a consequent normalisation of the A−V SO_2 difference, makes our

porcine vascular model highly predictive of antimigraine activity (Saxena, 1987; 1995; De Vries *et al*., 1999). In the present study, i.c. infusions of capsaicin significantly decreased A−V SO_2 difference together with dilatation of carotid arteriovenous anastomoses. Since both these effects of capsaicin were effectively blocked by BIBN4096BS, it confirms that capsaicin-induced responses are mediated *via* the release of CGRP. Indeed, CGRP also decreases A−V SO_2 difference, and this effect is antagonised by BIBN4096BS (Kapoor *et al*., 2003).

Plasma concentrations of CGRP

The release of CGRP by capsaicin is mediated by selective activation of the Aδ- and C-fibre sensory neurones *via* vanilloid receptors (Caterina *et al*., 1997; Ebersberger *et al*., 1999; Eltorp *et al*., 2000). Our results showing an increase in plasma concentrations of CGRP after capsaicin (see Figure 6) are consistent with the above observations. Interestingly, not only did BIBN4096BS fail to block capsaicin-induced CGRP release, but also there was a modest enhancement of CGRP release. There is evidence for uptake of CGRP into perivascular, capsaicin-sensitive neurones in the guinea-pig isolated basilar artery (Sams-Nielsen *et al*., 2001). Therefore, it may well be that blockade of prejunctional 'inhibitory' CGRP autoreceptors by BIBN4096BS led to increased release of CGRP by capsaicin, similar to the modulation of sympathetic neurotransmission by presynaptic α-adrenoceptors (Langer, 1980).

It may be noted that plasma CGRP concentrations measured by us at baseline (27 ± 2 pmol ml^{-1}, $n = 12$) as well as after capsaicin treatment (58 ± 5 pmol ml^{-1}, $n = 12$) are in agreement with those previously reported in pigs (Table 1) (Alving *et al*., 1991; Arden *et al*., 1994; Kallner *et al*., 1998).

Table 1 Plasma CGRP concentration range (pg ml^{-1}) in pigs

Baseline	*Capsaicin*	*Sampled from*	*Reference*
10	36	Femoral artery	Alving *et al*. (1991)
11–16	Not measured	Femoral artery and interventricular vein	Kallner *et al*. (1998)
4–12	Not measured	Carotid artery	Arden *et al*. (1994)
14–38	27–88	External jugular vein	Present investigation

Possible clinical implications

Lastly, we would like to consider the possible clinical implications of our results with BIBN4096BS within the context of antimigraine therapy. Indeed, the trigeminovascular system, a functional network of cranial blood vessels and their trigeminal innervation, seems to be activated during migraine (Goadsby *et al*., 2002), thereby provoking CGRP release and cranial blood vessel dilatation. Thus, a blockade of the release and/or the effects of CGRP are likely to provide novel avenues for developing antimigraine drugs without associated vasoconstriction. BIBN4096BS may be such a compound, and the present findings demonstrating that it effectively antagonises the carotid vasodilator responses elicited by capsaicin are indeed encouraging. Obviously, the results of currently undergoing clinical trials with BIBN4096BS are awaited with great interest; these would be crucial in determining not only the role of CGRP in the pathophysiology of migraine, but also of such compounds as therapeutic agents.

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