A helical segment makes potassium channels go-go

Lucie Parent

From the Département de Pharmacologie et Physiologie, Faculté de Médecine, Centre de recherche de l’Institut de Cardiologie de Montréal, Université de Montréal, Montréal, Québec H3C 3J7, Canada

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More than 500 variants in the KCNH2 gene, which encodes the cardiac human ether-a-go-go (hERG) ion channel, have been associated with sudden cardiac death, but only a subset of these variants have been investigated. Matthew D. Perry and colleagues now combine NMR spectroscopy and electrophysiological experiments to explore the functional properties of mutations within an overlooked hERG helix, finding important contributions to channel function.

Congenital loss-of-function mutations in the KCNH2-encoded hERG channel expressed in cardiomyocytes cause a reduction in the rapid repolarizing potassium current IKr and a significant increase in the heart repolarization QT interval (Fig. 1A) (1). This genetic disorder associated with life-threatening arrhythmias belongs to the LQTS2 family of channelopathies (2, 3). The hERG channel (also known as Kv11.1) is characterized by six transmembrane-spanning helices (S1–S6) with S1–S4 forming the voltage-sensor domain (Fig. 1B). Four of these proteins need to assemble to form a functional channel with a unique ion-conduction pathway lined by the four S6 helices. Similar to other voltage-gated ion channels, the flow of ions is controlled by the movement of an activation gate, formed by the cytoplasmic ends of the four S6 helices, and the movement of an inactivation gate located at the selectivity filter. Nonetheless, the rates of activation (transition from the closed to the open state), deactivation (transition from the open to the closed state), and inactivation (transition from the open to the inactivated states) of Kv11.1 channels are distinctly different from those of other voltage-gated potassium channels. Their trademark signature, slow activation kinetics coupled with fast inactivation kinetics, makes Kv11.1 channels ideally suited to regulate the heart rhythm (4).

LQTS2-associated mutations have been reported in almost every transmembrane helix as well as in the intracellular N and C termini (5). However, mutations found before or within the S1 helix have been largely overlooked, leaving an important gap now being filled by Phan et al. (6). It has been argued that residues located in the pre-S1 helix contribute to biogenesis, protein folding, and/or inter-subunit interactions in related ion channels while the S1 helix is anchored within the voltage-sensing module where it contributes to channel function. As a first step in addressing the functional role of the LQTS2 mutations, the authors delineated the boundaries of the S1 helix, a region that is poorly conserved, by identifying the amino acids found in the hydrophobic (membrane) versus hydrophilic (aqueous) regions. This was achieved by implementing a de novo structural approach based on proton NMR spectroscopy. The NMR experiments were performed using a S1-like 45-residue synthetic peptide reconstituted in sodium dodecyl sulfate micelles in the presence of Gd3+, a paramagnetic agent, which suppresses signals from residues located within the micelle environment (7). Using this clever approach, it was noted that Gd3+ suppressed signals in the region Arg-394 to Ala-408 (not in a transmembrane domain), whereas the signals for residues Trp-410 to Glu-435 were largely unaltered, suggesting that they form the transmembrane portion of S1. The data do not support the presence of a pre-S1 helical segment in agreement with the three-dimensional structure of the homologous rEAG channel obtained by cryo-electron microscopy (8) but in contradiction with previous suggestions.

With the alignment of the helix defined, the authors then applied an alanine-scanning mutagenesis approach to examine the functional role of the 45 residues in and around the S1 helix. The authors used electrophysiological recordings to measure the changes in the activation energies associated with the individual mutants expressed in recombinant systems. From the absence of correlation between the rates of deactivation and activation, the authors concluded that the channel transits through conformational states that are not simply mirroring one another. Furthermore, given that S1 mutants were more likely to accelerate deactivation than activation kinetics, the authors concluded that the S1 helical segment may contribute to the slow deactivation of kinetics. This second series of experiments on its own represents an impressive amount of work and supports a role for S1 in the channel gating.

Having established that the S1 helix is functionally important, the authors were then curious to assess the functional impact of the LQTS2-associated mutations. Experiments performed with six mutations located in the pre-S1 loop (I400N, H402R) or the S1 helix (W410S, Y420C, T421M, S428L) showed some degree of reduction in the membrane expression of the channel protein (as previously shown for the T421M mutation (5)), as well as changes in the kinetics of transitions between the channel conformational states. These effects are congruent with the increase in the repolarizing interval associated with the mutations. However, the extent of these changes may not be sufficiently important to cause lethal arrhythmias in...
the current set of data establishes that the LQTS2 mutations identified in the first transmembrane domain S1 in Kv11.1 may prevent repolarizing outward currents in cardiomyocytes (Fig. 1C).

Even more than demonstrating a role for S1 in modulating the kinetics of Kv11.1 channels, the study by Phan et al. (6) illustrates the power of embracing multimodal approaches to solve lingering issues in membrane transport. This approach could be extended to study the biochemical properties of many other disease-causing mutations, which in turn may account for changes in channel function, not only in Kv11.1 but in other ion channels. Identification of protein surfaces by NMR spectroscopy can be seen as an emerging strategy to study transmembrane domains in integral membrane proteins. In this regard, the work by the group of Matthew D. Perry is opening up new and exciting avenues of research.

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Figure 1. The ABC of cardiac electrophysiology. A, changes in the electrical activity of the heart are recorded as variations in the waves forming the electrocardiogram (ECG) (top trace). The heart repolarization QT interval (measured from the beginning of the QRS complex to the end of the T wave) is significantly increased as a result of congenital long QT syndrome such as the long QT syndrome 2 (LQTS2) (2nd trace shown in light blue). The normal (black) and longer LQTS2 (blue) action potentials are aligned with their approximate duration during the ECG (3rd trace). The time course of the hERG channel (IKr current) is significantly increased in LQTS2 probands (blue). B, the hERG channel is an integral membrane protein formed by the folding of 6 transmembrane helices (S1 to S6). Four of these subunits are required to form a unique aqueous permeation pathway. LQTS2-associated mutations in the S1 helix are identified. C, cartoon of a cardiomyocyte (gray) with some of the ion channels that are present in the cell membrane. Openings of sodium (Nav1.5) and calcium (Cav1.2) channels carry depolarizing currents. Repolarizing potassium currents (Kv7.1 and Kv11.1) bring the cell back to its resting membrane potential while Kir2.1 stabilizes the membrane potential around the equilibrium potential for potassium ions. Mutations in the S1 helix of Kv11.1 (in green) could prevent the fluxes of potassium ions through hERG (shown by straight red line) and increase the repolarization interval. Auxiliary subunits are not pictured. This figure was adapted with permission from research originally published in *Heart*. Wilde, A. A. M., and Bezzina, C. R. Genetics of cardiac arrhythmias. *Heart*. 2005; 91:1352–1358. © British Cardiovascular Society.