

# Pituitary cyclase-activating polypeptide targeted treatments for the treatment of primary headache disorders

Nazia Karsan<sup>1</sup>, Lars Edvinsson<sup>2</sup>, Laszlo Vecsei<sup>3</sup> & Peter J Goadsby<sup>1,4</sup> 

<sup>1</sup>Headache Group, The Wolfson Sensory, Pain and Regeneration Centre (SPaRC), NIHR King's Clinical Research Facility and SLaM Biomedical Research Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

<sup>2</sup>Department of Medicine, Institute of Clinical Sciences, Lund University, 221 84, Lund, Sweden

<sup>3</sup>Department of Neurology, Albert Szent-Györgyi Medical School, and HUN-REN-SZTE Neuroscience Research Group, Hungarian Research Network, University of Szeged, Semmelweis u. 6, Szeged, H-6725, Hungary

<sup>4</sup>Department of Neurology, University of California, Los Angeles, California, USA

## Correspondence

Peter J. Goadsby, Wellcome Foundation Building, King's College Hospital, London SE5 9PJ, UK. Tel: +442032993106; E-mail: [peter.goadsby@kcl.ac.uk](mailto:peter.goadsby@kcl.ac.uk),

Received: 20 May 2024; Accepted: 23 May 2024

doi: 10.1002/acn3.52119

## Abstract

**Objective:** Migraine is a complex and disabling neurological disorder. Recent years have witnessed the development and emergence of novel treatments for the condition, namely those targeting calcitonin gene-related peptide (CGRP). However, there remains a substantial need for further treatments for those unresponsive to current therapies. Targeting pituitary adenylate cyclase-activating polypeptide (PACAP) as a possible therapeutic strategy in the primary headache disorders has gained interest over recent years. **Methods:** This review will summarize what we know about PACAP to date: its expression, receptors, roles in migraine and cluster headache biology, insights gained from preclinical and clinical models of migraine, and therapeutic scope. **Results:** PACAP shares homology with vasoactive intestinal polypeptide (VIP) and is one of several vasoactive neuropeptides along with CGRP and VIP, which has been implicated in migraine neurobiology. PACAP is widely expressed in areas of interest in migraine pathophysiology, such as the thalamus, trigeminal nucleus caudalis, and sphenopalatine ganglion. Preclinical evidence suggests a role for PACAP in trigeminovascular sensitization, while clinical evidence shows ictal release of PACAP in migraine and intravenous infusion of PACAP triggering attacks in susceptible individuals. PACAP leads to dural vasodilatation and secondary central phenomena via its binding to different G-protein-coupled receptors, and intracellular downstream effects through cyclic adenosine monophosphate (cAMP) and phosphokinase C (PKC). Targeting PACAP as a therapeutic strategy in headache has been explored using monoclonal antibodies developed against PACAP and against the PAC1 receptor, with initial positive results. **Interpretation:** Future clinical trials hold considerable promise for a new therapeutic approach using PACAP-targeted therapies in both migraine and cluster headache.

## Introduction to Migraine Biology

Migraine is a complex and heterogeneous brain disorder, involving dysfunction of sensory, limbic, and homeostatic regulation.<sup>1,2</sup> It represents the most common and disabling disorder presenting to secondary care and headache clinics worldwide, thus there is a considerable need for effective and tolerated acute and preventive therapies for its management.<sup>3</sup> Widespread dynamic and oscillating

changes in brain function throughout the migraine cycle<sup>4–10</sup> mediate a multisymptomatic clinical phenotype involving headache, but also including symptoms such as cognitive dysfunction, sensory sensitivities, altered arousal, and mood change.<sup>11</sup> Certainly, pain processing brain areas are involved in migraine, including anterior cingulate cortex (ACC), periaqueductal grey (PAG), prefrontal cortex (PFC), and thalamus.<sup>12,13</sup> In addition, areas distinct from other pain syndromes have been shown to be involved on

functional imaging studies, including hypothalamus,<sup>9,12–14</sup> ventral tegmentum (VTA),<sup>12,13</sup> and the pons.<sup>13,15–17</sup> These areas are distinct from those involved in provoked cranial pain,<sup>18</sup> while sharing overlap with other primary headache disorders, such as cluster headache<sup>19</sup> and hemicrania continua,<sup>20</sup> suggesting at some level shared neurobiology between these primary headache disorders, although certainly, of them, we understand the most about migraine thus far.

Migraine is well accepted to be a neurovascular disorder<sup>21</sup>; in that the interaction between sensory afferents supplying the intra- and extracranial vasculature, craniofacial structures, and intracranial pain processing areas, are involved in the pathophysiology. However, the drivers for migraine initiation and maintenance remain debated.<sup>2</sup> It has become apparent that early symptoms preceding headache during the attack have feasible neural substrates, supporting a theory of central rather than peripheral attack initiation.<sup>12,13,22,23</sup> In addition, in those with migraine with aura, which is 20–30% of all migraine patients depending on age,<sup>24</sup> the aura typically precedes headache, and aura is thought to be caused by cortical spreading depression (CSD), a wave of cortical depolarization followed by repolarization, occurring mainly over visual cortex.<sup>25–29</sup> This may manifest as cortical hypoperfusion followed by hyperperfusion.<sup>25,28</sup> How, and whether this phenomenon is related to migraine headache remains unclear, as does whether CSD occurs in migraine without aura in an asymptomatic way, but clearly central changes precede headache. The persistence of brainstem imaging changes following successful abortion of headache with sumatriptan, suggests that these areas may be important to both headache initiation and maintenance.<sup>15</sup>

## Neuropeptides and primary headache disorders

The nociceptive innervation of the intracranial vessels and the meninges is from C- and A $\delta$  fibers, primarily through the first division of the trigeminal nerve (V1), and to a lesser extent from V2 and V3, as well cervical dorsal root ganglia which supply the dura mater.<sup>2,30</sup> Axonal innervation of the dura mater involves several vasoactive neuropeptides, such as calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating polypeptide (PACAP), which are released on neuronal stimulation and cause vasodilatation.<sup>1,21</sup> Studies in migraine have shown that activation of perivascular nociceptive nerve fibers intra- and extracranially causes headache very similar to migraine, and with associated symptoms like nausea and light aversion,<sup>31</sup> whereas stimulation further away from the vasculature is less nociceptive. Additionally, stimulation of dural vasculature in animals

causes activation in the trigeminocervical complex (TCC) in the brainstem, an area where sensory afferent input from the trigeminal ganglion converges with that from the upper cervical cord.<sup>32–35</sup> The involvement of this area likely accounts for the typical pain distribution of migraine, involving the occipital region and neck.<sup>36</sup> In cluster headache and the other trigeminal autonomic cephalalgias (TAC's), unilateral activation of the trigemino-vascular pathway, with prominent activation of the parasympathetic trigeminal autonomic reflex via V1 is a key feature of the pathophysiology,<sup>37,38</sup> as is hypothalamic involvement.<sup>39</sup> It is unclear whether activation of dural nociceptive fibers is the driver of migraine headache and if the brain changes are secondary to this, or if, perhaps, the brain changes in migraine cause a susceptibility to nociceptive pathway activation: such that usually innocuous stimuli, such as dural pulsation for example, may be perceived as painful, in the same way as unchanged light (photic hypersensitivity) and sound intensities (phonophobia) become uncomfortable during an attack. From the TCC, ascending connections to other brain areas like hypothalamus, thalamus and cerebral cortex enable pain processing and cause other migraine-associated symptoms such as cognitive and homeostatic changes.<sup>40</sup> Similarly, in the TAC's, unilateral hypothalamic involvement has been demonstrated on functional MRI studies,<sup>19,20,41,42</sup> but the link between peripheral dural nociceptor activation and central brain changes in these conditions is also unknown. Most recently evidence of premonitory symptoms occurring in cluster headache has emerged,<sup>43–45</sup> as has evidence of cranial autonomic symptoms (CAS) occurring before pain onset,<sup>43,44</sup> both of which necessitate some reappraisal of the mechanisms involved. Taken together, the new data suggest cluster headache also involves central brain changes mediating premonitory symptoms. These are likely to occur before peripheral dural nociceptor activation, such that even activation of the parasympathetic autonomic reflex via the sphenopalatine ganglion (SPG) and superior salivatory nucleus (SSN) does not require peripheral dural afferent activation and can occur prior to headache.

## Serotonin-based treatments for migraine

While vasodilatation may be a feature of the migraine attack, it does not seem to be significant enough to occur, at least on a level that can be captured by currently available imaging techniques, during spontaneous attacks.<sup>46</sup> There is a suggestion of intracranial<sup>38</sup> and extracranial vasodilatation<sup>47</sup> on imaging in cluster headache. In addition, vasoconstriction for attack abortion is not necessary<sup>48,49</sup> nor temporally associated<sup>50</sup> in both migraine and cluster headache. This understanding and the appreciation that even the

triptans: serotonin 5-hydroxytryptamine (5-HT)<sub>1B/1D</sub> receptor agonists, which are the most widely used abortive therapy in migraine, and in cluster headache along with oxygen therapy, have a largely neural basis for their action.<sup>51</sup> Similarly, ergotamine, a more historic serotonergic treatment used in the management of both disorders has a plausible neural action.<sup>51</sup> Increased understanding of this biology has broadened the scope of therapeutic treatment targets in migraine and cluster headache, both within the serotonergic system and outside. While vasodilatation mediated by vasoactive neuropeptides is a feature of both disorders, it is not the only feature and central changes are likely to be equally if not more important.<sup>2</sup>

A series of randomized controlled trials of lasmiditan, a specific 5-HT<sub>1F</sub> receptor agonist, for the acute treatment of migraine have demonstrated efficacy,<sup>48,49,52,53</sup> even in those with difficult to treat attacks<sup>54</sup> and triptan non-responders.<sup>55</sup> These studies only identified post-dose dizziness as an adverse effect that could limit use in some settings.<sup>56</sup> Providing a novel approach, this agent does not cause vasoconstriction and therefore provides a safe treatment option for the elderly<sup>57</sup> and in those with cardiovascular risk,<sup>58</sup> patient groups that have historically been underserved by the triptans and by the ergot alkaloids. Lasmiditan is now US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved and is providing an option for acute treatment for many migraine patients. However, for a condition which affects more than 1 billion people worldwide, and has such global impact,<sup>3</sup> there remains a paucity of targeted and effective treatments in migraine, and an ever increasing need to develop more. In particular, there is an emerging need to develop non-vasoactive drugs to treat the disorder, given that vasoconstriction is not needed for an acute therapeutic effect, and ideally ones that do not contribute to medication overuse, which forms another challenge in the acute management of migraine.

### New treatments are still required for migraine

Understanding the many facets to migraine and primary headache neurobiology is important to allow therapeutic development strategies to be explored. As peptidergic neurotransmission at the level of the dura mater is involved in migraine and cluster headache, and results in vasodilatation and secondary central phenomena,<sup>59,60</sup> targeting vasoactive neuropeptides has become a substantial area of interest in migraine and cluster headache therapeutics. Recently, over three decades of research into CGRP's role in migraine as one of these neuropeptides,<sup>61</sup> has led to the emergence of small molecule receptor antagonists (gepants) and monoclonal antibodies against

the CGRP peptide or the receptor (mAbs) becoming part of our clinical practice and transforming the lives of many with migraine.<sup>62</sup> All the phase 3 trials of the four different mAbs and seven gepants have shown consistent efficacy and good tolerability; however, these drugs are not useful for everyone and in some, may be contraindicated by active vascular disease because of theoretical concerns about inhibition of CGRP causing vasoconstriction. There is suggestion that there is an association between erenumab response and triptan response,<sup>63</sup> and an increased response to triptans in mAb super-responders,<sup>64</sup> while increasing numbers of previous preventives tried negatively impacts mAb efficacy.<sup>65</sup> The need for additional therapies for groups like triptan non-responders, who may be less likely to respond to CGRP-targeted therapies, and those with more refractory disease with several failed preventives, is therefore real. The use of treatment prediction and treatment biomarkers is an area in migraine that has been disappointing thus far, so identifying targets with distinct downstream intracellular signaling mechanisms that lack vasoactive effects is crucial to advance therapeutics in this area.

One such strategy that has been explored has been targeting PACAP.<sup>66</sup>

## Pituitary Cyclase-Activating Polypeptide 38 (PACAP38)

### Structure, function, and distribution in the nervous system

PACAP<sup>67</sup> belongs to the vasoactive intestinal polypeptide (VIP)–secretin–growth hormone-releasing hormone–glucagon superfamily,<sup>68,69</sup> and is found as a 38-amino acid peptide (PACAP38) and a truncated 27-amino-acid peptide (PACAP27).<sup>70</sup> PACAP shares 68% of its amino acid homology with VIP and therefore these two peptides share four receptors: PAC<sub>1</sub>, VPAC<sub>1</sub>, VPAC<sub>2</sub>, and a Mas-related G-protein-coupled receptor (member B2 in mouse and X2 in humans).<sup>71</sup>

PACAP38 is the predominant mammalian peptide and represents more than 90% of the total PACAP content in most tissues, including the central nervous system (CNS)<sup>68</sup>; but both isoforms have similar functions and receptor binding affinity.<sup>71</sup> PACAP was first identified in bovine hypothalamus in 1989. It was found to localize in sensory neurons<sup>72</sup> and could depress C-fiber-evoked responses.<sup>73</sup> Furthermore, it was demonstrated that PACAP extract could stimulate anterior pituitary cells in rats.<sup>74</sup> PACAP was subsequently found to be involved in many physiological and endocrinological functions, including vasodilatation,<sup>75,76</sup> and circadian rhythm<sup>77</sup> and feeding regulation.<sup>78</sup>

## PACAP expression

Peripherally, PACAP is expressed in several cranial ganglia relevant to migraine and cluster headache biology, including the sphenopalatine ganglion (SPG)<sup>79–82</sup> and trigeminal ganglion,<sup>83,84</sup> which have been examined the most closely with regards to co-expression of CGRP and PACAP.<sup>85,86</sup> PACAP mRNA for three PACAP receptors is found in middle meningeal artery.<sup>87</sup> Whereas CGRP is widely expressed on trigeminal neurons within the trigeminal ganglion, PACAP38 is found in far fewer trigeminal neurons,<sup>72,81</sup> but is more widely distributed in parasympathetic neurons, leading to interest in the involvement of this peptide in both migraine and the TACs; the latter include prominent cranial autonomic symptoms in their canonical phenotypes. VIP has been historically been implicated in the biology of cluster headache, because of its role in mediating cranial autonomic symptoms, and a study suggesting its release during spontaneous cluster attacks.<sup>88</sup> It has been hypothesized that via PACAP stimulating trigeminal CGRP release, the sensory and autonomic systems interact in migraine and cluster headache biology.<sup>89,90</sup> Centrally, PACAP and its receptors, like CGRP, are also widely expressed in brain areas of interest in migraine, such as thalamus, locus coeruleus, parabrachial nucleus and TNC. Recent work showed that PACAP38 had a weak expression with CGRP in trigeminal neurons; experiments showed that PACAP38 was only released from the trigeminal neurons and not the trigeminal nerve fibers and that its receptors were observed only on the adjacent satellite glial cells,<sup>91</sup> suggesting a somewhat distinct role in pain processing as compared to that of CGRP. A summary of the main areas of PACAP expression of interest in the primary headache disorders is shown in Figure 1.

## PACAP receptors

PACAP mediates its actions via three G-protein-coupled receptors: VPAC<sub>1</sub> and VPAC<sub>2</sub> receptors, for which PACAP and VIP have equal affinity, and the PAC<sub>1</sub> receptor, which is about 100-fold more PACAP specific.<sup>92</sup> Receptor activation causes cAMP release, which is among one of the mechanisms responsible for the intracellular and physiological effects of PACAP38. The PAC<sub>1</sub> receptor also stimulates intracellular calcium and protein kinase C activation.<sup>93</sup> Experimental animal models have shown that elevated cAMP can sensitize trigeminal neurons.<sup>94</sup> In addition, recent evidence has shown PACAP38 can activate the meningeal mast cells through Mas-related G-protein coupled receptor member B2 (MrgprB2) and promote pain behavior in mouse through the mouse homologue receptor to the human MRGPRX2 receptor,<sup>67</sup>

so this has emerged as a fourth PACAP receptor of interest. See Figure 2 for a summary of the four PACAP receptors, and their downstream intracellular signaling mechanisms by which receptor activation causes physiological effects of PACAP.

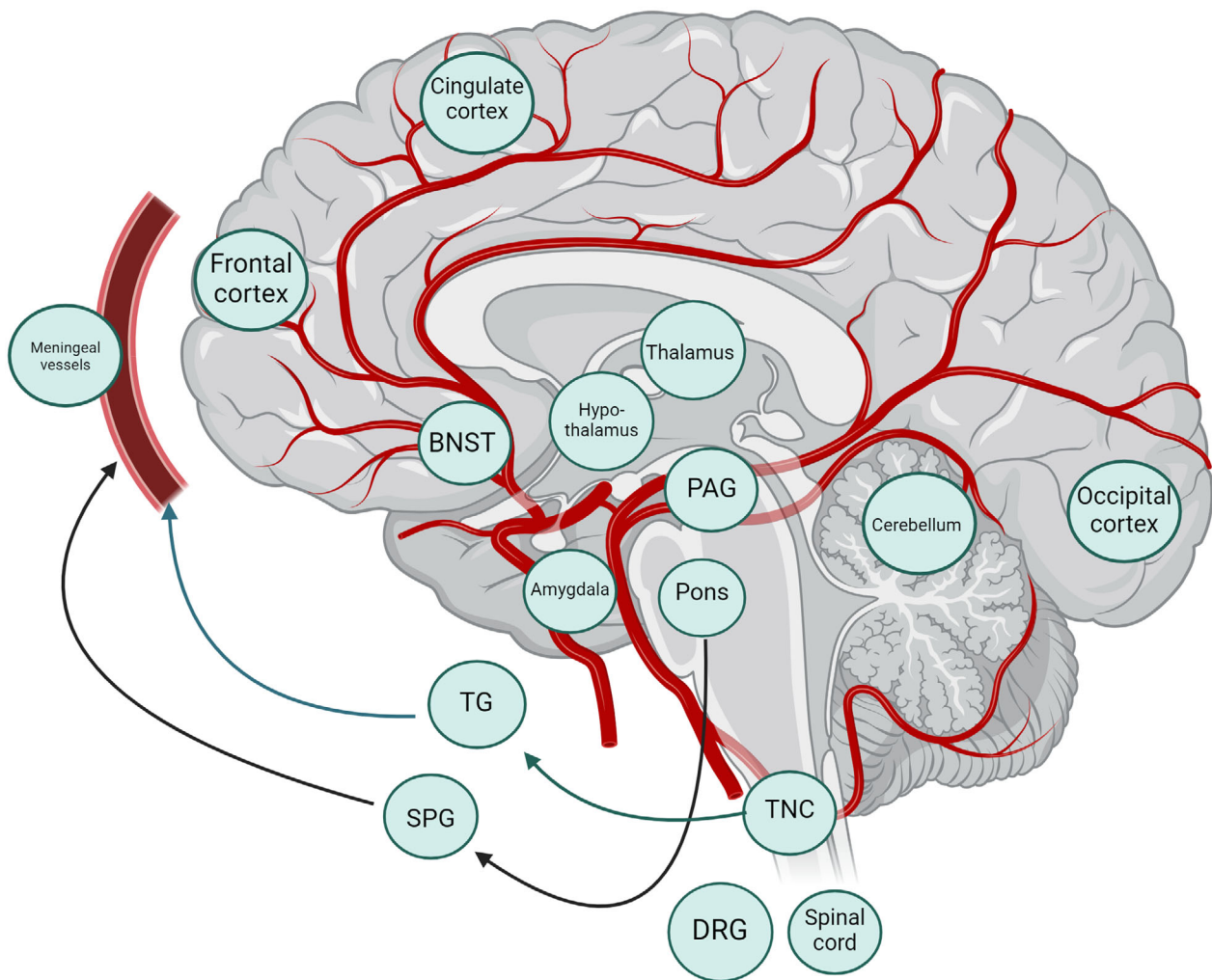
Each of the PAC<sub>1</sub>, VPAC<sub>1</sub>, and VPAC<sub>2</sub> receptors and their mRNA are expressed on human cerebral arteries<sup>87</sup> and cranial sensory and autonomic ganglia.<sup>95</sup> In an experimental migraine model, superior sagittal sinus stimulation causes PACAP release into the extracerebral circulation, where it mediates vasodilatation.<sup>96,97</sup> Interestingly, this is only reversed by VPAC<sub>1</sub> antagonism, suggesting that PAC<sub>1</sub> agonism does not contribute to vasodilatation.<sup>98</sup> In another study, both VIP and PACAP-38 caused short-lived meningeal vasodilatation mediated by the VPAC<sub>2</sub> receptor independently of activation of central trigeminovascular neurons. Intravenous delivery of a PAC<sub>1</sub> receptor antagonist specifically inhibited the meningeal vasodilatory effects of dural trigeminovascular activation, whereas only central (intracerebroventricular) administration of the drug inhibited dural nociceptive-evoked action potentials in central trigeminovascular neurons, suggesting that a central role of PACAP in migraine is likely in addition to the primary afferent innervation in the meninges.<sup>99</sup> In another animal model, middle meningeal artery dilatation was only inhibited via the PAC<sub>1</sub> receptor, and not via VPAC<sub>1</sub> or VPAC<sub>2</sub>,<sup>87</sup> again suggesting that PAC<sub>1</sub> may be a target without vasoconstrictive effects. Notably, in the sphenopalatine ganglion only the VPAC<sub>1</sub> receptor was found and not VPAC<sub>2</sub> or PAC<sub>1</sub>,<sup>95</sup> which has led to interest in this receptor in particular in migraine and cluster headache therapy.

## PACAP38—Preclinical Evidence in Migraine and Cluster Headache

The PAC<sub>1</sub> receptor is more specific to PACAP compared to VIP, and has therefore had the most interest in migraine,<sup>100</sup> particularly given initial suggestions that VIP was not implicated in the pain part of migraine,<sup>101</sup> although there are suggestions of its role in inducing episodic cluster headache in bout when administered intravenously.<sup>102</sup> PACAP and VIP, as well as being from the same neuropeptide family and acting on similar receptors, also functionally interact. PACAP fibers innervate VIP neurons<sup>103</sup> and PACAP promotes VIP gene expression<sup>104</sup> and VIP release,<sup>105</sup> suggesting a possible role of both peptides in both migraine and cluster headache.

Unlike CGRP, which is a somewhat larger molecule peptide and is thought to not penetrate the blood–brain barrier in significant concentrations, PACAP has a transporter pump offering the ability to cross the blood–brain barrier.<sup>106</sup> This may explain why premonitory symptoms



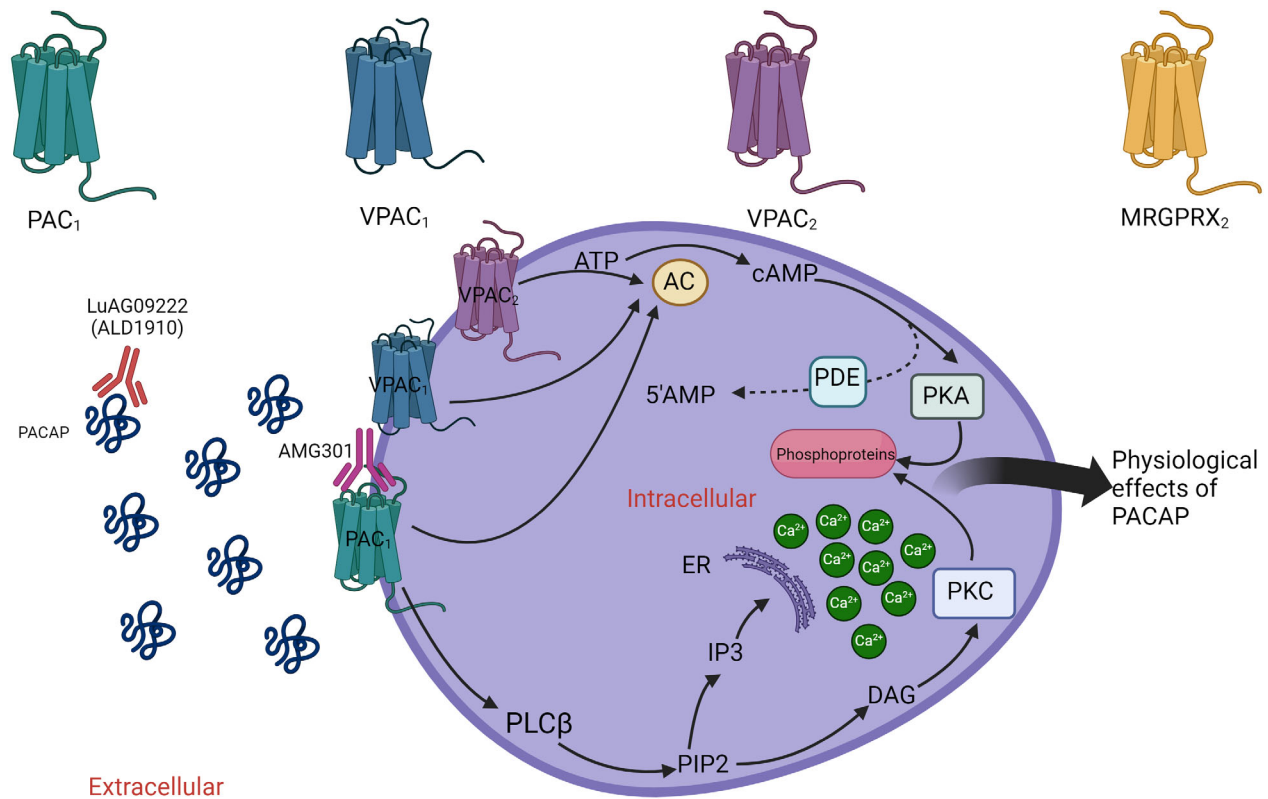


**Figure 1.** Summary of the main expression sites of PACAP and its receptors within the brain, cranial and spinal ganglia, spinal cord, and extracranial vasculature. DRG; dorsal root ganglion, PAG; periaqueductal grey, SPG; sphenopalatine ganglion, TG; trigeminal ganglion, TNC; trigeminal nucleus caudalis. Image created using [BioRender.com](https://www.biorender.com).

are less readily clinically provoked by CGRP compared to NTG and PACAP.<sup>107–110</sup> PACAP is heavily expressed in the hypothalamus,<sup>71</sup> and this area of the brain is thought to be crucial in mediating premonitory symptoms.<sup>8,13,111</sup> Moreover, these symptoms have also been more recently identified as occurring in cluster headache.<sup>12,43–45</sup> Interestingly, PACAP is only released in the trigeminal ganglion and not from the sensory nerve fibers or in the dura mater of rat.<sup>91</sup> PACAP receptors: PAC<sub>1</sub> and VPAC<sub>1/2</sub>, are found on trigeminal ganglion neurons and satellite glial cells.<sup>112</sup> Taken together, these data suggest a potential therapeutic target against PACAP may need to include the central nervous system to some extent.<sup>85,99</sup> Germane to this, post-traumatic stress disorder is associated with PACAP, particularly via PAC<sub>1</sub>-mediated mechanisms.<sup>113,114</sup>

### PACAP and the trigeminovascular system

In animal models, PACAP has a role in trigeminovascular sensitization and photophobia.<sup>115</sup> PACAP and VIP both cause vasodilatation,<sup>87</sup> but only PACAP causes TCC activation and hypersensitivity to somatosensory cranial stimulation.<sup>99</sup> When PACAP is locally applied to meningeal afferents, it promotes the central release of CGRP from the TNC, whereas VIP has no effect.<sup>89</sup> In line with these observations, PACAP38-induced migraine-like attacks do not involve changes in blood CGRP levels.<sup>116</sup> When complete Freund's adjuvant is applied to rat dura mater or orofacially to provoke inflammation, tissue necrosis, and ulceration, PACAP immunoreactivity was increased in the TNC.<sup>117,118</sup> PACAP also causes mast cell degranulation, which may contribute to its effect in



**Figure 2.** Summary of the intracellular downstream effects of PACAP. PACAP binds to four different G-protein-coupled receptors (PAC<sub>1</sub>, VPAC<sub>1</sub>, VPAC<sub>2</sub>, and MRGPRX<sub>2</sub>) to exert its effects. Receptor activation of PAC<sub>1</sub>, VPAC<sub>1</sub>, and VPAC<sub>2</sub> causes release of intracellular cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) via adenylate cyclase (AC). cAMP is degraded to 5'adenosine monophosphate (5'7AMP) via phosphodiesterases (PDE). cAMP causes protein kinase A (PKA) activation and phosphoprotein production. Activation of PAC<sub>1</sub> also causes release phospholipase C $\beta$  (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) and produces intracellular mediators inositol triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG), which lead to increased intracellular calcium (Ca<sup>2+</sup>) release from the endoplasmic reticulum (ER) and phosphoprotein production via protein kinase C (PKC), respectively. These are responsible for the physiological effects of PACAP, including trigeminal neuronal sensitization. ALD1910 and AMG301 are two mAbs developed for migraine treatment. Lu AG09222 (ALD1910) binds to the PACAP ligand, and AMG301 binds to the PAC<sub>1</sub> receptor. Both hold therapeutic scope for migraine. Image created using BioRender.com.

migraine.<sup>119</sup> PACAP is elevated in the TNC in rat after nitroglycerin (NTG) injection,<sup>120</sup> and NTG is a commonly used migraine trigger in experimental study of migraine in humans and in animal models of migraine.

### PACAP centrally

NTG promotes PACAP immunoreactivity in the TNC in a rodent model,<sup>115,120</sup> and nitric oxide mechanisms may therefore play a role in neuronal sensitization via PACAP in migraine. Unlike CGRP which is pro-nociceptive, PACAP has pro- and antinociceptive properties and can reduce TCC responses to dural stimulation when locally injected into the VTA parabrachial pigmented nucleus.<sup>121</sup> PACAP-deficient mice exhibit increased thermal allodynia (antinociceptive), but centrally PACAP mediates neuronal excitation in areas like PAG and somatosensory cortex

(pro-nociceptive), so there are divergent effects peripherally and centrally.<sup>122,123</sup> Central excitatory effects of PACAP have been demonstrated in other studies, via areas of interest in migraine such as the hypothalamus,<sup>124</sup> and the TCC.<sup>99</sup> A rodent study of nociceptive trigemino-vascular activation demonstrated that PACAP causes activation of trigeminocervical neurons with sensitization and that this effect is reversed only through a centrally administered PAC<sub>1</sub> receptor antagonist. The same drug administered intravenously reduced the peripheral result of central trigemino-vascular activation, with reduced meningeal vasodilatation. This study, therefore, suggested that a central PAC<sub>1</sub> mechanism is important in migraine and could be exploited for clinical therapeutics.<sup>99</sup> PACAP may also have a modulatory role in facilitating trigemino-vascular nociception at the level of the hypothalamus via a PAC<sub>1</sub> mechanism.<sup>124</sup> In addition, kynurenic acid<sup>125</sup>

inhibits increased expression of PACAP in the trigeminal nucleus caudalis evoked by trigeminal ganglion stimulation,<sup>126</sup> suggesting that PACAP may be mediating its role in migraine via glutamatergic neurotransmission.

### **PACAP, photophobia, and allodynia**

A shared role of CGRP and PACAP in migraine biology is in mediating photophobia. However, these mechanisms are likely to be distinct, given that both can trigger light aversive behaviors in animal models, but CGRP-mediated light aversion could only be inhibited by a CGRP monoclonal antibody and not a PACAP one, and vice versa.<sup>127</sup> PACAP can also provoke periorbital allodynia in animal models, and this can be reversed with a PACAP receptor antagonist.<sup>128</sup> Other mouse models have also suggested distinct roles of CGRP and PACAP in mediating migraine-like behaviors, such as hypersensitivity as a correlate for allodynia.<sup>129</sup> Mice pretreated with an anti-CGRP antibody and receptor activity-modifying protein 1 (RAMP-1) knockout mice lacking CGRP receptors can still display allodynic behaviors in response to PACAP, but NTG-mediated allodynia could be prevented by anti-CGRP antibodies and in the RAMP-1 knockout mice. NTG and CGRP, therefore, are likely to act via shared mechanisms and these are distinct from PACAP in mediating sensory sensitivities, but the interactions of these molecules in the wider migraine phenotype remain unclear. A role of PACAP in intracellular signaling pathways, some shared with CGRP given the co-expression of both peptides in many brain regions involved in migraine, like those via cAMP, is also likely.<sup>130</sup>

## **PACAP38—Clinical Evidence in Migraine and Cluster Headache**

### **PACAP is released in migraine and cluster headache**

PACAP, like CGRP, is released during acute migraine in both experimental and clinical settings, and blood levels are reduced following sumatriptan administration.<sup>96</sup> PACAP is also released into the circulation during episodic cluster headache bouts compared to the interbout period.<sup>131</sup> There is also suggestion that blood levels of PACAP fluctuate in a dynamic fashion during different phases of migraine and may actually be lower in patients with migraine compared to healthy controls interictally, but peak ictally.<sup>132</sup> Interestingly, PACAP levels are elevated in migraine attacks, but VIP levels are only elevated if there are cranial autonomic symptoms associated,<sup>59</sup> and both are elevated in the cranial circulation in cluster headache,<sup>88,131</sup> a condition in which CAS are necessary

for diagnosis. This suggests that PACAP and VIP are both involved in the mediation of CAS, with PACAP having additional roles in trigeminovascular nociceptive processing. Inter-attack PACAP levels in migraine patients correlate with mean diffusivity in white matter,<sup>133</sup> so these may serve a biomarker role as therapeutic avenues are explored.

### **PACAP administration can trigger migraine and cluster headache attacks**

PACAP38 can also trigger migraine-like attacks,<sup>134</sup> cluster headache attacks,<sup>102,135</sup> and premonitory symptoms associated with migraine<sup>107</sup> when infused intravenously into patients with migraine, cluster headache in bout or chronic cluster headache. PACAP38 triggers migraine when infused more readily than VIP in a double-blind crossover head-to-head study (73% vs. 18%).<sup>136</sup> More recently, a prolonged VIP infusion has been shown to provoke migraine attacks in 71% in one study (compared to 5% with placebo),<sup>137</sup> and VIP infusion was associated with prolonged extracranial vasodilatation.<sup>138</sup> An earlier study had suggested no difference in headache reporting following VIP and placebo, despite marked extracranial vasodilatation after VIP.<sup>101</sup> PACAP and VIP triggering rates in episodic cluster headache in bout and in chronic cluster headaches were equivalent between the two peptides in one study.<sup>102</sup> VIP-targeted treatments have thus far not been explored in migraine or cluster headache.

Interestingly, PACAP38 infusion is not associated with regional cerebral blood flow changes in human healthy volunteers,<sup>139</sup> but PACAP-triggered migraine can be prevented with pre-medication with sumatriptan.<sup>140</sup> Angiographic imaging has shown that like NTG and CGRP, PACAP is a vasodilator of the extracranial vasculature and does not affect intracranial arteries.<sup>136,141,142</sup> PACAP-triggered migraine is associated with widespread functional brain network changes.<sup>143</sup> There is a suggestion that PACAP27 can also trigger migraine,<sup>144</sup> and cause extracranial vasodilatation.<sup>145</sup>

A summary of the similarities and differences between CGRP and PACAP receptors, expression and mechanisms in migraine is shown in Table 1.

## **PACAP—Therapeutic Scope in Migraine**

### **Targeting the PAC1 receptor**

A rodent-specific PAC<sub>1</sub> receptor antibody reduced TCC firing in response to stimulation, mediated via its binding at the trigeminal and sphenopalatine ganglia, without central binding.<sup>146</sup> This study further supported the role

**Table 1.** A summary of some of the similarities and differences between CGRP and PACAP in migraine biology (developed using<sup>129</sup>).

	CGRP	PACAP
Receptors (all G-protein-coupled)	Canonical CGRP receptor (GPCR calcitonin receptor-like receptor CLR and RAMP1) AMY <sub>1</sub> with equal affinity Lower affinity for CLR/RAMP2 and CLR/RAMP3 adrenomedullin receptors	PAC <sub>1</sub> VPAC <sub>1</sub> VPAC <sub>2</sub> MRGPRX2
Migraine mechanisms	Vasodilation, dural mast cell degranulation, pro-nociceptive	Vasodilation, dural mast cell degranulation, peripherally antinociceptive, centrally nociceptive
Infusion triggers migraine-like headache	63%	72%
Infusion triggers premonitory symptoms before migraine-like headache	9%	48%
Vasodilatory side effects of infusion (flushing, feeling hot, palpitations, and light-headedness)	Yes	Yes
Nervous system distribution	Shared other than CGRP more in trigeminal ganglion	Shared other than PACAP more in sphenopalatine ganglion
Role in anxiety behaviours	Yes	Yes
Role in light aversion	Yes	Yes (mechanism distinct to CGRP)
Role in allodynia	Yes	Yes (although perhaps via mechanisms distinct to CGRP/nitric oxide)
Intracellular mechanisms	Less robust intracellular mechanisms via inositol 1,4,5-triphosphate	More robust intracellular mechanisms via inositol 1,4,5-triphosphate (via non-canonical cAMP mechanisms and exchange protein directly activated by cAMP (EPAC's)
Action on ATP-sensitive potassium channels	Yes	Only partial effect

of targeting the PAC<sub>1</sub> receptor in migraine therapeutics. A subsequent phase 2 clinical study unfortunately showed that a PAC<sub>1</sub> receptor antibody: AMG 301, was ineffective in migraine prevention at different doses.<sup>147</sup>

A preclinical study used a PAC<sub>1</sub> receptor antibody to map PAC<sub>1</sub> receptor expression in the trigeminal-autonomic system in both rat and man using immunohistochemistry and in situ hybridization.<sup>81</sup> The study demonstrated PAC<sub>1</sub> immunoreactivity in SPG with only weak expression in trigeminal ganglion, and strong immunoreactivity in spinal trigeminal nucleus, but no expression in dura mater vessels, and these findings were confirmed with in situ hybridization.<sup>81</sup> This finding was consistent with previous data and suggests that the vascular effects of PACAP are mediated by the VPAC receptors rather than the PAC<sub>1</sub> receptor,<sup>95</sup> making targeting PAC<sub>1</sub> an exciting strategy for migraine potentially without substantial vascular effects, akin to specific targeting of the 5HT<sub>1F</sub> receptor in the serotonergic system.

The disappointing lack of success of a PAC<sub>1</sub> receptor antibody led to the development of strategies to target the PACAP ligand, and also raised the possibility of targeting VPAC<sub>1/2</sub> receptors as an approach in migraine. This work has also led to consideration of the concept that PACAP may be mediating its actions in migraine, such as direct actions at the TNC, to stimulate CGRP release where

PACAP and CGRP are co-localized,<sup>89</sup> or via its effect on mast cells,<sup>67</sup> or at alternative yet-to-be identified receptors.

Recently, PAC<sub>1</sub> small molecule antagonists have been identified, one of which seems to show anti-allodynic effects in an animal neuropathic pain model when administered orally or intrathecally,<sup>148</sup> and another showing anti-nociceptive effects in a rodent inflammatory pain model.<sup>149</sup> Interestingly, a role of these agents in anxiety behaviors has also been identified.<sup>150</sup> Whether these are tested in migraine models remains to be seen.

### Targeting PACAP directly

Lu AG09222 (ALD1910) is a high-affinity PACAP-neutralizing antibody which binds the ligand. A phase 1 double-blind parallel-group placebo-controlled study showed it could inhibit cephalic vasodilatation induced by PACAP in healthy volunteers, as well as reduce PACAP- and VIP-induced facial vasodilatation and mild headache.<sup>151</sup> Subsequently, a phase 2 double-blind placebo-controlled trial showed a high dose (750 mg) of Lu AG09222 compared to a low dose (100 mg) and to placebo reduced migraine frequency over a 4-week endpoint in participants with 2–4 previous preventive treatment failures.<sup>152</sup> High dose Lu AG0222 reduced monthly



migraine days by  $-6.2$  (compared to placebo  $-4.2$ ), with a baseline migraine frequency across the groups of mixed episodic and chronic migraine, of 16.7 migraine days per month.<sup>153</sup> This study has paved the way for further development of approaches to this target.

## PACAP—Therapeutic Scope in Cluster Headache

While no dedicated studies using PACAP-targeted treatments have been conducted in cluster headache, PACAP targeting poses an exciting opportunity for treating cluster headache based on the known pathophysiology. Features of cluster headache include the prominent cranial autonomic symptoms are experienced by most cluster headache patients, and the equivalent triggering of cluster headache attacks by VIP and PACAP38, which share receptors, as well as the shared neurobiology between migraine and cluster headache suggest that targeting PACAP may hold promise in cluster headache in the future. If phase 3 studies with Lu AG09222 are successful in migraine, this could lead to dedicated cluster headache trials, for a population of patients who have very few available treatment options.

## The Need for More

While PACAP poses an exciting therapeutic avenue in migraine and cluster headache, thus far we only have a failed phase 2 trial of a PAC<sub>1</sub> receptor antibody and an encouraging phase 2 trial of a ligand PACAP antibody with results available. Given emerging evidence that VIP may have a role in migraine induction, based on the more recent triggering data,<sup>137</sup> and demonstration of elevated blood levels interictally in migraine,<sup>154</sup> as well as ictally in both blood and saliva,<sup>155</sup> contrary to what had been thought previously, further targeting of VPAC<sub>1/2</sub> receptors is likely to be an avenue of future interest. While treatment prediction and biomarkers of treatment response are not currently available, the emergence of targeted peptidergic therapies and the ability of these peptides to trigger migraine and cluster attacks in some patients may allow us to witness a time where triggering efficacy allows treatment efficacy to be estimated. This would ideally lead to personalization of treatment with potentially high-cost drugs.

The CGRP-related peptides; adrenomedullin and amylin, both of which are able to provoke migraine attacks in patients via the amylin analog pramlintide (with equivalent efficacy to CGRP),<sup>156</sup> and intravenous adrenomedullin,<sup>157</sup> have also gained interest as possible therapeutic substrates in migraine, providing alternative receptors as targets. The CGRP-targeted therapies used in

migraine management may have weak effects at the amylin receptor AMY<sub>1</sub>,<sup>158–160</sup> and also alter adrenomedullin signaling at the canonical CGRP receptor.<sup>161</sup>

As well as targeting neuropeptides, intracellular targets such as nitric oxide, and ion channel targets like potassium channels, are also of interest in advancing migraine therapeutics.<sup>162</sup>

## Conclusion

Targeting the PACAP pathway, as a neuropeptide pathway distinct from CGRP, holds therapeutic promise in migraine therapeutics going forwards. Further targeted treatment development against the VPAC<sub>1/2</sub> receptors may yield exciting results in this area. Moreover, the presence of cranial autonomic symptoms may serve as a future biomarker for response to targeted therapeutics against the cranial parasympathetic projection, modulated via PACAP.<sup>163</sup>

In migraine therapeutics development, there has not yet been a false-positive phase 2 study, so it is hopeful that PACAP-targeted therapies will be the next big advance in migraine therapeutics, offering some mechanisms distinct from CGRP to help those underserved by these therapies. This effect may be translated to other primary headache disorders, including cluster headache and the other TAC's in due course, exploiting cranial autonomic symptoms as a potential biomarker. However, there will always remain a need for the identification of new targets to help deal with the burden that is migraine.

## AUTHOR CONTRIBUTIONS

LE, LV, and PJG were involved in conceptualization. NK, LE, LV, and PJG were involved in the literature review, analysis and interpretation of the results, and manuscript preparation.

## Acknowledgments

None.

## Funding Information

None.

## Conflict of Interest Statement

NK has nothing to declare. LE has given talks sponsored by Pfizer, Abbvie, TEVA, and Novartis. LV has nothing to declare. PJG reports, over the last 36 months, grants from Celgene and Kallyope, and personal fees from Aeon Biopharma, Abbvie, Aurene, CoolTech LLC, Dr Reddy's, Eli

Lilly and Company, Linpharma, Lundbeck, Pfizer, Pure-Tech Health LLC, Satsuma, Shiratronics, Teva Pharmaceuticals, Tremereau, and Vial, and personal fees for advice through Gerson Lehrman Group, Guidepoint, SAI Med Partners, Vector Metric, and fees for educational materials from CME Outfitters and WebMD, and publishing royalties or fees from Massachusetts Medical Society, Oxford University Press, UptoDate and Wolters Kluwer, and a patent magnetic stimulation for headache (No. WO2016090333 A1) assigned to eNeura without fee.

## References

- Karsan N, Gosalia H, Goadsby PJ. Molecular mechanisms of migraine: nitric oxide synthase and neuropeptides. *Int J Mol Sci.* 2023;24:11993.
- Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S. Pathophysiology of migraine: a disorder of sensory processing. *Physiol Rev.* 2017;97:553-622.
- Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Lancet.* 2020;396:1204-1222.
- Meylakh N, Marciszewski KK, di Pietro F, Macefield VG, Macey PM, Henderson LA. Brainstem functional oscillations across the migraine cycle: a longitudinal investigation. *NeuroImage Clinical.* 2021;30:102630.
- Marciszewski KK, Meylakh N, di Pietro F, et al. Changes in brainstem pain modulation circuitry function over the migraine cycle. *J Neurosci.* 2018;38:10479-10488.
- Marciszewski KK, Meylakh N, di Pietro F, Macefield VG, Macey PM, Henderson LA. Fluctuating regional brainstem diffusion imaging measures of microstructure across the migraine cycle. *eNeuro.* 2019;6:1-11.
- Stankewitz A, Schulz E. Intrinsic network connectivity reflects the cyclic trajectory of migraine attacks. *Neurobiol Pain.* 2022;11:100085.
- Schulte LH, Mehnert J, May A. Longitudinal neuroimaging over 30 days: temporal characteristics of migraine. *Ann Neurol.* 2020;87:646-651.
- Schulte LH, May A. The migraine generator revisited: continuous scanning of the migraine cycle over 30 days and three spontaneous attacks. *Brain.* 2016;139:1987-1993.
- Stankewitz A, Keidel L, Rehm M, et al. Migraine attacks as a result of hypothalamic loss of control. *NeuroImage Clinical.* 2021;32:102784.
- Karsan N, Goadsby PJ. Biological insights from the premonitory symptoms of migraine. *Nat Rev Neurol.* 2018;14:699-710.
- Karsan N, Bose RP, O'Daly O, Zelaya F, Goadsby PJ. Regional cerebral perfusion during the premonitory phase of triggered migraine: a double-blind randomized placebo-controlled functional imaging study using pseudo-continuous arterial spin labeling. *Headache.* 2023;63:771-787.
- Maniyar FH, Sprenger T, Monteith T, Schankin C, Goadsby PJ. Brain activations in the premonitory phase of nitroglycerin-triggered migraine attacks. *Brain.* 2014;137:232-241.
- Denuelle M, Fabre N, Payoux P, Chollet F, Geraud G. Hypothalamic activation in spontaneous migraine attacks. *Headache.* 2007;47:1418-1426.
- Weiller C, May A, Limmroth V, et al. Brain stem activation in spontaneous human migraine attacks. *Nat Med.* 1995;1:658-660.
- Bahra A, Matharu MS, Buchel C, Frackowiak RS, Goadsby PJ. Brainstem activation specific to migraine headache. *Lancet.* 2001;357:1016-1017.
- Afridi SK, Matharu MS, Lee L, et al. A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate. *Brain.* 2005;128:932-939.
- May A, Kaube H, Büchel C, et al. Experimental cranial pain elicited by capsaicin: a PET study. *Pain.* 1998;74:61-66.
- May A, Bahra A, Buchel C, Frackowiak RS, Goadsby PJ. Hypothalamic activation in cluster headache attacks. *Lancet.* 1998;352:275-278.
- Matharu MS, Cohen AS, McGonigle DJ, Ward N, Frackowiak RS, Goadsby PJ. Posterior hypothalamic and brainstem activation in hemicrania continua. *Headache.* 2004;44:747-761.
- Edvinsson L, Edvinsson JCA, Haanes KA. Biological and small molecule strategies in migraine therapy with relation to the calcitonin gene-related peptide family of peptides. *Br J Pharmacol.* 2022;179:371-380.
- Maniyar FH, Sprenger T, Schankin C, Goadsby PJ. The origin of nausea in migraine—a PET study. *J Headache Pain.* 2014;15:84.
- Maniyar FH, Sprenger T, Schankin C, Goadsby PJ. Photic hypersensitivity in the premonitory phase of migraine—a positron emission tomography study. *Eur J Neurol.* 2014;21:1178-1183.
- Rasmussen BK, Olesen J. Migraine with aura and migraine without aura: an epidemiological study. *Cephalalgia.* 1992;12:221-228; discussion 186.
- Cao Y, Welch KM, Aurora S, Vikingstad EM. Functional MRI-BOLD of visually triggered headache in patients with migraine. *Arch Neurol.* 1999;56:548-554.
- Lauritzen M, Olesen J. Regional cerebral blood flow during migraine attacks by Xenon-133 inhalation and emission tomography. *Brain.* 1984;107(Pt 2):447-461.
- Leao A. Spreading depression of activity in the cerebral cortex. *J Neurophysiol.* 1944;7:359-390.
- Hadjikhani N, Sanchez del Rio M, Wu O, et al. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc Natl Acad Sci U S A.* 2001;98:4687-4692.

29. Olesen J, Larsen B, Lauritzen M. Focal hyperemia followed by spreading oligemia and impaired activation of rCBF in classic migraine. *Ann Neurol*. 1981;9:344-352.
30. Goadsby PJ, Hoskin KL. The distribution of trigeminovascular afferents in the nonhuman primate brain *Macaca nemestrina*: a c-fos immunocytochemical study. *J Anat*. 1997;190(Pt 3):367-375.
31. Ray BS, Wolff HG. Experimental studies on headache. Pain sensitive structures of the head and their significance in headache. *Arch Surg*. 1940;51:813-856.
32. Kaube H, Keay KA, Hoskin KL, Bandler R, Goadsby PJ. Expression of c-Fos-like immunoreactivity in the caudal medulla and upper cervical spinal cord following stimulation of the superior sagittal sinus in the cat. *Brain Res*. 1993;629:95-102.
33. Bartsch T, Goadsby PJ. Increased responses in trigeminocervical nociceptive neurons to cervical input after stimulation of the dura mater. *Brain*. 2003;126:1801-1813.
34. Liu Y, Broman J, Edvinsson L. Central projections of the sensory innervation of the rat middle meningeal artery. *Brain Res*. 2008;1208:103-110.
35. Liu Y, Broman J, Edvinsson L. Central projections of sensory innervation of the rat superior sagittal sinus. *Neuroscience*. 2004;129:431-437.
36. Bartsch T, Goadsby PJ. Anatomy and physiology of pain referral patterns in primary and cervicogenic headache disorders. *Headache Currents*. 2005;2:42-48.
37. May A, Goadsby PJ. The trigeminovascular system in humans: pathophysiologic implications for primary headache syndromes of the neural influences on the cerebral circulation. *Journal of Cerebral Blood Flow and Metabolism*. 1999;19:115-127.
38. May A, Bahra A, Buchel C, Frackowiak RS, Goadsby PJ. PET and MRA findings in cluster headache and MRA in experimental pain. *Neurology*. 2000;55:1328-1335.
39. Hoffmann J, May A. Diagnosis, pathophysiology, and management of cluster headache. *Lancet Neurol*. 2018;17:75-83.
40. Liu Y, Broman J, Zhang M, Edvinsson L. Brainstem and thalamic projections from a craniovascular sensory nervous centre in the rostral cervical spinal dorsal horn of rats. *Cephalalgia*. 2009;29:935-948.
41. Wei DY, O'Daly O, Zelaya FO, Goadsby PJ. Areas of cerebral blood flow changes on arterial spin labelling with the use of symmetric template during nitroglycerin triggered cluster headache attacks. *NeuroImage Clinical*. 2022;33:102920.
42. May A, Bahra A, Buchel C, Turner R, Goadsby PJ. Functional magnetic resonance imaging in spontaneous attacks of SUNCT: short-lasting neuralgiform headache with conjunctival injection and tearing. *Ann Neurol*. 1999;46:791-794.
43. Snoer A, Lund N, Beske R, Jensen R, Barloese M. Pre-attack signs and symptoms in cluster headache: characteristics and time profile. *Cephalalgia*. 2018;38:1128-1137.
44. Snoer A, Lund N, Beske R, Hagedorn A, Jensen RH, Barloese M. Cluster headache beyond the pain phase: a prospective study of 500 attacks. *Neurology*. 2018;91:e822-e831.
45. Pedersen AS, Snoer A, Barloese M, Petersen A, Jensen RH. Prevalence of pre-cluster symptoms in episodic cluster headache: is it possible to predict an upcoming bout? *Cephalalgia*. 2021;41:799-809.
46. Amin FM, Asghar MS, Hougaard A, et al. Magnetic resonance angiography of intracranial and extracranial arteries in patients with spontaneous migraine without aura: a cross-sectional study. *Lancet Neurol*. 2013;12:454-461.
47. Nielsen TH, Tfelt-Hansen P, Iversen HK. Asymmetry of temporal artery diameters during spontaneous attacks of cluster headache. *Headache*. 2009;49:383-385.
48. Kuca B, Silberstein SD, Wietecha L, et al. Lasmiditan is an effective acute treatment for migraine: a phase 3 randomized study. *Neurology*. 2018;91:e2222-e2232.
49. Goadsby PJ, Wietecha LA, Dennehy EB, et al. Phase 3 randomized, placebo-controlled, double-blind study of lasmiditan for acute treatment of migraine. *Brain*. 2019;142:1894-1904.
50. Limmroth V, May A, Auerbach P, Wosnitza G, Eppe T, Diener HC. Changes in cerebral blood flow velocity after treatment with sumatriptan or placebo and implications for the pathophysiology of migraine. *J Neurol Sci*. 1996;138:60-65.
51. Moskowitz MA. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. *Trends Pharmacol Sci*. 1992;13:307-311.
52. Ashina M, Reuter U, Smith T, et al. Randomized, controlled trial of lasmiditan over four migraine attacks: findings from the CENTURION study. *Cephalalgia*. 2021;41:294-304.
53. Färkkilä M, Diener HC, Géraud G, et al. Efficacy and tolerability of lasmiditan, an oral 5-HT<sub>1F</sub> receptor agonist, for the acute treatment of migraine: a phase 2 randomised, placebo-controlled, parallel-group, dose-ranging study. *Lancet Neurol*. 2012;11:405-413.
54. Tepper SJ, Vasudeva R, Krege JH, et al. Evaluation of 2-hour post-dose efficacy of lasmiditan for the acute treatment of difficult-to-treat migraine attacks. *Headache*. 2020;60:1601-1615.
55. Reuter U, Krege JH, Lombard L, et al. Lasmiditan efficacy in the acute treatment of migraine was independent of prior response to triptans: findings from the CENTURION study. *Cephalalgia*. 2022;42:20-30.
56. Tepper SJ, Krege JH, Lombard L, et al. Characterization of dizziness after Lasmiditan usage: findings from the SAMURAI and SPARTAN acute migraine treatment randomized trials. *Headache*. 2019;59:1052-1062.

57. Martin VT, Ahmed Z, Hochstetler HM, et al. Tolerability and safety of Lasmiditan treatment in elderly patients with migraine: post hoc analyses from randomized studies. *Clin Ther.* 2021;43:1066-1078.
58. Shapiro RE, Hochstetler HM, Dennehy EB, et al. Lasmiditan for acute treatment of migraine in patients with cardiovascular risk factors: post-hoc analysis of pooled results from 2 randomized, double-blind, placebo-controlled, phase 3 trials. *J Headache Pain.* 2019;20:90.
59. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol.* 1990;28:183-187.
60. Goadsby PJ, Edvinsson L. The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol.* 1993;33:48-56.
61. Edvinsson L, Haanes KA, Warfvinge K, Krause DN. CGRP as the target of new migraine therapies - successful translation from bench to clinic. *Nat Rev Neurol.* 2018;14:338-350.
62. Silvestro M, Orologio I, Siciliano M, et al. Emerging drugs for the preventive treatment of migraine: a review of CGRP monoclonal antibodies and gepants trials. *Expert Opin Emerg Drugs.* 2023;28:79-96.
63. Frattale I, Caponnetto V, Casalena A, et al. Association between response to triptans and response to erenumab: real-life data. *J Headache Pain.* 2021;22:1.
64. Raffaelli B, Fitzek M, Overeem LH, Storch E, Terhart M, Reuter U. Clinical evaluation of super-responders vs. non-responders to CGRP(-receptor) monoclonal antibodies: a real-world experience. *J Headache Pain.* 2023;24:16.
65. Hong JB, Lange KS, Overeem LH, Triller P, Raffaelli B, Reuter U. A scoping review and meta-analysis of anti-CGRP monoclonal antibodies: predicting response. *Pharmaceuticals (Basel).* 2023;16:1-19.
66. Tajti J, Tuka B, Botz B, Helyes Z, Vecsei L. Role of pituitary adenylate cyclase-activating polypeptide in nociception and migraine. *CNS Neurol Disord Drug Targets.* 2015;14:540-553.
67. Sbei S, Moncrief T, Limjunyawong N, Zeng Y, Green DP. PACAP activates MRGPRX2 on meningeal mast cells to drive migraine-like pain. *Sci Rep.* 2023;13:12302.
68. Arimura A, Shioda S. Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors: neuroendocrine and endocrine interaction. *Front Neuroendocrinol.* 1995;16:53-88.
69. Arimura A. Pituitary adenylate cyclase activating polypeptide (PACAP): discovery and current status of research. *Regul Pept.* 1992;37:287-303.
70. Miyata A, Jiang L, Dahl RD, et al. Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). *Biochem Biophys Res Commun.* 1990;170:643-648.
71. Vaudry D, Falluel-Morel A, Bourgault S, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol Rev.* 2009;61:283-357.
72. Mulder H, Uddman R, Moller K, et al. Pituitary adenylate cyclase activating polypeptide expression in sensory neurons. *Neuroscience.* 1994;63:307-312.
73. Zhang YZ, Sjölund B, Moller K, Håkanson R, Sundler F. Pituitary adenylate cyclase activating peptide produces a marked and long-lasting depression of a C-fibre-evoked flexion reflex. *Neuroscience.* 1993;57:733-737.
74. Miyata A, Arimura A, Dahl RR, et al. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun.* 1989;164:567-574.
75. Syed AU, Koide M, Braas KM, May V, Wellman GC. Pituitary adenylate cyclase-activating polypeptide (PACAP) potently dilates middle meningeal arteries: implications for migraine. *J Mol Neurosci.* 2012;48:574-583.
76. Uddman R, Goadsby PJ, Jansen I, Edvinsson L. PACAP, a VIP-like peptide: immunohistochemical localization and effect upon cat pial arteries and cerebral blood flow. *Journal of Cerebral Blood Flow and Metabolism.* 1993;13:291-297.
77. Holland PR, Barloese M, Fahrenkrug J. PACAP in hypothalamic regulation of sleep and circadian rhythm: importance for headache. *J Headache Pain.* 2018;19:20.
78. Maunze B, Choi S. Hypothalamic PACAP/PAC1R involvement in feeding and body weight regulation. *Endocrinology.* 2023;164:1-9.
79. Uddman R, Tajti J, Möller S, Sundler F, Edvinsson L. Neuronal messengers and peptide receptors in the human sphenopalatine and otic ganglia. *Brain Res.* 1999;826:193-199.
80. Steinberg A, Frederiksen SD, Blixt FW, Warfvinge K, Edvinsson L. Expression of messenger molecules and receptors in rat and human sphenopalatine ganglion indicating therapeutic targets. *J Headache Pain.* 2016;17:78.
81. Hensley K, Pretorius J, Chan B, et al. PAC1 receptor mRNA and protein distribution in rat and human trigeminal and sphenopalatine ganglia, spinal trigeminal nucleus and in dura mater. *Cephalalgia.* 2019;39:827-840.
82. Csati A, Tajti J, Kuris A, Tuka B, Edvinsson L, Warfvinge K. Distribution of vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide, nitric oxide synthase, and their receptors in human and rat sphenopalatine ganglion. *Neuroscience.* 2012;202:158-168.
83. Eftekhari S, Salvatore CA, Johansson S, Chen TB, Zeng Z, Edvinsson L. Localization of CGRP, CGRP receptor,



- PACAP and glutamate in trigeminal ganglion. Relation to the blood-brain barrier. *Brain Res.* 2015;1600:93-109.
84. Tajti J, Uddman R, Möller S, Sundler F, Edvinsson L. Messenger molecules and receptor mRNA in the human trigeminal ganglion. *J Auton Nerv Syst.* 1999;76:176-183.
  85. Warfvinge K, Edvinsson L. Distribution of CGRP and CGRP receptor components in the rat brain. *Cephalalgia.* 2019;39:342-353.
  86. Warfvinge K, Edvinsson L. Cellular distribution of PACAP-38 and PACAP receptors in the rat brain: relation to migraine activated regions. *Cephalalgia.* 2020;40:527-542.
  87. Boni LJ, Ploug KB, Olesen J, Jansen-Olesen I, Gupta S. The in vivo effect of VIP, PACAP-38 and PACAP-27 and mRNA expression of their receptors in rat middle meningeal artery. *Cephalalgia.* 2009;29:837-847.
  88. Goadsby PJ, Edvinsson L. Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies. *Brain.* 1994;117(Pt 3):427-434.
  89. Jansen-Olesen I, Baun M, Amrutkar DV, Ramachandran R, Christophersen DV, Olesen J. PACAP-38 but not VIP induces release of CGRP from trigeminal nucleus caudalis via a receptor distinct from the PAC1 receptor. *Neuropeptides.* 2014;48:53-64.
  90. Ingram SL, Williams JT. Modulation of the hyperpolarization-activated current (I<sub>h</sub>) by cyclic nucleotides in Guinea-pig primary afferent neurons. *J Physiol.* 1996;492(Pt 1):97-106.
  91. Edvinsson JCA, Grell AS, Warfvinge K, Sheykhzade M, Edvinsson L, Haanes KA. Differences in pituitary adenylate cyclase-activating peptide and calcitonin gene-related peptide release in the trigeminovascular system. *Cephalalgia.* 2020;40:1296-1309.
  92. Harmar AJ, Arimura A, Gozes I, et al. International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *Pharmacol Rev.* 1998;50:265-270.
  93. Grimaldi M, Cavallaro S. Functional and molecular diversity of PACAP/VIP receptors in cortical neurons and type I astrocytes. *Eur J Neurosci.* 1999;11:2767-2772.
  94. Levy D, Strassman AM. Distinct sensitizing effects of the cAMP-PKA second messenger cascade on rat dural mechanonociceptors. *J Physiol.* 2002;538:483-493.
  95. Knutsson M, Edvinsson L. Distribution of mRNA for VIP and PACAP receptors in human cerebral arteries and cranial ganglia. *Neuroreport.* 2002;13:507-509.
  96. Zagami AS, Edvinsson L, Goadsby PJ. Pituitary adenylate cyclase activating polypeptide and migraine. *Ann Clin Transl Neurol.* 2014;1:1036-1040.
  97. Zagami AS, Goadsby PJ, Edvinsson L. Stimulation of the superior sagittal sinus in the cat causes release of vasoactive peptides. *Neuropeptides.* 1990;16:69-75.
  98. Chan KY, Baun M, Vries R, et al. Pharmacological characterization of VIP and PACAP receptors in the human meningeal and coronary artery. *Cephalalgia.* 2011;31:181-189.
  99. Akerman S, Goadsby PJ. Neuronal PAC1 receptors mediate delayed activation and sensitization of trigeminocervical neurons: relevance to migraine. *Sci Transl Med.* 2015;7:308ra157.
  100. Edvinsson L. Role of VIP/PACAP in primary headaches. *Cephalalgia.* 2013;33:1070-1072.
  101. Rahmann A, Wienecke T, Hansen JM, Fahrenkrug J, Olesen J, Ashina M. Vasoactive intestinal peptide causes marked cephalic vasodilation, but does not induce migraine. *Cephalalgia.* 2008;28:226-236.
  102. Vollesen ALH, Snoer A, Chaudhry B, et al. The effect of pituitary adenylate cyclase-activating peptide-38 and vasoactive intestinal peptide in cluster headache. *Cephalalgia.* 2020;40:1474-1488.
  103. Falktoft B, Georg B, Fahrenkrug J. Signaling pathways in PACAP regulation of VIP gene expression in human neuroblastoma cells. *Neuropeptides.* 2009;43:387-396.
  104. Georg B, Fahrenkrug J. Pituitary adenylate cyclase-activating peptide is an activator of vasoactive intestinal polypeptide gene transcription in human neuroblastoma cells. *Brain Res Mol Brain Res.* 2000;79:67-76.
  105. Guo S, Vollesen ALH, Hansen RD, et al. Part I: pituitary adenylate cyclase-activating polypeptide-38 induced migraine-like attacks in patients with and without familial aggregation of migraine. *Cephalalgia.* 2017;37:125-135.
  106. Banks WA, Kastin AJ, Komaki G, Arimura A. Passage of pituitary adenylate cyclase activating polypeptide1-27 and pituitary adenylate cyclase activating polypeptide1-38 across the blood-brain barrier. *J Pharmacol Exp Ther.* 1993;267:690-696.
  107. Guo S, Vollesen AL, Olesen J, Ashina M. Premonitory and nonheadache symptoms induced by CGRP and PACAP38 in patients with migraine. *Pain.* 2016;157:2773-2781.
  108. Afridi SK, Kaube H, Goadsby PJ. Glyceryl trinitrate triggers premonitory symptoms in migraineurs. *Pain.* 2004;110:675-680.
  109. Karsan N, Bose PR, Thompson C, Newman J, Goadsby PJ. Headache and non-headache symptoms provoked by nitroglycerin in migraineurs: a human pharmacological triggering study. *Cephalalgia.* 2020;40:828-841.
  110. Onderwater GLJ, Dool J, Ferrari MD, Terwindt GM. Premonitory symptoms in glyceryl trinitrate triggered migraine attacks: a case-control study. *Pain.* 2020;161:2058-2067.
  111. Schulte LH, Menz MM, Haaker J, May A. The migraineur's brain networks: continuous resting state fMRI over 30 days. *Cephalalgia.* 2020;40:1614-1621.

112. Frederiksen SD, Warfvinge K, Ohlsson L, Edvinsson L. Expression of pituitary adenylate cyclase-activating peptide, calcitonin gene-related peptide and headache targets in the trigeminal ganglia of rats and humans. *Neuroscience*. 2018;393:319-332.
113. Ressler KJ, Mercer KB, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature*. 2011;470:492-497.
114. Dias BG, Ressler KJ. PACAP and the PAC1 receptor in post-traumatic stress disorder. *Neuropsychopharmacology*. 2013;38:245-246.
115. Markovics A, Kormos V, Gaszner B, et al. Pituitary adenylate cyclase-activating polypeptide plays a key role in nitroglycerol-induced trigeminovascular activation in mice. *Neurobiol Dis*. 2012;45:633-644.
116. Guo S, Vollesen ALH, Hansen YBL, et al. Part II: biochemical changes after pituitary adenylate cyclase-activating polypeptide-38 infusion in migraine patients. *Cephalalgia*. 2017;37:136-147.
117. Lukács M, Warfvinge K, Tajti J, et al. Topical dura mater application of CFA induces enhanced expression of c-fos and glutamate in rat trigeminal nucleus caudalis: attenuated by KYNA derivative (SZR72). *J Headache Pain*. 2017;18:39.
118. Körtési T, Tuka B, Nyári A, Vécsei L, Tajti J. The effect of orofacial complete Freund's adjuvant treatment on the expression of migraine-related molecules. *J Headache Pain*. 2019;20:43.
119. Baun M, Pedersen MH, Olesen J, Jansen-Olesen I. Dural mast cell degranulation is a putative mechanism for headache induced by PACAP-38. *Cephalalgia*. 2012;32:337-345.
120. Tuka B, Helyes Z, Markovics A, et al. Peripheral and central alterations of pituitary adenylate cyclase activating polypeptide-like immunoreactivity in the rat in response to activation of the trigeminovascular system. *Peptides*. 2012;33:307-316.
121. Martins-Oliveira M, Akerman S, Holland PR, Tavares I, Goadsby PJ. Pharmacological modulation of ventral tegmental area neurons elicits changes in trigeminovascular sensory processing and is accompanied by glycemic changes: implications for migraine. *Cephalalgia*. 2022;42:1359-1374.
122. Sándor K, Kormos V, Botz B, et al. Impaired nocifensive behaviours and mechanical hyperalgesia, but enhanced thermal allodynia in pituitary adenylate cyclase-activating polypeptide deficient mice. *Neuropeptides*. 2010;44:363-371.
123. Sándor K, Bölcskei K, McDougall JJ, et al. Divergent peripheral effects of pituitary adenylate cyclase-activating polypeptide-38 on nociception in rats and mice. *Pain*. 2009;141:143-150.
124. Robert C, Bourgeois L, Arreto CD, et al. Paraventricular hypothalamic regulation of trigeminovascular mechanisms involved in headaches. *J Neurosci*. 2013;33:8827-8840.
125. Tuka B, Nyári A, Cseh EK, et al. Clinical relevance of depressed kynurenine pathway in episodic migraine patients: potential prognostic markers in the peripheral plasma during the interictal period. *J Headache Pain*. 2021;22:60.
126. Körtési T, Tuka B, Tajti J, et al. Kynurenic acid inhibits the electrical stimulation induced elevated pituitary adenylate cyclase-activating polypeptide expression in the TNC. *Front Neurol*. 2017;8:745.
127. Kuburas A, Mason BN, Hing B, et al. PACAP induces light aversion in mice by an inheritable mechanism independent of CGRP. *J Neurosci*. 2021;41:4697-4715.
128. de Logu F, Landini L, Janal MN, et al. Migraine-provoking substances evoke periorbital allodynia in mice. *J Headache Pain*. 2019;20:18.
129. Ernstsen C, Christensen SL, Rasmussen RH, et al. The PACAP pathway is independent of CGRP in mouse models of migraine: possible new drug target? *Brain*. 2022;145:2450-2460.
130. Emery AC, Eiden LE. Signaling through the neuropeptide GPCR PAC<sub>1</sub> induces neuritogenesis via a single linear cAMP- and ERK-dependent pathway using a novel cAMP sensor. *FASEB J*. 2012;26:3199-3211.
131. Tuka B, Szabó N, Tóth E, et al. Release of PACAP-38 in episodic cluster headache patients - an exploratory study. *J Headache Pain*. 2016;17:69.
132. Tuka B, Helyes Z, Markovics A, et al. Alterations in PACAP-38-like immunoreactivity in the plasma during ictal and interictal periods of migraine patients. *Cephalalgia*. 2013;33:1085-1095.
133. Veréb D, Szabó N, Tuka B, et al. Correlation of neurochemical and imaging markers in migraine: PACAP38 and DTI measures. *Neurology*. 2018;91:e1166-e1174.
134. Schytz HW, Birk S, Wienecke T, Kruuse C, Olesen J, Ashina M. PACAP38 induces migraine-like attacks in patients with migraine without aura. *Brain*. 2009;132:16-25.
135. Deligianni C, Pellesi L, Chaudhry BA, et al. Plasma levels of VIP are not elevated during PACAP- and VIP-induced cluster headache attacks: an exploratory study. *Front Neurol*. 2023;14:1135246.
136. Amin FM, Hougaard A, Schytz HW, et al. Investigation of the pathophysiological mechanisms of migraine attacks induced by pituitary adenylate cyclase-activating polypeptide-38. *Brain*. 2014;137:779-794.
137. Pellesi L, al-Karagholi MAM, de Icco R, et al. Effect of vasoactive intestinal polypeptide on development of migraine headaches: a randomized clinical trial. *JAMA Netw Open*. 2021;4:e2118543.
138. Pellesi L, al-Karagholi MAM, Chaudhry BA, et al. Two-hour infusion of vasoactive intestinal polypeptide induces

- delayed headache and extracranial vasodilation in healthy volunteers. *Cephalalgia*. 2020;40:1212-1223.
139. Birk S, Sitarz JT, Petersen KA, et al. The effect of intravenous PACAP38 on cerebral hemodynamics in healthy volunteers. *Regul Pept*. 2007;140:185-191.
  140. Wienholtz NKF, Christensen CE, Zhang DG, et al. Early treatment with sumatriptan prevents PACAP38-induced migraine: a randomised clinical trial. *Cephalalgia*. 2021;41:731-748.
  141. Amin FM, Asghar MS, Guo S, et al. Headache and prolonged dilatation of the middle meningeal artery by PACAP38 in healthy volunteers. *Cephalalgia*. 2012;32:140-149.
  142. Gründe G, Labrujere S, Haanes KA, MaassenVanDenBrink A, Edvinsson L. Comparison of the vasodilator responses of isolated human and rat middle meningeal arteries to migraine related compounds. *J Headache Pain*. 2014;15:22.
  143. Amin FM, Hougaard A, Magon S, et al. Change in brain network connectivity during PACAP38-induced migraine attacks: a resting-state functional MRI study. *Neurology*. 2016;86:180-187.
  144. Ghanizada H, Al-Karaghali MA, Arngrim N, Olesen J, Ashina M. PACAP27 induces migraine-like attacks in migraine patients. *Cephalalgia*. 2020;40:57-67.
  145. Ghanizada H, al-Karaghali MAM, Arngrim N, et al. Effect of pituitary adenylate cyclase-activating polypeptide-27 on cerebral hemodynamics in healthy volunteers: a 3T MRI study. *Peptides*. 2019;121:170134.
  146. Hoffmann J, Miller S, Martins-Oliveira M, et al. PAC1 receptor blockade reduces central nociceptive activity: new approach for primary headache? *Pain*. 2020;161:1670-1681.
  147. Ashina M, Doležil D, Bonner JH, et al. A phase 2, randomized, double-blind, placebo-controlled trial of AMG 301, a pituitary adenylate cyclase-activating polypeptide PAC1 receptor monoclonal antibody for migraine prevention. *Cephalalgia*. 2021;41:33-44.
  148. Takasaki I, Ogashi H, Okada T, et al. Synthesis of a novel and potent small-molecule antagonist of PAC1 receptor for the treatment of neuropathic pain. *Eur J Med Chem*. 2020;186:111902.
  149. Takasaki I, Nakamura K, Shimodaira A, et al. The novel small-molecule antagonist of PAC1 receptor attenuates formalin-induced inflammatory pain behaviors in mice. *J Pharmacol Sci*. 2019;139:129-132.
  150. Shintani Y, Hayata-Takano A, Yamano Y, et al. Small-molecule non-peptide antagonists of the PACAP receptor attenuate acute restraint stress-induced anxiety-like behaviors in mice. *Biochem Biophys Res Commun*. 2022;631:146-151.
  151. Rasmussen NB, Deligianni C, Christensen CE, et al. The effect of Lu AG09222 on PACAP38- and VIP-induced vasodilation, heart rate increase, and headache in healthy subjects: an interventional, randomized, double-blind, parallel-group, placebo-controlled study. *J Headache Pain*. 2023;24:60.
  152. Ashina M, Phul R, Khodaie M, Florea I. Efficacy and safety of Lu AG09222 for migraine prevention in patients with 2-4 previous preventive treatment failures: HOPE, an interventional, randomized, double-blind, parallel-group, placebo-controlled phase 2 trial. *Cephalalgia*. 2023;43:8.
  153. Lundbeck. Lundbeck announces positive phase II proof of concept results with Lu AG09222 in migraine prevention. 1-3; 2023. <https://news.cision.com/h--lundbeck-a-s/r/lundbeck-announces-positive-phase-ii-proof-of-concept-results-with-lu-ag09222-in-migraine-prevention,c3754245>.
  154. Cernuda-Morollón E, Larrosa D, Ramón C, Vega J, Martínez-Cambor P, Pascual J. Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine. *Neurology*. 2013;81:1191-1196.
  155. Tajti J, Szok D, Majláth Z, Tuka B, Csáti A, Vécsei L. Migraine and neuropeptides. *Neuropeptides*. 2015;52:19-30.
  156. Ghanizada H, al-Karaghali MAM, Walker CS, et al. Amylin analog pramlintide induces migraine-like attacks in patients. *Ann Neurol*. 2021;89:1157-1171.
  157. Ghanizada H, al-Karaghali MA, Arngrim N, et al. Effect of adrenomedullin on migraine-like attacks in patients with migraine: a randomized crossover study. *Neurology*. 2021;96:e2488-e2499.
  158. Pan KS, Siow A, Hay DL, Walker CS. Antagonism of CGRP signaling by rimegepant at two receptors. *Front Pharmacol*. 2020;11:1240.
  159. Garelja ML, Walker CS, Hay DL. CGRP receptor antagonists for migraine. Are they also AMY(1) receptor antagonists? *Br J Pharmacol*. 2022;179:454-459.
  160. Hage la Cour S, Juhler K, Kogelman LJA, et al. Characterization of erenumab and rimegepant on calcitonin gene-related peptide induced responses in *Xenopus laevis* oocytes expressing the calcitonin gene-related peptide receptor and the amylin-1 receptor. *J Headache Pain*. 2022;23:59.
  161. Bhakta M, Vuong T, Taura T, Wilson DS, Stratton JR, Mackenzie KD. Migraine therapeutics differentially modulate the CGRP pathway. *Cephalalgia*. 2021;41:499-514.
  162. Ferrari MD, Goadsby PJ, Burstein R, et al. Migraine. *Nat Rev Dis Primers*. 2022;8:2.
  163. Akerman S, Goadsby PJ, Romero-Reyes M. PACAP-38 related modulation of the cranial parasympathetic projection: a novel mechanism and therapeutic target in severe primary headache. *Br J Pharmacol*. 2024;181:480-494.