Inhibitory effect of BIBN4096BS on cephalic vasodilatation induced by CGRP or transcranial electrical stimulation in the rat

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1 Calcitonin gene-related peptide (CGRP) is believed to play a pivotal role in the pathogenesis of migraine *via* activation of CGRP receptors in the trigeminovascular system. The CGRP receptor antagonist, BIBN4096BS, has proven efficacy in the acute treatment of migraine attacks and represents a new therapeutic principle.

2 We used an improved closed cranial window model to measure changes of the middle meningeal artery (MMA) and cortical pial artery/arteriole diameter (PA) and changes in local cortical cerebral blood flow ($LCBF_{Flux}$) in anaesthetised artificially ventilated rats.

3 The ability of BIBN4096BS (i.v.) to prevent the vasodilatatory actions of rat-αCGRP, βCGRP and endogenously released CGRP following transcranial electrical stimulation (TES) was investigated.

4 BIBN4096BS was *per se* without vasoactive effect on any of the measured variables and significantly inhibited the hypotension induced by both types of CGRP ($P<0.001$).

5 The αCGRP induced MMA dilatation was reduced from 97.4 ± 14 to $2.1\pm1.3\%$ ($P<0.001$) and the βCGRP induced dilatation was fully blocked by BIBN4096BS. ID_{50} was $5.4\pm1.6\,\mu g\,kg^{-1}$ for αCGRP and $16.3\pm1.6\,\mu g\,kg^{-1}$ for βCGRP.

6 Transcranial electrical stimulation induced a $119.1\pm6.9\%$ increase in MMA diameter. BIBN4096BS ($333\,\mu g\,kg^{-1}$) attenuated this increase ($19.8\pm2.1\%$) ($P<0.001$).

7 Systemic CGRP and TES induced an increase in PA diameter that was not significantly inhibited by BIBN4096BS. The CGRP induced increase in $LCBF_{Flux}$ was similar not prevented by the antagonist.

8 We suggest that systemic BIBN4096BS exerts its inhibitory action mainly on large dural blood vessels (MMA).

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Introduction

Trigeminal sensory C-fibres innervating the cranial vessels contain several vasoactive peptides such as calcitonin gene-related peptide (CGRP), neurokinin A, substance P and pituitary adenylate cyclase activating peptide (PACAP) (Gulbenkian *et al.*, 2001). Electrical stimulation of the trigeminal ganglion liberates vasoactive peptides into the perivascular space. CGRP is a potent dilator of cerebral and dural blood vessels (Williamson *et al.*, 1997a; Jansen-Olesen *et al.*, 2003) and may interact not only with smooth muscle cells, but also with presynaptic receptors on the perivascular nerves (Williamson *et al.*, 1997b). CGRP plasma levels are increased during acute attacks of migraine and cluster head-

ache (Goadsby *et al.*, 1990; Goadsby & Edvinsson, 1994) and infusion of CGRP induces headache and migraine attacks in migraine patients (Lassen *et al.*, 2002).

The peptide fragment of CGRP ($CGRP_{8-37}$) and compounds such as 'compound 1' and SB-273779 are effective in antagonising CGRP-induced responses *in vitro* and *in vivo* (Williamson *et al.*, 1997b; Aiyar *et al.*, 2001; Edvinsson *et al.*, 2001b). These antagonists are, however, not suitable for human use. BIBN4096BS is a well-characterised antagonist, which is highly selective and effective in animal and human experiments (Doods *et al.*, 2000; Wu *et al.*, 2000; Edvinsson *et al.*, 2002; Petersen *et al.*, 2003). It is safe for human administration and in a proof of concept phase-II study, it was found to be effective in aborting acute migraine attacks (Olesen *et al.*, 2004).

In the present study, we used a closed cranial window model often applied in migraine research (Williamson *et al.*, 1997a;

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Akerman *et al.*, 2002). The experimental set-up allows investigation of cerebrovascular responses to transcranial electrical stimulation (TES) and systemic administration of CGRP (Williamson *et al.*, 1997a). In previous studies using this method, only data on the reactivity of the middle meningeal artery (MMA) was reported (Williamson *et al.*, 1997a; Akerman *et al.*, 2002). We have verified the method and further demonstrated the possibility of measuring diameter changes of cortical pial arteries (PA) and local cortical cerebral blood flow (LCBF$_{Flux}$) by laser Doppler flowmetry (LDF) (Petersen *et al.*, 2004a).

We have currently used this technique to investigate the ability of BIBN4096BS to inhibit the effect of αCGRP, βCGRP and TES on MMA, PA, LCBF$_{Flux}$ and mean arterial blood pressure (MABP). The study contributes to a further understanding of the antimigraine effect of BIBN4096BS.

Methods

Surgical preparation

All experiments were performed in accordance with national guidelines and regulations for animal care and treatment. The study protocol was approved by the Danish Animal Experimental Inspectorate (file: 2001/561–390).

Male Sprague–Dawley rats (300–400 g) were anaesthetised throughout the study with pentobarbital (Mebumal®, 60 mg kg^{-1}, i.p. for induction and 20 mg kg^{-1} h^{-1} i.v. for maintenance). A tracheotomy was performed and the animals were artificially ventilated (Abovent 7025, Ugo Basil, Italy) with a 30/70% air mixture of O_2/N_2O (stroke rate 60–65 min^{-1} and a stroke volume of 3.5–4.0 ml). The body temperature was kept at 37.0–37.5°C using an automatic regulated heating plate (Letica HB101, Panlab, Spain). The femoral artery and vein were cannulated bilateral with polythene catheters (Portex® polyethylene catheters ID 0.4, Astratech, Denmark) for measurement of MABP (Transducer TCM4-7, World Precision Instruments Inc., U.S.A.), arterial blood samples, infusion of anaesthetics and test substances. All data were continuously displayed on a computer monitor by the data acquisition and analysis software Perisoft® (Perisoft® for Windows 2.0, Perimed AB, Sweden). Arterial blood analysis (ABL520, Radiometer A/S, Denmark) was performed at least three times during each study. $PaCO_2$, PaO_2 and pH were kept within normal limits (pH 7.40–7.42, $PaCO_2$ 36.5–38.7 mmHg and PaO_2 112.5–121.9 mmHg). The inspiratory and expiratory air was continually monitored by a capnograph (Capnomac AGM103, Datex-Ohmeda, Finland). The animal was placed in a stereotactic frame. Skin and connective tissue were removed from the dorsal side of the skull. The right parietal bone was thinned until transparency using a dental drill. During the drilling, cooling was obtained by local application of ice-cold isotonic saline.

Video microscopy

The cranial window was covered with mineral oil (37°C), a branch of the MMA and a PA were visualised using a video-microscope (Sony DSP digital camera, MS50 objective, Japan). The real-time image was displayed on a TV-screen. The diameter of the vessels was continuously measured by a video dimension analyser (V94, Living Systems Instrumentation Inc., U.S.A.). LCBF$_{Flux}$ was measured continuously using LDF (Perimed PF4001, Perimed AB, Sweden). The probe (Perimed 410, fibre separation 0.25 mm, Perimed AB, Sweden) was placed using a micromanipulator and microscope in an area with no or few observable vessels. The probe was placed free of the bone, but in touch with the mineral oil, which enhances the accuracy of the LDF measurement (Gerrits *et al.*, 1998).

Experimental protocols

Dose–effect relationship of r-αCGRP and βCGRP   After preparation, the rat rested for 1–1½h before the effect of the two forms of CGRP on MMA, PA, LCBF$_{Flux}$ and MABP was determined by cumulative infusions of semilogarithmic increasing doses. The CGRP (0.01 ng kg^{-1}–3 μg kg^{-1}) was administered as a bolus injection of 100 μl succeeded by a 150 μl isotonic saline (0.9% NaCl) flush, at 10 min intervals. Only one dose–effect curve was obtained in each animal.

Repeated infusions of αCGRP and βCGRP   Previously repeated exposure to CGRP (four to seven times) did not induce tolerance or tachyphylaxia (Foulkes *et al.*, 1991; Wu, 1999; Akerman *et al.*, 2002). To study, if tolerance or tachyphylaxia developed with αCGRP or βCGRP (0.3 μg kg^{-1}), infusions of the peptides were repeated seven times with a 15 min interval between infusions, as in the agonist–antagonist experiments described below.

Effect of BIBN4096BS   BIBN4096BS (1–333 μg kg^{-1}) was infused in cumulative semilogarithmic increasing doses with a 15 min interval to investigate a possible vasoactivity.

Electrical stimulation parameters   To investigate whether neurogenically induced vasodilatation was inhibited by BIBN4096BS, a bipolar stimulation electrode (NE-200x, Harvard Instruments, U.K.) was placed on the surface of the cranial window approximately 200 μm from the investigated arteries. Stimulations at 5 Hz, 1 ms pulse width and of 10 s duration were applied (Grass Stimulator S48, Grass Instruments, U.S.A.). Stimulations were done with increasing voltage until maximum dilation was observed. The same voltage was used for the subsequent TES in the same animal (Williamson *et al.*, 1997a).

A control experiment was performed to elucidate the reproducibility of seven consecutive electrically evoked responses in each animal. The seven stimulations were applied with 25 min intervals, as in the pharmacological experiments.

Effect of BIBN4096BS on αCGRP and βCGRP-induced hypotension and vasodilatation   In each rat, a control response to αCGRP or βCGRP (0.3 μg kg^{-1}) was first recorded. The first dose of BIBN4096BS (1 μg kg^{-1}) was administered 10 min later and followed by a second bolus of CGRP 5 min later. An increased dose of BIBN4096BS (3.3 μg kg^{-1}) was given after another 10 min, and again followed by a CGRP bolus after 5 min This sequence was repeated six times in all in each animal with increasing doses of BIBN4096BS until 333 μg kg^{-1}.
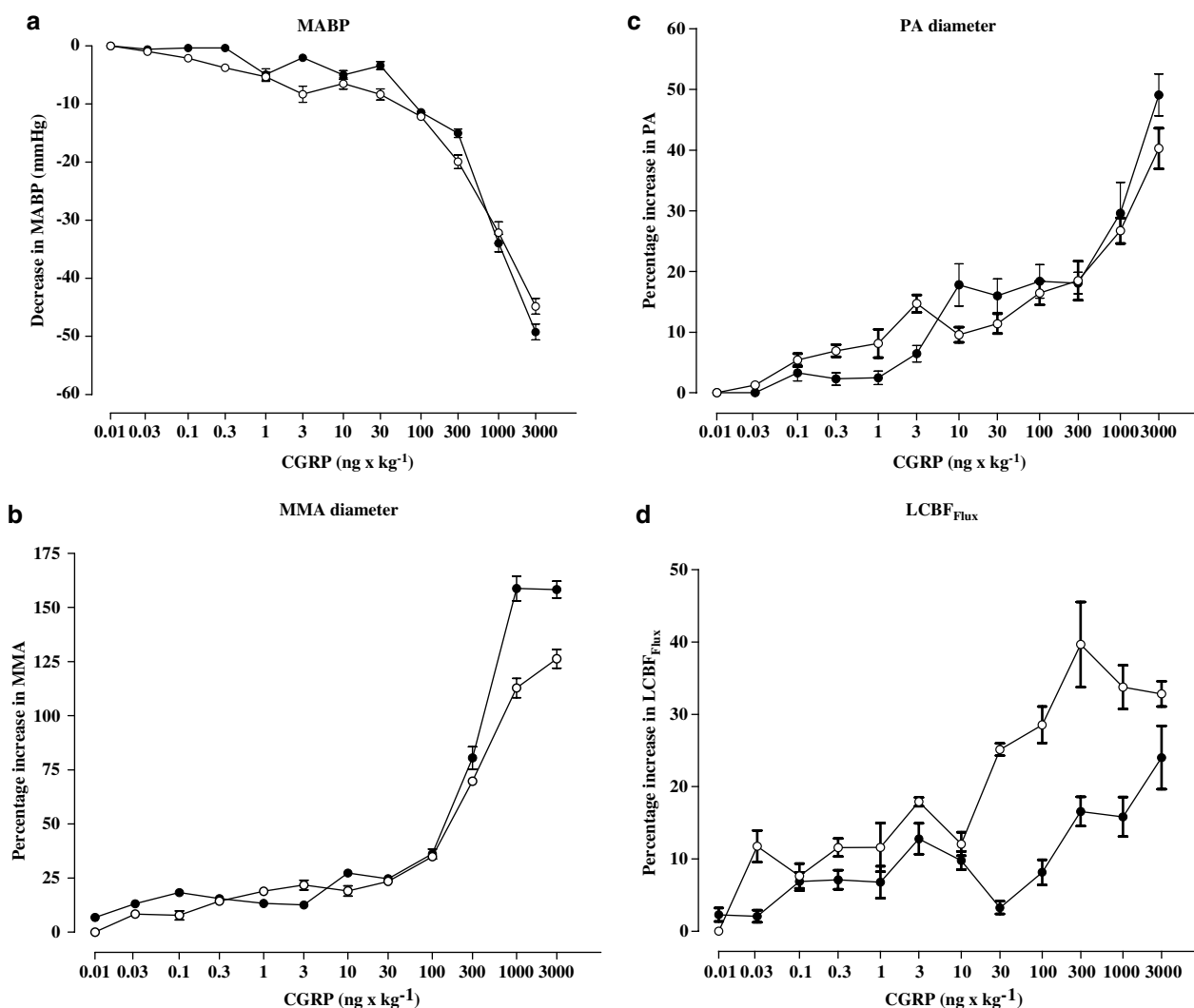
Figure 1 (a–d) Dose–effect curves of semilogarithmic increasing dose of αCGRP ($\bigcirc$, $n=6$) and βCGRP ($\bullet$, $n=6$). The dose–effect curves are displayed for (a) mean arterial blood pressure, (b) middle meningeal artery, (c) pial arteriole and (d) local cortical cerebral blood flow. Values are mean$\pm$s.e.m. (a) and (c) with permission Petersen *et al.*, 2004b.

Effect of BIBN4096BS on neurogenically induced vaso-dilatation The same protocol as for the CGRP application was used. However, the interval between the TES was 25 min, while the interval from administration of the antagonist to renewed stimulation was 5 min. In this protocol, $LCBF_{Flux}$ was not measured due to technical reasons.

Data analysis

The maximum effect (peak) of CGRP or applied TES on MMA, PA and $LCBF_{Flux}$ was calculated as the percentage increase or decrease from the baseline. The prestimulation baseline was defined as the average of the 60 s preceding the stimulation. The MMA and PA diameter were measured in arbitrary units, since the experimental set-up was dependent on the individual course of the arteries chosen for each experiment and a different magnification was used to optimise the image and analytic possibilities of the video dimension analyser. A standardised artery measurement would therefore be im-practical. Laser Doppler flowmetry was measured in

tissue perfusion units (arbitrary). The unit of the mean arterial blood pressure was mmHg and changes expressed as Δ mmHg. All listed data are mean$\pm$s.e.m. and a significance level of $P < 0.05$ was applied. Statistical analysis was performed using ANOVA. In the analytic design, the dose and animal were included as factors. If significance was found, a *post hoc* analysis was performed using Dunnett's multiple comparison tests with the initial stimulation or infusion as control. Comparison between the different groups of experiments with the different agonists was performed using an unpaired *t*-test. All statistical analyses were performed using SPSS version 10.0 (Chicago, IL, U.S.A.). Graphs and estimation of ID_{50}-values were done using GraphPrism$^{\copyright}$, version 3 (GraphPad, U.S.A.).

Drugs

Rat-α and β-calcitonin gene-related peptide (Neosystem, France) was dissolved in distilled water. BIBN4096BS was synthesised by Boehringer Ingelheim Pharma GmbH & Co.

KG Germany, and provided as a gift. BIBN4096BS was dissolved in a small volume (20 μl) of 1 N HCl, further diluted with saline (0.9% NaCl), and finally adjusted to pH 6.5–7.0 by 1 N NaOH. Stock solutions of each compound were aliquotted and frozen at $-20°$C. Before infusion all solutions were diluted to the final concentration using isotonic saline.

Results

Dose–effect relationship of αCGRP and βCGRP

Figure 1a–d illustrates the dose–effect curves obtained for MABP, MMA, PA and LCBF$_{Flux}$ ($n = 6$). There were no significant difference in maximal responses to αCGRP and βCGRP on MABP ($P = 0.4$), MMA diameter ($P = 0.06$) and PA diameter ($P = 0.5$). Data on MABP and PA have been published previously (Petersen *et al*., 2004b) LCBF$_{Flux}$ increased more after αCGRP than βCGRP at doses above 10 ng kg^{-1}.

Control of repeated infusions or TES

Both forms of CGRP resulted in consistent and reproducible responses in all measured parameters, when CGRP was administered seven times as an intravenous bolus at 15 min intervals (Table 1). After an initial voltage determination, it was possible to evoke the same dilatatory response to TES of both MMA and PA diameter seven times without any significant differences (Table 1).

Effect of BIBN4096BS per se

In the dose–effect studies of BIBN4096BS ($n = 3$, Table 2), doses up to the maximum tested (1–333 μg kg^{-1}) had no effect on MABP ($P = 0.12$), MMA ($P = 0.8$), PA ($P = 0.5$) or LCBF$_{Flux}$ ($P = 0.6$).

Effect of BIBN4096BS on αCGRP-induced hypotension and vasodilatation

The maximum hypotensive response to αCGRP was -24.7 ± 4.6 mmHg and the decrease in MABP after pretreatment with the highest dose of the antagonist was -0.9 ± 1.8 mmHg, $P < 0.001$. The ID$_{50}$ of BIBN4096BS was 21.3 ± 1.4 μg kg^{-1}. αCGRP caused a relaxation of the MMA (97.4 $\pm$ 14%). BIBN4096BS inhibited this dilatation (2.1 $\pm$ 1.3%, $P < 0.001$). The ID$_{50}$ of BIBN4096BS was 5.4 ± 1.6 μg kg^{-1}. αCGRP also induced a dilatation of the PA and an increase in LCBF$_{Flux}$. The PA and LCBF$_{Flux}$ responses were attenuated dose-dependently, but not significantly by the antagonist. The control response of the PA was 26 $\pm$ 4.9% and after the maximum dose of BIBN4096BS this was reduced to 6.3 $\pm$ 3.1% ($P = 0.08$). The increase in LCBF$_{Flux}$ was reduced from 19.3 $\pm$ 3.4 to 7.7 $\pm$ 3.7% ($P = 0.08$, Figure 2a–d).

Effect of BIBN4096BS on βCGRP-induced hypotension and vasodilatation

The βCGRP-induced hypotension and MMA vasodilatation were significantly inhibited by BIBN4096BS ($P < 0.001$). The hypotensive response to βCGRP was -15.5 ± 1.7 mmHg and the decrease in MABP after pretreatment with the maximum dose of BIBN4096BS was completely blocked

Table 2 Effect of BIBN4096BS *per se* on the measured variables compared to preinfusion baseline, ($n = 3$)

Dose (μg kg^{-1})	MABP (mmHg)	MMA (%)	PA (%)	LCBF$_{Flux}$ (%)
1	1.1 $\pm$ 0.7	0	0	1.8[a]
3.3	2.9 $\pm$ 1.6	0	0	0
10	3.7 $\pm$ 0.6	1.3[a]	0	2.9[a]
33	2.7 $\pm$ 1.5	0	0	6.5 $\pm$ 4.3
100	-2.2 ± 2.6	6.3[a]	2.5[a]	7.4 $\pm$ 3.7
333	-2.4 ± 3.5	6.9 $\pm$ 10.9	0	8.6 $\pm$ 4.8

[a]Denotes changes only seen in one animal.

Table 1 Repeated CGRP infusion and TES

Infusion or TES no.	1	2	3	4	5	6	7	P =
r-αCGRP ($n = 4$)								
ΔMABP (mmHg)	-25.6 ± 4.4	-23.1 ± 4.6	-22.5 ± 4	-23.9 ± 0.7	-26.8 ± 4.1	-28.5 ± 3.9	-29.6 ± 6	0.3
ΔDura (%)	88.5 $\pm$ 21.7	91.6 $\pm$ 22.8	86.6 $\pm$ 20.4	83.2 $\pm$ 18	82.3 $\pm$ 15.5	90.7 $\pm$ 21.5	89.1 $\pm$ 21.3	0.5
ΔPia (%)	28.6 $\pm$ 11.6	32.6 $\pm$ 15.2	30.4 $\pm$ 12.6	32.4 $\pm$ 17.8	29.3 $\pm$ 9.8	26.8 $\pm$ 8.7	34.3 $\pm$ 17.9	0.8
ΔLCBF$_{Flux}$(%)	30.5 $\pm$ 8.5	31.5 $\pm$ 7.9	28.6 $\pm$ 9.3	29.9 $\pm$ 8.9	28 $\pm$ 5.7	26.7 $\pm$ 6.6	27.9 $\pm$ 6.5	0.5
r-βCGRP ($n = 6$)								
ΔMABP (mmHg)	-15.4 ± 1.6	-18.6 ± 3.2	-17.8 ± 2.8	-21.5 ± 2.7	-17.3 ± 2.3	-20.4 ± 3.2	-20.8 ± 3	0.2
ΔDura (%)	105.9 $\pm$ 16.8	105.5 $\pm$ 19.5	108.7 $\pm$ 18.1	104.5 $\pm$ 13.6	109.7 $\pm$ 13.5	113.9 $\pm$ 18.7	112.3 $\pm$ 14.9	0.9
ΔPia (%)	16.7 $\pm$ 2.7	18.9 $\pm$ 3	15.4 $\pm$ 2.5	21.1 $\pm$ 4.3	20 $\pm$ 4.1	17.3 $\pm$ 2.7	22 $\pm$ 5.2	0.4
ΔLCBF$_{Flux}$(%)	17.4 $\pm$ 1.5	19.4 $\pm$ 4.1	20.1 $\pm$ 2.8	25 $\pm$ 3.1	20.1 $\pm$ 2.5	20.5 $\pm$ 1.7	20.3 $\pm$ 1.6	0.2
TES ($n = 6$)								
ΔMABP (mmHg)	-3.6 ± 2.4	-4.9 ± 2.3	-2.5 ± 1.9	-3.3 ± 2	-4.9 ± 2.2	-3.2 ± 2.6	-2.6 ± 2.5	0.2
ΔDura (%)	131.8 $\pm$ 13.3	125.2 $\pm$ 12	130.9 $\pm$ 14.1	140.5 $\pm$ 10.9	137.5 $\pm$ 13.4	130.5 $\pm$ 9.4	133.5 $\pm$ 11.1	0.5
ΔPia (%)	69 $\pm$ 13.7	66.4 $\pm$ 13.5	72.9 $\pm$ 16.9	68.9 $\pm$ 13.5	64.4 $\pm$ 14.2	57 $\pm$ 16.6	56 $\pm$ 14.4	0.2

Percentual or mmHg change from baseline of the seven consecutive infusions of αCGRP, βCGRP or stimulations. Data are listed as mean $\pm$ s.e.m. The *P*-values are the statistical comparison between the different infusions and stimulations (ANOVA).
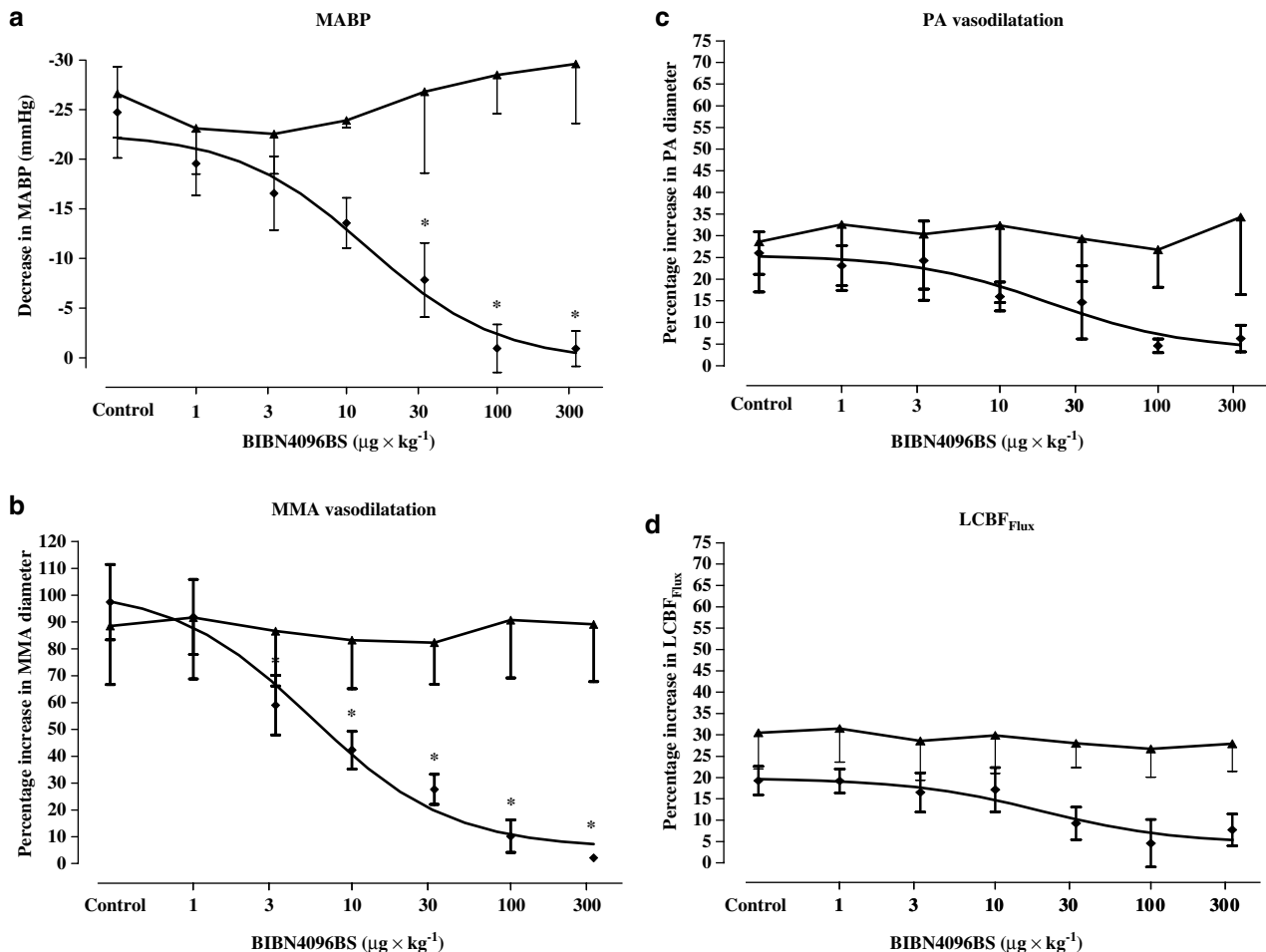
Figure 2 (a–d) Effect of semilogarithmic increasing doses of BIBN4096BS on αCGRP induced changes ($n = 6$, ($\blacklozenge$)). $* = P < 0.05$ (Dunnett's test). In addition, the challenge of the seven repeated infusions of αCGRP ($0.3\,\mu g\,kg^{-1}$, $n = 6$) from the control experiment are displayed as the upper line in the figure ($\blacktriangle$), data shown in detail in Table 1.

($P < 0.001$). The ID_{50} was $24.7 \pm 1.3\,\mu g\,kg^{-1}$. βCGRP relaxed the MMA by $74.6 \pm 13.6\%$, a dilatation fully blocked by the maximum dose of BIBN4096BS ($P < 0.001$). The ID_{50} was $16.3 \pm 1.6\,\mu g\,kg^{-1}$ (Figure 3a and b). The βCGRP-induced PA dilatation (11.8 ± 3.2) was not affected by BIBN4096BS ($333\,\mu g\,kg^{-1}$, $8.8 \pm 2.3\%$, $P = 0.4$). The βCGRP induced increase in $LCBF_{Flux}$ ($13.8 \pm 1.6\%$) was not altered by the maximum dose of BIBN4096BS ($8.2 \pm 4.1\%$, $P = 0.7$).

Effect of BIBN4096BS on neurogenic vasodilatation

TES increased MMA diameter by $119.1 \pm 6.9\%$, which was reduced to $19.8 \pm 2.1\%$ after BIBN4096BS ($P < 0.001$, $n = 6$, Figure 4a). The ID_{50} of BIBN4096BS on neurogenic dural vasodilatation was $48.3 \pm 1.9\,\mu g\,kg^{-1}$. TES increased PA diameter by $96.4 \pm 14.2\%$, however was not significantly reduced by BIBN4096BS ($P = 0.2$, Figure 4b).

Discussion

The present series of experiments provide data on the *in vivo* effectiveness of the CGRP receptor antagonist BIBN4096BS

and illustrates its possible site of action. The importance of BIBN4096BS resides in the fact that it is the first CGRP receptor antagonist proven to be effective in the treatment of acute migraine and thus represents a new principle in migraine therapy (Olesen *et al.*, 2004).

Effects of αCGRP and βCGRP on the cranial circulation

We studied whether rat-αCGRP and βCGRP elicited the same effect on the peripheral and cerebral hemodynamics and investigated the ability of BIBN4096BS to prevent their vasodilatatory properties. A dose of $0.3\,\mu g\,kg^{-1}$ of CGRP was chosen for the inhibition studies. The choice was based on the dose–effect relationship data obtained in this study (Figure 1) and existing knowledge of a submaximal and reproducible effect on the MMA of this dose (Williamson *et al.*, 1997b; Honey *et al.*, 2002). In the present study, αCGRP and βCGRP were equally potent and dose-dependently dilated both the MMA and the PA. This in agreement with previous findings showing that there are no major differences in the response of cranial arteries (human or animal) to the different subtypes of CGRP (Jansen-Olesen *et al.*, 2003). The somewhat less-pronounced relaxation of the PAs by CGRP may be
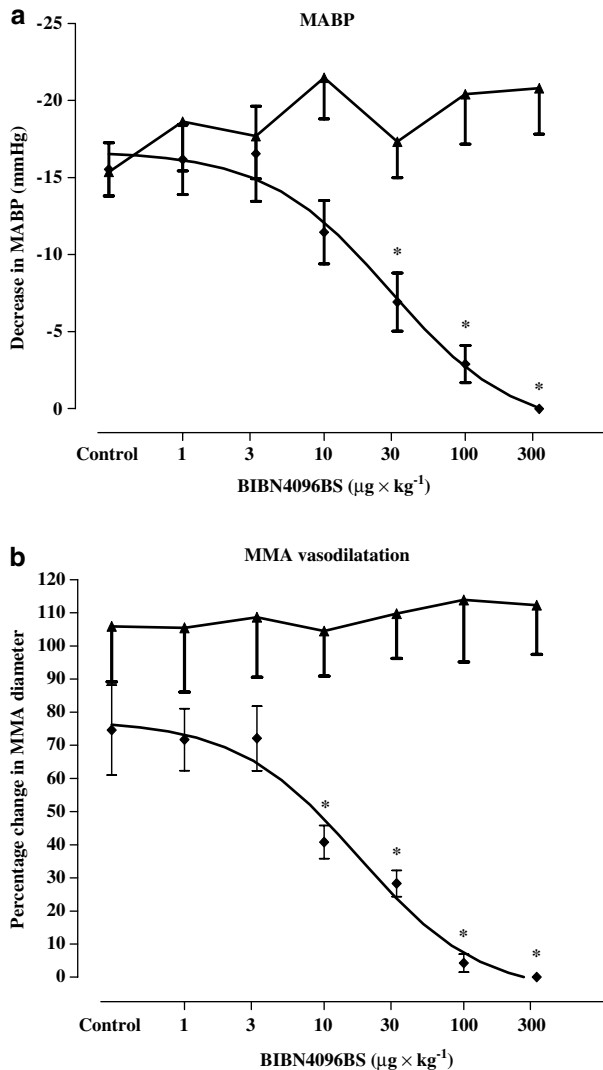
a

Figure 3 (a–b) Effect of semilogarithmic increasing doses of BIBN4096BS on βCGRP-induced changes ($n = 6$, (◆)). * = $P < 0.05$ (Dunnett's test). In addition, the challenge of the seven repeated infusions of βCGRP ($0.3\,\mu g\,kg^{-1}$, $n = 6$) from the control experiment are displayed as the upper line in the figure (▲), data shown in detail in Table 1.

explained by the concomitant hypotension, since we and others have documented a correlation between PA diameter and hemorrhagic-induced hypotension (Kontos *et al.*, 1978; Petersen *et al.*, 2004a).

Studies investigating the effect of systemic CGRP on CBF have revealed conflicting results (Beattie *et al.*, 1993; Baskaya *et al.*, 1995). In most species including the rat, the peptide may increase CBF in response to i.v. administration (Suzuki *et al.*, 1989; Baskaya *et al.*, 1995). Our data supported this and showed that infusion of αCGRP or βCGRP, the former being more potent, dose-dependently increased $LCBF_{Flux}$.

Mechanism and site of action of BIBN4096BS

BIBN4096BS is a relatively large hydrophilic compound and is therefore unlikely to pass the blood–brain barrier (BBB) in acute experiments. However, no direct data exist to support this. In contrast to cerebral vessels, the meningeal arteries have
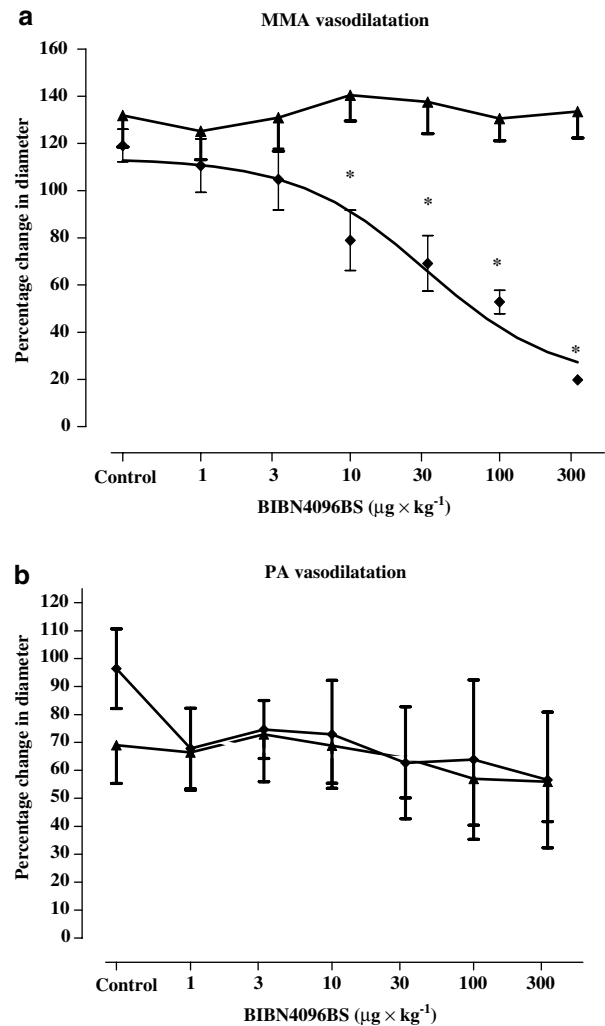
Figure 4 (a–b) Effect of semilogarithmic increasing doses of BIBN4096BS on neurogenic TES-induced vasodilatation ($n = 6$, (◆)). * = $P < 0.05$ (Dunnett's test). In addition, the result of the seven repeated neurogenic vasodilatations from the control experiment are displayed as the second line in the figure (▲), data shown in detail in Table 1.

no BBB and BIBN4096BS is likely to diffuse freely into the wall of the MMA (Knudsen *et al.*, 1988; Faraci *et al.*, 1989). BIBN4096BS has been shown to have a high affinity for the $CGRP_1$ receptor *in vitro* (Doods *et al.*, 2000; Edvinsson *et al.*, 2001b; 2002; Schindler & Doods, 2002). Since receptors with a $CGRP_1$ pharmacological profile are primarily situated on the smooth muscle cells of cerebral arteries (Edvinsson *et al.*, 2002; Oliver *et al.*, 2002; Petersen *et al.*, 2004b), it is not clear whether BIBN4096BS has any effect on cerebral arteries *in vivo*. However, an endothelial CGRP receptor may exist, since CRLR, RAMP1, RAMP2 and receptor component protein are expressed in microvascular endothelial cells and in human cerebral arteries, in addition RAMP3 (Edvinsson *et al.*, 2002; Moreno *et al.*, 2002; Oliver *et al.*, 2002; Jansen-Olesen *et al.*, 2003), but there are no functional data to support this.

We found that the MMA dilatation in response to systemically infused CGRP was blocked and the vasodilatation induced by TES (endogenously released CGRP) was markedly inhibited. Activation of the trigeminal ganglion by electrical stimulation releases primarily CGRP, but also, to

a minor extent, substance P and PACAP and other peptides to the abluminal side of the vessels (Goadsby *et al.*, 1988; Zagami *et al.*, 1990; Williamson *et al.*, 1997b). These co-released peptides may account for the remaining 20% of the dilatation not inhibited by BIBN4096BS. A higher local CGRP concentration and unspecific effects of TES are alternative explanations for the lack of complete inhibition. The inhibitory effect of BIBN4096BS was equally potent towards the αCGRP and βCGRP response, this in disagreement with previous *in vivo* findings (Wu *et al.*, 2000), however in agreement with the larger part of existing *in vitro* data (Jansen-Olesen *et al.*, 2003).

The PA investigated did not exceed a size of $100 \mu m$ and can be defined as second-order arterioles (Harper *et al.*, 1984). The αCGRP-induced PA vasodilatation was only nonsignificantly reduced after the highest doses of BIBN4096BS. The antagonist did not inhibit βCGRP and the neurogenically evoked PA dilatation. Furthermore, BIBN4096BS did not significantly inhibit the increase in $LCBF_{Flux}$ seen after intravenously administration of CGRP.

The difference in inhibitory effect of BIBN4096BS on MMA and PA raises an interesting subject for further discussion. As outlined previously, the two types of arteries express a different vessel wall structure. The PA directly investigated and the smaller cerebral vessels indirectly measured by LDF are believed to posses a BBB. In theory, this would mean that BIBN4096BS could not pass the BBB and hence could not bind to the cerebrovascular CGRP receptors (Petersen *et al.*, 2004b). The influence of the BBB was supported by the inability of BIBN4096BS to block the effect of TES on PA vasodilatation. With increasing doses of BIBN4096BS, the αCGRP-induced PA dilatation decreased. The reduction in PA dilatation is most likely to be secondary to the prevention of the induced hypotension. It is unlikely to be a direct effect of the antagonist. Since βCGRP induced a less-pronounced degree of hypotension (approximately 10 mmHg), a similar reduction in PA dilatation was not seen for βCGRP. The

origin of the minor dilatation seen in PA after infusion of the lower doses of CGRP with only limited effect on the blood pressure is not readily explained. The application of TES induced a dilatation of PA without any effect on the MABP.

It has been proposed (Markowitz *et al.*, 1988; Williamson *et al.*, 1997b) that the dilatation of MMA induced by electrical stimulation mainly is mediated by CGRP. This was further supported by the use of BIBN4096BS in this study. Electrical stimulation of the superior sagital sinus in the cat (Goadsby *et al.*, 1991) and the dura mater in rats (Kurosawa *et al.*, 1995) leads to an increase in cerebral blood flow, an increase found to be mediated by CGRP. Based on existing data, it is known that PAs are innervated by CGRP containing C-fibres originating in the trigeminal ganglion (Edvinsson *et al.*, 2001a) and furthermore that PA dilatation is mediated through CGRP receptors (McCulloch *et al.*, 1986; Wei *et al.*, 1992; Hong *et al.*, 1994). It is therefore most likely that the TES-induced PA dilatation in this study was caused by CGRP release, however no direct evidence is obtained due to the ineffectiveness of BIBN4096BS.

The results from these experiments contribute to the understanding of the site of action for BIBN4096BS. It seems that BIBN4096BS does not pass the BBB in the rat, but is very effective in preventing CGRP-induced vasodilatation in vessels without a BBB.

The present study strongly suggest that the clinically effective migraine drug BIBN4096BS (Olesen *et al.*, 2004) does not cross the BBB. With the caution of species differences in BBB function or the possible occurrence of transient BBB changes during the migraine attack, this indicates that dural arteries may play an important role in migraine pathogenesis.

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