Dynamics of inactivation of hERG potassium channels

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Potassium ion channels encoded by the human ether-a-go-go-related gene (hERG) have very unusual kinetic behavior, characterized by slow activation but very rapid inactivation. The unusually rapid and voltage-dependent inactivation of hERG is crucial for normal cardiac repolarization and suppression of propagation of premature beats. Inactivation of hERG channels result from conformational changes in the outer pore domain of the channel, however, the characteristics of the paths connecting the open conformation and inactive conformation of the channel remain largely unexplored. The aim of this study was to reveal the structural and temporal sequence of domain motions during hERG inactivation using the technique of rate-equilibrium linear free energy relationship (REFER, or phi-value) analysis. Wild type or mutant hERG cRNA was injected into *Xenopus laevis* oocytes and currents recorded using two electrode voltage clamp techniques. Rates of inactivation and recovery from inactivation were analysed at voltages in the range from −200 mV to +80 mV (the precise voltage range varied for each mutant) by fitting single exponential functions to the current traces recorded during double or triple pulse protocols. From these data we interpolated a value for the forward and reverse rate constants as well as the equilibrium constant for inactivation at 0 mV. The phi-value for each mutant was calculated as the ratio of the change in the log of the forward rate constant versus the change in the log of the equilibrium constant. Mutations in the S4, S5P and S5 domains all give phi-values of ~0.6, suggesting that mutations to these domains perturb early steps in the open to inactive state transition. In contrast, mutations to S631 have phi values of ~0.3, so mutations to S631 perturb a later step in the open-inactive state transition. Lastly, mutation to S624 gives a phi value of 0.01, so it clearly perturbs a very late step in the open-inactive state transition. These data suggests that inactivation involves a conformational wave of domain motions with peripheral domains moving earlier than more proximal domains and the final transition occurring at the interacellular end of the selectivity filter.