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COMMENTARY

Sensing its own permeant ion: KCNQ1 channel inhibition by external K^+

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Ion channels, including voltage-gated potassium Kv channels, are essential membrane proteins responsible for regulating and maintaining cellular homeostasis. These channels play central roles in controlling key physiological processes, such as excitability and rhythmicity throughout the nervous and cardiovascular systems, as well as electrolyte transport through epithelia. As such, ion channels have evolved an exquisite degree of control allowing them to fine tune the cellular response to different stimuli, including alterations in temperature, chemicals, physical forces, and voltage (Hille, 2001). One family of Kv channels, the Kv7 family, has increasingly received attention due to their central role in physiological and pathophysiological conditions. Failure to regulate Kv7 potassium channel gating, either by inherited or acquired defects, results in a wide variety of debilitating and/or life-threatening disorders, including epilepsy, chronic pain, autism, cancer, secretory diarrhea, and cardiac arrythmias (reviewed in Liin et al., 2015 and Jespersen et al., 2005). Therefore, understanding the gating mechanisms of Kv7 channels and how this gating is regulated by different physiological and pharmacological activators and inhibitors is of utmost importance. However, a detailed understanding of the mechanistic basis underlying Kv7 channel inhibition, including those by the permeant ion, K^+, has not yet been achieved. In this issue of the *Journal of General Physiology*, Abrahamyan et al. (2023) used systematic alanine scanning mutagenesis, whole cell and single channel electrophysiology, and molecular dynamic (MD) simulation approaches to systematically investigate the molecular mechanism and structural components of Kv7.1 inhibition by external K^+ and unraveled the role of the selectivity filter (SF; at the most extracellular K^+ site, S0, in particular) in this process.

The voltage-gated Kv7 channel family comprises five members, Kv7.1–Kv7.5, encoded by the KCNQ genes (1–5; Abbott and Pitt, 2014; Jentsch, 2000; Jespersen et al., 2005). Different heterotetrameric combinations of Kv7.2, Kv7.3, and Kv7.5 form the neuronal "M-current," a major repolarizing potassium current expressed throughout the peripheral and central nervous systems which plays a crucial role in controlling neuronal excitability (Brown and Adams, 1980; Halliwell and Adams, 1982; Schroeder et al., 2000; Wang et al., 1998). Kv7.4 channels, mainly expressed in the outer hair cells of the inner ear and in the brainstem, play a prominent role in controlling potassium homeostasis (Jentsch, 2000; Jentsch et al., 2000; Kubisch et al., 1999). The Kv7.1 channel, also referred to as KCNQ1 or KvLQT1, is probably the most extensively investigated member of the Kv7 channel family and the only one that coassembles with accessory β subunits (KCNE1–5; Jespersen et al., 2005). The capability of KCNQ1 to associate with other proteins confers a high degree of gating flexibility allowing KCNQ1 to play diverse physiological roles in a variety of excitable and non-excitable tissues, including the heart, inner ear, pancreas, thyroid gland, airways, and the gastrointestinal system (reviewed in Jespersen et al., 2005 and Liin et al., 2015). In cardiomyocytes, KCNQ1 associates with the accessory subunit KCNE1 to form the I_{Ks} potassium current, a slowly repolarizing current that contributes to the regulation of the duration of cardiac action potentials (Barhanin et al., 1996; Sanguinetti et al., 1996). In the inner ear and the intestinal epithelia, KCNQ1 associates with KCNE1, KCNE2, or KCNE3 to regulate K^+ homeostasis needed for electrolyte and hormone transport (Liin et al., 2015; Maljevic et al., 2010). Unsurprisingly, inherited or acquired defects in KCNQ1 channel function are associated with a range of disorders, including fatal cardiac arrhythmias and deafness (Anantharam et al., 2003; Goldenberg et al., 2008; Maljevic et al., 2010; Nerbonne and Kass, 2005). Therefore, gaining deeper insights into the biophysical properties governing KCNQ1 channel gating, as well as understanding how these membrane proteins are regulated by physiological and pharmacological modulators is central to understand their function in health and disease. This knowledge will put us a step closer to the development of therapeutic strategies to alleviate or treat disorders associated with KCNQ1 channel dysfunction.

Besides changes in membrane voltage, KCNQ1 gating is regulated by a wide range of intracellular signaling molecules and pharmacological agents including phosphatidylinositol-4,5-bisphosphate (PIP2), ATP, protein kinase A (PKA), ML277,

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polyunsaturated fatty acids (PUFAs), and Ca^{2+} through calmodulin. A comprehensive review of the abovementioned modulation of KCNQ1 is beyond the scope of this commentary and can be found elsewhere (Liin et al., 2015; Jones et al., 2021). Instead, this commentary focuses on the less-well understood mechanisms by which extracellular potassium ions (K^+_o) inhibit KCNQ1 channel conductance, investigated in a comprehensive study by Abrahamyan and co-workers (Abrahamyan et al., 2023). It was previously shown that extracellular potassium can inhibit KCNQ1 channels by a mechanism that differed from the classical C-type inactivation seen in other Kv channels (Larsen et al., 2011). That study showed that external potassium exerted its effect through stabilization of the inactivated state of the channel (Larsen et al., 2011), likely through a negatively charged glutamate residue at position 290 (E290; Wang et al., 2015), located in the turret of KCNQ1. E290 was proposed to serve as a negative potential sink attracting potassium ions to the vicinity of the external pore. However, the detailed mechanisms by which K^+_o modulates KCNQ1 channel and the structural components involved in such regulation were not completely understood.

In the current article, Abrahamyan and coworkers (Abrahamyan et al, 2023) tackle this question directly by combining systematic alanine scanning mutagenesis with a method to estimate the fractional fast inactivation in KCNQ1 channels (Tristani-Firouzi and Sanguinetti, 1998). To address whether the electronegative potential produced by the negatively charged residues surrounding the extracellular surface of the pore acts as the K^+_o regulatory site, the authors first mutated to alanine all these residues, which were identified based on the published high-resolution structure of KCNQ1 (Sun and MacKinnon, 2017). They found that the E290A/S291A/E295A triple mutant displayed a similar degree of K^+-induced inhibition of the currents as that seen in wt-KCNQ1 channel. Furthermore, substitution of the E290 residue with different polar, non-polar, and charge-reversed amino acids unequivocally confirmed that the negatively charged extracellular amino acids, including the previously reported E290 residue, play only a minimal role in the K^+_o-dependent inactivation process. These results motivated the authors to extend their search to residues in the SF and the pore gate as new putative regulatory site for external K^+. They compared mutations reported to have a marked reduction (e.g., F351A and L271A) or enhancement (e.g., I274A) of inactivation with the inactivation of the wt-KCNQ1 channels. Extracellular exposure to increasingly higher K^+ concentrations inhibited the ionic current of the F351A and L271A mutants similarly to that seen in the wt-KCNQ1 channel. Single-channel recordings showed that contrary to the stabilizing effect of K^+_o on the SF seen in most K-channels, K^+_o produced a marked reduction in the unitary conductance of the KCNQ1 channel. Further alanine scanning mutagenesis along the S6 gate revealed varying degrees of K^+_o-sensitivity, with some mutations right below the SF, such as V310A, F339A, and F340A, producing a surprising enhancement of current in response to K^+ exposure. Surprisingly, at the single-channel level, KCNQ1 bearing the F339A mutant exhibited current amplitudes that were higher than those seen with the wt-KCNQ1 channel, but similar under conditions of low and high external potassium. The inconsistency between the macroscopic current enhancement and unaltered unitary current under high K^+_o conditions in the F339A mutant was attributed to allosteric changes in other channel regions. While these data pointed toward an allosteric coupling effect of the inner gate of the KCNQ1 channel toward the SF, further studies will be needed to reconcile these intriguing findings. Together, these results suggested that external potassium directly inhibits KCNQ1 channel conductance by a novel mechanism in which the SF and the inner gate play synergistic modulatory roles in K^+_o-dependent inhibition of KCNQ1.

To gain further insights into the molecular underpinnings of K-induced inhibition, long-timescale atomistic simulations of the channel incorporated in a model membrane under voltage and ion gradients were employed (Fig. 1). The simulations in low external K^+ concentrations in KCNQ1 showed that, like most prototypic K-channels (Fig. 1 A), the K^+ knock on of the K^+ at the most intracellular K^+ site, S4, triggers the release of the K^+ bound at the most extracellular K^+ site, S0, into the external side of the pore (Fig. 1 B, left). However, unlike most channels, 40% of the K^+ permeation in KCNQ1 seems to follow another mechanism in which the release of K^+ from the S0 site into the external side of the pore occurs spontaneously without involving knock on of K^+ at S4 site in the inner side of the SF (Fig. 1 B, right). Simulations in high external potassium, however, showed that K^+ binds more frequently and spends longer time at S0 site, thereby reducing the outward permeation events because of a delay in the (spontaneous) K^+ release from S0 site to the external solution (Fig. 1 D). This important finding helped explain why under high external K^+ conditions both macroscopic and unitary K^+ currents are inhibited. This mechanism differs from the stabilizing effect of K^+_o on the conductive conformation of the SF seen in most potassium channels, as exemplified by the attenuation of C-type inactivation by external potassium seen in many studies (Fig. 1, A', A'', and C; Baukrowitz and Yellen, 1995; Cuello et al., 2010; Lopez-Barneo et al., 1993). Interestingly, recent structural work in a mutated Shaker channel suggested that C-type inactivation entails the dilation of the ion selectivity filter (Fig. 1 A''; Reddi et al., 2022; Tan et al., 2022). High resolution structural work under conditions of low and high external potassium will be needed to investigate whether K^+_o-mediated inhibition of KCNQ1 channels would involve neither dilation nor constriction of the external pore as predicted for a C-type inactivated filter. In any case, the model proposed by Abrahamyan et al. (2023) suggests that the ionic current inhibition in KCNQ1 channels results from an increased occupancy of the filter S0 site by external potassium. This is an important finding with implications toward our understanding of the modulation of KCNQ1 or other related channels.

Since KCNQ1 channels are expressed in various tissues (reviewed in Jespersen et al., 2005; Abbott and Pitt, 2014) where they control processes as diverse as action potentials in the heart or electrolyte and hormone transport in epithelia, high extracellular potassium might have significant physiological implications. In higher organisms, the potassium concentration found in interstitial spaces is tightly controlled within strict

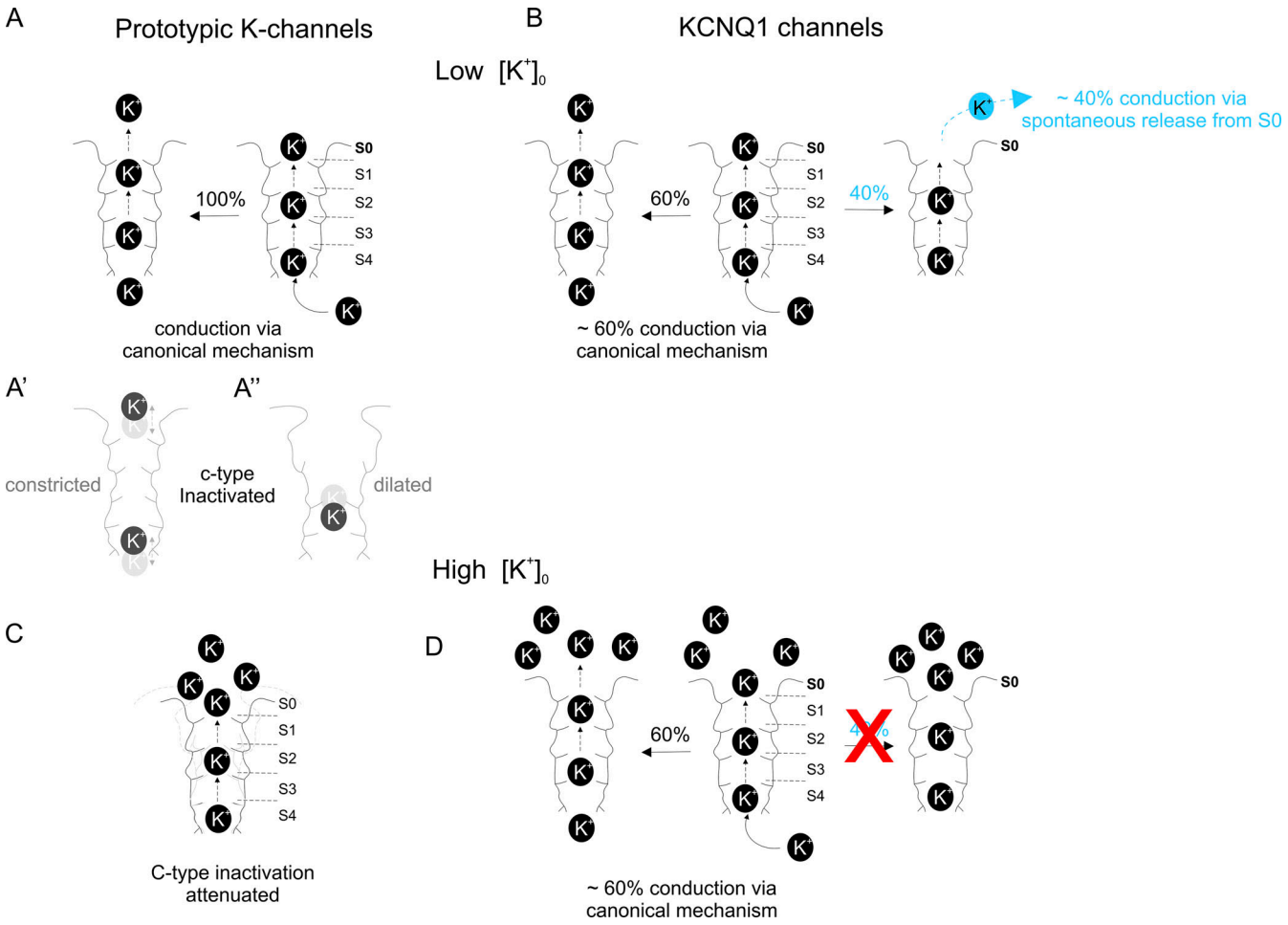

Figure 1. **K-channel inhibition by extracellular potassium.** Cartoon depicting proposed model of K^+_o inhibition of prototypic K-channels (A, A', A'', and C) and KCNQ1 channels (B and D). **(B)** In KCNQ1 channels, unlike for most K channels, under low $[K^+]_o$ conditions, there is a 40% spontaneous release of K^+ out of the S0 site of the selectivity filter. **(D)** In KCNQ1 channels, (bottom) high $[K^+]_o$ causes an increase in the frequency and duration of K^+_o occupancy in S0 site that leads to a reduction of the spontaneous K^+ permeation through S0 site. **(A–A'' and C)** Cartoons depicting C-type inactivation like that shown in KcsA (A'), Shaker Kv channels (A''), and attenuation of C-type inactivation under high $[K^+]_o$ (C).

physiological ranges since small variations in extracellular potassium levels can alter key physiological functions, including electrolyte homeostasis, resting membrane potential, cell division, or even drive cells into apoptosis (Bortner et al., 1997; DeCoursey et al., 1984). In contrast to the heart, where interstitial K^+ rarely exceeds 4–5 mM, extracellular K^+ concentrations in the intestinal and tracheal epithelia increase several folds, reaching values as high as 150 mM in the endolymph of the inner ear. Therefore, in theory K^+_o inhibition of the I_{Ks} (KCNQ1/KCNE1 complex) channels could disrupt the endocochlear potential (Nin et al., 2008), thereby compromising hearing and balance functions. Likewise, in basolateral membranes of intestinal and tracheal epithelial cells (Preston et al., 2010), where KCNQ1 co-assembles with KCNE3 to form voltage-independent KCNQ1/KCNE3 potassium channels, extracellular K^+ inhibition might impact apical water and Cl^- secretion with pathophysiological consequences such as promoting inflammatory bowel disease. Nevertheless, as highlighted by the current study of Abrahamyan and co-workers (Abrahamyan et al, 2023), the degree of K^+_o-dependent channel inhibition not only depends on the type of β subunit with which KCNQ1 associates, but also on the number of accessory subunits assembled. Thus, while heteromeric KCNQ1/KCNE1 channels with saturating KCNE1 content (4:4) are insensitive to external K^+, KCNQ1/KCNE1 complexes at lower stoichiometric rates (and heteromeric KCNQ1/KCNE3 channels irrespective of the amount of KCNE3 subunits) would be more significantly impacted by external K^+. The dynamic expression of varying KCNQ1/KCNE1 subunit assembly might be physiologically relevant during development as transepithelial potassium secretion from marginal cells of the stria vascularis to the endolymph changes from relatively low to high K^+. Indeed, the mechanism of K^+_o inhibition unraveled in the current study might offer an interesting novel tool that could be used to estimate stoichiometric ratios of KCNQ1/KCNEx subunit in different tissues, as homomeric KCNQ1, KCNQ1/KCNE3, but not KCNQ1/KCNE1 or KCNQ1/KCNE2 channels, would be sensitive to external K^+.

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