Review

Cephalalgia
An International Journal of Headache

International Headache Society

Cephalalgia
2019, Vol. 39(3) 403–419

DOI: 10.1177/0333102418765787
journals.sagepub.com/home/cep

SAGE

Molecular studies of CGRP and the CGRP family of peptides in the central nervous system

Erica R Hendrikse[1], Rebekah L Bower[1,+], Debbie L Hay[1,2] and Christopher S Walker[1]

Abstract

Background: Calcitonin gene-related peptide is an important target for migraine and other painful neurovascular conditions. Understanding the normal biological functions of calcitonin gene-related peptide is critical to understand the mechanisms of calcitonin gene-related peptide-blocking therapies as well as engineering improvements to these medications. Calcitonin gene-related peptide is closely related to other peptides in the calcitonin gene-related peptide family of peptides, including amylin. Relatedness in peptide sequence and in receptor biology makes it difficult to tease apart the contributions that each peptide and receptor makes to physiological processes and to disorders.

Summary: The focus of this review is the expression of calcitonin gene-related peptide, related peptides and their receptors in the central nervous system. Calcitonin gene-related peptide is expressed throughout the nervous system, whereas amylin and adrenomedullin have only limited expression at discrete sites in the brain. The components of two receptors that respond to calcitonin gene-related peptide, the calcitonin gene-related peptide receptor (calcitonin receptor-like receptor with receptor activity-modifying protein 1) and the AMY_1 receptor (calcitonin receptor with receptor activity-modifying protein 1), are expressed throughout the nervous system. Understanding expression of the peptides and their receptors lays the foundation for more deeply understanding their physiology, pathophysiology and therapeutic use.

Keywords

Amylin, adrenomedullin, calcitonin, CGRP, central nervous system, migraine

Date received: 18 November 2017; revised: 19 February 2018; accepted: 26 February 2018

Introduction

Calcitonin gene-related peptide (CGRP) is an important sensory neuropeptide that is involved in pain modulation (1,2). CGRP has attracted particular interest in regard to its role in migraine and likely plays a role in other primary headache disorders and painful conditions (2,3). Presently, three investigational classes of drug aim to reduce CGRP activity to prevent and treat migraine (3). These are small molecule antagonists against a CGRP receptor and antibodies that either block receptor activity or bind directly to the CGRP peptide. This 'first generation' of CGRP-based treatments will likely lead to further drugs over time, which will result from a deeper knowledge of the CGRP system at a cellular and molecular level and a greater understanding of the molecular neuroanatomy of CGRP and its receptors. This information guides understanding of the relative role of central and peripheral processes in which CGRP participates in the pathophysiology of migraine, and other disorders.

Understanding CGRP biology is complex due to the existence of similar peptides in the same family, and shared receptor complexes. The aim of this review is

[1]School of Biological Sciences, University of Auckland, Auckland, New Zealand
[2]Centre for Brain Research, University of Auckland, Auckland, New Zealand
[+]Current address: Department of Pharmacology and Toxicology, The University of Otago, Dunedin, New Zealand

Corresponding author:
Christopher S Walker, School of Biological Sciences, University of Auckland, Private Bag 92 019, Auckland 1142, New Zealand.
Email: cs.walker@auckland.ac.nz

to summarize information about the expression of CGRP and to compare this to its binding sites and molecularly defined receptors. We will detail what is known about the expression of CGRP and related peptides, where binding sites for labelled peptides are found, and what information is available for the molecular correlate(s) to that binding. We focus our attention on the central nervous system (CNS) and peripheral ganglia, with emphasis on brain regions that are of particular relevance to migraine. The sensory circumventricular organs (CVOs) will also be considered, as circulating factors can act directly on these sites within the brain.

Migraine and the nervous system

Historically, there has been considerable debate around the importance of central and peripheral mechanisms of migraine. This initially took the form of the competing vascular and neuronal hypotheses for migraine. It is now clear, based on recent clinical trials, that migraine can be treated through peripheral blockade of CGRP action (3,4). This does not preclude a central origin of migraine. Although the peripheral aspects of the trigeminovascular system are important in migraine, it is still worthwhile to consider central contributions (5). Numerous regions of the CNS are activated during a migraine attack and central phenomena are associated with migraine symptoms, such as the link between cortical spreading depression and migraine aura (3). It is unclear whether blocking central CGRP action may be beneficial or perhaps a hindrance in treating migraine. However, central CGRP receptors should be an important consideration for developing a 'second generation' of CGRP system-based treatments with potentially greater effectiveness or fewer side effects.

This review will focus on the craniofacial pain pathway and other brain regions associated with migraine. The craniofacial pain pathway begins with Aδ and C fibres of the trigeminal nerve, whose cell bodies are located peripherally in the trigeminal ganglia and project centrally primarily into the spinal trigeminal nucleus (STN) of the brainstem and into the C1/C2 levels of the spinal cord. The STN is proposed as a possible site of migraine initiation and displays increased activity immediately prior to a migraine attack (6). Higher order processing of painful signals primarily involves the thalamus, insular cortex and somatosensory cortex. Interconnected with these regions are other sites, including the amygdala, raphe nuclei, periaqueductal gray (PAG), parabrachial area, gracile nucleus and locus coeruleus, which play roles in pain processing, proprioception, stress or aversion. Interestingly, in migraine patients, altered connectivity between these regions is reported, such as from the thalamus to the insular cortex and somatosensory cortex or from the PAG to the cortex and amygdala (7,8). Other brain regions involved in migraine may include the hypothalamus, implicated in attack initiation; the hippocampus, which displays greater pain-induced activity in migraine patients; and the cortex, cerebellum and visual network, which may be involved in cortical spreading depression and symptoms of migraine aura (9–11).

The calcitonin gene-related peptide family of peptides

CGRP belongs to a small family of structurally related peptides (Figure 1). There are two forms of CGRP, α and β. The broad term "CGRP" is used for either α or β, unless specifically noted. The other major members of this family are amylin, adrenomedullin (AM) and adrenomedullin 2 (AM2). Another member of this family, the calcitonin receptor-stimulating peptide (CRSP) was reported in some mammals (12); however, CRSP is not expressed in primates or rodents. Each of these peptides contains a conserved pair of cysteine

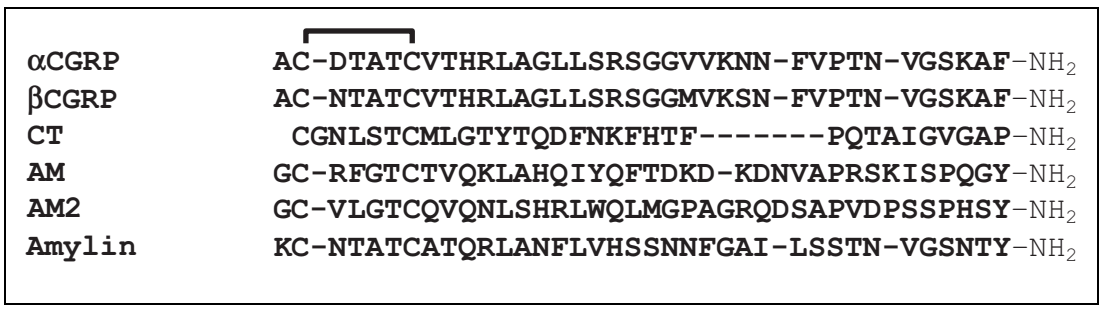

Figure 1. Amino acid sequences of calcitonin gene-related peptide family members. Alignment of the amino acid sequences for human αCGRP, βCGRP, calcitonin (CT), AM, AM2 and amylin using the single letter code. AM and AM2 have been truncated at the N-terminal. All the peptides have a C-terminal amide (-NH_2). A conserved disulfide bond between the two N-terminal cysteine residues is indicated by a solid line.

residues that form a disulfide bond, creating a loop. They also all contain a C-terminal amide. Amylin and CGRP are the most closely related peptides in terms of amino acid sequence, resulting in overlapping actions in pain modulation and nutrient balance (13,14). The similarities between the peptides within the CGRP family causes significant overlap in their ability to activate each other's receptors. This "blurred" receptor pharmacology is particularly evident at rodent receptors, where there is noticeable cross-reactivity. This makes working with this peptide family challenging because it is often very difficult to determine which of the many possible molecularly defined receptors actually mediates an effect for a given peptide (15).

Receptor composition and pharmacology

The molecular composition of the CGRP family of receptors is illustrated in Figure 2, which highlights the overlapping activity of the peptides at the different receptors. Overlap occurs, not only because the peptides have shared features but also because the receptors themselves have shared and closely related components. All of the receptors within this family are G protein-coupled receptors (GPCRs). Two specific GPCRs form high affinity receptors for the different peptides due to their association with accessory proteins. There are three of these accessory proteins, receptor activity-modifying protein (RAMP) 1, 2 and 3. The CGRP, AM_1 and AM_2 receptors consist of the calcitonin receptor-like receptor (CLR) and RAMP1,

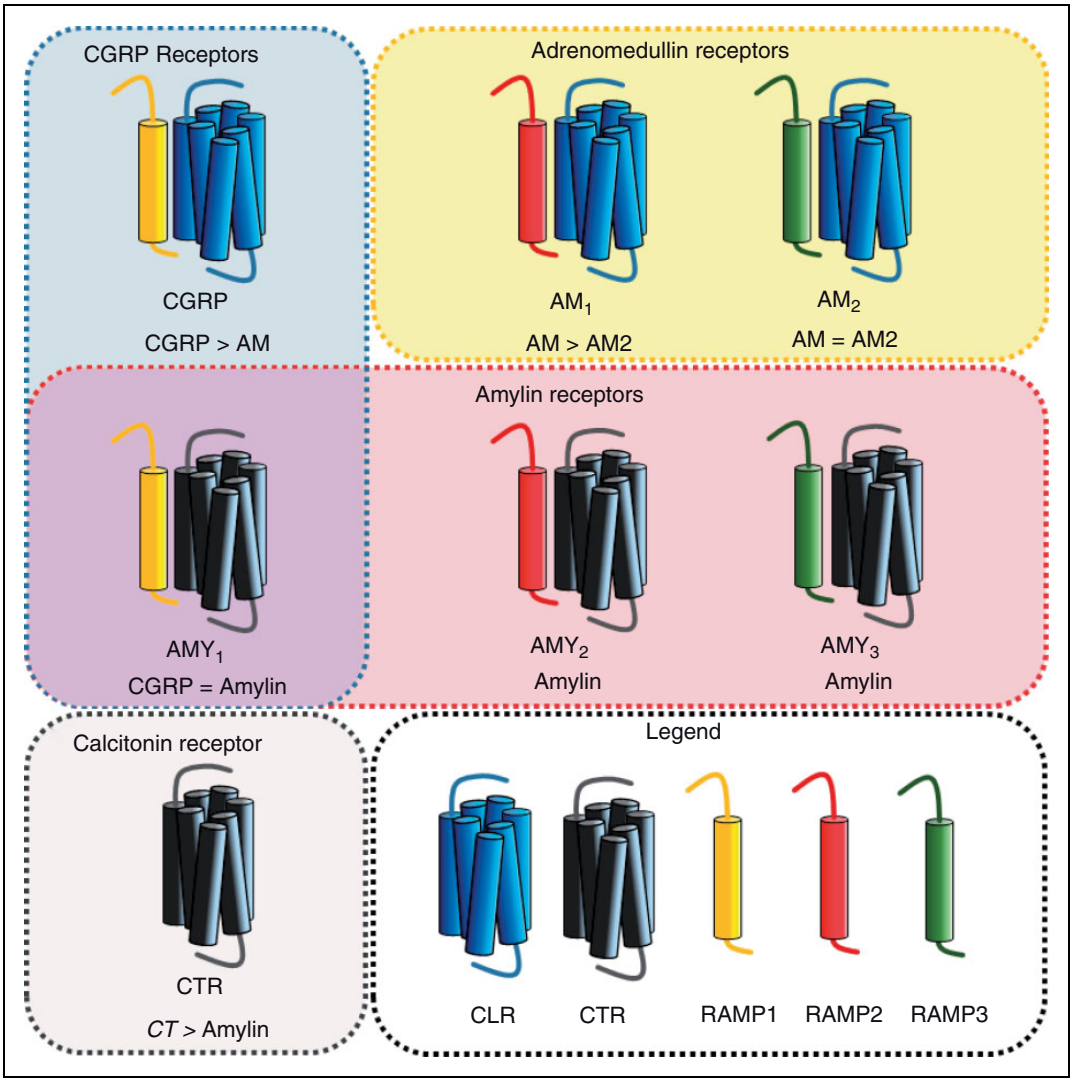

Figure 2. The calcitonin gene-related peptide (CGRP) receptor family. The relative potency of CGRP, calcitonin (CT), AM, AM2 or amylin is shown below a schematic representation of the appropriate receptor complex (17). The potential overlap between the CGRP and amylin receptors is highlighted in purple. The receptor components are described in the legend.

RAMP2 or RAMP3, respectively (16,17). The AMY_1, AMY_2 and AMY_3 receptors consist of the calcitonin receptor (CTR) with each RAMP (17,18). CLR alone does not appear to act as a functional receptor, whereas CTR is the receptor for calcitonin (16,17). Figure 2 shows that CGRP can activate both CLR/RAMP1 and CTR/RAMP1 equally. CGRP is weaker at other human receptor complexes, but there are clear deviations in other species (15). At rat CLR and CTR-based receptors, CGRP is potent at both RAMP3-based receptors (19). Amylin can potently activate CTR/RAMP1, CTR/RAMP2 and CTR/RAMP3 but not the CLR-based receptors. AM activates all three CLR/RAMP complexes but has a preference for CLR/RAMP2 and 3 (17).

One pharmacological method for teasing apart closely-related receptors with overlapping peptide binding parameters is to use specific antagonists. Although several antagonists have been reported that are capable of antagonizing members of the CGRP receptor family, they generally lack the required specificity. For example, although $CGRP_{8-37}$, an N-terminally truncated form of CGRP, is often cited as a specific CGRP receptor antagonist, it does not display sufficient specificity to be practically useful. $CGRP_{8-37}$ is less than 10-fold selective for CGRP receptors over AMY_1 and is capable of antagonizing AM_2 and AMY_3 receptors (19–22). Similarly, CTR and amylin receptor antagonists including truncated salmon calcitonin (sCT_{8-32}) and AC187 are potent antagonists, but do not distinguish between amylin receptor subtypes (19,21). The development of the small molecule "gepant" class of drugs has yielded some useful tools for separating the CGRP receptor from AM receptors and, if used carefully, can tell apart the CGRP and AMY_1 receptors (Table 1). For instance, olcegepant displays greater than 10,000-fold selectivity for the CGRP over AM receptors and 100–200 fold selectivity for the CGRP receptor over the AMY_1 receptor (23–25). However, other gepants including telcagepant and MK-3207 are less selective and the degree of selectivity measured may depend on other variables, including the signaling pathway measured (23,26). With the careful selection of several different agonist and antagonist concentrations, it may be possible to pharmacologically characterize members of the CGRP receptor family present in a particular cell line or tissue. However, for practical use in *in vivo* systems more specific pharmacological tools are required.

Challenges associated with determining the molecular target for a given peptide. To understand the biology of the CGRP peptide family, it is crucial to define the molecular identity of the peptide and receptor responsible for a biological effect in physiological systems. The overlapping pharmacology described above represents a major challenge associated with studying this important family of receptors. Therefore, pharmacological approaches should be complemented with other methods. The measurement of mRNA can be a useful guide. However, the detection of mRNA does not necessarily mean that protein is present, especially in neurons where proteins can be transported to projections distant from the cell body (27). Stable membrane proteins including CLR, CTR and RAMPs may not need high levels of mRNA expression to maintain protein expression in steady-state cells. This has been illustrated where moderate to high levels of immunoreactivity were observed in regions where the corresponding mRNA was low or not detected (28,29).

The development of techniques including high-resolution confocal imaging has allowed researchers to detect the precise cellular localization of a receptor using antibodies. However, when used to directly detect protein expression, antibodies also have limitations. They require comprehensive validation to confirm specificity and selectivity for their targets (30). In many cases, antibodies are used without sufficient evidence of validation and it may not be possible to draw the conclusions that the authors suggest. For example, studies may simply lack the required information regarding the antibodies used to allow evaluation of

Table 1. Summary of small molecule CGRP receptor antagonists (gepants) for migraine treatment.

Small molecule	Olcegepant	Telcagepant	MK-3207	BHV-5000	Atogepant	Ubrogepant	Rimegepant
Blocks CGRP receptor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Blocks AMY_1 receptor[1]	Yes (~100–200 fold lower)	Yes (~50–100 fold lower)	Yes (~50 fold lower)	No data	No data	No data	No data
Clinical development	Development stopped	Development stopped	Development stopped	Preclinical	Phase 2	Phase 3	Phase 3

No data, no published data is available.
[1]The relative affinity or potency for the AMY_1 receptor compared to the CGRP receptor is reported in parenthesis (20,23,26,154).

the data (31) or the antibodies used have been shown to recognize multiple proteins. This prevents observed immunoreactivity from being conclusively associated with a single protein (30). The latter situation has been a significant issue for antibodies designed to detect RAMPs (32).

Even if a receptor subunit has been identified with a well-validated antibody, this alone is not meaningful for this receptor family, except when considering calcitonin activity at CTR alone. Expression of CLR or CTR by themselves says little about the receptor phenotype without further examining potential co-localization with a RAMP. RAMPs are also known to modify the signaling of other receptors, forming alternative complexes that complicate interpretation (33). However, valuable information has been obtained from studies showing co-localization between CLR and receptor-component protein or between CLR and RAMP1, indicating the likely presence of the CGRP receptor (34–36). The ideal solution maybe to develop antibodies that specifically target the heteromeric complex. This strategy was employed to detect the CLR/RAMP1 complex in the trigeminal ganglia, dura mater and the spinal trigeminal nucleus (37). However, antibodies that recognize heteromeric complexes are difficult to generate and characterize. Thus, protein expression data should be used alongside other experimental observations. For example, mRNA and peptide binding correlations were informative in early studies validating CLR/RAMP complexes as receptors for CGRP and AM (38). Another consideration relates to the level of expression reported. For many receptors, there is considerable amplification in signal following ligand-receptor binding. Hence, relatively low expression does not automatically translate into little function.

Regardless of method, much research relies on qualitative description of the biological target within anatomical locations. This is a highly subjective process, and is open to individual interpretation of intensity and location. Collating data, improving its accessibility, and standardisation all help to address these issues. For example, the *Human Protein Atlas*, the *Allen Brain Atlas* and other web-based tools provide excellent resources for expression data, assisting identification of patterns (39–41).

Peptide accessibility to the CNS and relevance of receptors in different locations. The blood-brain barrier (BBB) is an important consideration when looking at the origin, expression and activity of neuropeptides in the CNS. Peptides present in the blood may cross the BBB either through active transport or passive diffusion (42,43). Alternatively, circulating peptides can interact directly with CVOs. CVOs are highly vascularized, with fenestrated capillaries, allowing peptides access to these discrete parts of the CNS. The CGRP family of peptides do not freely cross the BBB (44). The proportion of peripheral amylin that can cross the BBB is low and is unlikely to be sufficient to activate receptors inside the BBB at physiological concentrations (45,46). Interestingly, the highest amount of labelled amylin that was reported to penetrate the brain was observed in the hypothalamus and medulla (46), which are associated with sensory CVOs and thus are permeable to circulating hormones. Hence, relatively high levels of amylin experimentally reaching these regions is not surprising. The reported actions of exogenous amylin at sites inside the BBB, such as the ventral tegmental area, are unlikely to be explained by amylin crossing the BBB (47–49). Alternative explanations could include local production of amylin or another member of the CGRP peptide family triggering receptor activation in these regions. The BBB penetrance of CGRP and AM have been examined primarily in vascular models, which suggest that neither peptide can cross the BBB when the vascular endothelium is intact (50–52). A more definitive study has been performed for AM, which, under normal conditions, does not appear to cross the BBB and penetrate the brain (53). Curiously, definitive experiments have not been performed for CGRP. However, given the lack of brain penetrance displayed by calcitonin, amylin and AM and data from vascular models, significant crossing of the BBB by CGRP seems unlikely. Overall, the low BBB permeability for CGRP family peptides suggests that CNS expression of these peptides is required for them to have activity inside the BBB.

AM and AM2

AM and AM2 expression in the CNS. AM is best known as a regulator of the cardiovascular and lymphatic systems. It is a potent vasodilator and is highly expressed in the vascular endothelium (54,55). Vascular AM may be involved in maintaining the BBB, cerebral circulation and the volume of cerebrovascular fluid (56–58). Expression in the vasculature may complicate expression analysis that relies on homogenized tissue, including mRNA analysis and membrane binding. mRNA and immunoreactivity for AM has been reported in the human and rat brain, localized to the vasculature, the choroid plexus and in neurons and glia of the hypothalamus, cerebellum and medulla (59–63). However, the cerebellum, which has been implicated in AM-regulated blood pressure control, was the only brain region where mature AM peptide was detected (59,64). Interestingly, in a genetic mouse model that was modified to lack AM expression in the CNS, altered responses to pain, anxiety and stress were

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.