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Ion channels and migraine

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Abstract

Migraine is one of the most common neurological disorders. Despite its prevalence, the basic physiology of the molecules and mechanisms that contribute to migraine headache is still poorly understood, making the discovery of more effective treatments extremely difficult. The consistent presence of head-specific pain during migraine suggests an important role for activation of the peripheral nociceptors localized to the head. Accordingly, this review will cover the current understanding of the biological mechanisms leading to episodic activation and sensitization of the trigeminovascular pain pathway, focusing on recent advances regarding activation and modulation of ion channels.

Keywords

dural afferents; TRESK; ASICs; TRPA1; TRPV1; TRPV4; TRPM8; $P2X_3$

Introduction

Migraine is a complex neurologic disorder characterized by episodic headache and several associated manifestations, including nausea, vomiting, phonophobia and/or photophobia.[1] In one third of patients, attacks of disabling headaches are preceded by a focal, transient neurological phenomenon termed ‘aura’ (migraine with aura).[2] According to the American Migraine Prevalence and Prevention study, the cumulative incidence is 43% in women and 18% in men.[3] Given that the prevalence of migraine is highest during the ages of 25 to 55,[4] often the peak ages of productivity, the disorder imposes a huge burden on patients, their families, employers and society. Despite its prevalence, the basic physiology of the molecules and mechanisms that contribute to migraine headache is still poorly understood, making the discovery of more effective treatments extremely difficult.

The pain of migraine likely requires activation of primary afferent neurons that innervate cranial tissues,[5] although this is still a matter of debate within the field. Migraine aura,

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however, is more widely accepted to arise from cortical spreading depression (CSD) in cerebral sensory cortices.[6] Migraine is known to be trigged by specific factors in approximately 75% of patients.[7] These factors include stress, menstruation, noise, odors, heat, head/neck movement, neck pain, and certain foods.[8, 9] It is not clear how these factors bring on migraine attacks. Psychophysical, evoked potentials, transcranial magnetic stimulation (TMS), and magnetoencephalographic studies indicate enhanced cortical responsiveness to diverse stimuli in migraineurs.[10–12] Most studies observe a lowered threshold for neuronal activation in migraineurs compared with controls, which suggest that migraine may result from an altered state of neuronal excitability. Whether this occurs within central as well as peripheral neurons is unclear. A contribution from peripheral neurons innervating the cranial region provides the simplest physiological mechanism for the head-specific pain of migraine. But one interesting and unresolved question is whether there is increased peripheral input during migraine or whether increased neuronal excitability within the CNS allows pain to develop in response to tonic activity in peripheral neurons. This review will focus on ion channel-based mechanisms that activate trigeminal sensory afferents under normal and sensitized conditions. Although a simple interpretation for a role of these channels is that they mediate increased neuronal activity from peripheral tissues, their activation may also be important for tonic activity.

Ion channels are pore-forming transmembrane proteins that allow the passage of ions into and out of a cell. They mediate cell excitability and are essential for proper signaling and cell function. Therefore, dysfunction or aberrant regulation of ion channels could result in disruption of excitation-inhibition balance, neuronal excitability and peripheral or central sensitization. In support of this view, several prophylactic drugs for migraine work (albeit non-selectively) by inhibiting the function of ion channels such as voltage-gated sodium channels or voltage-gated calcium channels.[13] Although the site of action of these drugs is not clear, modulation of ion channels leading to amelioration of neuronal hyperexcitability nonetheless represents a currently utilized therapeutic approach and an attractive strategy for migraine prophylactic drug discovery. In the peripheral nervous system, considerable progress has been made in understanding the role of ion channels that may participate in the pathophysiology of migraine. Fluorescent retrograde labeling methods have allowed the study of the properties of primary afferent neurons innervating the dura (i.e. dural afferents), which are likely to be key players in transmitting peripheral input contributing to the pain of migraine. This review will focus on (1) ion channels expressed on dural afferents and (2) recent advances regarding activation and modulation of the ion channels on dural afferents. We have also included the TRESK potassium channel, which has recently been linked with inherited migraine. Although the site of action of TRESK channels in migraine pathophysiology is not clear, recent studies indicate that this channel might be important for regulating excitability of primary afferent neurons.

The Properties of Dural Afferents

Advances in histological, electrophysiological and behavioral techniques have allowed a better understanding of the signaling cascades that may underlie migraine pathophysiology. The trigeminal sensory system is likely a key component in pain initiation and transmission in migraine.[14, 15] Trigeminal nerves (primary afferent neurons) have cell bodies located in

the trigeminal ganglion and they detect mechanical, chemical and temperature changes from intracranial and extracranial tissues, and send the information into the CNS. Primary afferent neurons mainly project to the upper cervical and medullary dorsal horn,[16, 17] and sensory information then travels to higher brain regions. Anatomical studies demonstrate that only the meninges and large cranial vessels, but not the brain itself or its intrinsic blood vessels, are densely innervated by sensory fibers originating primarily from trigeminal ganglion.[14, 18, 19] These data indicate that primary afferent neurons have the potential to detect and transmit noxious stimuli from cranial tissues. *In vivo* electrophysiological recording studies have demonstrated the ability of primary afferent neurons to respond to mechanical stimuli, such as punctate probing and stroking,[20] thermal stimuli[21] and chemical stimuli, such as KCl, capsaicin, acidic buffers and a mixture of inflammatory mediators (histamine, bradykinin, serotonin, and prostaglandin E2 at pH 5).[20, 21] In addition, the only sensation that is evoked following stimulation of large cranial vessels and the meninges in humans is pain,[22] indicating that the sensory innervation of the meninges is purely nociceptive. Therefore, similar to afferents innervating other tissues, meningeal sensory afferents are able to transmit and encode noxious stimuli, pointing to their important roles in headache pain processing.

One major question is where the noxious stimuli that can activate primary afferent trigeminal neurons originate. Preclinical studies show that primary afferent neurons can be sensitized by inflammatory mediators, allowing previously innocuous stimuli to become noxious or allowing spontaneous activity to develop. This hypothesis is supported by clinical observations that intracranial and circulating levels of various inflammatory mediators are significantly higher during migraine attacks.[23, 24] Preclinical studies have also identified dural mast cells as a potential source for proinflammatory mediators. In addition to sensory fibers, the meninges are highly populated with mast cells.[25, 26] These granulated cells reside within the dura and are in close proximity to blood vessels and nerve terminals.[27] Stress (via the release of corticotrophin releasing factor or CRF),[28] CGRP release,[29] nitroglycerin infusion[30] and increased estrogen levels,[27] all factors associated with migraine in humans are known to trigger mast cell degranulation.[31] Activation/degranulation of dural mast cells could therefore be a key event which links several seemingly unrelated triggers to the emergence of episodic headache. Preclinical studies also provide direct evidence demonstrating that mast cell degranulation by itself can activate meningeal nociceptors.[32] Mast cell mediators, including serotonin (5-HT), prostaglandin I_2 (PGI_2), and to a lesser extent histamine can promote a robust sensitization and activation of meningeal nociceptors.[33] Exposure of the dura to mast cell mediators *in vivo* leads to headache-like responses to normally subthreshold pH changes in a rat behavioral model of headache.[34] These studies implicate dural mast cells as both “sensor” and “effector” cells that participate in detecting changes in the meninges induced by migraine triggers and promote the development of neurogenic inflammation. There could be a cycle occurring within the meninges where substances such as serotonin, histamine, and PGI_2 (among others) activate/sensitize the afferents,[33] initiate neurogenic inflammation which could then lead to more afferent activation causing further release of inflammatory mediators. The net outcome of this cycle is an amplified inflammatory cascade, greater activation/sensitization of dural afferents and a larger magnitude response.

Changes in the number of dural mast cell might contribute to headache attacks in migraine patients. In support of this hypothesis, a high prevalence of headache is observed in patients with mastocytosis, a disorder characterized by an increased number of tissue mast cells.[35] Estrogen has been shown to promote an increase in dural mast cell density through recruitment of mast cells from spleen, which might contribute to the higher incidence of migraine in women.[36] Taken together, clinical observations, preclinical studies, and anatomical and physiological characteristics of dural mast cells all point to an important role of these cells in migraine pathophysiology.

Another endogenous process proposed to promote meningeal inflammation is cortical spreading depression (CSD), thought to be the underlying mechanism for migraine aura. CSD is associated with a massive efflux of potassium,[37] hydrogen ions,[38] and neurotransmitters such as glutamate[39] into extracellular space, which can cause a multitude of changes in expressions of growth factors and proinflammatory mediators, such as tumor necrosis factor-α (TNF-α) and IL-1β.[40] These mediators have the potential to activate or sensitize meningeal nociceptors. Consistent with this hypothesis, preclinical studies confirm that CSD is able to induce a long lasting blood flow increase in the middle meningeal artery, cause plasma protein extravasation in dura mater, increase c-Fos staining in the ipsilateral trigeminal nucleus caudalis[41] and cause delayed and long-term activation of meningeal nociceptors.[42] One recent study demonstrated that following CSD, high-mobility group box 1 protein is released from cortical astrocytes through pannexin 1 channels, leading to increased blood flow in the middle meningeal artery, dural mast cell degranulation, and pain behaviors in mice.[43] Although it is known that meningeal nociceptors can be activated or sensitized by proinflammatory mediators[20, 33, 44, 45] or following CSD events[42, 43] and mast cell degranulation,[32] the mechanisms by which these events produce excitation of dural afferents are still not entirely clear. Multiple channels can contribute to activation of dural afferents (as with any population of nociceptor) in response to factors released during mast cell degranulation or CSD. The next section will focus on the current understanding of ionic mechanisms underlying activation and sensitization of dural afferents.

Ion Channels in Dural Afferents

Retrograde labeling techniques have been recently used in migraine research to study properties of afferents innervating the dura.[44, 46, 47] Briefly, retrograde tracers, either 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI) or Fluorogold (FG), are applied to a section of dura surrounding the superior sagittal sinus bilaterally to label the nociceptors innervating the dura (i.e. dural afferents). This method provides specific labeling[46] and the labeling process itself does not change the properties of the neurons.[47] Utilizing this method, ion channels expressed on dural afferents have been identified and their properties have been studied (Table 1.).

1. Acid Sensing Ion Channels (ASICs)

ASICs are voltage-insensitive cationic channels activated by increases in the concentration of extracellular protons.[48] The ASIC family consists of 4 members, ASIC1-4, with several splice variants.[49] Functional ASIC channels are assembled as heteromeric or homomultimeric channels. Different subunit composition of these channels gives rise to

different pharmacology, current kinetics and sensitivity to pH changes.[49] ASICs are widely expressed in the nervous system. ASIC1a and ASIC2 subunits are expressed in the central nervous system,[50–53] while almost all ASIC subunits are found in peripheral sensory neurons.[52, 54, 55]

Among all subunits, the ASIC3 subunit is of particular interest for several reasons. ASIC3 exhibits a sensitivity that allows it to respond to pH values at or near neutral pH. In addition to transient peak current, ASIC3 is also able to generate a non-desensitizing current in response to a physiologically relevant pH,[56] making it suitable to detect prolonged and slow acidification. ASIC3 is highly expressed in sensory neurons and largely restricted to the periphery,[49, 57] consistent with its role in detecting pH changes in peripheral tissues. Peripheral sensory neurons expressing ASIC3 innervate visceral organs including the colon and heart as well as skeletal muscles.[58–61] ASIC3 channels have been proposed to modulate painful conditions associated with these tissues, including angina, postoperative pain, various GI disorders and muscle pain.[56, 58, 60–63]

With respect to migraine, ASICs on dural afferents have been proposed as a sensor of decreased extracellular pH within the dura.[64] ASIC3 expression is found in most trigeminal neurons and approximately 80% of dural afferents express immunolabeling for ASIC3.[34, 65] A recent electrophysiology study in dural afferents demonstrated that in response to acidic solution, 80% of dural afferents generate rapidly activating and rapidly desensitizing currents through activation of ASICs.[47] These currents led to action potentials in dural afferents at physiologically relevant pH ranging from pH 6.8 to pH 7.0.[47] Moreover, biophysical characterization indicated that ASIC-like currents in dural afferents are mediated by ASIC3 homomeric or heteromeric channels.[47] In awake animals, dural application of acidic solution elicited migraine-related pain behavior through the activation of ASICs, providing support for their potential role in initiating migraine headache.[47] This study demonstrated for the first time that small changes in extracellular pH can directly excite dural afferents via the opening of ASICs. Following pH stimulation, CGRP release is also increased in trigeminal ganglion neurons via activation of ASIC3,[65] which can result in neurogenic inflammation and headache progression. A contribution of other ASIC subunits in migraine has also been reported. Amiloride was shown to block cortical spreading depression and inhibit trigeminal activation through an ASIC1-dependent mechanism in a preclinical study.[66] Importantly, in a small clinical study of intractable migraine patients, amiloride reduced both aura and headache severity in 4 of 7 patients.[66] This finding suggests the need for a larger-scale trial in a more general population of migraine patients. Further, since amiloride is a non-specific blocker of ASICs, the development of more selective pharmacological tools is necessary to clarify the role of ASIC subunits in human migraine pathophysiology.

One logical question surrounding an ASIC mechanism for migraine is where the pH drop would originate in the dura. The intragranular pH of isolated mast cells is approximately 5.5.[67] Dural mast cells located in close proximity to nerve endings, and therefore mast cell degranulation secondary to migraine-related triggers, could acidify the surrounding local environment and directly activate dural afferents. Alternatively, CSD is shown to be accompanied by ischemia,[68] which could induce a pH drop in the dura. Given the fact that

ASICs are extremely sensitive to pH change and dural afferents express a high density of ASICs, it is possible that even small pH changes can activate these neurons and initiate headache. Prior studies show that CSD events and mast cell degranulation can activate dural afferents in anesthetized animals.[32, 42] Further studies are essential to elucidate whether there is a role of ASICs in these pathophysiological conditions by utilizing either specific ASIC blockers or ASIC knockout animals.

2. Transient receptor potential channels (TRP channels)

TRP channels are a group of ion channels expressed on the cellular membrane as well as the endoplasmic reticulum that respond to a variety of stimuli, including heat, osmolarity changes, protons and various natural products.[69–71] The 28 mammalian TRPs can be divided into 6 subgroups: TRPC, TRPM, TRPV, TRPA, TRPP, TRPML based on their primary amino acid sequences.[72] Activation of TRPs allows the influx of Ca^{2+} and Na^{+} and depolarization of the cell.[69] TRP channels have been demonstrated to participate in sensing visual, gustatory, olfactory, auditory, mechanical, thermal, osmotic stimuli and environmental irritants.[73] Therefore, TRP channels seem to be particularly well-suited candidates for transducing and encoding noxious stimuli in migraine pathophysiology.

2.1 Transient Receptor Potential Cation Channel A1 (TRPA1)—Expressed on sensory neurons[74] as well as neurons in the airways,[75, 76] TRPA1 is thought to mediate neuronal responses to a series of chemically diverse and highly reactive environmental agents such as formaldehyde,[77] acrolein,[78] chlorine,[79] and cigarette smoke extract,[80] various electrophilic natural products, including pungent plant derivatives like isothiocyanates,[74] cinnamaldehyde,[81] and allicin[82] and by-products of oxidative and nitrative stress, such as nitrooleic acid,[83] 4-hydroxynonenal[84] and reactive prostaglandins.[85] A substantial amount of preclinical work has established a role for TRPA1 in pain transduction.[86] Activation of the TRPA1 channel produces nociceptive behavior in rodents[78] and a gain-of-function mutation of TRPA1 is linked with a heritable episodic pain syndrome in humans.[87] TRPA1 is also thought to be involved in modulation of mechanotransduction in sensory neurons.[88–90]

Recent evidence also proposes a role for TRPA1 in migraine pathophysiology, among which are two separate studies demonstrating TRPA1 expression on dural afferents. In mice, 5.7% of identified dural afferents expressed TRPA1,[46] while in rats, two TRPA1 agonists, mustard oil (MO) and the environmental irritant umbellulone, evoked TRPA1-like currents in approximately 42% and 38% of dural afferents, respectively.[91] The difference between the sensitivity of the detection methods, immunohistochemistry vs. electrophysiology, or species may account for the discrepancy in expression here. Umbellulone is the major volatile constituent of *U. californica*, a tree known to trigger violent headache crises.[92] Recent studies demonstrated that exposure to umbellulone evokes nociceptive behavior, meningeal vasodilation and calcitonin gene-related peptide (CGRP) release in rodents through activation of TRPA1.[92] Furthermore, dural application of mustard oil and umbellulone produced robust time-related tactile facial and hind paw allodynia and reduce exploratory activity in a TRPA1-dependent manner.[91] Preclinical studies also found that intranasal delivery of the TRPA1 agonists mustard oil or acrolein results in meningeal

vasodilatation via a TRPA1-dependent mechanism.[78] It is not clear whether this is due to direct access to dural afferents through nasal delivery or as speculated, cross-excitation between nasal afferents and dural afferents through intraganglionic neurotransmission following nasal application of TRPA1 agoinsts.[46] In accordance with this hypothesis, TRPA1+ neurons are found to be clustered around some dural afferents, indicating the possibilities of cross-excitation in the trigeminal ganglion.[46] Activation/desensitization of TRPA1 was also shown to modulate the function of trigeminal ganglion neurons. Parthenolide is a major constituent of feverfew, which has long been used to treat headache.[93] A recent study found that parthenolide behaves as a partial agonist for TRPA1.[93] Following its initial activation, parthenolide causes desensitization of CGRP release from trigeminal neurons and renders TRPA1-expressing nerve terminals unresponsive to other stimuli.[93] This study opens up new opportunities to develop migraine treatments targeting TRPA1, possibly via desensitization.

Many identified TRPA1 agonists, including formaldehyde, ammonium chloride, cigarette smoke, and umbellulone are chemical irritants and they have long been known to trigger migraine headache in susceptible individuals.[7, 92, 94, 95] However, patients with gain-of-function mutations in TRPA1 have spontaneous limb pain with premonitory symptoms but no migraine,[87] and inhalation of many TRPA1 agonists can cause severe headaches but not always migraine. The channel may be hyperactive with the mutation, but still in need of local activators (just lower levels of activators). Those activators may be present in sufficient concentrations in some people in the meninges, but in others they may be present in other tissues. Further investigation is required to evaluate how TRPA1 can contribute to headache in some conditions and migraine in others.

2.2 Transient Receptor Potential Cation Channel V1 (TRPV1)—Located on small and medium-sized neurons, either unmyelinated C fibers or thinly myelinated A fibers, in trigeminal and dorsal root ganglion neurons,[96, 97] TRPV1 is activated directly by capsaicin and noxious temperatures (above 42 °C).[98] Additionally, extracellular protons dramatically potentiate the response of TPRV1 to capsaicin and noxious temperature during tissue acidosis, such as that associated with arthritis, inflammation, and other forms of injury.[99] Numerous studies focus on the role of TRPV1 in the generation and pathophysiology of migraine. A two-stage genetic association study showed that single nucleotide polymorphisms in the TRPV1 gene contribute to the genetic susceptibility to migraine in a Spanish population.[100] An immunohistological study found that nerve fibers in the dura mater express TRPV1.[101] In line with this finding, approximately 24% of identified dural afferents express TRPV1.[46]

Functional studies also elucidate the interplay between TRPV1 and the release of CGRP. CGRP, released at nerve terminals upon afferent activation, plays an important role in the generation of migraine headache. Infusion of CGRP can trigger a migraine attack and CGRP is also a potent vasodilator.[102] Bolus injections of capsaicin induce dural vessel dilation through TRPV1-mediated release of CGRP.[103] Ethanol, a well-known inducer of headache, is able to stimulate TRPV1 on primary afferent neurons, promoting neurogenic inflammation and CGRP-mediated coronary dilation.[104] However, it is not clear how TRPV1 is activated within the dura. A growing list of endogenous or exogenous mediators

of inflammation[105] have been shown to either activate TRPV1 directly such as anandamide (an endogenous cannabinoid neurotransmitter),[106] N-arachidonoyl-dopamine (NADA) (endovanilloids),[107] lipoxygenase products,[108] polyamines,[109] and venoms from jellyfish[110] and spiders[111], or lower its activation threshold, including ethanol,[112] protons,[99] bradykinin (an endogenous inflammatory peptide),[113] nerve-growth factor,[113] prostaglandins,[114] ATP,[115] and prokineticins.[116] Therefore, numerous mechanisms exist by which dural TRPV1 may be activated and sensitized following meningeal inflammation but which of these occurs before, or during migraine is not clear.

Multiple TRPV1-targeted therapies have been developed to potentially treat migraine. An intranasal TRPV1 agonist, Civamide has been used to deplete neuropeptides such as CGRP within the trigeminal system, thereby preventing migraine headache.[117] Clinical studies demonstrate that Civamide Nasal Solution can also substantially reduce the frequency of another headache disorder, cluster headache, following a single one-week course of treatment.[117] Another TRPV1 agonist under study is capsaicin. Capsaicin is known to desensitize TRPV1-expressing nerve endings not only to capsaicin but to other stimuli as well.[118] Repeated intranasal capsaicin applications produce significant amelioration of migraine attacks.[119] However, the initial activation of TRPV1 and subsequent peripheral neuropeptide release related with this group of drugs cause rather uncomfortable side effects.[117] The mechanism by which TRPV1 agonists treat headache attacks is not clear and surprisingly headache is not reported as a side effect after initial administration of these compounds.[117] The side effects of nasal TRPV1 agonists, including nasal burning, lacrimation and rhinorrhoea, indicating activation of nasal afferents.[117] It may be speculated that the thresholds for activation, desensitization, and neuropeptide depletion between nasal afferents and dural afferents are different or the local concentration of TRPV1 agonists at the nerve endings are not equal and thus response properties and side effects are not identical in the territories supplied by these neuronal populations.

Despite a seemingly suggestive role for TRPV1 in migraine based on the studies described above, the ultimate efficacy of a TRPV1-based therapy for migraine is questionable. TRPV1 antagonists have been developed for various types of pain but side effects have so far prevented their progress beyond early stage clinical trials. AMG-517, a TRPV1 antagonist, was discontinued in clinical trials due to the incidence of long-lasting hyperthermia in susceptible humans with a maximal body temperature surpassing 40 °C,[120] and similar reports of increased body temperature were reported with other TRPV1 antagonists. Further, the effects of TRPV1 antagonists are inconsistent in various studies.[103, 120–123] Capsazepine was able to inhibit the capsaicin-induced vasodilation[103] and administration of SB-705498, a specific TRPV1 antagonist, was able to suppress the responses of second order neurons in nucleus caudalis due to the electric stimulation of the dura in cats.[122] However in another study, A993610, a specific TRPV1 antagonist, had no effect on capsaicin-induced vasodilation and it did not affect the firing of second order neurons following electric stimulation of the middle meningeal artery in rats.[121] Therefore, the clinical relevance of TRPV1 antagonists in the treatment of migraine remains to be established.

2.3 Transient Receptor Potential Cation Channel V4 (TRPV4)—Prior work has found that dural afferent nociceptors are mechanically sensitive.[20, 22, 124, 125] Trigeminal

afferent nociceptors can also be activated by dural application of solutions with either increased or decreased osmolarity.[20] The most likely channel mediating these effects is the mechano-sensitive TRPV4. TRPV4 is activated by hypotonic challenge as well as by mechanical stimulation[126–129] and mice lacking TRPV4 exhibit reduced pressure and osmotic sensitivity.[130, 131] Thus, TRPV4 represents a possible candidate for directly mediating the mechanosensitivity of dural afferent nociceptors. Expression of mRNA for TRPV4 has been found in the trigeminal ganglion[132] and both hypotonic solutions and the TRPV4 activator 4α-PDD were shown to excite 56% and 49% of identified dural afferents respectively.[133] In awake animals, dural application of hypotonic solutions or 4α-PDD resulted in migraine-related pain behavior through the activation of TRPV4.[133] Similar to TRPV1, the endogenous mechanism for activation of TRPV4 is not clear. Currently there is no evidence for a decrease in plasma osmolarity before or during a migraine attack. However, there could be mechanical stimulation of the meninges following sudden intracranial pressure changes due to head jolts or rotation, breath-holding, sneezing or coughing etc. It has been reported that mechano-stimulation such as rapid head movements and coughing can worsen headache pain in migraine patients,[134] suggesting increased mechanosensitivity of meningeal afferents. One possible basis for such symptoms could be sensitization of TRPV4. TRPV4 can be sensitized by pro-inflammatory mediators, including those released following mast cell degranulation e.g. PGE2, PAR2 etc.[135–138] Following sensitization, the threshold for TRPV4 activation in response to mechano-stimuli may decrease for dural afferents, contributing to intracranial mechanical hypersensitivity and this may also contribute to the throbbing pain of migraine. Therefore, TRPV4 is a promising candidate for transducing mechano-stimuli within the dura but this possibility merits further investigation.

2.4 Transient receptor potential melastatin 8 (TRPM8)—TRPM8 channels are activated by chemical cooling agents, including menthol and icilin and temperatures below 26 °C and therefore it has been proposed to sense cold stimuli. In support of this hypothesis, several studies have confirmed that TRPM8 knockout mice display severe behavioral deficits in response to cold stimuli, including diminished responses to acetone application to the hindpaw,[139–141] an impaired ability to discriminate warm and cold surfaces,[139, 141] and a reduction in icilin-induced jumping.[140] Cold allodynia is a symptom reported by patients during migraine attacks,[142] which could suggest the involvement of TRPM8, but activation of TRPM8 on dural afferents is unlikely to mediate this effect as channel activity would seemingly be required at the location of allodynia. The strongest evidence thus far for a role of TRPM8 in migraine comes from three separate genome-wide association analyses performed on 3 different groups of migraine patients, all of which identified that a TRPM8 gene variant (2q37.1, rs10166942) is associated with increased susceptibility to common migraine.[143–145] Studies have yet to identify how this gene variant alters channel function or expression if at all. And although intriguing, it is important to confirm that the identified gene variant is causally related to migraine and to explore how this channel contributes to pathophysiology. This is particularly important in light of potentially limited expression of TRPM8 in dural afferents as one study found a lack of TRPM8 expression in dural afferents,[46] while another study found sparse innervation of the dura with not all regions of the meninges containing TRPM8-positive afferents.[146]

3. P2X channels

P2X receptors are ligand-gated ion channels that activate in response to binding of extracellular ATP.[147] Seven P2X subtypes have been identified and functional channels are assembled as homomeric and heteromultimeric channels.[147] Among the 7 subtypes, $P2X_3$ has been widely implicated in various pain conditions[148] since it is highly expressed by peripheral sensory afferents.[149–151] Approximately 50% of dural afferents express $P2X_2$ or $P2X_3$ or both receptors[152] and 40% of the trigeminal sensory neurons expressing $P2X_3$ receptors also express $P2X_2$ receptors and vice versa, indicating the existence of $P2X_{2/3}$ heteromultimeric channels.[152] $P2X_3$-expressing trigeminal sensory neurons exhibit some unique neurochemical properties compared with those in dorsal root ganglion neurons. In contrast to almost complete co-localization between $P2X_3$ and IB4 in dorsal root ganglia (98–99%),[153, 154] only 64% of $P2X_3$-expressing neurons are IB4 positive in trigeminal ganglion.[152, 155] This suggests that $P2X_3$ receptors are also expressed on peptidergic afferents in trigeminal ganglion and may contribute to increased CGRP release.

Increased expression of $P2X_3$ channels may also be linked to migraine as $P2X_3$ receptors can be up-regulated by several migraine-related mediators. The neurotrophin nerve growth factor (NGF) is an important mediator of inflammatory pain conditions. A clinical study found higher levels of NGF in the cerebrospinal fluid of patients with chronic headache, supporting its involvement in chronic head pain.[24] NGF is able to enhance $P2X_3$ currents in trigeminal neurons[156] and neutralization of endogenous NGF decreases $P2X_3$ receptor-mediated currents and delays their recovery from desensitization via a PKC-dependent pathway.[157] CGRP has been demonstrated to up-regulate the membrane expression and activity of $P2X_3$ receptors[158] through enhancing gene transcription.[159] And acidosis may further enhance ATP response through interacting with $P2X_{2/3}$ heteromultimeric channels as $P2X_2$ is strongly pH-sensitive.[160, 161] $P2X_{2/3}$ activity may be enhanced during acidosis conditions, such as those following mast cell degranulation or meningeal inflammation. Interestingly, $P2X_3$ receptors might also be involved in the pathophysiology of familial hemiplegic migraine-1 with the R192Q missense mutation in the $\alpha 1$ subunit of $Ca_V2.1$ channels.[162] Neurons expressing this mutation possess more abundant lipid raft compartments containing a larger fraction of $P2X_3$ receptors at the membrane level.[162] This pathological change could lead to enhanced functional responses of $P2X_3$ receptors and contribute to migraine pathophysiology by enhancing ATP-mediated responses.[162] Together, these studies link $P2X_3$ to a variety of conditions implicated in migraine and suggest that the channel may play a role in migraine pathophysiology.

The source of ATP required to activate meningeal afferents is not clear. It has been shown that during a CSD event, ATP is released into the extracellular space at levels exceeding 100 mM,[163] but given the instability of ATP in the extracellular space, the likelihood of ATP migrating to the meninges before being degraded is low. There may also be ATP release in the meninges following sympathetic outflow as ATP is present with norepinephrine in vesicles released by sympathetic neurons. As yet, there are no conclusive studies demonstrating an increase or a source of ATP within the meninges so this remains a matter of speculation. Ultimately, efficacy of P2X antagonists in preclinical headache models or in humans with migraine, particularly if these compounds are peripherally restricted, would

lend support for a purinergic mechanism in migraine. Although specific antagonists for P2X3 receptors have not yet been tested for migraine, a recent study found that dihydroergotamine (DHE), an ergot alkaloid derivative used extensively in the acute treatment of migraine, represses ATP-mediated sensitization of trigeminal neurons via down regulation of $P2X_3$ receptors.[164] It is difficult to speculate that treatment antagonizing $P2X_3$ receptor could be useful for migraine prevention based on this finding given the non-selectivity of DHE, but some of the efficacy of DHE could be due to purinergic actions. As mentioned above, this hypothesis awaits testing with more selective compounds.

4. Calcium-activated Potassium Channel (BK_{Ca} or MaxiK)

In addition to ion channels expressed on dural afferent nerve endings which could directly initiate the activation of nociceptive signaling, other channels expressed in the trigeminal signaling pathway may also contribute to the hyperactivity of the trigeminocervical complex. One example is large conductance calcium-activated potassium channels, also known as BKCa or MaxiK. The α subunit of MaxiK is encoded by the *slowpoke* (*Slo*) gene.[165] Splice variants of *Slo* and co-association of *Slo* with its β subunit could give rise to a diversity of MaxiK channels.[165] The MaxiK channel is widely expressed in the brain, both in soma, dendrites and axonal terminals.[165] The highest expression level is observed in axonal terminals, suggesting that the neuronal MaxiK protein is most abundant in synaptic terminals.[165] Activation of neuronal cell body MaxiK channels induces K^+ efflux, hyperpolarization of neurons, and therefore reduces neuronal excitability.[165] In addition, MaxiK located at axonal terminals has been found to provide finely tuned modulation of neurotransmitter release through modulation of Ca^{2+} entry.[165] Located in close proximity to presynaptic voltage-gated calcium channels, MaxiK channels are thought to narrow the presynaptic action potential upon activation and therefore decrease presynaptic Ca^{2+} entry, resulting in reduced neurotransmitter release.[166] Thus, MaxiK might play a key role in modulation of pain transmission from the peripheral to the higher centers of central nervous system.

With respect to migraine, activation of MaxiK is hypothesized to work on both synaptic terminals and cell bodies to decrease neuronal firing by preventing transmitter release and subsequent activation of dorsal horn neurons.[167] Intravenous application of NS1619, an activator and opener of neuronal MaxiK channels, dose-dependently inhibited neurogenic dural vasodilation in a model of trigeminovascular nociception.[167] This suggests that activation of MaxiK channels is able to reduce neuronal firing and potentially the release of neurotransmitters from trigeminal neurons that innervate the dura.[167] In the CNS, MaxiK is expressed throughout the trigeminal nucleus caudalis.[167] Intravenous application of NS1619 had no effect on the firing of caudalis neurons,[167] whereas application of NS1619 directly onto the caudalis was able to inhibit neuronal firing induced by current injection or L-glutamate application,[167] indicating that NS1619 is not able to cross the blood-brain barrier. Thus, although MaxiK is expressed within the CNS, effects of systemic administration of NS1619 on dural vasodilation is likely occurring via peripheral action. The expression of the MaxiK channel is also found in sensory neurons, mainly in DRG neurons with small and medium diameters.[168] Mice with sensory neuron-specific knockout of MaxiK channels exhibited increased inflammatory pain, while acute nociceptive responses and neuropathic

pain conditions were unaffected.[168] These date suggest that MaxiK channels expressed on sensory neurons could play an important role in modulation of inflammatory pain conditions. Further investigation into the effects of MaxiK openers on trigeminal neurons, trigeminal nucleus caudalis neurons, and neurotransmitter release will help further clarify the role of MaxiK channels in migraine pathophysiology. The studies described above nonetheless suggest that manipulation of MaxiK channels may represent a novel therapeutic target for migraine treatment by affecting trigeminovascular neuronal firing in response to dural stimulation.

Sensitization of Dural Afferents

In vivo electrophysiological recording studies have found that dural afferents exhibit other properties of nociceptors, including sensitization.[20, 22, 125, 169] Transient and recurrent release of inflammatory mediators within the dura and resultant sensitization of dural sensory nerve endings might be relevant to symptoms that are characteristic of migraine, such as the presence of exaggerated intracranial mechanosensitivity. Therefore, a key question is whether dural afferents are similar to nociceptors innervating other tissues or alternatively, whether they have unique sensitization properties that might be of significance for headache pathogenesis.

A combination of inflammatory mediators (IM), consisting of PGE2, bradykinin, and histamine, was used to compare the differences in sensitization between dural afferents and afferents innervating temporalis muscle (TM). Interestingly, 100% of dural afferents were sensitized by IM application, compared to only 28.5% of TM afferents.[44] IM also depolarized the resting membrane potential in dural afferents, but not TM afferents.[44] To understand the unique sensitization properties of dural afferents, the ionic mechanisms underlying the sensitization were investigated. First, IM was able to increase tetrodotoxin-resistant voltage-gated sodium currents in dural afferents, which most likely underlies the IM-induced decreases in action potential threshold.[170] Most interestingly, IM application induced a depolarizing Cl^- current, which is most likely responsible for IM-induced membrane depolarization in dural afferents.[170] This IM-sensitive Cl^- current has never been reported in other sensory afferents, indicating that it might be unique to dural afferents.[44, 171] The ion channel mediating the IM-sensitive Cl^- current is yet to be determined but this channel could represent a significant new target for migraine therapeutics, particularly if there is a selective expression pattern within dural afferents.

In addition to above-mentioned inflammatory mediators, sensitizing effects of Interleukin-6 (IL-6) on dural afferents were also studied. IL-6 levels are elevated during migraine attacks[172, 173] and IL-6 levels are strongly correlated with stress, a reliable migraine trigger. Stress is capable of evoking IL-6 release in a mast cell dependent manner.[174] Thus, accumulating evidence points to IL-6 as a contributing factor in migraine. Following acute IL-6 application, trigeminal ganglion neurons were found to display phosphorylation of extracellular signal-regulated protein kinase (ERK), a neuronal activation marker,[175] indicating that these neurons respond to IL-6 through the Mitogen-Activated Protein Kinase (MAPK) signaling pathway. In awake animals, direct application of IL-6 to the dura produced dose-dependent facial and hindpaw allodynia via activation of the ERK signaling

pathway.[176] In dural afferents, IL-6 application decreased the current threshold for action potential firing, indicating an increased excitability,[176] and phosphorylation of the voltage-gated sodium channel Nav1.7 is most likely to contribute to these effects.[176] These data not only provide a cellular mechanism by which inflammatory mediators in the meninges cause sensitization of dural afferents, but also suggest that the ionic channels, such as the IM-sensitive Cl^- current or the voltage-gated sodium channel Nav1.7 might represent novel targets for developing antimigraine therapies.

Apart from the difference of sensitization in subpopulations of dural afferents, there is also a gender difference in IM-induced sensitization of dural afferents. The American Migraine study reports that migraine incidence is approximately three times higher in women than in men.[177, 178] Reasons for gender difference in migraine prevalence are not clear. Excitability and sensitization were compared between male and female dural afferents in rats and there was no difference between male and female dural afferents with respect to baseline excitability.[179] However, a significantly higher proportion of dural afferent from female rats (100%) than from male rats (50%) were sensitized,[179] which could contribute to higher susceptibility to migraine in women. The mechanisms for these differences are not yet clear but future studies should examine expression levels of IL-6 activated pathways, Nav1.7, and the IM-sensitive Cl^- current. Identification of sex differences in dural-afferent signaling will open up the possibility of developing different approaches for the treatment of migraine tailored specifically to men and women.

Inherited Migraine as a Channelopathy

Familial hemiplegic migraine is a rare inherited type of migraine with aura. Mutations in the genes CACNA1A at chromosome 19p13, encoding the α1 subunit of P/Q voltage-gated calcium channel Cav2. 1,[180, 181] ATP1A2 at 1q23, encoding the α2 subunit of the Na^+, K^+ adenosinetriphosphatase (ATPase),[182] and SCNA1A at 2q24, encoding α1 subunit of the votage-gated sodium channel Nav1.1[183] have been linked to FHM1,2,3, respectively (reviewed [181, 184, 185]). Recently, another susceptibility gene KCNK18, which encodes a two-pore domain (K2P) potassium channel, TWIK-related spinal cord potassium channel or TRESK, was linked to inherited migraine with aura.[186]

A negative resting membrane potential is critical for proper electrical signaling in excitable tissues. The resting membrane is mainly permeable to potassium ions due to the presence of background (leak) K^+ currents.[187] Only recently have several molecular entities responsible for leak K^+ currents been identified.[188] The family of cloned mammalian background potassium channels, named ‘two pore domain’ potassium channels (K2P), includes 15 related members.[188] Based on their sequence similarities, the K2P channel family was further divided into six subfamilies, including TWIK, TREK, TASK, TALK, THIK and TRESK,[188] all of which are characterized by a similar molecular architecture where each channel subunit contains two pore loop forming domain instead of one. K2P channel subunits dimerize to form functional channels. The electrophysiological features of K2P channels are also unique. Compared with other K^+ channel families, the opening of K2P channels is less dependent on voltage, making them ideal candidates for generating background K^+ currents and regulating the resting membrane potential.[189]

Among the K2P families, TRESK (TWIK-related spinal cord potassium channel) is the first member linked to a human disorder.[190] Genetic screening studies have demonstrated a link between a frameshift mutation in the KCNK18 gene, denoted F139wfsX24, with migraine with aura.[186] The mutation was discovered in a patient suffering from migraine with aura and 7 of the patient's relatives who are also migraine sufferers but was absent from 8 relatives who do not have migraine.[186] The F139wfsX24 mutation leads to a prematurely truncated and non-functional TRESK channel that can also suppress wild-type channel function through a dominant-negative fashion. However, it is unknown whether the aura or the migraine attacks in these patients are directly related to dysfunction of TRESK or due to other factors.

Several lines of preclinical evidence describe how TRESK may contribute to migraine pathophysiology. The expression of KCNK18 appears (in mice) in the trigeminal ganglion (TG) and dorsal root ganglion (DRG) at embryonic day 15.5 and reaches a peak in newborns.[186, 190] In adult mice, KCNK18 expression is highest in trigeminal ganglion.[186] Similarly, strong expression of TRESK is also found in human trigeminal ganglion neurons.[186] Trigeminal ganglion neurons expressing mutant TRESK subunits exhibit a lower threshold for activation and a higher spike frequency in response to supra-threshold stimuli.[191] Functional knockout of TRESK in mice results in a lowered threshold for activation, reduced action potential duration and slightly higher amplitudes of after-hyperpolarization in DRG neurons.[192] Taken together, these studies suggest that TRESK channel is important for regulating excitability of primary afferent neurons. However, at this point, the site of action of this channel in migraine pathophysiology is not clear and this is a question that merits further investigation. The presence of aura in affected family members suggests that decreasing TRESK activity contributes to aura, possibly through lowering cortical spreading depression (CSD) threshold,[186] which would suggest a CNS role for these channels in migraine. Regardless of the site of expression, these studies suggest that increasing TRESK activity will theoretically protect against migraine. Although not used for migraine, volatile anesthetics, such as isoflurane and halothane increase TRESK currents up to 3-fold at their clinical concentrations.[193] Since the TRESK channel is distantly related to other K2P channels,[188] it may be possible to develop novel molecules selectively targeting the TRESK channel that can be used as abortive or prophylactic drugs for migraine treatment.

Despite the interesting potential role for TRESK described above, a direct causative link between non-functional TRESK channels and migraine is questioned by a recent study showing that a loss of function mutation (C110R variant) of TRESK is found in both migraine and control cohorts.[194] This study argues that non-functional TRESK channels alone are not sufficient to cause typical migraine. Several reasons might account for this discrepancy. Among K2P channel families, TREK, TALK, and TASK channels exhibit several splice variants[188] and the expression pattern of the splice variants shows significant tissue specificity.[195, 196] The splice variants of TRESK channels have never been examined. It is possible that the C110R variant and F139wfsX24 mutation are differentially expressed, with the F139wfsX24 mutation expressed in migraine-related tissues. Furthermore, migraine has now generally been recognized as a polygenic disorder and thus it is dependent upon gene-gene interaction as well as environmental triggers instead of a single channel

mutation.[197] Therefore, the TRESK channel is a novel and interesting new component in the migraine pathophysiology pathway but its role in neuronal excitability and migraine signaling requires more study.

Conclusions

Taken together, these studies indicate that ion channels can play an important role in migraine pathophysiology. Mutations of certain ion channels, such as TRESK and TRPM8, are linked with inherited migraine. Activation of primary afferent neurons is likely a critical step for the pain of migraine and multiple ion channels expressed on dural afferents can contribute to afferent input by sensing environmental changes in the meninges following CSD, ischemic, or inflammatory events etc. Future studies should more closely examine the role of ASICs in migraine by expanding the patient size of amiloride clinical trials for migraine and the development of more selective tools to better probe for a role of ASICs in migraine. It is also important to explore the endogenous mechanisms leading to the activation of TRP channels within the meninges. Knowledge of which and how specific TRP channels contribute to migraine triggered by specific factors will be critical in the development of effective therapy tailored to the individual migraine patient. Although genome-wide association studies have yielded plausible susceptibility genes in migraine, further work is required to determine whether the association is causal and functional studies are necessary to dissect the exact underlying molecular pathways of TRPM8 and TRESK channels.

Current therapies for the treatment of migraine have been restricted to drug classes such as NSAIDs, triptans, tricyclic antidepressants and anticonvulsants. However, such compounds provide limited benefit to many migraine patients. The studies described here provide several targets for therapeutics that are not manipulated by currently available drugs. A greater understanding of the role of ion channels in the mechanisms contributing to headache initiation can ultimately make it possible to develop novel ion-channel based migraine therapies with increased efficacy over current therapeutics.

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Abbreviations

CSD	cortical spreading depression
FHM	familial hemiplegic migraine
K2P	a two-pore domain potassium channel
TRESK	TWIK-related spinal cord potassium channel
TG	trigeminal ganglion
ASICs	acid sensing ion channels

TRP	transient receptor potential channels
TRPA1	transient receptor potential cation channel A1
TRPV1	transient receptor potential cation channel V1
TRPV4	transient receptor potential cation channel V4
TRPM8	transient receptor potential melastatin 8
BKCa or MaxiK	calcium-activated potassium channel
TM	temporalis muscle
IM	inflammatory mediators
IL-6	Interleukin 6

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Table 1

Dural Afferent Transducers

Dural Afferent Transducers	Evidence		Potential Stimulating Events within the Dura	Ref
ASICs	**1**	80% of dural afferents respond to acidic solution with ASIC3-like currents.	mast cell degranulation secondary to stress, CGRP release, nitroglycerin infusion, increased estrogen level and CSD events	47
	2	Dural application of acidic solution induces migraine-related pain behavior through activation of ASICs.		
TRPA1	**1**	5.7% of identified dural afferents express TRPA1.	exposure to a series of chemically diverse and highly reactive environmental agents and various electrophilic natural products or mechanical stimuli	46, 91, 92
	2	TRPA1 agonists, mustard oil and the umbellulone evoke TRPA1-like currents in approximately 42% and 38% of dural afferents, respectively.		
	3	Dural application of TRPA1 agonists produces migraine-related behaviors in a TRPA1-dependent manner.		
TRPV1	**1**	Nerve fibers in the dura mater exhibit TRPV1-immunoreactivity.	Endogenous or exogenous mediators of inflammation have been shown to activate TRPV1 directly or lowered the activation threshold of TRPV1.	46, 101
	2	23.7% of identified dural afferents generate TRPV1 current.		
TRPV4	**1**	56% and 49% of dural afferents generate currents in response to hypotonic solutions and the TRPV4 activator 4α-PDD.	sudden intracranial pressure change due to head jolts or rotation, breath-holding, sneezing or coughing	133
	2	Dural application of hypotonic solution or 4α-PDD results in migraine-related pain behavior through activation of TRPV4.		
P2X	52% of dural afferents express $P2X_2$ or $P2X_3$ or both receptors.		During CSD event, ATP is released into the extracellular space at levels exceeding 100 μM.	152