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COMMENTARY

Unconventional voltage sensing in an inwardly rectifying potassium channel

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Moment-to-moment regulation of ion channels by membrane voltage has been among the most intensely studied topics related to ion channel function. Our tendency may be to immediately turn our minds to questions related to voltage-sensing domains and their coupling to channel pores in formally categorized voltage-gated channels (i.e., Kv, Nav, or Cav channels with similar tetrameric architecture). However, other channel subtypes have evolved alternative mechanisms to respond to voltage despite lacking a canonical voltage-sensing domain. In a recent study in the *Journal of General Physiology*, Marmolejo-Murillo and colleagues report unexpected and previously unrecognized intrinsic voltage dependence of inwardly rectifying potassium (Kir) channels comprised of Kir4.1 and Kir5.1 (Marmolejo-Murillo et al., 2021). These findings test previously held assumptions related to mechanisms of inward rectification—which have been primarily studied in a few model systems, with findings frequently generalized to other members of the Kir channel family (Hibino et al., 2010; Guo and Lu, 2003; Kurata et al., 2010b). Moreover, their findings highlight the diversity of mechanisms that may generate voltage-dependent gating in the absence of an apparent voltage-sensing domain.

The property of Kir channels to exhibit voltage dependence and preferentially conduct inward currents has long been attributed to their sensitivity to blockade by divalent cations and especially intracellular polyamines (Lopatin et al., 1994, 1995; Hibino et al., 2010). These multivalent organic cations are present in all cells, arising as byproducts of amino acid metabolism with intracellular concentrations of several hundred micromolar/liter, although ∼90% of cellular polyamines are bound to DNA, RNA, and phospholipids. Among their widespread biological roles is the ability to generate steeply voltage-dependent block of certain Kir channels. In terms of Kir channel block, the most potent endogenous polyamine is spermine. In addition to its high affinity, spermine block can exhibit an effective valence approaching 5 e_0 in certain Kir subtypes, including the Kir2.1 channel which has served as a prominent model for study of mechanisms of inward rectification (Guo and Lu, 2003; Lopatin et al., 1995). Although there have been varying reports over the years regarding residual gating properties of Kir channels in polyamine-free conditions (Sigg et al., 2018), many espouse a model describing Kir channels as devoid of meaningful voltage dependence when polyamines are absent (Guo and Lu, 2002; Kurata et al., 2010b). In my own experiences working as a post-doc with inside-out patch recordings of the strong inward rectifier Kir2.1, challenges including patch geometry (i.e., patches that are protected/recessed within the recording pipette), or contaminants in buffer solutions, often lead to experimental difficulties arising from residual channel blockade. However, many published recordings have shown that, in optimal experimental conditions, Kir2.1 channels devoid of polyamines do not exhibit gating behaviors over a very wide voltage range (Guo and Lu, 2002; Kurata et al., 2010b).

With this background in mind, the results presented by Marmolejo-Murillo et al. (2021) are interesting and surprising, as they demonstrate that prominent inward rectification persists in Kir4.1/Kir5.1 heteromeric channels, even in the absence of polyamines. These channels are indeed polyamine-sensitive, although not to the same extent as the Kir2.1 channel mentioned above. This is because in Kir4.1/Kir5.1 heteromers only the Kir4.1 subunit possesses an acidic residue (Kir4.1 E158) in the inner cavity, which is recognized as an important determinant for sensitivity to steeply voltage-dependent polyamine block. In contrast, Kir5.1 possesses a polar but neutral asparagine at this position (similar to weakly rectifying channels such as Kir1.1 and Kir6.2), and so the heteromeric channel combination yields intermediate polyamine sensitivity. Prior descriptions of Kir4.1/Kir5.1 heteromers have previously reported a slow time-dependent "activation" of current at negative voltages (Pessia et al., 1996; Casamassima et al., 2003), and while the underlying mechanism was unclear, a reasonable assumption would have been that it somehow involved relief of polyamine block.

Over time, optimization of conditions to study Kir channels have generated several tried and true approaches to minimize block by residual polyamines, as employed in this study by Marmolejo-Murillo et al. (2021). This includes replacing organic buffers such as HEPES with phosphate buffers, and including chelating agents such as EDTA (incidentally, most of my

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electrophysiologist colleagues of course add divalent ions to their recording solutions and believe it is futile to try to record in EDTA, but it works well in my experience). Despite these measures, along with extensive perfusion of patches, rectifying features of Kir4.1/Kir5.1 seem to clearly persist. These findings strongly suggest that the previously mentioned activation process involves an unanticipated intrinsic gating mechanism in these channels, perhaps operating alongside their modulation by polyamines (Marmolejo-Murillo et al., 2021).

The residual "intrinsic" gating mechanism that is revealed in Kir4.1/Kir5.1 heteromers clearly differs from voltage-dependent polyamine block, even though in this case it leads to a grossly similar outcome of attenuating outward K^+ currents. While polyamine block typically causes a steep cutoff of current over a narrow voltage range (reflecting a large effective valence associated with blockade), the residual voltage-dependence of Kir4.1/Kir5.1 is weaker and leads to a more gradual inhibition of current at depolarized voltages. With experiments reported thus far, the voltage dependence is difficult to accurately quantify but is certainly smaller than is typically seen for polyamine block; it is apparent in experimental records that even the most negative test voltages (approximately −140 mV in most cases) do not fully activate channels, suggesting a graded channel activity over a very broad voltage range (Marmolejo-Murillo et al., 2021). Interestingly, homomeric Kir4.1 channels do not appear to exhibit any prominent intrinsic voltage dependence, and so it seems likely that the intrinsic gating mechanism must arise from the Kir5.1 subunit. This is difficult to directly confirm as Kir5.1 is challenging to study in its homomeric form, but one older study has reported enhanced expression of Kir5.1 by coexpression with PSD-95, and recording of currents that exhibited fairly gradual rectification (Tanemoto et al., 2002). In terms of establishing this as a bona fide gating mechanism, the strongest evidence thus far is that the persistent voltage dependence of Kir4.1/Kir5.1 heteromers is markedly attenuated by phosphatidyl inositol-4,5,-bisphosphate (PIP2). This weakened ability of Kir4.1/Kir5.1 to close is consistent with the well-understood effects of PIP2 to stabilize the open state of all eukaryotic Kir channels including Kir4.1/Kir5.1 (Rapedius et al., 2007).

It is useful to consider this unique gating behavior in the context of other mechanisms of noncanonical voltage dependence. In channels equipped with voltage-sensing domains, there are rare cases where idiosyncratic features have enabled inward rectification, as seen for HCN or HERG channels. In these cases, unusual mechanisms of coupling or combinations of voltage-dependent transitions lead to enhanced channel activity at hyperpolarized voltages (Smith et al., 1996; Spector et al., 1996; Kasimova et al., 2019; Lee and MacKinnon, 2019). In contrast, Kir4.1/Kir5.1 heteromers are not equipped with an obvious structural mechanism for voltage sensing, and so an alternative mechanism must underlie their voltage dependence. Other closely related "sensor-less" channels have been reported to exhibit a voltage-dependent gating mechanism. In my own research, I stumbled across multiple examples of apparent voltage-dependent behavior of Kir6.2 mutants, which exhibited either outward rectification (Kir6.2[L157E] behaved much like a slowly activating Kv channel) or inward rectification (Kir6.2[F168E] exhibits gating properties very similar to the Kir4.1/Kir5.1 channels reported here; Kurata et al., 2010a; Vilin et al., 2013). Through that work we speculated that there may be a latent mechanism of voltage sensing in Kir channels that can be enhanced by certain mutations, but the Kir4.1/Kir5.1 heteromer is the clearest example of such a mechanism in a wild-type Kir channel. Another example of voltage dependence that emerges in a sensor-less channel is the prominent outward rectification apparent in many two-pore domain (K2P) channels (Schewe et al., 2016). Interestingly, as alluded to above for Kir4.1/Kir5.1, in many cases where lipid activation is possible (e.g., PIP2 for Kir channels, and arachadonic acid for TRAAK K2P channels), open-state stabilization at all voltages leads to significant attenuation of voltage dependence, suggesting a mechanism by which the voltage-dependent properties of these channels may be modulated physiologically (Kurata et al., 2010a; Schewe et al., 2016; Marmolejo-Murillo et al., 2021). Beyond the common tetrameric architecture of potassium channels, other channel families also offer examples of voltage sensing without a clear structural correlate. For example, CLC, TMEM16A, and TMEM16B channels exhibit clear voltage-dependent behavior in the absence of a distinct voltage-sensing module (Elvington et al., 2009; Peters et al., 2018).

A common thread in these diverse mechanisms of voltage dependence is the involvement of permeating ions. Although details vary between different channel types, a general theme is that voltage-dependent changes in the distribution of ions within the permeation pathway (or altered occupancy of regulatory sites) are coupled to gating. Consistent with this, Marmolejo-Murillo et al. (2021) demonstrate that the kinetics and overall voltage dependence of Kir4.1/Kir5.1 heteromers is markedly influenced by potassium concentration on both the intracellular and extracellular sides. Future experiments that systematically investigate how this gating process depends on the species and gradients of permeating ions will likely yield deeper mechanistic insights and models. For now, the authors propose a general model in which gating of Kir4.1/Kir5.1 heteromers is coupled to the direction of ion flux, but more subtle details as to how this may occur will certainly be interesting to uncover.

In summary, the recent study by Marmolejo-Murillo et al. (2021) establishes a clear polyamine-independent mechanism of voltage dependence in a Kir channel. This surprising finding stands as an outlier among previously studied models of inward rectification and adds to a growing list of channel types with unconventional mechanisms of voltage sensing that depend on their charged substrates.

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