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New discoveries in migraine mechanisms and therapeutic targets

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Migraine is among the most common and most disabling disorders worldwide, yet its underlying pathophysiology is among the most poorly understood. New information continues to emerge on mechanisms within the central and peripheral nervous systems that may contribute to migraine attacks. Additionally, new therapeutics have recently become available and along with much needed relief for many patients, these drugs provide insight into the disorder based on their mechanism of action. This review will cover new findings within the last several years that add to the understanding of migraine pathophysiology, including those related to the vasculature, calcitonin gene-related peptide (CGRP), and mechanisms within the cortex and meninges that may contribute to attacks. Discussion will also cover recent findings on novel therapeutic targets, several of which continue to show promise in new preclinical studies, including acid-sensing ion channels (ASICs) and the delta-opioid receptor (DOR).

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Introduction

Migraine is a neurovascular disorder consisting of numerous symptoms including premonitory signs, aura, nausea, vomiting, disabling headache lasting 4–72 hours, and hypersensitivity to light, sound, smell, and touch [1]. It is 2–3 times more common in women, particularly during reproductive years [2], although the reasons for the female bias remain unclear. It is also the second-most disabling disease worldwide according to a recent analysis of the Global Burden of Disease Study from the World Health Organization [3]. One of the primary reasons for the significant disability of migraine is the lack of effective treatment options for many patients, which is due in part, to poor understanding of the underlying pathophysiology.

The hypothalamus and development of attacks

While the exact origin of migraine attacks within the nervous system remains a mystery, there may be a role for the hypothalamus in the early stages. A study with a single migraine patient who was scanned daily for 30-days using functional MRI found alterations in both hypothalamic responses to trigeminal noxious stimulation (e.g. intranasal ammonia) and hypothalamic coupling to the trigeminal nucleus caudalis and dorsal rostral pons [4]. These effects were most pronounced in the 24-hours before a migraine attack; similar findings have since been published by an independent group [5]. Greater changes in hypothalamic activity following noxious trigeminal stimulation were also found in chronic migraine patients compared to healthy controls [6]. However, these findings do not prove that changes in hypothalamic activity are the origin of attacks as events may first occur elsewhere in the body, which then subsequently influence hypothalamic activity. Given the list of common triggers of migraines, including stress, lack of sleep, too much sleep, skipping meals, intense exercise/overheating, hormonal changes, and the impact that these triggers can have on the hypothalamus, these triggering events may occur before altered hypothalamic function early in the migraine attack. See Ref. [7] for a more extensive discussion of this topic.

Contributions of CGRP and the vasculature to migraine

There are now three monoclonal antibodies (mAbs) targeting calcitonin-gene related peptide (CGRP) signaling approved for use in humans, two of those antibodies target the peptide itself and the third targets the CGRP receptor [8]. A role for CGRP in migraine had been proposed for several decades, but the efficacy of the mAbs as well as several small-molecule receptor antagonists in clinical trials, has solidified the concept that CGRP plays an important role in the disorder, at least for many patients. These CGRP mAbs provide much needed relief for patients suffering from both episodic and chronic migraine (the latter differentiated by >15 headache days/month where at least 8 of those days have migraine features). Additionally, the efficacy of the mAbs provides new clues into the pathophysiology of migraine since these biologics are too large to cross the blood-brain barrier (BBB). Systemic administration of galcanezumab (one of the approved CGRP mAbs) resulted in concentrations of antibody within the CNS that were at most 0.34% of the plasma concentration; percentages in dura mater and trigeminal ganglia were 11% and 5% of plasma concentration, respectively [9]. While animal models

show that the BBB can open under conditions relevant for migraine allowing passage of small-molecule therapeutics and even albumin [10,11] (albumin complexed with Evans blue dye is approximately 70 kDa), human studies have shown no changes in the BBB during migraine with or without aura [12•,13•], and it is unclear whether there would be opening sufficient to allow mAbs (approximately 150 kDa) to gain access to the brain. Their efficacy despite being restricted to the periphery argues strongly that attacks can be driven by events outside the central nervous system, an issue that has been a matter of intense debate within the field. Thus, while the purpose for their development was ultimately to treat patients, their success has simultaneously led to important data about the pathophysiology of the disorder itself.

The contribution of vasodilation to migraine has been intensely debated over the last ten years following conflicting studies using imaging of meningeal and cortical vessels in humans during migraine attacks provoked with pharmacological triggers [14]. However, the vasodilation hypotheses appeared to suffer a fatal blow with the observation of little to no dilation of meningeal or cortical vessels in humans with spontaneous migraine attacks (i.e. attacks not triggered pharmacologically) [15]. The idea has recently been revived given demonstration that sildenafil and CGRP, both known migraine triggers, dilate blood vessels in the dura mater [16] and the finding that there is dilation of the middle meningeal artery (MMA) during cilostazol-triggered attacks, initially only on the side of the head where pain is present and later bilaterally [17•]. These recent findings re-invigorate interest into dural vasodilation, although they do not prove that vessels have a causative role in migraine. Additional support for a vascular contribution to migraine comes from meta-analyses of genome-wide association studies (GWAS) consisting of 60 000 migraine patients and over 300 000 controls. Using advanced bioinformatics, the authors found that the largest category of migraine-related genes were associated with vascular disease, blood vessels, or smooth muscle [18], providing suggestive genetic evidence for a role of the vasculature in migraine. There are also recent reports of genetic variants in endothelin-1 that are linked to migraine [19]; endothelin is among the vasoactive peptides proposed to contribute to the disorder [20]. And although speculative, a recently published metabolomic study on 2800 migraine patients and over 7000 controls found an association between migraine and decreased apolipoprotein A1 as well as decreased cholesterol content in high-density lipoproteins (HDLs) [21]. The authors hypothesized that these changes may contribute to migraine via endothelial dysfunction but this of course would require more specific testing. There may ultimately be a complex role of the vasculature where dilation is not the critical component in migraine; it may rather be contribution from the cells within the vasculature that release factors that initiate bi-directional communication with sensory, sympathetic, and parasympathetic neurons that densely innervate tissues like the meninges [14].

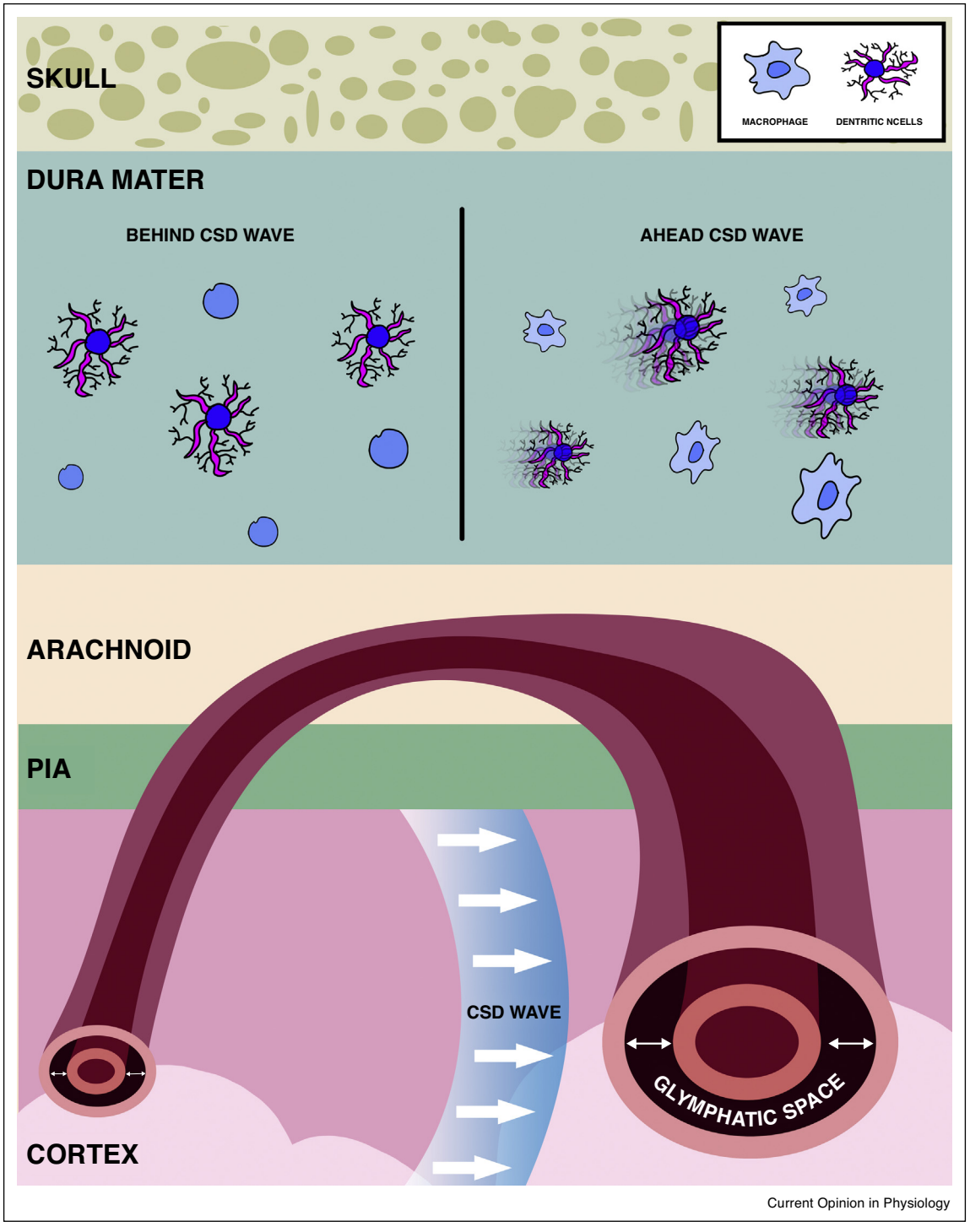
Other neuropeptides beyond CGRP in migraine: a continually emerging role for PACAP

Pituitary adenylate-cyclase activating polypeptide (PACAP) is a neuropeptide that has also been investigated for its role in migraine, and in many cases, findings with PACAP in both preclinical and clinical studies are very similar to those of CGRP. Like CGRP, PACAP38 (the 38-amino acid version of the peptide) causes attacks when infused into migraine without aura patients [22]. Also like CGRP, studies have described similar expression patterns of PACAP and its receptors on neurons and blood vessels within the trigeminovascular system; they have shown effects on vessel diameter, on nerve fibers, and on mast cells; and they have found changes in plasma levels of PACAP in association with migraine attacks and treatment [23]. Recent studies have now found that PACAP27 can also induce attacks in migraine without aura patients [24] and it dilates meningeal and extracerebral arteries (although it constricted cerebral arteries) [25]. Thus, multiple members of the PACAP family may contribute to migraine. How PACAP contributes to the disorder is not yet clear, but degranulation of meningeal mast cells has been proposed as at least one mechanism [26,27]; human mast cells were also recently shown to release PACAP [28]. Intriguingly, PACAP38 may not cause degranulation of mast cells via its canonical receptor system (PAC and VPAC receptors), but via a novel mechanism dependent on activation of the orphan MrgB3 receptor [29]. These latter data suggest that targeting traditional PACAP receptors with monoclonal antibodies or antagonists may yield different therapeutic effects than targeting the peptide itself. Both receptor and peptide antibodies are currently being developed as migraine therapeutics.

New insights into CSD and migraine

Among the many proposed mechanisms contributing to migraine, cortical-spreading depression (CSD) is one of the most extensively studied in this context [30–32]. CSD is a wave of altered electrical activity that propagates across the cortex at 2–5 mm per minute and it is thought to be the underlying basis of aura. While CSD is known to influence cortical neuronal function, and see a recent study showing an impact of CSD on both pre- and post-synaptic cortical mechanisms [33], whether and how it influences other cortical/brain physiology is not clear. Using *in vivo* multiphoton imaging of living mice, it was recently shown that CSD leads to a closure of paravascular space within the cortex and an impairment of glymphatic flow [34•] (Figure 1). Given the proposed role of glymphatic drainage of the brain in clearing harmful metabolites [35], this study suggests that the buildup of

Figure 1



CSD closes glymphatic space in the cortex and activates immune cells in the meninges. A CSD event is shown in blue in the cortex. Ahead of the CSD wave (on the right side of the figure), vessels in the cortex have open paravascular glymphatic space, allowing movement/drainage of fluid. After the CSD wave passes (on the left side), these cortical vessels close their glymphatic space, restricting fluid draining in the cortex. Additional events occur along with CSD in the meninges, shown here in the dura mater. Ahead of the CSD wave, dendritic cells are mobile and macrophages are ramified. Once the CSD wave passes, dendritic cells halt movement and macrophages become circular, suggesting activation of these cells by a signal during CSD.

these metabolites may have some role in migraine attacks, potentially contributing to the cortical inflammatory process that have been identified in CSD models [36–38]. Another study demonstrated that inadequate sleep (glymphatic flow is typically active during sleep) lowered the threshold for CSD induction due to lack of adequate glycogen supply [39•]. Inadequate sleep (with inadequate glymphatic flow) may ultimately contribute to CSD and CSD may then lead to impaired glymphatic flow, creating a cycle of changes in the cortex that contribute to migraine pathophysiology.

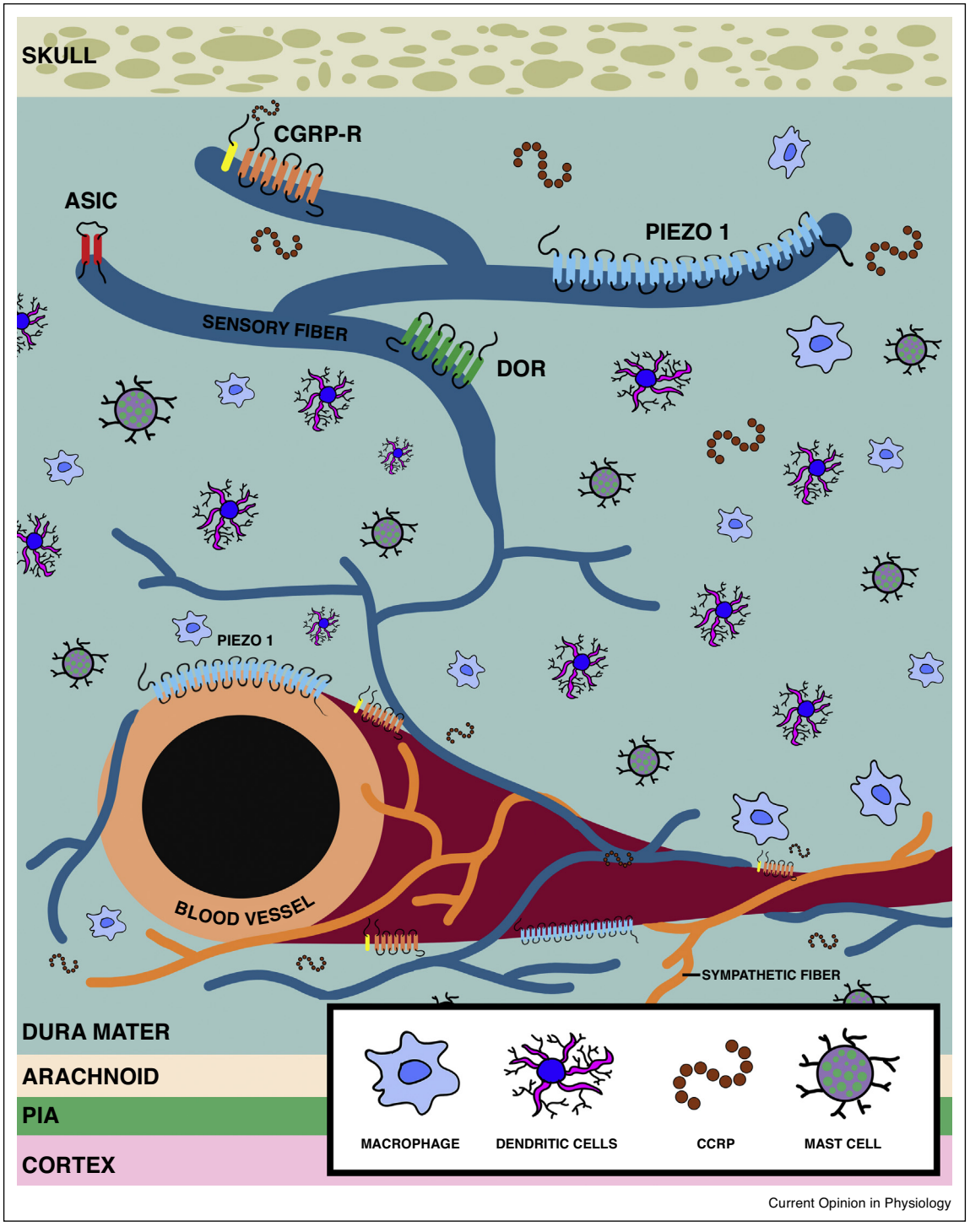
The link between CSD and headache is still a matter of debate within the field. Several recent papers confirm earlier work showing that CSD activates meningeal nociceptors [40,41] but mechanisms by which this occurs remain unclear. CSD causes changes in dural macrophages and dendritic cell morphology and migration that are consistent with activation of these cell types [42•] (Figure 1). Changes in dural immune cells could ultimately lead to activation of neighboring nociceptors and subsequently to headache [43] (see Ref. [44] for the effects of stress on dural immune cell populations). However, the signal between the cortex and these nerve endings remains enigmatic. There does not appear to be a relationship between CSD with its resulting cortical hypoperfusion/decreased oxygen content and activation of meningeal nociceptors, as increasing blood flow to the cortex before/during CSD did not prevent nociceptor activation [45•]. CSD can also increase the expression of CGRP in the cortex [46]. It was previously shown that CSD increased cortical CGRP release and blocking CGRP receptors decreased the cortical area impacted by CSDs [47]; recent reports have also shown a modulatory role of CGRP on CSD [48,49]. These studies show that CGRP has a role in migraine-relevant processes within the brain (and processes well beyond the cortex, see Refs. [50,51]), but whether CGRP can migrate into the meninges following release in the cortex is not known. CGRP receptor antagonists were unable to block the sensitization of meningeal nociceptors following CSD in one study [52] but blocked cFos expression in the nucleus caudalis and thalamus due to CSD in another [53], so it remains unclear whether this peptide is among the signaling molecules that connects the events in the cortex and meninges. Alternatively, CSD may not require nociceptor activation to cause headache. Projections from the cortex to the trigeminocervical complex (the region of the brainstem where meningeal afferents project) have been demonstrated; these pathways can modulate incoming sensory input from the meninges following CSD. Further, CSD can lead to sensory remapping of the cortex as well as alterations in cortical response and adaptation to sensory input (the prior two studies are covered in this recent review [31]). As described above, CGRP may play a role in these events within the brain, contributing to headache independent of any direct connection between CSD and meningeal nociceptor activation. Consistent with this possibility is a recent finding of headache-related behavioral responses following intracerebroventricular (but not intraperitoneal) injection of CGRP in mice overexpressing the CGRP receptor hRAMP1 in the nervous system [54]. Nonetheless, while CSD is thought to be the mechanism of aura, less than 30% of migraine patients have aura [1]. Whether migraine-without-aura patients have silent CSDs is unknown.

New insights into meningeal nociceptor activation and migraine

While still debated within the field, the pain phase of migraine (i.e. the headache) almost certainly requires activation of meningeal nociceptors [55], but the mechanisms that lead to activation of these neurons during spontaneous migraine attacks remain unclear. Lists of mechanisms have been proposed and demonstrated in animal models (see Ref. [43] for a recent review), but conclusive evidence from human studies is largely nonexistent. Nonetheless, there are several recent studies that shed new light on the potential mechanisms that may contribute to the headache phase of migraine. It has been repeatedly documented that meningeal nociceptors are mechanically sensitive, but the mechanisms by which pressure activates these neurons has remained unknown. Recent work now implicates the mechanically-sensitive ion channel Piezo1 since the agonist of this channel, Yoda1, activated meningeal afferents in an *ex vivo* hemi-skull preparation and also caused the release of CGRP from this same preparation [56•]. This channel (as well as Piezo2) was also found in the most extensive transcriptome analysis published thus far on RNA-sequencing data from the human trigeminal ganglia [57•], a study that was an important contribution to the migraine literature independent of this context. And although not focused on meningeal input, a recent study found larger rod-driven b waves from the retina of migraine patients compared to controls [58•], providing important new evidence for sensitization of peripheral inputs in migraine. Unfortunately, similar data will be difficult to obtain for human meningeal afferents given the inability to easily access the trigeminal ganglia and the meninges themselves in experimental studies.

As discussed above, a role for CGRP in migraine pathophysiology is clear; however, the location of CGRP action is not [59]. It has been known since the early 2000's that intravenous administration of CGRP provokes attacks in migraine patients [8] and given the poor BBB permeability of CGRP (a 37-amino acid peptide), the site of action has been speculated to be in the periphery (consistent with efficacy of the peripherally-restricted mAbs discussed above). The dura mater would otherwise seem to be a primary location, but this has been questioned since a 2005 study showed that dural application of CGRP

Figure 2



Meningeal nociceptors, blood vessels, and immune cells may all interact within the dura mater to initiate afferent input leading to headache. Expression of the Piezo1 receptor on meningeal afferents allows these neurons to respond to mechanical force (Piezo receptors are also found on endothelial cells in blood vessels, which is not discussed in this review). CGRP (as well as PACAP, not shown) can have direct actions in the meninges leading to headache, whether directly acting on CGRP receptors expressed on neurons, or activating CGRP receptors on the vasculature, with subsequent indirect activation of nociceptors. Activation of immune cells in the meninges, including dendritic cells, macrophages, and mast cells, by CSD or other events, can lead to signaling to blood vessels and sensory neurons initiating afferent input. Sensory afferent endings can be activated by ASICs following a drop in pH and can be inhibited by activation of DOR. Vasodilation, while observed in human meninges during provoked migraine attacks, may lead to activation of nociceptors or may simply be a result of nociceptor activation and the release of CGRP from sensory nerve endings.

caused vasodilation, but no activation or sensitization of meningeal nociceptors [60]. More recent work shows that systemic administration of CGRP causes spontaneous pain in mice (consistent with headache provocation in humans) [61], but systemic administration does not identify the site of action. However, it was recently shown that direct application of CGRP to the rat or mouse meninges does in fact cause behavioral responses consistent with headache, but only in female animals [62•]. This finding establishes that the meninges are a potential site of action of CGRP in migraine and also demonstrates that CGRP may have differential actions in females versus males, providing one mechanism that may help explain the higher prevalence of migraine in women.

Several recent studies have expanded the knowledge of meningeal innervation by both trigeminal and upper cervical sensory neurons. An elegant examination of the neuroanatomy of the rat dura mater found that the non-arterial innervation expresses noradrenergic α1 receptors, providing a potential basis for interaction with nearby sympathetic fibers expressing norepinephrine [63] and explaining the earlier observation that dural application of norepinephrine causes headache responses in rats [64]. This study also found expression of the 5HT1D receptor on these neurons, which is a target of triptan drugs, and expression of δ-opioid receptors (DORs), potentially providing a new therapeutic target (see below for more discussion). In another elegant meningeal-innervation study, the upper cervical dorsal root ganglia at C2 and C3 level were found to innervate the meninges over the cerebellum [65], suggesting that the cerebellar dura may contribute to headaches, likely those in the occipital region. This study also found that stimulation of the cerebellar dura caused sensitization in neck muscles and occipital skin, potentially explaining the tenderness and allodynia experienced by many migraine patients.

New therapeutic opportunities for migraine

In parallel to the mechanistic studies described above, there has been progress in preclinical studies identifying new therapeutic targets. One promising target that has now shown efficacy in multiple preclinical headache models is DOR. Activation of DOR with multiple agonists was first shown in 2014 to prevent hyperalgesia and conditioned-place aversion following administration of nitroglycerin, a common migraine trigger recently validated as a translational tool between rodents and humans [66•], and activation of DOR also inhibited evoked-CSD frequency [67]. Since this initial publication, activation of DOR has also shown efficacy in preclinical chronic migraine, medication-overuse headache (MOH), and post-traumatic headache models [68,69]. These data across multiple preclinical models argue strongly that DOR should be further investigated in humans as a potential therapeutic target for migraine. Additionally, the κ-opioid receptor (KOR) has emerged as a potential migraine target but in this case, antagonists of KOR given systemically or into the right central nucleus of the amygdala (CeA) blocked priming to stress in a rat sumatriptan model of MOH [70]. Finally, acid-sensing ion channels (ASICs) have been proposed previously as potential targets for migraine given their role in preclinical models and efficacy of amiloride, an ASIC blocker, in a small human trial of treatment-resistant migraine patients [71]. Additional preclinical studies continue to support the role of ASICs in migraine since repeated stimulation of the rat dura with inflammatory soup caused an increase in ASIC3 expression in the nucleus caudalis [72] and peripheral blockade of ASIC1a/ASIC1b with amiloride and mambalgin-1 prevented and reversed behavioral responses to acute and chronic administration of a nitric oxide donor in rats [73]. Like DOR, ASICs have now been shown to contribute to migraine-related processes in multiple laboratories and these data argue strongly that blockers of ASICs should be developed for testing in humans. Finally, there is a continually-emerging literature investigating the endocannabinoid system in migraine. Studies show that anandamide, either by direct administration or inhibition of its breakdown, is efficacious in animal migraine models, data are beginning to emerge on other endocannabinoids in preclinical models, and evidence from humans supports decreased levels o endocannabinoids in migraine patients, supporting the idea that migraine may involve deficiencies in endocannabinoid tone [74,75]. This system deserves additional exploration for its potential to treat migraine, whether via natural plant-based derivatives or via synthetic modulators of receptors and metabolic enzymes.

Conclusions

While there is currently a great deal of excitement in the migraine field over the CGRP-based mAbs, there remains a need for new therapeutics. These new therapeutics will likely come from better understanding of the disorder. New insights into the contribution of the hypothalamus early in attacks, continued suggestion of a vascular contribution to migraine, the impact of CSD on the brain and meninges, and additional discoveries of meningeal afferent properties, can all provide increased knowledge of the underlying pathophysiology of the disorder and may identify new treatment avenues. At the same time, there continue to be developments related to novel therapeutic targets for migraine where DOR and ASIC remain among the most widely published targets that have shown efficacy across multiple models. Together, there is much to be excited about in the migraine research community and this new knowledge should provide hope for patients that additional therapeutics are on the horizon.

Conflict of interest statement

Nothing declared.

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