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CALCITONIN GENE-RELATED PEPTIDE (CGRP):

A New Target for Migraine

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Abstract

Migraine is a neurological disorder that manifests as a debilitating headache associated with altered sensory perception. The neuropeptide calcitonin gene-related peptide (CGRP) is now firmly established as a key player in migraine. Clinical trials carried out during the past decade have proved that CGRP receptor antagonists are effective for treating migraine, and antibodies to the receptor and CGRP are currently under investigation. Despite this progress in the clinical arena, the mechanisms by which CGRP triggers migraine remain uncertain. This review discusses mechanisms whereby CGRP enhances sensitivity to sensory input at multiple levels in both the periphery and central nervous system. Future studies on epistatic and epigenetic regulators of CGRP actions are expected to shed further light on CGRP actions in migraine. In conclusion, targeting CGRP represents an approachable therapeutic strategy for migraine.

Keywords

trigeminovasculature; neuropeptide; photophobia; neuromodulation; sensitization

MIGRAINE: MUCH MORE THAN A HEADACHE

Migraine is not just another headache. It is a complex neurological disorder in which a debilitating headache is accompanied by sensory alterations (1, 2). Electrophysiological and imaging studies of evoked and reflex responses during migraine have revealed underlying changes in far-flung neural networks within the central nervous systems (CNS) (3), including the cerebral cortex, brainstem, hypothalamus, and thalamus. Also affected are the peripheral and central portions of the trigeminovascular system, which relays head pain signals to the brain. This review discusses potential contributions from the neuropeptide calcitonin gene-related peptide (CGRP) in many of these regions.

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The International Headache Society defines migraine as a headache that lasts for 4 to 72 h and has at least two of the following characteristics: unilateral localization, pulsating quality, moderate to severe pain intensity, and aggravation by movement (4). In addition, the headache must be accompanied by at least one of the following two symptoms: nausea and/or vomiting, or photophobia and phonophobia (4). Migraine is typically episodic; however, many migraineurs experience chronic daily headaches occurring at least 15 days per month, often with migrainous characteristics. Migraine headache is almost always preceded by a premonitory phase that can last hours before the headache begins (2, 4). The most commonly reported symptoms are fatigue, gastrointestinal issues, and mood changes, and these can persist for the entire migraine attack. About 20% of migraineurs also experience an aura, consisting of visual, sensory, or motor disturbances. The headache is often followed by a recovery or postdrome phase, which is characterized by fatigue and continued sensory disturbances (2). In summary, migraineurs are often incapacitated for extended periods of time---before, during, and after the headache.

One of the most striking features of migraine is its prevalence. Migraine is estimated to affect up to one in four households, with 6--8% of men and 15--25% of women suffering from it (5). Even more sobering is that the prevalence of migraine over an entire lifetime is estimated to be 43% in women and 18% in men (6). As expected from these numbers, the economic burden of migraine is very high, with annual direct health-care costs estimated at $13 billion in the United States (7). Incredibly, the World Health Organization ranks severe migraine in the highest disability group, along with quadriplegia, dementia, and active psychosis (8). Consequently, migraine has a significant impact not only on the individual but also on society as a whole.

Despite the importance of migraine, little advancement has been made in treating this condition over the past 20 years. Many migraineurs depend on oral triptans, 5-$HT_{1B/D}$ receptor agonists that were developed in the early 1990s. Indeed, these remain the gold standard in migraine-abortive therapy, even though the response rate is only about 60%, and they often have adverse effects, including potential cardiovascular complications (9). Because of these side effects, as well as cost issues, many migraineurs rely on nonsteroidal anti-inflammatory drugs, which fortunately can often provide some pain relief (10). Drugs that can act as prophylactics (e.g., propranolol, topiramate) are generally not very effective and far from ideal because of many undesirable side effects (1). Recently, botulinum toxin has also been approved as a preventative for chronic migraine (11). Nevertheless, many migraineurs do not respond to any of these medications, either prophylactic or acute, and many develop chronic daily headaches because of acute medication overuse (12). Hence, there is a pressing need to develop new therapeutics for migraine.

Migraine and the Trigeminovascular System

So what is the cause of migraine? The growing view over the past two decades has been that migraine is a neural as opposed to a vascular disorder; any vascular changes are now considered an epiphenomenon (13--15). Yet it is still premature to rule out a vascular component (16). The evidence for and against neural and vascular models is beyond the scope of this review but is nicely summarized in two opposing articles (17, 18). In brief, the

unfinished story suggests that vascular events alone are neither necessary nor sufficient to induce migraine, but they may contribute to migraine, perhaps by sustaining CGRP synthesis and release and thus modulating nociceptor signaling to the brainstem (19).

Regardless of the initial trigger of migraine, the headache is generally thought to involve the trigeminovascular system (1, 20, 21) (Figure 1). This system centers on the trigeminal nerve, whose primary afferents innervate pial and dural meningeal vessels and whose efferent projections synapse with second-order neurons in the trigeminal nucleus caudalis (TNC) of the brainstem. Neurons in the TNC project to the thalamus, where ascending input is integrated and relayed to higher cortical areas. Parasympathetic output by sphenopalatine ganglia contributes to the system via reflex communication with trigeminal ganglia (22). Activation of the trigeminovascular system most likely involves peripheral mechanisms, as described below. These include inflammatory mediators and agents that are released during neurogenic inflammation, cortical spreading depression (CSD), or both (22). According to an alternative central mechanism, defective processing in the CNS could lead to the perception of non-noxious trigeminovascular input as painful (23). Within the trigeminal nerve, the most abundant neuropeptide is CGRP, which is expressed in 35--50% of neurons in the trigeminal ganglia (24).

WHAT IS CGRP?

CGRP is a multifunctional neuropeptide. CGRP's initial claim to fame was its origin as an alternatively spliced transcript, which at the time was only the second example from a cellular gene (25). CGRP immunoreactivity was quickly identified in discrete regions of the central and peripheral nervous system that suggested activities in cardiovascular, integrative, and gastrointestinal systems (26). We now know that CGRP has diverse activities in these and other systems and that CGRP-containing nerve fibers innervate every major organ system of the body. Most relevant to migraine, CGRP is known to regulate the cardiovascular system, mediate neurogenic inflammation, and modulate nociceptive input (19, 27--31).

The mature form of CGRP is a 37--amino acid peptide with an N-terminal disulfide bond and amidated C terminus (Figure 2*a*). Both regions are required for receptor activation, as discussed below. The peptide has an amphiphilic α-helical structure between residues 8--18, based on nuclear magnetic resonance spectra (32). This structure is conserved across the six recognized members of the CGRP gene family [calcitonin, α- and β-CGRP, amylin, adrenomedullin, and adrenomedullin 2 (also known as intermedin)]. These peptides share some biological activities but generally have distinct functions and expression patterns (27, 33). The α- and β-isoforms of CGRP are expressed from two genes: *CALCA* encodes α-CGRP, which we refer to simply as CGRP and is the predominant form expressed in trigeminal ganglia (34, 35); and *CALCB*, which encodes β-CGRP and differs from α-CGRP by only 1--3 amino acids in different species. The two peptides have nearly indistinguishable activities, yet they are differentially regulated and expressed in a distinct but overlapping pattern (34--36). The CGRP family also includes some related peptides not found in rodents or humans, along with other unidentified immunoreactive peptides (27). Researchers also question whether the precursor peptide of calcitonin, procalcitonin, should

also be considered a family member because it can act as a partial agonist at the CGRP receptor (37).

The CGRP receptor is an unusual G protein--coupled receptor (Figure 2*b*). It is composed of three subunits: calcitonin-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1), and receptor component protein (RCP) (33). The seven-transmembrane CLR protein requires RAMP1 for both its trafficking to the plasma membrane and its binding to CGRP, and RCP facilitates coupling of Gαs. RAMP1 appears to be the rate-limiting subunit of the receptor (38, 39). Kinetic and biophysical evidence indicates that two RAMP1 subunits bind to a CLR dimer, which allows positive cooperativity (39, 40). The CGRP receptor generally activates a cyclic adenosine monophosphate (cAMP)-signaling pathway (although other pathways can be recruited) to modulate gene expression and regulate receptor and ion channel activity (41). Some structural requirements for CGRP binding to its receptor have been identified (42). CGRP C-terminal residues are likely to bind a pocket formed by the N-terminal extracellular domain of CLR and RAMP1, followed by binding of N-terminal CGRP residues to the juxtamembrane domain to allow receptor activation. The classical CGRP receptor antagonist is the C-terminal fragment containing residues 8--37, which binds but does not activate the receptor (33). Recently, the CLR/RAMP1 ectodomain complex was crystallized, which confirmed that the small-molecule antagonists act by blocking the peptide-binding cleft at the interface of CLR and RAMP1 (43).

CGRP can also bind receptors for two CGRP-related peptides, adrenomedullin and amylin. The adrenomedullin receptor is formed by CLR and RAMP2 or RAMP3, and the amylin receptor is formed by the calcitonin receptor and RAMP1 (33). The fact that these receptors are found in the trigeminovascular system (44) raises the question of whether they may also be activated in migraine. However, they are generally thought to be less likely to play a major role because the clinically effective small-molecule antagonists have remarkable selectivity for the complex of CLR and human RAMP1, and injection of adrenomedullin, unlike CGRP, fails to cause migraine (45). Nonetheless, researchers have speculated that the amylin receptor is a second physiological CGRP receptor (46). Thus, an interesting possibility remains that activation of amylin receptors by CGRP may contribute to migraine. Future studies should elucidate potential contributions of noncanonical CGRP receptors to migraine.

CLINICAL EVIDENCE IMPLICATING CGRP IN MIGRAINE

Over the past two decades, investigators have found that CGRP plays a key role in migraine pathogenesis (47, 48). Three lines of clinical evidence support this conclusion. The first hint came from a pioneering study in 1990 that reported elevated CGRP levels in the jugular outflow during migraine attacks (49). Since then, elevated CGRP levels have been reported in serum and saliva during both spontaneous and nitric oxide (NO)-induced migraine attacks (48, 50). Importantly, CGRP levels were reduced by triptans, coincident with pain relief. An elevation in serum CGRP levels has also been reported between attacks for both episodic (51) and chronic migraine (52). However, one well-controlled study did not observe elevation of CGRP in the jugular blood during migraine (53). Thus, although elevated

CGRP in migraine seems likely, it remains controversial whether that elevation can be reliably detected in the circulation (50).

The second indication of a link between CGRP and migraine was that intravenous injection of the peptide caused moderate to severe headaches, which often met the criteria for experimentally induced migraine (16, 54, 55). Notably, delayed, migraine-like headaches were seen only in migraineurs; nonmigraineurs experienced only an initial nonmigraine headache or fullness-of-head sensation (55, 56). This suggested that migraineurs are unusually sensitive to CGRP actions and provided the rationale for designing the CGRP-sensitized mouse model discussed below (57, 58). CGRP-induced migraines were also reversed by a triptan (16). Although two other vasodilators---NO and pituitary adenylate cyclase-activating polypeptide (PACAP)---can also induce delayed, migraine-like headaches similarly to those induced by CGRP, this is not a general property of vasodilators (59).

The third set of findings implicating CGRP in migraine relates to selective CGRP receptor antagonists being effective in the treatment of migraine, as discussed below. These drugs relieve both the pain and associated symptoms of migraine, providing clear proof of principle that CGRP is a valid target for treating migraine. We do not know the site(s) of action of these drugs (19, 47, 48, 60, 61). Because they are not very CNS-penetrant (61, 62), it is possible that peripheral inhibition of CGRP is sufficient to treat migraine. However, other evidence suggests a central site of action, and it is possible that sufficient amounts can enter the CNS (60). Possible sites of action are discussed below. Ironically, this uncertainty regarding site of action is not limited to CGRP-targeting drugs. Notably, the triptans have been in use for over 20 years, yet their site(s) of action remain controversial (9).

CGRP AS A THERAPEUTIC TARGET FOR MIGRAINE

In the late 1990s, the hints that CGRP might play a role in migraine prompted a few pharmaceutical companies to begin developing therapeutic receptor antagonists. In 2004, Boehringer Ingelheim released the results of a Phase II proof-of-concept trial that established the clinical validity of CGRP as a therapeutic target, and this opened the floodgates (63, 64).

Receptor Antagonists

To date, six CGRP receptor antagonists have been clinically tested, and others are in early stages of development (65, 66). The first was olcegepant (BIBN4096BS) (64). At a 2.5-mg intravenous dose, this drug showed an optimal response rate of 66% reduced headache pain, compared to 27% for placebo (63). It was also superior to placebo with respect to a 2-h pain-free rate as well as reduced photophobia, phonophobia, and nausea. The response rate and time to onset were comparable to the triptans, and adverse effects were minimal (63). However, further development was limited because the drug could not be taken orally.

The next major advance was the development of the oral antagonist telcagepant (MK-0974) by Merck in 2007. Pain relief from telcagepant (600 mg) at 2 h was 68% relative to 70% for rizatriptan (an oral triptan) and 46% for placebo (67). Although migraine clinical trials tend to have relatively high and variable placebo effects (68), the drug response rate was

significantly greater than placebo. In addition, side effects were comparable to placebo. Additional Phase III trials confirmed the efficacy of telcagepant, including one with over 1,000 patients in which 300 mg telcagepant was found to be superior to placebo at 2 h, with a response rate similar to zolmitriptan and similar side effects as placebo (69, 70). In a large, long-term trial, subjects were allowed to use up to 8 doses of telcagepant per month for acute treatment of migraine attacks (71). After 18 months of this regimen (mean number of 31 treatments per subject), patients treated with telcagepant experienced fewer drug-related adverse effects than those taking rizatriptan. A meta-analysis of eight clinical trials concluded that telcagepant is effective and safe, with generally similar pain-relief response rates as triptans (72).

Unfortunately, further development of telcagepant was discontinued in 2011 following the discovery that it led to elevated liver transaminases, an indicator of liver toxicity, in two patients (72a). This occurred in a Phase II trial designed to test the drug for twice-daily use over three months as a prophylactic (http://clinicaltrials.gov identifier NCT00797667). A third antagonist, MK-3207 (200 mg, oral), was also effective, resulting in pain relief in 69% of patients compared to 36% with placebo, and had excellent tolerability (73). However, as with telcagepant, development was discontinued because of concerns about liver toxicity (65). Presumably, the toxicity is a consequence of drug metabolism, so tests of new antagonists should help resolve this issue.

Other small-molecule antagonists have been tested, but plans for their further development are not clear. For example, a fourth antagonist, BI 44370 TA (400 mg, oral), was shown in a Phase II trial to be better than placebo with comparable efficacy as eletriptan and excellent tolerability (74). Whether this drug shows liver toxicity is not known. Similarly, a fifth antagonist, BMS-927711 (75--300 mg, oral), developed by Bristol-Myers Squibb, also performed well in a Phase II trial (75), but its development status is not clear. Finally, a sixth antagonist, MK-1602, has been tested in two Phase II trials, but the results have not been reported (NCT01657370, NCT01613248).

At least two other receptor antagonists have been developed but not clinically tested. Amgen has made a modified peptide antagonist with improved pharmacokinetics (76). Vertex has made a CNS-penetrant small-molecule inhibitor (77). Although the reason the pipeline has slowed down is not known, it could reflect toxicity concerns and perhaps financial reasons (i.e., none of the antagonists had an efficacy significantly greater than the triptans) (66). Nonetheless, from a scientific perspective, the clinical trials have proved that CGRP is a valid therapeutic target for migraine and justify further studies targeting CGRP.

Monoclonal Antibodies

A promising complementary strategy to CGRP receptor antagonists is the use of monoclonal antibodies (mAbs) as biological drugs directed against CGRP or its receptor. An essential therapeutic advance was the development of humanized mAbs that could avoid the host immune response and have a long half-life (65). Because of their longevity, humanized mAbs have tremendous potential as prophylactic drugs to prevent migraine.

Currently, four mAbs are under development for preventing migraine. A humanized antibody against CGRP (ALD403, Alder Biopharmaceuticals) has completed a Phase I trial for safety and tolerability (NCT01579383). Results from a second Phase I trial for efficacy as a prophylactic treatment for frequent episodic migraine (4--14 attacks per month) is expected soon (NCT01772524).

A second antibody directed against CGRP (LY2951742, Eli Lilly) has completed a Phase I safety trial, and a Phase II trial testing efficacy at preventing migraine has just been completed (NCT01625988). Researchers reported it met primary and secondary endpoints and that this CGRP antibody may be beneficial for preventing migraine (78). Interestingly, this antibody was also effective in relieving pain-related behavior in rat models of osteoarthritis (79). This activity is consistent with preclinical observations with olcegepant and the predicted role of CGRP in joint pain (80).

A third CGRP antibody (LBR-101, Labrys Biologics) has been tested in Phase I trials and was well tolerated at all doses (65). It is currently in additional Phase I (NCT01991509) and Phase II trials for frequent episodic migraine (NCT02025556) and chronic migraine (>15 attacks per month) (NCT02021773). At present, this is the only trial targeting chronic migraine.

A fourth antibody was developed using a different strategy of targeting the CGRP receptor instead of the ligand. This antibody (AMG 334, Amgen) has completed Phase I safety and tolerability trials (NCT01688739), and Phase II trials are planned (NCT01952574). It is also being tested in menopausal women to reduce hot flashes (NCT01890109).

An underlying concern about the mAb approach is whether chronic antagonism of CGRP will have pathological consequences (65, 66). This is particularly important given CGRP's widespread multiple activities, especially as a potent vasodilator. Although the jury is still out, early safety studies are encouraging and in agreement with the paucity of adverse side effects seen in the trials of small-molecule CGRP receptor antagonists in the treatment of acute migraine, especially with the lack of coronary vasospasm. This safety profile is consistent with CGRP being primarily a compensatory peptide with modulatory roles in the body. However, a recent report serves as a good reminder that CGRP has long-term protective effects in the context of prolonged hypertension (81), and CGRP antagonist antibodies might be contraindicated in hypertensive individuals. Investigators have recently reported results from some of these clinical trials at scientific meetings. These reports indicate that CGRP-blocking antibodies show promise for preventing migraine. The efficacy of antibody therapy suggests a peripheral site of CGRP action in migraine and/or the possibility that CGRP might act at circumventricular organs of the brain that lie outside the blood brain barrier. Further studies should prove interesting from both clinical and basic science perspectives.

HOW DOES CGRP CONTRIBUTE TO MIGRAINE?

This review explores the possibility that elevated CGRP increases sensory activity at multiple levels in migraine (Figure 3) (19, 48). CGRP can act in both the periphery to enhance nociceptor sensitization and the CNS to enhance sensory input, thereby heightening

pain perception. CGRP enhancement of sensory neurotransmission appears to have been highly conserved. A recent study on the modulatory role of neuropeptides in arousal behavior using a zebrafish model system revealed that conditional overexpression of CGRP increased responses to all sensory stimuli (82). Furthermore, a similar phenotype was induced by the peptide PACAP, which researchers speculate is a potential epistatic modulator of CGRP in migraine, as discussed below.

It seems likely that researchers will find that CGRP plays important roles in both the periphery and CNS. In the periphery, CGRP is released from afferent endings of sensory neurons that primarily innervate blood vessels in nearly every organ system (19, 27, 29, 83). Although often overlooked, CGRP is also expressed in the enteric nervous system, primarily as β-CGRP, where it helps regulate gastrointestinal motility and secretions (35). These actions possibly contribute to gastroparesis and other gastrointestinal problems associated with migraine, although this remains to be seen. Trigeminal CGRP and its roles in vasodilation, neurogenic inflammation, and peripheral sensitization are likely to be the most relevant peripheral actions in migraine. In the CNS, both CGRP and its receptor are localized to many discrete regions (19, 27--30). The relatively wide distribution pattern provides numerous possible CGRP targets where it could act as a neuromodulator of light aversion, central sensitization, and CSD.

Vasodilation

A prominent effect of CGRP in the periphery is the dilation of vascular beds. Indeed, it is the most potent vasodilatory peptide known and is particularly potent in intracranial arteries (83). One study indicated that it induces dilation of the middle cerebral artery and middle meningeal artery in migraineurs, coincident with induction of migraine (16), although this has not been found in other studies (13, 14). Furthermore, the dilatory effect of CGRP infusion is immediate, but the migraine-like headache is delayed (54).

In most vessels, CGRP causes endothelium- and NO-independent vasodilation through a direct action on smooth muscle cells. This involves activation of protein kinase A and adenosine triphosphate (ATP)-dependent K^+ channels. CGRP can also stimulate endothelial production of NO, thereby contributing to vasodilation in certain vessels (83). The vascular actions of CGRP are not further described here; instead, the reader is referred to an excellent comprehensive review (83).

Neurogenic Inflammation and Peripheral Sensitization

CGRP contributes to both neurogenic inflammation and peripheral sensitization of nociceptive neurons, and it does so at multiple levels. There is good evidence for peripheral sensitization in migraine, and a likely mechanism involves neurogenic inflammation (84). The role of neurogenic inflammation in peripheral sensitization has been well studied (85, 86) and covered in recent reviews (19, 31, 87). Neurogenic inflammation is a sterile, neural-driven inflammatory process of vasodilation, plasma extravasation, and release of inflammatory agents, which together can activate meningeal nociceptors (20). The role of CGRP in this process is commonly thought to be only in vasodilation, as discussed above. However, CGRP also plays an indirect role in plasma extravasation, which is primarily

caused by substance P and neurokinin A. These peptides are often coreleased with CGRP, and CGRP can further increase substance P release, leading to plasma extravasation (39).

A third and probably the most important peripheral role of CGRP is that it can trigger mast cell degranulation, an event that releases proinflammatory and inflammatory compounds (88, 89). A direct role for CGRP in degranulation is supported by the identification of CGRP receptors on dural mast cells (90). In addition, glia of the trigeminal ganglia and nerve contain CGRP receptors (24, 90), and CGRP-induced release of proinflammatory cytokines from glia leads to sensitization of sensory neurons (91--93). However, despite many animal studies, whether neurogenic inflammation plays a role in migraine remains controversial, in large part because of the lack of substance P receptor antagonist efficacy in clinical trials (94). Another caveat that must be kept in mind is that CGRP administration fails to cause nociceptor activation, and dural administration of a receptor antagonist does not prevent activation of meningeal nociceptors (62, 95--97). Thus, CGRP in the periphery may play more of a long-term modulatory role by regulating expression of genes that would reinforce nociceptive signals, such as the purine receptor $P2X_3$ and CGRP itself.

CGRP increases $P2X_3$ gene expression in nociceptive trigeminal ganglia neurons (98, 99). $P2X_3$ is gated by extracellular ATP, promotes depolarization of primary trigeminal afferents, and transmits nociceptive stimuli (100). CGRP contributes to this process by initiating a cAMP-signaling cascade that activates the $P2X_3$ gene directly in an autocrine manner and indirectly through paracrine activation of the neurotrophin brain-derived neurotrophic factor (BDNF) gene. BDNF is also involved in nociception (101) and upregulates $P2X_3$ expression in CGRP-containing trigeminal neurons (99). Like CGRP, BDNF is elevated during migraine (102). Researchers have not tested whether BDNF or $P2X_3$ receptors then feed back to increase CGRP synthesis, but it seems likely given their signaling activities and colocalization with CGRP.

CGRP induces its own synthesis in trigeminal ganglia neurons by paracrine and autocrine mechanisms. Neuronal release of CGRP induces release of tumor necrosis factor-α (TNF-α) from satellite glia (91), which feeds back onto the neurons to activate CGRP transcription (103). Direct autocrine regulation of the CGRP gene in trigeminal ganglia was demonstrated in primary cultures (39) and is supported by colocalization of CGRP and its receptor in presynaptic central terminals in situ (24, 90, 104). Investigators have also speculated that autocrine regulation of CGRP transcription occurs in the cerebellar Purkinje neurons (105). These positive feedback loops may explain why transgenic mice overexpressing the RAMP1 receptor subunit had elevated CGRP levels in cerebrospinal fluid (57). Intriguingly, although colocalization was only rarely seen in the cell bodies of ganglia (24, 90, 104), application of the known migraine trigger NO in rats led to an increase in the number of cell bodies expressing the RAMP1 receptor subunit (106). Dynamic regulation of CGRP receptor subunits by other migraine-relevant stimuli (e.g., stress and hypoxia) has been reported (107). The possibility of increased CGRP synthesis in response to migraine triggers is further discussed below.

Light Aversion

Photophobia is a subjective experience in which normal levels of light are perceived as unpleasant or painful (108). It is a common and often debilitating feature of migraine (109, 110). A universal aspect of photophobia is light aversion. Hence, preclinical studies have used light aversion as a surrogate for photophobia. A series of experiments in mice demonstrated that CGRP plays a key role in light-aversive behavior. In mice, such behavior is measured as a balance between their innate preference for dark versus their desire to explore the lit zone.

The first studies used transgenic mice sensitized to CGRP. These mice were designed based on the aforementioned clinical evidence suggesting that migraineurs are unusually sensitive to CGRP. Sensitized mice were generated by overexpressing the rate-limiting RAMP1 subunit of the CGRP receptor in the nervous system. When CGRP was administered by intracerebroventricular injection, the transgenic mice spent significantly less time in the light compared to either vehicle-treated or CGRP-treated control mice (57, 111). This treatment also decreased movement, but only in the dark zone, possibly reflecting movement-exacerbated pain that is often experienced during a migraine (111). Wild-type mice also displayed these CGRP-induced behaviors but required much brighter light and habituation (to reduce exploratory drive) (112). The wild-type phenotype demonstrates that endogenous CGRP receptors are sufficient to convey light-aversive behavior. Importantly, triptan antimigraine drugs attenuated the CGRP-induced behaviors. Notably, although CGRP was centrally administered, these experiments do not rule out a peripheral site of action (112).

Given the ability of CGRP to induce light-aversive behavior, where might it be acting? Over the past decade, several neural networks have been implicated in the enhanced light sensitivity and pain of photophobia (109, 113). These paths include trigeminal afferents in the eye and dura, second-order neurons in the TNC, third-order neurons in the posterior thalamus, modulatory neurons in the hypothalamus, and fourth-order neurons in the visual and somatosensory cortices. Of particular interest is the posterior thalamus, where Burstein and colleagues (109, 114, 115) showed convergence of signals from the dura and melanopsin-containing retinal ganglion cells. Moreover, there are CGRP receptors on neurons responsive to nociceptive trigeminovascular input in the ventroposteromedial thalamus (116), an area activated by the convergent trigeminal and light signals (114). Whether these same neurons are modulated by CGRP remains to be tested.

Researchers have suggested that photophobia involves not only pain pathways but also limbic system pathways that superimpose an emotional processing of discomfort, leading migraineurs to seek the dark (108). These pathways have recently been reviewed (19, 31). Perhaps an analogous circuit contributes to photophobia. The interaction of pain and limbic pathways is especially intriguing given the neuromodulatory role of CGRP in nociceptive processing in the amygdala described below. Thus, CGRP may play a role in photophobia at multiple levels.

Central Sensitization

CGRP is a neuromodulator that can enhance synaptic transmission mediated by glutamatergic signaling (Figure 4) (19, 30, 48). This has been studied mainly in the central terminals of spinal and trigeminal sensory neurons and in the amygdala. This neuromodulation can result in central sensitization. The phenomena of central sensitization in migraine has been extensively studied by Burstein and colleagues and covered in a recent review (87). In the dorsal horn, CGRP can enhance glutamate transmission by presynaptic mechanisms, leading to central sensitization (117, 118). In the trigeminal nucleus, CGRP release from central terminals of the trigeminal nerve can modulate second-order nociceptive neurons in the TNC (119). Expression studies revealed that the TNC CGRP receptors are present on presynaptic afferent terminals but not on postsynaptic second-order neurons (90). Thus, CGRP may modulate only presynaptic activity in the trigeminal nucleus (20, 90).

CGRP receptors are also found on postsynaptic spinothalamic neurons, where they are colocalized with (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) AMPA glutamate receptors (120). In this role, CGRP increases responsiveness of AMPA receptors and potentiates the effects of substance P on AMPA and *N*-methyl-D-aspartate (NMDA) receptors (118). This could potentially lead to central sensitization manifested as mechanical allodynia (121). Recently, a cooperative interaction between CGRP and the vesicular glutamate transporter VGLUT2 was reported to promote development of heat hyperalgesia (122). In general, the effects of CGRP are mediated by cAMP-dependent pathways (123), which, along with other changes in gene expression, underlie central sensitization (118).

There is evidence for CGRP neuromodulation elsewhere in the CNS. Most relevant to migraine are CGRP-mediated synaptic transmissions to the amygdala and nearby bed nucleus of the stria terminalis that cause fear and anxiety-like responses (124). CGRP transmission between the lateral parabrachial nucleus and the central nucleus of the amygdala (CeA) is associated with central sensitization and pain-related behavior (125). Within the CeA, sensitization involves phosphorylation of the NR1 subunit of the NMDA receptors (126). CGRP is also involved in efferent pathways from the raphe and posterior hypothalamus that modulate nociception (127, 128). Thus, CGRP is involved in nociceptive central sensitization at multiple levels within the CNS.

Cortical Spreading Depression

Another migraine phenomenon that may involve CGRP is CSD, which is associated with the aura phase of migraine (129). CSD is a self-propagating wave of neuronal and glial depolarization that slowly spreads over the cortex, followed by a prolonged suppression of neuronal activity. CSD has been shown to activate meningeal nociceptors (22, 130, 131), presumably by diffusion of substances released from the cortex, such as glutamate, K^+, H^+, and ATP. In this mechanism, CGRP would be released from peripheral terminals in the pia and help trigger neurogenic inflammation in the dura (132). This mechanism is consistent with the loss of CSD-induced neurogenic inflammation by sensory denervation of the meninges (22) and with evidence that trigeminal fibers have collaterals that innervate both the pia and dura (133).

CGRP may contribute to CSD by promoting the initial transient hyperemia response. In animal studies, transection of the trigeminal nerve reduced this hyperperfusion (134), and topical administration of a CGRP receptor antagonist attenuated the pial dilation induced by CSD (135, 136). A recent study using rat cortical brain slices revealed that endogenous CGRP was released during CSD, and, interestingly, CGRP receptor antagonists significantly reduced the magnitude of the CSD effect in vitro (137). It remains to be seen whether this effect is observed in vivo. If so, this will imply that CGRP may have a neuronal or glial role in CSD in addition to its vascular actions.

CSD also increases the levels of reactive oxygen species (ROS) in the cortex and trigeminal ganglia in rats (138, 139). Surprisingly, a long-lived lipid peroxidation marker of ROS was observed not only in the ipsilateral cortex but also in the meninges and trigeminal ganglia of rats (139). The significance of trigeminal ROS is supported by the ability of hydrogen peroxide to trigger CGRP release from cultured dorsal root ganglia neurons (139). ROS production following CSD could potentially be involved in epigenetic activation of the CGRP gene, as ROS can activate the gene in cultured trigeminal ganglia (140). Interestingly, the primary product of the CGRP gene in glia is procalcitonin, a CGRP-related peptide that has been reported to be elevated in migraine (141). Researchers are studying whether CSD can activate glial CGRP gene expression. An elevation of CGRP synthesis in the context of CSD might contribute to an increased susceptibility to migraine by creating a positive feedback loop, with CSD promoting CGRP synthesis and release and this in turn increasing the likelihood of subsequent CSD events and migraine.

FUTURE DIRECTIONS: EPISTATIC PARTNERS AND EPIGENETIC REGULATORS OF CGRP

Although this review is centered on CGRP, it alone cannot be the one ring that rules migraine. At best, only about two-thirds of migraineurs respond to CGRP receptor antagonists, which suggests that a large percentage of migraines have other causes. Like most disorders, migraine is defined by its symptoms, so a diagnosis of migraine likely represents an amalgamation of related disorders with similar outcomes but different etiologies, genetic underpinnings, and environmental influences. Moreover, CGRP is not the only agent known to trigger migraine. Finally, migraine clearly has a strong genetic component and is a complex polygenic disorder. Indeed, the number of genes suspected to be involved in migraine increases each year as additional genes are implicated by genome-wide association studies (142, 143) and genetic studies of rare familial disorders (144). Thus, there is a growing list of genes suspected to be involved in migraine, which are covered in an excellent review (145). Surprisingly, none of the genetic screens carried out to date have identified either CGRP or its receptor (146).

How might we position CGRP into the network of genes implicated in migraine? One approach would be a study of epistasis relationships and epigenetic regulators of CGRP. Epistasis is defined as the interaction between two genes and has been considered in migraine genetics (145). Examples of epistatic genes that may partner with CGRP would be genes that encode a peptide that synergizes with CGRP or an enzyme that degrades CGRP. Possible epistatic cooperation between CGRP and the peptide PACAP are especially

interesting. PACAP levels are elevated in migraine, and PACAP injection can induce migraine, similar to CGRP. PACAP also triggers CGRP release in the TNC (147), which further supports a cooperative action of these peptides. For a more complete comparison of CGRP and PACAP activities relevant to migraine, see Reference 112. Another example of epistatic interactions would be genes that enhance cAMP signals, which are known to increase CGRP expression and actions. With this goal, an ongoing clinical trial is testing CGRP injections in migraineurs that have a genetic locus (rs13208321) near a gene encoding a LIM-domain protein (FHL5) believed to act as a transcriptional coactivator of cAMP response elements (NCT01924052). It will be interesting to know if this population has altered susceptibility to CGRP.

Epigenetics is defined as the regulation of gene expression in the absence of changes in DNA sequence and has also been considered in the context of migraine (148). Examples of epigenetic genes that may regulate CGRP would be genes encoding transcription factors that bind the CGRP gene enhancer or enzymes that modify chromatin structure. The ability of epigenetic reprogramming to activate the CGRP gene in trigeminal glia is supported by cell and organ culture studies (140, 141). In this regard, it may be relevant that valproate, an approved prophylactic drug for migraine (149), can modulate chromatin structure (150). Future studies using CGRP as a platform may provide insight into how epigenetic and epistatic genetic interactions contribute to migraine.

CONCLUSION: CGRP IS AN APPROACHABLE TARGET FOR MIGRAINE

Migraine is a severe headache with associated sensory disturbances that may in part be attributable to the neuropeptide CGRP. CGRP is released during migraine, and injection of CGRP can induce a migraine-like state in migraineurs. Moreover, blocking the actions of CGRP can effectively treat migraine symptoms. A common theme among CGRP actions appears to be enhancement of signaling from sensory input. CGRP actions in both the periphery and CNS are well positioned to contribute to migraine symptoms. Although the critical sites of action remain controversial, it seems likely that both central and peripheral sites may be important in the initiation and treatment of migraine. In this regard, researchers should explore the possibility of CGRP actions at CNS circumventricular organs. In the future, it seems likely that large genetic screens will identify epistatic and epigenetic modifiers that act in concert with CGRP. Continued development of small-molecule and mAb therapeutics targeting CGRP action are likely to open the door to a much-needed new generation of migraine therapeutics.

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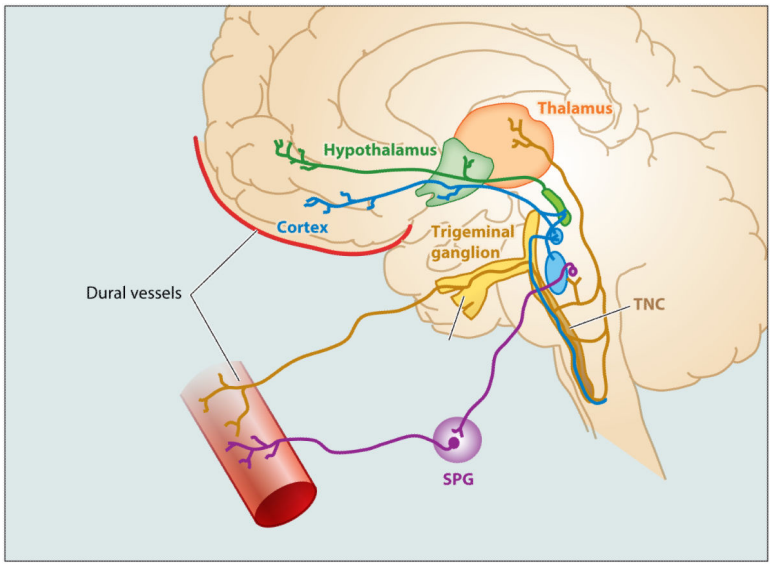

Figure 1.

Trigeminovascular system. Primary afferents of neurons in the trigeminal ganglion extend from the meningeal vasculature to central terminals in the TNC (*brown*). Second-order neurons of the TNC, in turn, project to the posterior thalamus. The SPG (*purple*) also provides reflex parasympathetic innervation to meningeal vessels. Abbreviations: SPG, sphenopalatine ganglion; TNC, trigeminal nucleus caudalis. Figure adapted from Reference 31 with permission; originally adapted from Reference 1.

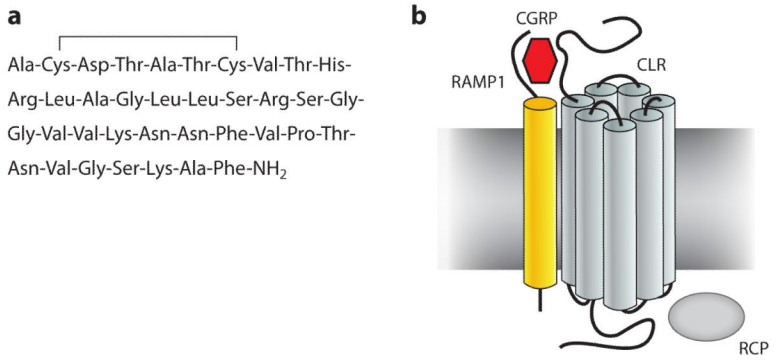

Figure 2.

CGRP and its receptor. (*a*) Human α-CGRP sequence with an amidated C terminus and N-terminal disulfide bond, indicated by the bracket. (*b*) The CGRP receptor complex, which contains three subunits: CLR, RAMP1, and RCP. Abbreviations: CGRP, calcitonin gene-related peptide; CLR, calcitonin-like receptor; RAMP1, receptor activity-modifying protein 1; RCP, receptor component protein.

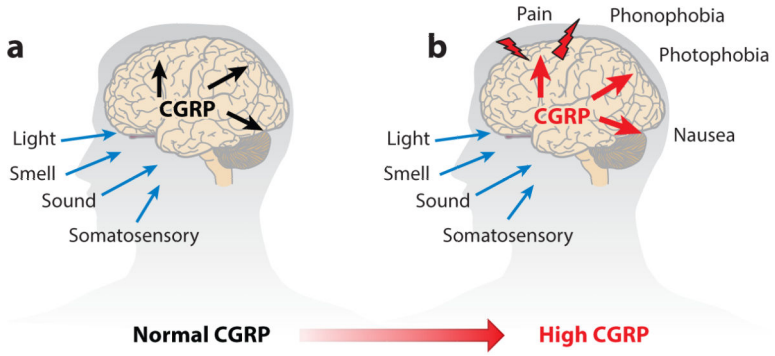

Figure 3.

CGRP-induced hypersensitivity to sensory stimuli. (*a*) Under normal conditions, CGRP levels are relatively low, neurotransmission is normal, and sensory input is properly filtered. Triggers of migraine lead to an increase in CGRP levels (*b*), causing enhanced synaptic transmission and thereby pain and altered sensory perception. Abbreviation: CGRP, calcitonin gene-related peptide. Figure modified from Reference 19 with permission; originally adapted from Reference 48.

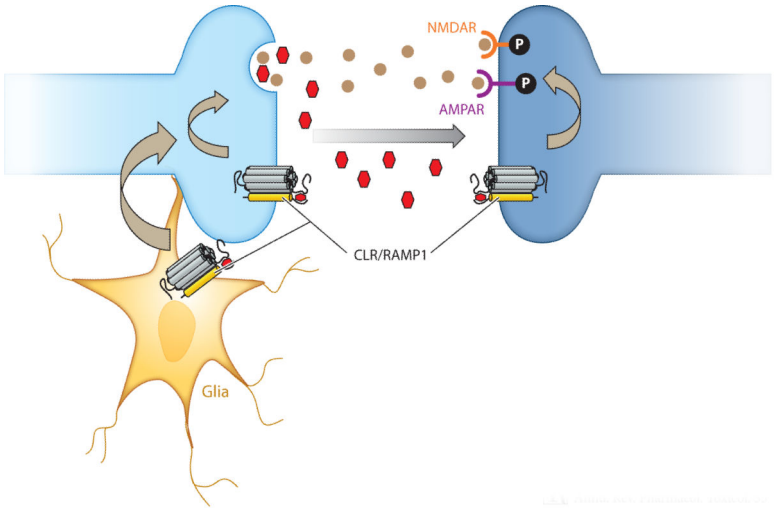

Figure 4.

CGRP facilitates synaptic transmission as a neuromodulator of glutamate release and receptor activation. CGRP (*red circles*) released from presynaptic terminals (*light blue*) leads to additional release of glutamate (*brown circles*) and CGRP. At postsynaptic terminals (*dark blue*), CGRP increases neuronal excitability by cAMP-dependent phosphorylation of AMPARs and NMDARs, leading to increased conductance and synaptic facilitation. In addition, neuronal CGRP may act in a paracrine fashion on nearby glia (*yellow*) and dural mast cells (not shown) to indirectly influence the neurons. Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; cAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene-related peptide; CLR, calcitonin-like receptor; NMDAR, *N*-methyl-D-aspartate receptor; RAMP1, receptor activity-modifying protein re modified from Reference 58 with permission.