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Review Article

The role of calcitonin gene-related peptide (CGRP) in the pathogenesis of primary headache

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Abstract

The activity of neurons in the spinal trigeminal nucleus (STN) is thought to reflect the activity of central trigeminal nociceptive pathways that underlie facial pain and headache in man. About 40% of human trigeminal neurons contain the neuropeptide calcitonin gene-related peptide (CGRP), which is released from these primary afferents upon activation. The CGRP levels in the jugular venous blood are elevated during migraine attacks and cluster headache, and the time course of CGRP plasma levels parallels headache intensity. The functional relevance of mechanisms downstream of CGRP has been demonstrated by headache generation following CGRP infusion and the successful treatment of spontaneous migraine by a CGRP receptor antagonist. The functional relevance of CGRP in the pathophysiology of headache is further supported by animal experiments in which a CGRP receptor antagonist reduced nociceptive signaling in the STN.

Calcitonin gene-related peptide (CGRP) and its receptors

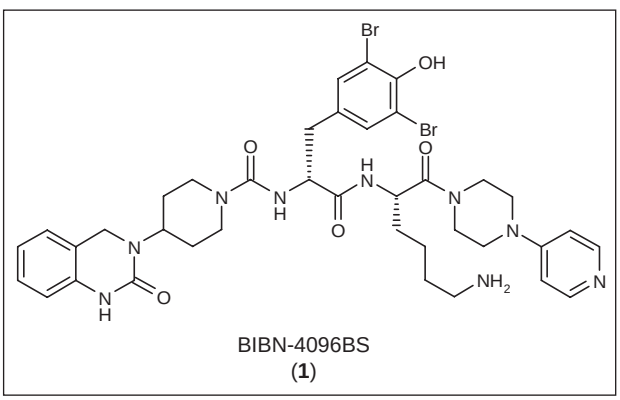
Calcitonin gene-related peptide (CGRP) is a 37-amino-acid peptide that was first described in rats as a splice variant of calcitonin (1). The protein family generated by alternative splicing includes calcitonin, amylin, adrenomedullin and α/βCGRP. Human αCGRP differs from rat αCGRP by only a single amino acid (2). Human βCGRP is encoded on a different chromosome and differs by three amino acids from αCGRP (3, 4). αCGRP is found in a subset of polymodal nociceptive afferents, including cerebrovascular nerve fibers (5). Motor neurons, some of which contain CGRP (6), lack intracranial innervation. βCGRP is found predominantly in the enteric nervous system (6). CGRP can be measured in human plasma (7). CGRP plasma levels of about 2-30 pmol/l were reported for healthy subjects, and the considerable variability in the literature was attributed to the analytical method applied (6). Primary afferents are the source for more than half of the CGRP plasma levels (8).

Functional CGRP receptors are composed of several proteins that determine their selectivity. The receptors are heteromers of the calcitonin receptor-like receptor CRLR protein (9), the receptor activity-modifying protein RAMP (10, 11) and the receptor component protein RCP (12-14). RAMP proteins are located close to the binding pocket for CGRP and determine the receptor specificity due to protein-protein interactions (15). Three RAMP proteins have been found in human cerebral arteries (16). RAMP1 has the highest affinity for CGRP, while RAMP2 and RAMP3 have higher affinities for amylin and adrenomedullin, respectively (10). The sensitivity to agonists and antagonists has significant variation among different species, which is also determined by the species-specific structure of RAMP1 (17, 18). Structure and structure-activity relationships (SAR) have been reviewed elsewhere (19). Briefly, there is a receptor-activating domain including amino acids 1-7 and two receptor-binding domains including amino acids 8-18 and 28-37. CGRP receptors are coupled to a stimulating type II G-protein, which increases adenylate cyclase activity.

A classification into two CGRP receptors has been proposed, and the arguments for separation of the two types have also been reviewed (20). This classification is only based on pharmacological evidence, in particular agonist and antagonist potencies in atrial *versus* vas deferens vessels of truncated or otherwise modified CGRP molecules (21). There are no reports on the possible molecular nature of this differentiation. In the nucleus accumbens, a receptor had higher affinity for salmon calcitonin than other characterized receptors, and a further receptor subtype was proposed (22).

On the other hand, the separation into two receptors has been criticized, since more recent data investigating different vessel types show a continuum rather than two distinct levels of inhibitory potency (23). No differences in expression of the known receptor components have been reported. Tissue factors have been proposed as an alternative hypothesis for the observed binding potencies (24). In summary, several questions regarding the molecular nature of CGRP receptors remain unanswered.

$CGRP_{8-37}$ lacks the first 7 amino acids of CGRP and is a full antagonist up to a concentration of 10 μM (25). The nonpeptide CGRP receptor antagonist BIBN-4096BS (olcegepant [**1**]) is a new pharmacological tool that can be used to investigate the properties of CGRP receptors (26) and CGRP receptor-dependent mechanisms. BIBN-4096BS and a truncated molecule are highly selective for CGRP receptors and have therefore contributed to the functional differentiation of the receptor types (26, 27). SB-273779 is a another CGRP receptor antagonist with lower affinity but irreversible binding (28). Using amino acid substitution and conjugation, a selective CGRP2 receptor agonist has been reported, although its selectivity was recently questioned (29, 30).



BIBN-4096BS
(**1**)

Evidence for the involvement of CGRP in primary headache

Multiple factors may play an essential role in primary headache and have therefore been the subject of focused reviews. The aim of this manuscript is to demonstrate the role of CGRP in primary headache. Other mediators are only mentioned for comparison to CGRP.

The involvement of CGRP in primary headache is based on the following lines of evidence:

1) Activation of trigeminal nerve fibers along meningeal and large cerebral vessels leads to headache

2) A significant proportion of primary trigeminal afferents contain CGRP

3) Activation of primary trigeminal afferents releases CGRP

4) Effects of CGRP receptor activation

5) CGRP infusion generates headache and migraine attacks can be treated successfully with a CGRP receptor antagonist

Headache is perceived as intracranial pain that cannot be precisely localized. The central nervous system (CNS) is devoid of nociceptive afferents and is therefore not painful, while the stimulation of meningeal structures leads to the perception of pain. Our knowledge of these structures is mainly derived from brain surgery on conscious patients under local anesthesia (31-34). Potentially damaging stimulation, such as faradic current, burning, distention, stretch or crush applied to meningeal arteries and large intracerebral arteries around the circle of Willis, but not to other intracranial structures, has been shown to cause headache-like pain. Application of a local anesthetic eliminates sensitivity along the vessels distal to that point.

Pain-sensitive intracranial vessels are supplied by sensory, sympathetic and parasympathetic nerve fibers. Neurons of sympathetic origin contain mainly the neurotransmitters noradrenaline and neuropeptide Y (NPY). Parasympathetic nerve fibers contain acetylcholine, vasoactive intestinal polypeptide (VIP), and less frequently, pituitary adenylate cyclase-activating polypeptide (PACAP). CGRP is not found in sympathetic or parasympathetic fibers.

In the intracranial nerves, CGRP is only found in primary sensory afferents, both in the somata within the trigeminal ganglion and in the peripheral and central projections (35, 36). Meningeal (dural and pial) blood vessels are supplied with a dense network of sensory fibers, mainly from the ophthalmic division of the trigeminal nerve. A major proportion of these nerves contain CGRP in mammalian species (37, 38), including humans (5, 39). Ultrastructural examination of CGRP-containing afferents in the rat dura has demonstrated that most fibers run along larger blood vessels and terminate in the vicinity of large and small arteries (40). In human trigeminal ganglion, CGRP immunoreactivity was found in 40% of neurons, compared to 18% of fibers with immunoreactivity for substance P (41). Similar percentages were found in nerve fibers surrounding large cerebral arteries of the rat (42).

Shortly after the discovery of CGRP, its spontaneous and stimulated release from trigeminal ganglion cell cultures was reported (43). Trigeminal neuralgia patients underwent thermocoagulation of the trigeminal ganglion. This allowed observation of activation of trigeminal neurons in humans. During this procedure, some of the patients became flushed. CGRP levels were determined during the procedure and were determined to be elevated only in the flushing patients (44, 45). CGRP is dose-dependently released from human cerebral artery preparations upon activation with capsaicin (46).

In cerebral vessels of cats, stimulation of the nasociliary nerve leads to a CGRP-dependent vasodilatation (47). More intact tissue preparations containing meningeal afferents are available in animal studies. In a hemisected skull preparation, CGRP released from meningeal afferents can be directly eluted (48), and its pharmacological modulation has been studied (49, 50). CGRP release upon stimulation has been demonstrated

from both the peripheral and central processes of these trigeminal afferents (51, 52).

Experiments with cultured trigeminal ganglia have also been used for stimulated CGRP release (53). However, functional findings for the exclusively nociceptive meningeal afferents might differ from trigeminal ganglion neuron cultures, which also contain other neurons. Infusion of a nitric oxide (NO) donor induced genuine migraine attacks in migraineurs (54) and cluster headache in the interval of an active headache period (55). Interestingly, NO stimulates CGRP release from meningeal afferents (49), and induces NO synthase (iNOS) in mononuclear cells in the meninges, which can release NO for a longer period (56).

Taken together, CGRP may therefore be regarded as a gross marker for trigeminal activation. Activated intracerebral afferents release CGRP, which can be measured in cerebrospinal fluid (CSF) or plasma, although this matter has been challenged recently (57). CGRP was elevated during migraine attacks (58), as well as cluster headache (59), in plasma samples from the jugular vein. CGRP plasma levels peaked 1 h (measured at 0.5, 1, 2 and 4 h in the cubital vein) after the beginning of spontaneous migraine attacks (60). The lower concentration and poorer detection limit for substance P may explain why increases in substance P levels were only detected after trigeminal ganglion stimulation, but never coincident with elevated CGRP plasma concentrations during migraine attacks or cluster headache. No changes in plasma levels of other neuropeptides were found in several studies, the only exception being VIP, which is released from parasympathetic fibers during cluster headache.

CGRP is mainly contained in transient receptor potential vanilloid 1 TRPV1-positive sensory neurons. Repeated activation of trigeminal afferents via TRPV1 with intranasal capsaicin application had a therapeutic effect in patients with cluster headache (61). It may be hypothesized that this effect is based on repeated activation and consequent depletion of neurotransmitters, including CGRP.

Upon stimulation of CGRP receptors, intracellular cAMP production is upregulated. The most extensively studied effect of CGRP receptor activation is vasodilatation. Potential antimigraine substances are often assessed according to their ability to counter vasodilatation, because this effect was found to be associated with antimigraine efficacy. However, it has never been shown that vasodilatation *per se* is pronociceptive. If a vasodilatation model is predictive for the antimigraine efficacy of a drug, this is probably based on the fact that vasodilatation serves as an index of trigeminal activation (62). The location and hypothetical disease-modifying effect of CGRP receptors in the vicinity of intracerebral vessels are unknown. Capsaicin injection into the forehead, but not into the chin or leg, increased blood flow in the ipsilateral internal carotid artery (63). This shows again that intracranial vessel dilatation is a consequence of trigeminal activation, but not necessarily the other way round. Furthermore, it should be mentioned that several drugs have therapeutic effects in primary headache but have little or no vasoconstricting action. Infusion of the CGRP receptor antagonist BIBN-4096BS demonstrated that unstimulated CGRP plasma levels have no effect on basal hemodynamics or blood pressure (64).

Apart from vasodilatation, CGRP has further important effects which should not be underestimated. CGRP competes with substance P for enzymatic degradation (65) and these neuropeptides influence each other (66). Due to the belief that CGRP may be a regulated inhibitor for substance P degradation, it was disregarded for a considerable period (67, 68). At central afferent terminals, CGRP is an excitatory co-transmitter modifying glutamatergic signal transmission to central neurons (69). CGRP has also been shown to modulate the release of neurotransmitters from primary afferents in spinal dorsal horn slices (70). CGRP release from afferents in the dorsal horn is known to enhance thermal and mechanical nociceptive sensitivity (71, 72). Most recently, CGRP receptor-dependent sensitization of tetrodotoxin (TTX)-resistant sodium currents was demonstrated in cultured dorsal root ganglia (73). Neurotrophic functions of CGRP at the neuromuscular junction have been reported (74). In peripheral tissues, CGRP released from perivascular afferents contributes to the development and maintenance of neurogenic inflammation (75-77). CGRP exhibited a pronociceptive modulation of transmission in a delayed-effect opioid tolerance model, although no immediate effects of a CGRP antagonist were seen with this experimental design (78).

If CGRP has a key function in headache, infusion of the peptide might be sufficient to cause headache. In early studies, only the cardiovascular effects of CGRP infusion were investigated in healthy volunteers. In a study using a prolonged infusion of effective cardiovascular doses, early or delayed headache would have been expected to be reported if this was a common side effect (79). In contrast, migraineurs developed a slight immediate headache and a delayed migraine attack upon infusion of 2 μg/min of CGRP for 20 min, a higher dose than that used in the aforementioned cardiovascular investigations (80). Thus, given the necessary susceptibility, CGRP can trigger a primary headache. In an animal model of meningeal nociception, CGRP was recently shown to increase the activity of central trigeminal neurons (69).

After successful provocation with CGRP, the second key pharmacological experiment is to demonstrate the efficacy of a CGRP antagonist. In its first clinical trial, BIBN-4096BS was shown to be superior to placebo in the treatment of acute migraine (81). Moreover, its efficacy was in the range reported for triptans. In a recent study, BIBN-4096BS was also able to prevent headache induced by 1.5 μg/kg αCGRP infusion in a control group (82).

The demonstrated efficacy of BIBN-4096BS needs to be considered in light of the failed clinical studies for drugs that were effective in certain basic research models. This sheds light on the validity of the animal model

used. Inhibition of NK_1 receptors (83), the endothelin antagonist bosentan (84) and the sumatriptan analogue CP-122288 (85) failed in the treatment of acute migraine, although these substances were effective in a neurogenic inflammation model of trigeminal nociception using plasma extravasation as an indicator of efficacy (86, 87). Additionally, using the neurogenic vasodilatation model, several substances show a mismatch between therapeutic potential for migraine and efficacy in the animal model. For example, valproate is effective in the treatment of acute migraine (88) but does not affect neurogenic vasodilatation (89). Intravenous valproate inhibited neuronal activation in the trigeminal nucleus caudalis (90).

The efficacy of BIBN-4096BS in the treatment of migraine attacks raises questions about the site of action. Extracellular recordings of trigeminal nucleus caudalis neurons with meningeal input in animals were used to investigate the relevance of a central site. In cats, microiontophoresis of BIBN-4096BS into the STN has been shown to reduce the activity of central trigeminal neurons evoked by glutamate application at the same site or by electrical stimulation of the superior sagittal sinus (69). Intravenous application of BIBN-4096BS reduced the spontaneous and heat-evoked activity of spinal trigeminal neurons with meningeal input in a dose-dependent manner (91). In summary, sensitization of trigeminal afferents at both a peripheral and a central site can contribute to increased signal transmission within the central neurons. The relative significance of both sites for the human pathophysiology of headache remains to be determined. In contrast to a central site, a peripheral sensitization by CGRP has not been demonstrated in animal experiments.

In a model of NO-induced migraine (92), the time course (onset, peak, and cessation) of headache intensity was paralleled by plasma CGRP levels (54), and absolute CGRP plasma levels were correlated with headache intensity. The significance of CGRP is also substantiated by the main effects of the antimigraine drug family of triptans. Sumatriptan has been shown to reduce CGRP release concomitant with the relief of migraine pain and other forms of primary headache (93). Triptans inhibit CGRP release by activating $5\text{-}HT_{1D/1F}$ receptors located on trigeminal afferents, as well as constricting meningeal arterial vessels by activating vascular $5\text{-}HT_{1B}$ receptors (94). The α and β subtypes of $5\text{-}HT_{1D}$ receptors are differentially expressed in smooth muscle cells and trigeminal afferents (95, 96). Newly developed selective agonists for these subtypes will help to clarify the still unresolved issue regarding the site of action.

The known relevance of CGRP for certain forms of primary headache has been illustrated above. On the other hand, it should be pointed out that CGRP does not appear to be of major importance for all kinds of primary headache. Especially for the most common type, tension-type headache, no special role for CGRP could be established (97). Although this article focuses on primary headache, it should be noted that CGRP content was also reduced, probably depleted, in the primary afferents of patients with bacterial meningitis (98). Triptans inhibited the inflammatory response in these patients (99) and a similar effect can be speculated for CGRP receptor antagonists.

Conclusions

CGRP has a major role in some forms of primary headache. CGRP plasma levels are elevated during migraine attacks and a correlation with headache intensity was found. With the demonstration of CGRP-induced headache and headache alleviation by CGRP receptor antagonism, a new therapeutic principle has been confirmed. Development of a CGRP receptor antagonist with sufficient oral bioavailability may have a major impact on the therapy of migraine and other forms of primary headache.

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