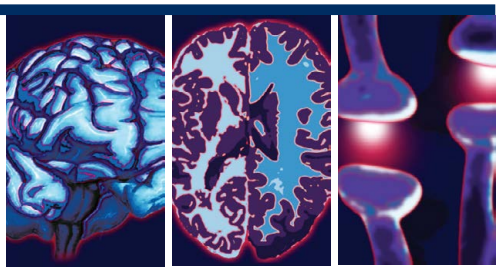
Review



Current insight into the role of voltage-gated potassiumion channel 7 (Kv7) channels: an emerging therapy target against epilepsy

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ABSTRACT

Kv channels, the voltage-gated potassium channels, are encoded by KCN gene family. Kv channels are important in maintaining the resting membrane potential and spiking threshold, deciding the firing frequency and nerve hyperexcitability, and inducing after hyperpolarization after burst firing. In this family, Kv7 is a special one for it is activated at sub-threshold potentials. Because of its specific characteristics, Kv7 channels show pronounced control over the excitability of neurons and often inhibit neuronal excitability. Moreover, it is also documented that mutations of Kv7 channels are associated with the occurrence of epilepsy, deafness and cardiac arrhythmia. In this review, we discussed the characteristics and functions of Kv channels especially the Kv7 channels, which determined their targeting role in treatment against epilepsy.

Keywords

Neuron; Voltage-gated potassium ion channel (Kv channel); Epilepsy

Introduction

Bioelectricity, the electrical activity existing in all the organs of higher animals is essential for mammals' physiological processes such as nerve excitability transmission. And all the electrical activities are caused by electrical signals generated through ion channels. Ions cross the channels distributed on the membranes to conduct electrical signals selectively and rapidly, which can modulate physiological and pathological activities of cells. Among all these ion channels, potassium channels possess the most extensive distribution. Littleton et al. reported that ion channels, especially potassium channels were the largest ion channel family in invertebrates and involved in governing neural functions such as synaptic organization and neuronal signaling [1].

The ion concentrations in internal environment homeostasis are very important for maintaining the function of the brain. The maintaining of the ion homeostasis depends on ATP consumption. In mammals, although the brain is a little part of the body, it will need 20% of basal O_2 consumption. A neuron will maintain high intracellular K^+ concentration, low Na^+ concentration, and very low or even"free"Ca^{2+} concentration via making use of ATP produced from O_2 by mitochondria. It is addressed Kv channels contribute to this environment homeostasis of the brain, and the dysfunction of Kv channels is detrimental for the brain, so it is of great importance to explore the characteristics, and the functions of Kv [2].

Neuronal K^+ channels can modulate membrane excitability, for example setting the resting potential, and holding short and timing

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interspike intervals in action potentials [3]. There are four kinds of K^+ channels: inwardly rectifying potassium channel (K_{IR}), calcium-activated potassium channel (K_{Ca}), voltage-gated potassium channel (K_V) and tandem pore domain potassium channel (K_{2p}). Increased activity of plasma membrane voltage-gated potassium (K_V) channels will reduce K^+ concentration in cytoplasm and will often trigger cell death process [4]. Pannaccione et al. reported that various K^+ channel blockers or the increase in extracellular K^+ concentration can inhibit K^+ outflow, and this can totally prevent cell death. It was indicated that K^+ currents were involved in neuronal apotheosis [3]. Moreover, if the hyperexcitation of K^+ channels occurs, epilepsy will follow behind it [5].

As for the Kv channels exhibit their importance in neuronal and synaptic activities, the dysfunction of them will result in many kinds of nervous diseases. Kv2 channels are linked with Alzheimer's disease (AD) [6], Kv4 channels have a close relationship with Parkinson's disease (PD) [7], and Kv7 channels are associated with neuronal hyperexcitability [8]. As neuronal hyperexcitability is considered the major cause for epilepsy [9], we lay emphasis on the role of Kv7 in epilepsy and will talk about its vital effect in epilepsy in this review.

Distribution and function of Voltage-gated K^+ Channels

Up to now, about 70 K^+ channel genes have been reported and the voltage-gated potassium channel forms the largest family in all the K^+ channels. Voltage-gated potassium channels family was formed by 12 subfamilies on the basis of sequence homology of the genes and their ability to form heteromultimeric channels such as KCNA, KCNB, KCNC, etc [1]. All Kv channels have important regulatory effects on diverse kinds of cellular processes, for example, neural KCNQ channels considered as therapy targets for epilepsy can be inhibited by stimulation of muscarinic acetycholine receptors (mAChR), so that improve the synaptic plasticity and cognitive functions [10].

Kv1-12 families can form a phylogenetic tree, including the genes that encode the twelve Kv channel proteins and their locations on the chromosomes. The amino acid sequence alignments used CLUSTALW and analysis by maximum parsimony using PAUP*[11].

The twelve subfamilies of Kv channels take effect differently in the regulation pathway. Each KCN gene encodes a peptide subunit and four subunit can assemble to a functional channel. Most of the Kv channels contribute to excitability of excitable cells [12]. The Kv1 encoded by KCNA has about 8 types including Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv1.6, Kv1.7 and Kv1.8. Kv1.3 alleviates regulatory volume decrease (RVD) when hypotonic shock occurs [13]. Kv2 channel paves the way to the cells' apoptotic death [14]. Four of the Kv gene families (Kv5, Kv6, Kv8, and Kv9) encode subunits acting as modifiers. They don't assemble to functional channels by themselves, but form heterotetramers with Kv2 family peptide subunits, which will add more diverse functions of this family [11]. Kv7 is also pronounced in nervous system for its close relationship with epilepsy [15]. The Kv channels take actions at both para-synaptic and synaptic sites; they will influence the synaptic efficiency by regulating the neurotransmitter release in neurons [16].

Voltage-gated K^+ Channels in Epilepsy

Among the 12 voltage-gated potassium channel families, one of them is a little different from others, that is Kv7 (KCNQ) family. KCNQ family is formed by Kv7.1–Kv7.5 and gained attention because the dysfunction of Kv7 will cause many diseases, such as epilepsy, deafness and cardiac arrhythmia [17]. The 5 kinds of Kv7 channels play different roles in mammals. Kv7.1 (KCNQ1) is the only channel that isn't expressed on neurons. It is activated in depolarization of cardiac action potentials and epithelial transport. Kv7.1 is often connected with the production of KCNE1 gene in order to form cardiac-delayed rectifier-like potassium currents in heart. If this channel gene mutates, one of the inherited long QT syndrome (LQT1) and the cardiac arrhythmia will be caused [18]. Meanwhile, KCNQ1 also has a close relationship with deafness, indicating that KCNQ1 is important in potassium recycling in the inner ear [19]. Besides Kv1, the heteromultimeric channels constituted by Kv7.2, Kv7.3, Kv7.4 and Kv7.5, especially Kv7.2 and Kv7.3 correlate with M-current (I_M). Having 40% homology with KCNQ1 [10], KCNQ2 and KCNQ3 heteromultimers are thought to have a close relation with M-current, which was first discovered in bull frog sympathetic ganglion cells as a type of noninactivating potassium current [20]. In the KCNQ family, KCNQ2 and KCNQ3 have the closest correlation with epilepsy; these two genes were identified by positional cloning

methods in families suffering from benign familial neonatal convulsions (BFNC) which is a neonatal form of epilepsy [21]. BFNC is a very rare genetic disease, while among 100,000 people only one person is attached. It will show effect in early life but most of the patients gain recovery within weeks or months of birth [19]. For years, it is reported that these subunits are mainly expressed in neuronal tissues [22,23], such as sympathetic ganglia [24]. Meanwhile, the expression patterns of these genes in the nervous system, especially in the brain, overlap extensively [22]. But, when they are hybridized in situ, it is concluded that they are not always expressed conformably since some neurons were stained only for one or the other subunit shown with the immunocytochemistry [25]. To sum, KCNQ2 and KCNQ3 are of importance in the maintenance of physiological functions of central nervous system (CNS).

KCNQ2 and KCNQ3 may combine and form a single channel because subunits expressed by them overlaps widely. Just for they are sensitive to inhibitors and the gating kinetics can be changed slightly, a larger current will be induced when these two subunits are expressed [22,24,26,27]. Furthermore, firstly in 1998, it has been put forward that once Kv7 subunits mutate, epilespy can be caused in humans [21]. After that, it has been found that Kv7.2 and Kv7.3 are expressed in CNS, such as the hippocampus and cortex, where seizure is strongly linked. Besides, Kv7 channels also foster the neuronal intrinsic firing activity in hippocampus [28] and the slow after hyperpolarization which mediates the neuronal excitability [29]. In recent years, a direct link between KCNQ channel function and epileptic activities are proved by knocking down the KCNQ3 gene [30]. So in sum, it could be concluded that the mutation of KCNQ2 and KCNQ3 associate with epilepsy strongly and the drug that can inhibit or activate these channels might all contribute to the treatment of epilepsy.

The KCNQ4 gene can take part in the encoding of molecular correlation of I_K, in outer hair cells (OHCs) of the cochlea and $I_{K,L}$ in Type I hair cells of the vestibular apparatus [19], moreover, mutations in these genes result in the inherited deafness. KCNQ4 in the CNS is expressed restrictively to certain structures in the brainstem, especially in nuclei and tracts of the central auditory pathway and trigeminal ganglia [31]. The KCNQ5 gene, which was identified in 2000, was expressed in skeletal muscle and brain. Mutations in KCNQ5 gene may induce retinopathy [32].

The Structure and Gating of Kv7 Channels

KCNQ proteins, which are related to Kv channels, always have six trans-membrane domains. The homology within Kv family branches is more than that between different KCNQ proteins. Compared with other Kv channels, there is also a single P-loop which forms the selectivity filter of the pore, a fourth trans-membrane domain (S4) that always serves as a voltage sensor, and intracellular amino and carboxy termini. But differently, KCNQ has a quite long C terminus. Once a short stretch happened, a conserved domain will be followed. In particular, the A domain that is highly expressed between different KCNQ proteins is often considered as an obvious characteristic of this family. Even though it has not been confirmed directly, it might be sure that 4 KCNQ peptide subunits combine with each other so that to form functional K^+ channels. These five KCNQ subunits could form HOMOMERIC CHANNELS in vitro, and it seems that the heteromers can only form several certain combinations restrictively [22,33,34].

KCNQ channels control several activities in the nervous system, such as somatic excitability, bursting and neurotransmitter release. These activities are modulated by different signaling pathways. In superior cervical ganglion sympathetic neurons, angiotensin II AT_1, bradykinin B_2, muscarinic M_1 and purinergic P2Y agonists inhibit the M-current. With the Probes of PLC, it has been shown that agonists of all 4 receptors can cause the hydrolyzation of robust phosphatidylinositol 4,5-bisphosphate (PIP2) [35]. Gq/11-mediated signaling ways have two basic types in SCG neurons. The first one need to stimulate M_1 angiotensin II AT_1 and mACh receptors, then PIP2 depletion will be induced principally [36]. The second one is caused by purinergic P2Y and bradykinin B_2 receptors. This way depends on the increase of IP3-regulated in intracellular Ca^{2+} concentration. Ca^{2+} stimulates PIP2 synthesis so that its depletion of PIP2 synthesis and subsequent Ca^{2+} binding to calmodulin (CaM) are prevented [37], which acts on the channels [38,39].

The Functions of Kv7 Channels in Epilepsy

Kv7 channels always open at subthreshold membrane potentials and induces outwardly

rectifying voltage-dependent potassium currents that is activated at -60 mV [19]. It is admitted that the Kv7 family (only Kv7.2-7.5) generates the M-currents, the K^+ currents between the thresholds and modulates the excitability of various peripheral and central neurons [40]. In recent reports, it is also suggested that KCNQ2 and KCNQ3 channels have been found at the nodes of Ranvier and at the axonal initial segments (AIS) in central nervous systems and peripheral nervous systems [41]. For the characteristic that Kv7.2-7.5 channels are slow activating and non-inactivating Kv channels existed in both central and peripheral nervous systems, the mutation of Kv7 channels may cause the membrane hyperexcitability. And it is also reported that the spontaneous mutation of Kv7 channels was an important cause for human neonatal epilepsy [42].

Kv7 channels are regulated by different kinds of G protein-coupled receptors and intracellular signal molecules. In hippocampal CA1 pyramidal neurons, axonal Kv7 channels can modulate RMP and the action potential threshold so that the inner spontaneous activity of these neurons could be inhibited. In 2008, it is found that mutant Kv7 channels do not bind ankyrin G and are not concentrated on the AIS. So it seems that in physiological conditions, if the axonal Kv7 channel function could be regulated, it may have a significant impact on the function of neurons [43]. In vivo, any spontaneous firing caused by axonal channel disruption might be intensified by the more global Kv7 inhibition, which is induced by stimulation of G-coupled receptors through afferent glutamatergic and cholinergic synaptic activity [44]. That XE-991 blocks the Kv7 channel resulting in the amplifying effect, will lead to convulsions. Therefore, much more AIS Kv7 channels are essential for modulating the activities of hippocampal pyramidal neurons and preserving neural network homeostasis [43].

Kv7 Channel in Neurology and Psychiatry

Different from other voltage-gated K^+ channels, Kv7 channels can be activated at -60mV. KCNQ2 and KCNQ3, which influence epilepsy a lot, can regulate M-currents, therefore M-current regulators could be important in treating epilepsy. In the meanwhile, inhibitors of M-currents such as XE-991 and linopirdine are often used as cognition enhancers [45], and with higher dosages, epilepsy could be induced. The retigabine which is also called Ezogabine in US activates KCNQ2 or KCNQ3 channels by shifting their voltage-dependence to more negative voltages [46].

Nowadays, because it is reported that the KCNQ2 and KCNQ3 are the genes closely related with some kind of human epilepsy, the anticonvulsive drug retigabine [N-(2-amino-4-[fluorobenzylamino]-phenyl) carbamic acid; D-23129] which can activate these channels are confirmed also effective against epilepsy [47]. Retigabine can affect the voltage- dependent Kv7 channels, so that the hyperpolarized membrane voltages could decrease to the resting potential of neurons and the epilepsy could be alleviated [48]. In addition, Retigabine has effect on KCNQ2–KCNQ5 channels but not KCNQ1 channels, which are only located in cardiac cells [48]. It was found that mutations to KCNQ2–KCNQ5 caused dysfunction, respectively including the long cytoplasmatic C-terminus, the voltage sensor S4 and the S1–S2 region of Kv7.2, and the pore regions (S5–S6 segments) of both Kv7.2 and Kv7.3 channels, which always resulted in epilepsy [49], However, there are some documented lines about the adverse effects of Retigabine. Ciliberto et al. reported its adverse effects in central nervous system, such as somnolence, dizziness, confusion, and fatigue [50]. Moreover, Garin Shkolnik et al. addressed another adverse effect, blue-gray mucocutaneous discoloration [51]. Although discontinuing ezogabine showed distinct improvement of the adverse effects, there is still no evidence that all the adverse effects are definitely reversible. So, Retigabine may be a promising medication with specific clinical effect against epilepsy but its clinical application should be monitored carefully.

Future Directions

As summarized, Kv7.2-7.5 channels show strong biological and pharmacological links to both neurological and psychiatric diseases, especially epilepsy. Just for Kv7 channels family in many parts of nervous system such as RMP, excitability of various peripheral and central neurons, etc., the mutations of different KCNQ genes can cause the epilepsy. Epilepsy has been treated with the drug targeting at Na^+ channels, Ca^{2+} channels or K^+ channels, but there still are about one third patients who accepted treatments but don't get alleviation. So in recent years, the drug targeting at Kv7 channels attracted more and

more attentions because its inhibition of the hyperpolarization could be beneficial for the alleviation of epilepsy. But up to now, the role of Kv7.2-7.5 in other nervous disease is still not elucidated yet. Since the Kv7 channels played the important role in Gq/11 mediated pathway and the Calcium-CaM pathway, we consider that the effect of some other mutation of KCNQ in related nervous diseases deserves a further exploration, which will clarify the functions of Kv7 and shed light on the novel therapy for the diseases. The systematic study of Kv7 channels can disclose its role in neuronal functions, which will contribute to the treatment of epilepsy, and support effective therapy targets for more nervous diseases.

Acknowledgments

This work was supported by the following grants: National Natural Science Foundation of China (31571162), Beijing Municipal Talents Project (2013D005018000010).

References

1. Littleton JT, Ganetzky B. Ion channels and synaptic organization: analysis of the Drosophila genome. *Neuron* 26(1), 35-43 (2000).
2. Emerit J, Edeas M Bricaire F. Neurodegenerative diseases and oxidative stress. *Biomed. Pharmacother* 58(1), 39-46 (2004).
3. Pannaccione A, Boscia F, Scorziello A, *et al.* Up-regulation and increased activity of KV3.4 channels and their accessory subunit MinK-related peptide 2 induced by amyloid peptide are involved in apoptotic neuronal death. *Mol. Pharmacol* 72(3), 665-673 (2007).
4. Bortner CD, Hughes FJ, Cidlowski JA. A primary role for K^+ and Na^+ efflux in the activation of apoptosis. *J. Biol. Chem* 272(51), 32436-32442 (1997).
5. Xiong Q, Gao Z, Wang W, *et al.* Activation of Kv7 (KCNQ) voltage-gated potassium channels by synthetic compounds. *Trends. Pharmacol. Sci* 29(2), 99-107 (2008).
6. Frazzini V, Guarnieri S, Bomba M, *et al.* Altered Kv2.1 functioning promotes increased excitability in hippocampal neurons of an Alzheimer's disease mouse model. *Cell. Death. Dis* 7(1), e2100 (2016).
7. Hall AM, Throesch BT, Buckingham SC, *et al.* Tau-dependent Kv4.2 depletion and dendritic hyperexcitability in a mouse model of Alzheimer's disease. *J. Neurosci* 35(15), 6221-6230 (2015).
8. Greene DL, Hoshi N. Modulation of Kv7 channels and excitability in the brain. *Cell. Mol. Life. Sci* (2016).
9. Grunnet M, Strøbæk D, Hougaard C, *et al.* Kv7 channels as targets for anti-epileptic and psychiatric drug-development. *Eur. J. Pharmacol* 726(1), 133-137 (2014).
10. Wang JJ, Li Y. KCNQ potassium channels in sensory system and neural circuits. *Acta. Pharmacol. Sin* 37(1), 25-33 (2016).
11. Gutman GA, Chandy KG, Grissmer S, *et al.* International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol. Rev* 57(4), 473-508 (2005).
12. Yellen G. The voltage-gated potassium channels and their relatives. *Nature* 419(6902), 35-42 (2002).
13. Deutsch C, Chen LQ. Heterologous expression of specific K^+ channels in T lymphocytes: functional consequences for volume regulation. *Proc. Natl. Acad. Sci. U S A* 90(21), 10036-10040 (1993).
14. Pal SK, Takimoto K, Aizenman E, *et al.* Apoptotic surface delivery of K^+ channels. *Cell. Death. Differ* 13(4), 661-667 (2006).
15. Li C, Huang P, Lu Q, *et al.* KCNQ/Kv7 channel activator flupirtine protects against acute stress-induced impairments of spatial memory retrieval and hippocampal LTP in rats. *Neuroscience* 280(1), 19-30 (2014).
16. Meneses D, Vega AV, Torres-Cruz FM, *et al.* KV1 and KV3 Potassium Channels Identified at Presynaptic Terminals of the Corticostriatal Synapses in Rat. *Neural. Plast* 8782518 (2016).
17. Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. *Nat. Rev. Neurosci* 1(1), 21-30 (2000).
18. Tyson J, Tranebjaerg L, McEntagart M, *et al.* Mutational spectrum in the cardioauditory syndrome of Jervell and Lange-Nielsen. *Hum. Genet* 107(5), 499-503 (2000).
19. Robbins J. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. *Pharmacol. Ther* 90(1), 1-19 (2001).
20. Brown DA, Adams PR. Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. *Nature* 283(5748), 673-676 (1980).
21. Singh NA, Charlier C, Stauffer D, *et al.* A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat. Genet* 18(1), 25-29 (1998).
22. Schroeder BC, Kubisch C, Stein V, *et al.* Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K^+ channels causes epilepsy. *Nature* 396(6712), 687-690 (1998).
23. Tinel N, Lauritzen I, Chouabe C, *et al.* The KCNQ2 potassium channel: splice variants, functional and developmental expression. Brain localization and comparison with KCNQ3. *FEBS Lett* 438(3), 171-176 (1998).
24. Wang HS, Pan Z, Shi W, *et al.* KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science* 282(5395), 1890-1893 (1998).
25. Cooper EC, Aldape KD, Abosch A, *et al.* Colocalization and coassembly of two human brain M-type potassium channel subunits that are mutated in epilepsy. *Proc. Natl. Acad. Sci. U S A* 97(9), 4914-4919 (2000).
26. Yang WP, Levesque PC, Little WA, *et al.* Functional expression of two KvLQT1-related potassium channels responsible for an inherited idiopathic epilepsy. *J. Biol. Chem* 273(31), 19419-19423 (1998).
27. Hadley JK, Noda M, Selyanko AA, *et al.* Differential tetraethylammonium sensitivity of KCNQ1-4 potassium channels. *Br. J. Pharmacol* 129(3), 413-415 (2000).
28. Shah MM, et al. Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. U S A* 105(22), 7869-7874 (2008).
29. Tzingounis AV, Heidenreich M, Kharkovets T, *et al.* 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 (2010).
30. Chege SW, Hortopan GA, T Dinday M, *et al.* Expression and function of KCNQ channels in larval zebrafish. *Dev. Neurobiol* 72(2), 186-198 (2012).
31. Kharkovets T, Hardelin JP, Safieddine S, *et al.* KCNQ4, a K+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway. *Proc. Natl. Acad. Sci. U S A* 97(8), 4333-4338 (2000).
32. Smith JS, Iannotti CA, Dargis P, *et al.* Differential expression of kcnq2 splice

variants: implications to m current function during neuronal development. *J. Neurosci* 21(4), 1096-1103 (2001).

33. Kubisch C, Schroeder BC, Friedrich T, *et al.* KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. *Cell* 96(3), 437-446 (1999).

34. Schroeder BC, Hechenberger M, Weinreich F, *et al.* KCNQ5, a novel potassium channel broadly expressed in brain, mediates M-type currents. *J. Biol. Chem* 75(31), 24089-24095 (2000).

35. Zhang H, Craciun LC, Mirshahi T, *et al.* PIP(2) activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. *Neuron* 37(6), 963-975 (2003).

36. Zaika O, Lara LS, Gamper N, *et al.* Angiotensin II regulates neuronal excitability via phosphatidylinositol 4,5-bisphosphate-dependent modulation of Kv7 (M-type) K+ channels. *J. Physiol* 575(1), 49-67 (2006).

37. Delmas P, Wanaverbecq N, Abogadie FC, *et al.* Signaling microdomains define the specificity of receptor-mediated InsP(3) pathways in neurons. *Neuron* 34(2), 209-220 (2002).

38. Zaika O, Tolstykh GP, Jaffe DB, *et al.* Inositol triphosphate-mediated Ca2+ signals direct purinergic P2Y receptor regulation of neuronal ion channels. *J. Neurosci* 27(33), 8914-8926 (2007).

39. Gamper N, Li Y, Shapiro MS. Structural requirements for differential sensitivity of KCNQ K+ channels to modulation by Ca2+/calmodulin. *Mol. Biol. Cell* 16(8), 3538-3551 (2005).

40. Delmas P, Brown DA. Pathways modulating neural KCNQ/M (Kv7) potassium channels. *Nat. Rev. Neurosci* 6(11), 850-862 (2005).

41. Abidi A, Devaux JJ, Molinari F, *et al.* A recurrent KCNQ2 pore mutation causing early onset epileptic encephalopathy has a moderate effect on M current but alters subcellular localization of Kv7 channels. *Neurobiol. Dis* 80(1), 80-92 (2015).

42. Shah MM, Huang Z, Martinello K. HCN and KV7 (M-) channels as targets for epilepsy treatment. *Neuropharmacology* 69(1), 75-81 (2013).

43. Shah, M.M., et al., Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. PNAS, 2008. 105(22): p. 7869-7874.

44. Wang HS, Pan Z, Shi W, *et al.* KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science* 282(5395), 1890-1893 (1998).

45. Zaczek R, Chorvat RJ, Saye JA, *et al.* Two new potent neurotransmitter release enhancers, 10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone and 10,10-bis(2-fluoro-4-pyridinylmethyl)-9(10H)-anthracenone: comparison to linopirdine. *J. Pharmacol. Exp. Ther* 285(2), 724-730 (1998).

46. Rundfeldt C, Netzer R. The novel anticonvulsant retigabine activates M-currents in Chinese hamster ovary-cells tranfected with human KCNQ2/3 subunits. *Neurosci. Lett* 282(1-2), 73-76 (2000).

47. Wickenden AD, Yu W, Zou A, *et al.* Retigabine, a novel anti-convulsant, enhances activation of KCNQ2/Q3 potassium channels. *Mol. Pharmacol* 58(3), 591-600 (2000).

48. Tatulian L, Delmas P, Abogadie FC, *et al.* Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. *J. Neurosci* 21(15), 5535-5545 (2001).

49. Maljevic S, Wuttke TV, Lerche H. Nervous system KV7 disorders: breakdown of a subthreshold brake. *J. Physiol* 586(7), 1791-1801 (2008).

50. Ciliberto MA, Weisenberg JL, Wong M. Clinical utility, safety, and tolerability of ezogabine (retigabine) in the treatment of epilepsy. *Drug. Healthc. Patient. Saf* 4(1), 81-86 (2012).

51. Garin Shkolnik T, Feuerman H, Didkovsky E, *et al.* Blue-gray mucocutaneous discoloration: a new adverse effect of ezogabine. *JAMA. Dermatol* 150(9), 984-989 (2014).