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Pain pharmacology in migraine: focus on CGRP and CGRP receptors

Abstract Over the last 100 years, the discovery of new analgesics has been a complex and difficult task. However, remarkable progress in the identification of novel molecular targets relevant for pain medicines has been reported. Here we will focus on the neuropeptide calcitonin gene-related peptide (CGRP) and its receptors (CGRP-R) because of their role in migraine mechanism and migraine therapy. Recent preclinical and clinical data on the localisation, regulation and plasma levels of CGRP and on the function of CGRP-R will be summarised. The reviewed findings highlight the major function of CGRP in migraine and the use of CGRP-R antagonists as a novel approach for the treatment of migraine attack and, perhaps, as migraine prophylactic medicines.

Key words Calcitonin gene-related peptide • Neurogenic vasodilatation • Migraine • Receptor antagonist • Meninges

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Introduction

The original proposal that neurogenic extravasation of plasma proteins in the dura mater, a phenomenon mediated by substance P (SP) released from perivascular trigeminal nerve endings and by the activation of NK1 receptors, could be responsible for the migraine attack [1], has been challenged by negative results obtained in migraine patients with NK1 receptor antagonists [2–4]. However, in the last few years both preclinical and clinical data are offering increasing support to the hypothesis that an additional component of neurogenic inflammation, calcitonin gene-related peptide (CGRP)-mediated neurogenic vasodilatation, makes a major contribution to migraine pathogenesis. Key findings in support of the role of CGRP in migraine are the observation that intravenous infusion of CGRP produces a migraine-like headache [5], and the clinical trial [6] that reported the efficacy of BIBN4096BS, a high-affinity, peptoid CGRP receptor (CGRP-R) antagonist [7], in relieving the pain and other symptoms of the migraine attack. Here, we will review some recent findings that further strengthen the role of CGRP and CGRP-R in migraine mechanism.

CGRP release and neurogenic inflammation

The working hypothesis regarding the role of CGRP in migraine is that this neuropeptide released by a large variety of stimuli from terminals of primary sensory neurons may cause effects relevant for the pathogenesis of migraine, and particularly neurogenic arterial vasodilatation. Together with this neurovascular effect there is now emerging evidence to support a role of CGRP in the activation and sensitisation of nociceptors at the peripheral and perhaps at the central level. One of the major mechanisms that promotes CGRP release is the activation of the

transient receptor potential vanilloid 1 (TRPV1) [8] channel. Capsaicin, the pungent principle contained in the plants of the genus *Capsicum*, because of its unique ability to activate TRPV1, has been for decades the instrument to understand the function of a subset of neuropeptide-containing, sensory neurons that mediate neurogenic inflammation [9]. Several chemical and physical agents have been shown to release CGRP from sensory neurons *via* the stimulation of receptors and channels expressed on the sensory nerve terminal [9]. Regulation of TRPV1 channels by a series of agents and mediators underlines the role of sensitisation in the modulation of the sensory and proinflammatory functions of nociceptors. In particular, it is believed that modulation of neuronal CGRP levels and of the receptors/channels that mediate CGRP release may be of relevance for migraine mechanism and migraine treatment.

Upregulation or downregulation of CGRP expression and release have been obtained with substances that are known to cause or to ameliorate migraine headaches, respectively. The triggering role of female sex hormones in migraine is well known. Recently, 17β-oestradiol was found to enhance neurogenic, CGRP-mediated vasodilatation, suggesting increased CGRP release from perivascular nerves, and that this may be one of the mechanisms through which 17β-oestradiol exacerbates migraine in women [10]. Topiramate, an antiepileptic drug recently introduced in migraine prophylaxis, directly repressed the stimulated CGRP release from sensory trigeminal neurons [11]. As topiramate is not able to act postsynaptically at the blood vessels, as it did not affect CGRP-induced dilation, inhibition by topiramate of neurogenic dural vasodilation may result from inhibition of CGRP release from trigeminal neurons, thus attenuating the dural vasodilatation [12]. The preventive antimigraine mechanism of action of topiramate may be at least in part due to this inhibitory effect on trigeminovascular activation [12]. It has been reported previously that nitric oxide (NO) donors release CGRP from sensory neurons. More recently, NO donors have also been reported to stimulate overexpression of the CGRP promoter [13]. Of relevance is the additional observation that the antimigraine drug sumatriptan greatly repressed NO-induced stimulation of CGRP promoter activity and secretion [13].

CGRP effects in experimental animals and man

CGRP is a potent vasodilator. However, there are emerging roles of CGRP, other than the vascular effects, that may contribute to the ability of this neuropeptide to provoke a migraine attack. CGRP is coexpressed with brain-derived neurotrophic factor (BDNF) in a large subset of adult rat trigeminal ganglion neurons *in vivo*, and it seems to regulate BDNF availability in these neurons, thus pointing to BDNF as a candidate mediator of trigeminal nociceptive plasticity [14]. Most trigeminal nociceptive neurons containing CGRP also express purinergic P_2X_3 receptors that are activated by ATP and that play a role in pain transduction. Prolonged, but not acute, exposure to CGRP changed P_2X_3 receptor function, as it enhanced the amplitude of P_2X_3 receptor currents and augmented P_2X_3 receptor expression. This mechanism might represent a model of neuronal plasticity that contributes to pain sensitisation in response to a migraine mediator [15]. Neuronal sensitisation has been proposed as a pivotal mechanism in migraine [16]. The efficacy of sumatriptan, and other triptans, in migraine treatment may reside, in addition to inhibition of neurotransmitter release, in their ability to reduce sensitisation. However, sumatriptan did not show any inhibitory effect, but rather produced a calcium-dependent discharge on sensory neurons. An index of the complexity of the system that promotes the sensitisation process is the observation that the vasodilator agent nitroprusside produced mixed effects on mechanosensitivity [17].

Peripheral CGRP producing meningeal vasodilatation could activate and sensitise nociceptors. However, in rats, administration of CGRP caused a significant increase in dural blood flow, but did not activate or sensitise meningeal nociceptors. Thus, it seems that meningeal vasodilatation is not sufficient to activate or sensitise meningeal nociceptors [18]. Additional unresolved issues relate to the selectivity of the effects of CGRP in the intracranial vessels *vs.* extracranial or other arteries. The forearm vascular response to NO and CGRP was not different in migraine patients and matched control subjects, indicating that migraine patients do not display generalised changes in vascular function [19]. BIBN4096BS almost completely diminished neurogenic facial vasodilatation in experimental animals [20]. Thus, it is possible that the vascular effects of CGRP are not limited to the intracranial meningeal vasculature, and that CGRP action in migraine may occur also at extracranial vessels.

CGRP levels in migraine and cluster headache

The seminal study reporting elevated cranial blood concentrations of CGRP during the headache phase of migraine [21] was soon followed by a series of additional findings. Thus, baseline CGRP levels in migraine patients were considerably higher than in controls, and the changes in plasma CGRP levels during migraine attacks significantly correlated with the headache intensity [22]. Increased CGRP levels were also found in interictal periods in migraine with or without aura as compared with controls [23]. One hour after rizatriptan administration, a decrease in CGRP levels was evident in the external jugu-

lar venous blood of patients, responders to rizatriptan, and this corresponded to significant pain relief and alleviation of the accompanying symptoms [24]. Baseline salivary levels of CGRP were significantly elevated between migraine attacks in migraineurs as compared to controls, and CGRP levels during a migraine headache were significantly reduced after sumatriptan treatment [25].

However, there are data that do not sustain the value of blood CGRP measurement as a marker of trigeminovascular activation in migraine. Basal plasma concentrations of CGRP were found to be higher in patients who suffer from dialysis headache, but dialysis significantly decreased CGRP concentrations in both groups [26]. This finding suggests that CGRP levels might be related more to the filtering procedure rather than to the migraine mechanism. No difference was found between CGRP levels assessed on days with and without headache in patients with cervicogenic headache [27]. Finally, a recent study shows that there was no difference between CGRP levels in external jugular or cubital fossa blood during and outside of migraine attacks. Failure to confirm previous findings of increased CGRP level in external jugular or cubital fossa venous blood suggests that CGRP cannot be used as a biomarker to validate human or animal models of migraine [28].

CGRP receptor localisation: relevance for migraine

CGRP-R family is distinctively complex, mainly because its activity is regulated by receptor activity-modifying protein (RAMP) [29]. In particular, RAMP type-1 (RAMP-1) is an obligatory subunit of the CGRP receptor. In addition to their expression and function in the vascular smooth muscle of arterial vessels, CGRP receptors are apparently expressed on cultured trigeminal ganglion neurons *in vitro* and *in vivo* and their expression is regulated by RAMP1. Thus, elevated RAMP1 might sensitise some individuals to CGRP actions in migraine [30]. Biological actions that follow CGRP-R activation are mediated by the resulting elevated intracellular concentration of the second messenger, cyclic adenosine monophosphate (cAMP). Indirect support to the role of CGRP in migraine is given by the observation that cilostazol, an inhibitor of cAMP degradation, caused a headache equal to or more severe than headache induced by glyceryl trinitrate [31].

Human isolated middle meningeal artery expresses subtype 1 CGRP-R, as evidenced from RT-PCR and *in vitro* functional studies [32]. Vasodilatation by CGRP, successfully inhibited by BIBN4096BS, is restricted to responses observed in rat meningeal arteries and not in cortical arteries [33], thus pointing to the meningeal subset of vessels as the target of the therapeutic action of BIBN4096BS. The efficacy of BIBN4096BS, which shows much greater selectivity for human CGRP-R 1 receptors compared to any other drug [34], suggests that this receptor subtype is principally involved in migraine mechanism, and that it may be the target of the beneficial effect of BIBN4096BS in migraine.

CGRP receptor antagonists

Pharmaceutical chemistry is actively working to produce CGRP-R antagonists with an optimised pharmacodynamic and pharmacokinetic profile, and specifically with high affinity and selectivity for their receptor coupled to oral bioavailability. The search encompasses diverse components. A better understanding of the interaction of the CGRP-R/RAMP element with antagonists seems a key factor. In this perspective, the importance of Trp74 in RAMP-1 for the interaction of BIBN4096BS with CGRP-R 1 and Glu74 in RAMP3 as the first amino acid in RAMP important for agonist interactions with calcitonin-family receptors have been identified [35]. The original discovery that CGRP(8-37) has antagonistic properties is still a source of key information. The structure–activity relationships exhibited by 26 analogues of CGRP (8-37) illustrated conformational requirements important for designing CGRP antagonists and underlined the importance for potency of β-turns centred at Gly33-Pro34 [36]. CGRP(8-37) had the same affinity for human and mouse CGRP-R, whereas bzl-CGRP(8-37) and bzl-bn-CGRP(8-37) displayed 6- and 80-fold higher affinities, respectively, for human CGRP-R [37]. However, the main focus for the discovery of clinically relevant medicines for the treatment of migraine attacks is on non-peptidic molecules. Incorporation of the piperidinyl-azabenzimidazolone and phenylimidazolinone structures into the benzodiazepine core produced potent CGRP receptor antagonists and the identification of a pharmacophore for the 4-substituted piperidine component of these CGRP-R antagonists [38].

A recent study has developed a pharmacokinetic/pharmacodynamic model resembling the mechanism of action of BIBN4096BS, with the aim to extract by the model-based simulations dosage formulations and pharmacodynamic properties to support the development of CGRP-R antagonists. Patients with an acute moderate to severe migraine attack lasting not more than 6 h were enrolled in this phase IIa study, and BIBN4096BS was given as a single intravenous 10-min infusion at different doses (0.25–10 mg). Outcome measures, including headache (up to 24 h), and time to rescue medication, were described as a function of the CGRP-R blocked by BIBN4096BS. Apart from indirectly confirming the efficacy of BIBN4096BS for the treatment of migraine, the study provides evidence that molecules with high-onset (k(on)) and low-offset (k(off)) values are the most promising [39].

After ergot derivatives and triptans, development of a third generation of specific anti-migraine drugs is progressing rapidly. Novel molecules and results will be

awaited with much expectation in the next few years or months. The possibility that antagonists of CGRP-R may be beneficial for the treatment of the migraine attack strengthens for the third time, after ergots and triptans, that migraine pain has a unique mechanism, which in part responds to classical analgesics, such as non-steroidal anti-inflammatory drugs, but principally responds to drugs that, apart from migraine headache, are not used for any other type of pain.

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