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# SMITten for KCNQ Channels

Anastasios V. Tzingounis[1,*]
[1]Department of Physiology and Neurobiology, University of Connecticut, Storrs, Connecticut

Over the last two decades, members of the KCNQ channel family of potassium channels (KCNQ1–5) have emerged as critical regulators of cardiac and neuronal excitability (1,2). KCNQ1 channels are the molecular determinants of the cardiac slow potassium current, $I_{KS}$ (1), which is partly responsible for the repolarization of the cardiac action potential. Loss-of-function variants of KCNQ1 channels lead to a rare heart condition, long QT syndrome, and gain-of-function variants lead to atrial fibrillation. In contrast, KCNQ2/3 channels are involved in neuronal activity. These channels are the molecular determinants of the M-current, a voltage-activated neuronal potassium current that exhibits slow activation-deactivation kinetics and no inactivation (2). KCNQ2/3 channels partially set the resting membrane potential of the axon initial segment, the site of action potential generation in neurons (2). Additionally, KCNQ2/3 channels contribute to the medium and slow afterhyperpolarization that follows a short burst or high frequency train of spiking activity, thus preventing excessive neuronal firing (2,3). Further highlighting the importance of these channels, a growing number of loss- and gain-of-function variants (primarily in *KCNQ2* and to a smaller extent in *KCNQ3)* have been reported in patients with severe neonatal and infantile epileptic encephalopathy.

The critical roles of KCNQ1 and KCNQ2/3 channels in physiology and disease have generated great interest toward understanding their properties. It is now well accepted that KCNQ channels require the presence of phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) to function (1). PIP2 exerts its effects on KCNQ1–3 channel activity by increasing the probability of channel opening and by strengthening the coupling between the voltage-sensor and the KCNQ pore. In addition to being regulated by PIP2, several studies have shown that KCNQ1 channel activity is controlled by a series of single transmembrane proteins, including KCNE1–3 (1).

More recently, the myo-inositol transporters SMIT1 and SMIT2 have been added to the pantheon of proteins that interact with KCNQ1–3 channels (4). SMIT1 and SMIT2 are symport carriers that use the downhill gradient of sodium ions to cotransport myo-inositol into cells. Myo-inositol is an osmolyte and a precursor to PIP2. In an earlier study, Neverisky and Abbott (4) found that SMIT1 and SMIT2 physically interact with KCNQ1–3 channels and reciprocally regulate their activity. For KCNQ2/3 channels, this interaction was thought to be functionally indirect, with SMIT1/2 bringing myo-inositol into cells, in turn leading to PIP2 formation and downstream activation of KCNQ2/3 channels (5). In the current study, Manville et al. (6) extended their previous work and revealed that SMIT1 not only physically interacts with KCNQ1–3 channels, but also alters their properties akin to a canonical auxiliary subunit, independent of changes in PIP2 concentration.

Manville et al. (6) first performed a series of tour-de-force biochemical experiments using truncated KCNQ2 channels to identify KCNQ2-SMIT1 interacting regions. Although the authors did not map the SMIT1 binding site, they found that SMIT1 interacts with the pore module, potentially by squeezing between two flanking S4 domains. This would position SMITs in a prime location to alter both the pore properties and the coupling between the S4 domain and pore gating. Indeed, the authors made several discoveries consistent with this model. Below, I briefly highlight the key findings of this study.

Although KCNQ2/3 channels are the molecular correlate of the M-current, when expressed in heterologous cells these channels do not recapitulate some of the M-current biophysical properties. For instance, the M-current has high permeability for cesium ($Cs^+$) ions in sympathetic neurons (7), unlike KCNQ2/3 channels expressed in heterologous cells (7). However, Manville et al. (6), following a second series of experiments using electrophysiology

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*Correspondence: anastasios.tzingounis@uconn.edu

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and ion substitution, found an increase in permeability for $Cs^+$ when KCNQ2/3 or KCNQ1 channels were coexpressed with SMIT1. These data suggest that SMIT1 binding to KCNQ channels indeed alters the conformation of the pore. In further support of this notion, coexpression with SMIT1 decreased the affinity of extracellular tetra-ethylammonium (TEA), a well-established probe for outer-pore conformations in potassium channels. Manville et al. (6) performed experiments using the traditional KCNQ channel inhibitor 10,10-*bis*(4-Pyrininylmethyl)-9(10*H*)-anthracenone dihydrochloride (XE991) that also supported the idea SMIT1-coexpression alters pore conformations. XE991 is a state-dependent blocker, preferably inhibiting KCNQ channels when they are in an activated open conformation (8); SMIT1 increased the blocking ability of XE991. Considering that SMIT1 also affects KCNQ channel voltage activation by increasing the G-V slope and activation kinetics, the data presented by Manville et al. (6) suggest that coexpression of SMIT1 along with KCNQ1–3 channels also alters the coupling between the voltage-sensing domain and the pore.

One possible explanation of the data is that KCNQ channels can exist between at least two pore conformations: "Cs impermeable" and "Cs permeable." In this scenario, the different conformations would result in different ion selectivity, XE991, and TEA pharmacological, and voltage activation properties. Based on this model, in the absence of SMIT1 KCNQ channels would be primarily found in a Cs-impermeable conformation; binding of SMIT1 to KCNQ channels would shift a high percentage of the KCNQ channels toward the Cs-permeable conformation. As the authors also found, in KCNQ1 channels, this conformation would partially counteract the inhibitory effects of increasing extracellular potassium concentrations on KCNQ1 currents, allowing them to maintain their activity even as the potassium gradient is decreased.

The observed changes by SMIT1 are reminiscent of a recent study showing that the auxiliary protein KCNE1 shifts the percentage of KCNQ1 channels between two different pore conformations, each exhibiting distinct pore properties (9). The current study found that SMIT1 regulates the effects of KCNE1 (and KCNE3) on KCNQ1 voltage activation, suggesting that SMIT1 and KCNE1 can form a macromolecular complex with KCNQ1. Therefore, the emergent model is that KCNQ1–3 can adapt multiple pore conformations whose distribution depends on an association with auxiliary subunits such as KCNEs and SMITs.

These new data also raise new questions. For instance, what is the channel-transporter stoichiometry in native cells? Although some of the observed properties such as increased $Cs^+$ permeability suggest functional coexpression of SMITs and KCNQ channels in vivo, some data suggest otherwise. For instance, the TEA affinity of the classically described M-current is ~5 mM, rather than the ~28 mM reported here with SMIT1-KCNQ2/3 channel coexpression. It is possible that in neurons the SMIT1-KCNQ2/3 channel stoichiometry is not fixed, giving rise to KCNQ-mediated current with variable properties. This might explain the two distinct TEA sensitivities of the M-current in cultured hippocampal neurons (10).

In addition, how do SMITs switch the conformation of the KCNQ channels from $Cs^+$ impermeable to $Cs^+$ permeable and how do the changes in KCNQ G-V and voltage activation take place? Considering previous work in KCNQ1 channels showing that distinct voltage-sensor domain conformations promote different pore conformations (9), SMIT1 might exert its effects by altering the link between the S4 domain and the KCNQ1–3 pore module. Another possibility is that SMIT1 might change the conformation of the KCNQ1–3 outer vestibule similar to what has been reported in Kv2.1 channels; Kv2.1 channels can adapt two open conformations, "TEA sensitive" and "TEA insensitive," based on the orientation of an outer vestibule lysine with regard to the pore axis (11). An analogous lysine is found in KCNQ3 channels, whereas KCNQ2 channels have a histidine in that position.

In sum, the work presented here is a leap forward for the field as it suggests that KCNQ1–3 channels can adapt multiple open channel conformations controlled by the presence of SMITs. It also raises the possibility that disease-related KCNQ variants might also perturb the KCNQ-SMIT interactions, which in turn could alter cardiac and neuronal excitability through changing PIP2 levels in cells. Consequently, to fully understand the spectrum of KCNQ2/3 and KCNQ1 channelopathies, future genotype-phenotype studies should also incorporate SMITs.

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