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The calcitonin gene-related peptide (CGRP) receptor antagonist BIBN4096BS reduces neurogenic increases in dural blood flow

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Abstract

In an *in vivo* preparation of the exposed rat cranial dura mater electrical field stimulation causes increases in blood flow that are mainly due to the vasodilatory effect of calcitonin gene-related peptide (CGRP) released from meningeal afferents. In this preparation the effect of BIBN4096BS, a non-peptide competitive antagonist of CGRP receptors, was examined. Additionally, in an *in vitro* preparation of the hemisected rat skull the effect of BIBN4096BS on CGRP release stimulated by activation of meningeal afferents was analysed. Injection of BIBN4096BS at cumulative doses of 300 μg/kg and 900 μg/kg caused dose-dependent inhibition of the electrically evoked blood flow increases. The basal blood flow and vital parameters were not significantly changed by any dose. In the hemisected skull BIBN4096BS at 10^{-6} M did not alter the CGRP release evoked by depolarizing K^+ concentrations or antidromic electrical stimulation of the trigeminal ganglion. We conclude that neurogenic increases in dural blood flow are reduced by BIBN4096BS without changing basal vascular parameters. This peripheral effect may be important with regard to CGRP receptor inhibition as an antimigraine strategy.

Keywords: Neurogenic vasodilatation; Neuropeptide release; Trigeminal; Dura mater encephali; Headache; (Rat)

1. Introduction

Calcitonin gene-related peptide (CGRP) is expressed in a major proportion of trigeminal afferents innervating intracranial tissues (Keller and Marfurt, 1991; Tsai et al., 1988). Release of CGRP upon activation of trigeminal afferents causes increased blood flow in trigeminal tissues (Escott et al., 1995; Kurosawa et al., 1995). At intracranial arteries CGRP is regarded to be the most potent vasodilatory neuropeptide (Edvinsson et al., 1987; McCulloch et al., 1986). It is therefore conceivable that CGRP plays a preferential role in the regulation of intracranial blood flow under pathological conditions that are associated with nociceptive activation and hence head pain (Hargreaves and Shepheard, 1999). Attacks of migraine and other severe primary headaches have been shown to be accompanied by significant elevations of CGRP concentrations in the venous outflow from the head (Goadsby et al., 1988; Edvinsson and Goadsby, 1995; Juhasz et al., 2003), apart from a recent report questioning this issue (Tvedskov et al., 2005). An important role for CGRP in the generation or maintenance of migraine is evidenced by some antimigraine effects of CGRP receptor inhibition, which have been demonstrated in first clinical studies using the non-peptide CGRP receptor antagonist BIBN4096BS (Olesen et al., 2004; Troconiz et al., 2006). Moreover, CGRP infusion has been shown to cause delayed migraine attacks in patients suffering from migraine (Lassen et al., 2002) but the manner in which CGRP affects the meningeal nociceptive system is presently unknown. Noxious stimulation of dural and large intracerebral blood vessels including mechanical distension of these vessels in humans is known to cause head pain (Ray and Wolff, 1940). Therefore rapid distension of intracranial arteries cannot be ruled out as a noxious stimulus. Massive release of CGRP, possibly together with other vasodilatory mediators, could facilitate arterial distension. On this background, regardless of whether CGRP is a direct nociceptive stimulus or not, measurements indicating vasomotor changes in the meninges caused by CGRP release upon trigeminal afferent activation are of considerable interest.

In several animal experiments the exposed cranial dura mater was used as a model to study the vasomotor effect caused by

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CGRP release, measured as changes in arterial diameter with intravital microscopy (Petersen et al., 2004; Williamson et al., 1997) or as changes in dural blood flow using laser Doppler flowmetry (Messlinger et al., 1995; Carmody et al., 1996; Dux et al., 2003). In a previous study our group has shown that local electrical stimulation of the exposed rat cranial dura mater evoked increases in dural blood flow that were dose-dependently inhibited by topical application of the CGRP receptor antagonist $CGRP_{8-37}$ (Kurosawa et al., 1995). The effect lasted for a few minutes at a $CGRP_{8-37}$ concentration of 10^{-7} M and for more than half an hour at 10^{-5} M. $CGRP_{8-37}$ was not applied systemically because it undergoes fast degradation in the blood plasma. The development of BIBN4096BS alleviates this degradation problem as it is a stable and long-acting competitive non-peptide CGRP receptor antagonist with high affinity and specificity for CGRP receptors (Doods et al., 2000; Schindler and Doods, 2002; Rudolf et al., 2005). In the present study we used our dural blood flow preparation to examine the effects of i.v. administered BIBN4096BS on neurogenic blood flow increases produced by local electrical stimulation of the rat cranial dura mater. An in vitro preparation for measuring CGRP release was used to examine whether BIBN4096BS can inhibit CGRP release.

2. Materials and methods

2.1. Anaesthesia and general preparation for in vivo measurements

Male Wistar rats (250–440 g) bred and housed in our Institute were used. The experiments were performed in accordance with the ethical guidelines of the International Association for the Study of Pain and the German laws of animal protection. The experimental procedure was reviewed by an ethics committee and approved by the local district government. The animals were anaesthetized with an initial intraperitoneal dose of 120–150 mg/kg thiopentone (Trapanal®, Byk Gulden, Konstanz, Germany), followed by additional doses of 25 mg/kg thiopentone when required. Depth of anaesthesia was routinely assessed and held at a level in which noxious stimuli (pinching of earlobes) failed to elicit motor reflexes or changes in systemic arterial pressure, which ranged from 80 to 120 mm Hg. The right femoral artery was cannulated to monitor systemic arterial blood pressure. Another catheter was inserted into the right femoral vein for infusion of solutions. The animals were tracheotomised and artificially ventilated with oxygen-enriched room air. Expiratory CO_2 was monitored and maintained at 4.5–5%, which suppressed spontaneous breathing. Body temperature was maintained at 37–37.5 °C with a feedback- controlled homoeothermic system (FMI, Germany). Vital parameters (blood pressure, heart rate, expiratory CO_2 level, and body temperature) were recorded continuously throughout the experiment.

2.2. Head surgery and blood flow recording

The preparation for dural blood flow recording has been reported previously (Kurosawa et al., 1995; Messlinger et al., 2000; Denekas et al., 2006). The animals were placed in a stereotaxic frame with the head held in a fixed position by ear bars. The eyes were covered with a protecting ointment (Bepanthen®, Roche). An incision was made along the midline of the scalp, and the left parietal region of the skull was exposed. Using a dental drill and liquid cooling with drops of saline, a cranial window of about 4×6 mm was drilled into the parietal bone to expose the dura mater and the middle meningeal artery. A second slit-like window (about 1.5×6 mm) was drilled apically along the superior sagittal sinus for electrical stimulation. In this stimulation window a pair of parallel wire electrodes (diameter 0.2 mm, length 5 mm, separation distance 1 mm) were placed on the dura mater and covered with mineral oil.

Dural blood flow was recorded as flux signal using a laser Doppler system (Moor Instruments, DRT4) at a sampling frequency of 10 Hz with needle type probes (tip diameter 0.8 mm) that were positioned directly over branches of the middle meningeal artery about 1–2 mm proximal to the stimulated area. In this position the dominant component of the flux signal comes from the artery, although minor contributions from adjacent venous vessels and capillaries in the dura and pia mater around the artery cannot be excluded. This has previously been shown by optical separation of the dura mater from the underlying cortical surface in similar measurements (Kurosawa et al., 1995). Flux is a product of mean blood cell velocity and the concentration of blood cells in the recording area. Increases in dural flow during electrical field stimulation are mainly due to increases in the velocity component of the flux signal, which can be specifically read out in the system used. Therefore increases in arterial blood velocity are most likely caused by vasodilatory processes distal to the site of measurement and the dural flow is an integrated measure of these processes.

The dura mater in the recording window was protected from drying with pieces of cotton soaked with isotonic saline arranged around the recording probe.

2.3. Stimulation and drug administration

The experiment was started not earlier than 60 min after trepaning the skull to ensure that the basal (unstimulated) blood flow was stable. The dura mater was stimulated at regular periods of 4 min for 30 s with rectangular pulses of 0.5 ms duration, 8–12 V at 10 Hz. The stimulus strength was optimised at the beginning of each experiment to elicit clear increases in blood flow without changing the systemic blood pressure or causing motor responses in head muscles. After 3 control periods with responses of similar size, 0.5 ml of physiological saline was i.v. injected over 30 s. After six stimulation periods, 300 μg/kg BIBN4096BS in 0.5 ml saline was slowly i.v. injected over 30 s, followed by six more stimulation periods and then injection of 900 μg/kg BIBN4096BS, followed by other six stimulation periods. 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(2H) quinazolinyl)-, [R-(R*,S*)]), which was generously provided by Dr. H. Doods (Boehringer Ingelheim, Germany) as a crystalline substance, was dissolved in acidic saline (pH 2.5–4.5)

at a concentration of 1 mg/ml, titrated to pH 6.8 and topped up to 0.5 ml with saline prior to injection.

2.4. Data collection and calculations

Blood flow (in arbitrary units) and vital parameter data were stored and processed with the DRTsoft program (Moore Instruments). Changes in blood flow caused by electrical stimulation were calculated as mean flow within a period of 60 s from the onset of stimulation (spanning over the 30 s stimulation period and the ensuing 30 s) from which the mean basal flow within a period of 60 s before each individual stimulation was subtracted. The three control values of stimulated flow (before application of the first substance) were averaged in each experiment, and all subsequent stimulated flow values were normalised to this mean. Normalised stimulated flow values of the subsequent stimulation periods were statistically compared using one-way analysis of variance (ANOVA) with repeated measurements followed by Fisher's least significance difference (LSD) test with multiple comparisons for post-hoc analysis. (Statistica® software package, StatSoft, USA). For a second calculation the data were grouped by substances and the order of stimulation interval after each substance administration and statistically analysed with two-way ANOVA followed by the Tukey's HSD post-hoc test. Significance was accepted at the 5% level. Basal flow values (mean flow within 60 s periods before stimulation) were also normalised to the control measurements and their variation statistically analysed using the same tests. All flow values are given as mean ± standard error of the mean (S.E.M.).

2.5. Preparation for CGRP release in vitro

The preparation has been described previously (Ebersberger et al., 1999; Strecker et al., 2002b). Heads from male Wistar rats (160–230 g) killed in a CO_2 atmosphere were skinned and divided into two equal halves along the sagittal plane. The brain was carefully removed, while the dura mater remained attached to the skull. The skull halves were washed for 30 min at room temperature with carbogen-gased synthetic interstitial fluid (SIF, pH 7.4) containing (mM): 108 NaCl, 3.48 KCl, 3.5 $MgSO_4$, 26 $NaHCO_3$, 11.7 NaH_2PO_4, 1.5 $CaCl_2$, 9.6 sodium gluconate, 5.55 glucose and 7.6 sucrose. The skull cavities were mounted in a water bath held at a constant temperature of 37 °C. In the course of the experiments the cavities were repetitively filled with SIF that completely covered the supratentorial dura mater. At intervals of 5 min the eluate was evacuated from the skull without touching the dura mater.

For electrical stimulation an isolated steel needle electrode (cathode, diameter 250 μm) was introduced with its blank tip to a depth of 1 mm into the trigeminal ganglion near to the branching of nerves V1 and V2/3. The anode was fixed to the tissue at the occipital bone. The skull cavity was filled with 300 μl SIF and 5 min later replaced with SIF (control experiments) or with BIBN4096BS (10^{-6} M), which was made from a stock solution of 10^{-3} M BIBN4096BS dissolved in acidic saline and diluted in SIF. Another 5 min later the skull was again filled either with SIF or BIBN4096BS and the trigeminal ganglion was stimulated with pulses of 0.5 ms, 40 V at 10 Hz for 5 min. Post-stimulation the same solutions were applied as in the pre-stimulation periods but in reverse order. For chemical instead of electrical stimulation, the skull was filled with SIF containing 60 mM KCl balanced with equimolar removal of NaCl.

2.6. CGRP immunoassay and data analysis

The method for detecting CGRP in the eluate with an enzyme-linked immunoassay (EIA) has been previously published in detail (Averbeck and Reeh, 2001). Briefly, the incubation fluids were immediately processed after the experiment using distinct volumes of commercial CGRP-EIA buffers containing peptidase inhibitors (Cayman, distributed by SPIbio, France). The antibody directed against human α/β-CGRP has 100% cross-reactivity to rat and mouse CGRP with a detection level of immunoreactive CGRP (iCGRP) of about 2 pg/ml. Values of iCGRP release from subsequent sampling periods were compared using one-way ANOVA with repeated measurements followed by the post-hoc LSD-test (Statistica® software package, StatSoft, USA). Concentrations of iCGRP between skull halves treated with BIBN4096BS and control halves (left and right from individual animals) were examined with the Student *t*-test. Differences were considered significant at $P<0.05$. All values are given as mean ± S.E.M.

3. Results

3.1. Basal blood flow and vital parameters

A total of 16 experiments were made with 21 repetitive stimulation periods at 4 min intervals. After three control stimulation periods saline (vehicle) was injected i.v., after which a further six stimulation periods followed before solutions of 300 μg/kg and finally 900 μg/kg BIBN4096BS were applied.

During the course of the experiments the basal blood flow (flow within the 60 s periods before electrical stimulation) did not significantly change (ANOVA with repeated measurements). After vehicle injection the mean basal flow (average of 6 measurements) was 105.4 ± 4.0% of the control level, after 300 μg/kg BIBN4096BS it was 106.3 ± 5.7% and after 900 μg/kg it was 111.1 ± 7.1% (Fig. 2B). To examine whether the injection of substances caused immediate changes, basal flow values in the 2 min period before and 2 min period after the injections were compared. Immediately after vehicle injection the basal flow increased by 2.6 ± 1.1% and after 300 μg/kg BIBN4096BS it increased by 4.8 ± 2.2%, which was a significant change ($P=0.03$; *t*-test of dependent samples); immediately after injection of 900 μg/kg BIBN4096BS the basal flow increased slightly by 1.3 ± 1.2% (not significant). These changes were not consistent, however, since in about half of the experiments there was nearly no difference or even a small decrease in basal flow after drug application.

The systemic arterial pressure did not significantly change in the course of the experiments (ANOVA with repeated measurements). The mean arterial pressure was 108.8 mm Hg in

the control period, 106.4 mm Hg after injection of saline, 106.1 mm Hg after 300 μg/kg and finally 102.0 mm Hg after 900 μg/kg BIBN44096BS (Fig. 2C). The values were not significantly different to control. Immediate changes in arterial pressure (2 min before vs. 2 min after injection of substances) were also not significantly different (1.7% after vehicle, 1.1% after 300 μg/kg and −2.2% after 900 μg/kg BIBN4096BS). Other vital parameters (body temperature, end-expiratory CO_2) that were held at physiological levels were not influenced by any of the drug injections.

3.2. Stimulated blood flow

Electrical field stimulation of the dura mater caused increases in blood flow that started after a latency of 5 s, increased to a maximum within 30 s and returned to the baseline within 20–40 s when the stimulus was switched off (Fig. 1A). These increases in flow are known to be largely independent of the basal flow and reproducible in size under repetitive stimulation for more than one hour (Kurosawa et al., 1995; Messlinger et al., 1997 and unpublished observations). During the control period the averaged increase in blood flow of three consecutive stimulations was 100.6±3.3% of the basal flow. The mean flow increase in the control period was set to 100% in each individual experiment (control value). Following slow i.v. injection of vehicle (saline), the stimulated flow did not significantly change compared to the control value. After i.v. injection of 300 μg/kg BIBN4096BS the stimulated flow decreased to 77.2±6.5% of the control. Some of the subsequent six stimulation values (at 40, 48, 56 and 60 min) were significantly different to the first control value and two values (at 56 and 60 min) were in addition significantly different to the second control value (one-way repeated measures ANOVA and LSD test; Fig. 1B). After additional injection of 900 μg/kg BIBN4096BS the stimulated flow decreased to 56.9±16.3% of the control level. The subsequent stimulated flow values were all significantly different to each of the control values and to each of the values after vehicle ($P<0.05$; one-way repeated measures ANOVA and LSD test; Fig. 1B).

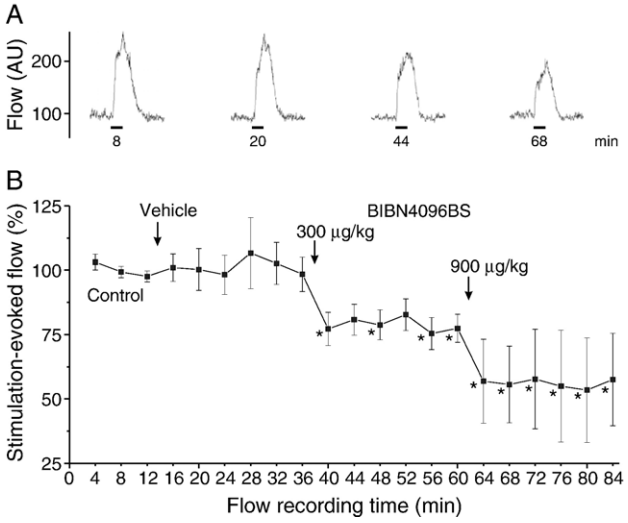

Fig. 1. Increases in meningeal blood flow caused by electrical field stimulation (30 s) of the exposed dura mater after vehicle (saline) and BIBN4096BS at two cumulative doses. (A) Sections from a continuous recording showing representative flow increases in different phases of the experiment corresponding to the time indicated by the stimulation bars. (B) Increases in flow (normalised to the mean of the control responses) and their variation after saline and BIBN4096BS; *significant differences in comparison to one or more control values ($P<0.05$, ANOVA with repeated measurements and LSD test).

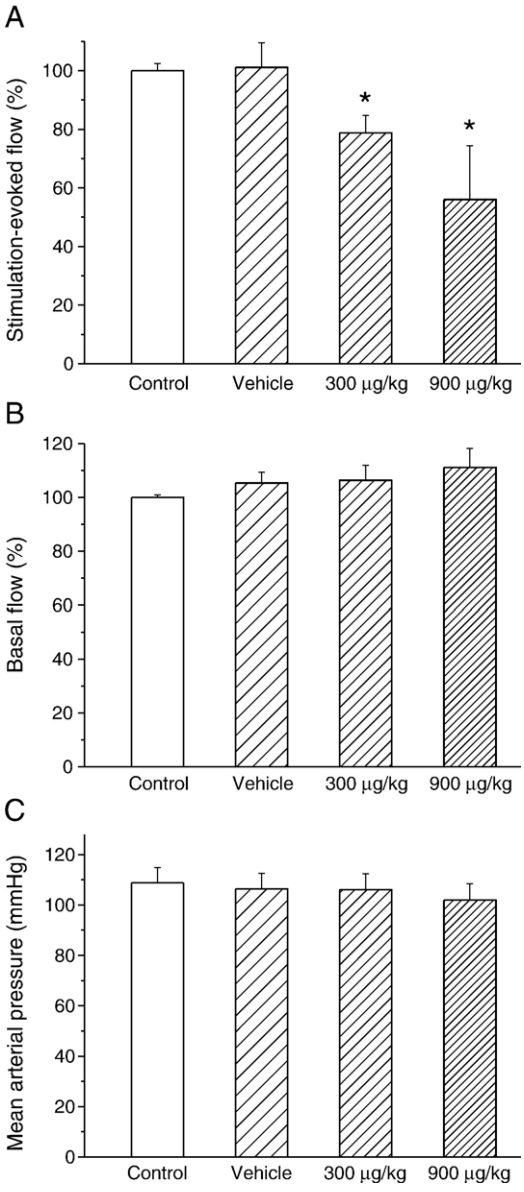

Fig. 2. Grand average of flow values and systemic arterial pressure during the course of the experiments. (A) Mean stimulated flow values (6 stimulation intervals after each treatment averaged) normalised to the control responses before vehicle. Values are significant (*) between both doses of BIBN4096BS and vehicle ($P<0.05$, ANOVA and HSD test). (B) Mean basal flow values (6 intervals after each treatment averaged) normalised to the control level before injection of vehicle. (C) Mean arterial pressure after each treatment during the experiment.

The mean values of stimulated blood flow (average of all 6 stimulation periods) were 101.2±8.3% after saline, 78.7±6.0% after 300 μg/kg and 56.0±18.4% after the additional 900 μg/kg BIBN4096BS (Fig. 2A). These mean values were significantly different and both differed significantly with respect to the mean

value after saline but there was no difference within the groups in the course of the flow measurements (two-way ANOVA and Tukey HSD test).

3.3. CGRP release in vitro

CGRP release in the presence of BIBN4096BS was examined in 16 skull halves, 8 of which were stimulated electrically and 8 chemically, and compared with the same number of skull halves without BIBN4096BS as control. The basal CGRP release (iCGRP concentration in the first and second 5 min eluates) was stable and not changed by 10^{-6} M BIBN4096BS (Fig. 3A,B). High K^+ concentrations caused increases in iCGRP concentration which were significantly different to the pre-and post-stimulation values (one-way ANOVA and LSD test; Fig. 3A) but there was no difference as to whether or not the high K^+ concentrations were applied in the presence of 10^{-6} M BIBN4096BS (*t*-test of dependent samples). Electrical stimulation (40 V) of the trigeminal ganglion caused significant increases in CGRP release in the control samples as well as in the BIBN4096BS treated skull halves (one-way ANOVA and LSD test; Fig. 3B) without significant difference between the samples pretreated with BIBN4096BS and the control ($P=0.08$, *t*-test of dependent samples). In the post-stimulation periods the iCGRP concentration fell without returning to the basal value but again there was no difference between BIBN4096BS treated samples and control samples (Fig. 3B).

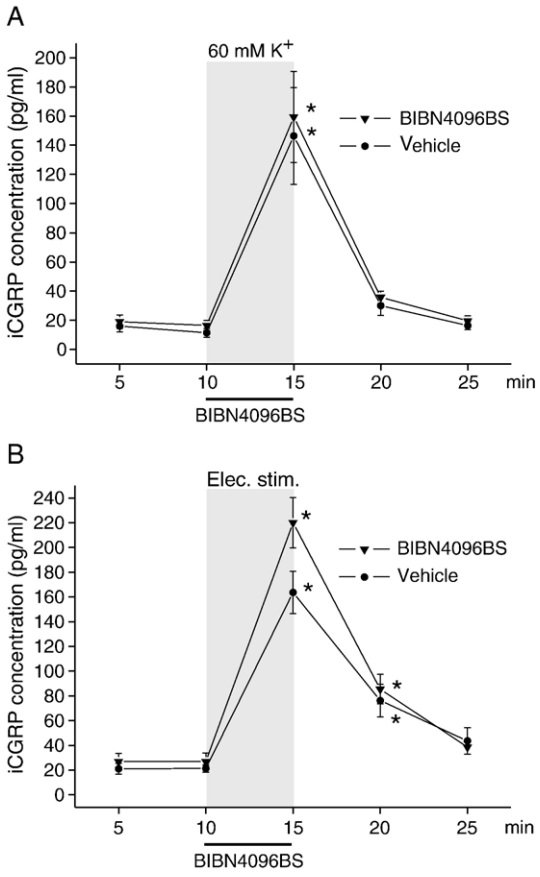

Fig. 3. Mean concentration of immunoreactive CGRP (iCGRP) in the eluate collected from skull cavities at intervals of 5 min as a measure for CGRP release from the cranial dura mater. (A) Chemical stimulation with 60 mM KCl caused significant increases in iCGRP (*) both in the presence of BIBN4096BS (10^{-6} M) and vehicle but no significant difference between samples treated with BIBN4096BS and vehicle (*t*-test of dependent samples). (B) Significant increases in CGRP release (*) during and after antidromic electrical stimulation of the trigeminal ganglion (40 V) but no significant difference between samples treated with BIBN4096BS (10^{-6} M) and vehicle (*t*-test of dependent samples). BIBN4096BS itself had no effect on basal CGRP release.

4. Discussion

Dural blood flow is neurogenically influenced by nerve fibres of different origin that innervate the cranial dura mater (Edvinsson and Uddman, 1981; Keller and Marfurt, 1991; Keller et al., 1989; Messlinger et al., 1993). Local electrical field stimulation of the dura mater activating these nerve fibres increases dural blood flow, which is thought to be mainly due to the vasodilatory action of CGRP released from the activated afferent fibres distal to the site of the flow probe (Kurosawa et al., 1995; Williamson et al., 1997).

In the present study we have applied a field stimulation technique with bipolar wire electrodes parallel to the superior sagittal sinus with the idea that this geometry would cause an optimal orthodromic activation of primary meningeal afferents that mostly spread along the middle meningeal artery towards the sagittal sinus (Messlinger et al., 1993). Using this technique, we have previously shown that stimulation-evoked increases in meningeal blood flow can be concentration-dependently inhibited in size and duration by local application of the CGRP receptor antagonist $CGRP_{8-37}$ onto the exposed dura mater (Kurosawa et al., 1995). The availability of BIBN4096BS, a biologically stable non-peptide CGRP receptor antagonist (Doods et al., 2000; Schindler and Doods, 2002) provided an opportunity to examine the effect of systemic CGRP receptor inhibition on neurogenic blood flow increases. We preferred systemic application of BIBN4096BS at doses of 300 and 900 μg/kg because these were known to inhibit dose-dependently the activity of neurons in the spinal trigeminal nucleus caudalis with meningeal input under similar conditions (Fischer et al., 2005). Topical application of BIBN4096BS onto the dura mater was not used because the drug was dissolved in acidic solution, which can influence meningeal blood flow unless it is buffered by a fluid like the blood (unpublished observations). In the present study we found dose-dependent inhibition of the evoked meningeal blood flow lasting at least half an hour without lowering the basal flow. The effect looks remarkably similar to the time course and the extent of inhibition observed for trigeminal neuron activity (Fischer et al., 2005).

With the same preparation as used in this study, we recently examined the effect of a specific CGRP-binding mirror-image RNA oligonucleotide, a so-called Spiegelmer, on stimulated meningeal blood flow (Denekas et al., 2006). Compared to BIBN4096BS, systemic application of the Spiegelmer, even at a high dose, was somewhat less effective in lowering evoked meningeal flow. This difference may be due to limited diffusion of the Spiegelmer compound into the perivascular tissue

because of its high molecular mass. The inhibitory principle of the Spiegelmer is to inactivate CGRP rather than inhibiting the CGRP receptor and, in addition, it seems to reduce CGRP release by an unknown mechanism. Because there is some evidence for CGRP autoreceptors on primary afferents (Ma et al., 2003; Segond et al., 2002; Cottrell et al., 2005), the possibility could not be ruled out that blockade of CGRP receptors modifies CGRP release. Therefore we used an in vitro preparation of the hemisected rat head to induce CGRP release from meningeal afferents either by antidromic electrical stimulation of the trigeminal ganglion (Ebersberger et al., 1999) or by superfusion of the dura mater with a depolarising solution containing 60 mM K^+. Pre-application of BIBN4096BS did not change the evoked CGRP release induced by either stimulus, making the involvement of CGRP autoreceptors in the control of CGRP release less likely.

In a closed cranial window preparation in SD rats, Petersen et al. (2004) measured the dilatation of the middle meningeal artery and of pial arteries as well as the local cortical blood flow induced by infusion of CGRP and by local transcranial electrical stimulation. BIBN4096BS at systemic doses (in the range of the lower dose used in our study) inhibited the dilatation of the middle meningeal artery nearly completely but had no significant effect on pial artery dilatation or cortical blood flow. In the study of Petersen et al. (2004) the vasodilatory reaction (peak value) was measured very close to the site of electrical stimulation within a period of 10 s, whereas the flow signal in the present study is an integrated measure of vascular responses within a wide area of stimulation over a period of 1 min. The nearly 50% inhibition of stimulated flow with the high dose of BIBN4096BS in our experiments lies between the effects on dural and pial vessels reported by Petersen et al. (2004), thus one may be tempted to conclude that part of the meningeal flow in our experiments could have been cortical flow recorded through the dura mater, a possibility that we cannot exclude. On the other hand, local increases in dural arterial diameter and dural blood flow provide two different readouts of vasodilatory effects, in which the flow is not only dependent on the blood cell concentration but also on blood flow velocity, the latter of which is mainly influenced by the vasodilatory state of arterial resistance vessels distal to the point of measurement. These arterioles are controlled by several vasodilatory mediators from different cellular sources, e.g. vasoactive intestinal polypeptide (Gottselig and Messlinger, 2004; Keller and Marfurt, 1991), nitric oxide (Akerman et al., 2002a; Strecker et al., 2002a), prostaglandins (Ebersberger et al., 1999, 2006) and histamine (Jansen-Olesen et al., 1997; Akerman et al., 2002b; Dimitriadou et al., 1997; Dux et al., 2002), all of which could be released during electrical field stimulation and contribute to an increase in flow.

There are certainly species differences in the affinity of different agonists for CGRP receptors that probably underlie the reported differences in efficacy of BIBN4096BS in the cerebrovascular system (Moreno et al., 2002). In pigs increases in carotid and capillary blood flow caused by capsaicin-induced release of CGRP were dose-dependently inhibited by i.v. BIBN4096BS but only the highest dose tested (1 mg/kg) was able to nearly reverse the increased flow (Kapoor et al., 2003). In rats no changes in heart rate, mean arterial pressure, systemic vascular conductance nor cardiac output were observed with BIBN4096BS at 3 mg/kg (Arulmani et al., 2004). Compared to rat CGRP receptors, human CGRP receptors have more than 100-fold the affinity for BIBN4096BS (Mallee et al., 2002). In the first clinical proof-of-concept study published by Olesen et al. (2004), in which migraine scores rather than vascular parameters were examined, the maximum response rate after i.v. administration of BIBN4096BS was already seen at a dose of about 70 μg/kg (Olesen et al., 2004). Considering the species differences in the efficacy of BIBN4096BS the cumulative dose of 1.2 mg/kg used in our experiments is not unreasonably high for rats.

It is interesting to compare our data with the findings of Petersen et al. (Petersen et al., 2004) in rats and those of corresponding examinations in humans (Petersen et al., 2005) under another aspect. The hemodynamically effective dose of BIBN4096BS seems to be much lower in man where it caused significant inhibition of α-CGRP-induced vasodilatation in human extracranial arteries such as the temporal artery. However, there was no evidence for an effect of the same dose of BIBN4096BS on either cortical artery diameter or cerebral blood flow. This may be due to the intact blood brain barrier that prevents BIBN4096BS reaching CGRP receptors on the vascular smooth muscle cells of intracerebral arteries. As pointed out by Petersen et al. (2004) and again evidenced by the present study, arteries in the dura mater like other extracerebral vessels seem not to have an effective barrier for substances like BIBN4096BS. Therefore, although the contribution of vascular changes to primary headaches is still an unresolved issue, the therapeutic effect of CGRP receptor inhibition in migraine pain (Olesen et al., 2004) and experimental headache (Petersen et al., 2005) argues for a contribution of extracranial and dural vessels rather than intracerebral arteries.

Taken together, regardless of whether neurogenic vasodilatation contributes to the generation of headaches or not, measurements of arterial vasodilatation and blood flow in the cranial dura mater can serve as models for meningeal nociceptive processes and have shown to be predictive for the effect of CGRP receptor inhibition as a new route for future headache therapies.

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