HEADACHE CURRENTS

A narrative review of the calcitonin peptide family and associated receptors as migraine targets: Calcitonin gene-related peptide and beyond

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Abstract

Objective: To summarize the pharmacology of the calcitonin peptide family of receptors and explore their relationship to migraine and current migraine therapies.

Background: Therapeutics that dampen calcitonin gene-related peptide (CGRP) signaling are now in clinical use to prevent or treat migraine. However, CGRP belongs to a broader peptide family, including the peptides amylin and adrenomedullin. Receptors for this family are complex, displaying overlapping pharmacologic profiles. Despite the focus on CGRP and the CGRP receptor in migraine research, recent evidence implicates related peptides and receptors in migraine.

Methods: This narrative review summarizes literature encompassing the current pharmacologic understanding of the calcitonin peptide family, and the evidence that links specific members of this family to migraine and migraine-like behaviors.

Results: Recent work links amylin and adrenomedullin to migraine-like behavior in rodent models and migraine-like attacks in individuals with migraine. We collate novel information that suggests females may be more sensitive to amylin and CGRP in the context of migraine-like behaviors. We report that drugs designed to antagonize the canonical CGRP receptor also antagonize a second CGRP-responsive receptor and speculate as to whether this influences therapeutic efficacy. We also discuss the specificity of current drugs with regards to CGRP isoforms and how this may influence therapeutic profiles. Lastly, we emphasize that receptors related to, but distinct from, the canonical CGRP receptor may represent underappreciated and novel drug targets.

Conclusion: Multiple peptides within the calcitonin family have been linked to migraine. The current focus on CGRP and its canonical receptor may be obscuring pathways to further therapeutics. Drug discovery schemes that take a wider view of the receptor family may lead to the development of new anti-migraine drugs with favorable clinical profiles. We also propose that understanding these related peptides and receptors may improve our interpretation regarding the mechanism of action of current drugs.

INTRODUCTION

Migraine and the calcitonin family of peptides

Migraine is a common and debilitating neurologic condition, with attacks being characterized by symptoms such as visual aura, sensitivity to light, nausea, and moderate to severe headache. This range of symptoms implicates many biological systems in the underlying mechanisms of migraine. Migraine is the leading cause of disability-adjusted life years in females aged 15–49 years and is the second highest cause of disability-adjusted life years in the general population.[1] Drugs designed to reduce the signaling of calcitonin gene-related peptide (CGRP) are now in clinical use for the prevention and treatment of migraine attacks. These drugs work by targeting either the CGRP peptide (galcanezumab, fremanezumab, eptinezumab), or CGRP-responsive receptors (erenumab, atogepant, ubrogepant, rimegepant). These drugs have been transformative for some patients; still, ~40% of patients do not respond to these treatments, indicating that there is substantial room to improve on the current therapeutic landscape.[2]

Calcitonin gene-related peptide belongs to a peptide family comprising calcitonin, amylin, adrenomedullin (AM), and adrenomedullin 2/intermedin (henceforth referred to as AM2). Additionally, CGRP itself exists in two forms, αCGRP and βCGRP (sometimes also known as CGRP-I and CGRP-II, respectively). Although the overall amino acid sequence identity within the family is low (Figure 1), all peptides share several features such as amidated C-termini, and a loop structure in the N-terminus created by a disulfide bond between the two conserved cysteine residues.[3]

These peptides have diverse physiologic functions; although CGRP is commonly associated with migraine it also plays roles in cardioprotection and regulation of gastrointestinal functions.[4] AM and AM2 have protective roles in the cardiovasculature, while also influencing reproductive and renal function.[5–7] Amylin is a metabolic hormone that reduces gastric emptying and regulates satiety.[8,9] Pramlintide, an amylin-mimetic, is used therapeutically in the management of diabetes; novel amylin-mimetics are also being explored for managing body weight and appetite.[10] Calcitonin stimulates bone growth; salmon calcitonin is used clinically for the treatment of osteoporosis.[11] Calcitonin analogs are also being developed for the management of obesity and diabetes.[12] CGRP is the most prominent member of this family currently linked to migraine, but growing evidence suggests that other peptides within the family could also play a role. In this review, we collate pharmacologic data regarding this peptide family and summarize evidence linking these peptides to migraine.

METHODS

We performed a narrative review of literature using PubMed, Google Scholar, and Scopus. The final searches were performed on April 28, 2022. Descriptions of receptors and peptides were derived from the International Union of Basic and Clinical Pharmacology maintained "Guide to Pharmacology" database (available at https://www.guide topharmacology.org/); this was supplemented by searching PubMed with search terms including "pharmacology" combined with "CGRP", "adrenomedullin", or "amylin". Results from in vivo studies using these peptides were also collated for this review. The search terms included "CGRP", "amylin", or "adrenomedullin" in combination with "pain", "migraine", "light aversion", or "hyperalgesia". Article titles and full texts were screened to determine relevance for inclusion. In addition, reference lists of these articles were examined to identify further studies for inclusion. We have focused on primary data sources in our citations; however, in certain sections we have also cited reviews to draw these to the reader's attention.

RESULTS

Pharmacology of the calcitonin family of receptors

Receptors for the calcitonin peptide family consist of two class B G protein-coupled receptors; the calcitonin receptor (CTR), and the calcitonin receptor-like receptor (CLR), both of which can interact


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h-Calcitonin   C G N L S T C M L G T Y T Q D F N K F – – – – – – – H T F P Q T A I G V G A P
h-Amylin     K C – N T A T C A T Q R L A N F L V H S S N N F G – – A I L S S T N V G S N T Y
h-αCGRP      A C – D T A T C V T H R L A G L L S R S G G V V K – – N N F V P T N V G S K A F
h-βCGRP      A C – N T A T C V T H R L A G L L S R S G G M V K – – S N F V P T N V G S K A F
h-AM         G C – R F G T C T V Q K L A H Q I Y Q F T D K D K – D N V A P R S K I S P Q G Y
h-AM2        G C – V L G T C Q V Q N L S H R L W Q L M G P A G R Q D S A P V D P S S P H S Y
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FIGURE 1 Amino acid sequence alignment of human peptides. Alignment performed using Clustal. The black background with white text indicates an exact match across peptides at a given position, dark gray with white text indicates >80% similarity across a position, and light gray with black text indicates 60%–80% similarity across a position; scoring was performed in Geneious using a BloSUM matrix of 80 and a threshold of 2. The adrenomedullin (AM) and AM2 sequences shown here omit the N-terminal 14 and 13 amino acids of each peptide, respectively. These N-terminal amino acids do not appear to be required for peptide function[3] and are thus omitted from the figure; however, they were included in the original alignment.

with accessory proteins known as receptor activity-modifying proteins (RAMPs).[3,13] These protein–protein interactions give rise to receptors that each have a distinct recognition profile for different combinations of calcitonin-family peptides. Thus, the interaction between CLR and RAMP1 creates the canonical CGRP receptor, which has high affinity for αCGRP and βCGRP.[3,14] This is the receptor that drug discovery programs leading to the development of receptor-targeting drugs, such as erenumab and the "gepants", have been targeted towards. Complexes comprising CLR and RAMP2 or RAMP3 create the AM$_1$ or AM$_2$ receptors, respectively; these receptors have high affinity for AM and AM2.[3,15] The CTR can also complex with RAMPs; these interactions give rise to the AMY$_1$ (CTR:RAMP1), AMY$_2$ (CTR:RAMP2), and AMY$_3$ (CTR:RAMP3) receptors.[13] These receptors all display a high affinity for amylin; however, the AMY$_1$ receptor also displays high affinity for CGRP.[3,16–18] CLR is unable to translocate to the cell surface or act as a peptide-ligand receptor on its own, this contrasts with CTR that can traffic to the cell surface and act as a receptor for calcitonin in the absence of RAMPs.[3] The ability of CTR to translocate to the cell surface alone means that any individual cell that expresses CTR and RAMPs could theoretically express a mixed population of CTR and AMY receptors on the cell surface. It is unclear whether this has physiologic implications in vivo; however, it does complicate the interpretation of in vitro work where it is likely that cells transfected with CTR and a RAMP express a combination of CTR and AMY receptors at the cell surface.[3] This has previously lead to the over-estimation of the potency of human calcitonin at AMY receptors.[3,16]

The nomenclature suggests that each of these receptors has clear-cut pharmacology, but there is actually substantial overlap in the ligand preference of each receptor.[3] This is illustrated in Figure 2 by showing that several human, rat, and mouse receptors are activated by CGRP.[3,19–21] This overlapping pharmacology means that each receptor could potentially function as a receptor for multiple distinct endogenous peptide ligands in vivo. This complex pharmacology also makes it difficult to directly link a given receptor complex to defined (patho)physiologic scenarios because administration of a single peptide can theoretically activate multiple receptors from this family. This problem is exacerbated in rodents, where each peptide tends to activate more individual receptors, or activate them more potently than human receptors (Figure 2). This means that peptide administration to humans or animals could activate a wide range of receptors. For example, in mice αCGRP has nanomolar potency at four of the seven receptors from this family.[19] This causes serious difficulties for the interpretation of in vivo results. Efforts are being made to probe the relative importance of individual receptors in different physiologic pathways using a combination of pharmacologic and genetic approaches.[22–25] More work like this is needed.

Migraine-provocation studies in which humans have been administered calcitonin-family peptides have offered great insight into the molecular mechanisms of migraine; however, they have also raised several questions. For instance, peptides such as CGRP and AM are rapidly metabolized by proteases and peptidases in blood,[5,26,27] so their long-lasting effects are difficult to explain. Additionally, intravenous administration delivers peptides to the peripheral blood supply. The blood–brain barrier excludes molecules of similar size and charge to CGRP, amylin, and AM,[28–30] and the penetrance from peripheral blood to central sites is very low for these peptides.[28,31–33] This means that it is unlikely that these peptides are reaching intracranial receptors and instead suggests that migraine-like attacks induced by these peptides given peripherally could be driven by activation of peripheral receptors. The trigeminovascular system is a conduit for orofacial pain and is thus intimately linked to migraine pathophysiology.[35,36] Importantly, the trigeminovascular system also receives blood from the peripheral supply,[28,30] leading to the idea that migraine-inducing agents, such as CGRP, act in the trigeminovascular system to cause migraine attacks.[28,34,37,38] The importance of these peripheral receptors is supported by the fact that antibody therapeutics have limited central penetrance,[39–41] but still have therapeutic efficacy in patients. More work investigating the relative importance of peripheral and central sites in migraine pathophysiology is needed.

αCGRP and βCGRP in migraine

There is a wealth of literature linking CGRP to migraine pathophysiology in humans and rodents, the findings of which have recently been reviewed.[34,35,37,42–44] These reviews effectively cover a lot of background, and it is not our intention to replicate them. This section will instead introduce methods and themes used in migraine research and highlight new insights in the field.

We will first consider preclinical rodent models. Rodents are often used to probe mechanisms of disease in a way that is not feasible in humans. Various rodent models have therefore been developed to interrogate the different facets of the human migraine experience. Facial signs of discomfort (that can be measured through grimace and squint scales) capture the spontaneous symptoms of pain and discomfort arising during migraine attacks such as headache and nausea; CGRP administration elicits both grimace and squint responses.[45–48] Mechanical and thermal assays that measure hyperalgesia and allodynia recapitulate the hypersensitivity commonly associated with migraine attacks; exogenous CGRP administration reduces pain thresholds in both mechanical and thermal assays.[49–52] Light aversive behaviors, in which rodents spend longer times resting in the dark and avoid illuminated spaces, recapitulate the hypersensitivity to light that is commonly associated with a migraine attack; CGRP administration to rodents elicits light aversive behaviors.[48,53,54] Collectively, these animal studies provide weight to the importance of CGRP in migraine pathophysiology.

Recent rodent work has also indicated that there may be a sex-specific element to CGRP action. For example, dural application of CGRP causes periorbital allodynia in female but not male rodents.[47,55] Intraperitoneal injections of CGRP cause light aversion and squint responses in both male and female mice,[45,48,56] though
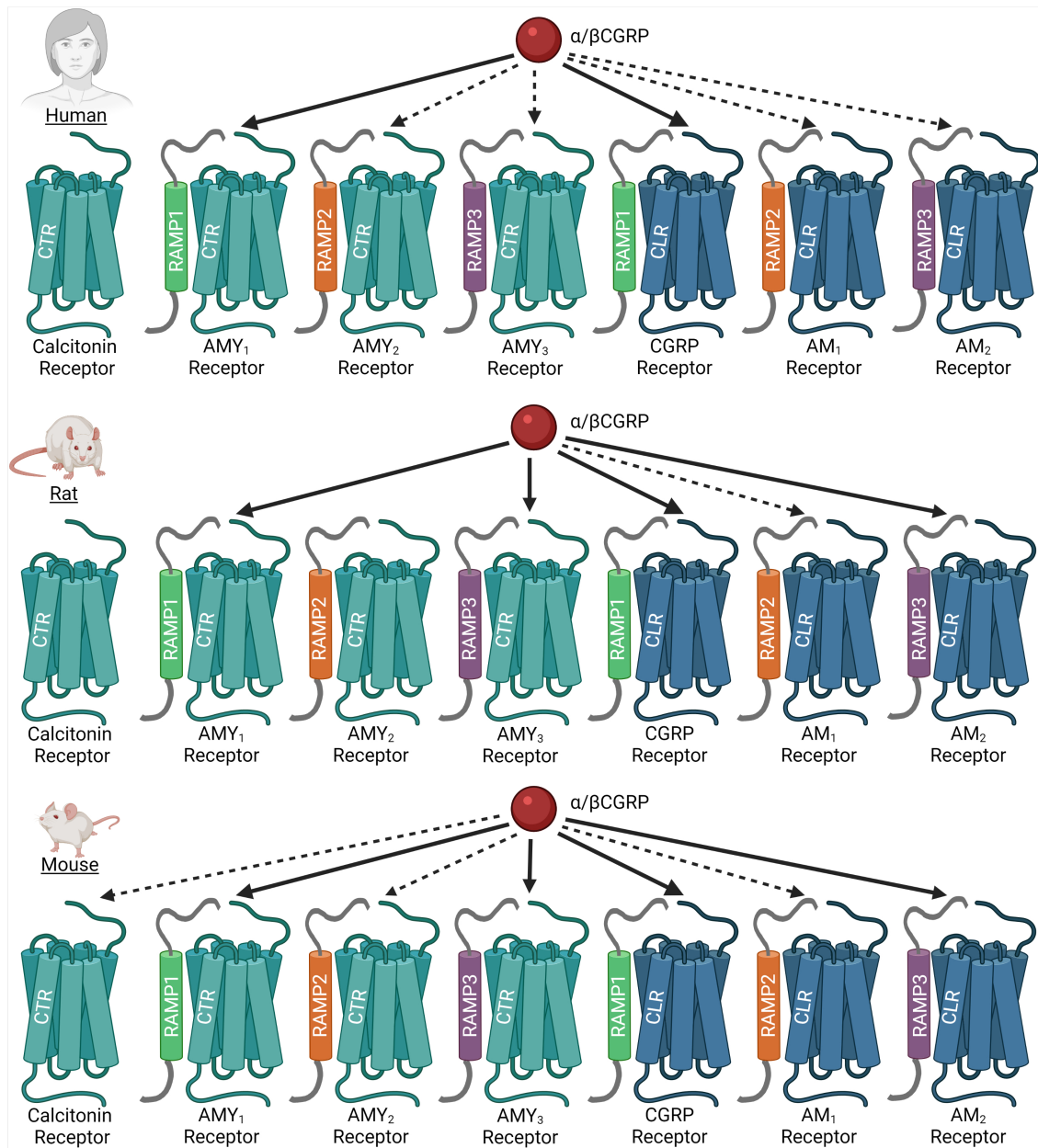
FIGURE 2 Pharmacology of calcitonin gene-related peptide (CGRP) at human, rat, and mouse calcitonin receptor (CTR)/calcitonin receptor-like receptor (CLR)-based receptors. Undashed arrows indicate receptor at which CGRP is either the most potent peptide, or where CGRP is <10-fold weaker than the most potent peptide. Dashed arrows indicate receptors at which CGRP is between ~10-fold and 100-fold weaker than the most potent peptide. Visualizations are approximate, please see references for a more in-depth discussion of results.[3,19,21] AM, adrenomedullin; AMY, amylin; RAMP, receptor activity-modifying protein. Image created using BioRender.com. [Color figure can be viewed at wileyonlinelibrary.com]

recent evidence suggests that these squint responses occur at lower doses in females.[45] These findings may be especially relevant given that migraine has a higher prevalence in women.[1] CGRP also appears to regulate other pain responses more potently in females than males, e.g., interleukin-6 primed mechanical hyperalgesia.[57] Thus, CGRP may also have a broader role in pain processing in a sex-dependent manner. This is an avenue worth investigating and future studies may extend the therapeutic repertoire of CGRP receptor antagonists beyond migraine to other chronic pain conditions.[57]

The clinical evidence implicating CGRP in the human migraine response has been extensively reviewed and thus will not be developed in detail in this review.[37,42,58–60] To summarize key points from this work: CGRP and its receptors are expressed in the trigemino-vascular system,[35,43,61–65] CGRP levels are increased in circulating bodily fluids during migraine attacks,[66,67] though this finding has not always been reproducible;[68] intravenous infusion of CGRP in individuals with migraine provokes migraine-like attacks;[69] and blockade of CGRP activity can reduce the incidence of migraine attacks in

individuals with migraine.[2,70–73] As a consequence of such observations and much other research, we are now in a position to have multiple therapeutics available that target either the CGRP peptide or receptor to reduce CGRP-mediated signaling. The efficacy of these drugs has further strengthened the evidence-base for CGRP being a key contributor to migraine symptoms. Although effective in many patients, they are not effective in all,[71–77] and some patients experience side-effects, such as constipation.[76,77]

Genome-wide association studies (GWAS) are starting to provide some insights. In one study, a novel migraine-associated risk locus included the genes for both αCGRP (*CALCA*) and βCGRP (*CALCB*), but the resolution of this study was not sufficient to distinguish between the two genes.[78] A second study, which was more targeted than the previous study, identified a strong link between the *CALCB* locus and migraine.[79] Additionally, a retrospective literature analysis identified a link between the *CALCA* locus and migraine though the clinical significance of this was unclear;[80] this study also identified a variant in the RAMP1 locus that is associated with the progression from episodic migraine to medication overuse headache and noted a potential link between the methylation state of the RAMP1 promoter region and migraine.[80,81] Thus, it appears that there may be subtle genetic susceptibilities in the CGRP-CGRP receptor pathway that could predispose individuals to migraine or affect the behavior of anti-CGRP therapeutics.

Calcitonin gene-related peptide is most commonly associated with the canonical CGRP receptor (CLR:RAMP1) but CGRP can also bind to and activate the AMY_1 receptor (CTR:RAMP1) with high affinity and potency (Figure 2).[3,17,18] The canonical CGRP receptor is often presumed to be the predominant CGRP-responsive receptor; however, CTR messenger RNA (mRNA) and protein expression is noted in the trigeminal ganglia in addition to CLR and RAMP1,[20,62,65,82] and CTR is found in other migraine-relevant locations of the brain such as the locus coeruleus and nucleus raphe magnus.[83,84] Thus, both CGRP-responsive receptors could play a role in migraine. It is prudent to add that some studies note low expression of CTR mRNA in sites such as the trigeminal ganglia,[82] but are able to detect CTR protein in the same tissue through immunohistochemical techniques.[20,82] This apparent contradiction could be explained by the fact that low levels of mRNA can still lead to protein production.[85,86] It could also be the result of mRNA being synthesized and translated in neuronal cell bodies, with the protein then being trafficked to a remote fiber, leading to protein expression at sites distant to the original mRNA synthesis.[85,86]

Adding weight to the idea that both CGRP-responsive receptors could be important to migraine is the mounting evidence showing that drugs designed to target the canonical CGRP receptor also have antagonist activity at the AMY_1 receptor.[20,87–90] The effects of these drugs could therefore be due in part to their activity at AMY_1 receptors. The molecular mechanism of this dual antagonism can be interpreted through crystal structures that have captured complexes of gepants and erenumab bound to the extracellular domains of CLR:RAMP1 [PDB 3N7R and PDB 6UMG, respectively].[91,92] Both drug types bind to the extracellular domain of the CGRP receptor,

making contacts with both CLR and RAMP1. It is thought that this occludes the CGRP binding pocket to prevent the ligand–receptor interaction.[91,92] Given that RAMP1 is shared between the CGRP and AMY_1 receptors, and that the extracellular domains of CLR and CTR have a similarity score of >70% (Blocks Substitution Matrix [BLoSUM] 75 matrix with threshold 0), it is perhaps not surprising that these drugs display some effect at the AMY_1 receptor.[87–90] Future studies investigating whether CGRP induces migraine-like symptoms via individual receptors or through a combination will be important for understanding and more effectively treating migraine.

Finally, it is worth noting that work in this space has generally focused on αCGRP. It is not known whether αCGRP and βCGRP have differential effects in migraine. Given that αCGRP and βCGRP are both expressed in the trigeminal ganglia,[93–95] are both identified in GWAS studies as linked to migraine,[78–80] and exhibit overlap in binding sites across the nervous system,[96,97] it is likely that βCGRP plays some role in migraine. Interestingly, βCGRP is often reported to be a little more potent than αCGRP in vitro,[3,98–100] has a higher affinity than αCGRP in the rat central amygdala,[97] and has a higher density of binding sites than αCGRP in the ventromedial hypothalamus,[96] therefore future studies should specifically investigate this peptide and its link to migraine.

Amylin and migraine

Amylin is a 37 amino acid hormone best known for its roles in glucoregulation and meal-ending satiation.[8,101] Amylin is expressed in islet β-cells in the pancreas and is released alongside insulin in response to glucose.[8,101] However, amylin expression has also been reported in a few other locations. Notably, its apparent expression in the dorsal root ganglia suggests a role for amylin in nociception.[102] A few studies have also reported amylin-like immunoreactivity in neurons of the trigeminal ganglia.[56,82,103] However, when CGRP and amylin immunoreactivity in normal trigeminal ganglia are directly compared, there are two important observations. First, the amylin-like immunoreactivity is much weaker, as compared to CGRP-like immunoreactivity,[56,82] and second, the amylin-like immunoreactivity that is present strongly overlaps with CGRP;[56,82] both points are made clear when comparing amylin and CGRP expression in a single section of tissue.[56] An explanation for this is that the "amylin" staining reported in the trigeminal ganglia most likely represents "off-target" detection of CGRP, which is expressed at high concentrations in densely packed vesicles in these neurons,[104] as opposed to actual amylin expression. This could occur because many "amylin-targeting" antibodies that have been used to detect amylin in the trigeminal ganglia also recognize CGRP,[105] which is perhaps not surprising given the high sequence similarities between the two (Figure 1). Considering this, further work is needed to scrutinize the potential expression of amylin outside of the pancreas, where mRNA for amylin is compared to amylin protein expression, and expression of amylin is directly compared to CGRP with well-validated antibodies.

Regardless of the ambiguity around its endogenous site(s) of expression, amylin has been functionally linked to pro-nociceptive and migraine-like behaviors in rodents. It is worth noting that amylin is a relatively potent agonist of several receptors in mice, including the mouse AM_2 receptor, as well as AMY receptors.[19] Therefore, any functional responses noted in mice could potentially be mediated by multiple receptors. Mice lacking amylin display reduced nociceptive behavior in response to intraplantar formalin injection.[102] In female mice, intraperitoneal administration of amylin decreases mechanical pain thresholds, reduces time spent in the light, and induces squint responses.[45,56] Interestingly, in male mice intraperitoneal amylin administration did not cause squint responses or light-aversive behaviors, but it did cause a reduction in plantar mechanical pain thresholds.[56] A recent study reported that subcutaneous amylin did not induce periorbital allodynia in male mice.[52] Given that migraine skews heavily female in human populations,[1] and that female mice appear to be more sensitive to amylin,[45,56] negative results obtained from male-only studies should be interpreted with caution. This also emphasizes the importance of including female rodents in preclinical studies, especially when studying a phenomenon such as migraine, which is more common in women. In contrast to the above nociceptive actions of amylin, other studies report anti-nociceptive effects of amylin; intraperitoneal administration of amylin reduces acetic acid-induced visceral pain, while intracerebroventricular injection reduces thermal nociception.[106,107] Thus, the effects of amylin may be dependent on sex, route of administration or nociceptive pathways measured. Another factor to consider is pharmacokinetics. When administering amylin via peripheral injection, it reaches a maximum plasma concentration within ~10 min and is rapidly cleared.[108] Interestingly, amylin causes a squint response at earlier time-points than CGRP.[45] Hence, in an acute study setting, selecting the correct time points is crucial. Additionally, amylin appears to require higher doses to elicit the same pain responses as CGRP,[45,56] and this should be factored into study design.

Despite mixed results in rodents, a recent human study reported that the amylin mimetic, pramlintide, induced migraine-like attacks in individuals with migraine. Using a double-blind crossover approach, individuals with migraine were administered CGRP (30 µg over 20 min) or pramlintide (120 µg over 20 min) and headache scores monitored. Pramlintide induced headache and migraine-like attacks in 88% and 41% of patients, respectively; this was not significantly different to CGRP, which induced headache and migraine-like attacks in 97% and 56% of patients, respectively.[56] Despite the rate of headache and migraine-like attacks being similar between peptides, CGRP-induced symptoms were significantly more painful than pramlintide-induced symptoms.[56] In all cases, the migraine-like attacks mimicked the patients' usual migraine symptoms.[56] Interestingly, there was a subset of patients who responded only to CGRP, but also a subset of patients who responded only to pramlintide[56] suggesting that some individuals with migraine could be hypersensitive to amylin in addition to, or instead of, CGRP. Given that pramlintide has only weak activity at the human CGRP receptor,[56,109]

it suggests that activation of AMY receptors is sufficient for induction of migraine-like attacks in some patients. When combined with other data showing that plasma amylin levels appear to be elevated in individuals with chronic migraine during the interictal phase of migraine,[110] it is possible that endogenous amylin plays a role in migraine. United States Food and Drug Administration (FDA) documents for pramlintide report that headache is a relatively common side-effect of pramlintide use;[111] however, the studies leading to this listing were not designed to measure headache or migraine intensity. Further studies with larger sample sizes, which investigate pramlintide as a migraine-inducing agent, and studies that investigate the relative contribution of each AMY receptor in the migraine response will be valuable. It is possible that AMY_2 and AMY_3 receptors play a role in migraine, as both RAMP2 and RAMP3 are expressed in the trigeminal ganglia;[34,82] however, at this time the role of each receptor remains an open question. It is very challenging to separate the roles of the different AMY receptors in human studies because we currently lack pharmacologic probes specific enough to distinguish individual AMY receptors. This means that we will likely need to rely, for now, on genetic manipulation of specific receptor components in rodents to link defined receptors to specific functional effects.

Adrenomedullin and migraine

Adrenomedullin is expressed at high concentrations by the vascular endothelium.[5,82,112,113] AM is a powerful vasodilator, playing important roles in vascular maintenance and regulation of the blood–brain barrier.[5,112–114] At human receptors, AM is most potent at AM_1 and AM_2 receptors; however, it also has activity at the CGRP receptor, being between 10-fold and 100-fold less potent than αCGRP itself.[3,14,99] Although not commonly associated with migraine, AM expression is noted in neurons of the dorsal root ganglia hinting at a role in pain processing pathways.[115] Within the trigeminal ganglia, AM expression has been reported in glial and Schwann cells.[82]

Consistent with other calcitonin-family peptides, AM has relatively potent activity at more receptors in mice than in humans. For example, the mouse AMY_3 receptor stands out as potentially mediating AM effects in mice.[19] Rodent studies investigating AM in nociception have been dependent on the pain pathway measured. AM knockout mice have increased tail-flick latency in response to heat; however, they also have decreased paw withdrawal latency in response to heat.[116] Intrathecal administration of AM induces mechanical hyperalgesia and inflammatory pain in the paw.[117,118] There has been no investigation into AM-induced non-nociceptive migraine-like responses such as light aversion. Administration of AM to male mice did not induce periorbital allodynia[52]; the equivalent experiments have not been performed using female mice.

The ability of AM to induce migraine-like attacks in humans has been explored with mixed results. The first investigation was a double-blinded placebo-controlled two-way crossover study that reported results from 12 participants; the AM dosage used in this study was 1.6 µg/kg over 20 min.[119] In this initial study 33.3% (four of 12) of patients

reported migraine-like attacks after AM administration; this was identical to the number of patients who reported migraine-like attacks after placebo administration.[119] More patients reported immediate and delayed headaches after AM (50% [six of 12] and 58.3% [seven of 12] of patients, respectively) than placebo (8.3% [one of 12] and 33.3% [four of 12] of patients, respectively), although this difference did not reach statistical significance.[119] It is worth noting that the nocebo migraine response rate in this study (33.3%) is higher than the average nocebo response rate in migraine provocation studies (95% confidence interval of 2.5%–15.5%)[120] and it is possible that this high nocebo rate may have confounded interpretation of results. Recently, a second double-blinded placebo-controlled two-way crossover study reported results from 20 patients; this study dosed AM at 2.4 μg/kg over 20 min.[121] In this study, 55% (11/20) of patients reported migraine-like attacks after AM infusion, which was significantly higher than the 15% (three of 20) of patients who reported migraine-like attacks after placebo.[121] Unlike the previous study, headache analysis was not separated into immediate and delayed phase, but similar to the previous study more patients reported headache after AM infusion than after placebo infusion (80% [16/20] and 55% [11/20] of patients, respectively).[121] When considering migraine-like attacks in the second study, the time to onset and distribution of pain around the head was similar between AM-induced and CGRP-induced migraine-like attacks.[121] Additionally, AM was associated with headache induction in other human studies designed to explore the vascular effects of AM.[122,123] Thus, it appears that at high concentrations AM can induce headache and migraine-like attacks, but whether this could occur at physiologically relevant concentrations is unclear. It is also unknown which AM-responsive receptor/s are responsible for this migraine-like response, as the estimated blood concentrations reached in these studies would likely be able to activate the CGRP receptor at least partially, as well as the AM_1 and AM_2 receptors.[121] To differentiate the involvement of specific receptors in humans, a gepant could be used to determine whether this was able to block AM-induced migraine-like headache. This experiment would be useful because at human receptors AM strongly favors CLR-based receptors, and gepants have very low affinity for AM receptors.[124–126] Thus, if blocked by a gepant, it would be reasonable to conclude that the canonical CGRP receptor was mediating the response to AM.

Migraine therapeutics in the context of the wider calcitonin family of peptides and receptors

Despite the undoubted success associated with developing drugs that modulate CGRP signaling, there are several questions that arise when considering targets beyond the canonical αCGRP-CLR:RAMP1 axis. For instance, the three CGRP-targeting drugs (eptinezumab, galcanezumab, fremanezumab) appear to bind αCGRP and βCGRP non-specifically.[127–129] The clinical relevance of this is unclear. αCGRP and βCGRP display different relative expression patterns in the body, with αCGRP being more highly expressed in the central nervous system and βCGRP being more highly expressed in the enteric nervous system; however, there is overlap in expression with βCGRP also being found in migraine-related tissues such as the trigeminal ganglia.[93,94,130] It is not known whether endogenous βCGRP plays a role in migraine, but given that the gene encoding βCGRP has been identified as potentially linked to migraine in GWAS work it is plausible.[79] It could be that the ability of these antibodies to recognize both CGRP isoforms is important for their clinical efficacy, alternatively, the inability of these antibodies to differentiate the two isoforms could be responsible for side-effects such as the constipation reported in ~20% of patients taking anti-CGRP antibodies.[77]

There are also important unanswered questions regarding the receptors that mediate the effects of CGRP in vivo. Although receptor-targeting drugs are often considered specific for the canonical CGRP receptor, there is clear ability of many of these drugs to also antagonize the AMY_1 receptor.[87–90] The "gepants" display an ~30–100-fold preference for the CGRP receptor over the AMY_1 receptor through the canonical cAMP signaling pathway;[87–89] this preference can be as low as 10-fold in other pathways.[88] This effect is not limited to small molecules as erenumab has been shown to antagonize both CGRP at the CGRP receptor and amylin at the AMY_1 receptor,[90] although the key experiment of erenumab antagonizing CGRP at the AMY_1 receptor has not been performed. This precludes a direct comparison of erenumab activity at both receptors; however, it suggests that erenumab can act at both CGRP-responsive receptors. The monoclonal antibodies that target the CGRP peptide by default reduce CGRP activity at both the CGRP and AMY_1 receptors. Thus, the pharmacologic effects of either class of drugs could be a result of their direct or indirect influence on CGRP and AMY_1 receptor activity. The relative contribution each receptor makes to the overall migraine response is unclear, meaning that we do not know whether this dual antagonism is beneficial for migraine therapies, whether we could reduce side-effects by targeting only one of these receptors, or whether this dual antagonism is incidental and irrelevant to the drug profile. Future studies that disentangle the role of each receptor in migraine will be invaluable to understanding the mechanism of action for these drugs.

An emerging phenomenon is co-administration of multiple classes of anti-CGRP pathway drugs.[131–134] Preliminary clinical reports show that these combinations can be safe and more effective than each approach individually.[131–134] Interestingly, the anti-migraine effects appear additive when gepants are combined with either peptide-targeting antibodies (e.g., galcanezumab),[131,132] or receptor-targeting antibodies (e.g., erenumab).[131,133] The former finding is somewhat intuitive; blockade of both parts of the CGRP:CGRP/AMY_1 receptor signaling pathway is likely to be more effective at reducing CGRP signaling than targeting a single component of the pathway. The latter finding is perhaps more interesting and suggests erenumab does not block 100% of the CGRP-responsive receptors in the body on its own. This additive effect could be explained by circulating concentrations of erenumab being too low to target all migraine-relevant receptors in the body, leaving some receptors to be blocked by the gepants. Alternatively, this could be due to the gepants and erenumab having different relative tissue distributions across the body. It is also possible that this additive effect is due to the gepants and erenumab having different relative abilities to antagonize the CGRP

TABLE 1 An overview of calcitonin-family peptides and their functional links to migraine

Peptide	Receptors activated (mouse)	Migraine-like behavior (mouse)	Receptors activated (rat)	Migraine-like behavior (rat)	Receptors activated (human)	Headache (human)	Migraine-like attack (human)
CGRP	**CGRP, AMY$_1$, AMY$_2$, AMY$_3$, AM$_2$**, CTR, AM$_1$	Y[44,45,47,52,54-56]	**CGRP, AMY$_1$, AMY$_3$, AMY$_2$, AM$_2$**, CTR, AM$_1$	Y[44,47,55,57]	**CGRP, AMY$_1$, AMY$_3$, AM$_1$, AM$_2$**	Y[36,37]	Y[35,37]
Amylin/ pramlintide	**AMY$_1$, AMY$_2$, AMY$_3$, AM$_2$,** CTR	Y[45,56,102]/N[52,106]	**AMY$_1$, AMY$_3$, AMY$_2$** CTR	Y[135]/N[107]	**AMY$_1$, AMY$_2$, AMY$_3$,** CTR	Y[56]	Y[56]
AM	**AM$_1$, AM$_2$, AMY$_3$,** AMY$_2$, CGRP	Y[116]/N[52,116]	**AM$_1$, AM$_2$,** CGRP, AMY$_1$, AMY$_3$	Y[115,117,118]	**AM$_1$, AM$_2$,** CGRP	Y[121-123]/N[119]	Y[121]/N[119]

Note: The table is divided into results from studies investigating mice, rats, or humans. Receptors in bold are those at which the named peptide is either the most potent peptide or has a potency within 10-fold of the most potent peptide, while receptors in italics are those at which the peptide has a potency between 10-fold and 100-fold lower than the most potent agonist (data are approximate only, please see references[3,19,21,136] for deeper discussion). Migraine-like behaviors in mice and rats include squint responses, light aversion, and hyperalgesia/allodynia (mechanical and thermal). Y, statistically significant induction of the symptom in response to peptide infusion; N, no statistically significant induction of symptoms.

Abbreviations: AM, adrenomedullin; AM$_1$, AM receptor subtype 1 (calcitonin receptor-like receptor [CLR]/RAMP2); AM$_2$, AM receptor subtype 2 (CLR/RAMP3); AMY$_{(1)(2)(3)}$, CTR:RAMP(1)(2)(3), respectively; CGRP, calcitonin gene-related peptide; CTR, calcitonin receptor; RAMP, receptor activity-modifying protein.

and AMY$_1$ receptors; full pharmacologic profiles of these compounds will help us understand whether this is a viable explanation.

CONCLUSION

Drugs targeting the CGRP system are success stories in the development of migraine therapeutics; however, there is still significant scope to improve upon these drugs to reach better clinical endpoints in larger proportions of the patient population. The focus on CGRP and CGRP receptor has led to the success we have today, although there is a growing body of evidence to suggest that receptors outside the canonical CGRP receptor play a role in migraine (Table 1). Taking a holistic view of this receptor/peptide family may unlock additional targets, leading to improvements in current therapies.

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