Biological and small molecule strategies in migraine therapy with relation to the calcitonin gene-related peptide family of peptides

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Funding information
Lundbeck Foundation, Grant/Award Number: R345-2020-1977

Migraine is one of the most common of neurological disorders with a global prevalence of up to 15%. One in five migraineurs have frequent episodic or chronic migraine requiring prophylactic treatment. In recent years, specific pharmacological treatments targeting calcitonin gene-related peptide (CGRP) signalling molecules have provided safe and effective treatments, monoclonal antibodies for prophylaxis and gepants for acute therapy. Albeit beneficial, it is important to understand the molecular mechanisms of these new drugs to better understand migraine pathophysiology and improve therapy. Here, we describe current views on the role of the CGRP family of peptides - CGRP, calcitonin, adrenomedullin, amylin - and their receptors in the trigeminovascular system. All these molecules are present within the trigeminovascular system but differ in expression and localization. It is likely that they have different roles, which can be utilized in providing additional drug targets.

KEYWORDS
adrenomedullin, amylin, calcitonin, CGRP, CLR, RAMPs, receptors

1 INTRODUCTION

Migraine is a complex disorder, known for centuries but still not fully understood. Affecting approximately 1 billion people, migraine is one of the most prevalent, disabling neurological disorders worldwide. Migraine has a female predominance with debilitating effects in the most active years (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017; Roberts et al., 2018). Currently, the migraine attack is proposed to start in the CNS, mainly involving regions such as the hypothalamus and brainstem (Goadsby et al., 2017). Evidence of migraine initiating in the CNS has been demonstrated in longitudinal neuroimaging studies over 30 days in spontaneous migraine attacks (Schulte, Mehnert, & May, 2020; Schulte, Menz, et al., 2020). These studies revealed that hypothalamic activation during the premonitory phase of a spontaneous migraine attack and ictally, there was activity in brainstem regions often discussed in migraine. However, the link with the trigeminovascular (TGV) system...
and the CNS is still unclear. The CNS is the sole activator of the trigeminal nucleus caudalis (TNC) and, subsequently, the TGV system or the incoming signals from the TGV system can be modified by brainstem nuclei. Following activation of the first-order neurons in the trigeminal ganglion (TG), the sensory nerve fibres transmit pain signals to the lower brainstem and spinal cord C1–C3, verified in tracing studies, and send signals to second order ascending neurons to various CNS regions such as brainstem nuclei and thalamus (Edvinsson, 2011).

An extracerebral site for initiation of migraine attacks has long been suggested, based on findings of dilated temporal arteries during attacks and alleviation by vasoconstrictor drugs, such as ergotamine. Further support for this hypothesis is given by current intravenous infusion of many vasodilator agents, “the provocation method,” where vasodilatation of cranial arteries is proposed to induce migraine attacks (Ashina et al., 2017). As neither of the drugs used passed the blood–brain barrier (BBB), migraine pain was proposed to originate in extracerebral vessel walls (Olesen et al., 2009). Another recurring proposal has been the neurogenic inflammation hypothesis. This takes place in the dura mater when the trigeminal system is activated, and subsequently, this process has been proposed to trigger migraine attacks (Pietrobon & Moskowitz, 2013). However, numerous drugs that block the plasma protein extravasation component of neurogenic inflammation in the dura of animals have been tested in clinical trials but have not exhibited anti-migraine efficacy. Moreover, the endogenous peptide CGRP does not induce neurogenic inflammation in humans or rodents but mediates only the vasodilatory aspect of inflammation (Levy et al., 2005; Schain et al., 2019). Although the idea of neurogenic inflammation has been discussed at length over the years, the role in migraine is still not clear, as not all parameters defining such inflammation appears to be present (Edvinsson, Haanes, & Warfvinge, 2019). Still the topic remains a focus for recent research (Hadjikhani et al., 2020; Khan et al., 2019).

Until 25 years ago, neurologists had few, and often ineffective, options to treat patients with acute migraine attacks, apart from general analgesics alone or in combinations. Understanding the mechanisms of ergotamine-related molecules resulted in development of the triptan group of acute medications, acting with high specificity on 5-HT1B and 5-HT1D receptors. The triptans may act via at least three sites: (i) They are cranial vasoconstrictors with possibility of cardiovascular side-effects (Saxena & Den Boer, 1991), (ii) they inhibit neuropeptide release (Amrutkar et al., 2012; Goadsby & Edvinsson, 1994), and (iii) they may inhibit second-order neurons of the TGV pain pathway (Goadsby & Knight, 1997).

Due to the high probability of cardiovascular adverse events, there was a need for new drugs without vasoconstrictor effects. This work lead to a new group of prophylactic agents, monoclonal antibodies (mAbs) towards CGRP itself or selective binding towards components of CGRP receptors. These new mAbs have since proven to be effective and have few side effects (Edvinsson et al., 2018). Currently, upcoming additions to acute anti-migraine pharmacotherapy includes the 5-HT1F receptor class of agonists, the ditans (Labastida-Ramirez et al., 2020), and the CGRP receptor antagonists, the gepants (Edvinsson et al., 2018).

This review provides a brief description of mechanistic actions of the molecules acting through CGRP-related mechanisms in relation to migraine therapy and discusses the actions based on available molecular data. In addition, we propose ways towards improving the outcomes of acute and prophylactic treatments of migraine, at sites involving other members of the CGRP family of peptides.

## 2 | THE CGRP SYSTEM AND MIGRAINE

CGRP and its receptor are extensively expressed in two separate neuron populations in the TGV system (Eftekhari et al., 2010). CGRP is released from the TGV system in conjunction of acute attacks of migraine and cluster headache (Ho et al., 2010). The trigeminal C-fibres and ganglia are the main source of stimulus-induced CGRP found in the jugular vein, as shown in man and cat (Goadsby et al., 1988) and rat (Hoffmann et al., 2012). The C-fibres have both afferent (collecting peripheral information) and efferent aspects: the afferent release of CGRP from perivascular fibres onto the vascular smooth muscle cells induces vasodilatation and could activate dural mast cells, at least in rats, which in turn contribute to the induction of neurogenic inflammation (Pietrobon & Moskowitz, 2013). Many outstanding questions remain, particularly regarding the other members of the CGRP family. This group of signalling peptides include calcitonin (CT), adrenomedullin (AM), and amylin and their receptors. A relevant question often asked is “Can other peptides be released in the TGV and will CGRP-acting drugs have effects that include other related receptor targets?” Below, we will present an overview on the localization of the CGRP family of peptides in the TGV system to pave the way for other anti-migraine targets.

### 2.1 | CGRP family; peptides and receptors

The CGRP family of peptides (α and β forms) are ligands for a closely related family of G protein-coupled receptors (GPCRs) with shared structural homology (Hay et al., 2014). The CGRP family of peptides and receptors show a varying degree of structural homology with CGRP and have a widespread distribution throughout the body (Hendrikse et al., 2018).

The receptors in this group are formed from either of two 7-transmembrane (TM) G-protein-coupled receptors (GPCRs), the calcitonin receptor-like receptor (CLR), or the calcitonin receptor (CTR), which interact with receptor activity-modifying protein (RAMPs) to form heterodimers (Gingell et al., 2019). The RAMPs are a small family of three proteins (RAMP 1–3) that are single TM-spanning proteins that can modify binding characteristics, pharmacology, functionality, and cell trafficking of the specific GPCRs (Hay & Pioszak, 2016). The currently most central is the 7-TM complex—CLR—which is a required element of receptors for CGRP and for adrenomedullin (AM1 and AM2). Early studies showed that transfecting cells with only CLR revealed no response to CGRP (McLatchie et al., 1998). It was only after the demonstration that the
dimerization of RAMP1 and CLR resulted in formation of a functional receptor to CGRP (Hay et al., 2018). When CLR couples to RAMP2 or RAMP3, it forms AM1 receptors (CLR/RAMP2) or AM2 receptors (CLR/RAMP3). The AMY receptors are formed by association of the calcitonin (CT) receptor (CTR) with RAMPs: AMY1 receptor (CTR/RAMP1), AMY2 receptor (CTR/RAMP2), and AMY3 receptor (CTR/RAMP3). The CTR can also signal on its own, as a calcitonin receptor (without any RAMP) (Poyner et al., 2002). Due to the complexity of this peptide-receptor system, their expression in the trigeminal system is still unclear, particularly in humans. In rats, the receptor localization has been described in detail (Edvinsson et al., 2020), but their full functional roles are yet to be resolved. We summarize the current findings on localization at a cellular level, in the trigeminal ganglion in Figure 1.

2.2 Localization of the CGRP family of peptides in relation to the TGV system

CGRP is expressed in a granular pattern in small-sized (<30 μm) to medium-sized (30–60 μm) TG neurons (Eftekhari et al., 2010; Lennerz et al., 2008) where CGRP is packed in vesicles that are transported in the fibres, most clearly seen in boutons (also known as varicosities) along the fibres (Edvinsson et al., 2019). In addition, the C-type of sensory unmyelinated nerves show pearl-like CGRP immunoreactivity (ir) in boutons. The myelinated fibres do not contain CGRP, as was reported in some early papers and sometimes appear in the literature (Eftekhari et al., 2013; Lennerz et al., 2008). The CGRP receptor components, CLR and RAMP1, are co-expressed in medium to large (>60 μm) diameter neurons (Eftekhari et al., 2010; Eftekhari et al., 2016). In addition, CLR and RAMP1 have been observed in satellite glial cells (SGCs) and in thinly myelinated fibres, typical for Aδ fibres in various parts of the TGV system such as the TG, dura mater, and root entry zone (Eftekhari et al., 2010; Lennerz et al., 2008; Miller et al., 2016). Early findings, which have subsequently been confirmed by many researchers, have stood the test of time and served to support the successful development of mAbs and the low MW antagonists of CGRP receptors, known as gepants (Edvinsson et al., 2018).

CT is a hormone produced mainly by C cells in the thyroid gland with a role to reduce plasma calcium and to promote bone formation (Findlay & Sexton, 2004). In the clinic, CT is used in treatment of bone disorders characterized by increased bone resorption, osteoporosis, and hypercalcaemia due to malignancy, with some pain relief (Findlay & Sexton, 2004). Thus, CT and its receptor CTR are seen in different cell types and tissues, which suggests a range of physiological roles, including bone metabolism (Findlay & Sexton, 2004). CT has not been shown to be expressed in the nervous system, although binding sites for CT are found in many brain structures (Hendrikse et al., 2018). In addition, a recent study has revealed the presence of the CT receptor in the human brainstem (Bower et al., 2016). Usually, CT is not expressed in nervous tissues, as it is just the essence of the altered expression of the CT gene to produce CGRP instead in neurons (Amara et al., 1982). The observation of a positive CT-ir might be due to cross-reactivity of the antibody with CGRP. Nevertheless, pro-CT is expressed in glial cells (Edvinsson et al., 2020; Tajti et al., 2011). The CTR receptor needs more analysis since it is a receptor on its own responding to CT in the circulation, but in, for example, the trigeminal system, it could be involved in binding to one of the RAMPs forming AMY receptors that can respond to CGRP or amylin either from the trigeminal neurons or from the circulation.

The peptide, amylin, was first isolated from human tissue in 1987 (Cooper et al., 1987). It is an endocrine hormone that signals to the brain and acts as a satiety factor (Mulder et al., 1995). The concentration of amylin in plasma is about 10 times higher than that of CGRP (Hay, 2017). Deposition of amylin in brain neurons has been discussed as a contributor to Alzheimer’s disease (Mietlicki-Baase et al., 2017), and this peptide altered the viability of human brain pericytes (Schultz et al., 2017). Early on, we reported that amylin was expressed in feline TG neurons and that the peptide relaxed cerebral vessels, in vitro and in vivo (Edvinsson et al., 2001). A detailed report showed expression of amylin in a few small to medium sized TG neurons which co-express CGRP and in C-fibres (Edvinsson et al., 2020). This agrees
with a recent report that demonstrated low to no expression of amylin-ir in the TG of mouse, rat, and man (Ghanizada, Al-Karagholi, et al., 2021). To us, this would suggest a minor role of amylin in the TGV. Because of the closeness of the CGRP family of peptides to each other, the possibility of cross-reactivity between antibodies to CGRP and amylin cannot be excluded (Rees et al., 2021).

Amylin is currently a focus of much migraine research, to some extent due to its molecular closeness to CGRP in terms of their signaling. Triggered by the fact that CGRP has strong affinity for the AMY$_1$ receptor (Hendrikse et al., 2018), comparison of the binding affinities of olcegepant and CGRP$_{8-37}$ to the AMY$_1$ and CGRP receptors revealed similarities in binding characteristics, as well as in cAMP production (Walker et al., 2015). The relevance to migraine was supported by the demonstration that there is some expression of amylin in the TG neurons; however, more recent studies have shown that amylin was sparse in the TG, probably due to newer and more selective antibodies (Edvinsson et al., 2020; Ghanizada, Al-Karagholi, et al., 2021). Binding studies by Hay et al. showed, not unexpectedly, that CGRP can bind to both the canonical CGRP receptor and the AMY$_1$ receptor. Respectively, rimegepant and erenumab show 30-fold and 5000-fold higher specificity for the CGRP receptor than for the AMY receptor (Pan et al., 2020; Shi et al., 2016). This agrees well with immunohistochemical findings that there is indeed co-localization of CTR and RAMP1 in the TG; however, it is difficult to quantify the degree of expression (Edvinsson et al., 2020). The interesting discussion is now whether the CGRP receptor alone is the key molecular site for anti-migraine medication, acting on both receptors or if the AMY$_1$ receptor is another important site for migraine pathophysiology with amylin and/or CGRP being the primary ligand agonists. This question is illustrated in a recent study with the amylin analogue pramlintide showing that intravenous infusion induced migraine-like attacks (Ghanizada, Al-Karagholi, et al., 2021). Amylin and pramlintide are weak agonists for the CGRP receptor and have around 100 times lower potency compared with CGRP at the canonical CGRP receptor. Amylin is known to be present in the circulation at levels 10 times higher than that of CGRP, and the expression of amylin and AMY$_1$ receptors in the TGV system is low. Hence, for amylin to activate the AMY$_1$ receptor would therefore most likely rely on a peripheral source of the peptide (Ghanizada, Al-Karagholi, et al., 2021). This is in marked contrast to activation by CGRP which, of course, is highly expressed in the TGV system and could be the main ligand. The intravenous infusion of pramlintide elicited significantly lower levels of palpitations, increase in heart rate, facial flushing, and heat sensations than CGRP (Ghanizada, Al-Karagholi, et al., 2021). Notably, CGRP resulted in significant drop in blood pressure and increased heart rate, while pramlintide showed a marked increase in blood pressure but lower level of heart rate response, which indicates the classical autonomic reflex. Thus, the dynamic modulations of the circulation due to infusion of potent vasoactive drugs needs to be understood in relation to migraine provocation with different vasoactive drugs. Receptors for CGRP and amylin are related and share components, CLR/RAMP1 and CTR/RAMP1, respectively. Given the close relationship between amylin and CGRP and as release of the peptides may act on either or both receptors, it is clear that further experiments with AMY receptor ligands are needed (Ghanizada, Al-Karagholi, et al., 2021). It would be interesting to see if pramlintide could induce migraine in patients pretreated with erenumab.

The peptide AM is most often considered an endothelial peptide and was first isolated in 1993 (Kitamura et al., 1993). AM is widely expressed and participates in a variety of physiological functions including vasodilation, bronchodilatation, growth and hormone regulation (Ferrero, Larrayoz, Martisova, et al., 2018). Furthermore, AM is involved in pathophysiological processes such as hypertension, retinopathy and tumour genesis (Ferrero, Larrayoz, Gil-Bea, et al., 2018). In mammals,

![Diagram of the differential distribution of the CGRP family of peptides](image-url)

**FIGURE 2** Diagram of the differential distribution of the CGRP family of peptides. As in Figure 1, the large pink cell represents a large neuron, which is surrounded by much smaller SGCs (orange); the cell (purple) on the left of the large neuron represents a small neuron CGRP is expressed in the small to medium sized neurons. The data on amylin is not fully established but some studies show co-localization with CGRP in small to medium sized neurons. AM and pro-CT are expressed in SGCs. The pattern of expression shown here is based on a number of immunological studies cited in the text.
endothelial AM-ir is present in low concentrations in the vascular endothelium (Satoh et al., 1996). AM is also found in neurons and glial cells (Serrano et al., 2000). We have reported the presence of AM-ir in the thin cytoplasm of the glial cells, SGCs, and cells enveloping neuronal processes, probably myelinating cells. In addition, AM-ir has been noted in cranial vascular endothelium (Edvinsson et al., 2020). Its molecular relation to CGRP has resulted in the suggestion that AM may have a role in migraine pathophysiology (Juhl et al., 2006). However, intravenous infusion of AM did not cause migraine-like attacks in man (Ashina et al., 2017). This view is in line with our demonstration that there is no AM in TG neurons but it is present in glial cells and vascular endothelium (Edvinsson et al., 2020). Interestingly, recent experimental results contrast with the earlier data. Infusing adrenomedullin intravenously for 20 min resulted in migraine attacks in 55% of migraine patients, whereas only 15% got a migraine attack after infusion of placebo (Ghanizada, Al-Mahdi Al-Karakahi, et al., 2021). The infusion of AM, as expected, increased heart rate, palpitations, and facial flushing and heat sensations. However, the symptoms were very overt and clearly unblinds the study. We summarize the expression of the CGRP family of neuropeptides in Figure 2.

3 | Site of Action of CGRP and the Drugs Acting on CGRP Receptors

CGRP antagonistic molecules have by now a history of two decades, with olcegepant and telcagepant as the first low MW compounds that competitively antagonize the vasodilator effect of CGRP in human arteries (Edvinsson et al., 2002). They were studied in several clinical trials, but the program for telcagepant was halted because of hepatotoxicity. Following molecular modifications, three other gepants have now completed Phase III trials and two are approved by the FDA; these are ubrogepant (Rubio-Beltran et al., 2020) and rimegepant (I. A. Mulder et al., 2020). The drugs were initially designed for acute therapy, but they are now also studied for prophylaxis, for example, atogepant. The other class of antagonistic molecules are humanized monoclonal antibodies directed towards different parts of the CGRP molecule itself (eptinezumab, fremanezumab, galcanezumab) or a human antibody, which binds to the N-terminals of CLR and RAMP1 (erenumab). They have all passed extensive clinical studies with significant beneficial effects and minor adverse events; consequently, they are now approved for migraine prophylaxis. While these molecules are effective, their respective site of action remains unclear, although the expanded research in this field also highlights putative sites-of-action for other members of the CGRP family.

3.1 | Action on intracranial blood vessels

Dilatation of the middle meningeal arteries and neurogenic inflammation in the dura mater, have been suggested as a triggering mechanism regarding migraine pathophysiology. The direct activation of trigeminal afferents in the dura causes a painful headache in humans; this observation was first made in 1940 via stimulation of these vascular nerves during surgery and extended more recently to include pia mater and small pial vessels (Fontaine et al., 2018). Similarities of the occurring headache with a migraine headache led to the vascular theory of migraine, which postulates that the headaches are a disorder triggered by dilation of intracranial or extracranial blood vessels. One aspect worth considering is that nearly all vasodilators given in a peripheral vein results in “migraine-like headache or migraine” (Ashina et al., 2017). However, this view is disputed as the same group of researchers did not find dilatation of cerebral and middle meningeal arteries in genuine migraine attacks (Amin et al., 2013).

The first study of CGRP on intracranial arteries revealed very potent vasodilatation, independent of endothelial action and associated with activation of adenyl cyclase in the vascular smooth cells (Edvinsson et al., 1985) and with a parallel reduction in intracellular calcium ion concentration (Erdling et al., 2017). Subsequent studies revealed that other members of the CGRP family of peptides also were vasodilators but had lower potency. While the perivascular sensory nerves had a rich supply of CGRP-containing fibres, there is less expression of amylin and no AM in nerve fibres on cerebral vessels. A subpopulation of CGRP-positive TG neurons were also positive for amylin, while no AM-ir was seen (Edvinsson et al., 2001). The receptor components CLR and RAMP1–3 were demonstrated with qPCR, suggesting that AMY receptors might be present. In agreement, human cerebral pial arteries, middle cerebral, and superficial temporal and middle meningeal arteries have CLR, RAMP1, RAMP2, and RAMP3 in the smooth muscle layers (Oliver et al., 2002). The vascular endothelium contained CLR and RAMP2, but no RAMP1 or RAMP3. Functional study of cerebral arteries revealed strong vasodilator effects by CGRP or amylin (with or without endothelium) while only at very high concentration did AM show a relaxant effect; CGRP_{B-37} blocked the relaxant response. Intracortical administration of CGRP, amylin and AM caused increased local cerebral blood flow by 42%, 32%, and 15%, respectively (Edvinsson et al., 2001). The responses to CGRP and amylin were blocked by CGRP_{B-37}, suggesting that the vasodilatation is mediated via CGRP receptors (both mRNA for CLR and RAMP1 were seen in the MCA) (Edvinsson et al., 2001). Early studies of CT revealed very low relaxant effect on brain vessels (Edvinsson et al., 1987) and more recent preliminary studies have not shown CT receptor-ir in the vessel walls, while the CGRP receptor components CLR and RAMP1 are present in human cerebral and middle meningeal arteries (Edvinsson et al., 2010). While gepants showed competitive inhibition (Edvinsson et al., 2010; Rubio-Beltran et al., 2020), the monoclonal antibodies (mAbs) showed competitive antagonism with no depression of maximum CGRP-induced relaxation in human middle meningeal and cerebral arteries (Ohlsson et al., 2018; Ohlsson et al., 2019). The data for the vasculature support a strong role for CGRP, but the lack of the AMY receptor component CTR, and the few AM receptors make their role in induction of a migraine attack unlikely, if vasodilatation is the key migraine mechanism (Ashina et al., 2017; Walker et al., 2015). This view is compatible with that of others who stated that the TGV system does not require a peripheral sensory input to be activated (Goadsby & Akerman, 2012).
3.2 | Relation to the BBB

Considering a possible vascular mechanism for the anti-migraine drugs, the possible relation of CGRP or its antagonists and the effect on cerebral arteries must be considered. Importantly, the endothelium in these vessels restricts passage of molecules from the vessel lumen to the smooth muscle layers of the vascular wall containing CGRP nerve endings. The hypothesis was tested in isolated rat middle cerebral arteries that were cannulated and luminally perfused, enabling application of drugs to either the lumen or directly to the smooth muscle layers on the abluminal side of the artery (Edvinsson et al., 2007). In these experiments, CGRP as well as AM, amylin and CT only produced arterial relaxation when applied to the abluminal surface of the cerebral artery (Edvinsson et al., 2007). Moreover, neither CGRP receptor antagonists (olcegepant and telcagepant) nor anti-CGRP antibodies blocked CGRP-mediated dilatation when applied to the lumen; they were effective only when applied to the abluminal side. On this basis, circulating CGRP receptor antagonists and mAbs against CGRP and the CGRP receptor, all of which are effective in migraine, do not seem to be able to cross the endothelial barrier to access targets in the brain or its vasculature.

In light of the BBB, the question remains “where do the gepants and mAbs act” to relieve migraine headache. Looking at the dura mater, the cerebral circulation, the brain, and the TG after administration of tracers, such as dye Evans Blue or radioactive compounds, to calculate the permeability surface area-product (PS) showed that the dura and the TG were freely accessible to circulating non-BBB penetrating molecules (e.g., triptans and CGRP-mAbs; Eftekhar et al., 2015; Lundblad et al., 2015). Induction of neurogenic inflammation using Freund’s adjuvant or the “inflammation soup” revealed activation within the TG but no quantitative increase in PS into the CNS. Subsequent measurements revealed minor passage by gepants (2%) or none of the mAbs (<0.01%) (Johnson et al., 2019). This is in support of a more recent study by Noseda et al. (2020) showing that labelled fremanezumab was distributed to cranial sensory and autonomic ganglia and to the dura mater but not to the CNS in rats with uncompromised BBB. As the TG and its peripheral ramifications (the TGV system) are outside the protection of the BBB, it is likely that the anti-migraine site(s) of action of the CGRP group of anti-migraine drugs reside here. This is also the reason why there are few CNS-related side effects of these anti-migraine agents.

3.3 | Action on neuron cell bodies and nociceptive fibres

The trigeminal system provides the link between the peripheral primary afferents and the central terminals of the TNC. Activation of this pathway may result in sensitization within second-order neurons and drive the CNS aspects of the migraine attacks (May & Burstein, 2019). Also, activation of the hypothalamus is an early (prodomal) event and this may define the site where the migraine attack starts (May & Burstein, 2019; Schulte, Mehnert, & May, 2020; Schulte, Menz, et al., 2020). Connectivity studies have revealed that other CNS regions are subsequently activated, including the brainstem, from which links are available to activate or modulate the function of the trigeminal system (May & Burstein, 2019). In both hypotheses, the trigeminal system plays a key role, and currently available mAbs towards CGRP and the CGRP receptor are effective (Edvinsson et al., 2018) despite their inability to penetrate the BBB (Lundblad et al., 2015; Noseda et al., 2020).

Each of the peptides of the CGRP family exhibits a distinct selection of biological actions (Poyner et al., 2002). CGRP and amylin are the most closely related peptides in terms of amino acid sequence, which may cause an overlap in their ability to activate their receptors. These two peptides have effects related to pain, though there are still limited data and it is unclear how much overlap exists, because the peptides are usually not studied simultaneously. The relative potency of CGRP, CT, amylin and AM at the different receptor complexes is complicated and challenging because of cross-reactivity (Hay et al., 2018; Hendrikse et al., 2018). In addition, release of a peptide from nerve endings will result in a very high concentration just at the receptor site, while circulating levels vary considerably.

The possibility of intercellular cross-talk in the TG was recently proposed to result in a feedback loop sensitizing neurons (Messlinger et al., 2020). Apart from direct gap-junction communication between SGCs and neurons, paracrine signalling may also occur. CGRP locally released by neurons or C-fibres could potentially activate receptors located on SGC, other neurons, or auto-receptors. This activation may in turn affect gene expression, neurotropic factors, neuropeptides or receptor regulation.

There are only a few direct functional studies on the various cells in the TG. Hypothetically, there is a range of plausible targets, available to circulating drugs with low BBB permeability: (i) peripheral terminals of nociceptive Aδ-fibres and C-fibres innervating various cranial structures, (ii) receptors expressed at or proximal to the nodes of Ranvier on Aδ-fibres (see below) (Edvinsson, Warfvinge, et al., 2019), (iii) neuron cell bodies (notably, in the TG), these are vigorously enveloped by SGCs, which may act as gate-keepers to systemically introduced drugs, and (iv) the SGCs and Schwann cells in the TG (more likely to be a complex signalling relationship between these cells and proximal neurons/axons; Messlinger et al., 2020).

Both receptor activation pathways lead to an upregulation of cAMP, which in turn could further sensitize neurons and axons to noxious stimuli. Non-myelinating Schwann cells form Remak bundles around parts of C-fibre axons. They are vital in providing trophic factors and regenerating damaged C-fibres (Murinson & Griffin, 2004). Disruption of ErbB signalling in non-myelinating Schwann cell in mice resulted in a progressive sensory loss. This suggests intercellular signalling between non-myelinating Schwann cells and unmyelinated sensory fibres and is critical for C-fibre and Schwann cell survival. The involvement of SGC and Schwann cell interactions with neurons is a field of research not yet fully understood. This gap in our knowledge is, in part, due to difficulties in studying the structures in vitro without disrupting the neuron-glia architecture.
3.4  Action in the nodes of Ranvier—Axon–axon interaction

The exploration of CGRP and its receptor have so far yielded successful scientific breakthroughs and therapies. RAMP1 and CLR are co-expressed mainly in larger neurons and in the thinly myelinated fibres, typically identified as Aβ-fibres (Edvinsson, Warfvinge, et al., 2019; Eftekhar et al., 2010). CGRP receptors are found distally in the dura mater, associated both with the vessels and at avascular sites. In larger arteries, these fibres run in the adventitia, close to the C-fibres. Interestingly, the C-fibres storing CGRP could align with adjacent nodes of Ranvier in the Aβ-fibres (Edvinsson, Warfvinge, et al., 2019). This led to the suggestion that at the nodes of Ranvier, local release of CGRP may act on CGRP receptors on the Aβ-fibres to modify the sensory signalling. In this region, CLR/RAMP1 as well as a complete CGRP receptor antibody immunoreactivity were observed on the Aβ-fibres and associated with protein kinase A, which provides a link towards the possible phosphorylation of ion channels in Aβ-fibres (Edvinsson, Warfvinge, et al., 2019). This mechanism may alter ion channel activity in the Aβ-fibres and contribute to the understanding of pain signalling and perhaps also the sensitization processes. This may be a site for the gepants and mAbs to interact with the trigeminal system and the perception of pain.

4  FUTURE PERSPECTIVES

Further research should aim to compare quantitatively the expression of the peptides and receptors discussed above, particularly in human samples and with focus on sex and age. Preliminary expression studies suggest that (i) CGRP and pro-CT are the most expressed of this group of peptides, (ii) CGRP receptor and CTR are the most frequently expressed receptor types and limited expression of AMY1 receptors, and (iii) an expression of AM2 and AMY3 receptors may occur in rat TG, but is mainly localized to the SGCs. The demonstration of the specific ligands and receptor sites in TG neurons highlights the importance of CGRP and the CGRP receptor as viable contributors in primary headache disorders. Future investigations of the expression of the CGRP family of peptides near the node of Ranvier is warranted. Understanding the location and distribution is important in deciding on how to identify novel targets for anti-migraine medications, and this will facilitate future drug development.

4.1  Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Fabbro et al., 2019a, b)

ACKNOWLEDGEMENTS

KAH was supported by a Lundbeck Foundation Fellowship (R345-2020-1977). The funders had no role in the decision to publish or the preparation of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

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REFERENCES

Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharan, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019). THE CONCISE GUIDE TO PHARMACOLOGY 2019: G protein-coupled receptors. British Journal of Pharmacology, 176, S21–S141. https://doi.org/10.1111/bjp.14748

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharan, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019a). THE CONCISE GUIDE TO PHARMACOLOGY 2019: Catalytic receptors. British Journal of Pharmacology, 176, S247–S296. https://doi.org/10.1111/bjp.14751

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharan, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019b). THE CONCISE GUIDE TO PHARMACOLOGY 2019: Enzymes. British Journal of Pharmacology, 176, S297–S396. https://doi.org/10.1111/bjp.14752

Amara, S. G., Jonas, V., Rosenfeld, M. G., Ong, E. S., & Evans, R. M. (1982). Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature, 298(5871), 240–244. https://www.ncbi.nlm.nih.gov/pubmed/6283379

Amin, F. M., Asghar, M. S., Hougaard, A., Hansen, A. E., Larsen, V. A., de Koning, P. J., Larsson, H. B. W., Olesen, J., & Ashina, M. (2013). Magnetic resonance angiography of intracranial and extracranial arteries in patients with spontaneous migraine without aura: A cross-sectional study. Lancet Neurology, 12(5), 454–461. https://doi.org/10.1016/S1474-4422(13)70067-X

Amrutkar, D. V., Ploug, K. B., Hay-Schmidt, A., Porreca, F., Olesen, J., & Jansen-Olesen, I. (2012). mRNA expression of 5-hydroxytryptamine 1B, 1D, and 1F receptors and their role in controlling the release of calcitonin gene-related peptide in the rat trigeminovascular system. Pain, 153(4), 830–838. https://doi.org/10.1016/j.pain.2012.01.005

Ashina, M., Hansen, J. M., Bo, A. D., & Olesen, J. (2017). Human models of migraine—Short-term pain for long-term gain. Nature Reviews. Neurology, 13(12), 713–724. https://doi.org/10.1038/nrneurol.2017.137

Bower, R. L., Eftekhar, S., Waldvogel, H. J., Faul, R. L., Tajti, J., Edvinsson, L., Hay, D. L., & Walker, C. S. (2016). Mapping the calcitonin receptor in human brain stem. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 310(9), R788–R793. https://doi.org/10.1152/ajpregu.00539.2015

Cooper, G. J., Willis, A. C., Clark, A., Turner, R. C., Sim, R. B., & Reid, K. B. (1987). Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proceedings of the National Academy of Sciences of the United States of America, 84(23), 8628–8632. https://www.ncbi.nlm.nih.gov/pubmed/3317417

Edvinsson, J. C. A., Warfvinge, K., Krause, D. N., Blixt, F. W., Sheykzhade, M., Edvinsson, L., & Haanes, K. A. (2019). C-fibers may
receptor and receptor activity-modifying proteins in the human cerebrovascularature. *Journal of Cerebral Blood Flow and Metabolism*, 22(5), 620–629. https://doi.org/10.1097/00004647-200205000-00014

Pan, K. S., Slow, A., Hay, D. L., & Walker, C. S. (2020). Antagonism of CGRP signaling by rimegepant at two receptors. *Frontiers in Pharmacology*, 11, 1240. https://doi.org/10.3389/fphar.2020.01240

Pietrobon, D., & Moskowitz, M. A. (2013). Pathophysiology of migraine. *Annual Review of Physiology*, 75, 365–391. https://doi.org/10.1146/annurev-physiol-030212-183717

Poyner, D. R., Sexton, P. M., Marshall, I., Smith, D. M., Quirion, R., Born, W., Muff, R., Fischer, J. A., & Foord, S. M. (2002). International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacological Reviews*, 54(2), 233–246. https://www.ncbi.nlm.nih.gov/pubmed/12037140

Rees, T. A., Hay, D. L., & Walker, C. S. (2021). Amylin antibodies frequently display cross-reactivity with CGRP: Characterization of eight amylin antibodies. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 320(5), R697–R703. https://doi.org/10.1152/ajpregu.00338.2020

Roberts, N. L., Mountjoy-Venning, W. C., Anjomshoa, M., Banoub, J. A., Yasin, Y. J., & GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the global burden of disease study 2017. *Lancet*, 392(10159), 1789–1858. https://doi.org/10.1016/s0140-6736(18)32279-7

Rubio-Beltran, E., Chan, K. Y., Dancer, A. J., MaassenVanDenBrink, A., & Edvinsson, L. (2020). Characterisation of the calcitonin gene-related peptide receptor antagonists ubrogepant and atogepant in human isolated coronary, cerebral and middle meningeal arteries. *Journal of Neurology*, 238(Suppl 1), S28–S32. https://doi.org/10.1007/BF01642903

Sato, Y., Kuroda, K., Totsune, K., Sone, M., Ohneda, M., Sasano, H., & Mouri, T. (1996). Immunocytochemical localization of adrenomedullin-like immunoreactivity in the human hypothalamus and the adrenal gland. *Neuroscience Letters*, 203(3), 207–210. https://www.ncbi.nlm.nih.gov/pubmed/8742029

Saxena, P. R., & Den Boer, M. O. (1991). Pharmacology of antimigraine drugs. *Journal of Neurology*, 238(Suppl 1), S28–S32. https://doi.org/10.1007/BF01642903

Schain, A. J., Melo-Carrillo, A., Stratten, J., Strassman, A. M., & Burstein, R. (2019). CSD-induced arterial dilatation and plasma protein extravasation are unaffected by Fremanezumab: Implications for CGRPs role in migraine with aura. *The Journal of Neuroscience*, 39(30), 6001–6011. https://doi.org/10.1523/JNEUROSCI.0232-19.2019

Schulte, L. H., Mehnert, J., & May, A. (2020). Longitudinal neuroimaging over 30 days: Temporal characteristics of migraine. *Annals of Neurology*, 87(4), 646–651. https://doi.org/10.1002/ana.25697

Schulte, L. H., Menz, M. M., Haake, J., & May, A. (2020). The migraineur’s brain networks: Continuous resting state fMRI over 30 days. *Cephalalgia*, 40(14), 1614–1621. https://doi.org/10.1177/033102420951465

Schultz, N., Byman, E., Fex, M., & Wennstrom, M. (2017). Amylin alters human brain pericyte viability and NG2 expression. *Journal of Cerebral Blood Flow and Metabolism*, 37(4), 1470–1482. https://doi.org/10.1177/0271678X16657093

Serrano, J., Uttenthal, L. O., Martinez, A., Fernandez, A. P., de Velasco, J. M., Alonso, D., Bentura, M. L., Santacana, M., Gallardo, J. R., Martinez-Murillo, R., & Cuttitta, F. (2000). Distribution of adrenomedullin-like immunoreactivity in the rat central nervous system by light and electron microscopy. *Brain Research*, 853(2), 245–268. https://www.ncbi.nlm.nih.gov/pubmed/10640622

Shi, L., Lehto, S. G., Zhu, D. X., Sun, H., Zhang, J., Smith, B. P., Immke, D. C., Wild, K. D., & Xu, C. (2016). Pharmacologic characterization of AMG 334, a potent and selective human monoclonal antibody against the calcitonin gene-related peptide receptor. *The Journal of Pharmacology and Experimental Therapeutics*, 356(1), 223–231. https://doi.org/10.1124/jpet.115.227793

Tajti, J., Kuris, A., Vecsei, L., Xu, C. B., & Edvinsson, L. (2011). Organ culture of the trigeminal ganglion induces enhanced expression of calcitonin gene-related peptide via activation of extracellular signal-regulated protein kinase 1/2. *Cephalalgia*, 31(1), 95–105. https://doi.org/10.1177/0333102410382796

Walker, C. S., Eftekhar, S., Bower, R. L., Wilderman, A., Insel, P. A., Edvinsson, L., Waldvogel, H. J., Jamaldinid, M. A., Russo, A. F., & Hay, D. L. (2015). A second trigeminal CGRP receptor: Function and expression of the AMY1 receptor. *Annals of Clinical Translational Neurology*, 2(6), 595–608. https://doi.org/10.1002/acn3.197

How to cite this article: Edvinsson, L., Edvinsson, J. C. A., & Haanes, K. A. (2022). Biological and small molecule strategies in migraine therapy with relation to the calcitonin gene-related peptide family of peptides. *British Journal of Pharmacology*, 179(3), 371–380. https://doi.org/10.1111/bph.15669