



SPECIAL REPORT

Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist

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Calcitonin gene-related peptide (CGRP) is one of the most potent endogenous vasodilators known. This peptide is increased during migraine attacks and has been implicated in the pathogenesis of migraine headache. Here we report on the first small molecule selective CGRP antagonist: BIBN4096BS. *In vitro*, this compound is extremely potent at primate CGRP receptors exhibiting an affinity (K_i) for human CGRP receptors of 14.4 ± 6.3 ($n=4$) pM. In an *in vivo* model, BIBN4096BS in doses between 1 and $30 \mu\text{g kg}^{-1}$ (i.v.) inhibited the effects of CGRP, released by stimulation of the trigeminal ganglion, on facial blood flow in marmoset monkeys. It is concluded that BIBN4096BS is a potent and selective CGRP antagonist.

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Abbreviations: 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*)]-; cAMP, adenosine 3'5'-cyclic monophosphate; CGRP, calcitonin gene-related peptide; EDTA, ethylenediaminetetraacetic acid; LDF, laser doppler flow; SK-N-MC, neuroblastoma cell line of human origin; 5-HT, 5-hydroxytryptamine

Introduction CGRP, a 37 amino acid neuropeptide first identified in 1982 (Amara *et al.*, 1982) belongs to a family of peptides including calcitonin, adrenomedullin and amylin. Localization studies have shown a wide distribution of CGRP-like immunoreactive structures including receptors in the periphery and central nervous system (Sexton, 1991; Brain & Cambridge, 1996). In many tissues, CGRP containing nerves are closely associated with blood vessels. Although CGRP has a number of effects, its most pronounced action is vasodilation. It is one of the most potent endogenous vasodilators known and its vasodilatory effects have been shown in a variety of vessels, e.g. CGRP released from sensory fibres originating in the trigeminal ganglia dilates cerebral vessels (Goadsby & Edvinsson, 1993).

Migraine headache is thought to be associated with dilatation of cranial vessels and activation of the trigemino-vascular system (Moskowitz & Macfarlane, 1993; Moskowitz, 1984; Goadsby *et al.*, 1991). In man stimulation of the trigeminal ganglion results in the release of CGRP (Goadsby *et al.*, 1988). Moreover, during a migraine attack levels of CGRP are increased (Goadsby *et al.*, 1990; Gallai *et al.*, 1995). Thus, CGRP has been implicated in the pathogenesis of migraine headache. Accordingly, inhibition of the CGRP-induced vasodilatation could be expected to attenuate migraine symptoms.

So far, only C-terminal fragments, example given CGRP(8–37), have been described to act as antagonists (Chiba *et al.*, 1989; Rist *et al.*, 1998), however these compounds are not very potent and their peptidic nature limit their use, especially for *in vivo* or clinical investigations. Our goal was to identify small molecule antagonists for the CGRP receptor and to use them to assess the role of CGRP in migraine headache.

Our high throughput screening led to the identification of dipeptide-like compounds that showed weak but unequivocal

inhibition of binding to the CGRP receptor. Lead optimization led to potent CGRP antagonists, the prototype being 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*)]- (Figure 1). The present study was undertaken to examine the pharmacological profile of BIBN4096BS and to investigate its potential as an anti-migraine drug.

Methods *In-vitro experiments* Binding studies investigating CGRP-receptors from human or rat origin were performed using SK-N-MC (neuroblastoma cell line of human origin) cell membranes (approximately 0.175 Mio cells) and rat spleen homogenates (approximately 50 μg of protein per assay). ^{125}I -hCGRP was used as the radioligand. The incubation buffer contained (in mM): Tris 50, NaCl 150, MgCl_2 5 and EDTA 1, (ethylene diamine tetra-acetic acid) pH 7.4. Membrane homogenates were incubated for 180 min at room temperature with 50 pM ^{125}I -hCGRP and increasing concentrations of displacing compound. The incubation was terminated by filtration through GF/B glass fibre filters using a cell harvester. The protein-bound radioactivity was determined in a gamma counter. The nonspecific binding was defined as radioactivity bound in the presence of 1 μM CGRP. The IC_{50} values were obtained by non-linear regression analysis on the basis of a one binding site model. K_i values were calculated according to the equation $K_i = \text{IC}_{50}/(1 + L/K_d)$, according to Cheng & Prusoff (1973), where L and K_d represent the concentration and the dissociation constant of the radioligand. The K_d was 0.05 nM and 0.12 nM for the human and rat CGRP receptor, as determined by saturation binding experiments with 0.5–500 pM ^{125}I -CGRP.

Antagonism of BIBN4096BS was determined by measuring the formation of cyclic AMP in SK-N-MC cells. For cyclic AMP (adenosine 3'5'-cyclic monophosphate) measurements SK-N-MC cells were incubated with CGRP alone or in the

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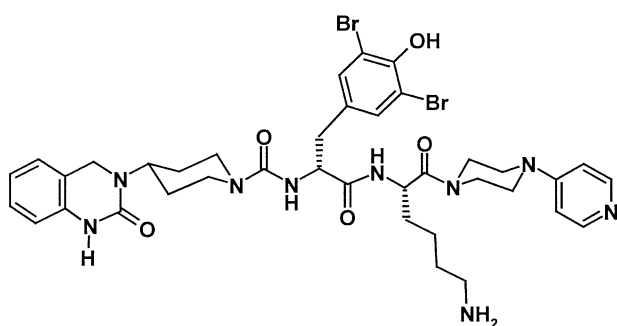


Figure 1 Structural formula of BIBN4096BS.

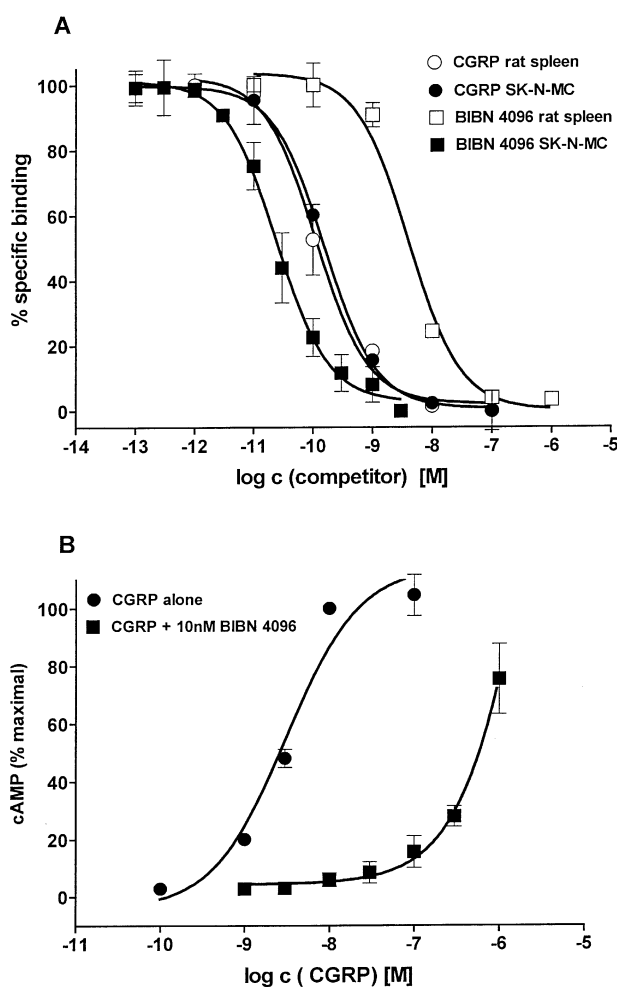


Figure 2 (A) Concentration-dependent inhibition of BIBN4096BS and CGRP of the specific binding of ^{125}I -CGRP to membranes obtained from the human neuroblastoma cell line SK-N-MC or from rat spleen. (B) Stimulation of cyclic AMP formation in SK-N-MC cells by CGRP alone and in the presence of 10 nM BIBN4096BS. Data are means \pm s.e.mean.

presence of 10 nM BIBN4096BS for 15 min at 37°C. Cyclic AMP was extracted with 0.1 M HCl and determined by radioimmunoassay.

An apparent pK_b value was calculated by the equation $pK_b = -\log[B] + \log(CR-1)$ (Schild, 1949), where $[B]$ is the molar concentration of the antagonist and CR is the ratio of the EC_{50} values in the presence and absence of the antagonist.

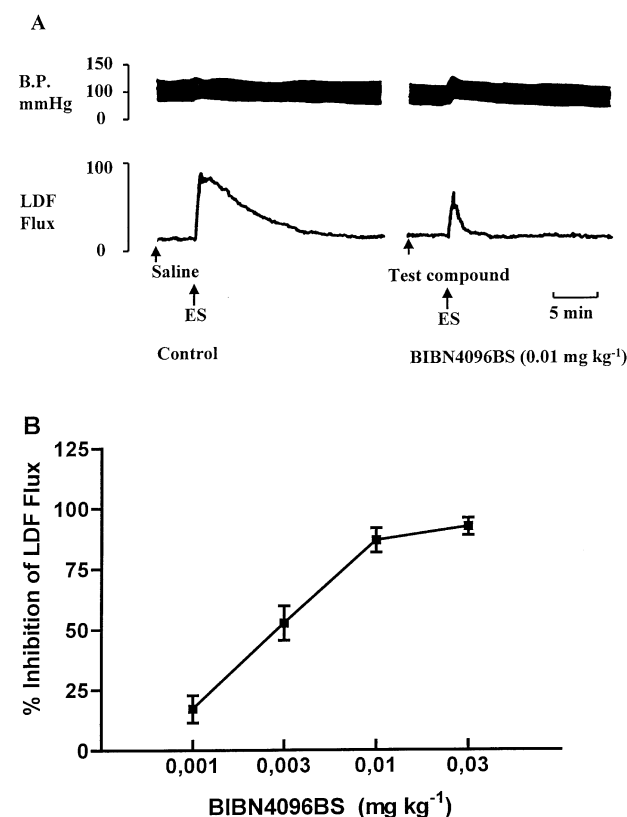


Figure 3 (A) Representative recording to illustrate the changes in blood pressure and LDF-Flux following electrical trigeminal ganglion stimulation. (B) Dose-dependent inhibition of the facial skin vasodilation induced by trigeminal ganglion stimulation in the marmoset by BIBN4096BS. Data are means \pm s.e.mean.

In vivo experiments Marmosets of either sex (300–400 g) were anaesthetized with sodium pentobarbitone (induction with 30 mg kg⁻¹, i.p. and maintained with an infusion of 6 mg kg⁻¹ h⁻¹, i.m). Body temperature was kept at 37°C by automatic control of a heating pad. The animals were placed in a stereotaxic frame and a longitudinal incision was made in the scalp. A small hole was drilled in the skull and a bipolar electrode (Rhodes SNEX 100) was lowered, using a micro-manipulator, in the trigeminal ganglion.

We used a radiographic approach to identify bony landmarks as guide in placing probes in the ganglion. An X-ray was taken using a Trophy CCX digital X-Ray apparatus and the Pars petrosa was identified as guide for placing the electrodes. The positions of the electrodes in the trigeminal ganglion were checked visually at the end of each experiment following removal of the brain. The trigeminal ganglion was stimulated at 10 Hz, 2 mA, 2 ms for 30 s using a Hugo Sachs Elektronik stimulator. Neuromuscular blockade, 15 min before electrical stimulation, was achieved using pancuronium bromide (initial 1 mg kg⁻¹, i.v., followed by 0.5 mg kg⁻¹ every hour).

Microvascular blood flow changes in the facial skin were measured by Laser Doppler flowmetry with a PeriFlux laser doppler system (wave length 780 nm; Time Constant 3 s) as flux in arbitrary units and expressed as area under the flux-curve (mm²) according to Escott *et al.*, 1995.

Animals were subjected to two or three periods of electrical stimulations, separated by a 30 min interval. The first stimulation was a control for the subsequent stimulations. Test compounds were administered intravenously 5 min prior to the second or third stimulation.

Results BIBN4096BS completely inhibited the specific binding of ^{125}I -CGRP to SK-N-MC cells and displayed an affinity (K_i) of 14.4 ± 6.3 pM ($n=4$) for the human CGRP receptor (Figure 2A). The endogenous ligand CGRP itself and the peptidic antagonist CGRP(8-37) displayed affinities (K_i) of 31.7 ± 1.6 pM ($n=15$) and 3.6 ± 0.7 nM ($n=4$), respectively. Employing the same cell line it was shown that BIBN4096BS is a pure antagonist. In the presence of antagonist the concentration-response curve of CGRP to increase cyclic AMP levels was shifted to the right in a parallel fashion, indicating competitive antagonism (Figure 2B). The pK_b value calculated at an antagonist concentration of 10 nM was 11.0 ± 0.3 ($n=3$). In addition, concentrations of BIBN4096BS up to 100 μM did not show any agonistic effects. The affinity (K_i) for CGRP binding sites in rat spleen was 3.4 ± 0.5 nM ($n=3$) and 96 ± 4 pM ($n=9$) for BIBN4096BS and CGRP, respectively. Accordingly, BIBN4096BS possessed a 236 fold higher affinity for human (SK-N-MC cells) than for rat (spleen) receptors.

Electrical stimulation of the trigeminal ganglion in marmosets caused an ipsilateral increase in facial skin blood flow. Following stimulation there was a rapid increase in flow which returned to base line values after approximately 10 min. This response was obtained three times in the same animal with no significant difference between the first and third stimulation. The area under the curve for the increase in flux observed was used to determine the effects of BIBN4096BS and amounted to 112 ± 11 mm². Administration of BIBN4096BS significantly and dose-dependently inhibited the response evoked by trigeminal ganglion stimulation (Figure 3). A 50% inhibition was achieved with a dose of approximately $3 \mu\text{g kg}^{-1}$ ($n=4$) applied intravenously. An almost complete blockade was observed with a dose of $30 \mu\text{g kg}^{-1}$ (i.v.). BIBN4096BS in doses up to 1 mg kg^{-1} (i.v.) had no intrinsic cardiovascular effects.

Discussion In order to determine the affinity of BIBN4096BS for human CGRP receptors we decided to use SK-N-MC cells since the receptor expressed in this cell line is identical to the cloned human CGRP receptor (Aiyar *et al.*, 1996). The affinity of BIBN4096BS for the human CGRP receptor is even higher than that of the endogenous ligand CGRP itself and 150 fold higher compared to the peptidic antagonist CGRP(8-37). Moreover, BIBN4096BS proved to be a competitive antagonist without any intrinsic agonistic activity. BIBN4096BS had no significant affinity for a set of 75 different receptor or enzyme system ($\text{IC}_{50} > 1000$ nM) including calcitonin, amylin or adrenomedullin receptors (for complete list of assays, see Rudolf *et al.*, 1997). Therefore, it can be concluded that BIBN4096BS is a selective CGRP antagonist with high affinity. We also demonstrated that BIBN4096BS possesses a remark-

able species selectivity, exhibiting an approximately 200 fold higher affinity for primate CGRP receptors compared to rat CGRP receptors. The same was observed for other non-primate species, such as dog, guinea-pig and rabbit. Having established that BIBN4096BS is a potent and selective CGRP antagonist we addressed the question whether BIBN4096BS has the potential to be an anti-migraine drug. Measuring facial blood flow following antidromic stimulation of the trigeminal ganglion has been suggested as a model to evaluate drugs that target the trigemino-vascular system during migraine headache (Escott *et al.*, 1995). Antidromic stimulation will result in the release of neuropeptides such as CGRP. Concomitantly, an increase in blood flow will be observed in blood vessel innervated by sensory nerves. As the facial skin is innervated by the trigeminal nerve, we measured the blood flow in this area. Because BIBN4096BS has a much higher affinity for primate CGRP receptors compared to rat CGRP receptors, we used marmosets. BIBN4096BS completely inhibited the neurogenic vasodilation, demonstrating that CGRP plays a major role in the vasodilation observed following stimulation of the trigeminal nerve in the marmoset. This is in agreement with experiments performed in the rat by Escott *et al.* (1995). Although CGRP itself is a strong vasodilator, i.v. administration of the antagonist had no effect on basal blood pressure and heart rate. This might indicate that CGRP plays a minor role in basal cardiovascular homeostasis under normal conditions, and that CGRP is only involved in (human) pathophysiology. Moreover, it is of interest to mention that in our model anti-migraine drugs like sumatriptan and zolmitriptan, both 5-HT_{1B/D} agonists, inhibited the increase in facial blood flow due to trigeminal stimulation not completely, but only approximately seventy per cent (data not shown). Accordingly, it could be hypothesized that blockade of postsynaptically located vascular CGRP receptors is a more powerful way to attenuate CGRP mediated effects than inhibiting the release of the peptide by stimulating presynaptic 5-HT-receptors.

In conclusion, our results demonstrate that BIBN4096BS is a high affinity and selective CGRP antagonist. Moreover, BIBN4096BS is a very potent inhibitor of neurogenic vasodilation. Since several lines of evidence indicate that CGRP might be a key factor in the initiation of migraine headache, we expect that CGRP antagonists will be effective anti-migraine drugs. Much remains to be investigated to understand the origin of migraine and to validate the concept that CGRP is a major player in its pathophysiology. The availability of a potent and well tolerated CGRP antagonist is a prerequisite to examine whether CGRP antagonism is a new therapeutic concept. BIBN4096BS is such a tool which is presently under clinical investigation for the acute treatment of migraine headache.

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