Review

The CNS as a primary target for migraine therapeutics

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ABSTRACT

Migraine is a debilitating neurological disorder characterized by recurring headache attacks lasting 4-72 hours. Although migraine is common, the pathophysiological causes of this disorder remain unknown. Evidence suggests the importance of the central nervous system (CNS) in the pathology of all migraine variants; however, drug development remains focused on peripheral targets. Migraine therapeutics including triptans, calcitonin gene-related peptide (CGRP) antagonists, and monoclonal antibodies against CGRP and its receptor are not thought to cross the blood-brain barrier (BBB). Nonetheless, triptans and CGRP antagonists have been shown experimentally to bind to and exert actions in migraine-relevant regions of the CNS. Some studies have observed increased permeability of the BBB in migraine, which may partially account for the CNS activity of these drugs. Adding to the complexity of the disorder, different migraine variants such as familial hemiplegic migraine (FHM), migraine with aura, and migraine without aura have different underlying pathological mechanisms. In FHM and migraine with aura, cortical spreading depression (CSD) is thought to trigger both the aura phase as well as meningeal primary nociceptor activation in migraine. The cause of CSD in patients experiencing migraine with aura is largely unknown; however studies indicate that sex hormones, corticosteroids, inflammatory mediators such as CGRP, and genetic factors play an important role. Furthermore, CSD has also been correlated with an increase in BBB permeability, strengthening the argument for migraine drug activity in the CNS. It is clear from the mounting evidence, both preclinical and clinical, that CNS mechanisms play an important role in the underlying pathology of migraine. It is therefore vital that future studies continue to investigate these CNS mechanisms in order to create new, more effective treatments to treat and prevent migraine.

INTRODUCTION

Migraine is a common, complex neurological disease affecting approximately 15 percent of the world population [1]. Although migraine is a primary headache disorder, migraine sufferers experience a host of symptoms during attacks including nausea, vomiting, and hypersensitivity to sensory stimuli including visual, auditory, and olfactory [2]. Additionally, approximately 25 percent of migraineurs experience aura, a preceding sensory disturbance (usually visual) about one hour prior to the headache phase of migraine. The headache phase of the attack typically includes a throbbing, usually unilateral headache lasting 4-72 hours [2].

The first known record of migraine dates back to ancient Egypt, thousands of years ago [3]. Despite its long-standing prevalence in human society, most of the relevant advances in migraine knowledge and therapy did not occur until the early 20th century. Early studies by Graham and Wolff in the 1930s described migraine as a purely

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vascular disease caused by the painful dilation and stretching of the cranial arterial walls [4]. This notion persisted for several decades, until modern technological advances allowed for the examination of genetic and neurologic contributions to migraine pathogenesis.

Migraine is now generally accepted as a multifaceted neurological disorder, likely arising from a combination of vascular, neural, and genetic factors [5]. Despite advances in our knowledge of migraine pathogenesis, the exact physiological origin of migraine has yet to be fully described. This is evident in our apparent lack of universally effective migraine therapies. Studies report that as many as 50 percent of migraine patients are currently unsatisfied with their migraine treatment regimen [6].

As a result of inadequate management, more than 90 percent of migraine sufferers report being unable to work or function normally. In fact, migraine and chronic headache was found to be the second most frequently identified cause of short-term absence in employees, comprising 47% of the examined reports [7, 8]. This loss in productivity, combined with the disabling pain and accompanying symptoms experienced by the migraineurs, qualify migraine as a global health issue. In fact, the World Health Organization has classified migraine as one of the most disabling illnesses in the world [1]. From these figures it is clear that more research on the underlying cause of migraine, as well as on the creation of new therapies, is vital to address this disabling and poorly managed disease.

Most currently available anti-migraine medications target peripheral sites to stop headache pain after it has already begun [9]. Although this method is effective for a number of migraineurs, it is important to explore other potentially relevant target sites, including areas of the CNS, in order to fill in the gaps in our current care of migraine patients. Considering that the underlying cause of migraine is poorly understood, current migraine therapeutics target only the symptoms, leading to the apparent lack of highly effective medications to treat and prevent migraine.

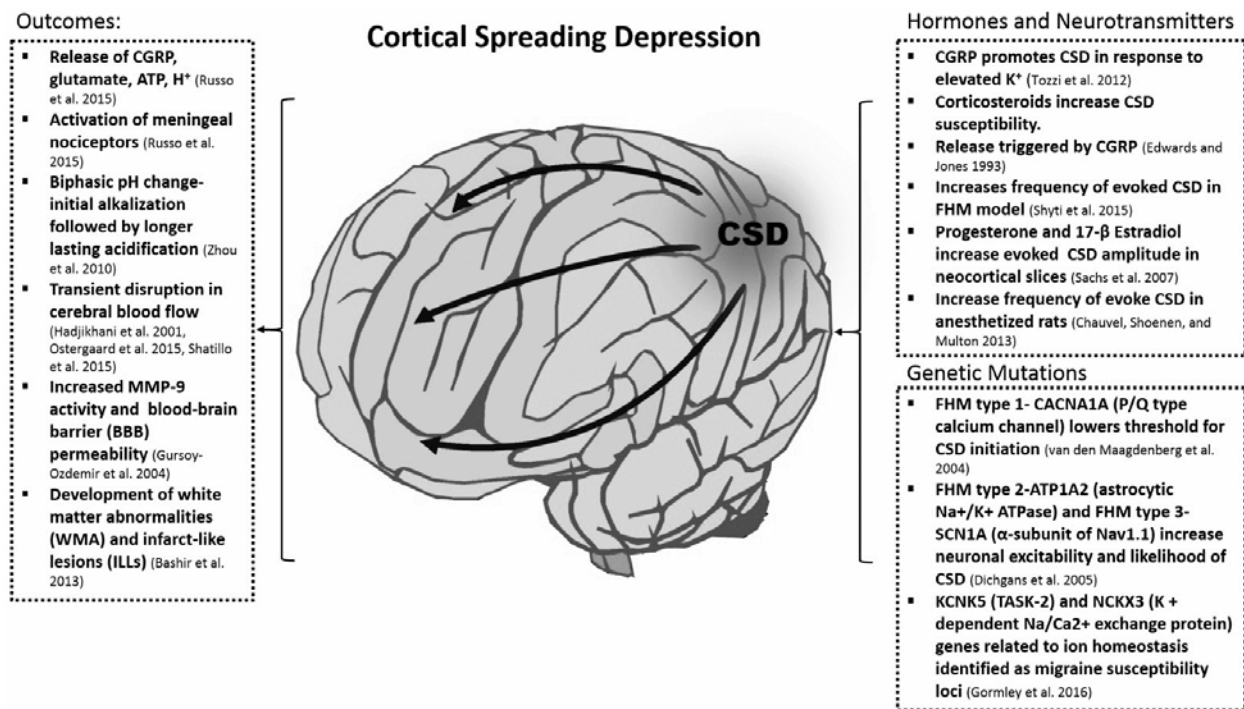In this review, we will present the central nervous system (CNS) as a primary site in the underlying pathogenesis of migraine. Overarching goals of this review are to discuss the known functional and molecular changes in the CNS in migraine, as well as the potential for migraine drugs such as CGRP antagonists, neutralizing antibodies, and triptans to act in these CNS regions associated with migraine. Lastly, this review will detail the obstacles faced in delivering migraine drugs to the CNS including the necessity to overcome the blood-brain barrier (BBB) and the potential for CNS-mediated side effects.

1. Mechanisms of migraine pain

The trigeminal nerve contains the major primary afferents for the relay of facial and scalp sensory information, including pain [10]. The trigeminal nerve separates into three branches: the ophthalmic nerve, the maxillary nerve, and the mandibular nerve. The ophthalmic and maxillary nerves solely transmit sensory information, while the mandibular nerve performs both motor and sensory functions [10]. Several mechanisms by which the nociceptive fibers of the trigeminal nerve are activated during migraine have been proposed including neurogenic inflammation of the dura mater and the occurrence of a cortical spreading depression (CSD) (Figure 1).

Neurogenic inflammation involves the release of endogenous inflammatory mediators [11]. In migraine, this phenomenon is most often attributed to degranulation of mast cells in the dura, leading to activation of meningeal afferents in the trigeminovascular system [12]. Experimentally, it is known that noxious stimuli in the primary afferents of the trigeminovascular system lead to the release of vasoactive neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) [13, 14].

CGRP, in particular, has been shown to play a central role in migraine pathology [14, 15], though it performs many functions in the body. In regard to migraine, CGRP is both a potent vasodilator and nociceptive neurotransmitter acting at first-order meningeal neurons; it also activates second-order neurons in the spinal cord and trigeminal nucleus caudalis (TNC) [15, 16]. Vasodilation of meningeal arteries *via* CGRP actions prior to migraine attacks may cause extravasation of inflammatory mediators onto dural afferents, leading to their activation [14]. Evidence also

Outcomes:

- **Release of CGRP, glutamate, ATP, H⁺** (Russo et al. 2015)
- **Activation of meningeal nociceptors** (Russo et al. 2015)
- **Biphasic pH change-initial alkalization followed by longer lasting acidification** (Zhou et al. 2010)
- **Transient disruption in cerebral blood flow** (Hadjikhani 2001, Ostergaard et al. 2015, Shatillo et al. 2015)
- **Increased MMP-9 activity and blood-brain barrier (BBB) permeability** (Gursoy-Ozdemir et al. 2004)
- **Development of white matter abnormalities (WMA) and infarct-like lesions (ILLs)** (Bashir et al. 2013)

Cortical Spreading Depression

CSD

Hormones and Neurotransmitters

- **CGRP promotes CSD in response to elevated K⁺** (Tozzi et al. 2012)
- **Corticosteroids increase CSD susceptibility.**
- **Release triggered by CGRP** (Edwards and Jones 1993)
- **Increases frequency of evoked CSD in FHM model** (Shyti et al. 2015)
- **Progesterone and 17-β Estradiol increase evoked CSD amplitude in neocortical slices** (Sachs et al. 2007)
- **Increase frequency of evoke CSD in anesthetized rats** (Chauvel, Shoenen, and Multon 2013)

Genetic Mutations

- **FHM type 1- CACNA1A (P/Q type calcium channel) lowers threshold for CSD initiation** (van den Maagdenberg et al. 2004)
- **FHM type 2-ATP1A2 (astrocytic Na+/K+ ATPase) and FHM type 3-SCN1A (α-subunit of Nav1.1) increase neuronal excitability and likelihood of CSD** (Dichgans et al. 2005)
- **KCNK5 (TASK-2) and NCKX3 (K + dependent Na/Ca2+ exchange protein) genes related to ion homeostasis identified as migraine susceptibility loci** (Gormley et al. 2016)

Figure 1. Representation of contributing factors and outcomes of CSD in migraine with aura. CSD is provoked by abnormalities in the cellular ionic gradient. Several hormones and neurotransmitters including CGRP, corticosteroids, and sex hormones such as progesterone and 17-β estradiol increase CSD susceptibility and frequency of CSD triggered by elevated extracellular K^+. Mutations in genes encoding for ion channels or proteins related to ion homeostasis also increase CSD susceptibility. As a result of CSD, numerous inflammatory molecules are released and meningeal nociceptors are activated. Additionally, changes in pH, blood-brain barrier permeability, and cerebral blood flow have been shown to occur in response to CSD. Long-term structural anomalies such as white matter lesions and infarct-like lesions are thought to occur due to repeated CSDs.

suggests that CGRP is able to directly cause mast cell degranulation which, among other things, can lead to additional CGRP release and further activation of trigeminal nociceptors [17]; these studies have yet to be confirmed clinically. Studies on CGRP levels in serum, cerebral spinal fluid (CSF), and saliva in migraine patients have shown elevated levels both during and between attacks as compared to healthy controls [14, 18-20]. Therefore CGRP can be seen as partly responsible for the initiation as well as the progression of migraine pain.

Although studies done on neurogenic inflammation and CGRP involvement thus far provide a compelling explanation to some aspects of migraine pain, the underlying mechanism for episodic mast cell degranulation and meningeal nociceptor activation remains to be elucidated. Infusion of CGRP in migraine-susceptible individuals reproducibly induces a migraine-like attack several hours post

infusion [15]. However, the endogenous cause of increased CGRP levels in migraine is unknown. It is clear from these observations that additional mechanisms must be involved in the underlying cause of migraine attacks.

2. CNS origins of migraine

Peripheral trigeminal nociceptors originating from the dura project through the trigeminal tract to the caudal medulla and the upper cervical spinal cord [21-24]. These second-order neurons originating largely from the spinal trigeminal nucleus caudalis (TNC) convey nociceptive information to several brainstem relay centers, including the rostral ventromedial medulla (RVM) and periaqueductal gray (PAG) [25, 26]. Nociceptive signals from the TNC are also sent to the hypothalamus and thalamus for further processing and relay to higher order cortical centers [26-28].

Following transmission to brain and brainstem processing centers, nociceptive signals originating from meningeal nociceptors can be modulated *via* descending facilitation or inhibition [29, 30]. Experimental evidence suggests the importance of the PAG and RVM in descending pain modulation [31]. Opioid, serotonergic, and noradrenergic pathways have all been implicated in descending pain inhibition [32]. Recently, migraine patients have been shown to have atypical connectivity in pain modulatory regions in the brainstem [33]. Interestingly, migraine patients have been shown to exhibit reduced functional connectivity between the PAG and key brain regions associated with descending pain inhibition, namely the rostral anterior cingulate cortex and medial prefrontal cortex [34]. The extent of these changes in connectivity was found to be correlated to migraine headache intensity [34]. These data suggest that migraine patients may have deficits in pain modulation through alterations in descending pain inhibition. These issues in functional connectivity likely compound with other influencing factors in migraine such as sensitization, triggers, inflammation, and CNS abnormalities, to account for the recurrent/remitting features of migraine in humans.

2.1. Cortical spreading depression

Imaging studies in humans have provided evidence for both structural and functional anomalies in the CNS in migraine patients. Structural anomalies including white matter abnormalities (WMA), infarct-like lesions (ILL), and changes in gray matter and white matter volume have been observed in population-based and cross-sectional human neuroimaging studies [35]. Although the findings were not universal in each study, several meta-analyses have provided evidence that migraineurs, especially those with aura, have a higher prevalence of these CNS structural abnormalities than healthy controls suggesting a neuroanatomical component of the pathology [35].

The functional outcome of these structural differences in migraine has not been definitively determined. Some structural changes, such as ILL and WMA may occur as a consequence of migraine; these may occur due to migraine-related disruption of cerebral blood flow resulting from

cortical spreading depression [36]. Cortical spreading depression (CSD) originating from the CNS is another postulated mechanism by which the trigeminal nerve is activated during migraine.

CSD is described as a wave of self-propagating depolarization followed by a wave of inhibition across the cortex. CSD is thought by many to be the underlying cause of the aura phase of migraine [37]. Some evidence also suggests that the CSD itself may be contributing to the activation of meningeal nociceptors [38]. CSD has been shown to cause the release of numerous pronociceptive substances including CGRP, glutamate, ATP, and hydrogen ions in the cortex that can diffuse to and activate meningeal nociceptors [39]. Activation of these nociceptors can elicit neurogenic inflammation through mast cell degranulation and vasodilation of meningeal blood vessels causing subsequent diffusion of inflammatory mediators onto meningeal afferents as a positive feedback that exacerbates migraine and other headache disorders [12].

2.2. CNS neurotransmitter, neuropeptide, and hormone involvement in CSD

Mechanisms of CSD initiation in migraine have not been fully elucidated; however, aberrations in neurotransmitter and neuropeptide release and function have been suggested as major contributors to CSD propagation (Figure 1). Supporting the idea that CGRP has a prominent role in migraine, CGRP in rat neocortical slices has also been shown to promote CSD in response to elevated extracellular K^+ [40]. The same study demonstrated a dose-dependent inhibition of CSD with three different CGRP receptor antagonists [40]. It is known that CGRP is elevated in serum, saliva, and CSF in migraineurs both ictally and interictally [14, 18, 19]. Therefore it is plausible that elevated CGRP levels in these patients can trigger a CSD and resulting migraine when combined with other migraine triggers.

Stress is one of the most commonly reported migraine triggers. Some studies suggest that the increased cortisol levels brought on by stress can increase susceptibility to CSD initiation [41]. In a mouse model of familial hemiplegic migraine, for instance, pre-treatment of mice with corticosterone increased the frequency of CSD events in response to elevated extracellular K^+ [41]. Another study

examining the effect of environmental stress on trigeminal activation in a rat model of medication-overuse headache found that bright-light stress significantly increases TNC Fos staining, indicating an increase in trigeminal afferent activation [42]. Additionally, it has been demonstrated that CGRP can stimulate corticosteroid release [43]. Conversely, high levels of cortisol have been shown to reduce CGRP plasma levels in patients with cluster headache; however it is unknown if a similar effect would be seen in migraine [44]. If true in migraineurs, this may partially explain why many patients experience migraines at the resolution of a period of high stress. Together, these studies validate stress as a potential trigger for CSD initiation in migraine patients.

In addition to cortisol, steroid sex hormones such as estrogen and progesterone have been implicated in migraine pathogenesis. Approximately 3 out of 4 migraineurs are women [8]. Of these women who experience migraine with aura, many experience migraines during discrete times in their menstrual cycle, either when their female sex hormones are at their lowest or their highest [2]. One study demonstrated the ability of both 17-β estradiol and progesterone to increase the amplitude of high K^+-induced CSDs in rat somatosensory neocortical slices [45]. Additionally, this study showed enhanced long-term potentiation (LTP) in these slices with high levels of female sex hormones [45].

More recently, an *in vivo* study demonstrated the ability of 17-β estradiol and progesterone to increase the frequency of K^+-induced CSDs in anesthetized rats [46]. Furthermore, the male sex hormone testosterone has been shown to decrease CSD susceptibility in a mouse model of familial hemiplegic migraine [47]. These reported effects of sex hormones suggest that female sex hormones can both increase the risk of CSD as well as modulate cortical synaptic transmission.

2.3. Genetic contributions to CSD

The influence of genetic factors on CSD in migraine has not been fully established, although several gene loci are implicated in disease susceptibility. In familial hemiplegic migraine (FHM), CSD is potentially caused by defects in ion channels critical for the maintenance of neuronal activity homeostasis [48]. FHM is a genetic variant of migraine with aura which, in addition to classical migraine symptoms, includes weakness on one side of the body as a symptom during attacks. Migraine attacks in FHM have been more strongly linked to CSD than in classical migraine with aura, making it a useful disorder for studying potential causes of CSD in migraine [49].

The most common form of FHM (type 1) is caused by a mutation in the CACNA1A gene encoding the pore-forming subunit of P/Q type calcium channels. This mutation enables these channels to open at more negative membrane potentials resulting in a lower threshold for CSD initiation [50]. The less common forms of FHM also involve mutations of critical ion channels such as ATP1A2 (type 2), encoding the astrocytic Na^+/K^+ ATPase, and SCN1A (type 3) which encodes the alpha subunit of the voltage-gated sodium channel Nav1.1. It is thought that all of these mutations increase neuronal excitability leading to an increased likelihood of CSD initiation, thus establishing CSD as a key component in FHM [51].

Evidence suggests that genetic factors contribute greatly to migraine susceptibility in both migraine, with and without aura, though identification of the discrete loci is in its infancy. A recent meta-analysis of genes related to migraine susceptibility uncovered few genes related to ion transport, leading to the conclusion that other mechanisms must also be involved in CSD initiation and/or trigeminal activation in the common forms of migraine with aura [52]. Nonetheless, the ion channel-associated genes related to migraine do provide some link between common forms of migraine and CSD. Thirty-eight migraine susceptibility loci were identified in a recent meta-analysis; two contained genes for ion channels (KCNK5 and TRPM8) and three contained genes related to ion homeostasis (SLC24A3, ITPK1, and GJA1) [52]. Of these genes, KCNK5 and SLC24A3, stand out as being potentially pertinent to CSD.

KCNK5 is a protein-encoding gene for a two-pore domain acid-sensitive potassium channel TASK-2 [53]. Immunostaining of TASK-2 in the rat CNS indicates that this channel is highly expressed in many migraine-relevant regions including the hypothalamus and amygdala in the brain as well as

the periaqueductal grey, locus coeruleus, and trigeminal brainstem complex [54]. The functional outcome of specific mutations found in this channel in migraine is unknown; however, its role in migraine can be postulated based on the channels' function. TASK channels, including TASK-2, contribute to the potassium leak current in neurons and are involved in setting and modulating the membrane potential and controlling neuronal firing [53, 54]. Therefore, down-regulation in the function or expression of the TASK-2 channel could lead to conditions favoring CSD induction.

Additionally, TASK-2 has been implicated in adaptive responses to seizure activity through rapid upregulation leading to hyperpolarization [55]. This upregulation is likely due to extracellular pH changes resulting from seizure activity. It is known that CSDs result in a biphasic pH change within the cortex with an initial alkalization followed by a longer-lasting acidification [56]. The activity of TASK channels is highly regulated by pH, where lowering pH increasingly inhibits their activity [53]. It is therefore possible that defects in the ability of TASK-2 to upregulate in response to the acidic environment following CSD in migraine may contribute to an impaired ability to adapt to the CSD, eventually leading to a strong enough stimuli to activate meningeal nociceptors. Although probable, these hypothesized roles of TASK-2 in migraine have yet to be effectively studied experimentally.

Most CSD research focuses on the contribution of extracellular K^+ accumulation to CSD induction and propagation. It is clear that K^+ plays a vital role in CSD progression, although other ions including Ca^{2+} may also significantly contribute to CSD in migraine [57]. Increased Ca^{2+} influx has been shown in rats to assist in the propagation and acceleration of CSD [57]. One of the migraine-susceptible genes, SLC24A3, encodes for the K^+-dependent Na^+/Ca^{2+} exchanger NCKX3 [52]. NCKX3, along with the other Na^+/Ca^{2+} exchange proteins is prominently involved in the regulation of Ca^{2+} homeostasis in neurons; functionally it exchanges one intracellular Ca^{2+} and K^+ for four extracellular Na^+ [58].

NCKX3 is broadly expressed throughout the CNS [59]. Interestingly, the expression of NCKX3 in the uterus has been shown to be significantly increased with increasing 17β-estradiol levels [60]. This is particularly relevant to migraine as approximately 75 percent of migraineurs are female, many of whom experience migraines during or near the time of their menstrual period when estrogen levels are at their highest or lowest [2, 8]. It is possible that circulating estrogen levels may more broadly influence NCKX3 levels in CNS regions, and that increased NCKX3 function or expression in migraineurs may contribute to aberrant regulation of Ca^{2+} homeostasis. As with TASK-2, however, the function of NCKX3 in migraine has not been experimentally validated. Thus more research is warranted to determine the contribution of these genetic polymorphisms to CSD and migraine.

2.4. Central sensitization in migraine

In addition to CSD, many other CNS processes and changes are thought to contribute to migraine pain. One persisting hypothesis behind the chronic and recurring nature of migraine and other pain states is the development of central sensitization [61]. The development of central sensitization with regard to pain has been observed and studied in numerous animal models and human pain conditions [62]. Central sensitization involves the ability of second-order neurons to be activated in response to previously subthreshold, nociceptive stimuli. This occurs due to strengthening of these synapses leading to increased excitability [24]. These changes manifest as allodynia and hypersensitivity in both humans and animals [24, 63].

Transmission of head and facial pain begins in nociceptive trigeminal primary afferents, which synapse in the spinal trigeminal nucleus in the medulla. Persistent sensitization of these medullary dorsal horn neurons has been shown to occur in response to dural inflammation in rats, providing evidence for the development of central sensitization in migraine [24]. Increased circulating levels of pro-inflammatory molecules in migraine patients are thought to contribute to central sensitization in migraine. Interleukin-6 (IL-6), which has been shown to be elevated in migraine patients, has been demonstrated to contribute to priming and sensitization of second-order neurons in the TNC [64-66]. This effect was shown to be dependent

on centrally-acting brain-derived neurotrophic factor (BDNF) within the brainstem [64]. CGRP is also thought to play a key role in central sensitization in response to migraine. CGRP is co-released with glutamate from trigeminal primary afferents onto second-order neurons in the TNC, thus lowering the activation threshold for glutamate on the second order neuron [67]. Additionally, this increase in central CGRP can stimulate glial cells in the CNS to release inflammatory mediators, further perpetuating this inflammatory state and central sensitization [68].

Further evidence for central sensitization in migraine comes from observations in human migraine patients. Many migraineurs experience hypersensitivity on their face and scalp during migraine attacks which persists after the primary migraine pain has subsided. In these patients, even common grooming activities such as combing their hair can become painful [69].

3. Migraine drugs and challenges of drug delivery to the CNS

Due to a lack of therapeutic options, treatment of migraine clinically is still quite limited. Pharmacological treatments include non-steroidal anti-inflammatory drugs (NSAIDs), opioids, ergotamines, triptan compounds, and prophylactic therapies [70, 71]. NSAIDs are most commonly used as abortive treatments for mild to moderate migraine; however they are often not efficacious and their sustained use may lead to adverse effects such as gastrointestinal (GI) irritation [70]. Although opioids are currently the most frequently used medications for chronic pain conditions, their use in migraine is restricted due to the development of medication-overuse headache as well as their high abuse potential [71, 72].

Very few migraine-specific drugs exist, making the treatment of more severe cases of migraine headache difficult. Triptan compounds represent the first-line of migraine-specific abortive drugs [70]. Although triptans are generally safe, with minimal major adverse reactions, they are reported to be ineffective in fully relieving migraine pain in as many as 55 percent of patients [7, 70, 73]. In order to fill this large therapeutic gap, targeting of CGRP and its receptor continues to be thoroughly researched as a new therapeutic option for the treatment of migraine [68, 74, 75]. These new drugs have proved to be efficacious as both preventative and abortive treatments [75]. The location of action of these and other migraine drugs, either central or peripheral, is not completely established [75]. Although research suggests that both triptans and CGRP-targeting compounds exert their actions in the periphery, both have the potential to exert migraine-relevant effects in the CNS [75, 76] (Figure 2). Additionally, some evidence suggests increased BBB permeability in migraine, which may facilitate CNS uptake of these drugs during migraine attacks [77, 78]. Therefore, a more in-depth assessment of actions of migraine drugs in the CNS is warranted in order to further understand their mechanism of action as well as to aid in the development of new, more efficacious drugs.

3.1. Triptans

Triptans are a class of drugs used as primary abortive agents for the treatment of migraine. The anti-migraine effects of triptans arise from their agonistic interaction with $5\text{-HT}_{1B/1D/1F}$ serotonergic receptors [73, 76]. The 5HT1 class of receptors, including those acted upon by triptans, are $G_{\alpha i/o}$-coupled G-protein coupled receptors (GPCRs) which are negatively coupled to adenylate cyclase [79]. These receptors are believed to function primarily as auto-receptors, inhibiting the release of serotonin from central and peripheral termini when active, and thereby reducing glutamatergic transmission [73, 76]; thus, synaptic transmission from peripheral nociceptors in the dura to the TNC is halted.

Triptans have traditionally been thought to exert their effects only in the periphery, as studies have shown minimal crossing of triptans through the blood-brain barrier (BBB) [76]. It is known, however, that $5\text{-HT}_{1B/1D/1F}$ serotonergic receptors are highly expressed in the CNS and have the potential to produce functionally relevant changes in the context of migraine [80]. Inhibition of second-order neurons in the TNC by triptans may prevent synaptic relay of nociceptive signals to higher order processing centers [81]. It has widely been assumed that this inhibition occurs as a consequence of presynaptic actions of triptans at
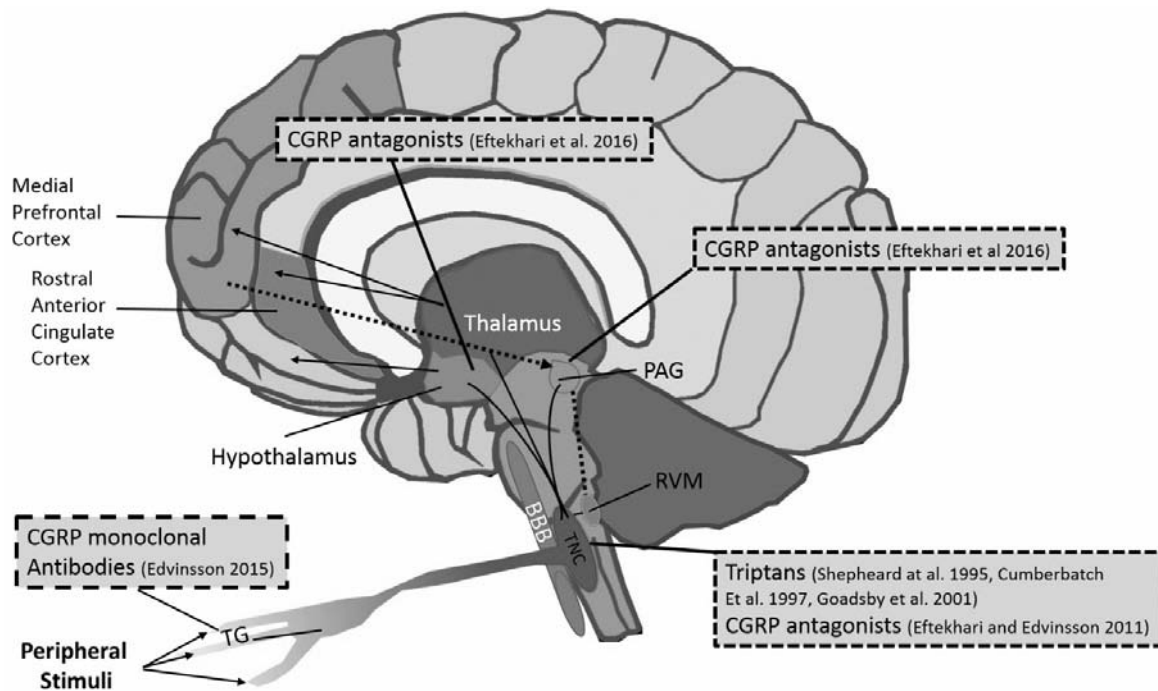
Figure 2. General overview of pain transmission through the CNS in migraine. Transmissions from peripheral nociceptors pass through trigeminal ganglia (TG) to second-order neurons in the TNC. Here they are sent to relay centers in the brainstem including the RVM and PAG. Nociceptive transmissions are also sent to the thalamus and hypothalamus for relay to higher order cortical regions including the medial prefrontal cortex and the rostral anterior cingulate cortex. From here, descending pain inhibition can occur through efferent connections with the PAG. These descending connections have been shown to be impaired in migraine patients. Migraine drugs including triptans and CGRP antagonists have been shown to bind to and act in several migraine-relevant regions of the CNS including the TNC (triptans and CGRP antagonists), PAG (CGRP antagonists), and hypothalamus (CGRP antagonists). Conversely, CGRP monoclonal antibodies are thought to only act in the periphery due to their inability to cross the BBB. Some evidence suggests the opening of the BBB during migraine attacks, however, increasing the possibility for CNS activity of migraine therapeutics.

the primary nociceptor. Experimental evidence, however, supports the additional function of triptans as direct inhibitors of second-order neurons in the TNC [81, 82]. Studies investigating the influence of sumatriptan on neuronal activity of second-order neurons in the TNC revealed a decrease in *Fos* immunostaining following electrical stimulation of primary afferents. This effect was only seen following BBB opening *via* mannitol infusion, suggesting the ability of triptans to inhibit second-order neurons through direct mechanisms [22, 83]. As increased BBB permeability during migraine attacks has been demonstrated in some studies, the ability of triptans to inhibit second-order neurons in the TNC may represent a therapeutically relevant mechanism of action for triptans [77, 78].

The therapeutic effects, if any, of triptans in the brain are unknown; however CNS side effects such as dizziness and drowsiness are often reported indicating their action in the brain [84]. A recent systematic review of studies on serotonergic mechanisms in the migraine brain revealed some relatively consistent aberrations in brain serotonergic transmission. In general, serotonin levels in the brain were found to be lower between migraine attacks and increased during attacks [85]. The functional outcome of increased brain serotonin in regard to migraine is unknown; however, serotonin has been shown to be released by the brain onto the central terminals of the trigeminal nerve in chronic pain. There, 5-HT increases the activity of TRPV1 receptors leading to more frequent firing of trigeminal nociceptors and increased pain

signals [86]. Acute systemic administration of triptans has been shown to reduce serotonin synthesis in the brain in rats, which could potentially lead to decreased serotonin activity on trigeminal nerves. Interestingly, sustained systemic administration of triptans has been shown to increase serotonin synthesis in the brain, potentially explaining the occurrence of medication-overuse headache seen in chronic triptan use in humans [87]. Thus, it is likely that triptans have a central site of action in addition to their well-characterized peripheral mechanisms.

3.2. CGRP antagonists and monoclonal antibody treatments

In recent years, researchers have provided overwhelming experimental evidence suggesting CGRP as a critical component in migraine pathogenesis. These observations have generated much interest in the development of CGRP-antagonizing drugs. The first orally available small molecule CGRP antagonist, telcagepant was developed by Merck Research Laboratories [75]. In clinical trials, telcagepant was found to be as efficacious as triptans in aborting migraine headaches while displaying minimal acute side effects [75]; continued analysis of its long-term side effect profile, however, revealed liver toxicity in some patients [88]. Due to this finding, Merck Research Laboratories suspended the development of telcagepant.

Although the small-molecule CGRP-antagonizing drugs failed to reach the clinic, their efficacy in relieving migraine pain encouraged researchers to continue searching for new safe and efficacious methods for blocking CGRP's effects. As a result of these efforts, monoclonal antibodies (mAbs) against CGRP and its receptor were developed [75]. These mAbs have proved thus far in clinical trials to be highly efficacious and safe as migraine preventatives.

Small-molecule CGRP receptor antagonists and monoclonal antibodies are primarily thought to exert their action in the periphery due to their apparent inability to cross the BBB [75]. Monoclonal antibodies against CGRP largely preclude the need for CNS penetration as their mechanism of action relies on their ability to neutralize excess CGRP, thus preventing it from

reaching its sites of action [75]. This does not preclude CGRP actions in the CNS resulting from central release. Consequently, in the presence of CGRP-neutralizing antibodies, CGRP may still be able to exert actions in the CNS including modulation and sensitization of second-order neurons in the TNC [89], the clinical outcome of which may be incomplete relief or a rebound of migraine in response to continued central sensitization.

Small-molecule CGRP inhibitors, on the other hand, may work directly at central sites. Detailed imaging analysis of CGRP receptor expression in the rhesus monkey brainstem has revealed expression in a number of migraine-relevant areas in the CNS [90]. In particular, several areas found were directly correlated with nociceptive transmission in migraine including the PAG, locus coeruleus (LC), and posterior hypothalamus. Additionally, the selective CGRP antagonist MK-3207 was found to bind in the PAG and posterior hypothalamus in this study, indicating the potential for CNS activity [90]. Another study examining expression of CGRP binding in the human CNS also found high levels of CGRP binding at all levels of the spinal cord. The CGRP receptor has been found in the human spinal trigeminal nucleus (STN) in the medulla. Following stimulation, CGRP is released from primary afferents where it acts post-synaptically on these second-order neurons in the STN [16]. Although CGRP and its receptor are seen in the CNS, the role that they may be playing in migraine is not well-established. More research is needed to establish if centrally-mediated actions of CGRP and thus CGRP-targeting agents play a role in migraine and its management, respectively.

3.3. Overcoming the blood-brain barrier in migraine treatment

Based on research findings to date, it is clear that CNS mechanisms are important in the pathogenesis of migraine headache. Development of new, CNS penetrant migraine drugs is warranted to combat this component of migraine. Although new, peripherally targeted therapeutics such as CGRP monoclonal antibodies hold great promise, many migraineurs do not respond to these treatments [74], and are left with little to no relief from their symptoms or therapy-induced side effects (i.e., dizziness, somnolence, etc.). While development

of CNS-active drugs may provide additional relief to migraine patients, it also provides unique and challenging hurdles for researchers to overcome.

The most significant obstacle in the development of CNS-penetrating drugs is the need to bypass the BBB. The BBB represents the dynamic physical interface between circulating blood and the brain. The BBB is composed of specialized endothelial cells containing tight junctions at cell-cell contact points. These tight junctions severely restrict the ability of molecules to pass through the brain vasculature by paracellular means [91]. In addition, these endothelial cells contain numerous influx and efflux transporters, which tightly regulate the substances which are allowed to pass from the general circulation into the brain [92]. Further regulation of the BBB is provided by the other components of the neurovascular unit (NVU). In addition to brain endothelial cells, neurons, astrocytes, pericytes, and microglia in close proximity to the brain vasculature comprise the NVU. These interactions allow the BBB to respond to changes in brain activity or disease [93].

Evidence provided over the last several decades indicates the ability of the BBB to change in response to painful stimuli [94]. Studies have shown that peripheral inflammatory pain transiently induces increased leakiness at the BBB, which can be blocked with the peripheral application of bupivacaine [95]. Research in the migraine field, however, has proved to be less conclusive in regard to BBB changes. One study in humans has shown increased matrix metalloproteinase-9 (MMP-9) activity in migraine patients during headache attacks, indicating a breakdown of the BBB [77]. Additionally, a study done in rats showed increased MMP-9 activity as well as increased brain permeability to Evans blue-albumin in response to KCl-induced CSD [78]. This model has also been shown to cause migraine-like pain behaviors in rats in a separate study [96]. On the other hand, many studies have shown explicit evidence against BBB disruption. One study in rats showed no change in BBB permeability to [(51)Cr]-EDTA in response to dural inflammation induced by either complete Freund's adjuvant (CFA) or inflammatory soup (IS) [97]. Additionally, a recent positron emission tomography (PET) imaging study in migraine patients determined no increase in BBB permeability to ^{11}C-dihydroergotamine in response to glyceryl trinitrate (GTN)-induced migraine [98].

Although these results on BBB changes in migraine are conflicting, there may be a logical explanation as to their difference. This explanation likely lies in the differences between the preclinical and clinical models used to study BBB in migraine. For example, while studies finding an increase in MMP-9 activity were performed during spontaneously occurring migraine headache, no change in BBB permeability in migraine patients was detected under the GTN artificial induction system. It is possible that the GTN-induced migraine model, while useful in many cases, is not able to fully recapitulate the complex neurological events found during spontaneously occurring migraine attacks. Furthermore, the conflicting evidence seen in the KCl-induced migraine model compared with the model of dural inflammation suggests that there may be differences in BBB response, both to different migraine variants (such as migraine with aura) as well as to the models utilized. Currently, the preclinical models of migraine used to assess BBB integrity involve acute application of an inflammatory or CSD-inducing compound through a large window in the skull [78, 97]. This causes damage to the skull and may cause inflammation and additional activation of dural nociceptors, leading to difficulties in distinguishing between surgical and model-induced effects on the BBB paracellular permeability; less invasive models for BBB assessment in migraine are needed to combat this research obstacle.

Drugs can also access the CNS *via* transcellular mechanisms including uptake by drug influx transporters [92, 99]. Very few studies have examined the ability of migraine drugs to act as substrates for uptake transporters. One study found triptans to act as substrates for the influx transporter organic anion-transporting polypeptide (OATP) 1A2 [100]. Increases in the functional expression of Oatp1a4, the rodent homolog of OATP1A2, have been observed at the BBB in response to inflammatory pain as well as hypoxia/reoxygenation stress [101, 102]. It is unknown, however, if migraine produces similar increases in Oatp1a4 expression. Such changes

would further strengthen the argument for centrally mediated actions of triptans; however, studies that assess changes in drug influx transporters that may influence the uptake and CNS efficacy of antimigraine agents during episodes are lacking.

Imaging studies on brain structure in migraine patients, particularly in patients experiencing aura, show structural anomalies including WMAs and ILLs [35]. These lesions have been shown in other models to occur as a result of BBB disruption caused by chronic cerebral hypoperfusion [103]. CSD has been shown to temporarily disrupt cerebral blood flow, the outcome of which may be similar to those seen as a result of chronic hypoperfusion [104-106]. Of note, these lesions are also seen in a variety of brain disorders causing BBB disruption including ischemic stroke and traumatic brain disorders (TBI) [107, 108]. Recently, a significant association between migraine with visual aura and the occurrence of ischemic stroke has been shown, indicating a greater risk for ischemic stroke in this subset of migraineurs [109]. It is clear that these disorders share common features, including the development of white matter lesions. It is possible that in migraine with aura, as in other disorders causing white matter lesions, these abnormalities arise in response to alterations in cerebral blood flow and BBB disruption brought on during attacks.

CONCLUSION

Migraine is a complex disorder. It is clear from available research on pathology that many different factors influence the development of migraine. Migraine pathogenesis is likely caused by a combination of vascular, neurological, and genetic factors. Additionally, it is relatively safe to assume that all migraines do not occur from the same underlying cause. Rather, it is very probable that many migraine variants exist, which manifest similarly in the clinic.

Numerous studies have suggested an important role of the CNS in migraine. Here we have examined potential contributions of the CNS to the pathogenesis of migraine headache in order to encourage further research into this topic of interest. Although there is vast evidence for a central involvement of the CNS in migraine, drug

development continues to focus on creating peripherally active drugs. This is likely due to a number of factors including difficulties in overcoming the BBB as well as the potential for more serious CNS-related side effects to occur. The use of strategies such as development of drugs that are substrates for BBB influx transporters may help combat some of the issues with drug delivery to the CNS. Moreover, research suggests that in at least some variants of migraine the BBB is impaired during the attack [77, 78]. This may inadvertently increase the ability of migraine drugs to reach CNS sites during attacks. Future research in the migraine field, particularly in drug development, should continue to consider CNS-mediated mechanisms in order to gain a more complete picture of the complex variables involved in migraine development in humans.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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