Re-examining the role of the voltage sensor in hERG channel inactivation

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The human ether-a-go-go related gene (hERG) potassium channel plays an important role in repolarization of the cardiac ventricular action potential. A reduction in the current passed through hERG channels (I_{Kr}) prolongs the action potential duration resulting in a disorder known as Long QT syndrome (LQTS). This disorder can, in turn, lead to life-threatening arrhythmias. HERG channels are part of a family of voltage gated potassium channels (K_v) which have six transmembrane spanning segments in the protein. The fourth segment (S4) is highly conserved among all family members and has been identified as the voltage sensing region for activation. However, hERG channels have gating properties that are unique from other K_v family members. In particular transition from the open to inactivated states occurs much more rapidly and is intrinsically voltage dependent. We have used the technique of ϕ-value analysis to investigate whether the S4 segment also plays a role in hERG channel inactivation.

ϕ-value analysis allows us to determine the temporal order of conformational changes at given sites within the channel protein in response to a single transition step (*i.e.* from open to inactivated). Point mutations were introduced into the channel protein and the kinetic rates for the onset and recovery from inactivation were determined. The ϕ-value was then calculated by comparing perturbations to the energetics of the transition state (derived from $\Delta\log(k_f)$ for mutant relative to WT where k_f is the forward rate constant at 0mV) to that of the stable end states (derived from $\Delta\log(K_{eq})$ for mutant relative to WT where K_{eq} is the equilibrium between open and inactivated states at 0mV). ϕ-values are calculated from the ratio of $\Delta\log(k_f)$ to $\Delta\log(K_{eq})$. As $\Delta\log(K_{eq})$ is the denominator for calculating a ϕ-value, small values for $\Delta\log(K_{eq})$ can give inaccurate results (Cymes, Grosman & Auerbach, 2002). Previous analysis have shown that $\Delta\log(K_{eq})$ should be at least 0.5 in order for us to confidently accept the derived ϕ-value of a residue. ϕ-values in the range of 0 to 1 have been interpreted as indicating the extent to which the reaction has occurred, with the value of 0 indicating the last step in the pathway and a value of 1 indicating a change at the start of the reaction. We have previously used this technique to demonstrate the relative contribution of regions of the hERG channel protein to the inactivation process (Wang *et al.*, 2010). However, point mutations of charged residues within the S4 segment did not provide sufficient perturbations in the inactivation process for an accurate ϕ-value to be calculated.

In the present study, a tryptophan, alanine and serine mutagenesis scan was conducted for all non-charged residues within the S4 segment. Electrophysiological recordings were then carried out using the two-electrode voltage-clamp technique. The most dramatic effect of all the mutant channels studied was found at residue V535. The average shift in $\log(K_{eq})$ of V535 mutations to Trp or Ser were found to be 0.91±0.10 (n=7) and 0.84±0.11 (n=7) respectively. Derived mean ϕ-values for V535W and V535S were 0.49±0.03 (n=7) and 0.57±0.01 (n=7). The overall ϕ-value for all V535 mutants studied (W, S, I) is thus 0.53 (R^2=0.98). These results reveal that a conformational change in the S4 segment occurs midway through the inactivation process. Other residues showed only mild changes in $\log(K_{eq})$ and thus were not considered reliable enough to give an accurate ϕ-value.

For the first time we have identified a residue (V535) in the S4 segment of the hERG protein that when mutated enables an accurate ϕ-value determination. From this we can determine that a conformational change in the vicinity of V535 in the S4 segment occurs approximately halfway through the temporal sequence of events that mediates the interconversion of the open and inactivated states of the hERG K^+ channel. This result clearly indicates that the S4 segment plays an important role in the inactivation process. However, whether the S4 segment functions as the voltage sensor for inactivation, remains to be determined.

Cymes GD, Grosman C, Auerbach A. (2002) Structure of the transition state of gating in the acetylcholine receptor channel pore: a ϕ-value analysis. *Biochemistry* **41:** 5548-5555.

Wang D, Hill AP, Mann SA, Tan PS, Vandenberg JI. (2010) Mapping the sequence of conformational changes underlying selectivity filter gating in the hERG potassium channel. *Nature Structural and Molecular Biology* provisionally accepted 4 September 2010.