

Activation of Kv7 (KCNQ) voltage-gated potassium channels by synthetic compounds

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Voltage-gated Kv7 (or KCNQ) channels play a pivotal role in controlling membrane excitability. Like typical voltage-gated ion channels, Kv7 channels undergo a closed-to-open transition by sensing changes in transmembrane potential, and thereby mediate inhibitory K⁺ currents to reduce membrane excitability. Reduction of Kv7 channel activity as a result of genetic mutation is responsible for various human diseases due to membrane hyperexcitability, including epilepsy, arrhythmia and deafness. As a result, the discovery of small compounds that activate voltage-gated ion channels is an important strategy for clinical intervention in such disorders. Because ligand binding can induce a conformational change leading to subthreshold channel opening, there is considerable interest in understanding the molecular basis of these ‘gain-of-function’ molecules. Although small-molecule activators of cation channels are rare, several novel compounds that activate Kv7 voltage-gated channels have been identified. Recent advances in defining the activator-binding sites and in understanding their mechanism of action have begun to provide insight into the activation of voltage-gated channels by synthetic compounds.

Introduction

Membrane hyperexcitability is a common feature of various neurological diseases [1]. Although the cause for excessive membrane excitation varies, an increase in inhibitory activity such as K⁺ channel conductance might provide a beneficial counter force, and thus therapeutic benefit. K⁺ channels function in controlling membrane excitability [1]. Because they are selective to K⁺ ions, their roles in neurons commonly involve repolarizing the membrane during action potential firing. A typical neuron has a specific voltage threshold for firing, and opening of K⁺ channels below this threshold (known as subthreshold activation) will inhibit initiation of the action potential.

Potassium channels can be classified according to the primary source of energy for their gating, in other words, their closed-to-open transition. The three most common classes are voltage-gated channels, ligand-gated channels and mechanosensitive channels [2–4]. Extensive studies of channel physiology in both native and recombinant

systems have led to the appreciation that the gating of a given channel can be influenced by multiple factors including ligands and voltage. For example, a group of ligand-gated K⁺ channels known as Ca²⁺-activated K⁺ channels have been classified according to their single-channel conductance: namely, small-, medium- and large-conductance Ca²⁺-activated K⁺ channels [5]. Among these channels, the large-conductance Ca²⁺-activated K⁺ channels are best known for their gating by both transmembrane voltage and intracellular Ca²⁺. High concentrations of Ca²⁺ can induce channel opening at the resting potential [6,7], providing conceptual evidence that chemical and physical energy sources can function both independently and in combination to cause the transition between the closed and the open state.

Many K⁺ channels are voltage-gated and can be divided into several Kv subfamilies (Kv1.x to Kv12.x) according to sequence homology and their ability to assemble into heteromultimeric channels [8]. The Kv7 family has five members termed Kv7.1 to Kv7.5 (or KCNQ1 to KCNQ5). These channels are activated at subthreshold membrane potentials [9], and the strong inhibitory K⁺ currents that they produce below the threshold potential are useful to reduce or to prevent undesirable membrane excitation. Neuronal Kv7 channels are distributed throughout the central nervous system and the peripheral nerves within, for example, hippocampal and dorsal root ganglion neurons. As a result, potentiation of these channels by synthetic compounds is beneficial in treating diseases involving neuronal hyperexcitability, such as epilepsy and neuropathic pain [10,11].

All Kv channels including Kv7 channels share a typical topological design, consisting of a functional channel formed by four subunits, each of which comprises six transmembrane segments termed S1 to S6 (Figure 1). Of particular importance, the S4 segment has alternating positively charged residues, which plays an essential role in sensing membrane potential and triggering conformational changes [12–18]. Under physiological conditions, Kv7 channels are activated only by membrane depolarization. Interestingly, a few Kv channel activators have been found. Some of these activators seem to be specific to Kv7 channels and have been shown to open the channels at resting potentials. In this review, we summarize key aspects of the neurophysiology of Kv7 channels and also

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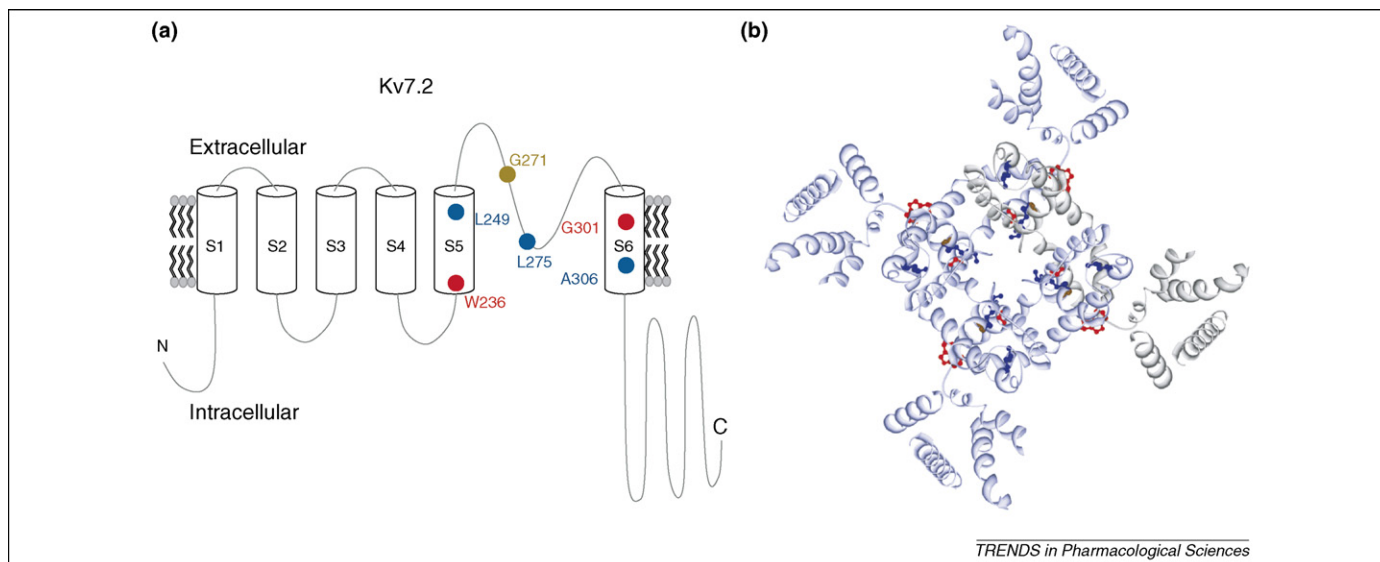


Figure 1. Structure of Kv7.2 channels. **(a)** Structure of Kv7.2 showing residues essential for modulation of channel activity by R-L3 (brown), retigabine (red) and ZnPy (blue). Some of these residues have been identified in other Kv7 channels. For comparison, the corresponding positions in Kv7.2 have been highlighted (Gly271 on Kv7.2 is equivalent to Gly306 on Kv7.1). **(b)** Tetrameric structure of Kv7.2 modeled according to the Kv1.2 structure. One of the four subunits is colored grey. Three-dimensional structural models of the KCNQ2 S1–S6 segments were generated by using the solved crystal structure of Kv1.2 (Protein Data Bank code 2A79) as a template. The corresponding domains between KCNQ2 and Kv1.2 were aligned with DNASTAR MegAlign using standard parameters. The KCNQ2 models were constructed with DeepView/SWISSPdbViewer (<http://ca.expasy.org/spdbv>) [55]. The structural representation was performed with PovRay (<http://www.povray.org>).

the different classes of compounds that have been reported to activate Kv7 channels. By discussing the results of recent studies, we hope to identify some of the important outstanding issues concerning the mechanisms of action of these activators. A better understanding of how these activators function in regulating Kv channels might facilitate the development of therapeutics for various neuronal and other related diseases.

Kv7 channels

Low-threshold non-inactivating K^+ channels formed by Kv7 subunits are characterized by being potently inhibited by the activation of muscarinic receptors; as a result, they are also known as ‘M channels’ [19,20]. Their biophysical properties, together with their specific protein subcellular localization, facilitate powerful control over the firing rate of a neuron [21–23]. Because M channels are inhibited by the neurotransmitter-activated G-protein-coupled signaling pathway, the downregulation of their K^+ current or ‘M current’ by G-protein signaling can in turn reduce conductance of the inhibitory M current and thus potentiate membrane excitability. These opposing regulatory mechanisms are an important aspect of M-current physiology.

Molecular cloning and recombinant expression have identified five genes (*KCNQ1* to *KCNQ5*) that encode the Kv7.1 to Kv7.5 pore-forming α -subunits [8,24–28], and at least five genes (*KCNE1* to *KCNE5*) that encode the KCNE1 to KCNE5 accessory β subunits [29]. The diversity of Kv7 currents is much enhanced as a result of the formation of heteromultimeric channels from different pore-forming α -subunits and additional accessory β -subunits. For example, The Kv7.1–KCNE heteromultimeric complex mediates cardiac I_{Ks} , a slowly activating component of delayed rectifier K^+ current [30,31]. Much evidence indicates that the heteromultimeric channels formed by Kv7.2, Kv7.3, Kv7.4 and Kv7.5, and in particular

Kv7.2–Kv7.3, represent the molecular correlates of M currents. First, channels formed in heterologous systems by the coexpression of Kv7.2 and Kv7.3 channels possess biophysical and pharmacological properties reminiscent of those of the M channels [23,32]. Second, both Kv7.2 and Kv7.3 mRNA and protein show substantial overlap in tissue distribution and in subcellular localization. Furthermore, they are coimmunoprecipitated by Kv7.2- or Kv7.3-specific antibodies [33]. Third, mutations of either Kv7.2 or Kv7.3 are linked to the same disease – namely, benign familial neonatal convulsions [27,34]. Fourth, conditional suppression of the Kv7.2 current in mouse brain at different developmental periods causes epilepsy, hyperactivity and abnormal morphology of the hippocampus – features that are shared with human diseases [35]. Genetic mutations of Kv7 channels are linked to human disorders arising from hyperexcitability including epilepsy, arrhythmia, deafness and neuromyotonia [24,25,27,28,34,36,37]. As a result, Kv7 channels are important regulatory proteins for controlling electrical excitability [38–40].

Under physiological conditions, Kv7 channels and their corresponding M currents are activated by membrane depolarization and can be regulated by various signal transduction pathways. Most prominently, the currents from both heterologously expressed Kv7 channels and wild-type channels can be suppressed by the activation of G-protein coupled receptors, particularly the muscarinic acetylcholine receptor [19,20,41]. This suppression is caused by interactions with intracellular signaling molecules such as Ca^{2+} , phosphatidylinositol bisphosphate, and the protein kinases A and C [22,42,43]. In addition, several reports have provided strong evidence that redox potential modulates channel activity. Cysteine-modifying reagents such as *N*-ethylmaleimide (NEM) also react with Kv7.2 to Kv7.5 subunits to enhance the corresponding channel activities [44,45]. In addition, hydrogen peroxide

Table 1. Summary of Kv7 channel activators

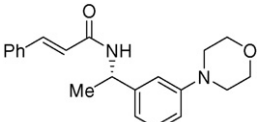
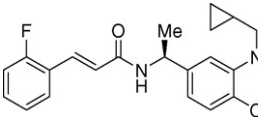
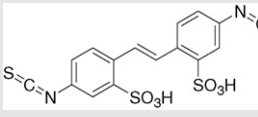
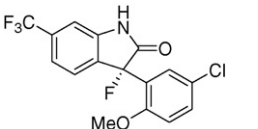
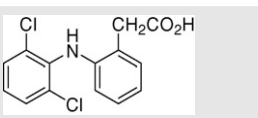
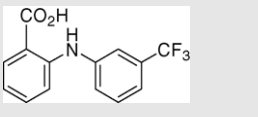
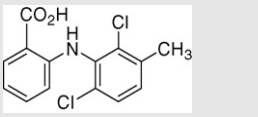
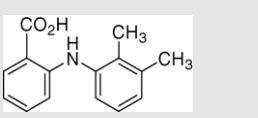
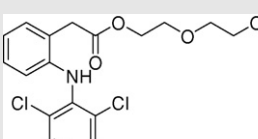
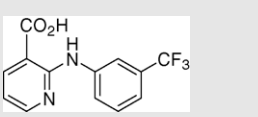
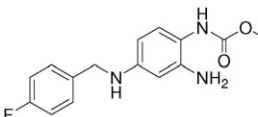
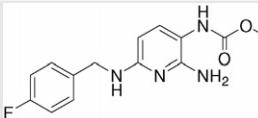
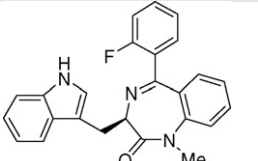
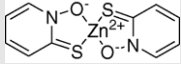
Compound name	Structure	Target genes	Effects	Refs	
Acrylamides	Acrylamide (S)-1		Kv7.2, 7.3, 7.4, 7.5 and Kv7.2–Kv7.3	Hyperpolarizing shift of $V_{1/2}$ (Kv7.2, 7.4, 7.5 and Kv7.2–Kv7.3).	[67]
	Acrylamide (S)-2		Kv7.2	Hyperpolarizing shift of $V_{1/2}$.	[66]
4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS)		Kv7.1–KCNE1	Decrease I_{Ks} deactivation rate. Increase the time-dependent outward current.	[77,84]	
Maxipost (BMS-204352)		Kv7.2, 7.3, 7.4 and 7.5	Hyperpolarizing shift of $V_{1/2}$ (Kv7.2, 7.3, 7.4, Kv7.2–Kv7.3, Kv7.3–Kv7.4 and Kv7.3–Kv7.5). Slow activation and deactivation rates of the Kv7.5 currents.	[53,56,57]	
N-phenylanthranilic acid derivatives	Diclofenac		Kv7.2, 7.3 and Kv7.2–Kv7.3	Hyperpolarizing shift of $V_{1/2}$ (Kv7.2–Kv7.3). Slow deactivation rate.	[63]
	Flufenamic acid		Kv7.1–KCNE1	Decrease I_{Ks} deactivation rate. Decrease the time-dependent outward current.	[77,84]
	Meclofenamic acid		Kv7.2, 7.3 and Kv7.2–Kv7.3	Hyperpolarizing shift of $V_{1/2}$ (Kv7.2–Kv7.3). Slow deactivation rate.	[63]
	Mefenamic acid		Kv7.1–KCNE1	Decrease I_{Ks} deactivation rate. Increase the time-dependent outward current.	[77,84]
	NH6		Kv7.2–Kv7.3	Hyperpolarizing shift of $V_{1/2}$ (Kv7.2–Kv7.3). Slow deactivation rate.	[64]
	Niflumic acid		Kv7.1–KCNE1	Hyperpolarizing shift of $V_{1/2}$. Decrease I_{Ks} deactivation rate. Decrease the time-dependent outward current.	[77,84]
Retigabine		Kv7.2, 7.3, 7.4, 7.5 and Kv7.2–Kv7.3	Hyperpolarizing shift of $V_{1/2}$. Accelerate activation rate and slow deactivation rate.	[50–52,70,71]	
(Retigabine analog) Flupirtine		Kv7.2	Hyperpolarizing shift of $V_{1/2}$.	[85]	
L-364 373 [(3-R)-1, 3-dihydro-5-(2-fluorophenyl)-3-(1H-indol-3-ylmethyl)-1-methyl-2H-1,4-benzodiazepin-2-one (R-L3)]		Kv7.1, Kv7.1–KCNE1	Hyperpolarizing shift of $V_{1/2}$, slow I_{Ks} deactivation rate.	[61,62]	

Table 1 (Continued)

Compound name	Structure	Target genes	Effects	Refs
Zinc pyrithione		Kv7.1, 7.2, 7.4, 7.5 and Kv7.2–Kv7.3	Hyperpolarizing shift of $V_{1/2}$ (Kv7.2, Kv7.2–Kv7.3), slow deactivation rate and activation rate, increase G_{max} (Kv7.2, 7.2–7.3) and single channel open probability P_O (Kv7.2, 7.4).	[68]

strongly potentiates Kv7 channels through oxidative modification of the cysteine residues in Kv7.2, Kv7.4 and Kv7.5 channels [46]. Thus, Kv7 channels can be upregulated or downregulated by intracellular signaling or by covalent modification. These different pathways of modulation are important aspects of Kv7 physiology. Synthetic activators of Kv7 seem to function independently of the above modulation pathways by directly binding to the protein to cause a conformational change leading to channel opening. So far, no physiological activator has been reported to act in a similar manner.

Synthetic activators of Kv7 channels

In addition to compounds that modulate Kv7 channels by covalent modification such as NEM, several reversible synthetic activators of Kv7 channels have been identified (Table 1). These compounds, when acting on the channel, have different degrees of voltage dependence.

The first – and perhaps best-characterized – activator of Kv7 channels is retigabine or *N*-[2-amino-4-(4-fluorobenzylamino)-phenyl] carbamic acid ethyl ester [47–52]. Retigabine activates Kv7.2, Kv7.3, Kv7.2–Kv7.3, Kv7.4 and Kv7.5 channels, with an effective concentration for half-maximum response (EC_{50}) of 1.4 μ M at –30 mV for Kv7.2 [53]. It has served as an important chemical tool with which to study the biological function and therapeutic potential of Kv7 channel modulation [51,52], but it also potentiates other channels, including γ -aminobutyric acid receptor channels [54]. Its potential clinical applications include treatment of epilepsy, anxiety, neuropathic pain, neurodegenerative disorders, cancer, inflammation and ophthalmic diseases [55–59]. Limitations of retigabine include potential side-effects owing to its broad action on all neuronal Kv7 channels and other channels [60], its pharmacokinetics and its therapeutic potency [58].

Recent work has identified new chemical scaffolds that have expanded the repertoire of Kv7 activators. These scaffolds potentiate both recombinant Kv7 channels and native M channels by various mechanisms, such as lowering the activation threshold, increasing channel open probability, and stabilizing the open state of the channel (Table 1). For example, oxindole analogues, represented by BMS-204352 [(3*S*)-(+)-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2*H*-indol-2-one], are potent activators of Kv7 channels with an EC_{50} of 2.4 μ M for Kv7.5 at –30 mV. BMS-204352 also potentiates Kv7.2, Kv7.2–Kv7.3 and Kv7.3–Kv7.4 channels to a variable extent [53,56,57]. Both BMS-204352 and retigabine are anxiolytic [56], supporting the possibility that Kv7 channels might be targets for treating anxiety.

Compound R-L3 or L-364 373 [(3-*R*)-1, 3-dihydro-5-(2-fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-1-methyl-2*H*-1,

4-benzodiazepin-2-one)] is an activator of Kv7.1 channels. Intriguingly, the effect of R-L3 on Kv7.1 is diminished when Kv7.1 is coexpressed with its accessory subunit KCNE1 [61,62]. Although the mechanism of action is unclear, this potential modulation by KCNE subunits raises the issue of differential drug specificity for different heteromultimeric Kv7.1–KCNE complexes. Kv7.1 is expressed most abundantly in cardiac tissues, but is also found in other tissues and can coassemble with KCNE subunits in addition to KCNE1. Thus, this compound could be a useful pharmacological probe for studying Kv7.1 channels in cardiac and other tissues.

Fenamates are another important class of Kv7 channel activators. Meclofenamic acid and diclofenac are relatively potent activators of Kv7.2 and Kv7.3 with EC_{50} values of 25 and 2.6 μ M, respectively. Both compounds enhance M currents in rat cortical neurons known to coexpress Kv7.2 and Kv7.3 [63]. These compounds cause a hyperpolarizing shift of the voltage dependence of channel activation and slow the rate of channel closure (deactivation). Furthermore, diclofenac and, to a lesser extent, meclofenamic acid show robust antiepileptic properties *in vivo*. Attali and co-workers [64] have reported that fenamate NH6 has an activation mode similar to that of meclofenamic acid and diclofenac. The fenamates are widely used nonsteroidal anti-inflammatory drugs that act as nonselective inhibitors of the cyclooxygenases COX-1 and COX-2 [65]. Although the reported pharmacological effects on Kv7 channels are obtained at relative high concentrations of fenamates, the fenamate scaffold might nevertheless serve as a template for the development of Kv7 channel modulators useful for the treatment of neuronal hyperexcitability such as migraine, epilepsy and neuropathic pain. The channels targets of fenamates have not, however, been fully characterized. Their pharmacological effects might come from Kv7 and possibly other related channels.

Acrylamides, exemplified by acrylamides (*S*)-1 and (*S*)-2 [66,67], have also been reported to activate Kv7 channels. (*S*)-1 is a Kv7.2 activator with an EC_{50} value of 3.28 μ M at –40 mV, as determined by whole-cell patch-clamp assay. (*S*)-1 inhibits Kv7.1 but activates all other Kv7 channels. The effect of (*S*)-1 on Kv7.2 and Kv7.2–Kv7.3 is voltage dependent: it blocks the channel at high voltages and potentiates it at low voltages. By contrast, the effect of (*S*)-1 on Kv7.4 and Kv7.5 is potentiated at all voltages. The (*S*)-1 acrylamide shows significantly enhanced solubility and excellent oral bioavailability as compared with the original lead compound. (*S*)-2 is a conformationally restricted molecule and has an EC_{50} of 0.06 μ M, making it ~55 times more potent than (*S*)-1.

Recently, bis(1-hydroxy-2(1*H*)-pyridineselonato-O,S) zinc, commonly known as zinc pyrithione (ZnPy), has been

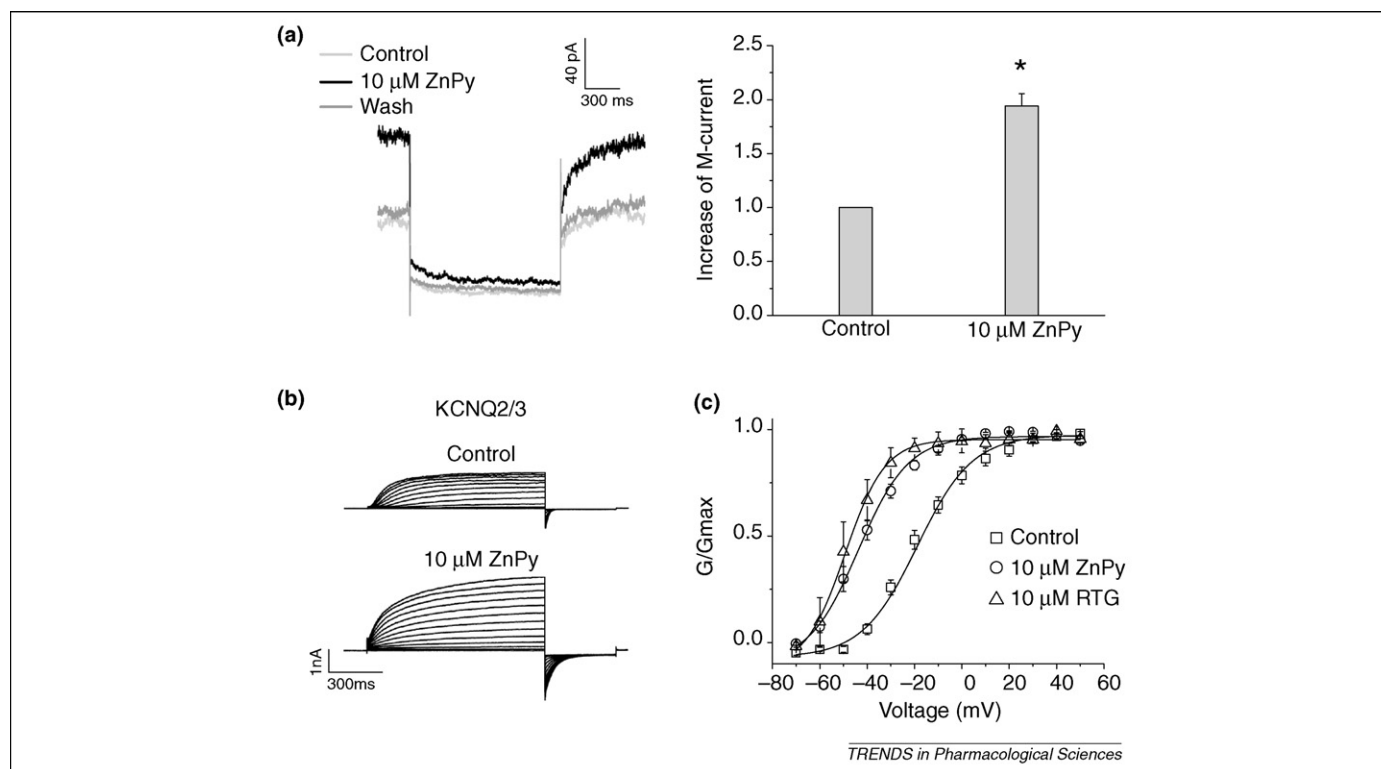


Figure 2. Potentiation of M and Kv7.2–Kv7.3 currents by activators. (a) Left, M currents recorded from rat dorsal root ganglion neurons in the absence and presence of 10 μM ZnPy. Right, histogram showing that 10 μM ZnPy causes an increase in M-current amplitude ($n = 10$, $*P < 0.001$) [68]. (b) Kv7.2–Kv7.3 heteromeric currents in the absence and presence of 10 μM ZnPy. (c) Conductance–voltage curves of Kv7.2–Kv7.3 channels under the indicated drug conditions. Panels (a) and (b) of this figure are adapted from Ref. [68].

shown to be a strong Kv7 activator, potentiating all Kv7 channels except Kv7.3 [68]. ZnPy has been used widely for controlling dandruff and treating psoriasis [69]. ZnPy has an EC_{50} of 1.7 μM for both recombinant Kv7.2–Kv7.3 and wild-type Kv7 channels. Although ZnPy strongly potentiates Kv7.1, it does not have an effect on the cardiac I_{Ks} current formed by Kv7.1 and KCNE1 subunits (Z.G. and M.L., unpublished). The effects of ZnPy on neuronal Kv7 channels include both a hyperpolarizing shift in the voltage dependence of activation and an increase in current amplitude (Figure 2). In dorsal root ganglion and hippocampal neurons, ZnPy causes hyperpolarization indicative of M channel opening at the resting potential [68]. Studies have found that this compound has a novel and unique mode of activation, because crucial regions required for ZnPy sensitivity are different from those required for earlier Kv7 activators such as retigabine. Furthermore, both Zn^{2+} and pyrithione are essential for activity, and the potency depends on the proper stoichiometry of zinc and pyrithione. Therefore, ZnPy represents a new probe for studying the function of Kv7 channels.

Ligand-binding sites on Kv7 channels

Binding of an activator to a channel can trigger numerous effects, including stabilizing the open conformation and facilitating a series of conformational changes to open the channel. As a result, a channel residue that affects efficacy of the activator might either alter the binding affinity of the activator or disrupt activator-induced conformational changes. Residues essential for activator efficacy can be identified by mutagenesis. The site of action of small

molecules can be divided into two types: the binding site and the active site. The former is required for the compound to bind, whereas the latter is not required for binding but is essential for activity of the compound. Currently, the primary strategy of trying to identify sites of action is through site-directed mutagenesis and recombinant chimeric channels. These experiments have been particularly effective in identifying key molecular determinants for drug effects [62,68,70,71] (Figure 3).

Initial efforts to identify residues essential for agonistic effects on Kv7 channels were based on alanine scanning mutagenesis studies [62]. Seeböhm, Sanguinetti and others [62] focused on R-L3 potentiation of Kv7.1. They identified a series of residues spanning from S5 to S6 that reduce the efficacy of R-L3-mediated potentiation [62]. Combined with molecular docking analyses, these experiments revealed a reasonable correlation between crucial residues identified by mutagenesis and residues in contact with R-L3.

Although the activity of retigabine on Kv7 was noted in the mid-1990s, the molecular determinants were not known until recently when systematic mutagenesis studies [70,71] identified a crucial tryptophan residue in S5 that is conserved from Kv7.2 to Kv7.5. (Figures 1 and 2, red). Interestingly, there is a leucine in the corresponding position in Kv7.1. This pattern of conservation correlates with the specificity of retigabine, which potentiates Kv7.2, Kv7.3, Kv7.4 and Kv7.5, but not Kv7.1, channels. Indeed, when the tryptophan is mutated to leucine, the four sensitive channels are no longer potentiated by retigabine [70,71]. Furthermore, when the corresponding leucine

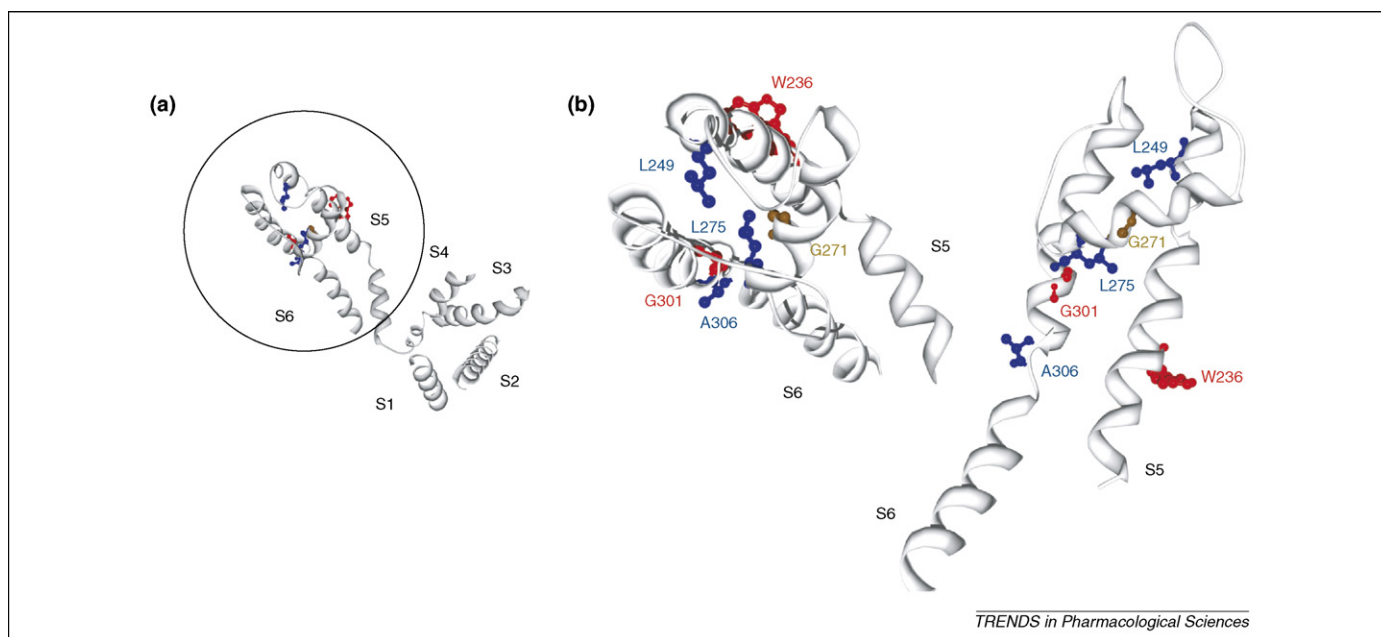


Figure 3. Structure of an isolated Kv7.2 subunit. (a) Structure of the S1–S6 segments. Residues essential for channel modulation by R-L3, retigabine and ZnPy are colored according to Figure 1. (b) Enlarged structures of the S5–S6 segment from different views.

residue in Kv7.1 is changed to tryptophan, the mutant Kv7.1 channel becomes sensitive to retigabine, although the effects of retigabine are complex and differ from its potentiating effects on Kv7.2, Kv7.3, Kv7.4 or Kv7.5 channels [70,71]. The conserved tryptophan is clearly necessary for retigabine-mediated potentiation. Additional residues have also been found to have effects on retigabine efficacy, including a glycine residue in S6 that forms the gating hinge essential for channel activation [71]. The lack of effect of retigabine on Kv7.1 is probably due to inefficient binding; however, direct experimental evidence has not been obtained.

The molecular determinants of ZnPy have also been reported, primarily from mutagenesis studies based on Kv7.2 channels [68]. The key determinants include a leucine residue in S5 (Leu249) and one within the linker (Leu275) between S5 and the pore region (Figures 1 and 2, residues labeled in blue). Mutation of either leucine causes significant reduction in the hyperpolarizing shift caused by ZnPy. For the double mutant channel, treatment with ZnPy does not result in a shift in voltage dependence but it does still lead to an increase in overall conductance. Conversely, mutation of Ala306 in segment S6 results in only a small ZnPy-induced increase in overall channel conductance but still leads to a hyperpolarizing shift. Thus, these residues represent key positions essential for the two aspects of ZnPy modulation.

Retigabine and ZnPy seem to act on two different sites because a W236L mutant of Kv7.2 that is insensitive to retigabine is fully sensitive to ZnPy, and the $V_{1/2}$ (voltage required for half maximum activation) of the ZnPy-insensitive L249A/L275A double mutant can be shifted by retigabine [68] (Q.X. and M.L., unpublished). Less information is available for other potentiating compounds. Because the conserved tryptophan is also required for acrylamide (S)-1- and BMS-204352-mediated potentiation of Kv7.4 [67], the data suggest that the three chemical scaffolds of acrylamide,

BMS-204352 and retigabine might function similarly on Kv7 channels and thus require the same molecular elements. Although these mutagenesis studies have not definitively pinpointed the molecular binding sites, they have provided both important information and useful reagents for future studies. For example, the specific mutant channels could be used to distinguish the modes of action of the different chemical activators.

Future directions

One of the well-characterized properties of Kv7 channels is their potentiation by covalent modification of cysteine residues [46,72]. So far, it is unclear whether, and if so to what extent, the two modes of K^+ channel activation – covalent modification and reversible binding – are mechanistically related. One approach to resolve this issue might be to examine channel sensitivity to a reversible activator after treatment with a saturating concentration of a covalent modification agent such as NEM. Because cysteine modifications can conceivably take place within the fluctuation window of native redox potentials induced by some cellular conditions [73,74], they might have physiological relevance. By contrast, there is no evidence for such a mechanism for reversible activators. The chemical structure of pyrithione shares some similarity with the side chain of histidine. It will be interesting to test whether either histidine or its metabolic derivatives in the presence of Zn^{2+} ions can potentiate native Kv7 channels. In preliminary studies, however, neither histidine alone nor histidine plus Zn^{2+} has been found to potentiate the Kv7.2 channel (Q.X. and M.L., unpublished), although the derivatives remain to be tested.

With respect to the channel activators discussed above, emerging evidence has begun to define the residues crucial for activity. By combining the atomic structure of Kv1.2 channels [75] with the abundance of mutagenesis data from structure–function studies, especially data concerning

disease relevance and inhibitory molecules [1,76], we can identify specific regions and can begin to construct models that might explain the mechanisms of potentiation (Figures 1 and 3). Indeed, like many other ion channels, Kv7 subunits can form heteromultimeric complexes by combination with different α and/or β subunits. Of particular interest, one study has shown that fenamates can affect Kv7 channel activity by modulating KCNE1 subunits that coassemble with Kv7.1 [77]. Most of the supporting evidence came from mutagenesis studies. It will be important to obtain direct binding data such that the experimental results can definitively distinguish those residues required for binding from those residues necessary for activity. To tackle this issue, it would be extremely useful to develop radioactive and photoactivated derivatives of the activators to dissect the molecular binding sites for agonistic activity.

Kv7 channels are modulated by various intracellular signaling factors and by coassembly with other subunits. Follow-up studies are essential to understand whether, and if so to what extent, the synthetic agonists function in the context of these modulators. Clearly, the resultant information is necessary for understanding the pharmacological potency of a given drug candidate. Mechanistically, there is considerable interest in defining the effects on Kv7 channels of intracellular Ca^{2+} , phosphatidylinositol biphosphate and phosphorylation. Knowledge of the role and potency of synthetic agonistic compounds would add another layer of interaction information useful in defining sites and modes of action. Along this line, it would be interesting to evaluate the functional stoichiometry required for the full effects of an activator and the influence of auxiliary subunits in determining compound sensitivity. For those activators with distinct molecular determinants, there might be compound effects if two activators are applied simultaneously.

In addition to traditional assays, new screening tools have been developed that could facilitate the isolation of both chronic and acute Kv7 modulators [78]. These modulators might affect channel activity by altering protein trafficking and surface density or by changing channel gating itself. Few activators of other cation channels have been identified, possibly owing to the nature of the channel structure and the mechanism of gating. Recently, however, several compounds that potentiate hERG (*ether-a-go-go*-related gene) channels have been discovered [79–83], in part because of the heightened interest in the function of the hERG channel in cardiotoxicity. Future studies that define the mechanism of action will provide considerable insight into how a ligand-mediated effect could circumvent voltage gating and result in conformational changes with an outcome similar to that normally caused by membrane depolarization. The list of potential clinical applications could become even longer as compounds with better specificity and potency emerge. Additional expansion of the uses of these compounds could come from a better understanding of the physiological function of Kv7 channels. Indeed, some of these compounds have already been shown to potentiate defective channels with mutations found in humans [62,68].

In summary, hyperexcitability is a common phenomenon found in various neurological diseases [1]. Although

the cause for excessive membrane excitation can vary, an increase in inhibitory activity such as K^+ channel conductance could provide a beneficial counter force, and thus therapeutic benefit. Because Kv7 channels are found in several different tissues – including the central nervous system, the peripheral nerves, cardiac tissue and some non-excitable tissues – the utility of Kv7 channel openers for treating various diseases should not be underestimated. Although several different chemical scaffolds have been reported, considerable work lies ahead to address issues such as subunit specificity, compound efficacy and therapeutic target choices. These channel activators serve as powerful pharmacological tools with which to study Kv7 function, as exemplified by retigabine and its analogue flupirtine. More importantly, they have been validated in clinical trials as potential therapeutic agents for the treatment of epilepsy; various types of pain including acute pain, neuropathic pain and migraine pain; and both neurodegenerative and psychiatric disorders [55–59].

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