Front. Biol. 2014, 9(3): 205–215
DOI 10.1007/s11515-014-1305-3

REVIEW

Role of calmodulin in neuronal Kv7/KCNQ potassium channels and epilepsy

Hee Jung CHUNG (✉)[1,2]

[1] *Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*
[2] *Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

Abstract **Neuronal Kv7/KCNQ channels are critical regulators of neuronal excitability since they potently suppress repetitive firing of action potentials. These voltage-dependent potassium channels are composed mostly of Kv7.2 / KCNQ2 and Kv7.3 / KCNQ3 subunits that show overlapping distribution throughout the brain and in the peripheral nervous system. They are also called 'M-channels' since their inhibition by muscarinic agonists leads to a profound increase in action potential firing. Consistent with their ability to suppress seizures and attenuate chronic inflammatory and neuropathic pain, mutations in the KCNQ2 and KCNQ3 genes are associated with benign familial neonatal convulsions, a dominantly-inherited epilepsy in infancy. Recently, *de novo* mutations in the KCNQ2 gene have been linked to early onset epileptic encephalopathy. Notably, some of these mutations are clustered in a region of the intracellular cytoplasmic tail of Kv7.2 that interacts with a ubiquitous calcium sensor, calmodulin. In this review, we highlight the recent advances in understanding the role of calmodulin in modulating physiological function of neuronal Kv7 channels including their biophysical properties, assembly, and trafficking. We also summarize recent studies that have investigated functional impact of epilepsy-associated mutations localized to the calmodulin binding domains of Kv7.2.**

Keywords calmodulin, Kv7, KCNQ, epilepsy, action potential, M-channel

Introduction

Kv7/KCNQ channels are voltage-dependent potassium channels (Kv) that play critical roles in the heart, brain, ear, and epithelia (Robbins, 2001). KCNQ1–5 genes encode Kv7.1–5 subunits, which assemble to form tetramers (Soldovieri et al., 2011). While Kv7.1 and Kv7.4 subunits are mainly expressed in the heart and inner ear, respectively, Kv7.2, Kv7.3, and Kv7.5 subunits are highly expressed in the central nervous system (Soldovieri et al., 2011). Similar to all Kv channels, each Kv7 subunit consists of six transmembrane domains (S1–S6), which are further divided into a voltage-sensor domain (S1–S4) and a pore domain (S5-pore-S6) (Robbins, 2001) (Fig. 1). Unlike *Shaker*-related Kv channels that have T1 tetramerization domains in their long intracellular N-termini (Lai and Jan, 2006), each Kv7 subunit possesses a short cytoplasmic N terminus that regulates surface expression and open probability of Kv7 channels (Etxeberria et al., 2004; Dahimene et al., 2006). Long cytoplasmic C-termini of Kv7 channels are critical for channel assembly, trafficking, and interaction with various signaling proteins (Haitin and Attali, 2008) (Fig. 1).

Neuronal Kv7 channels are mostly heterotetramers composed of Kv7.3 and Kv7.2 subunits (Wang et al., 1998) with a possible contribution of Kv7.5 subunits (Shah et al., 2002), and to a lesser extent, homomeric Kv7.2 subunits (Hadley et al., 2003; Schwarz et al., 2006). Localized throughout the brain and in the peripheral nervous system (Wang et al., 1998; Cooper et al., 2001; Roche et al., 2002; Devaux et al., 2004; Pan et al., 2006), they produce potassium (K^+) currents that activate slowly but show no inactivation (Robbins, 2001). They are activated at the membrane potentials that are more depolarized than action potential (AP) threshold much slowly than ion channels that underlie fast-propagating APs. Thus, they tend to allow the firing of a single AP, but effectively

Received April 1, 2014; accepted April 14, 2014
Correspondence: Hee Jung CHUNG
E-mail: chunghj@life.illinois.edu

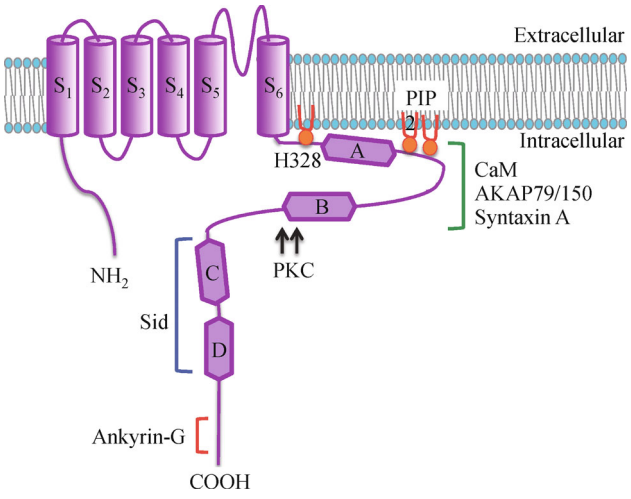

Figure 1 Schematic representation of a single Kv7.2 subunit. Schematic drawing (not to scale) of a Kv7.2 subunit (accession number: Y15065; 844 amino acids) showing a short N-terminal tail, 6 transmembrane domains (S1–6), and a long C-terminal tail that contains four predicted α-helical regions (A–D) and ankyrin-G binding domain (amino acids 833-842). The proximal C-terminal tail from helix A (amino acids 335–356) to helix B (amino acids 532-558) provides phosphorylation sites for protein kinase C (PKC) and tyrosine kinase Src (not shown) as well as binding sites for calmodulin (CaM), A-kinase-anchoring protein (AKAP79/150), and syntaxin 1A. A subunit interaction domain (Sid) spans from helix C (amino acids 574–588) to helix D (amino acids 619–653). The binding regions for PIP_2 (phosphatidylinositol-(4,5)-bisphosphate) are also indicated.

function as a brake for repetitive firing of APs, which is a hallmark of excessive neuronal excitability leading to seizure (Brown and Passmore, 2009). Originally named 'M-channels', stimulation of the muscarinic acetylcholine receptors inhibits their current, leading to a profound increase in AP firing in frog sympathetic neuron (Brown and Passmore, 2009). Similarly, pharmacological inhibition of Kv7 channels decreases the AP threshold and facilitates spontaneous and burst firing of APs in hippocampal pyramidal neurons (Yue and Yaari, 2004; Shah et al., 2008; Shah et al., 2011). Kv7 channels also contribute to resting membrane potential, spike frequency adaptation, spike after depolarization, and spike after hyperpolarization (Yue and Yaari, 2004; Gu et al., 2005; Peters et al., 2005; Yue and Yaari, 2006; Tzingounis and Nicoll, 2008; Tzingounis et al., 2010).

The Kv7.2 and Kv7.3 subunits are concentrated at the axonal initial segments (AIS) and nodes of Ranvier in myelinated axons (Devaux et al., 2004; Pan et al., 2006). In hippocampal neurons, Kv7.2/Kv7.3 channels are preferentially enriched at the plasma membrane of the axons rather than the soma and dendrites (Chung et al., 2006), consistent with their ability to regulate AP propagation along the axon (Dedek et al., 2001; Schwarz et al., 2006) and neurotransmitter release (Martire et al., 2004). The distal C-termini of Kv7.2 and Kv7.3 but not other Kv7 subunits bind to ankyrin-G (Chung et al., 2006; Pan et al., 2006; Rasmussen et al., 2007) (Fig. 1), an essential component of the AIS that maintains neuronal axon versus dendrite polarity (Song et al., 2009). Targeting of Kv7.2/Kv7.3 channels to the AIS is abolished by genetic ablation of ankyrin-G in mice or disruption of their ankyrin-G binding motifs (Chung et al., 2006; Pan et al., 2006; Rasmussen et al., 2007). Furthermore, spontaneous and burst firing of APs is increased by impairing the channel localization at the AIS (Shah et al., 2008), the critical site for AP initiation and regulation (Clark et al., 2009). Importantly, axonal Kv7 channels rather than dendritic Kv7 channels potently inhibit AP firing by regulating AP threshold and resting membrane potential (Shah et al., 2008; Shah et al., 2011).

Consistent with the ability of Kv7 channels to suppress burst and spontaneous firing of APs (Yue and Yaari, 2004; Shah et al., 2008; Shah et al., 2011), mice lacking a single copy of the KCNQ2 gene display increased susceptibility to convulsant agents (Watanabe et al., 2000). Inhibition of Kv7 currents during early neonatal development but not adulthood leads to abnormal development in hippocampus and enhanced spontaneous seizures and seizure susceptibility (Peters et al., 2005). In contrast, seizures are suppressed in animal models by retigabine (Dailey et al., 1995; Rostock et al., 1996; Tober et al., 1996), a potent and selective agonist of Kv7 channels (Surti and Jan, 2005). Importantly, mutations in the KCNQ2 and KCNQ3 genes have been linked to benign familial neonatal convulsion (BFNC) (Soldovieri et al., 2011), a rare dominantly-inherited epilepsy in infancy with penetrance of 85% but minimal effect on the psychomotor development (Maljevic et al., 2008). Although seizures in BFNC spontaneously remit after several weeks to months, the risk of recurring seizures later in life is about 15% (Psenka and Holden, 1996). Additionally, *de novo* missense mutations in KCNQ2 gene have been recently discovered to be associated with epileptic encephalopathy (Borgatti et al., 2004; Schmitt et al., 2005; Weckhuysen et al., 2012) including Ohtahara syndrome (Saitsu et al., 2012), severe symptomatic drug resistant epilepsy with poor prognosis often accompanied by psychomotor retardation (Ohtahara and Yamatogi, 2006).

Studies in the heterologous system revealed that BFNC mutations in the pore and voltage sensor regions of Kv7.2 and Kv7.3 results in a modest 20%–25% reduction in K^+ current (Schroeder et al., 1998; Lerche et al., 1999; Schwake et al., 2000; Schwake et al., 2003; Singh et al., 2003; Schwake et al., 2006). BFNC mutations in the cytoplasmic C-terminal tail of KCNQ2 causes mild to severe reduction in current and surface expression (Schwake et al., 2000; 2003) by impairing calmodulin (CaM) interaction (Richards et al., 2004) or protein stability (Soldovieri et al., 2006). In hippocampal neurons, BFNC mutations variably disrupt axonal enrichment of Kv7.2/Kv7.3 channels (Chung et al., 2006). Based on these findings, haploinsufficiency of Kv7 function has been suggested to cause BFNC. In contrast, *de novo* missense mutations in the pore and voltage sensor regions of Kv7.2

have been recently shown to cause a dominant-negative reduction of Kv7.2/Kv7.3 current at subthreshold membrane potentials (Orhan et al., 2013). These findings collectively indicate the critical roles of Kv7 channels in regulation of neuronal excitability, and suggest the inhibition of Kv7 channel function as a potential pathogenic mechanism for Kv7-associated epilepsy.

CaM binds to neuronal Kv7 channels

CaM is a ubiquitous intracellular Ca^{2+} binding protein consisting of two globular domains in the N- and C-termini (N- and C-lobes), each of which binds to two Ca^{2+} ions via two EF hand structures (Hoeflich and Ikura, 2002) (Fig. 2). In response to the changes in intracellular Ca^{2+} levels, CaM regulates voltage-gated ion channels critical for neuronal excitability in part by binding to classical CaM binding motifs within the channels such as the IQ motif, 1-5-10 motif, or 1-8-14 motif (Hoeflich and Ikura, 2002). Among four predicted helical regions known as helices A-D conserved in the intracellular cytoplasmic C-termini of all Kv7 subunits (Yus-Najera et al., 2002), helices A and B bind to CaM (Wen and Levitan, 2002; Yus-Najera et al., 2002) whereas helices C and D mediate tetramerization (Schwake et al., 2006) (Fig. 1). Helix A contains a consensus CaM binding IQ motif (IQXXXR) (Yus-Najera et al., 2002) and helix B harbors two 1-5-10 motifs and one 1-14 motif for binding Ca^{2+}-bound CaM (Ca^{2+}/CaM) (Yus-Najera et al., 2002; Xu et al., 2013) (Fig. 2). A number of co-immunoprecitation studies have revealed that both apoCaM and Ca^{2+}/CaM can bind to neuronal Kv7 channels (Wen and Levitan, 2002; Yus-Najera et al., 2002; Gamper and Shapiro, 2003; Etxeberria et al., 2008; Alaimo et al., 2009). Mutations disrupting the IQ motif in Kv7.2 or Kv7.3 abrogate apoCaM interaction with homomeric channels, and markedly reduce apoCaM binding to heteromeric Kv7.2/Kv7.3 channels (Wen and Levitan, 2002; Yus-Najera et al., 2002; Alaimo et al., 2009; Gomez-Posada et al., 2011).

In vitro binding studies using fusion proteins of the Kv7 cytoplasmic C-terminal tail have shown that Ca^{2+}/CaM favors binding to the B helices of Kv7.2–5 subunits (Yus-Najera et al., 2002; Gamper and Shapiro, 2003; Alaimo et al., 2012; Xu et al., 2013). ApoCaM interacts with both A and B helices of Kv7.1–5 subunits better than with either helix A or B alone (Xu et al., 2013). In contrast, Ca^{2+}/CaM binds only to the B helices of Kv7.1–5 subunits (Alaimo et al., 2012; Xu et al., 2013), suggesting that helix B is sufficient for Ca^{2+}/CaM binding. According to the X-ray crystal structure resolved at 2.60A resolution, Ca^{2+}/CaM interacts with the helix B of Kv7.4 in an antiparallel orientation in which the Ca^{2+}/C-lobe and Ca^{2+}/N-lobe bind to the N-terminal and C-terminal portions of the helix B, respectively (Xu et al., 2013). This binding occurs through a novel variation of the 1-14 Ca^{2+}/CaM binding motif instead of the two predicted 1-5-10 Ca^{2+}/CaM binding motifs (Xu et al., 2013). Equilibrium sedimentation experiments have further revealed a 4:4 stoichiometry for a Ca^{2+}/CaM complex with Kv7.4 helices B-D and a 1:1 stoichiometry for a Ca^{2+}/CaM complex with Kv7.4 helix B (Xu et al., 2013), suggesting that helix A is dispensable for Ca^{2+}-dependent CaM interaction with Kv7 channels. Based on these findings, it has been suggested that Ca^{2+} induces a conformational change from an apoCaM-bound state that bridges the A and B helices to a Ca^{2+}/CaM-bound state that

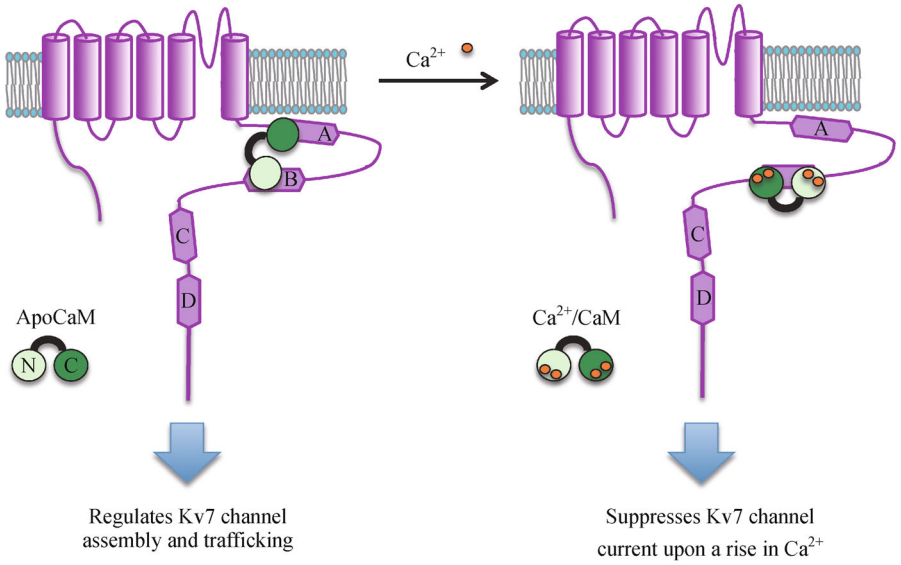

Figure 2 Ca^{2+}-dependent interactions of Kv7.2/Kv7.3 channels with CaM. In this model, one apoCaM binds to both helix A and helix B of a single Kv7.2 or Kv7.3 in 1:1 stoichiometry. The apoCaM binding regulates the assembly of Kv7.2/Kv7.3 channels and facilitates the channel exit from the ER. One Ca^{2+}-CaM binds only to helix B of a single Kv7.2 or Kv7.3 in 1:1 stoichiometry. The Ca^{2+}-CaM is critical for Ca^{2+}-dependent current suppression of Kv7.2/Kv7.3 channels.

only interacts with a single helix B (Xu et al., 2013)(Fig. 2).

CaM regulates the activity of neuronal Kv7 channels

Out of five Kv7 subunits, coexpression of Kv7.2 and Kv7.3 has been shown to produce a large current with kinetic and pharmacological properties that closely resemble those of the neuronal M-current (Wang et al., 1998; Schwake et al., 2000) whereas expression of Kv7.2 or Kv7.3 alone yields a very small K^+ current (Schwake et al., 2000). A yeast two-hybrid screen has identified CaM as a binding partner of the M-channel subunits, Kv7.2 and Kv7.3 (Wen and Levitan, 2002; Yus-Najera et al., 2002). Mutant Kv7.2 subunits deficient in CaM binding lack current although they assemble efficiently with Kv7.3 subunits in heterologous cells (Wen and Levitan, 2002), suggesting that functional expression of Kv7.2/Kv7.3 channels requires constitutive interaction of CaM with the IQ motif of Kv7.2. Furthermore, the Kv7.2/Kv7.3 current density is reduced in heterologous cells by co-expression of a fusion protein containing the CaM binding motif of Kv7.2, which competes with the full-length Kv7.2 channels for CaM binding (Shahidullah et al., 2005). Importantly, expression of this fusion protein also decreases M-current density and increases the number of evoked APs in hippocampal neurons (Shahidullah et al., 2005), suggesting that dissociation of CaM from Kv7.2 reduces hippocampal M-current and leads to neuronal hyperexcitability.

On the other hand, CaM also contributes to Ca^{2+}-dependent modulation of M-channels. In the inside-out membrane patches excised from sympathetic neurons, the Ca^{2+} levels just above the normal resting concentration potently inhibit native M-channels (Selyanko and Brown, 1996). Furthermore, IP3 receptor-mediated rise in the Ca^{2+} level upon bradykinin or purinergic receptor activation leads to suppression of Kv7.2/Kv7.3 channel activity in heterologous cells and M-current in sympathetic neurons via Ca^{2+}/CaM since this effect is prevented by overexpression of a Ca^{2+}-insensitive mutant of CaM (CaM1234) (Gamper and Shapiro, 2003; Gamper et al., 2005; Zaika et al., 2007). These results collectively support the notion that CaM serves as the Ca^{2+} sensor to modulate neuronal Kv7 channel activity (Fig. 2).

The Ca^{2+}/CaM-dependent suppression of Kv7 current occurs in a subunit-specific manner. Overexpression of CaM but not CaM1234 robustly reduces currents of Kv7.2/Kv7.3 channels and homomeric Kv7.2, Kv7.4, and Kv7.5 channels, but not those of homomeric Kv7.1 and Kv7.3 channels in CHO cells (Gamper et al., 2005). Recently, downregulation of the Kv7.2 channels by Ca^{2+}/CaM is shown to involve the linker between helices A and B of Kv7.2 and a stronger intramolecular interaction between N- and C terminus of Kv7.2 (Gamper et al., 2005; Etzioni et al., 2011), although precisely how Ca^{2+} binding to CaM/Kv7.2 complex mediate current suppression through the linker and intra-molecular interaction remains unknown.

CaM regulates the assembly of neuronal Kv7 channels

Helices C and D in the C-termini have been identified to mediate channel tetramerization and have been termed the "subunit interaction domain" (Sid) (Schwake et al., 2006) (Fig. 1). Helix C is highly conserved within the Kv7 subfamily whereas helix D displays significant sequence differences among the Kv7 sub family. Disruption of the coil-coil structure of helix C fails to form homomeric or heteromeric Kv7 channels and leads to no detectable K^+ current (Schwake et al., 2006). Mutations in helix D impair the formation of heteromeric but not homomeric Kv7 channels, reducing K^+ current (Schwake et al., 2006). Based on the high-resolution crystal structure of the helix D of Kv7.4 and electrophysiological recordings, it has been suggested that the polar residues at the subunit interface of helix D likely mediates subunit-specific assembly of Kv7 channels but not macroscopic current amplitudes (Etxeberria et al., 2004; Howard et al., 2007a; Zaika et al., 2007; Choveau et al., 2012).

Recent studies have found that the channel assembly between Kv7.2 and Kv7.3 subunits is regulated by CaM binding to the IQ motif of Kv7.3 but not Kv7.2 (Liu and Devaux, 2013). The mutations in the IQ motif of Kv7.2 abolish CaM binding but do not affect Kv7.2 association with Kv7.3 (Wen and Levitan, 2002; Yus-Najera et al., 2002; Etxeberria et al., 2008; Alaimo et al., 2009; Gomez-Posada et al., 2011; Liu and Devaux, 2013), suggesting that CaM interaction with the IQ motif of Kv7.2 is not required for the formation of Kv7.2/Kv7.3 channels. Conversely, the I342A mutation in the IQ motif of Kv7.3 disrupts CaM interaction with Kv7.3 and markedly reduces its heteromeric association with Kv7.2 in HEK293 cells (Liu and Devaux, 2013). Interestingly, apoCaM restores the assembly of Kv7.3 with the mutant Kv7.2 harboring the L638P mutation (Liu and Devaux, 2013) that blocks formation of heteromeric channels (Schwake et al., 2006). Given that Kv7.3 is the most promiscuous subunit that can assemble with Kv7.2, Kv7.4, and Kv7.5 (Schwake et al., 2003; Howard et al., 2007b), these findings support the idea that apoCaM facilitates the formation of heteromeric channels by binding to the IQ domain of Kv7.3 (Fig. 2). Curiously, CaM overexpression increases the association of Kv7.2 and the mutant Kv7.3-I342A and rescues the defects caused by the mutant Kv7.3-I342A (Liu and Devaux, 2013). This rescue is shown to be dependent on CaM binding to their B-helices (Liu and Devaux, 2013), suggesting a possibility that Ca^{2+}-dependent cross-talk between helix B and the Sid domain may promote the assembly of Kv7.2 and Kv7.3.

CaM regulates the trafficking of neuronal Kv7 channels

Biochemical studies have shown that mutations in the IQ motif of Kv7.2 disrupt CaM binding but not its ability to assemble with Kv7.3 (Wen and Levitan, 2002; Yus-Najera et al., 2002; Etxeberria et al., 2008; Alaimo et al., 2009; Gomez-Posada et al., 2011; Liu and Devaux, 2013). While these mutant subunits display a moderate reduction in Kv7.2 surface expression in tsA201 cells (Wen and Levitan, 2002), other studies in *Xenopus* oocytes and HEK293T cells have reported that disruption of CaM binding to Kv7.2 abolishes surface expression of Kv7.2/Kv7.3 channels and increases the retention of Kv7.2 in the endoplasmic reticulum (ER) (Etxeberria et al., 2008; Alaimo et al., 2009). CaM overexpression greatly reduces the fraction of wild-type Kv7.2 channels retained in the ER but not that of I340E mutant Kv7.2 channels that cannot bind to CaM (Etxeberria et al., 2008). Conversely, the ER retention of wild-type Kv7.2 channels increases upon sequestering endogenous CaM or overexpressing CaM1234 that cannot bind to Ca^{2+} but still associates with Kv7.2. (Etxeberria et al., 2008). These findings collectively indicate that CaM binding to helix A of Kv7.2 plays a critical role in the channel's exit from the ER and their subsequent expression at the plasma membrane (Fig. 2).

Interestingly, the removal of the linker between helix A and helix B of Kv7.2 increases the surface expression of not only wild-type Kv7.2 channels but also the I340Q mutant channels deficient in CaM binding (Aivar et al., 2012; Alaimo et al., 2012), suggesting that this interlinker region may likely contain an ER retention signal. Interestingly, the S511D mutation located in helix B of Kv7.2 abolishes CaM binding but not the surface expression or current of Kv7.2 channels and Kv7.2/Kv7.3 channels in the oocyte system (Gomez-Posada et al., 2011). Thus, the role of CaM in regulating the surface expression of neuronal Kv7 channels remains controversial. Further studies are necessary to unravel complex mechanisms by which CaM binding to helices A and/or B of Kv7.2 regulates the trafficking of neuronal Kv7 channels.

CaM modulates channel interaction with other signaling proteins and molecules

The helices A and B bind to a number of signaling molecules other than calmodulin, including phosphatidylinositol 4,5-bisphosphate (PIP_2), syntaxin 1A, A-kinase-anchoring protein (AKAP) 79 and AKAP150, a murine ortholog of human AKAP79 (Fig. 1). Helix A and, to a lesser extent, helix B of Kv7.2 interact with syntaxin 1A, a plasma membrane protein component of the exocytotic SNARE complex (Regev et al., 2009). In *Xenopus* oocytes, expression of syntaxin 1A attenuates voltage-dependent activation of macroscopic current of Kv7.2 but not Kv7.3 channels without altering their surface expression (Regev et al., 2009) by promoting the intramolecular interactions between the N- and C-termini in Kv7.2 channels (Etzioni et al., 2011). This syntaxin-mediated effect is abolished by coexpression of CaM or CaM1234 (Etzioni et al., 2011). Given that syntaxin 1A colocalizes with Kv7.2-containing channels at presynaptic terminals (Regev et al., 2009), the interplay between CaM and syntaxin 1A may likely regulate neurotransmitter release through modulation of presynaptic M-current (Martire et al., 2004; Peretz et al., 2007).

Helices A and B of Kv7.2 are also the sites of AKAP79 and PIP_2 modulation of Kv7 channels (Suh and Hille, 2002; Hoshi et al., 2003; Delmas and Brown, 2005; Hoshi et al., 2005; Suh and Hille, 2007; Hernandez et al., 2008). Elevation of PIP_2 levels increases the maximal open probability of Kv7.2 channels and Kv7.2/Kv7.3 channels (Li et al., 2005; Gamper and Shapiro, 2007) and prevents their rundown upon patch excision (Suh and Hille, 2002; Ford et al., 2003; Zhang et al., 2003). Conversely, PIP_2 scavengers lead to suppression of Kv7.2/Kv7.3 current (Suh and Hille, 2002; Ford et al., 2003; Zhang et al., 2003). Importantly, muscarinic receptor stimulation suppresses M-current via PIP_2 depletion (Suh et al., 2004; Higashida et al., 2005; Winks et al., 2005). Recent studies have shown that protein kinase C bound to AKAP79/150 mediates muscarinic inhibition of Kv7 current (Hoshi et al., 2003; Delmas and Brown, 2005; Higashida et al., 2005) via phosphorylation of serine residues located in helix B (Kosenko et al., 2012). Such phosphorylation has been shown to dissociate CaM from Kv7.2 channels, leading to a reduced affinity to PIP_2 and suppression of M-current (Kosenko et al., 2012), suggesting that CaM interaction facilitates the ability of PIP_2 to stabilize the open state of Kv7.2 channels. Furthermore, Ca^{2+}/ CaM binding disrupts the interaction between AKAP79 and Kv7.2–5 subunits and prevents AKAP79-mediated sensitization of Kv7.2/Kv7.3 channels to muscarinic inhibition (Bal et al., 2010). Given that AKAP79 mediates PKC-dependent inhibition of M-current via PIP_2 (Higashida et al., 2005; Kosenko et al., 2012) and serves as an adaptor protein for CaM and PIP_2 (Wong and Scott, 2004), it remains to be determined how helices A and B mediate and integrate physical and functional interaction of CaM, AKAP79, and PIP_2 to modulate M-current.

Disruption of CaM binding contributes to Kv7-associated epilepsy

Epilepsy is a common neurological disorder that strikes about 1% of the world population (Kwan and Brodie, 2000). It is caused by excessive neuronal excitability characterized by seizures, which are abnormal and uncontrolled discharges of APs (Kwan and Brodie, 2000). Neuronal Kv7 channels have emerged as critical players of epilepsy for two main reasons. First, specific agonists for Kv7 channels (ezogabine/retiga-

bine) have been approved as anti-epileptic drugs (Gunthorpe et al., 2012; Large et al., 2012). Second, mutations in Kv7 channels are associated with epileptic encephalopathy (Borgatti et al., 2004; Schmitt et al., 2005; Saitsu et al., 2012; Weckhuysen et al., 2012) and BFNC (Soldovieri et al., 2011) which is variably associated with the later development of more severe epilepsy that is resistant to anticonvulsants (Alfonso et al., 1997; Dedek et al., 2003; Borgatti et al., 2004), Rolandic epilepsy (Coppola et al., 2003), developmental delay (Schmitt et al., 2005), mental retardation (Borgatti et al., 2004), peripheral nerve hyperexcitability (Wuttke et al., 2007), and myokymia (Dedek et al., 2001). Interestingly, some of these BFNC and encephalopathy mutations are localized to helices A and B of Kv7.2 that interact with CaM (Fig. 3).

To date, 8 BFNC missence mutations in KCNQ2 gene have been found to localize to helices A and B (Richards et al., 2004; Soldovieri et al., 2014). Five BFNC mutations in Kv7.2 and one BFNC mutation in Kv7.3 are localized to helix A. The L339R mutation located in the IQ motif of Kv7.2 (Richards et al., 2004) abolishes CaM binding to Kv7.2, leading to a>80% reduction in current and surface expression of Kv7.2/Kv7.3 channels (Alaimo et al., 2009). The W344R mutation in the IQ motif of Kv7.2 fails to generate measurable K^+ currents (Soldovieri et al., 2014). Located distal to the IQ motif of Kv7.2, the L351F but not L351V mutation causes a significant reduction in the current densities of homomeric channels and Kv7.2/Kv7.3 channels (Soldovieri et al., 2014). Another BFNC mutation located distal to the IQ motif (R353G) reduces not only CaM binding to Kv7.2 channels (Richards et al., 2004; Etxeberria et al., 2008; Alaimo et al., 2009) but also their affinity to PIP_2 (Kosenko et al., 2012), an essential cofactor for M-current function (Suh and Hille, 2002; Zhang et al., 2003; Winks et al., 2005). A combination of reduced binding to CaM and PIP_2 could explain the observed reduction in current through Kv7.2-R353G/Kv7.3 mutant channels in heterologous cells (Etxeberria et al., 2008). In contrast, BFNC mutations located immediately distal to helix A of KCNQ2 (Y362C) and of KCNQ3 (A381V) caused no significant reduction in Kv7.2/Kv7.3 current (Soldovieri et al., 2014). Importantly, the L339R and R353G mutant channels located at/near the IQ motif display enhanced retention in the ER of HEK293T cells (Etxeberria et al., 2008; Alaimo et al., 2009), suggesting that disruption of CaM binding to IQ motif and subsequent channel retention in the ER likely serves as one pathogenic mechanism for BFNC.

Three BFNC mutations (K526N, R525Q, R553Q) are located in helix B of Kv7.2 (Moulard et al., 2001; Borgatti et al., 2004; Soldovieri et al., 2014). The K526N and R525Q mutations are predicted to disrupt the electrostatic interaction of Kv7.2 with N-lobe of Ca^{2+}/CaM (Xu et al., 2013) or disrupt the α-helical structure of helix B (Borgatti et al., 2004). The K526N mutation in KCNQ2 gene was found in all affected members in a BFNC family, who exhibited BFNC alone or together with either a severe epileptic encephalopathy or focal seizures with mental retardation (Borgatti et al., 2004). Although functional studies on the R525Q mutation have not been performed, a single K526N mutant subunit was sufficient to reduce the voltage-dependence of opening heteromeric Kv7.2/Kv7.3 channels without affecting their plasma membrane expression in CHO cells (Borgatti et al., 2004). In hippocampal neurons, the K526N mutation of KCNQ2 moderately reduced the targeting of Kv7.2/Kv7.3 channels to the plasma membrane of distal axon but not AIS (Chung et al., 2006). The R553Q mutation is shown to reduce the current of both homomeric Kv7.2 and heteromeric Kv7.2/

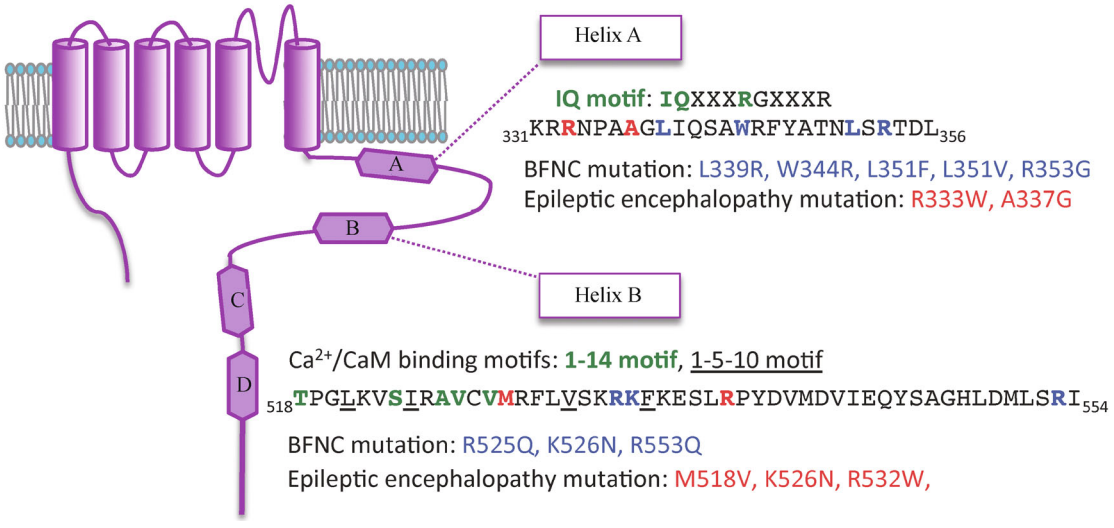

Figure 3 Location of the mutations associated with BFNC and epileptic encephalopathy in the helices A and B of Kv7.2. In this schematic representation of a single Kv7.2 subunit (accession number: Y15065), the CaM binding motifs in helices A and B are indicated. The amino acid residues mutated in BFNC and epileptic encephalopathy are indicated in red and blue, respectively.

Kv7.3 channels and abolish the ability of syntaxin 1A to inhibit Kv7.2 channels in CHO cells without affecting their interaction with syntaxin 1A (Soldovieri et al., 2014). Since the syntaxin-mediated Kv7.2 inhibition is abolished by an increase in CaM binding (Etzioni et al., 2011), it is tempting to speculate that the R553Q mutation may increase CaM binding to Kv7.2, similar to another BFNC-causing L619R mutation (Richards et al., 2004). Although further work should be done to directly test the effect of these mutations in CaM binding, these findings suggest that reduced Kv7.2 or Kv7.2/Kv7.3 current associated with the mutations in helix B may likely decrease the ability of the M-current to effectively dampen epileptiform discharges, leading to neuronal hyperexcitability in BFNC.

So far, 4 epileptic encephalopathy mutations have been identified in helices A and B of Kv7.2. Two *de novo* mutations (A337G and R333W) lie proximal to the IQ motif in helix A of Kv7.2 (Schmitt et al., 2005; Saitsu et al., 2012). The A337G mutation is associated with Ohtahara syndrome (Saitsu et al., 2012) while the R333W mutation was found in the boy who suffered from drug resistant epilepsy, Rolandic epilepsy, and psychomotor retardation (Schmitt et al., 2005). These mutations have not been analyzed for their effects on CaM binding and Kv7 channel function. Based on the functional studies of BFNC mutations localized to the IQ motif of Kv7.2 (Etxeberria et al., 2008; Alaimo et al., 2009), one could speculate that these mutations may likely disrupt CaM binding and cause the channel retention in the ER, ultimately leading to a severe reduction in the channel surface and current expression. Two de novo mutations (M518W and R532W) associated with epileptic encephalopathy lie in helix B of Kv7.2 (Weckhuysen et al., 2012). Electrophysiological recording in *Xenopus* oocytes has revealed that the M518W and R532W mutations markedly reduces current amplitude of Kv7.2 channels, but had no effect on heteromeric Kv7.2/Kv7.3 channels (Orhan et al., 2013), although the effect of these mutations on CaM binding to Kv7.2 remains unknown. Interestingly, only the R532W mutation caused a reduction in surface expression, a large depolarizing shift in the voltage dependence of channel activation, and a significant slowing of activation and acceleration of deactivation kinetics (Orhan et al., 2013).

Conclusion and future direction

In summary, CaM binding to neuronal Kv7 channels has two major functions. First, CaM binding to helix A of Kv7.2 and Kv7.3 plays a critical role in the assembly of Kv7.2/Kv7.3 channels and their ability to exit the ER and express at the plasma membrane. Second, CaM bound to helix B acts as a Ca^{2+} sensor to modulate biophysical properties of homomeric Kv7.2 channels and heteromeric Kv7.2/Kv7.3 channels. CaM binding to Kv7.2 is critical for hippocampal M-current and dampening neuronal excitability whereas Ca^{2+}-CaM binding mediates Ca^{2+}-dependent suppression of M-current. The studies in heterologous systems have provided evidence that epilepsy-associated mutations in helices A and B of Kv7.2 disrupt CaM binding and/or reduces function and trafficking of Kv7.2 channels and Kv7.2/Kv7.3 channels.

However, there are still many questions that need to be answered: What molecular mechanisms underly the CaM-dependent ER exit of the Kv7.2/Kv7.3 channels? How does CaM binding to helices A and B mediate both channel assembly and intramolecular interaction between N- and C-termini? In what neuronal environment are Kv7 channels mostly bound to apoCaM or Ca^{2+}/CaM or both? What conformational changes of intact Kv7 channels are induced by binding of apoCaM or Ca^{2+}/CaM? And when and how do apoCaM and Ca^{2+}/CaM functionally interact with AKAP79/150, syntaxin 1A, PIP_2 and CaM through helices A and B? Further studies to tackle these questions should advance our understanding of the roles that CaM play in mediating the physiological functions of Kv7 channels.

We look forward to the future studies that investigate on the effect of epilepsy-associated mutations on CaM binding and their functional impact on M-current and neuronal excitability. Such continuous efforts should facilitate the development of novel therapeutic interventions to restore channel interaction with CaM or correct channel mislocalization and dysfunction. These novel therapeutic interventions may provide more effective remediation or slowing of cognitive decline associated with the severe symptomatic epileptic encephalopathy, when combined with early screening for KCNQ2 mutation and treatment with ezogabine/retigabine. Considering that Kv7 channels are implicated in brain development (Peters et al., 2005), learning and memory (Peters et al., 2005) and other pathological conditions such as chronic inflammatory and neuropathic pain (Blackburn-Munro and Jensen, 2003; Passmore et al., 2003), anxiety (Korsgaard et al., 2005), mania (Dencker et al., 2008), and addiction (Hansen et al., 2007), these novel therapeutic interventions may also result in more efficacious therapy for a wide range of neurological diseases and disorders.

Abbreviations

voltage-dependent potassium channels (Kv), potassium (K^+), calcium (Ca^{2+}), action potential (AP), axonal initial segments (AIS), benign familial neonatal convulsion (BFNC), calmodulin (CaM), protein kinase C (PKC), A-kinase-anchoring protein (AKAP), PIP_2 (phosphatidylinositol-(4,5)-bisphosphate), endoplasmic reticulum (ER).

Acknowledgements

I thank John Cavaretta for his helpful comments on this manuscript.

Compliance with ethics guidelines

Hee Jung Chung declares that she has no conflict of interest. This review article does not contain any studies with human or animal subjects performed by the author.

References

Aivar P, Fernández-Orth J, Gomis-Perez C, Alberdi A, Alaimo A, Rodríguez M S, Giraldez T, Miranda P, Areso P, Villarroel A (2012). Surface expression and subunit specific control of steady protein levels by the Kv7.2 helix A-B linker. PLoS One, 7(10): e47263

Alaimo A, Alberdi A, Gomis-Perez C, Fernandez-Orth J, Gomez-Posada J C, Areso P, Villarroel A (2012). Cooperativity between calmodulin binding sites in Kv7.2 channels. J Cell Sci

Alaimo A, Gómez-Posada J C, Aivar P, Etxeberría A, Rodriguez-Alfaro J A, Areso P, Villarroel A (2009). Calmodulin activation limits the rate of KCNQ2 K^+ channel exit from the endoplasmic reticulum. J Biol Chem, 284(31): 20668–20675

Alfonso I, Hahn J S, Papazian O, Martinez Y L, Reyes M A, Aicardi J (1997). Bilateral tonic-clonic epileptic seizures in non-benign familial neonatal convulsions. Pediatr Neurol, 16(3): 249–251

Bal M, Zhang J, Hernandez CC, Zaika O, Shapiro MS (2010) Ca^{2+}/calmodulin disrupts AKAP79/150 interactions with KCNQ (M-Type) K^+ channels. The Journal of neuroscience: the official journal of the Society for Neuroscience30: 2311–2323.

Blackburn-Munro G, Jensen B S (2003). The anticonvulsant retigabine attenuates nociceptive behaviours in rat models of persistent and neuropathic pain. Eur J Pharmacol, 460(2–3): 109–116

Borgatti R, Zucca C, Cavallini A, Ferrario M, Panzeri C, Castaldo P, Soldovieri M V, Baschirotto C, Bresolin N, Dalla Bernardina B, Taglialatela M, Bassi M T (2004). A novel mutation in KCNQ2 associated with BFNC, drug resistant epilepsy, and mental retardation. Neurology, 63(1): 57–65

Brown D A, Passmore G M (2009). Neural KCNQ (Kv7) channels. Br J Pharmacol, 156(8): 1185–1195

Choveau F S, Bierbower S M, Shapiro M S (2012). Pore helix-S6 interactions are critical in governing current amplitudes of KCNQ3 K+ channels. Biophys J, 102(11): 2499–2509

Chung H J, Jan Y N, Jan L Y (2006). Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-terminal domains. Proc Natl Acad Sci U S A, 103(23): 8870–8875

Clark B D, Goldberg E M, Rudy B (2009). Electrogenic tuning of the axon initial segment. Neuroscientist, 15(6): 651–668

Cooper E C, Harrington E, Jan Y N, Jan L Y (2001) M channel KCNQ2 subunits are localized to key sites for control of neuronal network oscillations and synchronization in mouse brain. J Neurosci, 21: 9529–9540

Coppola G, Castaldo P, Miraglia del Giudice E, Bellini G, Galasso F, Soldovieri M V, Anzalone L, Sferro C, Annunziato L, Pascotto A, Taglialatela M (2003). A novel KCNQ2 K^+ channel mutation in benign neonatal convulsions and centrotemporal spikes. Neurology, 61(1): 131–134

Dahimène S, Alcoléa S, Naud P, Jourdon P, Escande D, Brasseur R, Thomas A, Baró I, Mérot J (2006). The N-terminal juxtamembranous domain of KCNQ1 is critical for channel surface expression: implications in the Romano-Ward LQT1 syndrome. Circ Res, 99 (10): 1076–1083

Dailey J W, Cheong J H, Ko K H, Adams-Curtis L E, Jobe P C (1995). Anticonvulsant properties of D-20443 in genetically epilepsy-prone rats: prediction of clinical response. Neurosci Lett, 195(2): 77–80

Dedek K, Fusco L, Teloy N, Steinlein O K (2003). Neonatal convulsions and epileptic encephalopathy in an Italian family with a missense mutation in the fifth transmembrane region of KCNQ2. Epilepsy Res, 54(1): 21–27

Dedek K, Kunath B, Kananura C, Reuner U, Jentsch T J, Steinlein O K (2001). Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K^+ channel. Proc Natl Acad Sci U S A, 98(21): 12272–12277

Delmas P, Brown D A (2005). Pathways modulating neural KCNQ/M (Kv7) potassium channels. Nat Rev Neurosci, 6(11): 850–862

Dencker D, Dias R, Pedersen M L, Husum H (2008). Effect of the new antiepileptic drug retigabine in a rodent model of mania. Epilepsy Behav, 12(1): 49–53

Devaux J J, Kleopa K A, Cooper E C, Scherer S S (2004). KCNQ2 is a nodal K^+ channel. J Neurosci, 24: 1236–1244

Etxeberria A, Aivar P, Rodriguez-Alfaro J A, AlaimoA, Villace P, Gomez-Posada J C, Areso P, Villarroel A (2008). Calmodulin regulates the trafficking of KCNQ2 potassium channels. FASEB J, 22: 1135–1143

Etxeberria A, Santana-Castro I, Regalado MP, Aivar P, Villarroel A (2004). Three mechanisms underlie KCNQ2/3 heteromeric potassium M-channel potentiation. J Neurosci, 24: 9146–9152

Etzioni A, Siloni S, Chikvashvilli D, Strulovich R, Sachyani D, Regev N, Greitzer-Antes D, Hirsch J A, Lotan I (2011). Regulation of neuronal M-channel gating in an isoform-specific manner: functional interplay between calmodulin and syntaxin 1A. J Neurosci, 31: 14158–14171

Ford C P, Stemkowski PL, Light P E, Smith P A (2003). Experiments to test the role of phosphatidylinositol 4,5-bisphosphate in neurotransmitter-induced M-channel closure in bullfrog sympathetic neurons. J Neurosci, 234931–4941

Gamper N, Li Y, Shapiro M S (2005). Structural requirements for differential sensitivity of KCNQ K^+ channels to modulation by Ca^{2+}/calmodulin. Mol Biol Cell, 16(8): 3538–3551

Gamper N, Shapiro M S (2003). Calmodulin mediates Ca^{2+}-dependent modulation of M-type K^+ channels. J Gen Physiol, 122(1): 17–31

Gamper N, Shapiro M S (2007). Regulation of ion transport proteins by membrane phosphoinositides. Nat Rev Neurosci, 8(12): 921–934

Gómez-Posada J C, Aivar P, Alberdi A, Alaimo A, Etxeberría A, Fernández-Orth J, Zamalloa T, Roura-Ferrer M, Villace P, Areso P, Casis O, Villarroel A (2011). Kv7 channels can function without constitutive calmodulin tethering. PLoS One, 6(9): e25508

Gu N, Vervaeke K, Hu H, Storm J F (2005). Kv7/KCNQ/M and HCN/h, but not KCa2/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells. J Physiol, 566(Pt 3): 689–715

Gunthorpe M J, Large C H, Sankar R (2012). The mechanism of action of retigabine (ezogabine), a first-in-class K^+ channel opener for the treatment of epilepsy. Epilepsia, 53(3): 412–424

Hadley J K, Passmore G M, Tatulian L, Al-Qatari M, Ye F, Wickenden A D, Brown D A (2003) Stoichiometry of expressed KCNQ2/KCNQ3

potassium channels and subunit composition of native ganglionic M channels deduced from block by tetraethylammonium. J Neurosci, 23: 5012–5019

Haitin Y, Attali B (2008). The C-terminus of Kv7 channels: a multifunctional module. J Physiol, 586(7): 1803–1810

Hansen H H, Andreasen J T, Weikop P, Mirza N, Scheel-Krüger J, Mikkelsen J D (2007). The neuronal KCNQ channel opener retigabine inhibits locomotor activity and reduces forebrain excitatory responses to the psychostimulants cocaine, methylphenidate and phencyclidine. Eur J Pharmacol, 570(1–3): 77–88

Hernandez C C, Zaika O, Shapiro M S (2008). A carboxy-terminal inter-helix linker as the site of phosphatidylinositol 4,5-bisphosphate action on Kv7 (M-type) K^+ channels. J Gen Physiol, 132(3): 361–381

Higashida H, Hoshi N, Zhang J S, Yokoyama S, Hashii M, Jin D, Noda M, Robbins J (2005). Protein kinase C bound with A-kinase anchoring protein is involved in muscarinic receptor-activated modulation of M-type KCNQ potassium channels. Neurosci Res, 51(3): 231–234

Hoeflich K P, Ikura M (2002). Calmodulin in action: diversity in target recognition and activation mechanisms. Cell, 108(6): 739–742

Hoshi N, Langeberg L K, Scott J D (2005). Distinct enzyme combinations in AKAP signalling complexes permit functional diversity. Nat Cell Biol, 7(11): 1066–1073

Hoshi N, Zhang J S, Omaki M, Takeuchi T, Yokoyama S, Wanaverbecq N, Langeberg L K, Yoneda Y, Scott J D, Brown D A, Higashida H (2003). AKAP150 signaling complex promotes suppression of the M-current by muscarinic agonists. Nat Neurosci, 6(6): 564–571

Howard A L, Neu A, Morgan R J, Echegoyen J C, Soltesz I (2007a). Opposing modifications in intrinsic currents and synaptic inputs in post-traumatic mossy cells: evidence for single-cell homeostasis in a hyperexcitable network. J Neurophysiol, 97(3): 2394–2409

Howard R J, Clark K A, Holton J M, Minor D L Jr (2007b). Structural insight into KCNQ (Kv7) channel assembly and channelopathy. Neuron, 53(5): 663–675

Korsgaard M P, Hartz B P, Brown W D, Ahring P K, Strøbaek D, Mirza N R (2005). Anxiolytic effects of Maxipost (BMS-204352) and retigabine via activation of neuronal Kv7 channels. J Pharmacol Exp Ther, 314(1): 282–292

Kosenko A, Kang S, Smith I M, Greene D L, Langeberg L K, Scott J D, Hoshi N (2012). Coordinated signal integration at the M-type potassium channel upon muscarinic stimulation. EMBO J, 31(14): 3147–3156

Kwan P, Brodie M J (2000). Epilepsy after the first drug fails: substitution or add-on? Seizure, 9(7): 464–468

Lai H C, Jan L Y (2006). The distribution and targeting of neuronal voltage-gated ion channels. Nat Rev Neurosci, 7(7): 548–562

Large C H, Sokal D M, Nehlig A, Gunthorpe M J, Sankar R, Crean C S, Vanlandingham K E, White H S (2012). The spectrum of anticonvulsant efficacy of retigabine (ezogabine) in animal models: implications for clinical use. Epilepsia, 53(3): 425–436

Lerche H, Biervert C, Alekov A K, Schleithoff L, Lindner M, Klinger W, Bretschneider F, Mitrovic N, Jurkat-Rott K, Bode H, Lehmann-Horn F, Steinlein O K (1999). A reduced K^+ current due to a novel mutation in KCNQ2 causes neonatal convulsions. Ann Neurol, 46 (3): 305–312

Li Y, Gamper N, Hilgemann DW, Shapiro MS (2005). Regulation of Kv7 (KCNQ) K^+ channel open probability by phosphatidylinositol 4,5-bisphosphate. J Neurosci, 25: 9825–9835

Liu W, Devaux J J (2014). Calmodulin orchestrates the heteromeric assembly and the trafficking of KCNQ2/3 (Kv7.2/3) channels in neurons. Mol Cell Neurosci, 58: 40–52

Maljevic S, Wuttke T V, Lerche H (2008). Nervous system KV7 disorders: breakdown of a subthreshold brake. J Physiol, 586(7): 1791–1801

Martire M, Castaldo P, D'Amico M, Preziosi P, Annunziato L, Taglialatela M (2004). M channels containing KCNQ2 subunits modulate norepinephrine, aspartate, and GABA release from hippocampal nerve terminals. J Neurosci, 24: 592–597

Moulard B, Picard F, le Hellard S, Agulhon C, Weiland S, Favre I, Bertrand S, Malafosse A, Bertrand D (2001). Ion channel variation causes epilepsies. Brain Res Brain Res Rev, 36(2–3): 275–284

Ohtahara S, Yamatogi Y (2006). Ohtahara syndrome: with special reference to its developmental aspects for differentiating from early myoclonic encephalopathy. Epilepsy Res, 70(Suppl 1): S58–S67

Orhan G, Bock M, Schepers D, Ilina E I, Reichel S N, Loffler H, Jezutkovic N, Weckhuysen S, Mandelstam S, Suls A, Danker T, Guenther E, Scheffer I E, Jonghe P D, Lerche H, Maljevic S (2013). Dominant-negative Effects of KCNQ2 mutations are associated with epileptic encephalopathy. Ann Neurol, 75(3): 382–394

Pan Z, Kao T, Horvath Z, Lemos J, Sul J Y, Cranstoun S D, Bennett V, Scherer S S, Cooper E C (2006). A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. J Neurosci, 26: 2599–2613

Passmore G M, Selyanko A A, Mistry M, Al-Qatari M, Marsh S J, Matthews E A, Dickenson AH, Brown T A, Burbidge S A, Main M, Brown D A (2003). KCNQ/M currents in sensory neurons: significance for pain therapy. J Neurosci, 23: 7227–7236

Peretz A, Sheinin A, Yue C, Degani-Katzav N, Gibor G, Nachman R, Gopin A, Tam E, Shabat D, Yaari Y, Attali B (2007). Pre- and postsynaptic activation of M-channels by a novel opener dampens neuronal firing and transmitter release. J Neurophysiol, 97(1): 283–295

Peters H C, Hu H, Pongs O, Storm J F, Isbrandt D (2005). Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. Nat Neurosci, 8(1): 51–60

Psenka T M, Holden K R (1996). Benign familial neonatal convulsions; psychosocial adjustment to the threat of recurrent seizures. Seizure, 5 (3): 243–245

Rasmussen H B, Frøkjaer-Jensen C, Jensen C S, Jensen H S, Jørgensen N K, Misonou H, Trimmer J S, Olesen S P, Schmitt N (2007). Requirement of subunit co-assembly and ankyrin-G for M-channel localization at the axon initial segment. J Cell Sci, 120(Pt 6): 953–963

Regev N, Degani-Katzav N, Korngreen A, Etzioni A, Siloni S, Alaimo A, Chikvashvili D, Villarroel A, Attali B, Lotan I (2009). Selective interaction of syntaxin 1A with KCNQ2: possible implications for specific modulation of presynaptic activity. PLoS One, 4(8): e6586

Richards M C, Heron S E, Spendlove H E, Scheffer I E, Grinton B, Berkovic S F, Mulley J C, Davy A (2004). Novel mutations in the KCNQ2 gene link epilepsy to a dysfunction of the KCNQ2-calmodulin interaction. J Med Genet, 41(3): e35

Robbins J (2001). KCNQ potassium channels: physiology, pathophy-

siology, and pharmacology. Pharmacol Ther, 90(1): 1–19

Roche J P, Westenbroek R, Sorom A J, Hille B, Mackie K, Shapiro M S (2002). Antibodies and a cysteine-modifying reagent show correspondence of M current in neurons to KCNQ2 and KCNQ3 K^+ channels. Br J Pharmacol, 137(8): 1173–1186

Rostock A, Tober C, Rundfeldt C, Bartsch R, Engel J, Polymeropoulos E E, Kutscher B, Löscher W, Hönack D, White H S, Wolf H H (1996). D-23129: a new anticonvulsant with a broad spectrum activity in animal models of epileptic seizures. Epilepsy Res, 23(3): 211–223

Saitsu H, Kato M, Koide A, Goto T, Fujita T, Nishiyama K, Tsurusaki Y, Doi H, Miyake N, Hayasaka K, Matsumoto N (2012). Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome. Ann Neurol, 72(2): 298–300

Schmitt B, Wohlrab G, Sander T, Steinlein O K, Hajnal B L (2005). Neonatal seizures with tonic clonic sequences and poor developmental outcome. Epilepsy Res, 65(3): 161–168

Schroeder B C, Kubisch C, Stein V, Jentsch T J (1998). Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K+ channels causes epilepsy. Nature, 396(6712): 687–690

Schwake M, Athanasiadu D, Beimgraben C, Blanz J, Beck C, Jentsch TJ, Saftig P, Friedrich T (2006). Structural determinants of M-type KCNQ (Kv7) K^+ channel assembly. J Neurosci, 26: 3757–3766

Schwake M, Jentsch T J, Friedrich T (2003). A carboxy-terminal domain determines the subunit specificity of KCNQ K+ channel assembly. EMBO Rep, 4(1): 76–81

Schwake M, Pusch M, Kharkovets T, Jentsch T J (2000). Surface expression and single channel properties of KCNQ2/KCNQ3, M-type K^+ channels involved in epilepsy. J Biol Chem, 275(18): 13343–13348

Schwarz J R, Glassmeier G, Cooper E C, Kao T C, Nodera H, Tabuena D, Kaji R, Bostock H (2006). KCNQ channels mediate IKs, a slow K^+ current regulating excitability in the rat node of Ranvier. J Physiol, 573(Pt 1): 17–34

Selyanko A A, Brown D A (1996). Intracellular calcium directly inhibits potassium M channels in excised membrane patches from rat sympathetic neurons. Neuron, 16(1): 151–162

Shah M M, Migliore M, Brown D A (2011). Differential effects of Kv7 (M-) channels on synaptic integration in distinct subcellular compartments of rat hippocampal pyramidal neurons. J Physiol, 589(Pt 24): 6029–6038

Shah M M, Migliore M, Valencia I, Cooper E C, Brown D A (2008). Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. Proc Natl Acad Sci U S A, 105(22): 7869–7874

Shah M, Mistry M, Marsh S J, Brown D A, Delmas P (2002). Molecular correlates of the M-current in cultured rat hippocampal neurons. J Physiol, 544(Pt 1): 29–37

Shahidullah M, Santarelli L C, Wen H, Levitan I B (2005). Expression of a calmodulin-binding KCNQ2 potassium channel fragment modulates neuronal M-current and membrane excitability. Proc Natl Acad Sci U S A, 102(45): 16454–16459

Singh NA, Westenskow P, Charlier C, Pappas C, Leslie J, Dillon J, Anderson VE, Sanguinetti MC, Leppert MF (2003) KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. Brain, 126: 2726–2737

Soldovieri M V, Boutry-Kryza N, Milh M, Doummar D, Heron B, Bourel E, Ambrosino P, Miceli F, De Maria M, Dorison N, Auvin S, Echenne B, Oertel J, Riquet A, Lambert L, Gerard M, Roubergue A, Calender A, Mignot C, Taglialatela M, Lesca G (2014). Novel KCNQ2 and KCNQ3 mutations in a large cohort of families with benign neonatal epilepsy: first evidence for an altered channel regulation by syntaxin-1A. Hum Mutat, 35(3): 356–367

Soldovieri M V, Castaldo P, Iodice L, Miceli F, Barrese V, Bellini G, Miraglia del Giudice E, Pascotto A, Bonatti S, Annunziato L, Taglialatela M (2006). Decreased subunit stability as a novel mechanism for potassium current impairment by a KCNQ2 C terminus mutation causing benign familial neonatal convulsions. J Biol Chem, 281(1): 418–428

Soldovieri M V, Miceli F, Taglialatela M (2011). Driving with no brakes: molecular pathophysiology of Kv7 potassium channels. Physiology (Bethesda), 26(5): 365–376

Song A H, Wang D, Chen G, Li Y, Luo J, Duan S, Poo M M (2009). A selective filter for cytoplasmic transport at the axon initial segment. Cell, 136(6): 1148–1160

Suh B C, Hille B (2002). Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. Neuron, 35(3): 507–520

Suh B C, Hille B (2007). Regulation of KCNQ channels by manipulation of phosphoinositides. J Physiol, 582(Pt 3): 911–916

Suh B C, Horowitz L F, Hirdes W, Mackie K, Hille B (2004). Regulation of KCNQ2/KCNQ3 current by G protein cycling: the kinetics of receptor-mediated signaling by Gq. J Gen Physiol, 123(6): 663–683

Surti T S, Jan L Y (2005). A potassium channel, the M-channel, as a therapeutic target. Curr Opin Investig Drugs, 6(7): 704–711

Tober C, Rostock A, Rundfeldt C, Bartsch R (1996). D-23129: a potent anticonvulsant in the amygdala kindling model of complex partial seizures. Eur J Pharmacol, 303(3): 163–169

Tzingounis A V, Heidenreich M, Kharkovets T, Spitzmaul G, Jensen H S, Nicoll R A, Jentsch T J (2010). The KCNQ5 potassium channel mediates a component of the afterhyperpolarization current in mouse hippocampus. Proc Natl Acad Sci U S A, 107(22): 10232–10237

Tzingounis A V, Nicoll R A (2008). Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents. Proc Natl Acad Sci U S A, 105(50): 19974–19979

Wang H S, Pan Z, Shi W, Brown B S, Wymore R S, Cohen I S, Dixon J E, McKinnon D (1998). KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science, 282(5395): 1890–1893

Watanabe H, Nagata E, Kosakai A, Nakamura M, Yokoyama M, Tanaka K, Sasai H (2000). Disruption of the epilepsy KCNQ2 gene results in neural hyperexcitability. J Neurochem, 75(1): 28–33

Weckhuysen S, Mandelstam S, Suls A, Audenaert D, Deconinck T, Claes L R, Deprez L, Smets K, Hristova D, Yordanova I, Jordanova A, Ceulemans B, Jansen A, Hasaerts D, Roelens F, Lagae L, Yendle S, Stanley T, Heron S E, Mulley J C, Berkovic S F, Scheffer I E, de Jonghe P (2012). KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. Ann Neurol, 71(1): 15–25

Wen H, Levitan IB (2002) Calmodulin is an auxiliary subunit of KCNQ2/3 potassium channels. J Neurosci, 22: 7991–8001

Winks JS, Hughes S, Filippov A K, TatulianL, Abogadie F C, Brown D A, Marsh S J (2005). Relationship between membrane phosphatidylinositol-4,5-bisphosphate and receptor-mediated inhibition of native

neuronal M channels. J Neurosci, 25: 3400–3413

Wong W, Scott J D (2004). AKAP signalling complexes: focal points in space and time. Nat Rev Mol Cell Biol, 5(12): 959–970

Wuttke T V, Jurkat-Rott K, Paulus W, Garncarek M, Lehmann-Horn F, Lerche H (2007). Peripheral nerve hyperexcitability due to dominant-negative KCNQ2 mutations. Neurology, 69(22): 2045–2053

Xu Q, Chang A, Tolia A, Minor D L Jr (2013). Structure of a Ca(2+)/CaM:Kv7.4 (KCNQ4) B-helix complex provides insight into M current modulation. J Mol Biol, 425(2): 378–394

Yue C, Yaari Y (2004) KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. J Neurosci, 24: 4614–4624

Yue C, Yaari Y (2006) Axo-somatic and apical dendritic Kv7/M channels differentially regulate the intrinsic excitability of adult rat CA1 pyramidal cells. J Neurophysiol, 95(6): 3480–3495

Yus-Najera E, Santana-Castro I, Villarroel A (2002). The identification and characterization of a noncontinuous calmodulin-binding site in noninactivating voltage-dependent KCNQ potassium channels. J Biol Chem, 277(32): 28545–28553

Zaika O, Tolstykh G P, Jaffe D B, Shapiro M S (2007) Inositol triphosphate-mediated Ca^{2+} signals direct purinergic P2Y receptor regulation of neuronal ion channels. J Neurosci, 27: 8914–8926

Zhang H, Craciun L C, Mirshahi T, Rohács T, Lopes C M, Jin T, Logothetis D E (2003). PIP(2) activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. Neuron, 37(6): 963–975