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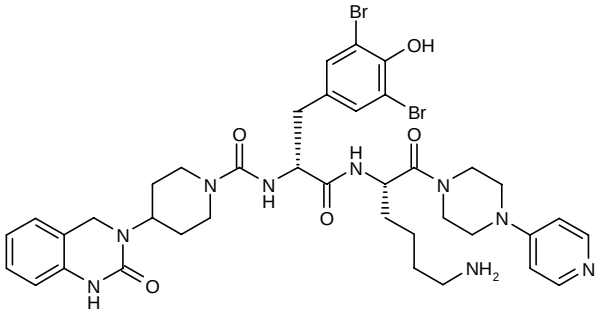
Olcegepant

Prop INN

Antimigraine Drug
CGRP Antagonist

BIBN-4096BS

N-[2-[5-Amino-1(*S*)-[4-(4-pyridinyl)piperazin-1-ylcarbonyl]pentylamino]-1(*R*)-(3,5-dibromo-4-hydroxybenzyl)-2-oxoethyl]-4-(2-oxo-1,2,3,4-tetrahydroquinazolin-3-yl)piperidine-1-carboxamide



C38 H47 Br2 N9 O5
Mol wt: 869.6553
CAS: 204697-65-4
EN: 285741

Abstract

Migraine involves dysfunction of brainstem pathways that normally modulate sensory input. The involvement of calcitonin gene-related peptide (CGRP) in migraine pathology is supported by both clinical and experimental evidence. The release of CGRP and other neuropeptides from trigeminal nerves is thought to mediate neurogenic inflammation within the meninges, which contributes to the generation of severe cerebral pain experienced during migraine attacks. Hence, structure-activity relationship studies have been conducted in an attempt to develop small molecules that behave as CGRP antagonists. Recently, the development of a potent, nonpeptide CGRP antagonist, BIBN-4096BS (olcegepant), represented a major breakthrough. BIBN-4096BS demonstrates very high affinity for the human CGRP receptor expressed in SK-N-MC cells and may be useful in the treatment of migraine, as well as providing new information on CGRP receptor subtypes.

Introduction

Migraine is defined as benign recurring headache and/or neurological dysfunction, usually accompanied by pain-free interludes and often provoked by stereotyped stimuli (1). It is more prevalent in females than in males. Although considerable progress has been made, the pathophysiology of migraine is still far from clear. As depicted in Figure 1, current theories propose that migraine-specific triggers (cause unknown) evoke changes in meningeal blood vessels causing dilatation and activation of perivascular trigeminal nerves (2-6). These nerves provide sensory information from the major blood vessels, such as cerebral arteries, that are responsible for regulating cerebral blood flow and from smaller blood vessels located within the pain-sensitive meninges (7, 8). Activated trigeminal nerve terminals release vasoactive neuropeptides such as calcitonin gene-related peptide (CGRP) within the meninges, mediating neurogenic inflammation that is characterized by vasodilatation, plasma extravasation and mast cell degranulation, releasing mediators such as histamine (8). Additionally, neuropeptides relay nociceptive transmission to the central nervous system (thalamus, hypothalamus, chemoreceptor trigger zone) via second-order neurons, leading to severe migraine pain, photophobia/phonophobia, nausea and vomiting, respectively.

Calcitonin gene-related peptide is one of several neuropeptides found in human trigeminal sensory neurons (2, 9-19). Present in both the pericranial vascular nerves and the trigeminal ganglion, it is a potent dilator of cerebral and dural vessels (20, 21) and is involved in meningeal dural vasodilatation (22). Cranial CGRP levels are elevated in patients with migraine (23, 24) and an infusion

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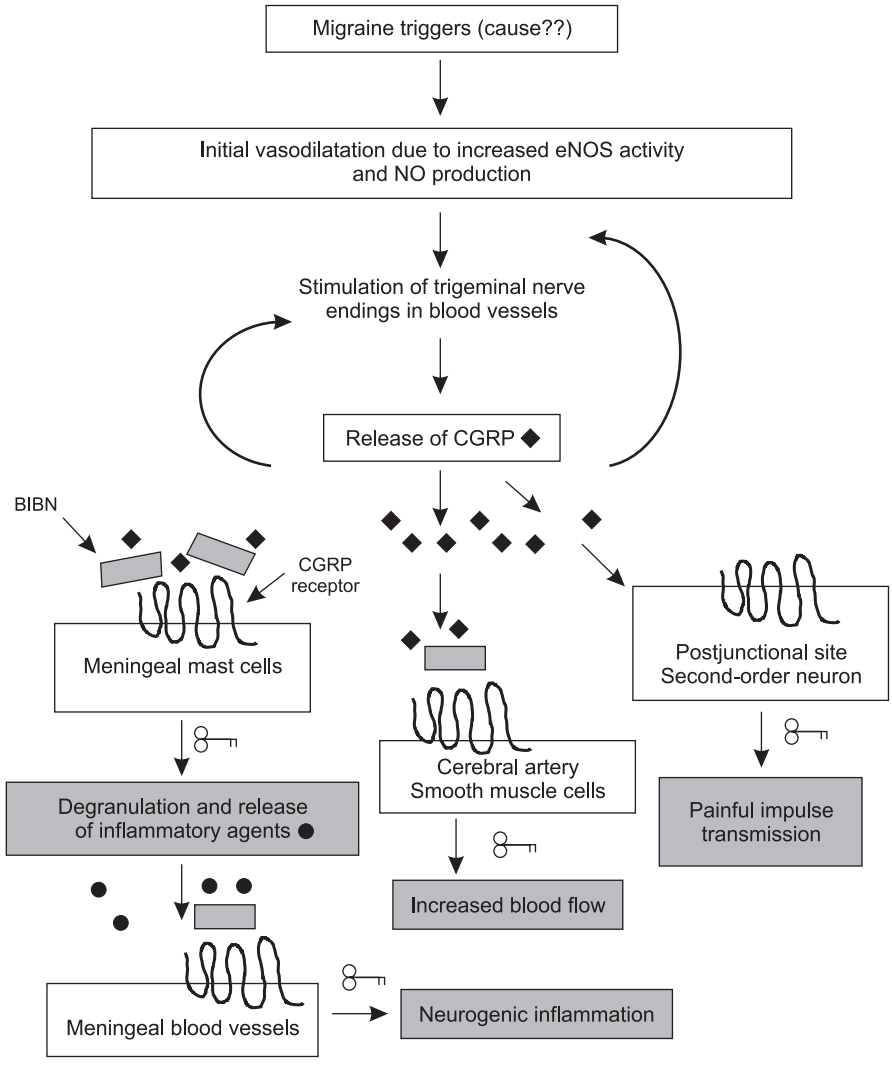

Fig. 1. Schematic representation of migraine pathophysiology and possible sites of action of BIBN-4096BS (●inflammatory products such as histamine; ◆ CGRP; ⊖inhibited).

of CGRP can trigger a migraine attack (25). It has therefore been hypothesized that CGRP antagonists might be effective in the treatment of acute migraine.

Calcitonin gene-related peptide is a 37-amino-acid peptide that is expressed by neurons and endocrine cells in various tissues. Two discrete isoforms have been described: αCGRP, which is produced by alternative splicing of a calcitonin gene transcript, and βCGRP, the product of a separate gene (1, 2). The two transcripts are highly similar, differing by 3 and 1 amino acids in humans and rats, respectively (9). For both CGRP isoforms, potent vasodilating activity has been demonstrated both *in vitro* and *in vivo* (10). The vasodilating activity of CGRP has recently gained importance in the pathogenesis of migraine (11).

At least two receptor types, $CGRP_1$ and $CGRP_2$, have been proposed from functional pharmacological experiments. The family of CGRP receptors that has been characterized molecularly consists of the 7-transmembrane G-protein-coupled calcitonin receptor-like receptor (CRLR) linked to one of three single membrane-spanning receptor activity-modifying proteins (RAMPs). RAMPs are required for both receptor trafficking and ligand binding. The association of CRLR with RAMP1 leads to a CGRP receptor that is antagonized by the CGRP fragment $CGRP_{8-37}$, whereas CRLR associated with RAMP2 produces an adrenomedullin receptor that is antagonized by $adrenomedullin_{22-52}$, and CRLR associated with RAMP3 gives a CGRP/adrenomedullin receptor.

Physiological studies have demonstrated that CGRP is a potent dilator of cerebral and dural vessels (15, 18, 26, 27), and therefore has an important function in regulating blood flow to the brain and pain-sensitive meninges, suggesting that CGRP receptor antagonists might be effective in treating migraine by blocking the

Table I: pA_2 values of BIBN-4096BS as compared to $CGRP_{8-37}$ (antagonists) in different cell lines expressing corresponding receptors, as measured by human αCGRP (agonist)-induced cAMP production.

Cell line	Receptor	pA_2 BIBN-4096BS	pA_2 $CGRP_{8-37}$	Ref.
Rat L6	CRLR + RAMP1 ($CGRP_1$)	9.25	7.81	31, 55
Human SK-N-MC		9.95	8.35	31, 55
	CRLR + RAMP1 ($CGRP_1$)	11	-	56
		11.2	-	37
Human colony Col-29	CRLR + RAMP2 ($hCGRP_2$)	9.98	7.34	31, 55
Rat2	CRLR + RAMP2 (ADM)	Unable to antagonize	6.72	31, 55

ADM, adrenomedullin; CGRP, calcitonin gene-related peptide; CRLR, calcitonin receptor-like receptor; RAMP, receptor-associated membrane protein.

actions of CGRP. Agents acting as CGRP receptor antagonists are likely to inhibit the dilatation of major cerebral vessels, diminish neurogenic inflammation within the meninges, and block the activation of nociceptive neural pathways. Thus, the ability of both triptans and CGRP receptor antagonists to alleviate migraine pain may be mediated by the same molecular targets activated following CGRP receptor activation.

Boehringer Ingelheim's BIBN-4096BS (olcegepant) is the first CGRP receptor antagonist for which significant efficacy has been demonstrated in the treatment of migraine, which in turn provides further evidence for the critical role of CGRP in the pathology of migraine. A non-peptide CGRP antagonist, BIBN-4096BS (28), has undergone clinical investigation (29) to assess the importance of CGRP in migraine headache and to ascertain whether the concept of CGRP antagonism may offer advantages, *e.g.*, higher efficacy, lower recurrence rate or improved side effect profile, compared to currently used antimigraine drugs.

Pharmacological Actions

Based on its antagonist properties, BIBN-4096BS has been used as a pharmacological tool to characterize cell lines endogenously expressing receptors of known composition. The pA_2 values of BIBN-4096BS and $CGRP_{8-37}$ for antagonism of hCGRP-induced cAMP production are shown in Table I. These studies describe the ability of BIBN-4096BS to interact with cell lines expressing human and rat $CGRP_1$ (CRLR/RAMP1) receptors (*i.e.*, SK-N-MC and L6 cells), rat adrenomedullin receptors (CRLR/RAMP2; *i.e.*, Rat2 cells) and a potential human $CGRP_2$-like receptor (Col-29 cells). The results clearly demonstrate that BIBN-4096BS shows preferential binding to the human CRLR/RAMP1 complex in functional assays and that the differences in affinity for CGRP receptors reported previously are due to species differences within the same receptor subtype. In another study, BIBN-4096BS was more potent than $CGRP_{8-37}$ in antagonizing agonist-induced cAMP production in smooth muscle cells, endothelial cells and astrocytes, with pIC_{50} values of 9.4, 10.9 and 13.8, respectively (30).

Surprisingly, BIBN-4096BS also acts as an antagonist at adrenomedullin receptors in rat vas deferens, suggesting an unlikely CRLR/RAMP2 receptor pharmacology (31). Additionally, characterization of CGRP receptor subtypes in rat left atrium and vas deferens employing CGRP as agonist demonstrated 10-fold higher affinity for BIBN-4096BS for rat atrium over rat vas deferens. BIBN-4096BS also exhibited 10-fold greater potency in antagonizing $(Cys(Et)^{2,7}$-human αCGRP- and human adrenomedullin-induced responses compared to CGRP-induced responses in rat vas deferens, indicating receptor heterogeneity in rat vas deferens (32). The selectivity of BIBN-4096BS for human over rat receptors is due to binding to residue 74 of human RAMP1 (33). The greater selectivity of BIBN-4096BS for CRLR/RAMP1 over CRLR/RAMP2 compared to $CGRP_{8-37}$ suggests that the latter compound undergoes greater interactions with CRLR (31). Recently, the affinity of BIBN-4096BS and $CGRP_{8-37}$ was compared on CRLR/RAMP2 and CRLR/RAMP3 complexes. BIBN-4096BS did not antagonize adrenomedullin at any of these receptors (34).

The isolated perfused murine mesenteric bed provides a model for direct measurement of microvascular vasodilatation. The vessels are preconstricted with noradrenaline prior to application of a vasodilator, and a decrease in perfusion pressure correlates with vasodilatation. BIBN-4096BS was found to be an effective antagonist of CGRP and adrenomedullin responses in the murine mesenteric and cutaneous microvasculature, and of CGRP in the murine aorta. Additionally, after local application, BIBN-4096BS selectively inhibited the potentiation of microvascular permeability in the cutaneous microvasculature by CGRP and adrenomedullin, with no effect on responses induced by other microvascular vasodilators. BIBN-4096BS reversed both newly developed and established vasoactive responses induced by CGRP (35).

It is unlikely that BIBN-4096BS is interacting with the CRLR/RAMP2 (AM1 receptor) or CRLR/RAMP3 (AM2 receptor) complexes, as it has a very low affinity for these receptors (34), but rather its effects appear to be

mediated via the CGRP receptor (35). This observation was initially confirmed in pig coronary and basilar arteries (36), where BIBN-4096BS was used as a pharmacological tool to determine the different CGRP receptors. It was evident from this study that αCGRP receptors are present in these arteries, which are potently blocked by BIBN-4096BS. In another study in bovine middle cerebral artery segments, BIBN-4096BS and $\alpha CGRP_{8-37}$ both blocked αCGRP-induced dilatation with respective pK_B values of 6.3 and 7.8.

BIBN-4096BS has been shown to possess potent competitive antagonist activity at human cerebral and coronary artery $CGRP_1$ receptors (37). The pA_2 values are similar to those obtained in the SK-N-MC cell line (see Table I), suggesting the involvement of a common receptor mechanism mediating the vasodilating effects of CGRP, *i.e.*, CRLR/RAMP1. Interestingly, CGRP was approximately 10-fold less potent on coronary than cerebral arteries (pEC_{50} values of 8.3 and 9.4, respectively), which was probably related to differences in receptor density in the two arteries (37, 38).

Antidromic stimulation of the trigeminal ganglion in marmosets is a model suggested to measure facial blood flow to evaluate drugs that target the trigemino-vascular system during migraine headache (39). Antidromic stimulation of this ganglion results in the release of neuropeptides, including CGRP, leading to an increase in the skin blood flow innervated by sensory nerves. BIBN-4096BS completely inhibited this response, demonstrating that CGRP plays a major role in the vasodilatation observed following stimulation of the trigeminal nerve in marmosets. Triptans such as sumatriptan and zolmitriptan partially inhibited the increase in facial blood flow due to trigeminal ganglion stimulation (28).

We have carried out different sets of experiments in a porcine model of migraine (5, 40) to study the hemodynamic effects of CGRP after endogenous release through capsaicin administration or exogenous administration of human αCGRP. Hemodynamic effects of exogenous intracarotid infusion of human αCGRP and the cardiovascular safety of BIBN-4096BS in pigs were initially assessed. Activation of CGRP receptors elicits dilatation in different vascular beds in several species (41-43). Consistent with these studies, our experiments show that intracarotid infusions of αCGRP produced a marked vasodilatation in the porcine carotid circulation, with an accompanying fall in systemic arterial blood pressure (Fig. 2). The fact that the animals were systematically vagosympathectomized may explain why the hypotension was not accompanied by a baroreflex-mediated tachycardia, as reported earlier (41-43). Interestingly, the ipsilateral skin redness, together with the marked decrease in arteriovenous oxygen saturation difference (A-V SO_2) induced by CGRP, indicate that porcine carotid arteriovenous anastomoses dilated in response to αCGRP (44). In this experimental study in anesthetized pigs, BIBN-4096BS proved to be an effective antagonist at the CGRP receptors mediating the systemic (hypotension), as well as the carotid (increased carotid conductance, pulsations and skin redness) hemodynamic responses to αCGRP.

The fact that BIBN-4096BS also abolished αCGRP-induced decreases in the A-V SO_2 difference suggests an action on carotid arteriovenous anastomoses (for further considerations, see 45). Chemical substances, such as capsaicin, release endogenously stored CGRP (3, 46), which in turn dilates the carotid vasculature, including carotid arteriovenous anastomoses (43). In our study, intracarotid administration of capsaicin dilated carotid arteriovenous anastomoses and arterioles, together with an increase in carotid pulsations (Fig. 3) and a narrowing of arteriovenous oxygen saturation difference (A-V SO_2), as well as an elevation in jugular vein plasma CGRP concentration (Figs. 4 and 5). These effects were dose-dependently antagonized by BIBN-4096BS, except jugular venous plasma CGRP concentrations which increased (47). We concluded that blockade of prejunctional 'inhibitory' CGRP autoreceptors by BIBN-4096BS led to increased release of CGRP by capsaicin, similar to the modulation of sympathetic neurotransmission by presynaptic α-adrenoceptors (48).

Although the exact nature of CGRP receptors that mediate porcine carotid vascular responses is not certain, cardiac inotropic and vasodilator responses are mediated predominantly by $CGRP_1$ receptors (49), for which BIBN-4096BS has very high affinity (28, 50). Although we cannot rule out the involvement of CGRP in certain other conditions, for example, in cardiac preconditioning or coronary artery disease (51-53), the present results suggest cardiovascular safety for BIBN-4096BS. Nevertheless, the role of CGRP in cardiovascular pathophysiology needs to be elucidated before it can be established whether or not CGRP receptor antagonists like BIBN-4096BS are completely safe in patients with cardiovascular disorders.

Pharmacokinetics

The safety, tolerability and pharmacokinetics of BIBN-4096BS have been evaluated in healthy young male and female volunteers in a double-blind, placebo-controlled, randomized first-in-human study using single rising doses (54). BIBN-4096BS was infused i.v. for 10 min at doses ranging from 0.1 to 10 mg. Blood and urine samples were collected at different time periods. More adverse effects were observed with the higher doses of 5 and 10 mg of BIBN-4096BS, while lower doses had incidence rates similar to placebo. Mild to moderate adverse effects included reactions at the injection site, fatigue, headache, diarrhea, flatulence, paresthesia, rhinitis, flushing and abdominal pain. BIBN-4096BS exhibited multicompartmental disposition characteristics following i.v. administration. The apparent volume of distribution at steady state (V_{ss}) was 20 l and the terminal half-life was 2.5 h. The total plasma clearance of BIBN-4096BS was 12 l/h and the mean renal clearance was 2 l/h, suggesting that elimination is mainly via nonrenal pathways.

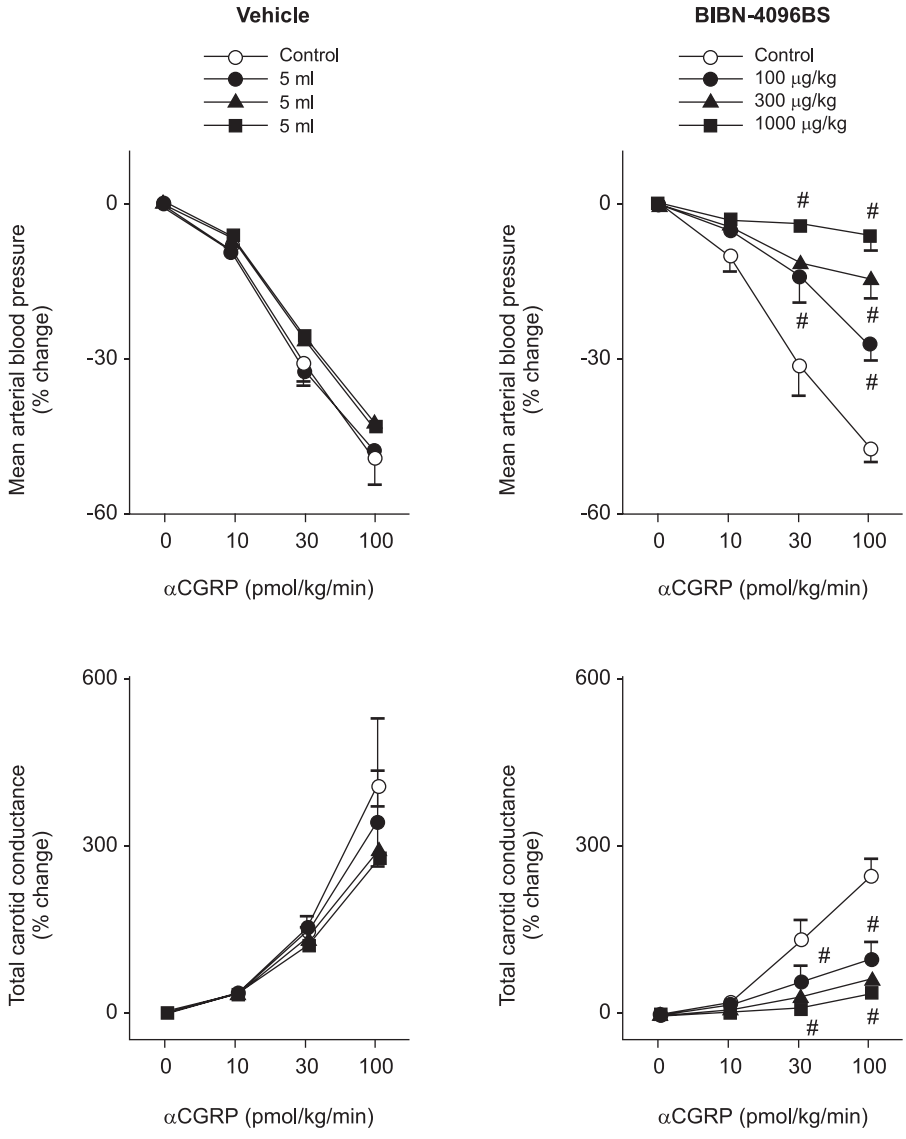

Fig. 2. Changes in mean arterial blood pressure and total carotid vascular conductance from baseline following intracarotid infusion of αCGRP in anesthetized pigs before (control) and after i.v. treatment with vehicle (n=6) or BIBN-4096BS (100, 300 and 1000 μg/kg; n=7). All values are expressed as mean ± s.e.m. The two highest doses of αCGRP significantly decreased mean arterial blood pressure and increased total carotid conductance (significance not shown for the sake of clarity). These effects of αCGRP were dose-dependently antagonized by BIBN-4096BS. #$p < 0.05$ *vs.* response after the corresponding volume of vehicle.

Clinical Studies

A multicenter, double-blind, randomized clinical trial of BIBN-4096BS was performed in patients with migraine receiving either placebo or 0.25, 0.5, 1, 2.5, 5 or 10 mg of BIBN-4096BS intravenously over a period of 10 min (29). BIBN-4096BS was effective in treating migraine headaches up to 6 h after onset. It had no vasoconstrictor effect, with paresthesia as the only adverse event. This confirmed the favorable safety and tolerability results reported earlier in the phase I study (54). Additionally, the rate of response to pain 2 h after treatment, the primary endpoint of the study, was significantly higher after infusion of BIBN-4096BS than after placebo.

Although these clinical findings are very encouraging, future research will be required to compare the safety and tolerability of BIBN-4096BS with the triptans and to develop more convenient formulations for the administration of nonpeptide CGRP receptor antagonists.

Acknowledgements

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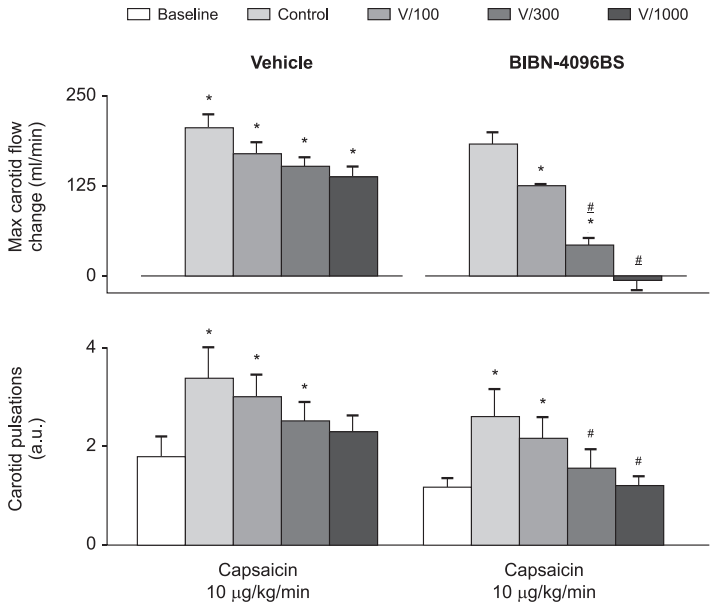

Fig. 3. Maximum changes in absolute carotid blood flow and carotid blood flow pulsations measured at baseline and following intracarotid infusions of capsaicin (10 μg/kg/min) in anesthetized pigs before (control) and after i.v. administration of vehicle (V; n=11) or BIBN-4096BS (100, 300 and 1000 μg/kg; n=11). 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. a.u., Arbitrary units.

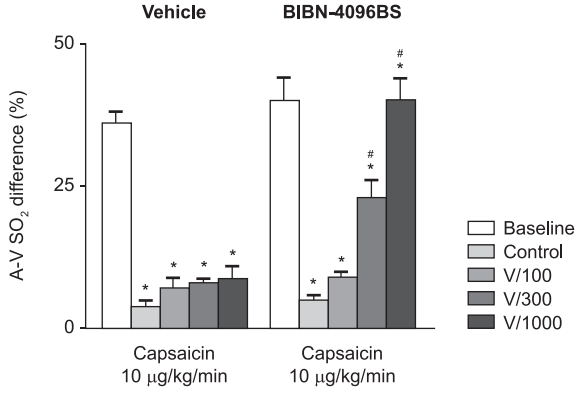

Fig. 4. Difference between arterial and jugular venous oxygen saturations (A-V SO_2 difference) measured at baseline and following intracarotid infusions of capsaicin (10 μg/kg/min) in anesthetized pigs before (control) and after i.v. administration of vehicle (V; n=11) or BIBN-4096BS (100, 399 and 1000 μg/kg; n=11). 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.

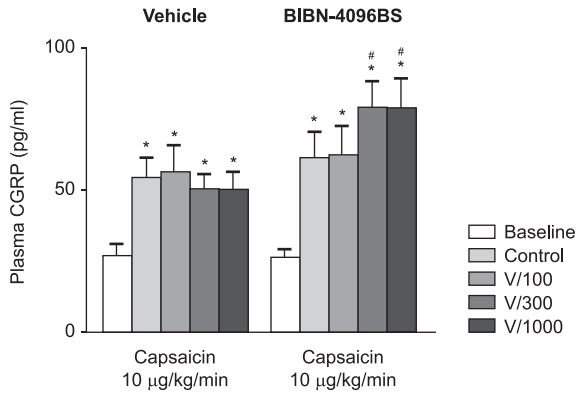

Fig. 5. Jugular vein plasma CGRP concentrations measured at baseline and after intracarotid infusions of capsaicin (10 μg/kg/min) in anesthetized pigs before (control) and after i.v. administration of vehicle (V; n=7) or BIBN-4096BS (100, 300 and 1000 μg/kg; n=6). All values are expressed as mean ± s.e.m. *$p < 0.05$ *vs.* baseline values; #$p < 0.05$ *vs.* response after the corresponding volume of vehicle.

Source

Boehringer Ingelheim Pharma GmbH & Co. KG (DE).

References

1. Raskin, N.H. *Diseases of the central nervous system.* In: Harrison's Principles of Internal Medicine. McGraw Hill, 1998, 2307-11.

2. Goadsby, P.J., Edvinsson, L. *The trigeminovascular system and migraine: Studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats.* Ann Neurol 1993, 33: 48-56.

3. Buzzi, M.G., Bonamini, M., Moskowitz, M.A. *Neurogenic model of migraine.* Cephalalgia 1995, 15: 277-80.

4. Doggrell, S.A. *Migraine and beyond: Cardiovascular therapeutic potential for CGRP modulators.* Expert Opin Invest Drugs 2001, 10: 1131-8.

5. De Vries, P., Villalon, C.M., Saxena, P.R. *Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy.* Eur J Pharmacol 1999, 375: 61-74.

6. Goadsby, P.J. *New directions in migraine research.* J Clin Neurosci 2002, 9: 368-73.

7. Kapoor, K. *Novel potential antimigraine compounds: Carotid and systemic haemodynamic effects in a porcine model of migraine.* In: Thesis Pharmacology. Rotterdam: Erasmus MC, 2003, 1-163.

8. Durham, P.L. *CGRP-receptor antagonists - A fresh approach to migraine therapy?* New Engl J Med 2004, 350: 1073-5.

9. Goadsby, P.J., Edvinsson, L. *Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies.* Brain 1994, 117: 427-34.

10. Goadsby, P.J. *Current concepts of the pathophysiology of migraine.* Neurol Clin 1997, 15: 27-42.

11. Hargreaves, R.J., Shepheard, S.L. *Pathophysiology of migraine - New insights.* Can J Neurol Sci 1999, 26: S12-9.

12. Poyner, D.R. *Calcitonin gene-related peptide: Multiple actions, multiple receptors.* Pharmacol Ther 1992, 56: 23-51.

13. Poyner, D. *Pharmacology of receptors for calcitonin gene-related peptide and amylin.* Trends Pharmacol Sci 1995, 16: 424-8.

14. Sams, A., Knyihar-Csillik, E., Engberg, J., Szok, D., Tatji, J., Bodi, I., Edvinsson, L., Vecsei, L., Jansen-Olesen, I. *CGRP and adrenomedullin receptor populations in human cerebral arteries: In vitro pharmacological and molecular investigations in different artery sizes.* Eur J Pharmacol 2000, 408: 183-93.

15. van Rossum, D., Hanisch, U.K., Quirion, R. *Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors.* Neurosci Biobehav Rev 1997, 21: 649-78.

16. Smith, D., Hill, R.G., Edvinsson, L., Longmore, J. *An immunocytochemical investigation of human trigeminal nucleus caudalis: CGRP, substance P and 5-HT_{1D}-receptor immunoreactivities are expressed by trigeminal sensory fibres.* Cephalalgia 2002, 22: 424-31.

17. Welch, K.M., Barkley, G.L., Tepley, N., Ramadan, N.M. *Central neurogenic mechanisms of migraine.* Neurology 1993, 43: S21-5.

18. Wimalawansa, S.J. *Calcitonin gene-related peptide and its receptors: Molecular genetics, physiology, pathophysiology, and therapeutic potentials.* Endocr Rev 1996, 17: 533-85.

19. Juaneda, C., Dumont, Y., Quirion, R. *The molecular pharmacology of CGRP and related peptide receptor subtypes.* Trends Pharmacol Sci 2000, 21: 432-8.

20. Goadsby, P.J., Duckworth, J.W. *Effect of stimulation of trigeminal ganglion on regional cerebral blood flow in cats.* Am J Physiol 1987, 253: R270-4.

21. Olesen, J.I., Mortensen, A., Edvinsson, L. *Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase.* Cephalalgia 1996, 16: 310-6.

22. Williamson, D.J., Hargreaves, R.J. *Neurogenic inflammation in the context of migraine.* Microsc Res Tech 2001, 53: 167-78.

23. Goadsby, P.J., Edvinsson, L., Ekman, R. *Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system.* Ann Neurol 1988, 23: 193-6.

24. Goadsby, P.J., Edvinsson, L., Ekman, R. *Vasoactive peptide release in the extracerebral circulation of humans during migraine headache.* Ann Neurol 1990, 28: 183-7.

25. Lassen, L.H., Haderslev, P.A., Jacobsen, V.B., Iversen, H.K., Sperling, B., Olesen, J. *CGRP may play a causative role in migraine.* Cephalalgia 2002, 22: 54-61.

26. Wisskirchen, F.M., Burt, R.P., Marshall, I. *Pharmacological characterization of CGRP receptors mediating relaxation of the rat pulmonary artery and inhibition of twitch responses of the rat vas deferens.* Br J Pharmacol 1998, 123: 1673-83.

27. Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R., MacIntyre, I. *Calcitonin gene-related peptide is a potent vasodilator.* Nature 1985, 313: 54-6.

28. Doods, H., Hallermayer, G., Wu, D., Entzeroth, M., Rudolf, K., Engel, W., Eberlein, W. *Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist.* Br J Pharmacol 2000, 129: 420-3.

29. Olesen, J., Diener, H.C., Husstedt, I.W. et al. *Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine.* New Engl J Med 2004, 350: 1104-10.

30. Moreno, M.J., Terrón, J.A., Stanimirovic, D.B., Doods, H., Hamel, E. *Characterization of calcitonin gene-related peptide (CGRP) receptors and their receptor-activity-modifying proteins (RAMPs) in human brain microvascular and astroglial cells in culture.* Neuropharmacology 2002, 42: 270-80.

31. Hay, D.L., Howitt, S.G., Conner, A.C., Doods, H., Schindler, M., Poyner, D.R. *A comparison of the actions of BIBN4096BS and $CGRP_{8-37}$ on CGRP and adrenomedullin receptors expressed on SK-N-MC, L6, Col 29 and Rat 2 cells.* Br J Pharmacol 2002, 137: 80-6.

32. Wu, D., Eberlein, W., Rudolf, K., Engel, W., Hallermayer, G., Doods, H. *Characterisation of calcitonin gene-related peptide receptors in rat atrium and vas deferens: Evidence for a (Cys(Et)(2,7))hCGRP-preferring receptor.* Eur J Pharmacol 2000, 400: 313-9.

33. Mallee, J.J., Salvatore, C.A., LeBourdelles, B., Oliver, K.R., Longmore, J., Koblan, K.S., Kane, S.A. *Receptor activity-modifying protein 1 determines the species selectivity of non-peptide CGRP receptor antagonists.* J Biol Chem 2002, 277: 14294-8.

34. Hay, D.L., Howitt, S.G., Conner, A.C., Schindler, M., Smith, D.M., Poyner, D.R. *CL/RAMP2 and CL/RAMP3 produce pharmacologically distinct adrenomedullin receptors: A comparison of effects of $adrenomedullin_{22-52}$, $CGRP_{8-37}$ and BIBN4096BS.* Br J Pharmacol 2003, 140: 477-86.

35. Grant, A.D., Tam, C.W., Lazar, Z., Shih, M.K., Brain, S.D. *The calcitonin gene-related peptide (CGRP) receptor antagonist BIBN4096BS blocks CGRP and adrenomedullin vasoactive responses in the microvasculature.* Br J Pharmacol 2004, 142: 1091-8.

36. Wu, D., Doods, H., Arndt, K., Schindler, M. *Development and potential of non-peptide antagonists for calcitonin-generelated peptide (CGRP) receptors: Evidence for CGRP receptor heterogeneity.* Biochem Soc Trans 2002, 30: 468-73.

37. Edvinsson, L., Alm, R., Shaw, D., Rutledge, R.Z., Koblan, K.S., Longmore, J., Kane, S.A. *Effect of the CGRP receptor*

antagonist BIBN4096BS in human cerebral, coronary and omental arteries and in SK-N-MC cells. Eur J Pharmacol 2002, 434: 49-53.

38. Moreno, M.J., Abounader, R., Hebert, E., Doods, H., Hamel, E. *Efficacy of the non-peptide CGRP receptor antagonist BIBN4096BS in blocking CGRP-induced dilations in human and bovine cerebral arteries: Potential implication in acute migraine treatment.* Neuropharmacology 2002, 42: 568-76.

39. Escott, K.J., Beattie, D.T., Connor, H.E., Brain, S.D. *Trigeminal ganglion stimulation increases facial skin blood flow in the rat: A major role for calcitonin gene-related peptide.* Brain Res 1995, 669: 93-9.

40. Saxena, P.R. *Cranial arteriovenous shunting, an in vivo animal model for migraine.* In: Experimental Headache Models. J. Olesen and M.A. Moskowitz (Eds), Lippincott-Raven Publishers, Philadelphia, 1995, 189-98.

41. Gardiner, S.M., Compton, A.M., Kemp, P.A., Bennett, T., Bose, C., Foulkes, R., Hughes, B. *Antagonistic effect of human α-CGRP$_{8-37}$ on the in vivo regional haemodynamic actions of human α-CGRP.* Biochem Biophys Res Commun 1990, 171: 938-43.

42. Shen, Y.-T., Pittman, T.J., Buie, P.S., Bolduc, D.L., Kane, S.A., Koblan, K.S., Gould, R.J., Lynch, J.J. Jr. *Functional role of α-calcitonin gene-related peptide in the regulation of the cardiovascular system.* J Pharmacol Exp Ther 2001, 298: 551-8.

43. Van Gelderen, E.M., Du, X.Y., Schoemaker, R.G., Saxena, P.R. *Carotid blood flow distribution, haemodynamics and inotropic responses following calcitonin gene-related peptide in the pig.* Eur J Pharmacol 1995, 284: 51-60.

44. Kapoor, K., Arulmani, U., Heiligers, J.P.C., Willems, E.W., Doods, H., Villalón, C.M., Saxena, P.R. *Effects of BIBN4096BS on cardiac output distribution and on CGRP induced carotid haemodynamic responses in the pig.* Eur J Pharmacol 2003, 475: 69-77.

45. Saxena, P.R. *Arteriovenous anastomoses and veins in migraine research.* In: Migraine, Clinical, Therapeutic, Conceptual and Research Aspects. J.N. Blau (Ed.), Chapman and Hall Medicine, London, 1987.

46. Eltorp, C.T., Olesen, J.I., Hansen, A.J. *Release of calcitonin gene-related peptide (CGRP) from guinea pig dura mater in vitro is inhibited by sumatriptan but unaffected by nitric oxide.* Cephalalgia 2000, 20: 838-44.

47. Kapoor, K., Arulmani, U., Heiligers, J.P.C., Garrelds, I.M., Willems, E.W., Doods, H., Villalón, C.M., Saxena, P.R. *Effects of the CGRP receptor antagonist BIBN4096BS on capsaicin-induced carotid haemodynamic changes in anaesthetised pigs.* Br J Pharmacol 2003, 140: 329-38.

48. Langer, S.Z. *Presynaptic regulation of catecholamine release.* Biochem Pharmacol 1974, 23: 1793-800.

49. Saetrum Opgaard, O., Hasbak, P., de Vries, R., Saxena, P.R., Edvinsson, L. *Positive inotropy mediated via CGRP receptors in isolated human myocardial trabeculae.* Eur J Pharmacol 2000, 397: 373-82.

50. Poyner, D., Marshall, I. *CGRP receptors: Beyond the CGRP1-CGRP2 subdivision?* Trends Pharmacol Sci 2001, 22: 223.

51. Lu, R., Li, Y.J., Deng, H.W. *Evidence for calcitonin gene-related peptide-mediated ischaemic preconditioning in the rat heart.* Regul Pept 1999, 82: 53-7.

52. Peng, J., Xiao, J., Ye, F., Deng, H.-W., Li, Y.-J. *Inhibition of cardiac tumor necrosis factor-α production by calcitonin gene-related peptide-mediated ischaemic preconditioning in isolated rat hearts.* Eur J Pharmacol 2000, 407: 303-8.

53. Wu, D.-M., van Zwieten, P.A., Doods, H.N. *Effects of calcitonin gene-related peptide and BIBN4096BS on myocardial ischaemia in anaesthetized rats.* Acta Pharmacol Sin 2001, 22: 588-94.

54. Iovino, M., Feifel, U., Yong, C.-L., Wolters, J.-M., Wallenstein, G. *Safety, tolerability and pharmacokinetics of BIBN 4096 BS, the first selective small molecule calcitonin gene-related peptide receptor antagonist, following single intravenous administration in healthy volunteers.* Cephalalgia 2004, 24: 645-56.

55. Choksi, T., Hay, D.L., Legon, L., Poyner, D.R., Hagner, S., Bloom, S.R., Smith, D.M. *Comparison of the expression of calcitonin receptor-like receptor (CRLR) and receptor activity modifying proteins (RAMPs) with CGRP and adrenomedullin binding in cell lines.* Br J Pharmacol 2002, 136: 784-92.

56. Doods, H. *Development of CGRP antagonists for the treatment of migraine.* Curr Opin Invest Drugs 2001, 2: 1261-8.