

COMMENTARY

Chemical regulation of Kv7 channels: Diverse scaffolds, sites, and mechanisms of action

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Among the voltage-gated potassium channels, members of the KCNQ/Kv7 subfamily stand out for their powerful multi-pronged modulation by diverse stimuli (Barrese et al., 2018). The Kv7 channels (Kv7.1–Kv7.5) share common features of regulation by phospholipids (PIP₂), voltage, and calmodulin, in addition to a variety of regulatory signaling cascades that influence electrical excitability of cells. This unique ion channel family was first identified based on the role of Kv7.1 in heritable long QT syndrome (Wang et al., 1996). In the heart, regulation of Kv7.1 by adrenergic signaling shortens the cardiac action potential duration, allowing our hearts to beat faster in times of stress. Mutations of other KCNQ genes (encoding Kv7.2–Kv7.5) are most commonly linked to neurological diseases including early onset epilepsy and progressive hearing loss, although they also function in many peripheral tissues (Allen et al., 2020). Heteromeric channels comprising Kv7.2 and Kv7.3 are the primary contributor to the neuronal “M-current,” which is prominently expressed throughout the central nervous system and can profoundly alter the firing patterns of neurons when Gq-coupled signals trigger depletion of PIP₂ (leading to KCNQ channel suppression; Delmas and Brown, 2005). Moment-to-moment regulation of Kv7 channels, by diverse signaling systems, lies at the core of many of their well understood physiological roles.

In addition to prominent physiological regulation, the KCNQ/Kv7 channels have an extraordinary pharmacological profile relative to other voltage-gated potassium channels. The most widely studied pharmacological modulator of Kv7 channels is retigabine, which, along with its close structural analogue flupirtine, are the only voltage-gated potassium channel activators approved for use in humans (Gunthorpe et al., 2012). Retigabine has remarkable functional effects on Kv7.2–Kv7.5 channels, most notably causing prominent shifts of the voltage-dependence of activation as large as ~60 mV (this depends on the combination of subunits and expression system). Although retigabine was not widely adopted in its original application as an anti-epileptic drug, its clinical efficacy has motivated ongoing efforts to discover and understand Kv7 channel activators. Today, ~30 yr after the discovery of retigabine, ~20 yr after the recognition of its mechanism of action, and ~9 yr

after its FDA approval, we have in hand a large set of synthetic and naturally occurring Kv7 activator compounds. These compounds are diverse in terms of their structural scaffold, mechanism of action, specificity for different Kv7 channel subtypes, and functional effects. Although a comprehensive description of this fascinating pharmacology is beyond the scope of this commentary, I would guide readers to a recent extensive review on this topic (Miceli et al., 2018). In this issue of the *Journal of General Physiology*, Larsson and colleagues expand our understanding of the diversity and mechanism of Kv7 activators in their report on the effects of a panel of endocannabinoid-like compounds (Larsson et al., 2020). Their findings reveal powerful functional effects, unexpected subtype specificity, and some of the chemical features that enable direct (cannabinoid receptor-independent) activation of Kv7 channels by some endocannabinoids. This comprehensive and interesting study provides valuable insights into the potential mechanisms of action of certain compounds like arachidonoyl-L-serine (ARA-S), which have endocannabinoid-like structures but weak affinity for the known native CB1 or CB2 cannabinoid receptors.

To place this work in context and highlight its significance, it is worthwhile to review in broad strokes what is known about mechanisms of activation of Kv7 channels by small molecules. The best recognized site of action for Kv7 modulation is a tryptophan residue in the S5 segment of the pore-forming domain, where retigabine, flupirtine, and many other KCNQ modulators are thought to bind (see Fig. 1, yellow highlight). The important role of this residue has been known for quite some time and was identified based on comparisons between Kv7.1 (retigabine insensitive) and Kv7.2 or Kv7.3 (retigabine sensitive; Schenzer et al., 2005). Mutation of this tryptophan, even to other aromatic side chains, abolishes retigabine sensitivity, and this is often used as a litmus test to distinguish whether a drug acts via this commonly targeted site (Kim et al., 2015).

Importantly, this is not the only mechanism underlying chemical activation of Kv7 channels. Subsequent drug screening efforts identified additional compound classes that retained the ability to activate Kv7.2 channels carrying a mutation of the

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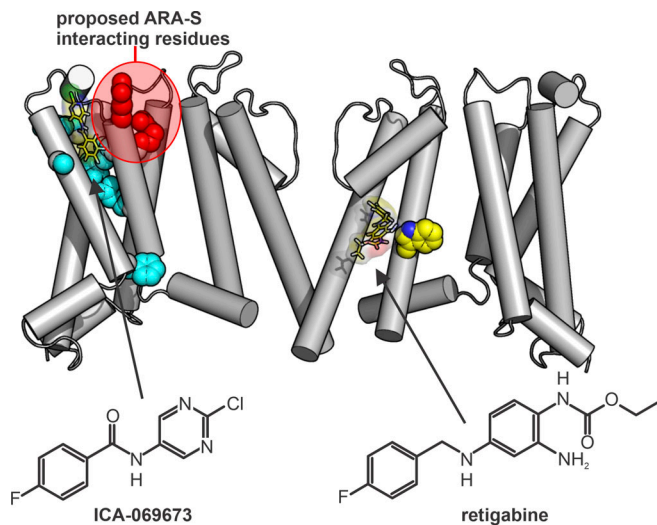


Figure 1. Distinct sites of action for Kv7 channel activators. Likely regions of binding and the location of implicated amino acid residues are highlighted on a homology model of Kv7.2 based on PDB accession no. 5VMS. The critical tryptophan in the retigabine binding site is highlighted in yellow (Schenzer et al., 2005). Voltage sensor residues that influence effects of ICA-069673 are highlighted in cyan (Peretz et al., 2010; Li et al., 2013). Voltage sensor residues that influence ARA-S effects are highlighted in red (Larsson et al., 2020).

critical tryptophan in the retigabine binding site (Padilla et al., 2009). This finding uncovered a second mechanism of chemical activation of KCNQ channels targeted toward the voltage sensor (Peretz et al., 2010; Li et al., 2013). These drugs directly stabilize the activated conformation of the voltage sensor, exhibiting strong state-dependent binding to activated channel subunits (Wang et al., 2018). Compared with the retigabine binding site, many details of this voltage sensor-targeted site remain unclear. Detailed binding models have been proposed for voltage sensor-targeted Kv7 drugs (see Fig. 1, cyan), but residues that influence sensitivity or subtype specificity of these drugs have been identified throughout the voltage sensor. Moreover, many of these residues are essential contributors to voltage-dependent gating and thus may have indirect effects on drug sensitivity. Besides these two prominent modes of Kv7 channel activator compounds, there have been other sporadic reports of compounds that are not well categorized into either group, such as Zinc-pyrithione (Xiong et al., 2007). Overall, while many powerful Kv7 activators have been discovered, a clear categorization and description of these drugs based on their sites and mechanisms of action remains a work in progress.

The report from Larsson et al. (2020) highlights the ability of certain endocannabinoid-like compounds to activate Kv7 channels. Some of the compounds tested are well recognized and prominent native agonists of cannabinoid receptors. For example, 2-arachidonoyl-glycerol has been reported to modulate some KCNQ channels by a cannabinoid receptor-mediated signaling cascade (Iannotti et al., 2014). Other endocannabinoid-like molecules have biological effects but more enigmatic signaling mechanisms, and it is one of these more obscure molecules (ARA-S) that stood out for its powerful actions on heteromeric Kv7.2/Kv7.3 channels. Although the authors judiciously point out that the physiological levels of ARA-S

are not known, it has been detected in brain lysates from other organisms and seem likely to be a physiologically generated compound. Thus, its reported ~ 20 – 30 mV shift in the voltage dependence of activation, in addition to prominent current enhancement, suggests a potentially large dynamic range for physiological channel modulation. Similarly, recent reports have highlighted unexpected direct chemical modulation of Kv7.2/Kv7.3 channels by GABA and other physiological compounds such as ketone bodies (likely acting through the retigabine site as they require the aforementioned S5 tryptophan; Manville et al., 2018, 2020).

The observations of direct activation of Kv7 channels by the ARA-S endocannabinoid have interesting implications in terms of drug mechanisms and physiology. The study findings indicate that actions of ARA-S are likely mediated by a noncanonical mechanism of action that is distinct from the two broad categories described above. Notably, ARA-S actions are resistant to mutation of the retigabine binding site in the Kv7 channel pore (W236 in Kv7.2, W265 in Kv7.3). Moreover, ARA-S also has prominent effects on KCNQ1 (which lacks this essential pore tryptophan). These findings provide concrete evidence that ARA-S does not act via the retigabine binding pocket. In addition, ARA-S is also resistant to a Kv7.2 mutation (F168L in the S2–S3 linker) that abolishes the actions of the most widely recognized/studied voltage sensor-targeted compounds (Wang et al., 2017). Overall, the absence of any effect of mutations that abolish sensitivity to established Kv7 activators suggests that ARA-S acts via a distinct mechanism.

Supporting the suggestion of a distinct mechanism for ARA-S, the authors describe several charge neutralization mutations on the extracellular side of Kv7.2 and Kv7.3 channels that attenuate the response to ARA-S. They identify positions on the extracellular side of S4 (Fig. 1, red highlight) that are close by but do not overlap with a “hot spot” of mutations in Kv7.2 that influence voltage sensor-targeted drugs (Peretz et al., 2010; Li et al., 2013). Additionally, they identify a positively charged residue in S6 that attenuates ARA-S mediated current potentiation, but not the shift of voltage-dependence. Overall, these effects provide some initial indication of a potential mechanism and draw similarity to previously published studies describing Kv channel modulation by structurally similar polyunsaturated fatty acids (Elinder and Liin, 2017). Specifically, based on structural differences between different endocannabinoids, the authors suggest a requirement for protonatable carboxylate headgroups (carrying a negative charge at physiological pH) to interact with these positively charged residues and facilitate movement of S4 and subsequent channel activation. Another important chemical feature of endocannabinoid and polyunsaturated fatty acid modulation of ion channels that has emerged is the degree of unsaturation of the acyl tail (Elinder and Liin, 2017), although it is still unclear how these chemical properties combine to determine endocannabinoid effects on Kv7 channels.

The authors extend their mechanistic investigation of endocannabinoids to describe coapplication of ARA-S with retigabine or ICA-069673. From a therapeutic perspective, the overall motivation of these experiments is the possibility that drugs with distinct mechanisms of action on a common receptor may interact and cause enhanced activation. This general principle is well-recognized in many ligand-gated ion channels, such as the synergistic effects of GABA, alcohol, and positive allosteric

modulators of the GABA_A receptor. However, in the context of Kv7 channel modulation, a mechanistic description of how different classes of Kv7 activators may interact has not been rigorously developed. In this study, the authors highlight the poor clinical performance of retigabine, which may be partially due to undesired side effects on peripheral Kv7 channels (in bladder smooth muscle, for example). Indeed, the lack of specificity of retigabine is frequently cited as a drawback, leading to efforts to engineer greater subtype specificity in emerging Kv7 activator compounds. An alternative approach is to use mixtures of drugs leading to a desirable combination of subtype specificity. In this way, the authors illustrate that combinations of low concentrations of ARA-S and retigabine can strongly activate Kv7.2/Kv7.3 heteromeric channels, while minimizing activation of Kv7.1 (due to channel insensitivity to retigabine) or Kv7.4 (due to channel insensitivity to ARA-S). An additional perspective on the importance of these findings lies in the growing number of naturally occurring compounds that can modulate Kv7 channel gating (Manville et al., 2020, 2018; Larsson et al., 2020). Although the magnitude of the effects of compounds including GABA, GABA metabolites, ketone bodies, and endocannabinoids are variable and depend on the Kv7 subtype being studied, this study highlights the possible interactions of these compounds as an area rich in possibility for future studies of Kv7 channel modulation. Lastly, another strong possibility is that individual differences in endogenous Kv7 modulator levels could significantly influence the effectiveness of retigabine or other Kv7-targeted drugs.

Overall, the Kv7 channels provide a fascinating system to study polymodal regulation of ion channel gating, as they are so dramatically regulated by a wide range of signaling systems and pharmacological tools. There has been an accelerating recognition of drugs and endogenous compounds, with diverse sites and mechanisms of action, which may interact and influence channel activity. Structures of Kv7 channels in complex with PIP2 and accessory proteins have begun to emerge (Sun and MacKinnon, 2020), so it is just a matter of time before we begin to benefit from high resolution structures of Kv7 channels bound to drugs. Further development of our understanding of chemical modulation of Kv7 channels will shed light on how these compounds can be used in a rational way to manipulate cellular electrical activity.

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