Alexandria Miller Chairman's Prize Essay

We recently published the first high-resolution three-dimensional structure of a human two pore domain (K2P) potassium channel family member, human K2P1. This structure represents one of only ~15 human membrane protein structures that have been currently solved. K2P channels represent a class of potassium (K^+) channels that function to establish and maintain the resting potential in eukaryotic cells. This process primes cells for diverse responses such as action potentials in excitatory cells and cell signaling cascades, which can direct growth and motility in non-excitable cell types.

K^+ channels are the largest family of ion channels in eukaryotes with over 80 genes in humans. K^+ channels are highly-selective for K^+ over other monovalent cations and, like other ion channels, switch between conductive (open) and non-conductive (closed) states through a process called gating. In the case of K2P channels, gating is modulated by a range of cell stimuli and pharmacological agents including temperature, pH, polyunsaturated fatty acids, mechanical stress, and anesthetics. Electrophysiological and K2P knockout mice studies suggest roles for these channels in neuroprotection, pain perception and anesthetic modulation through a common mechanism of hyperpolarization and decreased cell excitability. In humans, missense mutations within a K2P channel family member have been associated with typical familial migraine with aura. Although the therapeutic potential of K2P channels is evident, our limited understanding of how K2P channels gate and why this subfamily and not other K^+ channels respond to these stimuli has attenuated progress towards K2P channel drug discovery. Structural studies of K2P channels would provide insight into K2P channel gating and suggest strategies for drug design.

The K2P1 structure is dimeric and has three features that have not been previously observed in tetrameric K^+ channel structures. A 56 amino acid extracellular cap is positioned above the extracellular entryway and may serve as a molecular shield against K^+ channel protein toxins. The cap also creates an extracellular ion pathway with two side portals that can accommodate hydrated K^+ ions. In addition, we speculate that this may be a point of interaction with other extracellular proteins or ligands, which could provide another mechanism of K2P channel modulation that has not been studied. The extracellular cap is a feature that is well-conserved in the K2P channel family.

An amphipathic 'C-helix' runs parallel to the cytosolic face of the membrane. Its connection to transmembrane helix (TM) 4 and positioning near TM 2, both of which are helices that line the ion pore and have been previously implicated in K^+ channel gating, suggests that the C-helix may be involved in gating. Mutations to the C-helix in other K2P channels reduce the channel open probability, lending further evidence to its role in gating.

Finally, the K2P1 ion pore is exposed to the lipid bilayer through openings in the intramembrane molecular surface, accommodating electron density that we attribute to lipid or detergent alkyl chains. We speculate that this may be an access point for endogenous lipids as well as lipophilic compounds such as tetrahexylammonium, recently shown to be a K2P channel inhibitor.

Overall, the human K2P1 structure defines the molecular architecture of the K2P channel family and also lays a foundation for further investigation of how K2P channels are regulated by diverse stimuli. The structural features unique to K2P channels, and in particular the potential role of C-helix in gating, provide possible targets for K2P-specific therapeutics and an important direction for future work.

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