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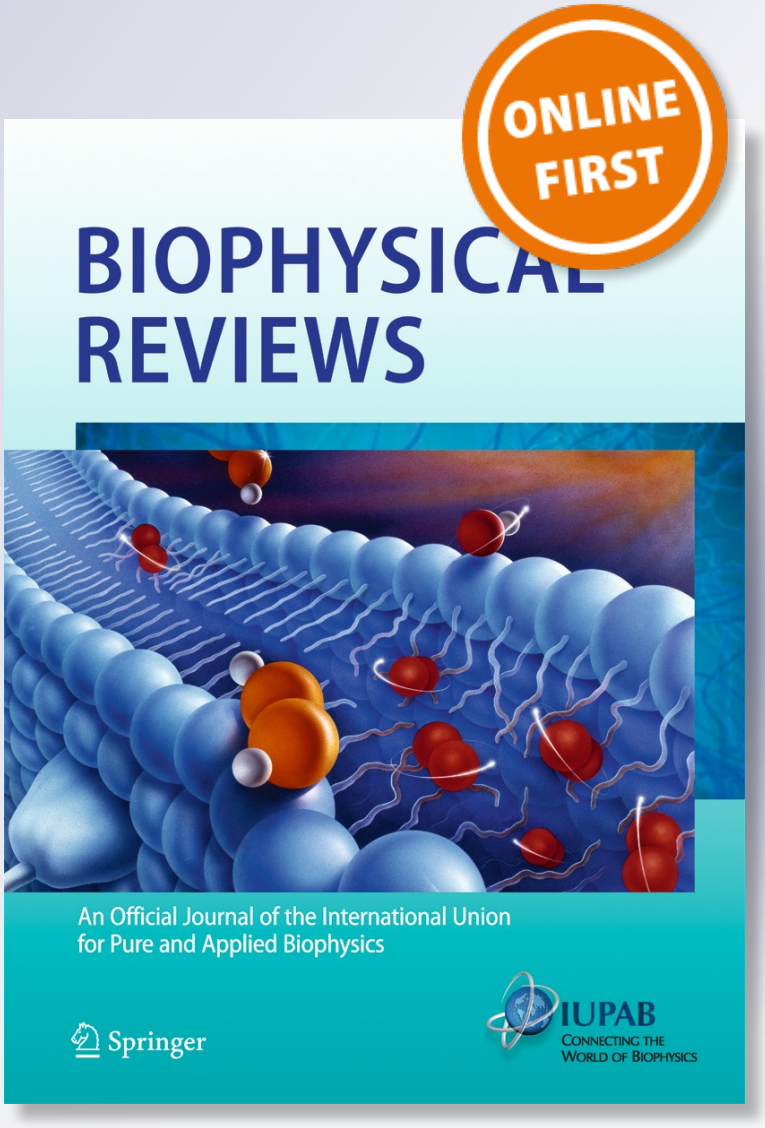

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REVIEW

Calcium channels and synaptic transmission in familial hemiplegic migraine type 1 animal models

Osvaldo D. Uchitel · Carlota González Inchauspe · Mariano N. Di Guilmi

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Abstract One of the outstanding developments in clinical neurology has been the identification of ion channel mutations as the origin of a wide variety of inherited disorders like migraine, epilepsy, and ataxia. The study of several channelopathies has provided crucial insights into the molecular mechanisms, pathogenesis, and therapeutic approaches to complex neurological diseases. This review addresses the mutations underlying familial hemiplegic migraine (FHM) with particular interest in Cav2.1 (i.e., P/Q-type) voltage-activated Ca^{2+} channel FHM type-1 mutations (FHM1). Transgenic mice harboring the human pathogenic FHM1 mutation R192Q or S218L (KI) have been used as models to study neurotransmission at several central and peripheral synapses. FHM1 KI mice are a powerful tool to explore presynaptic regulation associated with expression of Cav2.1 channels. FHM1 Cav2.1 channels activate at more hyperpolarizing potentials and show an increased open probability. These biophysical alterations may lead to a gain-of-function on synaptic transmission depending upon factors such as action potential waveform and/or Cav2.1 splice variants and auxiliary subunits. Analysis of FHM knock-in mouse models has demonstrated a deficient regulation of the cortical excitation/inhibition (E/I) balance. The resulting excessive increases in cortical excitation may be the mechanisms that underlie abnormal sensory processing together with an increase in the susceptibility to cortical spreading depression (CSD). Increasing evidence from FHM KI animal studies support the idea that CSD, the underlying mechanism of aura, can activate trigeminal nociception, and thus trigger the headache mechanisms.

Keywords Cav2.1 (P/Q-Type) Ca^{2+} channels · Familial hemiplegic migraine · R192Q and S218L knock in mice · Synaptic transmission · Cortical spreading depression (CSD)

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O. D. Uchitel (✉) · C. González Inchauspe · M. N. Di Guilmi
Instituto de Fisiología, Biología Molecular y Neurociencias (CONICET), Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, piso 2, Ciudad Universitaria, Buenos Aires 1428, Argentina
e-mail: odu@fbmc.fcen.uba.ar

Migraine and familial hemiplegic migraine (FHM)

Migraine is a chronic neurovascular disorder, typically characterized by disabling attacks of severe throbbing unilateral headache, accompanied by nausea and supersensitivity to sound and light. The etiology of migraine is multifactorial (for reviews, see Goadsby et al. 2002; Pietrobon and Striessnig 2003; Marmura and Silberstein 2011). The migraine pain is likely to be triggered by activation of the trigeminovascular system, which primarily modulates sensory signal transmission through the activation of trigeminal afferents to meningeal blood vessels, the trigeminal nerve, and brainstem nuclei (Olesen et al. 2009; Levy 2010).

In 20 % of cases, the migraine headache is preceded by a visual hallucination/illusion known as aura (Goadsby et al. 2002). Neuroimaging findings, from functional magnetic resonance imaging (fMRI) performed during migraine attacks indicate that the migraine aura is due to cortical spreading depression (CSD), a wave of transient intense spike activity that progresses slowly along the cortex and is followed by a long-lasting neuronal suppression (Pietrobon and Striessnig 2003). It is generally believed that cortical spreading depression (CSD) is the neurophysiological correlate of migraine aura (Ayata 2010). Animal studies support the idea that CSD may initiate the headache mechanisms, but the connection between CSD and headache in patients (particularly those with migraine without aura) remains an open question (Charles 2010).

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In experimental animals, CSD manifests as an intense and steady depolarization of neuronal and glial cell membranes which can spread to contiguous cortical areas of the brain at a rate of 3–5 mm/min, regardless of functional cortical divisions or arterial territories. CDS is evoked when local extracellular K^+ concentrations exceed a critical threshold, and is associated with the disruption of membrane ionic gradients, massive influxes of Ca^{2+} and Na^+, and massive K^+ efflux with concomitant release of glutamate, protons, nitric oxide, arachidonic acid, adenosine 5′-triphosphate and calcitonin gene-related peptide (CGRP) (Olesen et al. 2009; Bolay et al. 2002; Levy 2012; Tozzia et al. 2012). It is assumed that such an ionic unbalance could depolarize adjacent neurons and glia, thereby facilitating its propagation. An advancing wave of brief excitation would then be followed by a longer-lasting inhibition of spontaneous and evoked neuronal activity that traverses the cortex (Somjen 2001; Goadsby 2007; Hadjikhani et al. 2001; Bowyer et al. 2001). The release of mediators in the perivascular space that activate the trigeminocervical complex is not likely to be sustained for a headache lasting for hours. A recent study by Karatas et al. (2013) has shown that that intense depolarization and N-methyl-D-aspartate (NMDA) receptor overactivation open neuronal Pannexin1 (Panx1) megachannels. The induced Panx1 activation may cause a headache by releasing pro-inflammatory mediators, which initiate a parenchymal inflammatory response, leading to the sustained release of inflammatory mediators from glia limitans and, hence, prolonged trigeminal stimulation.

Genetic research in the field of migraines has mainly focused on the identification of genes involved in familial hemiplegic migraine (FHM), a rare monogenic subtype of migraines with aura. Genes for three monogenic subtypes of migraine (FHM1-3) have so far been identified (van den Maagdenberg et al. 2007).

FHM1 is an autosomal-dominant subtype of migraine with aura, caused by a spontaneous missense mutation in the *CACNA1A* gene encoding the ion-conducting, pore-forming α_{1A} subunit of Cav2.1 channels (Pietrobon 2005a; Ophoff et al. 1996). FHM1 missense mutations reported so far produce substitutions of conserved amino acids in important functional regions of the Cav2.1 channel including the pore lining and the voltage sensors (Cuenca-Leon et al. 2008; Pietrobon 2007; Thomsen et al. 2007) and have been associated with a wide range of clinical phenotypes (Haan et al. 2005; Kors et al. 2004; Alonso et al. 2004). Aside from the characteristic transient hemiparesis, typical attacks of FHM1 are identical to those of the common forms of migraine with aura (Pietrobon and Striessnig 2003; Pietrobon 2005b; Thomsen et al. 2002). Different clinical phenotypes include pure types of FHM1 (Ophoff et al. 1996), combinations of FHM1 with various degrees of cerebellar ataxia (Ducros et al. 2001; Ophoff et al. 1996), or fatal coma due to excessive cerebral edema (Kors et al. 2001, 2002). Calcium channels play a prominent role in initiating action potential-evoked neurotransmitter release both at peripheral neuromuscular junctions and central synapses, mainly within the cerebellum, brainstem, and cerebral cortex (Katz et al. 1996; Catterall 1998; Iwasaki et al. 2000). Even when pharmacological and electrophysiological studies in brain slices from rodents have revealed that P/Q-, N-, and R-type channels cooperate in controlling release at many central excitatory synapses, P/Q-type channels have a dominant role, at least because of their more efficient coupling to the exocytotic machinery (González Inchauspe et al. 2004; Li et al. 2007; Matsushita et al. 2002; Qian and Noebels 2001, 2000; Wu et al. 1999).

FHM2 is mainly caused by missense mutations in ATP1A2, the gene encoding the α_2 subunit of the Na+/K+ pump ATPase (De Fusco et al. 2003; Marconi et al. 2003; De Vries et al. 2009). In the adult brain, this isoform is expressed primarily in astrocytes. Its co-localization and functional coupling with glial glutamate transporters located in the astrocytic processes that surround glutamatergic synapses suggest a specific role in glutamate clearance (Cholet et al. 2002; Pellerin and Magistretti 1997; Rose et al. 2009). Deficiencies in the reuptake of potassium and glutamate from the synaptic cleft into the glia (Moskowitz et al. 2004) lead to an increased susceptibility to CSD (Koenderink et al. 2005; Pietrobon 2007). Leo et al. (2011) have reported the generation of the first mouse model of FHM2, a knock-in mutant harboring the human W887R-ATP1A2 mutation which decreases the induction threshold and increases the CSD velocity of propagation. The authors suggested that these modifications are due to an inefficient astrocyte clearance of glutamate and a consequent increase in cortical excitatory neurotransmission.

FHM3 is caused by missense mutations in SCN1A, the gene encoding the pore-forming subunit of neuronal Nav1.1 voltage-gated sodium channels (Dichgans et al. 2005; De Vries et al. 2009). These channels are highly expressed in particular inhibitory interneurons, where they play an important role in sustaining high-frequency firing (Catterall et al. 2010). Conflicting findings were obtained from the analysis of mutant recombinant human Nav1.1 channels expressed in non-neuronal cells, pointing to either gain- or loss-of-function effects of FHM3 mutations (Kahlig et al. 2008; Cestèle et al. 2008). Because Nav1.1 channels are crucial for the generation and propagation of action potentials, the overall effects of FHM3 mutations are most likely an increased frequency of neuronal firing plus an enhanced neuronal excitability and neurotransmitter release.

More recently, an homozygous mutation in the SLC4A4 gene (NBCe1 encoding the electrogenic Na(+)-HCO(3)(−) co-transporter) was found in some patients suffering from migraine (Suzuki et al. 2010). A link between a common form of migraine and a genetic mutation of the TWIK-related spinal cord (TRESK) two-pore domain potassium channel (K2P),

encoded by KCNK18, has also been recently reportd (Lafreniere et al. 2010).

A genome-wide association study in a large clinic-based sample of European individuals with migraine identified a genetic variant on chromosome 8q22.1 associated with migraine (Anttila et al. 2011). Furthermore, in a new study including 5,122 migraineurs and 18,108 non-migraineurs, several single nucleotide polymorphism associations (SPNs) specific for migraine were identified (Chasman et al. 2011), establishing a link between migraine with LRP1 (modulator of glutamate signaling) and TRPM8 receptors (related to neuropathic pain models).

Recently, the proline rich transmembrane protein 2 (PRRT2), an axonal protein associated with the exocytosis complex, has also been linked to FHM (Riant et al. 2012).

In a paper published in 2013, a case was reported where a family presented familial migraine with aura and their members exhibited circadian patterns consistent with familial advanced sleep phase syndrome, where individuals go to sleep unusually early in the evening and wake up early in the morning (Brennan et al. 2013). In this case, a mutation (threonine to alanine, T44A) in casein kinase Id (CKIδ) was found to be causing the circadian alteration. CKIδ is a ubiquitous serinethreonine kinase that phosphorylates the circadian clock protein Per2 and many other proteins involved in brain signaling. Mice engineered to carry the CKIδ-T44A allele were more sensitive to pain and also exhibited a reduced threshold for cortical spreading depression.

Voltage-gated calcium channels play an essential role in FHM type 1

Voltage-gated calcium channels can be classified based on their biophysical characteristics into high voltage-activated (HVA) and low voltage-activated (LVA) channels encoded by ten different CACANA1 genes.

LVA Ca^{2+} channels encompass the family of T-type channels with three members (Cav3.1–3.3). They activate at a relatively negative threshold of around −60 mV, have a small conductance, and inactivate rapidly. They are inhibited by drugs like mibefradil (Ro 40–5967) or 2-octanol (Llinás 1988; Perez-Reyes 2003; Bisagno et al. 2010). HVA Ca^{2+} channels include seven members (Cav1.1–1.4, Cav2.1–2.3) encoded by distinct genes each with multiple splice variants (Catterall 2000). HVA channels typically have an activation threshold of around −30 mV, with the exception of Cav1.3, which activates at around −50 mV (Xu and Lipscombe 2001). Cav1.1–1.4 channels, also referred to as L-type channels, are inhibited by three classes of drugs: dihydropyridines, phenylalkylamines, and benzothiazepines (Striessnig et al. 1998). Cav2.1, Cav2.2, and Cav2.3 are known as P/Q-type, N-type, and R-type, respectively. and they are selectively blocked by ω-agatoxin IVA (Mintz et al. 1992), ω-conotoxin GVIA (Plummer et al. 1989; Aosaki and Kasai 1989), and SNX-482 (Newcomb et al. 1998; Tottene et al. 2000), respectively. Ca^{2+} channels in the Cav2 subfamilies are composed of an α1 subunit, auxiliary subunits β, $\alpha_2\delta$, and sometimes γ subunits (Catterall 2011). The α1 subunit of 190–250 kDa includes the pore, voltage sensors, and gating apparatus, and most sites for channel regulation. The auxiliary subunits have an important influence on Ca^{2+} channel function (Catterall 2011).

At many central synapses, there is a developmental change in the Ca^{2+} channel types mediating synaptic transmission, whereby the relative contribution of Cav2.1 channels increases with post-natal age, until it becomes exclusively dependent on P/Q-type channels (Iwasaki et al. 2000). At certain synapses, N- or R-type Ca^{2+} channels fail to fully compensate the lack of Cav2.1 channels in Cav2.1−/− and other loss-of-function Cav2.1 mouse mutants (González Inchauspe et al. 2004, 2007, Giugovaz-Tropper et al. 2011; Pietrobon 2005a, b). Among the presynaptic Ca^{2+} channels, Cav2.1 channels are also unique in their capacity for interacting and being modulated in a complex manner by a number of Ca^{2+}-binding proteins (Catterall and Few 2008). Moreover, Ca^{2+}-dependent regulation of presynaptic Cav2.1 channels may play a crucial role in short-term synaptic plasticity during trains of action potentials (Adams et al. 2010; González Inchauspe et al. 2004; Mochida et al. 2008; Muller et al. 2008; Takago et al. 2005; Xu and Wu 2005; González Inchauspe et al. 2004; Tsujimoto et al. 2002; Cuttle et al. 1998; Takahashi et al. 1996).

Cav2.1 channels are expressed throughout the human and mammalian brain, with a higher concentration in the cerebellum, and are localized in most presynaptic terminals (Catterall 1998; Wu et al. 1999) as well as in the cell body and dendrites of many neurons (Volsen et al. 1995; Westenbroek et al. 1995). In many central synapses, Cav2.1 channels are preferentially located at the release sites and are more effectively coupled to neurotransmitter release than other Ca^{2+} channel types (Li et al. 2007; Matsushita et al. 2002; Qian and Noebels 2001, 2000; Dunlap et al. 1995; Wu et al. 1999; Iwasaki et al. 2000). At these synapses, the action potential-evoked Ca^{2+} influx and the local Ca^{2+} increase that triggers neurotransmitter release are mainly determined by the kinetics of opening and closing, the open probability, and the unitary conductance of Cav2.1 channels (Borst and Sakmann 1998; Sabatini and Regehr 1999; Meinrenken et al. 2002, 2003). In addition to the types of voltage-dependent Ca^{2+} channels mediating Ca^{2+} entry at the terminal, the relationship between presynaptic Ca^{2+} influx and neurotransmitter release is a critical aspect characterizing the Ca^{2+}-induced release of neurotransmitters. Neurotransmitter release is a power function of presynaptic Ca^{2+} current, with a power number between 3 and 4 (Dodge and Rahamimoff 1967; Bollmann et al. 2000; Schneggenburger and Neher 2000) so even small changes in

presynaptic Ca^{2+} influx have a large effect on the number of vesicles released by an action potential (Sabatini and Regehr 1999; Meinrenken et al. 2002). The impact of a relatively small change in the kinetics of activation of presynaptic Ca^{2+} channels on the Ca^{2+} current and neurotransmitter release evoked by an action potential at a central synapse has been shown by Borst and Sakmann (1998). The importance of the kinetics of activation of presynaptic Ca^{2+} channels in determining the fraction of channels that open during a short action potential and, consequently, in Ca^{2+} current waveform and neurotransmitter release, has been also described by Sabatini and Regehr (1999) and our group (González Inchauspe et al. 2010). Liu et al. (2003). We have shown that different kinetics of inactivation and especially voltage- dependence of steady-state inactivation of Ca^{2+} channels have a striking impact on the amount and temporal pattern of Ca^{2+} influx in response to repetitive firing waveforms.

Cav2.1 channels are expressed in those brain structures implicated in the pathogenesis of migraine, including the cerebral cortex, the trigeminal ganglia, and brainstem nuclei involved in the central control of nociception, their expression being particularly high in the cerebellum (Pietrobon and Striessnig 2003). In the cerebral cortex, excitatory synaptic transmission at pyramidal cell synapses in different cortical areas depends predominantly on Cav2.1 channels (Ali and Nelson 2006; Iwasaki et al. 2000; Koester and Sakmann 2000; Rozov et al. 2001; Tottene et al. 2009; Zaitsev et al. 2007). They also mediate about 40 % of the AP-evoked Ca^{2+} influx in dendritic spines and shaft of layer 2/3 cortical pyramidal neurons (Koester and Sakmann 2000), and contribute to the regulation of the intrinsic firing of the same neurons via activation of different Ca^{2+}-dependent K^+ (K_{Ca}) channels (Pineda et al. 1998). The somatodendritic localization of Cav2.1 channels points to additional postsynaptic roles, such as neural excitability (Pietrobon 2005b, 2010). However, it is still a central problem in Ca^{2+} channel neurobiology studies to be able to translate their genetic complexity to their functional role in synaptic transmission. The availability of several mouse models with mutations in Ca^{2+} channel genes have provided a valuable tool to define Ca^{2+} channel functions. It would be expected that changes in the functional properties of Cav2.1 channels alter the behavior of neuronal networks.

Functional consequences of FHM1 gene mutations

Studies in heterologous systems

Biophysical analysis of FHM1 Ca^{2+} channel dysfunction in heterologous systems show that the FHM1 mutations alter many biophysical properties of human Cav2.1 channels in a complex way, which is controversial since both loss-of-function and gain-of-function phenotypes have been reported (Cao et al. 2004; Barrett et al. 2005; Cao and Tsien 2005; Tottene et al. 2002; Kraus et al. 2000, 1998; Hans et al. 1999). The most consistent effect revealed by the analysis of single-channel properties of human Cav2.1 channels carrying FHM1 mutations was an increase in channel open probability and in single channel Ca^{2+} influx over a broad voltage range, mainly due to a shift of channel activation to more negative voltages (Hans et al. 1999; Tottene et al. 2002, 2005, 2009; van den Maagdenberg et al. 2004, 2010, González Inchauspe et al. 2010; Adams et al. 2010; Fioretti et al. 2011). Shifts to lower voltages of activation of the mutant channels were also revealed by measuring whole-cell currents in heterologous expression systems and transfected neurons (Adams et al. 2009; Melliti et al. 2003; Mullner et al. 2004; Serra et al. 2009; Tottene et al. 2002, 2005; Weiss et al. 2008). As a result, channels open with smaller depolarizations. However, the overall change in calcium influx at presynaptic terminals is difficult to predict since there have been contradictory results. For instance, Cao and colleagues (2004) found evidence for reduced calcium influx at the whole-cell level in transfections of cultured mouse hippocampal neurons. What happens in the mutant brain will therefore be determined by the delicate interplay between the functional effects of a particular mutation, the different channel properties and density, the type of channel subunits, and the direct and indirect cellular environment. Despite the discrepancies in current density through mutated Cav2.1 channels (Pietrobon 2010) and its effect on synaptic transmission, there is a consistent shift of the voltage dependence of Cav2.1-channel activation to more hyperpolarized values (Catterall et al. 2008). Since the biophysical effects of FHM1 mutations can be affected by a number of factors, including Cav2.1 splice-variation (Adams et al. 2009), β subunit co-expression (Mullner et al. 2004), and the nature of the expression system (Kraus et al. 1998), it is relevant to study the functional consequences of gene mutations on Cav2.1 Ca^{2+} channels in their native neuronal environment, that is, in knock-in mouse models carrying human pathogenic mutations. The generation of two knock-in FHM1 mouse models carrying either the human pathogenic R192Q or S218L missense mutations allowed the first analysis of the functional consequences of FHM1 mutations on Cav2.1 channels and synaptic transmission in neurons expressing the channels at the endogenous physiological level (van den Maagdenberg et al. 2010, 2004; Kaja et al. 2010; Tottene et al. 2009; González Inchauspe et al. 2010; Adams et al. 2010).

Studies in mouse models bearing CACNA1A human mutations

The R192Q KI mice exhibit no overt clinical phenotype or structural abnormalities. This situation is very similar in humans, in which the R192Q mutation causes only mild

FHM attacks, very similar to the common forms of migraine, albeit with hemiparesis, but without any other neurological symptoms. The S218L mutation causes a severe migraine phenotype combined with slowly progressive cerebellar ataxia and atrophy, epileptic seizures, coma or profound stupor, and severe, sometimes fatal, cerebral edema which can be triggered by a trivial head trauma (Chan et al. 2008; Kors et al. 2001; van den Maagdenberg et al. 2010).

Chanda et al. (2013) have recently reported an extensive behavioral characterization of 192Q mutant mice where they found that migraine headache-related behaviors triggered by stress are more frequent in mutants, more frequent and/or severe in S218L versus R192Q mutants, are predominant in females, and are reversed by analgesics effective against migraine in humans. Additionally, these migraine-like behaviors are associated with symptoms of migraine attacks in humans, such as photophobia.

As noted before, CSD is implicated in the pathophysiology of FHM1. In both R192Q and S218L KI mice, a lower threshold for CSD induction and an increased velocity of CSD propagation have been reported (van den Maagdenberg et al. 2004, 2010; Tottene et al. 2009; Eikermann-Haerter et al. 2009a). In agreement with the higher incidence of migraine in females, the velocity of propagation and the frequency of CSDs were larger in females than in males of both mutant strains (Eikermann-Haerter et al. 2009a, b). The sex difference was abrogated by ovariectomy and enhanced by orchiectomy, suggesting that female and male gonadal hormones exert reciprocal effects on CSD susceptibility. A female predominance is also described for familial (5:2 ratio) and sporadic (4.25:1 ratio) hemiplegic migraine (Eriksen et al. 2006; Thomsen et al. 2007, 2002). All these findings underscore the complex synergistic interactions between genetic and hormonal factors determining migraine susceptibility.

Accordingly to the larger gain-of-function observed in vitro, the S218L KI mice shows higher CSD susceptibility in vivo, compared to the R192Q mice. Experimentally induced CSD induces simple hemiplegia in R192Q mutant mice, whereas S218L mutants additionally develop coma and often fatal seizures (van den Maagdenberg et al. 2010; Eikermann-Haerter et al. 2009a, b). Recently, Eikermann-Haerter et al. (2011) have provided evidence for an enhanced subcortical SD susceptibility in both FHM1 mutant mice compared to WT. Whereas the facilitated subcortical spread appeared limited to the striatum in R192Q mutants, hippocampal and thalamic spread were detected in the S218L mutants with an allele-dosage effect. Their findings suggest a role for subcortical SD as a potential mechanism to explain hemiplegia, seizures, and coma in FHM1.

Other studies in knock-in animals revealed multiple gain-of-function effects. In cerebellar granule cells and cortical pyramidal cells of R192Q and S218L KI mice, the Cav2.1 Ca^{2+} current density was larger than in WT neurons in a wide range of relatively mild depolarizations. These findings show the activation of mutant mouse Cav2.1 channels at 8–9 mV more negative voltages than the corresponding WT channels. P/Q current densities were similar in KI and WT neurons at higher voltages (that elicit maximal Cav2.1 channel open probability), indicating similar densities of functional Cav2.1 channels (van den Maagdenberg et al. 2004, 2010; Tottene et al. 2009). Thus, in two different FHM1 mouse models and two different types of neurons, the functional consequences of the FHM1 mutations on native neuronal mouse Cav2.1 channels were quite similar to those on single recombinant human Cav2.1 channels (Tottene et al. 2002, 2005). In agreement with the lower threshold of activation of human S218L Cav2.1 channels compared to that of human R192Q CaV2.1 channels (Tottene et al. 2005), the gain of function of the P/Q current at low voltages was larger in S218L than R192Q KI mice (van den Maagdenberg et al. 2004, 2010). Similarly, a negative shift in the activation voltage of presynaptic Cav2.1 mutated channels was confirmed at the calyx of Held synapse of KI S218L mice (Di Guilmi et al., unpublished results).

Effects of FHM1 mutation on synaptic transmission

The first indication that the gain-of-function of Cav2.1 channels produced by FHM1 mutations could lead to enhanced evoked neurotransmitter release was obtained at the neuromuscular junction (NMJ), where evoked neurotransmission was unaltered at physiological Ca^{2+} ion concentrations but increased at 0.2 mM Ca^{2+} in KI mice (Kaja et al. 2005; van den Maagdenberg et al. 2004, 2010). Cortical excitatory neurotransmission was investigated in neuronal microcultures and in brain slices from homozygous R192Q KI mice (Tottene et al. 2009). The results show increased synaptic strength at physiological Ca^{2+} concentration, presumably due to enhanced action potential-evoked Ca^{2+} influx through mutant Cav2.1 channels and enhanced probability of glutamate release at cortical pyramidal cell (PC) synapses. Short-term synaptic depression during trains of action potentials was enhanced. Neither amplitude nor frequency of miniature postsynaptic currents were altered, indicating the absence of homeostatic compensatory mechanisms at excitatory synapses onto pyramidal cells (Tottene et al. 2009).

Characteristically, CSD arises spontaneously in response to specific triggers that somehow create in the cortex of migraineurs the conditions for initiating the positive feedback cycle that overwhelms the regulatory mechanisms controlling cortical $[K^+]_o$ and ignites CSD. Insights into how this might occur have been provided by the interesting finding that, in contrast with the enhanced glutamatergic excitatory neurotransmission at PC synapses, inhibitory GABAergic neurotransmission at fast spiking (FS) interneurons was not altered in R192Q KI mice, despite being mediated by Cav2.1 channels (Tottene et al. 2009). Given the evidence that the

magnitude (or even the presence) of the negative shift in activation of human Cav2.1 channels produced by FHM1 mutations may depend on the particular recombinant Cav2.1 $\alpha 1$ splice variant and/or Cav2.1 β subunit (Mullner et al. 2004; Adams et al. 2009), a possible explanation for the unaltered inhibitory transmission at the FS interneurons may be the presence of a Cav2.1 isoform that is little affected by the mutation, or a near saturation of the presynaptic Ca^{2+} sensors. Evoked transmitter release was also found to be normal at the neuromuscular junction and at the calyx of Held synapses of R192Q KI mice when studied in normal Ca/Mg concentration in the external solution (González Inchauspe et al. 2010). This finding was unexpected since mutated R192Q Cav2.1 channels at the calyx of Held nerve terminals are active at lower membrane potentials as predicted. Interestingly, transmitter release at the FS interneurons, at the neuromuscular junction, and at the calyx of Held is triggered by a relatively brief action potential. In each of these R192Q KI preparations, transmitter release is not affected. Thus, one factor that may be controlling the expression of a synaptic gain of function in the FHM1 KI mice is the duration of the action potential (AP) triggering transmitter release. To address this question we used the calyx of Held, a giant glutamatergic synapse in the mammalian auditory brainstem where, due to its size and accessibility, it is possible to make direct patch-clamp recordings from the presynaptic nerve terminal and its postsynaptic target, the principal neurons of the medial nucleus of the trapezoid body (MNTB). The large size of the calyx of Held allows it to harbor hundreds of active zones, and thus a single presynaptic AP releases hundreds of quanta, generating a large excitatory postsynaptic current (EPSC) that rapidly depolarizes the MNTB neuron to threshold. A single MNTB principal neuron receives input from only one calyx of Held presynaptic terminal, and glutamatergic synaptic transmission mediated by AMPA receptors is triggered almost exclusively by Cav2.1 channels in mature mice (for a review, see Schneggenburger and Forsythe 2006). The presynaptic Ca^{2+} currents (I_{pCa}) from the calyx of Held terminals recorded in brainstem slices also activate at more hyperpolarizing potentials in R192Q KI than in WT mice (González Inchauspe et al. 2010). Assuming presynaptic Ca^{2+} currents through Cav2.1 channels can be modeled by Hodgkin–Huxley equations, a shift to more negative activation voltages by the FHM1 mutation theoretically should generate larger Ca^{2+} currents during APs (Borst and Sakmann 1999). However, the short duration of the calyx of Held APs (half-width: 0.45±0.02 ms) elicited I_{pCa} with similar amplitudes in both WT and R192Q KI mice. Only when the AP duration was prolonged to 2 ms (typical mean half-width of pyramidal neuron APs), was the shift in the activation by the R192Q mutation sufficient to cause greater presynaptic Ca^{2+} influx (González Inchauspe et al. 2010). According to the similarity in I_{pCa} amplitudes evoked by physiological calyx of Held APs, there were no differences in mean amplitudes of EPSCs, or in release probability or EPSC pair pulse facilitation between WT and R192Q KI mice. However, when synaptic transmission was triggered by long duration APs (obtained by inhibiting K^+ channels with 4-Aminopyridine and tetraethylammonium chloride), FHM1 R192Q KI mice did show a significant increase in both amplitude and area of EPSCs compared to WT mice (González Inchauspe et al. 2012).

These findings strongly suggest that synapses driven by larger amplitude and short duration APs (e.g., calyx of Held and interneurons APs) are less affected by the mutation-induced hyperpolarizing shift in voltage-dependence of Ca^{2+} channel activation than those driven by longer duration APs (e.g., pyramidal neurons APs). The differences in AP durations that trigger cortical excitatory and inhibitory synapses might explain the unaltered inhibitory neurotransmission observed by Tottene et al. (2009) at the fast spiking (FS) interneuron–pyramidal cell (PC) synapses. It may also explain the gain-of- function observed at the PC–FS interneuron excitatory synapses, since several types of interneurons and other neurons that display fast spiking behavior have APs with short half-widths durations (Ali et al. 2007), while PCs depicts long APs. In this way, the expression of FHM1 mutations might vary according to the shape of the APs in charge of triggering synaptic transmission, adding to the complexity of the pathophysiology of migraine.

Effects of FHM1 mutation on synaptic plasticity

The dynamics and strength of neural circuits are essential for encoding and processing information in the CNS and rely on short- and long-term forms of synaptic plasticity. In several synapses, repetitive stimulation causes short-term depression (STD). Short-term depression of synaptic release has been traditionally accredited to depletion of the release-ready vesicle pool (von Gersdorff and Borst 2002; Wong et al. 2003; Zucker and Regehr 2002; Wang and Kaczmarek 1998), although other mechanisms like receptor desensitization (Wong et al. 2003) may be involved. At the calyx of Held synapse, it has been shown that short-term synaptic plasticity is also achieved by a mechanism involving the regulation of presynaptic Cav2.1 Ca^{2+} currents (Cuttle et al. 1998; Tsujimoto et al. 2002), like calcium channel inactivation (Xu and Wu 2005; Muller et al. 2008; Forsythe et al. 1998; Di Guilmi et al. 2011) and calcium channel inhibition by presynaptic metabotropic glutamate receptor (von Gersdorff et al. 1997; Takahashi et al. 1996) or AMPA receptors (Takago et al. 2005). Hennig et al. (2008) have developed a model of synaptic depression at the calyx of Held synaptic terminal that combines many of the mechanisms involved in short-term depression and plasticity, including vesicle recycling, facilitation, activity-dependent vesicle retrieval, and multiple mechanisms affecting calcium channel activity and release probability. The efficacy of

synaptic transmission during repetitive stimulation is also determined by the rate of recovery from STD, due to the replenishment of the readily releasable pool of synaptic vesicles, which is dynamically regulated by Ca^{2+} influx through voltage-gated Ca^{2+} channels in an activity-dependent manner (Zucker and Regehr 2002; Wang and Kaczmarek 1998). High-frequency stimulation of presynaptic terminals significantly enhances the rate of replenishment. Therefore, mutations or neuromodulators capable of altering the activation/inactivation properties of calcium channels (Di Guilmi et al. 2011; Uchitel et al. 2010) may also alter STD and/or vesicle replenishment. Recordings in connected MNTB neurons at the calyx of Held synapses show that, during high frequency activity, EPSC amplitudes depress to the same extent and with similar kinetics in WT and R192Q KI mice, but, interestingly, the rate of recovery of EPSCs amplitudes was significantly faster in R192Q KI compared to WT synapses (González Inchauspe et al. 2012).

Ca^{2+}-dependent facilitation (CDF) of Cav2.1 channels is an important mechanism required for normal synaptic plasticity at fast synapses in the mammalian CNS. As an example, at the calyx of Held synapse of Cav2.1 KO mice, lack of pair pulse facilitation of presynaptic Ca^{2+} channels was in correlation with absence of facilitation of synaptic transmission (González Inchauspe et al. 2004, 2007). In the same animal model, pair pulse facilitation of end plate potentials was also impaired at the neuromuscular junction (Urbano et al. 2003). Short-term facilitation of synaptic release has been attributed to enhanced vesicle release resulting from the accumulation of intracellular Ca^{2+} in presynaptic terminals during repetitive action potentials (APs), whereas the buildup of residual Ca^{2+} enhances, binding to those sensor proteins directly mediating vesicle fusion and transmitter release (Atluri and Regehr 1996, Zucker and Regehr 2002). There may exist at least one other mechanism of facilitation, driven by a Ca^{2+} sensor with high Ca^{2+} affinity, that can detect low, transient levels of Ca^{2+}, most likely near the pore of presynaptic Ca^{2+} channels (Atluri and Regehr 1996). A recent study using recombinant Cav2.1 channels expressed in cultured superior cervical ganglion neurons demonstrated that the Ca^{2+}-dependent facilitation (CDF) and Ca^{2+}-dependent inactivation (CDI) of Cav2.1 channels are mediated through neuronal Ca^{2+} sensor proteins (CaSs) like calmodulin (CaM), which binds the Cav2.1 subunit carboxyl terminus (Mochida et al. 2008). CaM-mediated CDF and CDI are robust forms of Cav2.1 channel modulation in which CaM interacts with the Cav2.1 carboxyl terminus in a bipartite regulatory processes where CDF is mediated by a local increase in Ca^{2+} and CDI through a global increase in Ca^{2+} (Tsujimoto et al. 2002; Chaudhuri et al. 2007). Adams et al. (2010) provide supporting evidence for the hypothesis that Ca^{2+} influx at Purkinje fiber boutons induces calcium-dependent facilitation of Cav2.1 channels as a mean to enhance Ca^{2+} influx during subsequent APs, achieving synaptic facilitation at the cerebellar parallel fiber (PF)-to-Purkinje cell (PC) central synapse. Interestingly, they demonstrated that mutations associated with human diseases affect this process. They observed that FHM1 gain-of-function missense mutations (R192Q and S218L) significantly occlude CDF of Cav2.1 currents in recombinant and native systems, and that this alteration correlates with reduced short-term synaptic facilitation at cerebellar PF-to-PC synapses. These findings suggest that CDF of Cav2.1 is an important mechanism required for normal synaptic plasticity at a fast synapse in the mammalian CNS. Additional evidence provided by two-photon imaging supported the fact that Cav2.1 FHM1 mutant presynaptic channels are in a constitutively facilitated state that basally increases presynaptic Ca^{2+} influx and transmitter release. Thus, observed changes in synaptic plasticity at the PF-to-PC synapse may result from a larger initial Ca^{2+} influx through basally facilitated mutant channels relative to non-facilitated WT channels in Purkinje fibers boutons. Overall, these results provide evidence that FHM1 mutations directly affect both Cav2.1 channel CDF and synaptic plasticity, and that together they are likely to contribute towards the pathophysiology underlying FHM1.

Aditional studies in mice with CACNA1A human mutations

The R192Q and S218L KI mice are being used as models for several studies on migraine diseases. Mutant Cav2.1 channels in R192Q KI mice are less susceptible to inhibition by G-proteins. Since A1 receptors modulate Cav2.1 channel functioning via G-protein inhibition and adenosine modulates sleep via A1 receptors, Deboer et al. (2013) proposed a possible role of the Cav2.1 channel in sleep regulation. They found that R192Q KI mice showed reduced susceptibility to inhibition by adenosine, which resulted in a sleep phenotype characterized by longer waking episodes in the dark period and less non-rapid eye movement (NREM) sleep, together with a reduced responsiveness to caffeine (adenosine receptor antagonist) and cyclopentyladenosine (CPA), an A1 receptor specific agonist which induces sleep.

Episodic ataxia is also linked to mutations in the CACNA1A gene that either decrease (Ophoff et al. 1996; van den Maagdenberg et al. 2007; Pietrobon 2010) or enhance Cav2.1-mediated Ca^{2+} influx (Kors et al. 2001; Tottene et al. 2005; Gao et al. 2012). In particular, Purkinje cells in mutant mice depict distinct irregular firing patterns, both in vitro and in vivo, that contribute to cerebellar ataxia in Cacna1a mutants characterized by either decreased (Hoebeek et al. 2005; Walter et al. 2006) or increased Cav2.1- mediated Ca^{2+} influx (Gao et al. 2012). In the S218L mouse, Gao et al. (2012) show that the mutation causes a negative shift of voltage dependence of Cav2.1 channels of mouse Purkinje cells and results in lowered thresholds for somatic action potentials and dendritic Ca^{2+} spikes as well as in disrupted firing patterns. Both the irregular Purkinje cell spiking and the ataxic motor

performance of the S218L mutation can be counteracted by activators of small-conductance Ca^{2+}-activated K^+ channels (SK channels): 1-EBIO and CHZ promote more regular simple spike firing by reducing the hyperexcitability of Cacna1a S218L Purkinje cells. Systemic CHZ application also improves the motor behavior of Cacna1a S218L mutants. These results demonstrate a narrow window in Ca^{2+} homeostasis: both sufficiently decreased or increased Ca^{2+} influx can induce ataxia. Targeting Ca^{2+}-dependent K^+ channels may be beneficial for treating ataxia not only in patients suffering from a decreased Ca^{2+} influx but also for those suffering from an increased Ca^{2+} influx in their Purkinje cells.

Summary

The role of Cav2.1 channels in controlling fast neurotransmitter release from central excitatory synapses suggest that the human and mouse Cav2.1 channelopathies and their episodic neurological symptoms (from migraine to absence epilepsy and ataxia) might be primarily synaptic diseases. These different disorders probably arise from a disruption of the finely tuned balance between excitation and inhibition in neuronal circuits of specific brain regions: the cortex for migraine, the thalamus for absence epilepsy, and the cerebellum for ataxia. Synapses are affected differently depending on their intrinsic differences, relative dependence on P/Q channels, and on the shape of their action potential.

The mutations in the Cav2.1 channels linked to FHM1 affect the biophysical properties and the density of presynaptic calcium currents as well as several other properties of synaptic transmission, like short-term plasticity. A gain of function was observed in R192Q and S218L KI mice, including an increased Ca^{2+} influx through Cav2.1 channels, as a consequence of mutant channels that open at lower voltages than WT ones. Given the three-fourth power dependence of neurotransmitter release on intracellular Ca^{2+} concentration, small changes in the amplitude or time course of Ca^{2+} influx at the release sites are expected to be very effective in modulating transmitter output at those synapses where the Ca^{2+} sensors are not saturated during an action potential. These characteristics predict that, at such synapses, Cav2.1 channels that open more readily and at lower voltages due to the FHM1 mutation will lead to an increased action potential-evoked Ca^{2+} influx and a consequent increase in neurotransmitter release and excitability.

Although an established model explaining migraine attacks is still lacking, a favored hypothesis considers that, due to excessive recurrent glutamatergic neurotransmission, there is an impairment of the cortical circuits that dynamically adjusts the E/I balance during cortical activity. This deterioration may be the basis for both the abnormal regulation of cortical function and the increased susceptibility for cortical spreading depression (CSD), which is believed to initiate the episodes of migraine with aura.

Novel therapeutic strategies should consider cortical E/I dysregulation and CSD as key targets for preventive migraine treatment. In particular, cortical glutamatergic synapses appear as key therapeutic targets for novel drugs aimed at counteracting excessive glutamatergic synaptic transmission in FHM and other migraine subtypes. Drugs that increase the CSD threshold independently of the specific cortical dysfunctions underlying the susceptibility to CSD in different migraineurs could be particularly effective.

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