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Modulation of Kv7 channels and excitability in the brain

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Running title: Kv7 channel modulation and neuromodulation

Non-standard abbreviations: PKC, protein kinase C; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; AKAP, A-kinase anchoring protein; PLC, phospholipase C; DAG, diacylglycerol; IP3, inositol triphosphate; PIP2, phosphatidylinositol 4,5- bis phosphate; CaM, calmodulin; RMP, resting membrane potential; AIS, axon initial segment; GSK3 β , glycogen synthase kinase 3 β ; CRMP-2, collapsin response mediator protein 2; Nav, voltage-gated sodium channel; AHP, afterhyperpolarization; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor ; EPSPs, excitatory post-synaptic potentials; ROS, reactive oxygen species; BFNC, benign familial neonatal convulsions.

Abstract

Neuronal Kv7 channels underlie a voltage-gated non-inactivating potassium current known as the M-current. Due to its particular characteristics, Kv7 channels show pronounced control over the excitability of neurons. We will discuss various factors that have been shown to drastically alter the activity of this channel such as protein and phospholipid interactions, phosphorylation, calcium, and numerous neurotransmitters. Kv7 channels locate to key areas for the control of action potential initiation and propagation. Moreover, we will explore the dynamic surface expression of the channel modulated by neurotransmitters and neural activity. We will also focus on known principle functions of neural Kv7 channels: control of resting membrane potential and spiking threshold, setting the firing frequency, afterhyperpolarization after burst firing, theta resonance, and transient hyperexcitability from neurotransmitter-induced suppression of the M-current. Finally, we will discuss the contribution of altered Kv7 activity to pathologies such as epilepsy and cognitive deficits.

Introduction

Potassium channels, being the most diverse of all ion channels, underlie a robust number of functions controlling the excitability of neurons. They are responsible for such aspects as setting the resting membrane potential, reducing excitability as well as controlling the duration, shape and firing frequency of action potentials. Potassium channels are localized to all subcellular compartments critical for electrical conduction of excitatory inputs. Therefore, it is not surprising that many of these channels are gated by specific activators (such as membrane potential or neurotransmitters) and regulated by complex molecular pathways.

Kv7 channels produce an outward potassium current, distinct from other voltage-gated potassium channels [1,2], that lacks inactivation, with slow gating kinetics (an order of magnitude slower than other voltage-gated potassium channels at room temperature as well as physiological temperatures [3,4]) and are activated near resting membrane potential [5,6]. Neuronal Kv7 subunits are widely expressed in the central as well as peripheral nervous system [7,8], where its steady outwardly rectifying current functions as “brakes” for neurons receiving persistent excitatory input. A somewhat unique characteristic of the M-current is derived from the circumstances by which it was discovered. Namely, upon activation of muscarinic acetylcholine receptors (M1 and M3), both of which being Gq-coupled receptors, there is subsequent robust suppression of the M-current, leading to a transient increase in excitability. Suppression of the M-current has been shown to lower the action potential threshold, increase afterdepolarization and depolarize axonal resting potential [2,9]. Over the past several years it has been revealed that activation of numerous Gq/11-coupled receptors suppress the channel [10-12].

Since the initial discovery of the M-current in bullfrog sympathetic ganglion neurons there have been numerous breakthroughs identifying neuronal types expressing the M-current as well as mediators of the channel [9,13]. However, it took nearly two decades to determine the molecular

identity [7]. The lapse in time that was required to elucidate Kv7 as the underlying subunits of the M-current was largely due to the lack of available tools able to link cloned channels to native M-current [14]. On the heels of this discovery numerous facets involved in the modulation of the Kv7 channel complex have also been uncovered. However, though the M-current has consistently been discussed as playing a pivotal role in neuroplasticity, due to its prominent expression throughout the brain and its pronounced suppression via neurotransmitters, only recently have we begun to understand how dynamic Kv7 channel function contributes to higher brain functions.

Components of the Kv7 channel complex

The M-current is conducted through a voltage-gated potassium channel comprised of tetrameric assemblies by members of Kv7 α subunit homomers or heteromers derived from KCNQ1-5 genes. Kv7.1 is the predominant subunit of the Kv7 family that is found in cardiomyocytes [15,16] (Fig 1A). Kv7.1 channel generates the slowly activating delayed rectifier current (I_{Ks}) that contributes to the repolarization phase after action potential initiation. Kv7.1 channel is also expressed in the cochlea of the inner ear [17], and vascular smooth muscles [18].

A wide variety of neurons in the CNS as well as peripheral neurons express Kv7.2, 7.3, and 7.5 subunits, which are considered to generate the neuronal M-current [1,2]. It is widely believed that heteromeric Kv7.2/3 or Kv7.3/5 channels are the dominant subunit composition (Fig 1B) [1,2] since Kv7.3 has been shown to facilitate surface expression of other Kv7 subunits especially in *Xenopus* oocytes [19] and immunohistochemical studies show co-localization of these subunits in many areas [8,20]. However, a recent study using conditional knock-out mice of KCNQ2 or 3 genes showed abolished M-current in Kv7.2 deficient neurons, but normal M-current in Kv7.3 deficient neurons, which suggests that Kv7.2 homomeric channels can be fully functional without Kv7.3 subunit in the CNS neurons [21].

Kv7.4 is a subtype selectively expressed in the auditory pathway including hair cells of the inner ear (Fig 1A) [22-24]. In addition to neurons, Kv7.4 and Kv7.5 are also expressed in various smooth muscle cells including vascular [18,25] as well as gastrointestinal tract (Fig 1A) [26,27].

With the advent of discovering the molecular identity of the M-current many aspects of the Kv7 channel complex have been brought to light (Fig. 1C-E): protein-protein and protein-lipid interactions, as well as channel phosphorylation [7,8,28]. Understanding the components of the Kv7 channel complex has provided the necessary insight for understanding the requirements for basal channel function, its modulation, as well as the pathways involved in neurotransmitter-induced M-current suppression.

Phosphatidylinositol-4,5-bisphosphate (PIP₂) is an anionic phospholipid found on the intracellular leaflet of the plasma membrane. PIP₂ is a co-factor for numerous ion channels and transporters [29], including Kv7, that alters channel function and often is obligatory for

activation [30-33]. While it has been known that PIP₂ interaction with Kv7 is mandatory for channel function [34,35], more recently it was shown that PIP₂ has multiple sites of interaction within the channel, with varying effects [36]. One such PIP₂ interaction modulates coupling the voltage-sensing to the pore-gating domain [37-40]. Functionally, PIP₂ interaction within the voltage-sensing domain of the S4-S5 linker has been attributed to increasing open probability thus voltage-conductance [40]. Another key interaction site lies within the proximal C-terminus of the Kv7.2 subunit that overlaps with the calmodulin (CaM) binding site, a key target for M-current suppression [39,41,42]. Notably, while depletion of PIP₂ in Kv7.2 and Kv7.5 homomeric channels leads to profound current suppression, heteromeric assemblies incorporating the Kv7.3 subunit, with its higher affinity to PIP₂, offers resistance to PIP₂ turnover [40].

Both N and C termini of Kv7 subunits locate within the intracellular side and are rich with sites of protein interaction. Within the amino terminus lies a consensus site for phosphorylation by protein kinase A, which in *Xenopus* oocytes has been shown to increase Kv7 currents [19]. Protein kinase CK2 and protein phosphatase 1 (PP1) also tether to Kv7 subunits (Fig. 1C), and modulate the channel through the phosphorylation state of an essential auxiliary unit CaM [43]. The carboxyl terminus is relatively long compared to other potassium channels, containing numerous sites of interaction [42]. Among these are the aforementioned sites for PIP₂, CaM, Ankyrin anchoring protein 5, AKAP5 (also known as AKAP79/150), as well as ankyrin G (Fig. 1D). Interaction of the channel with many of these factors is highly modulated by neurotransmitters activating Gq/11-coupled receptors [44,45].

AKAPs are facilitators of second messenger signaling events that tether signaling enzymes to target substrates at the plasma membrane, such as Kv7 channels (Fig.1C) [28,46,47]. This anchoring protein is located to the plasma membrane through PIP₂ binding and a dynamic post-translational fatty acid modification known as palmitoylation [48]. AKAP5-bound Protein kinase C (PKC) is kept in proximity to neuronal Kv7 channels, which has been shown to be important for the specificity of PKC-induced dissociation of CaM from Kv7.2 subunits [8,28,46].

CaM plays roles in the trafficking of Kv7 channels to the plasma membrane [49,50], functions as the Ca²⁺ sensor, and promotes channel interaction with PIP₂ (Fig 1C) [44,51]. CaM interacts with Kv7 subunits through two α helical regions (A and B) running antiparallel in the C-terminal tail. There has been controversy regarding whether calcium facilitates or reduces CaM binding to Kv7 [52,53]. A recent crystal structure study using Kv7.1 suggests that the story is not that simple [54]. The study shows that the calcium-free C-lobe of CaM interacts with helix A of Kv7 and the calcium-containing N-lobe of CaM interacts with helix B of Kv7 [54]. However, Kv7.2 has been shown to have higher affinity for CaM at helix B, regardless of [Ca²⁺] [55], whereas the C-lobe of CaM has higher affinity to [Ca²⁺] and is dynamically bound to helix A in a calcium-dependent manner [56].

Studies during last several years identified an increasing number of accessory proteins for the Kv7.2 subunit (Fig. 1D). One of the earliest examples is ankyrin G, which is an underlying molecule for anchoring Kv7.2 at the axon initial segment as well as the Node of Ranvier [57,58]. The SNARE protein syntaxin 1A is a plasma membrane protein, which serves as a docking site for synaptic vesicles. Syntaxin 1A has been demonstrated to interact with both the cytoplasmic carboxyl and amino termini of Kv7.2 subunit, which slows channel activation and decreases current amplitude (Fig. 1D) [59,60]. Nav β 1 was originally considered as a beta subunit unique to voltage-gated sodium channels (Nav) [61]. More recently the promiscuity of this auxiliary protein was uncovered, first in its modulation of Kv4 [62], and more recently Kv1 and Kv7 subfamilies [63] (Fig. 1D). Nav β 1 slows Kv7.2 channel activation at depolarized potentials [63]. An interesting addition to accessory proteins of the Kv7.2 subunit is β -site amyloid precursor protein cleaving enzyme 1 (BACE1), which was originally identified to produce neurotoxic β -amyloid [64] and known to cleave Nav β s and increases sodium currents [65]. Physical interaction of BACE1, even with enzymatic inactive BACE1, can change gating kinetics and functional expression of Kv7 channels with the exception of Kv7.3 homomeric channels [66]. However, effects through cleavage of Kv7.2-bound Nav β has not been characterized to date. Channel trafficking of Kv7 is another mechanism for regulating neuronal excitability (Fig. 1E). It has been noted that the majority of Kv7 channel subunits stay in cytoplasmic vesicles rather than being transported to the plasma membrane [49]. Detailed analyses of CaM-dependent regulation of Kv7 channel trafficking revealed that CaM regulates exit of Kv7 channels from ER [49,50]. In addition, our recent proteomic study identified tubulin dimer together with Collapsin response mediator protein-2 (CRMP-2) play a role in channel trafficking of Kv7.2 channels at post-Golgi vesicles (Fig. 1E) [67].

Integrated channel suppression

Various neurotransmitters activating Gq-protein coupled receptors induce profound transient suppression of the M-current [68-70]. It has been demonstrated that stimulation by luteinizing hormone releasing hormone [9], purinergic P2Y [13], substance P [71], 5-HT₂ serotonin [72], as well as activation of M1/M3 muscarinic receptors [5,73,74], metabotropic glutamate receptors [75], κ and δ opioid receptors [76], and AT1 angiotensin receptors [77,78] all suppress the M-current.

Gq/11 mediated pathway

Perhaps most well characterized is M-current suppression subsequent to activation of Gq/11-coupled muscarinic acetylcholine receptors [79,80]. It is well documented that reduction in PIP₂ leads to marked suppression of the M-current (Fig. 2A) [81-83]. Also, PKC was one of the first modulators proposed for neurotransmitter-induced M-current suppression [84]. This was for a time debated due to confounding and at times contradicting pharmacological data such as PKC inhibitors at times showing no interference with muscarinic suppression of the M-current

[12,85]. These reports were reconciled once it was determined that AKAP-tethered PKC is protected from certain PKC inhibitors [86,87]. Indeed, a key PKC phosphorylation site on the Kv7.2 subunit has been shown to induce inhibition equivalent to muscarinic suppression of the channel [28,44,88]. Therefore, both depletion of PIP₂ and activation of PKC are targeted downstream of Gq-coupled receptor activation.

The Gq-protein mediated pathway occurs as follows: subsequent to activation of Gq/11-coupled receptors, such as the M1 muscarinic receptor, comes activation of phospholipase C (PLC) causing hydrolysis of PIP₂ into diacylglycerol (DAG) and inositol triphosphate (IP3), leading to the activation of PKC. Activated PKC, tethered to the M-channel complex through AKAP5, phosphorylates the C-terminus of the Kv7.2 subunit, which overlaps with the channel's CaM binding site. Phosphorylation of the Kv7.2 subunit dissociates CaM from the channel, which destabilizes its interaction with PIP₂ along with the concomitant reduction in PIP₂ due to its hydrolysis. Together, these signaling pathways downstream of Gq/11 activation lead to an amplified response that synergistically suppresses the M-channel [44].

Calcium-CaM pathway

Another well established pathway of M-current suppression is induced by increases in intracellular calcium sensed by channel-bound CaM (Fig. 2B) [44,89,90]. Bradykinin receptors have been demonstrated to use this pathway [90].

The physical interaction of calcium-free CaM bound to helix A of Kv7 subunits is integral to maintaining channel affinity to PIP₂ [44]. Consequently, calcium-bound CaM decreases Kv7.2 channel efficacy for PIP₂, thereby inhibiting the M-current [44,90,91]. On the other hand, a recent study showed that splicing variants of Kv7.4 are differentially modulated by CaM [92]. This mechanism may explain why bradykinin induced suppression of the M-current is usually smaller than that by muscarinic agonists.

Kv7 channel physiology

The voltage dependent characteristics of the M-current lend it to various roles associated with controlling excitability in the brain. Kv7 channels activate within subthreshold potentials, approximately at -60 mV. Additionally, unlike many other voltage-gated potassium channels, Kv7 does not inactivate, therefore, as long as the membrane remains depolarized Kv7 current will persist [7]. The outcome is the effective stabilization of the membrane potential throughout the duration that neurons receive subthreshold excitatory inputs [93]. Relatively slow activation kinetics indicate that Kv7 channels do not appreciably alter single action potential amplitude or duration [94]; rather, the functional consequence of M-current is to clamp the membrane at more negative potentials, preventing repetitive action potential firing. Moreover, the persistent outward current functions to control numerous aspects of neuronal excitability as follows:

Setting membrane potential

Due to its activation at subthreshold potentials, Kv7 channels have been implicated in controlling the resting membrane potential (RMP) at the axon initial segment (AIS) and unmyelinated portions of the axons, the nodes of Ranvier as well as axon terminals. Examples have been shown in sympathetic, cortical and hippocampal neurons such as visceral sensory neurons and within the calyx of Held [95-97]. M-current control of RMP is also a major determinant for increasing the recovery of inactivated channels (i.e. axonal Na⁺ and A-type K⁺ channels) at the nodes of Ranvier, allowing for consecutive action potential spiking [4,97,98]. Therefore, axonal Kv7 channels at nodes of Ranvier exert two contradicting functions: increasing the action potential threshold but also increasing excitability by promoting recovery of sodium channels from inactivation [4,20]. In addition, Kv7 channels are known to reach axon terminal and regulate synaptic release through regulating RMP [97,99]. Confusing is that inhibition of Kv7 channel sometimes facilitate synaptic transmission such as in calyx of Held [97] and hippocampal synaptosomes [99], while suppressing synaptic transmission in Schaffer neurons [100]. These contradicting effects are assumed to be results of difference in inactivation of calcium channels and sodium channels [99,100]. On the other hand, post-synaptic responses can be also affected by perisomatic Kv7 channels by changing integration of excitatory postsynaptic potentials at the AIS, dampening synaptic transmission of prolonged subthreshold stimulus [101,102].

Afterhyperpolarization

As a consequence of continuous spiking, numerous types of neurons produce three types of an afterhyperpolarization (AHP) that contribute to refractory periods: fast AHP lasting 2-5 ms, medium AHP with durations ranging from 50-100 ms (mAHP), and a slow AHP lasting 0.1-2 s (sAHP).

Medium AHP is produced through slowly activating and long-lasting outward K⁺ current that is composed in part by a component that requires Ca²⁺ influx [103,104] and has been shown to control the time where neurons are refractory to further excitatory input. Channels contributing to the generation of mAHP are Kv7 channels as well as SK2 calcium-activated potassium channels and HCN channels [80,103,105]. However, it has been reported that distinct channels are responsible for mAHP depending on membrane potentials and neuronal types [104,106].

Slow AHP is involved in neuronal plasticity and is implicated as a major component during learning and establishing memory within the hippocampus [34,38]. The identity of the potassium channel responsible for the slow AHP had proven elusive until recently. While Kv7 has been suggested as a contributor of sAHP, as afferent cholinergic stimulation reduces effective sAHP amplitude [107], one confounding factor discredited its involvement: the sAHP is active at membrane potentials more negative than M-current activity is typically observed. Activation of the sAHP is known to have a Ca²⁺ dependent component, and neuronal calcium sensor proteins such as hippocalcin have been implicated in the activation of potassium channels that constitute

the sAHP [108,109]. A physiological consequence of hippocalcin activation is the downstream production of PIP₂. In a recent report evidence was given for shifting Kv7 open probability to more negative potentials through increased interaction with PIP₂ [40,110]. Further support comes from the observation that BACE1 knockout mice have reduced sAHP. As BACE1 has been shown to cause a leftward shift in Kv7 voltage activation, within the voltage range where sAHP is active [66].

Interspike interval

High frequency bursts of action potentials leads to Kv7 channel activation. Consequently, this gradually increases spike interval, which is also known as spike frequency adaptation [80,111]. Recently M-current was also shown to regulate the firing frequency of tonically firing neurons in rat entorhinal cortex layer II stellate cells [112], as well as neurons of the retrotrapezoid nucleus [113], which, during continuous current input, initially fire with short interspike intervals that quickly lead to refractory periods with minimal firing [112]. Furthermore, Kv7 current has also been shown to control the rate of firing in dopaminergic neurons of the ventral tegmental area [94] and hippocampal neurons [114]. Thus, the M-current robustly controls the frequency at which neurons are able to fire while receiving continuous excitatory input [115].

Theta-resonance

Another physiological function of Kv7 channels, in conjunction with HCN channels, is facilitating the responsiveness to oscillating subthreshold membrane potential within theta frequencies (2-7 Hz), which functions as a band pass filter [116]. This function has predominantly been characterized in pyramidal neurons in the hippocampus [116,117]. Interestingly, two distinct channel types with different ion species, gating kinetics, and subcellular localization (somatic Kv7 channels and dendritic HCN channels) produce a synergized function [116,117]. Theta resonance has been considered integral to inducing synchronous activity within the local circuit and shown to be a necessary component of neuroplasticity as well as learning and memory [118-120]. Network oscillations at the theta frequency have been shown to be important for hippocampal function, such as exploration [121] and working memory such as navigation of mazes [122,123]. As such, disruption of M-channel activity, such as conditional knockout of Kv7.2 subunits, reduces hippocampal theta resonance and consequently impairs animal performance in spatial memory tasks [124].

Transient neuronal hyperexcitability

Suppression of Kv7 current leads to the transient removal of above functions, leading to neural hyperexcitability (Fig. 3). Consequently, channel suppression allows for the accumulation of excitatory inputs that lead to burst firing or “complex-spikes” [125]. In addition, suppression of Kv7 channel leads to concomitant reduction in action potential threshold such as what is seen in dentate granule cells after stimulation by cholinergic fibers [126]. Thus, reduction in action potential threshold by Kv7 channel suppression makes action potential propagation more

permissive, where weaker stimuli can invoke neuronal spiking with higher probability, and increases the likelihood of spontaneous firing, which underlies burst firing in some neurons [4]. Alternatively, ablation of Kv7 current by conditional knock-out produces a similar effect [21]. Furthermore, since the M-current is activated during high frequency firing, M-current suppression allows neurons to respond to high frequency inputs. Moreover, This bursting has been shown to be one component of memory coding after learning in subcortical regions [14,127]. Suppression of axonal M-current also increases the likelihood of the back-propagation of action potential spikes into the apical dendrites, which has been demonstrated to promote long-term potentiation [101,128].

Kv7 channel augmentation

Just as there are multiple mediators of M-current suppression, so to for M-channel preservation as well as enhancers of M-current activity. Early examples include neurotransmitters such as somatostatin [129], corticostatin [130] and dynorphin [131]. Since phosphorylation of certain residues of Kv7 channels are known to cause robust suppression it follows that some protein phosphatases preserve neuronal Kv7 channel activity. One example is Protein Phosphatase 2A, (PP2A), which was demonstrated to counteract channel inhibition via phosphorylation by Glycogen Synthase Kinase 3 β , (GSK3 β) [132,133]. While phosphorylation of Kv7 subunits is typically associated with channel suppression, phosphorylation of Kv7-bound CaM by protein kinase CK2 has been shown to facilitate CaM interaction with the channel, increasing PIP₂ efficacy and increasing channel amplitude while remaining sensitive to increases in intracellular Ca²⁺ [43,134]. Another signaling pathway known to augment the M-current (specifically, channels containing Kv7.2/4/5 subunits) is through the increase in an intracellular reactive oxygen species, ROS [135,136]. It has been shown that the oxidation of three cysteine residues lying within the S2-S3 linker increase the P_o of Kv7 channels [135]. Another recently identified pathway for ROS induced augmentation is methylation of arginine residues of Kv7.2 subunit by arginine methyltransferase 1 (Prmt1), which promotes Kv7.2 interaction with PIP₂ [136]. This study also suggests that partial methylation of arginine residues in Kv7 channels is essential for maintaining basal M-current [136].

Auxiliary units of potassium channels such as members of the KCNE family are common co-factors that associate with pore-forming subunits and alter channel activity, such as voltage dependence of activation. Recently β -secretase BACE1 was revealed to associate with members of the Kv7 family in neurons (Fig. 1D). Interestingly, it is the physical interaction and not the enzymatic activity of BACE1 protein that is responsible for the leftward shift in the voltage conductance of the M-current. This leftward shift was accompanied by a change in Kv7 channel kinetics, accelerating activation as well as slowing channel deactivation [66]. While PIP₂ is necessary for M-current function, it has also been revealed that increasing PIP₂ levels caused Kv7 channels to open at deeper potentials, similar to the effect seen with BACE1 [40,110].

Interestingly, the combination of BACE1 interaction with the channel along with elevating PIP₂ levels causes even greater leftward shift in channel activation [40]. These revelations bring credence to the growing consensus that Kv7 channels are a major part of the burst firing-activated outward current underlying sAHP, which is known to be active at membrane potentials lower than where the M-current has been thought to be active.

Another association with Kv7.4 subunits has recently been uncovered. Kv7 channels in vascular smooth muscle are positively regulated by G-protein $\beta\gamma$ subunits, enhancing the rate of Kv7 channel activation and shifting the voltage dependence of activation so that the channel is open at deeper potentials [137]. While it is important to note that this phenomenon has yet to be confirmed in CNS neurons, G-protein $\beta\gamma$ subunits may prove to be an underlying factor responsible for enhancement of Kv7 activity by as seen with G-protein coupled receptor activation by somatostatin [129] and dynorphins [131].

Subcellular localization

A key characteristic determining the roles of neuronal Kv7 channels lies in their subcellular localization. Kv7 channels are highly concentrated at the distal end of the AIS and nodes of Ranvier by the protein ankyrin-G. The AIS functions as the gatekeeper of action potential initiation by controlling the threshold at which excitatory presynaptic potentials transmit action potentials down the axon. Ankyrin G also functions to localize Kv7.2 and to a lesser extent Kv7.3 channels at unmyelinated regions of the axon, known as Nodes of Ranvier [57,58,138]. At these nodes, Kv7 channels (Kv7.2/7.3) co-cluster with voltage gated sodium channels and are thought to underlie the slow outward potassium current, stabilizing RMP [4,98]. In agreement with its axonal localization, selective suppression of axonal Kv7 channel increases spike afterdepolarization, facilitating action potential firing [125]. Interestingly, in a study where myelination of the axon was removed, Kv7 channels dispersed along the axon, which had the net effect of increasing neural excitability [139]. This was postulated to be through stabilizing the RMP throughout the axon, thereby increasing the availability of Nav channels as previously described [4,20].

Kv7 channels have also been reported to be expressed in the perisomatic region outside the AIS [57,140,141]. Somatic function of the M-current is implicated in excitatory synaptic potential integration as well as counteracting afterdepolarization [57,142,143]. Perisomatic Kv7 channel has been shown to change the amplitude and shape of somatic EPSPs [102]. There is also accumulating evidence that Kv7 channels may be expressed at apical dendrites [140,142,144]. Due to their relative scarcity at this location, the function of Kv7 channels within dendrites is still controversial, though it has been proposed to contribute to conditions where there is dendritic hyperexcitability, as in the case of persistent excitatory presynaptic input [142,144], however, further clarification of Kv7 channel function outside the axon is still needed.

Finally, Kv7.2 has been shown to localize to presynaptic terminals [99]. Augmentation of Kv7 channels at this location attenuates neurotransmitter release [99] whereas suppression of Kv7 current by Gq activation increases activation of voltage-gated calcium channels leading to increased transmission [145].

Surface expression

Under basal conditions, Kv7 channels have very low turnover rate throughout the axon [146]. However, surface expression of Kv7 channels is upregulated after neuronal stimulation [147]. Paradoxically, persistent excitation of neurons leads to pronounced internalization of Kv7 channel at the AIS, which is largely irreversible [148]. Li and colleagues demonstrated that persistent activation through glutamate induced a Ca^{2+} and PKC-dependent internalization of Kv7 channel. Similar irreversible suppression of Kv7 channel was observed with cholinergic stimulation onto dentate granule cells, causing an increase in their intrinsic excitability [126]. It was revealed that cholinergic stimulation onto the AIS of dentate granule neurons leads to persistent activation of T-type Ca^{2+} channels, causing robust M-current suppression that does not recover after washout. Conversely, deprivation of afferent axonal stimulus, as shown in neurons of the avian cochlear nucleus leads to pronounced increase in surface expression of Kv7.2 channels and concomitant reduction of Kv1 channels at the AIS [149]. This reduces the fast activating Kv1 currents, thereby increasing excitability, while preserving axonal RMP by Kv7 channels.

CaM tethered to Kv7 subunits is important for of Kv7 channel exit from the endoplasmic reticulum and is necessary for basal surface expression of the channel at the plasma membrane [49,55]. Alternatively, a recent study uncovered a form of induced surface translocation subsequent to muscarinic suppression of the channel tested in a heterologous system in which Kv7 channels in post-Golgi vesicles were determined to be the predominant source of this form of over-recovery of the M-current, mediated through a CRMP-2 pathway [67] (Fig. 1E).

Pathology

The genes encoding Kv7.2 and Kv7.3 have been shown to have numerous mutations that induce epilepsies and encephalopathies. Most common of these pathologies is known as benign familial neonatal seizures (BFNS), a form of autosomal dominant idiopathic epileptic syndrome. The generalized seizures induced by these mutations occur within the first days after birth and have a high likelihood of spontaneously receding within the first two months [150-153]. Mutations leading to epileptic activity are predominantly autosomal dominant, inducing loss of channel function such as from missense, truncation, and non-native splice variants that reduce functional Kv7 channel at the plasma membrane [154,155]. Many of these mutations lead to reduction in

basal M-current and subsequent hyperexcitability [156]. This is also true in the cases of early onset epileptic encephalopathy, though usually with more severe channel suppression [152]. As of yet, encephalopathies have only been detected from missense mutations in KCNQ genes and tend to occur *de novo* [152,157,158]. One example is through an alternative substitution of a previously described select amino acid mutation, which is known to cause BFNS, instead leading to a severe form of encephalopathy. It was determined that the functional difference in this severe form of single amino acid mutation is derived from not only a reduction of functional expression of Kv7 channels but also loss of localization of Kv7 channels to the AIS [152]. As a consequence of the growing literature on pathologies associated with Kv7 mutations there has been an effort to develop genetically engineered animals to recapitulate and understand the pathologies of the numerous implicated mutations and better understand underlying causes [159,160] as well as the effectiveness of potential therapies [161].

M-current activity is an important obstacle for seizure onset [162,163]. However, gain of function mutations in Kv7 channels have also been uncovered in patients suffering from epileptic encephalopathies [164-166]. One such mutation was found within the voltage-sensing domain of Kv7.2 and Kv7.3 subunits, stabilizing the channel in the open conformation, reducing the voltage dependence of activation [165]. Interestingly, while these mutations decrease the intrinsic excitability in neurons where they are expressed, there is a marked increase in network excitability within the CA1 of the hippocampus. This suggests the grave importance in Kv7 channel control of action potential propagation, particularly at its axonal localization.

Conclusion

The specific characteristics of Kv7 channels lend it to function as powerful brakes against continuous neural activity. As an extreme example, retigabine-induced augmentation of the M-current has seen great success as an antiepileptic therapy [167], and more recently it was determined that one of the predominant antiepileptic drugs, valproic acid, owes part of its protective activity to the preservation of the M-current during seizures [168]. In contrast, removal of Kv7.2 increases susceptibility to seizures and leads to deficits in hippocampal-dependent spatial memory [124] as well as fear memory in mice [169]. However, continuous ablation of hyperactivity in neurons expressing Kv7 channels may disrupt cognitive functions such as memory encoding, indicating the importance of transient channel suppression. Neurotransmitters are intricately involved in higher brain functions and acetylcholine-induced suppression of the M-current was among the first examples of modulating neuronal excitability [79]. It would follow, therefore, that suppressing the activity of Kv7 channels such as what is seen by afferent cholinergic stimulation [126] would provide a means to allow plasticity in neural excitability with great spatial and temporal resolution. Even so, work is ongoing to determine the intricacies of M-current suppression in higher brain functions.

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Figure legends

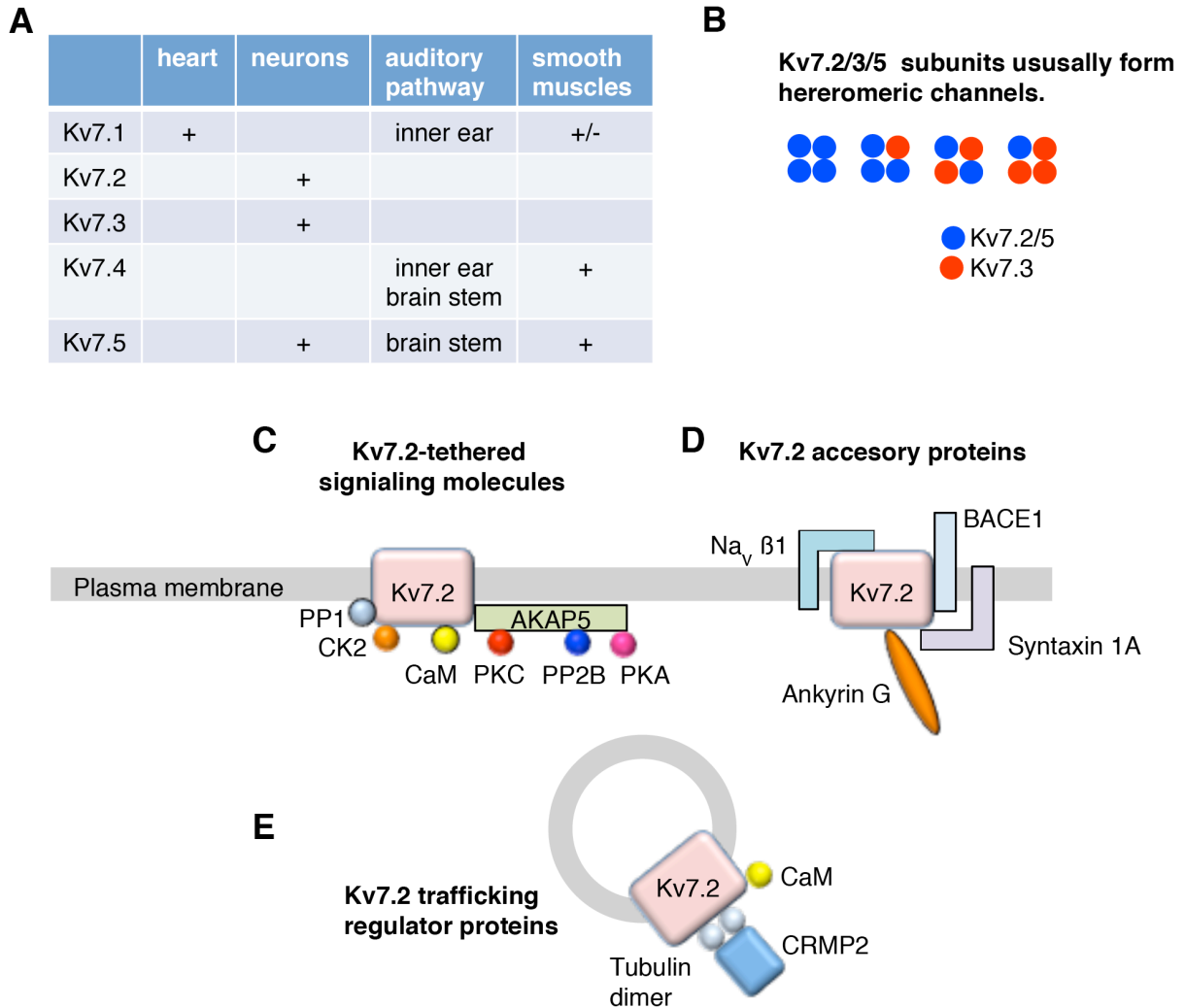


Figure 1. Summary of Kv7 channel family and their binding proteins. A) Subtypes of Kv7 channel family and their expression pattern. B) Summary of subunit composition with Kv7.2, 7.3 and 7.5 subunits, which is widely observed in neurons. C) Schematic summary of signaling proteins that are tethered to Kv7.2 subunit. PP1, protein phosphatase 1. CK2, CK2 protein kinase. CaM, calmodulin. PKC, protein kinase C. PP2B, protein phosphatase 2B. PKA, protein kinase A. D) Schematic summary of accessory proteins for Kv7.2 subunit. Nav β 1, sodium channel β subunit 1. BACE1, β -site amyloid precursor protein cleaving enzyme 1. E) Schematic summary of identified regulator proteins for Kv7.2 channel trafficking. CRMP2, collapsin response mediator protein 2.

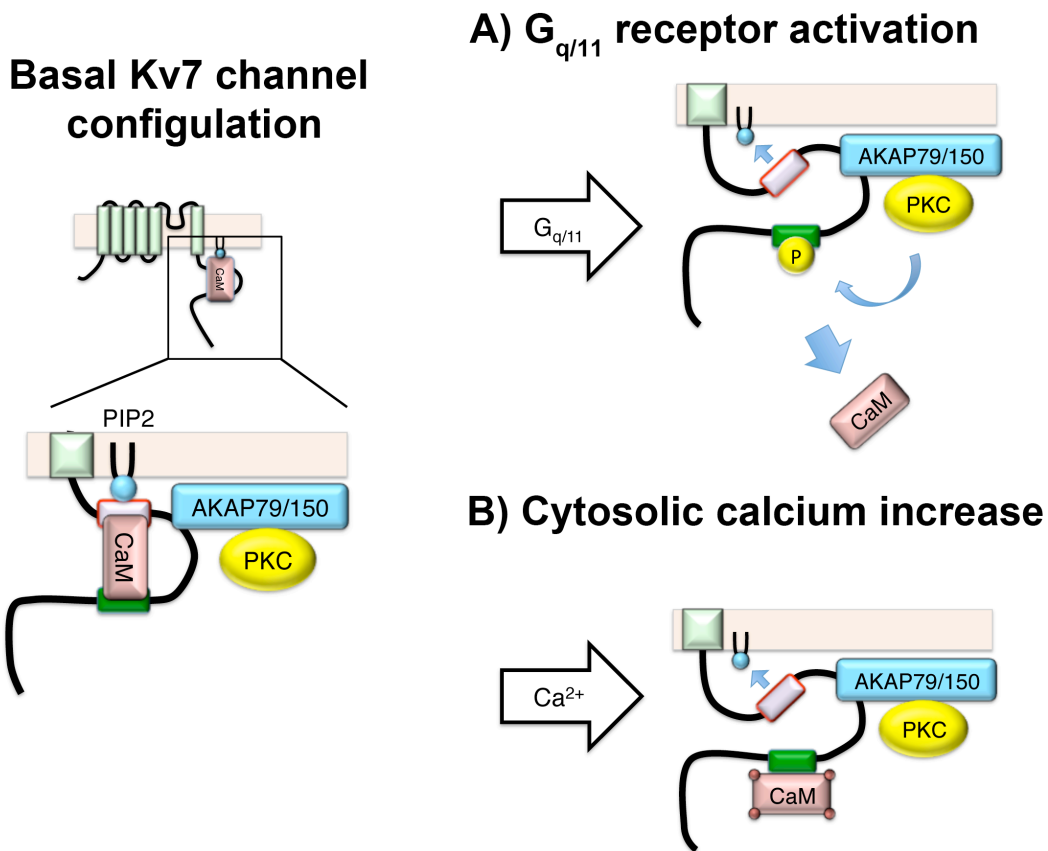


Figure 2. Schematic summary of molecular configuration of Kv7 channel and its modulation modified from ref 29. A) Activation of $G_{q/11}$ coupled receptor induces depletion of PIP2 as well as activation of PKC, which phosphorylates Kv7 channel leading to dissociation of CaM and unstabilized PIP2 interaction. B) Increase in cytosolic calcium induces change in CaM conformation, and configuration between CaM and Kv7 channel, which leads to unstabilized PIP2 interaction.

Physiological Relevance of neuronal Kv7 channels

- 1) Resting membrane potential
- 2) Afterhyperpolarization
- 3) Spike frequency adaptation
- 4) Theta-resonance



Kv7 current suppression

Neuronal hyperexcitability

Figure 3. Summary of physiological relevance of neuronal Kv7 channel and its modulation.