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Effects of BIBN4096BS on cardiac output distribution and on CGRP-induced carotid haemodynamic responses in the pig

Kapil Kapoor[a], Udayasankar Arulmani[a], Jan P.C. Heiligers[a], Edwin W. Willems[a], Henri Doods[b], Carlos M. Villalón[c], Pramod R. Saxena[a,*]

[a] *Department of Pharmacology, Erasmus MC, University Medical Center Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands*
[b] *Boehringer Ingelheim Pharma KG, Biberach, Germany*
[c] *Departamento de Farmacobiología, CINVESTAV-IPN, Czda. de los Tenorios 235, Col. Granjas-Coapa, 14330 Mexico D.F., Mexico*

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Abstract

Calcitonin gene related peptide (CGRP) seems to be involved in the pathogenesis of migraine, since plasma CGRP levels increase during the headache phase. In the present study, we investigated the effects of a novel CGRP receptor antagonist, 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**)]-), on the regional cardiac output distribution and on the carotid haemodynamic changes induced by α-CGRP in anaesthetised pigs. Treatment with BIBN4096BS (100, 300 and 1000 μg kg^{-1}, i.v.) did not affect the heart rate, mean arterial blood pressure or systemic vascular conductance, but a small decrease in cardiac output was noticed; the latter was, however, not significantly different from that in vehicle-treated animals. The highest dose of BIBN4096BS moderately decreased vascular conductance in the lungs, kidneys, spleen and adrenals. Vascular conductance in other tissues including the brain, heart, gastrointestinal system, skin and skeletal muscles remained unchanged. Intracarotid artery infusions of α-CGRP (10, 30 and 100 pmol kg^{-1} min^{-1} during 3 min) increased the total carotid blood flow and conductance, but decreased the arterial blood pressure. These responses were dose-dependently blocked by BIBN4096BS. The above results show that BIBN4096BS is a CGRP receptor antagonist in the porcine carotid and systemic circulations, but the endogenous CGRP does not seem to play an important physiological role in regulating basal vascular tone. These findings suggest that BIBN4096BS may have therapeutic usefulness in migraine.

Keywords: Arteriovenous anastomosis; BIBN4096BS; Carotid vasodilatation; Cardiac output; CGRP (calcitonin gene-related peptide); α-CGRP human; Migraine; (Pig)

1. Introduction

Calcitonin gene related peptide (CGRP), a 37 amino acid neuropeptide generated by alternative splicing of the calcitonin gene (Amara et al., 1982), is widely distributed in the body, including in trigeminal sensory nerve fibres innervating central and peripheral blood vessels, where it is co-localised with other vasoactive neuropeptides, such as substance P and neurokinin A (Gulbenkian et al., 1995, 2001). CGRP is a potent vasodilator agent in a wide variety of tissues (Brain et al., 1985; Juaneda et al., 2000; Poyner and Marshall, 2001; Van Rossum et al., 1997) and, although exogenous α-CGRP has potent systemic and regional haemodynamic effects (Gardiner et al., 1990), the physiological role of endogenous CGRP is not clear (Shen et al., 2001). This is mainly due to the unavailability of potent and selective CGRP receptor antagonists; the most widely used CGRP receptor antagonist thus far, CGRP-(8–37) is not very potent and displays partial agonist properties (Waugh et al., 1999; Wisskirchen et al., 1998). Clearly, the advent of 'silent', selective and potent non-peptide CGRP receptor antagonists would be valuable in this regard.

Interestingly, CGRP has been implicated in the pathogenesis of migraine (Ashina et al., 2000; Durham and Russo, 2002; Edvinsson, 2001; Goadsby et al., 1990), and it can mediate neurogenic dilatation of cranial blood vessels as well as sensory nerve transmission between the first

* Corresponding author. Tel.: +31-10-408-75-37; fax: +31-10-408-94-58.
E-mail address: p.saxena@erasmusmc.nl (P.R. Saxena).
URL: http://www.eur.nl/fgg/pharm/.

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and second order afferent input from these vessels during migraine headache (Goadsby et al., 2002; Gulbenkian et al., 2001; Smith et al., 2002; Williamson and Hargreaves, 2001). Significantly, plasma levels of CGRP, but not of other neurotransmitter (e.g. neuropeptide Y, vasoactive intestinal peptide or substance P), are elevated during migraine and, after sumatriptan, these levels are normalised paralleling the resolution of headache (Goadsby, 1999; Goadsby et al., 1990). Therefore, inhibition of α-CGRP release or blockade of α-CGRP-induced vasodilatation may be a novel approach in the management of acute migraine headache.

Doods et al. (2000) have recently described a small molecule CGRP receptor antagonist, BIBN4096BS (1-piperidinecarboxamide, *N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-pipera-zinyl]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**)]-), which possesses over 200 fold higher affinity for human (SK-N-MC cells; K_i: 14 pM) than for rat (spleen; K_i: 3.4 nM) CGRP receptors. BIBN4096BS as well as the endogenous ligand CGRP and its analogues concentration dependently displaces [^{3}H]BIBN4096BS from SK-N-MC cell membranes with the rank order of affinity: BIBN4096BS>human α-CGRP = human β-CGRP>[Cys(Et)^{2,7}] human α-CGRP>adrenomedullin (high affinity site) = human α-CGRP$_{8-37}$ = human β-CGRP$_{8-37}$ ≫ calcitonin = amylin (Schindler and Doods, 2002). The compound inhibits vasodilatation evoked by trigeminal ganglion stimulation in marmosets (Doods et al., 2000) and by CGRP in several human isolated blood vessels (Edvinsson et al., 2002; Moreno et al., 2002; Verheggen et al., 2002). The purpose of the present study in anaesthetised pigs was to investigate the effects of BIBN4096BS on: (i) the complete distribution of cardiac output to assess the potential role of endogenous CGRP in regulating basal vascular tone and thereby the cardiovascular safety of BIBN4096BS, and (ii) the haemodynamic responses produced by intracarotid arterial (i.c.) infusion of α-CGRP in a model predictive of antimigraine activity (De Vries et al., 1999; Saxena, 1995).

2. Materials and methods

2.1. General

After an overnight fast, 25 domestic pigs (Yorkshire x Landrace, females, 10–14 kg) were sedated with intramuscular injections of azaperone (120 mg) and midazolam hydrochloride (10 mg) and then anaesthetised with sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds, 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 mm Hg; pO_2: 100–120 mm Hg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (12–20 mg kg^{-1} h^{-1}). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. A catheter was placed in the inferior vena cava via the right femoral vein for the administration of vehicle and BIBN4096BS. Another catheter was placed in the aortic arch via the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). During the experiment, body temperature was kept around 37 °C and the animal was continuously infused with physiological saline to compensate for fluid losses.

Heart rate and systolic, diastolic and mean arterial blood pressure as well as the pulsatile and mean carotid artery blood flows (see later) were continuously monitored on a polygraph (CRW).

2.2. Cardiac output and its distribution

Cardiac output was measured by the thermodilution method using a 6F Swan–Ganz catheter (Braun Melsungen) introduced into the pulmonary artery via the left femoral vein.

The distribution of cardiac output was determined with 15.5 ± 0.1 (S.D.) μm diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, a suspension of about 1,000,000 microspheres, labelled with one of the isotopes, was injected into the left ventricle via a catheter guided by way of the left carotid artery. Starting 15 s before microsphere injection and lasting 70 s, a reference arterial blood sample was withdrawn (Withdrawal pump, Harvard Apparatus, Southnatick, Mass, USA; rate: 6 ml min^{-1}) via a catheter placed into the right femoral artery. An infusion of the corresponding volume of Haemaccel compensated blood loss during this procedure.

At the end of the experiment, the animal was killed using an overdose of pentobarbital. Subsequently, a number of tissues (lungs, kidneys, heart, stomach, small intestine, spleen, liver, adrenals, brain, skin and skeletal muscles) were dissected out, weighed and put into vials. The radioactivity in these vials was counted for 5 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000) using suitable windows for the discrimination of 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) using a personal computer. Tissue blood flows were calculated by multiplying the ratio of tissue and reference blood sample radioactivities by the blood withdrawal rate

(6 ml min^{-1}) and normalised to 100 g of tissue weight. Systemic and tissue vascular conductances were calculated by dividing cardiac output (ml min^{-1}) and tissue blood flows (ml.min^{-1}/100 g tissue), respectively, by mean arterial blood pressure (mm Hg). Radioactivity in the lungs mainly represents peripheral arteriovenous anastomotic blood flow (the non-nutrient part of the cardiac output), although a small part (1–1.5% of cardiac output) is derived from the bronchial arteries (Baile et al., 1982).

2.3. Carotid haemodynamic responses to CGRP

Both common carotid arteries and the external jugular veins were dissected free and the accompanying vagosympathetic trunks were cut between two ligatures in order to prevent a possible influence of CGRP via baroreceptor reflexes. Pulsatile and mean 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. Carotid vascular conductance was calculated by dividing the carotid blood flow (ml min^{-1}) by the mean arterial blood pressure (mm Hg).

The right external jugular vein was catheterised for obtaining jugular venous blood samples to determine blood gases. Two hub-less needles, connected to polyethylene tubes, were inserted into the right common carotid artery and used for intracarotid (i.c.) infusions of phenylephrine (α_1-adrenoceptor agonist) and α-CGRP, respectively. It should be noted that under pentobarbital anaesthesia, carotid arteriovenous anastomoses are dilated (Den Boer et al., 1993) and, therefore, to elicit vasodilator responses to CGRP, a continuous infusion of phenylephrine was used throughout the experiment. We have previously reported that phenylephrine decreases total carotid blood flow and conductance exclusively due to constriction of carotid arteriovenous anastomoses (Willems et al., 1999), resulting in an increase in the difference between arterial and jugular venous oxygen saturations (A-V SO_2 difference) (Saxena, 1987).

2.4. Experimental protocols

In the case of cardiac output distribution experiments ($n=12$), baseline values of heart rate, mean arterial blood pressure, cardiac output and its distribution to the various tissues (see above) were determined after a stabilisation period of at least 90 min. The animals were then divided into two groups ($n=6$ each) receiving three i.v. infusions (rate: 0.5 ml min^{-1}) of either BIBN4096BS (100, 300 and 1000 μg kg^{-1}) or its vehicle (5 ml of acidified distilled water); each dose was given over 10 min with an intervening period of 10 min before the next dose. At the end of each infusion, the above-mentioned haemodynamic variables were collated again. Lastly, the final measurements were made 40 min after the third dose of vehicle or BIBN4096BS (recovery).

In the case of the carotid artery experiments ($n=13$), phenylephrine (10 μg kg^{-1} min^{-1} for 10 min, followed by 3–6 μg kg^{-1} min^{-1} throughout the rest of the experiment) was infused into the right common carotid artery to maintain carotid blood flow at a constant low level. After a stabilisation period of at least 90 min, values of heart rate, arterial blood pressure, total carotid blood flow and A-V SO_2 difference were collated. The animal was then given three sequential i.c. infusions (rate: 0.083–1 ml min^{-1}, depending on the weight of the animal) of CGRP (10, 30 and 100 pmol kg^{-1} min^{-1}) for 3 min and the above variables (except the A-V SO_2 difference, which was determined only after the highest dose) were collated again. After the highest dose of α-CGRP, a recovery period of 20 min was allowed to elapse when all haemodynamic parameters returned to baseline levels. At this point, the animals were divided into two groups receiving three i.v. infusions (rate: 0.5 ml min^{-1}) of either BIBN4096BS (100, 300 and 1000 μg kg^{-1}; $n=7$) or its vehicle (5 ml of acidified distilled water; $n=6$); each dose was given over a period of 10 min with an intervening period of about 10 min before the next dose. Ten minutes after each treatment, the values of mean arterial blood pressure, heart rate, total carotid blood flow and A-V SO_2 difference were collated. CGRP was infused as above after each treatment and data were collated again.

It may be mentioned that the vehicle of α-CGRP (distilled water) was devoid of any systemic and carotid haemodynamic responses (data not shown).

2.5. Data presentation and statistical analysis

All data have been expressed as mean ± S.E.M., unless stated otherwise. The significance of changes from baseline values within one group (vehicle or BIBN4096BS) was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Saxena et al., 1980; Steel and Torrie, 1980). The differences in baseline haemodynamic values and percent change (from baseline values) in haemodynamic variables by corresponding doses of the vehicle and BIBN4096BS (between group comparisons) were evaluated by Student's unpaired *t*-test. Student's unpaired *t*-test was also applied to compare the changes in the effects of CGRP observed after different corresponding doses of the vehicle and BIBN4096BS. Statistical significance was accepted at $P<0.05$ (two-tailed).

2.6. Ethical approval

The Ethics Committee of the Erasmus MC, Rotterdam, dealing with the use of animals in scientific experiments,

approved investigation protocols, which adhere to EEC guidelines.

2.7. Compounds

The following compounds were used: azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), BIBN4096BS and human α-CGRP (Boehringer Ingelheim Pharma, Biberach, 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).

Phenylephrine and α-CGRP were dissolved in distilled water, while BIBN4096BS was initially dissolved in 0.5 ml of 1 N HCl and subsequently diluted with 4 ml of distilled water, and then adjusted to pH 6.5 with 1 N NaOH.

3. Results

3.1. Effect of BIBN4096BS on cardiac output and its distribution

3.1.1. Baseline values

Baseline values of heart rate, mean arterial blood pressure, cardiac output (expressed as cardiac index) and systemic vascular conductance in anaesthetised pigs ($n=12$) were: 108 ± 3 beats min^{-1}, 102 ± 2 mm Hg, 133 ± 4 ml min^{-1} kg^{-1} and 1491 ± 54 ml min^{-1} mm Hg^{-1}, respectively. Baseline values of regional vascular conductances (ml min^{-1} mm Hg^{-1}/100-g tissue) were: brain, 31 ± 3; heart, 104 ± 10; liver, 34 ± 8; stomach, 24 ± 2; lungs (mainly systemic arteriovenous anastomoses), 229 ± 37; adrenals, 138 ± 10; kidneys, 263 ± 14; spleen, 126 ± 15; skeletal muscles, 3.3 ± 0.3; and skin, 11 ± 2.

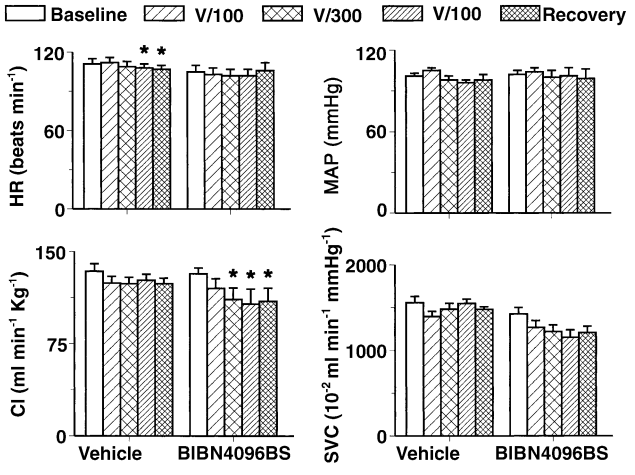

Fig. 1. Heart rate (HR), mean arterial blood pressure (MAP), cardiac index (CI) and systemic vascular conductance (SVC) measured at baseline, after i.v. treatments with either vehicle (V, three times 5 ml; $n=6$) or BIBN4096BS (BIBN; 100, 300 and 1000 μg kg^{-1}; $n=7$) and after 40 min of recovery. All values are presented as mean ± S.E.M. $^*P<0.05$ vs. baseline. The changes after BIBN4096BS are not significantly different from those in the corresponding vehicle group.

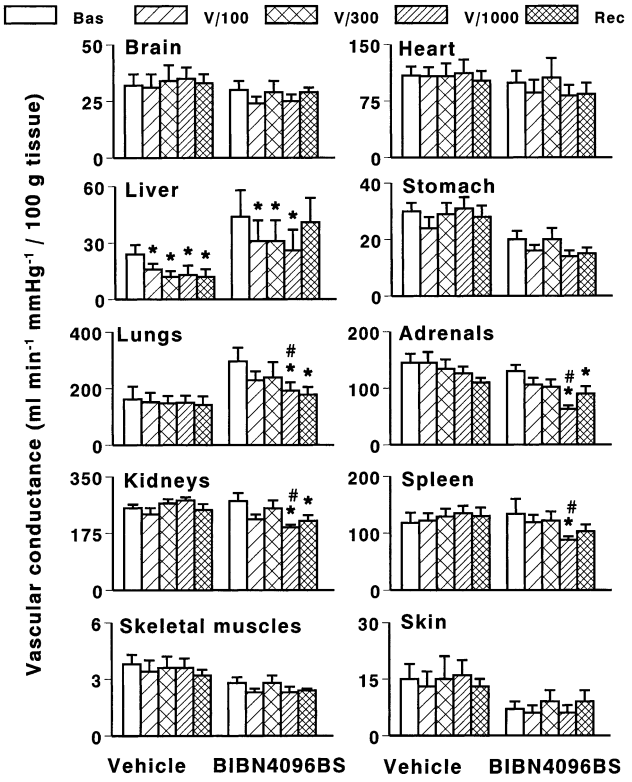

Fig. 2. Regional vascular conductances at baseline (Bas), after i.v. treatments with either vehicle (V, three times 5 ml; $n=6$) or BIBN4096BS (100, 300 and 1000 μg kg^{-1}, i.v.; $n=6$) and after 40 min of recovery (Rec). All values are presented as mean ± S.E.M. $^*P<0.05$ vs. baseline. $^\#P<0.05$ vs. the corresponding change in animals treated with vehicle.

3.1.2. Systemic and regional haemodynamic changes

Systemic haemodynamic values collated at baseline, after vehicle or BIBN4096BS (100, 300 and 1000 μg kg^{-1}, i.v.) and after a 40-min recovery period, are shown in Fig. 1. There were no statistically significant differences ($P>0.05$) in baseline values in the vehicle and BIBN4096BS groups. Except for small decreases in heart rate by the vehicle (maximum change: $4 \pm 1\%$) and cardiac index by BIBN4096BS (maximum change: $19 \pm 8\%$), no other changes were observed. The changes in cardiac index by BIBN4096BS did not differ significantly ($P>0.05$) from those in the vehicle-treated animals (maximum change: $7 \pm 3\%$).

Fig. 2 presents the regional vascular conductances in a number of tissues in animals treated with either vehicle or BIBN4096BS (100, 300 and 1000 μg kg^{-1}, i.v.). Baseline values in the two groups were not significantly different ($P>0.05$) in any of the tissues, including the liver, lungs and skin. Apart from decreases in liver conductance, no other

changes in regional vascular conductances were noticed in the vehicle-treated group. BIBN4096BS produced small decreases in vascular conductance to liver, and with the highest dose (1000 μg kg^{-1}) in lungs, adrenals, kidneys and spleen. Only the latter changes were significant when compared with the corresponding changes in the vehicle-treated animals.

3.2. Effect of BIBN4096BS on the haemodynamic responses to i.c. infusions of α-CGRP

3.2.1. Baseline values

Baseline values in anaesthetised pigs ($n = 13$) were: heart rate, 129 ± 5 beats min^{-1}; mean arterial blood pressure, 122 ± 4 mm Hg; carotid flow pulse, 1.7 ± 0.1 arbitrary units (a.u.); total carotid blood flow, 67 ± 7 ml min^{-1}; total carotid vascular conductance, 56 ± 5 10^{-2} ml min^{-1} mm Hg^{-1}; and A-V SO_2 difference, $26 \pm 3\%$. Baseline values in the two groups of animals (vehicle and BIBN4096BS) did not differ significantly.

3.2.2. Systemic and carotid haemodynamic responses

Fig. 3 shows the original tracings illustrating the systemic (blood pressure and heart rate) and carotid (flow pulse and total carotid blood flow) haemodynamic responses in anaesthetised pigs obtained with α-CGRP (10, 30 and 100 pmol kg^{-1} min^{-1}, i.c.) before and after i.v. treatments with three doses of vehicle (5 ml each time; upper panel) or BIBN4096BS (100, 300 and 1000 μg kg^{-1}; lower panel). The infusions of α-CGRP did not affect the heart rate, but decreased the arterial blood pressure and increased the carotid flow pulse and blood flow. These changes were accompanied by redness of head skin and ears on the side of infusion (not shown in the figure). The effects of α-CGRP were clearly attenuated in the animals receiving BIBN4096BS, but not in the ones treated with vehicle.

The effects of α-CGRP (10, 30 and 100 pmol kg^{-1} min^{-1}, i.c.) in the animals treated with vehicle or BIBN 4096BS (100, 300 and 1000 μg kg^{-1}, i.v.) were quantified as percent changes from baseline values (Fig. 4). In both groups, infusions of α-CGRP before treatments with vehicle or BIBN4096 (control infusions) produced dose-dependent decreases in mean arterial blood pressure and increases in total carotid blood flow (data not shown) and conductance; heart rate was not affected (data not shown). These responses to α-CGRP remained unaffected after vehicle, but, in contrast, were dose-dependently antagonised by BIBN4096BS (Fig. 4).

As shown in Fig. 5, infusions of α-CGRP (100 pmol kg^{-1} min^{-1}, i.c.) clearly increased carotid blood flow (depicted as the maximum changes) and carotid blood flow pulsations (compare baseline and control values). While there was little change in animals treated with vehicle, BIBN4096BS (100, 300 and 1000 μg kg^{-1}, i.v.) dose-dependently antagonised the responses to α-CGRP.

3.2.3. Changes in the A-V SO_2 difference

α-CGRP (100 pmol kg^{-1} min^{-1}, i.c.) produced a significant reduction in the A-V SO_2 difference in both

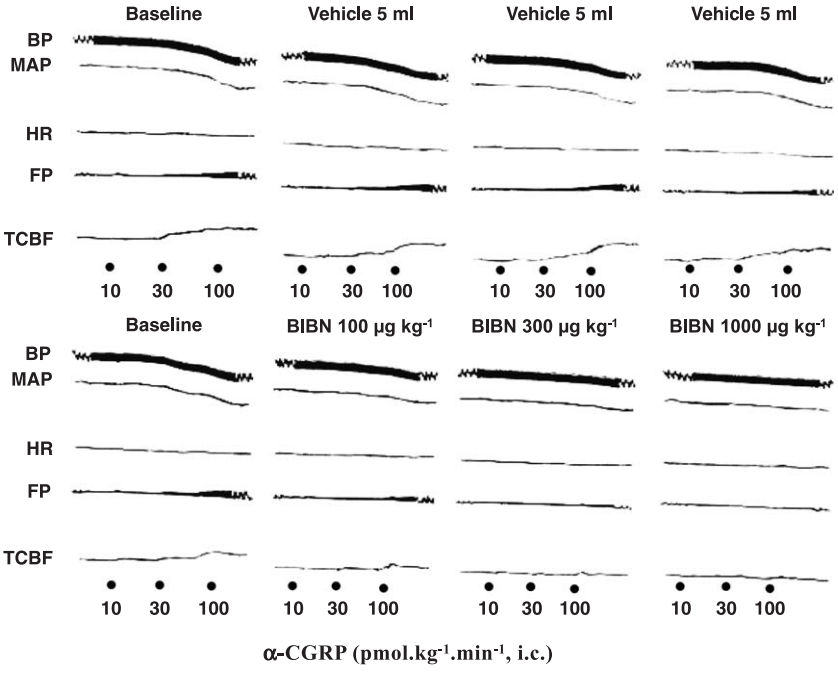

Fig. 3. Original tracings from experiments in anaesthetised pigs illustrating systemic and carotid haemodynamic responses to infusions of α-CGRP (●; 10, 30 or 100 pmol kg^{-1} min^{-1}, i.c.) given before and after i.v. treatments with either vehicle (three times 5 ml; upper panel) or BIBN4096BS (BIBN, 100, 300 and 1000 μg kg^{-1}; lower panel). BP; systolic and diastolic arterial blood pressures; MAP, mean arterial blood pressure; HR, heart rate; FP, carotid blood flow pulse; TCBF, total carotid blood flow.

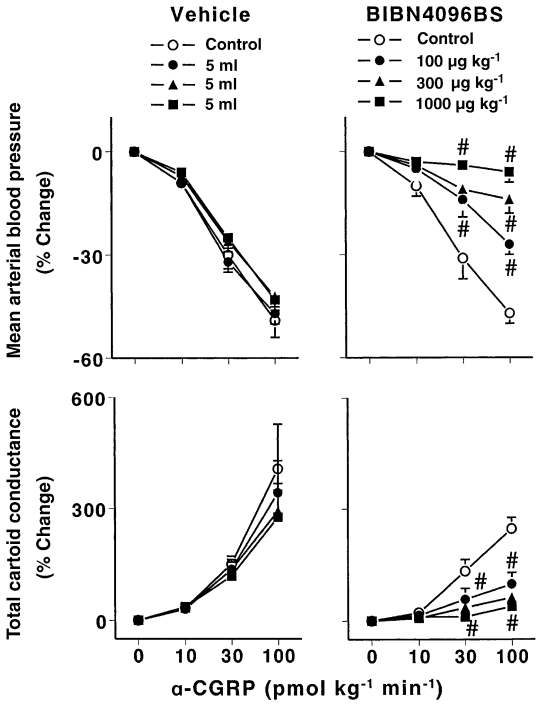

Fig. 4. Changes in mean arterial blood pressure and total carotid vascular conductance from baseline values by i.c. infusion of α-CGRP in anaesthetised pigs given before (Control) and after i.v. treatments with vehicle (three times 5 ml; $n=6$) or BIBN4096BS (100, 300 and 1000 µg kg^{-1}, $n=7$). All values are expressed as mean ± S.E.M. The two highest doses of α-CGRP significantly decreased the mean arterial blood pressure and increased the total carotid vascular conductance (significance not shown for the sake of clarity). These effects of α-CGRP were dose-dependently antagonised by BIBN4096BS. #$P<0.05$ vs. response after the corresponding volume of vehicle.

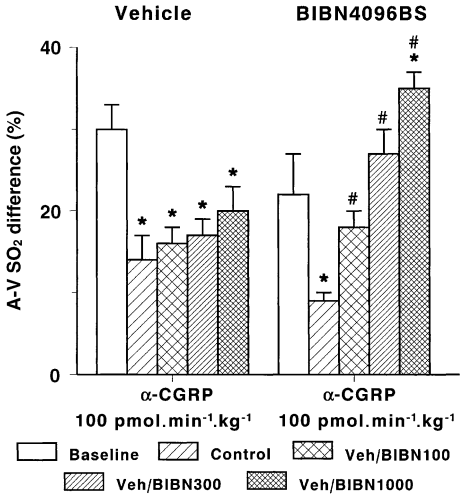

Fig. 6. Differences between arterial and jugular venous oxygen saturations (A − V SO_2 difference) measured at baseline and after infusions of α-CGRP (100 pmol kg^{-1} min^{-1}, i.c.) given in anaesthetised pigs before (Control) and after i.v. treatments with vehicle (V, 5 ml three times; $n=6$) or BIBN4096BS (100, 300 and 1000 µg kg^{-1}, $n=7$). 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.

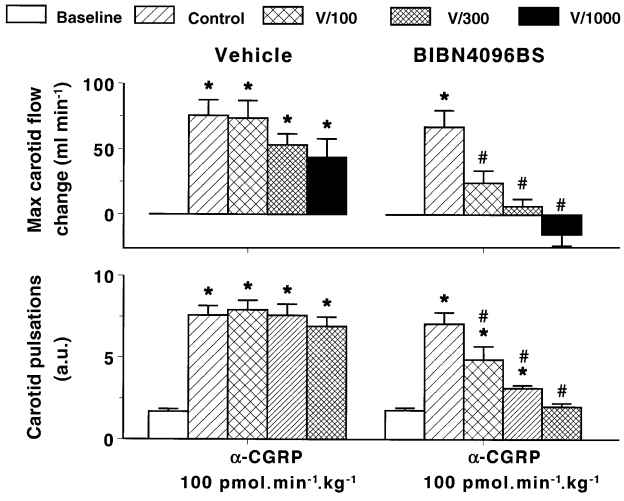

Fig. 5. Maximum carotid blood flow changes and carotid blood flow pulsations measured at baseline and following infusions of α-CGRP (100 pmol kg^{-1} min^{-1}, i.c.) given in anaesthetised pigs before (Control) and after i.v. treatments with vehicle (V, 5 ml three times; $n=6$) or BIBN4096BS (100, 300 and 1000 µg kg^{-1}, $n=7$). 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.

groups of animals (Fig. 6; compare baseline and control values). The response to CGRP remained largely unaffected after treatments with vehicle, but BIBN4096BS (100, 300 and 1000 µg.kg^{-1}, i.v.) dose-dependently blocked the reduction in the A-V SO_2 difference by α-CGRP. In fact, the CGRP-induced decrease in the A-V SO_2 difference was enhanced after the highest dose of BIBN4096BS (Fig. 6).

4. Discussion

4.1. General

Undoubtedly, a remarkable progress has been achieved in acute antimigraine therapy (De Vries et al., 1996). Notwithstanding, the exact pathophysiological mechanisms underlying migraine remain unclear. There is, however, evidence supporting the involvement of the trigeminovascular system in migraine pathophysiology (Goadsby, 1997, 1999; Hargreaves et al., 1999; Williamson and Hargreaves, 2001). Thus, activation of the trigeminovascular system leads to neuropeptide release, including that of CGRP, and neurogenic dural vasodilatation (Williamson and Hargreaves, 2001). Of particular relevance is the finding that plasma concentration of CGRP is elevated during the headache phase of migraine, and this is normalised after treatment with sumatriptan (Goadsby, 1997, 1999; Goadsby et al., 1990). Hence, it is reasonable to assume that a potent CGRP receptor antagonist, such as BIBN4096BS (Doods et al., 2000), might be useful in migraine therapy. BIBN4096BS behaves as a ‘silent’ competitive antagonist at CGRP receptors mediating relaxation of human temporal, cranial

and coronary arteries (Edvinsson et al., 2002; Moreno et al., 2002; Verheggen et al., 2002). The present study in anaesthetised pigs was designed: (i) to analyse, using BIBN4096BS, the potential role of endogenous CGRP in regulating vascular tone in vivo; and (ii) to investigate the effects of BIBN4096BS on the systemic and carotid haemodynamic responses produced by α-CGRP.

4.2. Systemic and regional haemodynamic effects of BIBN4096BS

It is well known that CGRP-immunoreactive nerve fibres are widely distributed in the cardiovascular system, with a higher preponderance in arteries than in veins (Bell and McDermott, 1996). CGRP decreases blood pressure and has positive inotropic and chronotropic effects on the heart (Wimalawansa, 1996), which are mainly mediated via $CGRP_1$ receptors (Bell and McDermott, 1996; Saetrum Opgaard et al., 1999, 2000). Though CGRP has diverse biological actions within the cardiovascular system, our experiments showing few systemic haemodynamic changes with BIBN4096BS do not support a major role for CGRP in the regulation of cardiovascular function in anaesthetised pig.

As far as regional haemodynamics is concerned, a moderate decrease (compared to vehicle) in vascular conductances in the lungs, adrenals, kidneys and spleen was observed with the highest dose (1000 μg kg^{-1}) of BIBN4096BS (Fig. 2). Similarly, renal vasoconstriction was noticed in conscious rats with a high (300 nmol kg^{-1} min^{-1}), but not with a low (30 nmol kg^{-1} min^{-1}) dose of CGRP-(8–37) (Gardiner et al., 1990). Since both BIBN4096BS and $CGRP_{8-37}$ caused renal changes only in doses that were considerably higher than those needed for CGRP antagonism, it does not appear that endogenous CGRP regulates renal vascular tone. Also, Shen et al. (2001) recently reported that 30 μg kg^{-1} min^{-1} (~ 10 nmol kg^{-1} min^{-1}) of CGRP-(8–37), which antagonised CGRP-induced haemodynamic responses, caused little regional haemodynamic effects in conscious dogs as well as anaesthetised rats, thereby not supporting an important physiological role for endogenous CGRP in regulating vascular tone. Although we cannot rule out the involvement of CGRP in certain other circumstances, for example, cardiac preconditioning or coronary artery disease (Lu et al., 1999; Peng et al., 2000; Wu et al., 2001), the present results imply cardiovascular safety of BIBN4096BS. Nevertheless, one will have to explore the role of CGRP in cardiovascular pathophysiology before establishing whether or not CGRP receptor antagonists are completely safe in patients afflicted with cardiovascular disorders.

4.3. CGRP-induced haemodynamic responses and antagonism by BIBN4096BS

Activation of CGRP receptors elicits dilatation in different vascular beds in several species (Gardiner et al., 1990; Shen et al., 2001; Van Gelderen et al., 1995). Consistent with these studies, our experiments show that i.c. infusions of α-CGRP produced a marked vasodilatation in porcine carotid circulation, with accompanying fall in arterial blood pressure. The fact that the animals were systematically vagosympathectomised may explain why the hypotension was not accompanied by a baroreflex-mediated tachycardia, as reported earlier (Van Gelderen et al., 1995). Interestingly, the ipsilateral skin redness, together with the marked decrease in A-V SO_2 difference by CGRP, indicates that porcine carotid arteriovenous anastomoses dilated in response to α-CGRP (Saxena, 1987). However, we previously reported that i.c. infusions of α-CGRP failed to increase porcine arteriovenous anastomotic blood flow, despite a marked increase in the total carotid and capillary blood flows (Van Gelderen et al., 1995). Admittedly, arteriovenous anastomotic blood flow was not directly measured in these experiments, but we have recently observed that i.c. infusions of capsaicin, which released CGRP, did increase carotid arteriovenous anastomotic blood flow with a concomitant decrease in the A-V SO_2 difference (Kapoor et al., 2003). Thus, it appears that the discrepancy between the two investigations may be due to different anaesthetic regimens employed (pentobarbital and fentanyl/thiopental, respectively) and, particularly, the use of phenylephrine in the present experiments. Phenylephrine potently constricts arteriovenous anastomoses (Willems et al., 1999).

In the present experimental study in anaesthetised pigs, BIBN4096BS proved to be an effective antagonist at the CGRP receptors mediating the systemic (hypotension) as well as the carotid (increased blood flow, pulsations and skin redness) haemodynamic responses to α-CGRP. The fact that BIBN4096BS also abolished α-CGRP-induced decreases in the A-V SO_2 difference suggests its action on carotid arteriovenous anastomoses; for further considerations, see Saxena (1987). Interestingly, BIBN4096BS also antagonised the capsaicin-induced increases in carotid arteriovenous anastomotic blood flow as well as decreases in the A-V SO_2 difference, but not the plasma CGRP concentrations (Kapoor et al., 2003).

One cannot be certain about the nature of CGRP receptors that mediate porcine carotid vascular responses, but cardiac inotropic and vasodilator responses are mediated predominantly by $CGRP_1$ receptors (Saetrum Opgaard et al., 1999), where BIBN4096BS has a very high affinity (Doods et al., 2000; Poyner and Marshall, 2001).

4.4. Potential therapeutic efficacy of BIBN4096BS in the treatment of migraine

Considering that plasma CGRP levels are elevated during the headache phase of migraine (Goadsby, 1997) and that BIBN4096BS dose-dependently blocked α-CGRP-induced carotid haemodynamic responses, it is likely that BIBN4096BS may be effective in migraine. The compound is presently under clinical investigation for the acute treat-

ment of migraine and the results are awaited with great interest.

In conclusion, our study clearly demonstrates that BIBN4096BS is an effective antagonist at vascular CGRP receptors in anaesthetised pigs, but has little haemodynamic effects of its own, a finding that negates a major physiological role for CGRP in cardiovascular regulation. The potent blockade of the carotid haemodynamic effects of CGRP does suggest that BIBN4096BS may be effective in migraine treatment.

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