Pharmacological characterization of calcitonin gene-related peptide receptors and BIBN4096BS -- a novel CRPG receptor antagonist

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CHAPTER 8
Effects of the CGRP antagonist BIBN4096BS on neurogenic vasodilation
—A migraine model involving trigeminal ganglion stimulation—

1. Introduction
It is believed that neurotransmitter release from trigeminal sensory afferent nerves and vasodilation of cerebral vessels are essential for the development of the headache phase of migraine (1). It has been found that plasma CGRP levels are increased during migraine attacks. However, there occur no changes in the levels of substance P, vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) (2-3). The cerebrovascular sensory nerves originate in the trigeminal ganglion (4-5). In patients with trigeminal neuralgia (tic douloureux), it was found that thermocoagulation of the trigeminal ganglion caused a significant release of CGRP and substance P into the external jugular vein (6). A recent study has shown that electrical stimulation of the trigeminal ganglion caused an increase in blood flow of the rat ipsilateral facial skin, a region innervated by the trigeminal nerve (7). The CGRP antagonist, h-αCGRP(8-37) markedly inhibited these responses induced by trigeminal ganglion stimulation, whereas neither the neurokinin-1 (NK1) receptor antagonist nor the vasoactive intestinal peptide (VIP) antagonist had any effect on these responses. These data suggest that CGRP is the major neuropeptide involved in the vasodilator response to trigeminal ganglion stimulation in rat facial skin (7). Extending these findings to humans may lead to the speculation that CGRP is involved in the headache phase in migraine attacks. Furthermore, CGRP antagonists could be thought of as potential therapeutic agents in the treatment of migraine.

Using a trigeminal ganglion stimulation model, the present study investigated the release of CGRP from the trigeminal ganglion and the effect of BIBN4096BS, a novel CGRP antagonist (8), on neurogenic vasodilation in anesthetized rats.

2. Materials and methods
2.1. Measurement of facial blood flow
Male Wistar rats (Chbb: Thom 280-320) which had been fasted for 1 day were anaesthetized with sodium pentobarbitone: induction with 60 mg/kg, i.p. and maintained with an infusion of 30 mg/kg/h, s.c. in the dorsal skin through a 23 G needle using a solution of 10 mg/ml. Both sides of the face were shaved and depilated with a commercial depilatory cream (Pilca, Schwarzkopf and Henkel, Germany). The trachea was cannulated and the animals were artificially respired (80 strokes/min) with room air supplemented by oxygen to maintain arterial blood gases within normal physiological limits. The body temperature was maintained at 37°C with a heating pad. The left femoral artery and right femoral vein were cannulated for the continuous measurement of arterial blood pressure and intravenous administration of test agents, respectively. The right jugular vein was cannulated for the administration of pancuronium bromide (1 mg/kg/0.5ml, 8 min prior to each stimulation for neuromuscular blockade). Heart rate was derived from the blood pressure signal.

The rat was placed in a stereotaxic frame and a longitudinal incision was made in the scalp. One burr hole was drilled in the skull (left or right) and a bipolar electrode (Rhodes SNEX 100) was lowered, using a micromanipulator, into the trigeminal ganglion (0.32 cm dorsal to bregma, +/-0.30 cm lateral from the midline and 0.95 cm below the dural surface). The trigeminal ganglion was stimulated at 10 Hz, 1ms, 1mA, for 30 seconds. Microvascular blood flow changes in the facial skin were measured by Laser Doppler flowmetry (PERIMED, PeriFLUX 4001 Master). Standard Laser Doppler probes (PROBE 408) were positioned on either side of the face approximately 0.5 cm below the centre of the eye, an area innervated by the maxillary branch (V2) of the trigeminal nerve. Blood flow changes were measured as flux (arbitrary units) (7). All the parameters were recorded on a 8 channel polygraph.

After 30 min of equilibration, the animals were subjected to three periods of electrical stimulation (10 Hz, 1 ms, 1 mA, 30 s), separated by 30 min intervals. The first stimulation served as a control for the second and third stimulations. Test agents or vehicles (saline) were administered intravenously 5 min prior to the second and third stimulations. Two different doses of the test compound were administered to each animal. Blood pressure and facial skin blood flow were measured continuously throughout the experiment.
2.2. Measurement of CGRP level

**Blood sampling:** In another separate group of animals external jugular vein blood samples were taken prior to trigeminal stimulation and 2-5 min after trigeminal stimulation (during the period of facial blood flow increase), respectively. The blood samples were promptly centrifuged at 12,500 rpm, 4°C, for 15 min, and the separated plasma was stored at -80°C until assay.

**Peptide extraction:** Plasma samples were mixed with a double volume of 0.1% trifluoroacetic acid (TFA) (v/v) and centrifuged at 12,500 rpm, 4°C, for 15 min. The supernatants were pooled and applied on preprimed C18 Sep-Pak cartridges (Waters Corporation, Massachusetts, USA). The Sep-Pak cartridges were washed with 20 ml 0.1% TFA at a flow rate of 3 ml/min, and then eluted with 3 ml of 60% acetonitril (v/v) containing 0.1% TFA (v/v) at a flow rate of 2 ml/min. The eluates were freeze dried, and stored at -80°C until the radioimmunoassay was performed. Recovery, determined by addition of 125I-rCGRP to plasma prior to extraction, it amounted to 75.4±1.1% (n=8).

**Radioimmunoassay:** A competitive radioimmunoassay was used to specifically measure rCGRP concentrations in the plasma extracts. The procedure was applied according to the instructions of rCGRP RIA kit. Plasma extracts were assayed in duplicate using an antibody raised against rCGRP. The label (125I-rCGRP) was added after the samples had been incubated with the antibody (rabbit anti-rCGRP) for 1 day. Following another day of incubation, the antibody bound 125I-rCGRP was separated using goat anti-rabbit antibody, and the radioactivity was counted on a gamma counter (Canberra-Packard GmbH, Germany). Rat CGRP was used as a standard.

2.3. Statistical analysis

Results are expressed as percent changes of the area under the flux curve, for periods up to 25 min after stimulation, compared to the first stimulation. All values are presented as means ± S.E.M.. Comparisons were carried out by means of analysis of variance (ANOVA). P values < 0.05 are considered to indicate significant differences.
2.4. Drugs used
The following drugs were used: Pancuronium was purchased from Organon Teknika Medizinische Produkte GmbH, Eppelheim, Germany. rCGRP RIA kit was purchased from (DRG Instruments GmbH, Marburg, Germany). BIBN4096BS was synthesized by Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany. BIBN4096BS was dissolved with a small volume (20μl) IN HCl, further diluted with saline, and then adjusted to pH 6.5-7.0 by 1 N NaOH. Solutions were diluted to final concentrations with saline.

![facial blood flow](image)

Fig. 1. Effects of BIBN4096BS on facial skin vasodilation induced by trigeminal ganglion stimulation. The results are expressed as percent change of the response compared to the first response, means±S.E.M., n=5-12. Asterisk indicates statistical significance at p<0.05.

3. Results
3.1. Responses to trigeminal stimulation
Electrical stimulation of the trigeminal ganglion caused a rise in facial skin blood flow ipsilateral to the side of stimulation, and a slight and more transient increase in blood flow of the contralateral facial skin. This stimulation also caused a small transient increase in mean arterial blood pressure and heart rate. These findings are in agreement with the results reported by Escott at al. (5). The reproducibility of trigeminal ganglion stimulation-induced increase in the ipsilateral facial blood flow was studied by three times repeated electrical...
stimulation, separated by 30 min intervals. This response was repeated three times in the same animal, and no significant difference was found as compared to the first stimulation. The second and third responses amounted to 102.0 ± 2.8% and 95.7 ± 5.2% (n=6) of the first effect, respectively.

3.2. Effect of BIBN4096BS
Administration of BIBN4096BS had no effect on blood pressure and resting facial blood flow values. BIBN4096BS dose-dependently inhibited the increase in facial blood flow provoked by stimulation of the trigeminal ganglion (fig 1). The ID50 value of the inhibitory effect was 0.052 mg/kg.

![Graph](image)

Fig. 2. CGRP levels (means± S.E.M., n= 6 ) in the external jugular vein of the rat before (control) and during 2-5 min trigeminal ganglion stimulation (TGS). Note the significant increase (asterisk) in the level of CGRP in rats after TGS. Data are expressed as pg CGRP/ml plasma.

3.3. Immunoreactive CGRP levels
There was a significant increase in CGRP plasma levels following trigeminal ganglion stimulation, that is from 65 ± 7.93 to 151 ± 17.65 pg/ml (p<0.05) (fig 2).

4. Disscusion
CGRP, a potent vasodilator of cerebral blood vessels, is assumed to play a neurotransmitter role in the trigeminal system (9-12). Stimulation of the trigeminal ganglion in cat or man leads to vasodilation in the cranial circulation (13-15). Furthermore, increased levels of
CGRP were also found in man during attacks of classical migraine in the jugular vein on the affected side (16). Several neuropeptides have been identified in the trigeminal nerves, such as substance P, vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) (17). However, there occur no changes in the levels of substance P, VIP or NPY in peripheral blood during headache in common or classical migraine (2-3). These findings suggest that CGRP is an important neuropeptide in migraine. The present study demonstrates that electrical stimulation of the trigeminal ganglion increased the level of immunoreactive CGRP in plasma sampled from the external jugular vein in the rat.

Antidromic electrical stimulation of the rat trigeminal ganglion is known to cause vasodilation in the facial skin. Escott et al. studied the role of several neuropeptides in these blood flow responses by using selective receptor antagonists (7). The CGRP antagonist, h-α-CGRP(8-37) markedly inhibited responses induced by trigeminal ganglion stimulation. However, neither the neurokinin-1 (NK₁) receptor antagonist nor the VIP antagonist had any effect on these responses, suggesting that CGRP is the major neuropeptide involved in the vasodilator response to trigeminal ganglion stimulation in rat facial skin. The present data show that the administration of BIBN4096BS, a novel CGRP antagonist dose-dependently counteracted the facial skin vasodilator responses to trigeminal ganglion stimulation and thus indicates a pivotal place for CGRP in the trigeminovascular system.

Several studies using different methods have confirmed that migraine (migraine with aura and also without aura) is accompanied by significant changes of the cerebral blood flow (CBF) which is reduced during the pre-headache phase but is increased during the headache phase. The reduction of CBF is due to intracerebral vasoconstriction, and the rise of CBF is a consequence of dilation of extra- or intracerebral arteries (18-21). Thus, BIBN4096BS could be a novel approach for the treatment of migraine, since it inhibits the neurogenic vasodilatation provoked by CGRP release.

In summary, the present study confirms that CGRP release plays an important role in facial skin vasodilation elicited by electrical stimulation of the trigeminal ganglion in rats. BIBN4096BS inhibited the vasodilation responses in the rat facial skin. Clinical studies which can evaluate the potential efficacy of BIBN4096BS in treatment of migraine may be worthwhile.
References


