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Restoration of Deactivation in N-truncated and LQTS HERG K^+ Channel Mutants by a Recombinant N-terminal Region Fragment

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The Human Ether-á-go-go Related Gene (HERG) encodes a voltage-activated K^+ channel, which is a primary component of the cardiac delayed rectifier K^+ current (I_{Kr}). Cardiac I_{Kr} contributes to the repolarization of the ventricular action potential by conducting an outward K^+ current whose amplitude is determined, in part, by the closing (deactivation) rate of the channel. Deletion of the HERG N-terminus (amino acids 2-354) leads to a channel (N-truncated) with very rapid deactivation kinetics compared to wild-type HERG channels. To investigate how N-terminal interactions regulate gating, we constructed a genetically encoded N-terminal region fragment, and co-expressed cRNAs encoding N-truncated HERG and this fragment in Xenopus oocytes. Electrophysiological recordings from these cells showed channels with a significant increase in the time constant for deactivation over that of the N-truncated channels alone. The time constant was not significantly different from that of wild-type HERG channels. These results demonstrate a restoration of slow deactivation to the N-truncated channel by the N-terminal region fragment. Förster resonance energy transfer (FRET) experiments showed that the N-terminal region fragment and the N-truncated channel are in proximity at the cell membrane. Interaction of the N-terminal region fragment with the channel was also demonstrated using biochemical techniques. We next co-expressed the N-terminal region fragment with HERG channels bearing N-terminal mutations which have been shown to underlie Type II Long QT Syndrome (LQTS), a cardiac arrhythmia characterized by a prolongation of the ventricular action potential. The N-terminal region fragment recovered slow deactivation in these mutant channels. In summary, a recombinant N-terminal region interacted directly with N-truncated and LQTS mutant HERG channels to restore slow deactivation gating.

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Functional Consequences of RNA Editing of the e*ag* Potassium Channel

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RNA editing is an enzymatic process that selectively alters one or more specific bases in a transcript. This change can potentially affect the function of a protein by introducing a point mutation. Four RNA editing sites in *eag*, a *Drosophila* voltage-gated potassium channel, result in such recoding. These sites and the editing mutations are K467R, Y548C, N567D and K699R. They are located at the top of the S6 segment (site 1, residue 467), and the cytoplasmic C-terminal domain including the putative cyclic nucleotide binding domain (sites 2-4). We examined the consequences of editing at these sites by using mutant constructs, each containing one edited site on the background of an all-genomic (unedited) channel. We characterized these channels using two-microelectrode voltage clamp in *Xenopus* oocytes. The fully edited construct (all four sites) had the slowest activation kinetics and a paucity of inactivation at depolarized voltages, whereas the fully unedited channel exhibited the fastest activation and most dramatic inactivation. Editing the first two sites slowed activation kinetics more so than at the other two sites. Site 1 plays an important role in inactivation; mutating this site from lysine (unedited) to arginine (edited) causes a 54% decrease in the steady-state (700 ms) fraction of inactivated current at +80 mV. Mutating this residue to alanine, glutamine, glutamate or cysteine resulted in intermediate inactivation phenotypes. These neutral or acidic side-chains also caused a leftward shift of the peak current-voltage relationship. Exposure of the cysteine mutant to a variety of methanethiosulfonate reagents had little effect on channel biophysics. These results show that the position of the editing site and the identity of the amino acid at that site are important for fine-tuning the channel's function.

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Developing In Silico Descriptions Of Herg Channel Gating

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The human ether a-go-go related gene (hERG) encodes the pore forming subunit of the cardiac delayed rectifier potassium channel. Mutations in hERG that disrupt gating can cause Long QT syndrome type 2, a disorder of cardiac repolarisation characterised by prolongation of the QT interval and risk of sudden cardiac death due to ventricular arrhythmias. There is therefore considerable interest in developing accurate mathematical descriptions of hERG gating in order to simulate the effect of hERG mutants on cardiac action potentials at both the cellular and tissue levels.

In the first part of this study we have used a 5 state Markov model (1) minimised to in vitro electrophysiological data to describe hERG ionic currents associated with two clinically relevant mutants: G584S and G572S. These Markov descriptions were incorporated into models of the human ventricular action potential (2) and used to simulate the effects of these mutants on action potential waveforms and responses to premature stimuli. The results of our simulations for each mutant correlated well with the reported clinical phenotypes and were able to show how compensatory currents can contribute to phenotypic variability between families carrying the same hERG mutation.

In the second part of this study we compare the abilities of several Markov schemes to reproduce hERG currents. In doing so we evaluate their suitability for our goal of incorporating mathematical descriptions of hERG into whole heart simulations as a way of assessing how altered hERG function predisposes to the development of arrhythmias triggered by premature beats.

Our results demonstrate how in silico techniques lend an additional level of insight into understanding and characterising clinical mutations.

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Simulation of Ion Channel Gating: from Energy Landscapes to Macroscopic Currents

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A modeling framework was developed to simulate the dynamics of tetrameric ion-channels gating based on their molecular structure, in order to enable analysis of the effects of mutations and drugs on their dynamics and electrical function. It has been shown that altering the membrane potential causes independent motion of voltage sensors of the four channel subunits (S4 segments). It is also known that channel opening involves a concerted transition of all subunits from a preopening conformation to open conformation. Monte-Carlo simulations using Molecular Dynamics simulation software compute up to one microsecond of protein movement and cannot simulate ion-channel conformational changes which occur over milliseconds.

In our approach, a configuration space was chosen based on experimentally suggested conformations of voltage sensors. The conformational changes occur under the influence of two force fields: a conservative force field associated with the potential energy of the voltage sensor compartment, and a stochastic force field representing thermal collisions. The stochastic force field was smoothed by averaging over a time window small enough to retain the stochastic nature. The potential energy was estimated by the electrostatic potential between charged residues and was calculated in configuration space (energy landscape). The governing equation for the probability of conformations was derived and solved to simulate the dynamics of voltage sensor motion in response to membrane potential during gating.

The concerted transition of all subunits to open state was solved analytically and included in the simulations to compute open probability. The open probabilities simulated using this molecular approach for a series of voltage clamp tests produced macroscopic current traces that are typical of tetrameric potassium channels. This modeling framework also reveals the importance of subunit cooperativity in magnifying the sensitivity of open probability to membrane potential.

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Molecular Dynamics Simulation of the Kv1.2 Potassium Channel

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Voltage-gated ion channels are membrane proteins that open and close in response to voltage changes across the membrane. Voltage-gated potassium (Kv) channels are composed of two structurally and functionally separate domains, a voltage-sensing domain (VSD) and the ion conducting pore (PD). The voltage-sensing domain is comprised of four transmembrane segments that are highly charged and move in response to changes in the electric potential resulting in opening of the channel. We have developed complete models of the Kv1.2 channel in the open and closed state using the homology, de novo, and domain assembly methods of the structure prediction program ROSETTA. Molecular dynamics (MD) simulations were carried out to refine selected candidates of the open and closed state models of Kv1.2 in an explicit water-membrane environment. Each model was simulated for 100ns in the presence of a positive or negative voltage using the program NAMD. The magnitude of the gating charge that is transferred across the membrane upon opening of the channel is calculated based on 500ns of MD simulations of the full-channel and the isolated VSDs. In addition FEP simulations were performed to calculate the free energy of neutralizing (transmembrane) charged residues on the voltage-sensing helices, and to determine the fraction of the transmembrane voltage acting on each residue. The contribution of several charged residues (on the S4 and