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Review

CGRP: from history to clinical application - A review

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Abstract

The role of calcitonin gene-related peptide (CGRP) and its receptor have played an important role in migraine for the last decades due to development of therapies that target their receptors at the trigeminal pain system, aiming at prevention or relief of acute migraine attacks. At first, CGRP receptor antagonists, called gepants have demonstrated appropriate effectiveness. In addition, they did not cause vasoconstriction, one of the drawbacks of triptans. However, their use had to be discontinued due to the risk of liver toxicity. Humanized monoclonal antibodies towards CGRP and the CGRP receptor have been developed as an alternative approach to block CGRP transmission. Still, there are some questions not fully answered as where CGRP and its receptor are located, how they influence the mechanisms of migraine attacks and if the blood brain barrier has any sort of importance. There is still much to learn about CGRP and migraine pathophysiology, especially its anatomical target sites and anti-CGRP agents. This paper presents a review of CGRP, including a brief history, focusing in CGRP mechanism, updates and future treatments.

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Introduction

Migraine is one of the oldest known diseases. The first report of migraine treatment comes from Egyptian medicine, about 4,000 years ago (Migraine - paradigm shift). The famous doctor Ibn Sina (Avicenna), born in 980, in the city of Bukhara (Uzbekistan) at his time already described local treatments for migraine, such as application of local anesthetic in the temporal region (Avicenna). The treatment of migraine has evolved over the years and, nowadays, molecular therapies have assumed an important role in this disease[1].

Actually, migraine is the third most prevalent disease in the world[2], and is also the third leading cause of disability in people under 50 years of age.[2]

Classically, migraine can be divided in different subtypes - migraine with aura and migraine without aura. However, a third type can be described according to the number of attacks per month - chronic migraine.[3]

The definition of migraine with aura (or classic migraine) is that of recurrent attacks lasting for minutes, of completely reversible neurological symptoms, such as visual, sensory or other symptoms from the central nervous system, which usually develop gradually and are usually followed by headache as well as other migraine symptoms (International Classification of Headache - third edition). It is worth mentioning that the aura usually lasts <60 minutes and that, if the duration is longer than this period, another diagnosis must be taken into account.[3]

Migraine without aura (or common migraine) is also a recurrent headache with attacks lasting from 4 hours to 72 hours, with unilateral, pulsatile, moderate to severe pain associated with nausea and/or photophobia and phonophobia, whose exacerbation of pain is caused by routine physical activity.[3]

Chronic migraine, on the other hand, can be defined as a headache that occurs on 15 days or more days per month for a period longer than 3 months, with at least 8 days per month of migraine headache - with or without aura.[3]

For the treatment of these conditions, CGRP (calcitonin gene-related peptide) and its receptor have gained an important field in the last decades, which is the focus of this article.

History

CGRP is a 37-amino acid neuropeptide and a potent endogenous vasodilator. It was first described in 1982 at the University of California by Ronald M. Evans, Susan G. Amara and his collaborators[4] and has since assumed an important role in studies addressing the trigeminovascular system. In 1985, Lars Edvinsson suggested that CGRP would be important in the regulation of cerebral blood flow and, consequently, in migraine.[5]

In 1988 with Goadsby et al.[6] the first study emerged that brought evidence that CGRP is increased in classic migraine (with aura) and common migraine (without aura) attacks to the detriment of other neuropeptides, thus suggesting CGRP as a therapeutic target in this condition. In the early 90s, triptans gained an important role in the treatment of migraine. Three years after that article, these same authors showed that sumatriptan prevented the increase in plasma CGRP while aborting headache attacks.[7]

In 1998 Linda M. McLatchie and Steven M. Foord and other collaborators promoted the characterization of the components of the CGRP receptor, which consists of CALCRL, RAMP1 and RCP - these will be covered in the subsequent topics.[8]

In 2000, a study published by a German team carried out the characterization of olcegepant (BIBN4096BS), the first blocker of the CGRP receptor.[9] Four years later, the study by Olesen et al.[10] showed that the intravenous infusion of olcegepant promoted relief of headache and improved functional capacity in patients with migraine (with or without aura). A year later, in 2005, the German industry Merck registered the patent for the use of anti-CGRP antibodies to treat migraine.

In the following years, trials involving anti-CGRP antibodies were developed, the main target of which was the use of medications for the prophylaxis of migraine attacks (galcanezumab, eptinezumab, fremanezumab, erenumab). However, it was only in 2018, 36 years after the description of the CGRP by Amara et al.[11] that the first drug, erenumab, was approved for use in the prevention of episodic migraine (<15 episodes/month).

CGRP peptide

The CGRP is a peptide that act as neurotransmitter and is produced in sensory neurons and numerous sites throughout the CNS. CGRP is produced in α and β forms. Seeing that the β form is not found in some species, the α form has been more studied. It is a 37-amino-acid peptide, produced through alternative splicing of RNA transcript from the calcitonin gene (located in chromosome 11) that results in production of distinct mRNAs encoding this peptide.[4,12] CGRP is generated as a pro-peptide, with endogenous cleavage sites and is broken

down by metalloproteinases.[13] With amidation of the carboxyl terminus it is less susceptible to degradation by proteases increasing its half-life. Afterwards, the peptides are packaged into storage vesicles transported to be released in the presynaptic terminals via calcium- dependent exocytosis.[14] CGRP release can be stimulated by capsaicin, while studies show that this constituent of some red peppers that has been useful as an experimental tool to deplete CGRP from its release site.[15]

Presynaptic receptors are located on trigeminal neurons where they regulate CGRP release. These are serotonin (5-hydroxytryptamine) 5-HT1B and 5-HT1D receptors, which play an important role in pathophysiology of migraine, since that when activated, they inhibit CGRP release.[7] Triptans are used directed to these receptors in order to treat acute migraine episodes. In 2017, the 5-HT1F receptor was identified as a target of interest in CGRP release suppression.[16,17] The specific 5-HT1F agonist lasmiditan, has efficacy as a treatment for acute migraine attacks and could serve as a treatment option for patients with a cardiovascular risk, based on its lack of vasoconstriction. Further limitations include CNS adverse event profile due to its fast penetration through the blood brain barrier.[18]

Studies suggest that after stimulation or coagulation of trigeminal ganglion, CGRP is released and can be measured in external jugular vein blood samples. In the same way, patients who had CGRP levels increased had flushed, suggesting that CGRP is spillover in blood from sites of neuronal release.[6]

A study to describe CGRP pharmacokinetics after infusion of CGRP estimated the plasma half-life between 7-26 min, depending on decay speed. Furthermore, this study showed the reduction of gastrointestinal hormones concentration.[19]

CGRP receptor and signaling

The CGRP is a member of the calcitonin/CGRP family of peptides (which includes calcitonin, a and β CGRP, amylin, adrenomedullin (AM) and adrenomedullin 2/intermedin (AM2/IMD), all of them act at class B G protein-coupled receptors (GPCRs). The calcitonine receptor-like receptor (CLR) act as main part of this protein complex.[20] In order to have specific affinity and functionality this protein is associated with a receptor activity modifying protein 1 (RAMP1), these single transmembrane protein also alter pharmacology and cell trafficking of this complex.[8]

In addition, the CLR/RAMP1 complex is coupled to a third cytoplasmatic protein named CGRP-receptor component protein (RCP) in order to improve signaling and CGRP efficacy by amplifying G protein activation.

CLR is coupled to a G protein that contains the G. subunit, which activates adenylyl cyclase and cAMP- dependent signaling pathways. Intracellular cAMP activate protein kinase A (PKA), which results in the phosphorylation of multiple targets[12]: including opening of potassium sensitive ATP channels (KATP channels), leading to vasodilation[21], extracellular signal-related kinases (ERKs) involved in protective pathways against apoptosis and transcription factors such as cAMP-responsive element-binding protein (CREB) indicating that CGRP is able to affect gene transcription.[22,23] In cerebrovascular smooth muscle, elevation of cAMP upon CGRP activation results in vasorelaxation and dilation of the blood vessel.[5] CGRP has also been shown to mediate endothelial-dependent vasodilation involving cAMP and a positive influence on the nitric oxide pathway in protection against aortic vascular hypertrophy and fibrosis in a model of hypertension.[24,25]

Endothelin converting enzyme-1 (ECE-1) is a metalloendopeptidase, being in early endosomes to degrade CGRP. After transient stimulation, CGRP co-internalizes with CLR, RAMP1, β-arrestin2 and ECE-1 to early endosomes. CGRP degradation promotes CLR/RAMP1 recycling and β-arrestin2 redistribution to the cytosol. Chronic exposure to CGRP initiates an internalization process that trafficks the receptor to lysosomes for degradation, probably as a desensitization mechanism.[26]

Interestingly, there is a receptor that responds equally well to amylin and CGRP, the amylin receptor AMY1, which consists of RAMP1 and the calcitonin receptor (CTR), another type of another type of GPCRs from the calcitonin/CGRP family (20). Although AMY1 was found in rats trigeminal ganglion, it appears to have a lesser role as is not blocked by drugs that target the CGRP receptor.[27]

Trigeminovascular system

The trigeminovascular system is involved in the regulation of the cranial vasculature and is a key element in transmission of pain. Immunohistochemical staining with anti- CGRP antibodies and in situ hybridization to localize CGRP mRNA have shown that approximately half of all neurons in the trigeminal ganglion express CGRP.[1–3] CGRP positive neurons were predominantly found in in C-type sensory pain fibers. In the other hand, CGRP receptor components were detected in larger neurons with thicker fibers which correspond to Aδ-sensory neurons, this could facilitate interaction between sensory nerves to amplify nociceptive signaling.[4] Also, CGRP receptors were found at satellite glial cells organized around neuronal cell bodies, this proximity suggests that neuron-glia communication exists, as some studies reveal. CGRP release activates inflammatory cytokines and nitric oxide liberation from glia cells and these substances enhance CGRP release

creating a positive feedback within the ganglion.[3,5–11]

5-HT_{1B} and 5-HT_{1D} receptors are expressed in the human trigeminal ganglion and they are mainly in medium-sized cells colocalized with CGRP. This relation suggests that triptans regulates CGRP release via inhibition of those receptors.[12,13]

Staining of CGRP and SNAP-25 (a presynaptic nerve terminal protein that has a role in synaptic vesicle fusion and exocytosis), showed coexpression in thin nerve fibers proximal to trigeminal ganglion cell bodies, suggesting paracrine signaling.[14]

Trigeminal peripheral targets

CGRP was found in perivascular trigeminal terminations, located at the adventitial vessel walls. After release, CGRP increases intracellular cAMP decreases intracellular Ca^{2+}, relaxing cerebral arteries with no effect on veins.[28,29] Interestingly, this peptide has no effect in endothelium. This was described in a study when after removing the endothelium the relaxation persisted.[5] In addition, in other study to examine the role of the endothelial barrier of brain vessels, luminal CGRP was applied with relaxation response. However luminal CGRP had minor effects on arteries. This suggests that the endothelium act as a barrier, confirmed with no response on vessels diameter after CGRP antagonist luminal administration.[30,31] Regardless, a study concluded that infusion of human CGRP may alter vasoconstriction in SAH. The CGRP role in the trigeminovascular vasodilatory reflex in vasoconstriction has also been described as a protective brain circulation response. After chronic trigeminal system lesion, perivascular CGRP disappeared and arteriolar response to noradrenaline vasoconstrictor was prolonged.[29]

Another key point to explain CGRP mechanism, is the presence of CGRP receptors in the smooth muscle of middle meningeal, middle cerebral, pial, and superficial temporal vessels where CGRP act to cause vasodilation.[10,32,33] Interestingly, no endothelial barrier is present in these vessels, allowing CGRP antagonists to act. It is known that activation of the trigeminovascular system by chemical stimulation of the dura also activates perivascular sensory fibers, leading to hypersensitivity to mechanical and thermal stimuli.[34,35] Likewise, experiments made after application of pain-inducing substances into the dura, induced enhanced expression of CGRP and activation of trigeminal ganglia unmyelinated fibers.[15] However, current studies seems to focus more on the neural mechanism of migraine trigger factors rather than on dural theories, since the dura mater does not seem to be the primary target to CGRP-target therapies[11,36] Nevertheless, this cannot be ruled out, due to the absence of endothelial barrier on this structure to this drugs act.

Trigeminal central targets

It is very important to identify the regions of the central nervous system that process nociceptive information from the trigeminovascular system. Immunohistochemistry studies showed the highest density of CGRP immunoreactive thin fibers were found at the Spinal Trigeminal Nucleus (STN) superficial laminae while CLR and RAMP 1 where predominantly found at the spinal trigeminal tract region, no colocalization was noted. In C1, CGRP was expressed in thicker fibers of laminae I and II, receptor components where found in the same site but in different fibers. Interestingly no cell bodies where positive, suggesting that CGRP acts to modify A-delta fibers.[37]

Other recent study investigating CGRP neuropeptide location, related to pain processing and others functions, showed immunoreactivity in most of the neurons of the cerebral cortex, hippocampus, cerebellum, thalamic nuclei, hypothalamic nuclei and brainstem nuclei.[38] In this way, In situ hybridization and immunofluorescence were performed to detect mRNA expression of RAMP1 and CLR, mRNA and protein expression were detected in the pineal gland, medial mammillary nucleus, median eminence, infundibular stem, periaqueductal gray, area postrema, pontine raphe nucleus, gracile nucleus, spinal trigeminal nucleus, and spinal cord.[39] The reduced passage into the brain of some drugs as gepants and antibodies, limits the possibility of the CNS as the main site of therapeutic target as no effective drug level is reached, so they could act outside the CNS.[40]

Table 1. Main sites of CGRP molecule and CGRP receptor

CGRP MOLECULE	CGRP RECEPTOR
• C-fibre trigeminal neuron (spinal trigeminal nucleus superficial laminae)	• A-delta fiber trigeminal neuron
• C1, C2 laminae I and II of the dorsal horn	• Trigeminal ganglion satellite glia
• Cerebral cortex, hippocampus, cerebellum, thalamic nuclei, hypothalamic nuclei.	• Spinal Trigeminal tract region
	• Smooth muscle vessels (middle meningeal, middle cerebral, pial and superficial temporal vessels)
	• Pineal gland, medial mammillary nucleus, median eminence, infundibular stem, periaqueductal gray, area postrema, pontine raphe nucleus, gracile nucleus.

CGRP related therapies

Currently, there are four drugs developed (or in the final stages of development), which work in the CGRP pathway (Table 2).

Erenumab (trade name Aimovig®) was evaluated in a study with 667 patients (placebo: 286, Erenumab 70 mg: 191, Erenumab 140 mg: 190). The use of Erenumab reduced the number of migraine attack days per month by 6.6 days (at doses of 70 mg and 140 mg) while the placebo group reduced it by 4.2 days. In the last 4 weeks of patient evaluation, 40% of the patients in the 70 mg group and 41% of the patients in the 140 mg group showed a 50% or more reduction in the number of migraine days per month, while in the placebo group only 23% showed such a reduction. The incidence of side effects was 39% in the placebo group, 44% in the 70 mg group and 47% in the 140 mg group. The most commonly reported adverse effects were pain at the injection site, upper airway infection, nausea, pharyngitis, constipation, muscle and migraine spasms.[41]

Fremanezumab (brand name Ajovy®) was evaluated in a study with 264 patients. In the study, doses of the drug (or placebo) were given every 28 days (one cycle) for 3 months (total of 3 cycles). Of these patients, 89 were randomized to receive placebo; 88 patients were selected for the 675/225 mg group (in which they received 675 mg in the first cycle and in the subsequent 2 cycles 225 mg); and 87 for the 900 mg group (in which they received 900 mg doses in the 3 cycles). During the last follow-up of the study (weeks 9 to 12), the reduction in the number of hours with headache was measured: the 675/225 mg group had an average reduction of 59.84 hours; the 900 mg group had an average reduction of 67.51 hours and the placebo group had an average reduction of 37.10 hours. The most common adverse events were pain at the injection site and itching. 40% of the patients in the placebo group, 53% in the 675/225 mg group and 48% of the patients in the 900 mg group had some type of adverse effects (42). More recently, Fremanezumab was evaluated in another study with 1,130 patients, who were divided into 3 groups and evaluated for 3 months – the first group with 376 patients, received a single dose of 675 mg at the beginning of treatment and then only placebo; the second group (379 patients) received a dose of 675 mg at the beginning of treatment and then two more doses of 225 mg (one each month); and finally, a third group (375 patients) who received only placebo. The percentage of patients who experienced a reduction of at least 50% in the average number of days with headache per month was 38% in the group that received a single dose of Fremanezumab 675 mg, 41% in the group that received the medication monthly and 18% in the placebo group. Regarding adverse effects, 70% of the patients in the group that received the 675 mg dose (in a single dose) had at least one adverse effect, 71% of the patients in the group that received the medication monthly and 64% of the patients that received only placebo had at least one side effect. Injection site pain was the most common effect reported in all groups.[43]

In relation to Eptinezumab, the company Alder® recently released the results of the phase 3 study PROMISE 2 (Prevention Of Migraine via Intravenous Eptinezumab Safety and Efficacy-2 Trial), in which 1072 patients were evaluated and randomized into 3 groups – the first group received a single dose of 300 mg of Eptinezumab every 12 weeks (for a total of two applications), the second group received 1 dose of 100 mg of the medication every 12 weeks (for a total of two applications) and the third group received only placebo. In the 300 mg group, the reduction in the number of days with headache in the month was 8.8 days at the end of the sixth month. In the 100 mg group this reduction was 8.1 days, while in the placebo group it was 6.1 days at the end of month 6. The side effects most related to Eptinezumab are upper respiratory tract infection and dizziness.[44]

Galcanezumab® was evaluated in a study with 1,113 patients – 558 patients received a placebo, 278 patients received a 120 mg monthly dose (with an initial dose of 240 mg – a total of 3 applications at the end of the 3 months), 277 patients received 240 mg monthly (3 applications at the end of 3 months). During the 3 months of treatment, the average rate of patients with ≥50% and ≥75% reduction from baseline in the number of days with headache per month were higher at both doses of galcanezumab than placebo; 27.6% (from the 120 mg group) and 27.5% (from the 240 mg group) of patients showed ≥50% improvement in the number of days with headache per month while the placebo group showed an improvement of 15.4%.[45]

Conclusion

The development of drug therapies for the treatment of headache has evolved greatly in recent decades. It is known that when there are several therapeutic modalities available for a given disease, what is true is that none of them is 100% effective. In the case of migraine it is no different. On the other hand, understanding the CGRP pathway and the development of therapies that work in this system is of fundamental importance for the treatment of migraine sufferers. The fact that the medications that act on the CGRP pathway did not promote the "cure of migraine" reinforces the theory - already well known - that the origin of pain is multifactorial and that this patient's approach must be multidisciplinary. Although we have not yet found the “cure”, we have gained an ally in the therapeutic arsenal to approach migraine sufferers.

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