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TRPS and Migraine

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Abstract: Migraine is a highly prevalent, disabling neurovascular disorder characterized by a combination of headache, nausea and altered sensory processing such as photophobia. Migraine has a strong genetic background but the molecular pathways that result in a migraine attack, and the role of various triggers, are poorly understood. The throbbing and pulsating pain associated with the headache phase of migraine attack implies an important role for the nociceptive activation of trigeminal intracranial afferents that contain calcitonin gene-related peptide (CGRP). Neurogenic inflammation triggered by the release of CGRP is now recognized as a significant underlying event in migraine. Indeed, CGRP receptor antagonists, the so-called "gepants", have already proved effective in clinical trials as novel, migraine-specific drugs. An alternative therapeutic approach is the modulation of CGRP release. As potential targets, the transient receptor potential (TRP) channels expressed by a subpopulation of CGRP-containing nociceptive primary sensory neurons are gaining increasing prominence, principally because of the recent discovery of a variety of endogenous and exogenous TRP agonists known to induce migraine attack as well as their emerging role in neuropeptide release. The present review focuses on the potential role of the different TRP channels, especially TRPV1, in the migraine mechanism.

Keywords: TRP channel, TRPV1, migraine, capsaicin, CGRP, neurogenic inflammation, clinical trials, heat, neuropeptides, geptins.

INTRODUCTION

Migraine is a common, disabling and economically costly [1, 2] neurovascular disorder that affects a significant portion of the population, an estimated 13 million Americans. Underlying migraine are neurovascular events that result in the dilatation of blood vessels and subsequent nerve activation and pain [3]. Disability due to migraine is severe. Although migraine involves patients from infancy through senescence, it is especially prevalent among women between the ages of 35 and 45 years, with one in five individuals reporting one or more migraine headaches per year in this age group. Migraine care is complicated by the fact that underlying triggers and pathways are poorly understood. Genetic abnormalities may initiate the alteration of the response threshold to migraine-specific triggers in the brain, but the specific gene(s) involved have not yet been determined [4]. The migraine attack can be precipitated by an extraordinary variety of stimuli, including psychological stress factors, excessive ingestion of alcoholic beverages, menstrual cycle, and changes in barometric pressure, anti-angina medicines, sleep deprivation, and other factors [5, 6]. The lack of knowledge about the pathways underlying migraine attack is one of the major obstacles to the discovery of better and safer drugs. Uncertainty also exists regarding the precise site of action and molecular targets of the various drugs used for both prophylaxis and symptomatic relief of migraine attack. However, in recent years much progress has been made to better understand the underlying mechanism of the disorder. There is growing evidence that the initiating event (though influenced by several contributing factors such as stress, environmental agents or hormones) occurs in the central nervous system (CNS) and somehow involves trigeminal neurons [4, 7].

In recent years, the hypothesis that the release of calcitonin gene-related peptide (CGRP) from sensory neurons stimulates sensory nerve transmission and dilates cranial blood vessels has gained experimental support [8], suggesting a major role of CGRP in the pathogenesis of migraine. In this perspective, CGRP receptor antagonists have become attractive as potential migraine-specific drugs. Moreover, it has been demonstrated that other brain regions with enriched CGRP receptor expression, such as the hypothalamus, are activated during spontaneous migraine attacks [9]. The first experimental observations that CGRP (8–37), a truncated form of CGRP [10], behaved as a competitive and selective antagonist of the biological effects of CGRP, including smooth muscle relaxation, were not pursued, mainly because of the short biological half-life of this peptide. More recently, the inhibition of CGRP receptors with two chemically unrelated CGRP receptor antagonists, telcagepant and olcegepant (collectively referred to as "gepants") [11, 12], has been successfully used to treat acute migraine [13-15].

CGRP is contained in neurons of the central and peripheral nervous system. In the periphery, except for intrinsic neurons of the gastrointestinal tract, CGRP is strictly confined to a subpopulation of somatosensory neurons with cell bodies located in the dorsal root (DRG), vagal (VG) and trigeminal (TG) ganglia. The release of sensory neuropeptides, including CGRP, undergoes fine tuning by a series of mediators and agents that act at

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prejunctional receptors/channels on sensory nerve terminals. A wide array of G protein-coupled receptors are involved in this process, including those for bradykinin, prostanoids, opioids, 5-hydroxytryptamine (5-HT_1 receptor), histamine (H_3 receptor), neuropeptide Y, somatostatin, vasoactive intestinal polypeptide (VIP), purines and galanin. One of the major mechanisms that promotes CGRP release from sensory neurons is the activation of members of the transient receptor potential (TRP) family of channels, in particular TRPV1 (Founding member of the Transient Receptor Potential Vanilloid family). The present review focuses on the distribution and activation mechanisms of the different TRP channels expressed by subsets of trigeminal primary sensory neurons, and discusses the potential of this receptor family in migraine mechanisms.

PRIMARY SENSORY NEURONS AND NEUROGENIC INFLAMMATION: ROLE IN MIGRAINE

More than 70 years ago, Sir Thomas Lewis, in his pioneering studies [16], precisely defined the dual 'nocifensor' role of a subset of primary sensory neurons. One segment of the widely branching sensory fiber network of this neuron responds to the injury and generates action potentials which are carried antidromically to collateral branches, where these branches release a chemical substance that causes a flare response and increases the sensitivity of other sensory fibers responsible for pain. It has been proposed that the neurons which mediate these responses belong to a previously unrecognized subgroup, and termed ‘nocifensors’ because of their dual function. The first function of these nocifensors is to sense nociceptive/pain stimuli. The second is to promote a first line of defense, mediated through neurovascular responses, which includes arterial vasodilatation, plasma protein extravasation, and other responses. All these responses are associated with the capacity to release neuropeptides from peripheral terminals of primary sensory neurons. There is now substantial evidence to suggest that a neurogenic component of inflammation exists whose main features have been described in details previously [17, 18].

In laboratory animals, neurogenic inflammation has been well documented in multiple tissues and organs, and its role has been robustly established in relevant models of human diseases. However, the hypothesis that this phenomenon contributes significantly to human diseases, and in particular to migraine, is still a matter of debate. However, recent clinical trials [19] have given credence to this hypothesis with neurochemical and pharmacological data supporting the key role that neurogenic inflammation plays in the mechanism of migraine [20].

Cerebral vessels, pial vessels, large venous sinuses and dura mater are surrounded by a dense plexus of unmyelinated fibers that arise from the ophthalmic division of the trigeminal ganglion. The trigeminal nerve also innervates extracranial tissues, including vessels. Altogether, this dense network of mainly perivascular fibers comprises the so called trigemino-vascular system [21]. Trigeminal fibers, arising from neurons in the trigeminal ganglion, contain CGRP and substance P (SP) [22]. The stimulation of trigeminal ganglia/nerve is considered a necessary step for the process that underlies pain [23] and the associated symptoms of migraine attack. A considerable number of animal and human studies have been carried out to better understand the physiology and pharmacology of the sensory innervation of the dura mater and cranial vessels. Evidence obtained in experimental animals suggests that the migraine aura is the clinical manifestation of a cortical spreading depression (CSD) [24, 25]. It has been proposed that the neural changes caused by the slow depolarizing wave of CSD eventually result in the painful symptoms associated with vasodilatation of the cranial blood vessels and other phenomena of the migraine attack [4]. However, while tonabersat, a drug specifically developed to block CSD, did abolish the aura, it failed to prevent the headache of migraine attack, in either an acute or a chronic setting [26-28]. This finding indicates that CSD and the resulting aura are epiphenomena, but not the cause of the pain of migraine attack.

In experimental animals, particularly rodents, stimulation of a subset of peptidergic somatosensory neurons leads to the release of various neuropeptides, specifically CGRP and the tachykinins SP and neurokinin A (NKA). Activation of CGRP receptors and tachykinin (NK1, NK2 and NK3) receptors located on effector cells at the peripheral level causes a series of inflammatory responses, collectively referred to as ‘neurogenic inflammation’ [17]. This term refers to a cascade of events that occur mainly, but not exclusively, at the perivascular level. Indeed, CGRP-, SP-, and NKA-release induce vasodilatation in arterial vessels, and plasma protein extravasation and leukocyte adhesion to the vascular endothelium of postcapillary venules [17]. These two latter responses are mediated by NK1 receptor activation on endothelial cells, which results in a calcium-dependent activation of intracellular contractile elements, leading to the opening of gaps between cells and the development of inflammatory edema. In the dura mater, neurogenic plasma extravasation mediated by NK1 receptors has been initially proposed as the main mechanism by which the trigeminovascular system contributes to the pathogenesis of the migraine attack [20]. Unfortunately, this exciting hypothesis has not been supported by successful clinical studies: selective and high affinity NK1 receptor blockers did not relieve migraine attacks [29-31]. The negative evidence from the clinic regarding a role of SP and NK1 receptors in migraine is in agreement with failure to demonstrate SP/NKA release from human tissues containing trigeminal nerve endings [32]. However, there is recent preclinical and clinical evidence pointing to the contribution of CGRP in migraine. This neuropeptide, released following exposure to a large variety of stimuli from terminals of primary sensory neurons, may cause a neurogenic arterial vasodilatation, or other effects relevant for the pathogenesis of migraine. In particular, evidence is now emerging for a role of CGRP in the activation and sensitization of nociceptors at the peripheral, and, perhaps, at the central level.

CGRP is synthesized and released from somatosensory neurons with small cell bodies and fibers that are unmyelinated (C-fibers) or thinly myelinated (Aδ-fibers). This extensive network of sensory nerves, found in virtually all tissue and organs, suggests a potential role for CGRP in diverse physiologic and pathophysiologic processes. The remarkable vasodilatation elicited by CGRP in the cerebral, coronary, and peripheral vasculature has led to its therapeutic evaluation in the treatment of cerebral vasospasm following subarachnoid hemorrhage, stable angina, and Raynaud's phenomenon [33]. The inotropic action and coronary vasodilatation have also led to a potential beneficial use of CGRP in congestive heart failure [34, 35]. CGRP interacts with specific G protein-coupled receptors to produce a vasodilatory effect which is more potent than that observed with other classical vasodilators, including prostaglandins, acetylcholine, adenosine, and SP. Except for a few vascular preparations, including the rat aorta and the human internal mammary artery, where the relaxation of CGRP depends on the presence of an intact endothelium and is attenuated by inhibitors of nitric oxide (NO) synthase [36-38], the relaxation induced by CGRP is mediated by an endothelium-independent phenomenon.

Trigeminal sensory nerve fibers that robustly stain with anti-CGRP antibodies are well represented at the intracranial and extracranial level [39]. The enriched expression of CGRP by trigeminal sensory neurons, and its release from these cells, has been demonstrated both in humans and animal models [40, 41]. Furthermore, recent evidence shows that in animal models and in man [12] the vasodilatation induced by depolarizing stimuli was inhibited by a CGRP-receptor antagonist, indicating that neurogenic vasodilatation is mediated by the release of CGRP from primary sensory neurons. Moreover, plasma concentrations of CGRP, but not of SP, were found to be elevated during spontaneous or nitroglycerine-provoked attacks of migraine [42] and cluster headache [43]. More importantly, the intravenous infusion of CGRP produced a migraine-like headache [44], and intravenous infusion of NO evoked a migraine-like headache with an associated increase in plasma CGRP levels in female migraineurs [45]. Finally, the CGRP plasma levels were more elevated in migraine patients, and changes in plasma CGRP levels during migraine attacks were related to the headache intensity [45]. Not only does it appear that CGRP release in cranial or systemic circulation is a suitable marker of trigemino-vascular activation in cluster headache and migraine [46], but the positive clinical trials with CGRP receptor antagonists also suggest that local CGRP secretion is likely causative factor in migraine and cluster headaches.

TRP CHANNELS IN PRIMARY SENSORY NEURONS

The stimulation of a subset of primary sensory neurons at the level of their peripheral terminals is not only associated with the induction of neurogenic inflammation, but also results in the transmission of nociceptive, or pain, signals. The neurons, which may mediate both the sensory, afferent response and the 'local' efferent effect, are those belonging to the sub-category with Aδ and C fibers, and are defined as polymodal nociceptors because they sense thermal, chemical, and high-threshold mechanical stimuli. A subset of these sensory neurons is also uniquely sensitive to capsaicin, the pungent principle contained in plants of the genus *Capsicum*. The sensation of burning pain that follows exposure of peripheral sensory neurons to capsaicin is mediated by TRPV1, the founding member of the Vanilloid subfamily of TRP ion channels [47]. TRPV1 is a polymodal sensor which, in mammals, has been proposed to play a crucial role in the hypersensitivity to thermal, chemical and mechanical stimuli associated with inflammatory and neuropathic pain.

TRPV1 is activated by three main pain-producing stimuli: (1) vanilloid compounds (capsaicin, resiniferatoxin), (2) noxious heat (≥43 °C), and (3) low pH (<5.9) [47, 48]. In addition, a series of endogenous molecules of lipid nature have been shown to activate the TRPV1 [48-51]. However, the physiologic relevance of these molecules has been questioned because it is unclear if their tissue concentrations found under either physiological or pathological conditions are sufficient to activate TRPV1. In addition to TRPV1 receptor, additional heat-sensitive TRPs ("thermoTRPs") have been described in TRPV1-expressing primary sensory neurons of the TG, VG and DRG, including TRPV2, TRPV3, TRPV4, and TRPA1. TRPM8, also known as the menthol receptor, is expressed by a different TRPV1-negative neuronal subpopulation. ThermoTRP channels are emerging as sensory transducers that may participate in the generation of pain sensations evoked by chemical, thermal and mechanical stimuli. There is also some evidence to suggest a role for TRPV4 in mechanosensation. However, the hallmark of TRP channels is their polymodality. While the precise distribution of each of these channels in sensory neurons is poorly understood, recent work has started to provide some clarity.

By using radial stretch in combination with live-cell calcium imaging, different mechanosensitive and insensitive sensory neuronal categories were identified [52]. A group of small-diameter stretch-sensitive cells could be further subdivided into (1) a cluster of small-diameter cells sensitive to both hydroxy-α-sanshool (a two pore K channel antagonist) and the TRPV1 agonist capsaicin, and (2) a cluster comprised of large-diameter cells that respond to hydroxy-α-sanshool, but not capsaicin. The former neuron type likely corresponds to high threshold nociceptors, and the latter to low threshold proprioceptors. Moreover, stretch insensitive neurons fall into two groups of small-diameter cells; the first group is composed by peptidergic neurons sensitive to capsaicin and to the TRPA1 selective agonist mustard oil, and a second group by a small cohort of menthol-sensitive cells [52]. Thus, TRPA1-expressing neurons, which obligatorily co-express TRPV1, are those apparently insensitive to mechanical stimulation which, because they contain neuropeptides, bring about neurogenic inflammation and neurogenic CGRP-mediated vasodilatation. (Parenthetically, recent data suggest a role for TRPA1 in mechanotransduction.) Finally, a subgroup of neurons, which does not contain neuropeptides, is uniquely sensitive to the excitatory action of menthol and express the relatively specific menthol-receptor TRPM8 [53], but not TRPV1 and/or TRPA1.

The study of peripheral sensory fibers has been facilitated by natural products, such as capsaicin, and these molecules have proven to be exquisite tools to probe the function of primary sensory neurons in a wide array of physiological processes, ranging from pain to neurogenic inflammation. But, sensory TRP channels are not simply natural product receptors. They are molecular sensors of an array of modalities (temperature, protein kinase activity, phospholipids, osmolarity and pH), which, leading to a common transduction process, elicit somatosensory responses. Therefore, the identification of TRP channels as the molecular transducers and integrators of a broad range of sensory modalities, best exemplified by TRPV1, has provided new insights into the physiological role of sensory nerve fibers.

ACTIVATION MECHANISMS OF TRP CHANNELS EXPRESSED IN TRIGEMINAL SENSORY NEURONS

Of the five heat-sensitive members of the TRP family [54, 55] that are co-expressed with TRPV1 in primary sensory neurons, three (TRPV2, TRPV3 and TRPV4) are gated by warm, non-noxious and noxious temperatures and small reductions in tonicity. In particular, TRPV4 activation has been associated with the release of neuropeptides and resulting neurogenic inflammation in peripheral tissues [56]. A more detailed pharmacological characterization of TRPV2, TRPV3 and TRPV4 has up to now been limited by the absence of selective activating and blocking compounds of these TRP subtypes. In particular, the lack of selective antagonists has narrowed the possibility to gain better knowledge on the role of these channels in health and disease. TRPM8 channels are activated by menthol and moderately low temperature [47, 53, 57-60].

It is well known that stimulation of TRPV1 by capsaicin produces a burning sensation and releases sensory neuropeptides such as CGRP. Because of the unique capability of capsaicin to activate and desensitize TRPV1 for decades it has been considered an instrument for better understanding the function of a subset of neuropeptide-containing sensory neurons that mediate neurogenic inflammation, as well as of their role in models of human diseases [17]. In addition to its initial excitatory effect, exposure to high concentrations/doses of capsaicin for a prolonged time induces a desensitization of the sensory neurons or nerve endings in a time- and concentration/dose-dependent manner, an effect that ultimately results in the inability of the nerve fibers to evoke pain and neurogenic inflammation [18, 61]. Moreover, because after capsaicin application the tissue becomes desensitized and irresponsive to a series of painful stimuli, this procedure has been used to successfully treat various painful conditions [62].

TRPV1 receptor activation is not only mediated by exogenous xenobiotics, such as capsaicin, but also by a series of endogenous agents, including low extracellular pH, anandamide, N-arachydonoil-dopamine, ecosanoids and other agents [48-51]. The major pro-inflammatory peptide bradykinin, indirectly (*via* activation of the B2 receptor) sensitizes TRPV1 by diverse intracellular mechanisms [63, 64]. Regulation of TRPV1 channels by a diverse array of signaling underlines the role of sensitization in the modulation of the sensory and proinflammatory functions of nociceptors. The finding that PAR-2 stimulation up-regulates the function of TRPV1 through a Protein Kinase C (PKC) -dependent mechanism adds PAR-2 to the list of G protein-coupled receptors that, by regulating TRPV1, orchestrate the neural components of the inflammatory response [65]. Sensitization of TRPV1 by PKC and cAMP-dependent protein kinase A (PKA) pathways seems to be promiscuously used by different stimuli, including capsaicin, anandamide, heat and protons [63,66-68], but it is not unique to endogenously generated agents. The common notion that exposure of mucosal surfaces or wounds to alcoholic tinctures causes burning pain has remained without an explanation until the observation that ethanol excites TRPV1-expressing rat sensory neurons [69]. TRPV1, usually stimulated at 42 °C, is activated by lower temperatures in the presence of ethanol, such as the physiological temperature of 37 °C, because ethanol lowers the threshold temperature for TRPV1 activation by about 8 °C [69]. In the presence of ethanol, the effects of TRPV1 agonists, including anandamide and protons, are markedly potentiated [69].

In the last few years, the role of the TRPA1 channel on primary sensory neurons has also been better defined. Shortly after the identification of TRPA1 receptor in human pulmonary fibroblasts [70] and in hair cells of the auditory system [71], abundant expression of TRPA1 was recognized in a subpopulation of peptidergic primary sensory neurons with C and Aδ fibers of the DRG, VG and TG that co-express TRPV1 [72]. TRPA1-expressing neurons contain and release the neuropeptides CGRP, SP and NKA from their terminals, which in peripheral tissues produce neurogenic inflammation [17]. Although the TRPA1 channel has been proposed to be involved in the transduction of thermal [73] and mechanical [74] stimuli, it is best characterized as a “chemosensor”, activated in response to many chemical agents (a large number produced by plants and still others synthetic) that, like capsaicin, activate peripheral C-fibers, thereby causing acute pain, thermal and mechanical hyperalgesia, and neurogenic inflammation.

Icilin, an activator of both TRPM8 and TRPA1 receptors, is in fact able to produce cold sensation [53, 60]. In contrast, other chemicals, including isothiocyanates (pungent ingredients of edibles like wasabi, mustard, and horseradish) show high selectivity for TRPA1. Cinnamaldehyde (contained in cinnamon), allicin and diallyl sulfides (garlic and onions), carvacrol (oregano), and polygodial (water pepper and Tasmanian pepper) are TRPA1-stimulating agents [54, 75]. Robust and conclusive evidence has been accumulated in the past 5 years with respect to the ability of TRPA1 to respond to a host of environmental irritants. These include acrolein (2-propenal), a highly reactive α,β-unsaturated aldehyde, produced endogenously or present in tear gas, vehicle exhaust, or smoke from burning vegetation (i.e., forest fires and cigarettes) [76] and other volatile irritants such as and formalin [77] and isocyanates [78]. TRPA1 has been also recognized as the target of a series of endogenous α,ß-unsaturated aldehydes, which are produced by lipid peroxidation in response to oxidative stress at sites of inflammation and tissue injury [76, 79, 80]. These aldehydes include 4-hydroxy-2-nonenal (HNE) which is produced by peroxidation of omega 6-polyunsaturated fatty acids, such as linoleic acid and arachidonic acid [81, 82] or 4-oxononenal [83]. Moreover, new reactive TRPA1 agonists have been discovered at a breathtaking pace, suggesting that, given appropriate conditions, almost all oxidizing or electrophilic chemicals will affect TRPA1 function. These include the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2) [78, 84, 85], superoxide (O^{2-}), hypochlorite (ClO^-) [86] and the reactive nitrative species (RNS) peroxynitrite ($ONOO^-$) [85]. Also, nitrooleic acid, a byproduct of nitrative stress, is a TRPA1 activator [87]. During inflammation, ROS are generated in abundance. Exposure of cellular membranes to ROS causes membrane lipid peroxidation, producing electrophilic reactive mediators such as cyclopentenone prostaglandins and isoprostanes, all compounds that have been recognized as TRPA1 agonists [88, 89]. Thus, byproducts of oxidative/nitrative stress converge on TRPA1 to alert the sensory system of the presence of inflammation or tissue damage. Similar to TRPV1, TRPA1 is sensitized by a number of proinflammatory stimuli.

Despite their different structures, all these stimuli are unified in their ability to form covalent adduct with the thiol group, a moiety that confers them the ability to activate TRPA1 receptor. Several known TRPA1 agonists, including acrolein and other α,ß-unsaturated aldehydes, possess an electrophilic carbon or sulphur atom that is subject to nucleophilic attack (Michael addition) [90] by cysteine, lysine or histidine of TRPA1. Mutagenesis studies have clarified that such reactivity promotes channel gating through covalent modification of residues within the cytoplasmic N-terminal domain of the channel [80, 91, 92].

RECENT ADVANCES IN MIGRAINE TREATMENT: CONTRIBUTION OF TRPV1/TRPA1 RECEPTORS AS POTENTIAL THERAPEUTICAL TARGET

Migraine is a disorder of the brain characterized by a complex sensory dysfunction. Unfortunately, precise mechanisms involved in this neuronal dysfunction are not well understood. Some studies define a central origin of headache pain [4], whereas others consider the activation and sensitization of peripheral nociceptors as the starting event of headache pain. More recently, the possibility that reduced inhibitory brainstem activity is sufficient to generate pain from otherwise silent nociceptors has been questioned [7]. Thus, if peripheral activation and sensitization of cranial nociceptors underlies migraine, it becomes important to identify the mediators that activate/sensitize perivascular nerve terminals that innervate cerebral, dural, and extracranial arteriole. In the last few decades, increased knowledge of the neurobiological and pharmacological components of a subset of trigeminal primary sensory neurons has provided key information for the development of effective molecules that specifically target the activation of the trigeminovascular system, and may represent a significant advancement in the treatment of the disease.

The prevention of migraine is a significant component of therapy aimed at reducing the severity and the frequency of the attack. Substances that have proven beneficial in migraine include beta-blockers (propranolol), antidepressants (amitriptyline), anticonvulsants (valproate, topiramate), calcium channel blockers (flunarizine) and serotonin antagonists (methysergide) [93]. According to the pathophysiological events involved in migraine attack, these drugs most probably target the activity of modulatory circuits, as well as the neuronal activity in afferent sensory pathways, such as the trigeminal system [94].

For severe migraine attacks, treatment with non-specific analgesic compounds, such as non steroidal anti-inflammatory drugs (NSAIDS), and more migraine-specific treatment approaches, such as ergot derivates and triptans, which are active at 5-HT$_1$ receptors, is currently considered to be the 'gold-standard' of care. In addition to their peripheral anti-inflammatory and analgesic effects, ergot derivatives and triptans also reduce neuronal activity at the trigeminocervical complex [95] and thalamic level [96]. Triptans, because of their superior tolerability, are the most effective medication chosen for severe migraine attacks replacing ergotamine in most cases [97]. However, the vasoconstrictive effect of triptans precludes their use in patients with cardiovascular disease [98].

To develop a therapy aimed at reducing attack frequency and severity of migraine in daily practice, new pharmacological and interventional therapies treatment strategies are being investigated. A major breakthrough in the field was the discovery of the peptoid CGRP receptor antagonist olcegepant [99], which, with high affinity and selectivity, inhibited neurogenic vasodilatation in various experimental animals and in humans [19]. More recently, the discovery of the orally available antagonist, telcagepant [100], has been reported, a compound which binds the CGRP receptor with high affinity and inhibits capsaicin-induced increases in forearm dermal blood flow in rhesus monkeys [12]. Telcagepant was able to abort acute migraine with significant effect at two hours [14]. Both drugs have been shown to reduce the pain and the associated symptoms of migraine attack with efficacy similar to triptans, but were safer. Moreover, the marked and prolonged efficacy of CGRP antagonists might represent a significant advancement for those patients who respond poorly to triptans. The success obtained in the relief of headache pain and other symptoms of the migraine attack with CGRP receptor antagonists reinforces a neurally-based approach to migraine. In this perspective, the identification of the molecular pathways that cause the release of CGRP from peptidergic sensory neurons is of primary interest. As peptidergic trigeminal neurons are labeled as TRPV1- and TRPA1-positive, major attention must be paid to these channel subtypes in order to provide a broader repertoire of migraine-specific drugs.

Many studies have shown the ability of repeated intranasal application with topical TRPV1 agonists to ameliorate migraine attack indicating that TRPV1-positive neurons play a significant role in the pathophysiology of migraine. Repeated capsaicin application to the nasal mucosa reduced the headache attacks of both chronic and episodic cluster headache [101, 102]. The contribution of TRP channels in triggering migraine attack is shown in recent studies with ethanol. Alcoholic beverages are known inducers of migraine attacks and, accordingly, migraineurs do not usually drink alcoholic beverages. However, it is not known why alcohol precipitates migraine attacks. Our recent studies demonstrate that ethanol (1-3% solution) reduces the threshold temperature for activation of the TRPV1 by 8 ºC (from ~43 to ~35 ºC) [69]. Thus, ethanol enables TRPV1 activation by normal body temperature, thereby producing the well know sensation of burning pain. The observation that ethanol stimulates TRPV1 channels and produces a CGRP-dependent vasodilatation of dural blood vessels [103] suggests the ability of alcoholic beverages to trigger the migraine attack. Previous pharmacological findings that low extracellular pH stimulates the TRPV1 [49] have been confirmed by genetic studies [48]. Ethanol increases by ~50 times the ability of low pH to activate the TRPV1 [69]. Thus, the TRPV1 sensitizing action of ethanol to the excitatory effect of a variety of normal or pathological stimuli (temperature, pH, anandamide) may contribute to the CGRP release that precipitates the migraine attack.

Our recent understanding of the pathways that produce TRP sensitization and hyperactivity may help explain the conflicting clinical evidence regarding the efficacy of NSAIDs in the treatment of headache syndromes including cluster headache, paroxysmal hemicrania, and acute migraine attacks. Most cluster headache patients are resistant to cyclooxygenase inhibitors. Conversely, paroxysmal hemicrania specifically responds to indomethacin, a drug that may also provide some temporary relief during mild migraine attacks [104]. Thus, the contribution of classical prostaglandins to migraine and other primary headaches such as paroxysmal hemicrania cannot be negated. There is increasing evidence linking the beneficial action of NSAIDs to TRPV1 and TRPA1. In animal models, TRPV1 expression is plastic and is up-regulated by neurotrophic factors, most notably NGF, during inflammation. In addition, TRPV1 is sensitized by both NGF and various pro-inflammatory mediators [63, 105]. These phenomena may also play a role in the pathogenesis of migraine. Indeed, prostaglandins (*via* prostaglandin-activated protein kinase A) have been reported to sensitize TRPV1 [106]. Moreover, low extracellular pH, which is often present at the site of inflammation, was shown to stimulate TRPV1 [48, 49] and to induce the release of sensory neuropeptides from the dura [107]. Thus, it may be speculated that blockade of TRPV1 activation and/or sensitization by prostaglandins and other agents in inflammatory soup contributes to the beneficial effect of NSAIDs in migraine. This model provided the foundation of the clinical trials with the potent, small molecule TRPV1 antagonist SB-705498 in treatment of migraine pain [108]. However, the results of this investigation, which ended in May 2009, have not been released yet.

In the past few years, the list of TRPA1 stimulants, including exogenous pungent compounds and irritants, endogenous pro-algesic agents, cold, mechanical displacement, bradykinin, and others, is increasing at an unprecedented pace, and many agents have been shown to release CGRP from DRG, VG or TG neurons [80, 89, 109]. The TRPA1 receptor, which is co-expressed with TRPV1 on sensory neurons [71, 72], is the sensor of oxidative and nitrative stress, because it can be activated by a variety of byproducts of ROS. These molecules include the metabolites of plasma membrane phospholipids, HNE, H_2O_2 and nitrolenic acid, or cyclopentenone prostaglandins and isoprostanes [80, 87, 89]. Similar to TRPV1, TRPA1 is sensitized by a number of proinflammatory stimuli. This novel inflammatory pathway encompasses cyclooxygenase-dependent (cyclopentenone prostaglandins) and independent (HNE, H_2O_2, cyclopentenone isoprostane) metabolites that *via* TRPA1 activation release CGRP. The release of CGRP and the ensuing neurogenic vasodilatation, through a cyclooxygenase-independent (and therefore NAIDS-resistant) pathway, renders this channel subtype a suitable target for further investigation in migraine.

CONCLUSION

The precise mechanism of migraine is still unknown. During the past 5 years, major progress has been made towards improving migraine therapy. This advancement also has an enormous impact on the understanding of the mechanism of the disease, and confirms that better knowledge of the pathophysiological and pharmacological aspects of trigeminal primary sensory neurons will accelerate the identification of novel therapeutics to treat migraine and other primary headaches. Recent clinical data obtained with chemically different CGRP antagonists identifies this neuropeptide, selectively released from a subset of nociceptive trigeminal nerve fibers co-expressing TRPV1 and TRPA1, as an important contributing molecule in the mechanism of migraine. Recently, a TRPV1 receptor antagonist (SB-705498) has been tested in migraine. Preclinical data have demonstrated that the inhibition of TRPV1 receptor with this molecule can both prevent and reverse established central sensitization [108].

Moreover, TRPA1 is also emerging as a potential target for the treatment of migraine. TRPA1 is expressed by a subset of nociceptive trigeminal nerve fibers that selectively release CGRP upon activation. This release causes neurogenic vasodilatation. The discovery of exogenous and, more importantly, endogenous activators of this channels that plays critical roles in inflammation further lend credence to the idea it could be involved in pain pathophysiology in general and migraine pathophysiology in specific. It is hoped that potent, small molecule TRPV1 and/or TRPA1 antagonists will broaden the therapeutic options in patients who cannot be adequately managed by available antimigraine medications.

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