Molecular Coupling in the Human ether-a-go-go-related gene-1 (hERG1) K+ Channel Inactivation Pathway

Tania Ferrer†, Julio F. Cordero-Morales‡, Marcelo Arias‡, Eckhard Ficker*, David Medovoy†, Eduardo Perozo‡, and Martin Tristani-Firouzi†‡**

From the †Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, Utah 84112, the ‡Department of Physiology, University of California, San Francisco, California 94143, the §Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western Reserve University, Cleveland, Ohio 44109, the ††Institute for Biophysical Dynamics, University of Chicago, Chicago, Illinois 60637, and the **Division of Pediatric Cardiology, University of Utah, Salt Lake City, Utah 84113

Background: Although fast inactivation gating of hERG1 K+ channels is crucial for maintaining electrical stability in the heart, the underlying molecular mechanisms remain unclear.

Results: Mutagenesis experiments and molecular dynamic simulations implicate coupling between the S5 segment and the pore helix during channel gating.

Conclusion: Coupling between the S5 segment and pore helix may participate in the inactivation process in hERG1 channels.

Significance: Elucidating the fundamental nature of hERG1 channel inactivation will advance our understanding of this clinically relevant channel.

Emerging evidence suggests that K+ channel inactivation involves coupling between residues in adjacent regions of the channel. Human ether-a-go-go-related gene-1 (hERG1) K+ channels undergo a fast inactivation gating process that is crucial for maintaining electrical stability in the heart. The molecular mechanisms that drive inactivation in hERG1 channels are unknown. Using alanine scanning mutagenesis, we show that a pore helix residue (Thr-618) that points toward the S5 segment is critical for normal inactivation gating. Amino acid substitutions at position 618 modulate the free energy of inactivation gating, causing enhanced or reduced inactivation. Mutation of an S5 residue that is predicted to be adjacent to Thr-618 (W568L), predicted to be adjacent to Thr-618, abolishes inactivation and altered ion selectivity. This data, derived from their unusual gating properties: slow activation and deactivation kinetics but very rapid C-type inactivation. Fast hERG1 inactivation produces marked inward rectification, a hallmark of hERG1 channels critical for their normal physiological function. The inactivated state also promotes the binding of common medications that cause the acquired form of LQTS (7). Thus, understanding the fundamental nature of C-type inactivation in hERG1 channels is interesting from both mechanistic and clinical standpoints.

C-type inactivation was first described in Shaker channels as a slow residual inactivation process revealed by the elimination of fast N-type inactivation (8). Recently, mechanistic insight into the structural basis of inactivation was provided by studies of the proton-gated K+ channel KcsA. Functional and structural data suggest that a multipoint hydrogen bond network between the selectivity filter and the pore helix plays an important role in determining the rate and extent of inactivation (9, 10). Additional evidence suggests that inner helix residues and the selectivity filter directly interact to couple opening of the activation gate to inactivation in the pore domain (11, 12).

Although hERG1 inactivation shares general features of C-type inactivation with other K+ channels, important differences exist. Like Shaker and KcsA, mutations in the outer mouth of the pore and changes in [K+], influence hERG1 inactivation (13, 14). However, hERG1 channel inactivation appears to be intrinsically voltage-sensitive and is not coupled to activation in the same way as in the Shaker family of K+ channels (15–17).

To further define the molecular basis of hERG1 inactivation, we performed an alanine scan of the hERG1 pore helix and showed that residue Thr-618, facing the S5 segment, is critical for normal inactivation gating. Amino acid substitutions at position 618 modulate the free energy of inactivation gating, causing enhanced or reduced inactivation. Mutation of an S5 residue (W568L), predicted to be adjacent to Thr-618, abolished inactivation and altered ion selectivity. This data,

---

**The on-line version of this article (available at http://www.jbc.org) contains supplemental Table 1.

† To whom correspondence should be addressed: Pediatric Cardiology, Suite 1500, PCMC, 100 N. Mario Capecchi Way, University of Utah School of Medicine, Salt Lake City, UT 84113. E-mail: mfirouzi@cvrti.utah.edu.
**Coupling in the hERG K⁺ Channel Inactivation Pathway**

Together with molecular dynamic simulations, demonstrated that the Thr-618-equivalent mutation in Kv channels stabilizes the inactivated state and markedly increases interaction energies between hERG1 618- and 568-equivalent positions. We suggest that hERG inactivation involves discreet molecular interactions between the S5 segment and the pore helix.

**EXPERIMENTAL PROCEDURES**

*Molecular Biology—*Site-directed mutagenesis, *in vitro* synthesis, and injection of cRNA into *Xenopus laevis* oocytes were performed as described (18). Oocytes were injected with 5–15 ng of hERG or Kv1.5 cRNA and incubated for 1–3 days at 19 °C.

*Electrophysiology—*Because of the rapid onset of inactivation relative to activation, the voltage dependence of hERG inactivation cannot be assessed using the traditional voltage protocols for the Shaker family of channels. Standard measures of hERG1 inactivation utilize a two-pulse or triple-pulse voltage protocol. Both protocols include a depolarizing voltage step to activate and inactivate channels. For the two-pulse protocol, the second voltage steps record peak current reflecting recovery from inactivation into the open state. Current magnitude is corrected for driving force and deviation from ohmic conduction to a rectification factor, which is plotted against the test potential (Vtest), yielding a half-point (V1/2) and valence (z) for hERG1 inactivation of −50 mV and 1.0, respectively, in physiological extracellular K⁺ (3). For the triple-pulse protocol, a brief (e.g. 10-ms) interpulse to a variable potential allows channels to recover from inactivation into the open state but is too short to allow channels to appreciably deactivate. A third test pulse is applied to a positive potential to measure the relative proportion of channels in the open state as a function of interpulse potential. Both protocols assess inactivation only after channels have been fully activated and measure the voltage dependence of the recovery from inactivation in isolation from early closed state transitions.

For the ease of screening a large number of mutants, we used the double-pulse voltage protocol and measured ionic currents with a GeneClamp 500B (Molecular Devices, Union City, CA) using the two microelectrode voltage clamp technique (19). Currents were filtered at 1 kHz with an eight-pole Bessel filter and then digitized at 2 kHz. To directly compare ionic and gating currents associated with inactivation (Fig. 5), we used the double-pulse voltage protocol and measured ionic currents using the two microelectrode voltage clamp technique when filled with 3 M KCl. All recordings were performed as described (18).

Coupling in the hERG1 inactivation of hERG demonstrates that hERG inactivation involves discreet molecular interactions between the S5 segment and the pore helix.

**Data Analysis—**To quantify the energetic perturbation produced by individual mutations, we calculated the free energy difference (ΔG) between the open inactivated transitions in WT channels and compared these values to the energy difference induced by the mutation. The difference in ΔG between WT and mutant channels (ΔΔG) reflects the perturbation in a gating transition produced by the mutation. The extent of inward rectification (rectification factor) was determined by the deviation of Irelmax obtained by the double-pulse protocol from a linear ohmic conductance and correcting for driving force (Vtest−Erev). The voltage dependence for steady-state hERG1 inactivation was obtained by plotting the rectification factor versus Vtest and fitting it to a Boltzmann function as in Equation 1,

\[
\frac{I_{rel}}{I_{max}} = \frac{1}{1 + e^{z(\frac{V_{test}}{T} - V_{0.5})}}
\]

where \(I_{max}\) is the maximum tail current, \(V_{t}\) is the test potential, \(V_{0.5}\) is the voltage required to elicit 0.5*\(I_{max}\), z is the effective valence, F is Faraday’s constant, R is the gas constant, and T is room temperature in °K. \(V_{0.5}\) and z values from the Boltzmann fitting were used to estimate the ΔG for inactivation using Equations 2 and 3,

\[
\Delta G = zFV_{0.5} - 0.02306zV_{0.5}
\]

where the conversion value 0.02306 kcal/(coulomb*mV*mol) incorporates the value of F.

\[
S_E,\Delta G = \frac{\Delta G}{2zS_E} + \frac{V_{0.5}V_{0.5}}{V_{0.5}V_{0.5}}
\]

where \(S_E\) refers to standard error. Perturbations in the ΔG (ΔΔG) were estimated for each mutation by Equation 4 and the standard error (\(S_E\)) calculated by Equation 5.
Coupling in the hERG1 K\(^+\) Channel Inactivation Pathway

\[
\Delta G = \Delta G_{\text{MUT}} - \Delta G_{\text{WT}} \quad \text{(Eq. 4)}
\]
\[
S_{\text{R},\Delta G} = \sqrt{S_{\text{R},\Delta G_{\text{WT}}}^2 + S_{\text{R},\Delta G_{\text{MUT}}}^2} \quad \text{(Eq. 5)}
\]

Data are expressed as mean ± SE (n = number of oocytes). Data plots, Boltzmann fits, and ΔΔG calculations were performed using Origin 7.0 (OriginLabs Corp., Northampton, MA). In earlier works (23, 24), we used a threshold value of ΔΔG\(_0\) \(\geq 0.5\) kcal-mol\(^{-1}\) to denote a significant mutation-induced perturbation in inactivation gating for residues distant from the selectivity filter/pore helix. Given the importance of the pore helix structure to inactivation gating, we reasoned that many mutations in this region might alter inactivation gating. As we were interested in mutations that markedly altered inactivation gating, we used a threshold value of ΔΔG\(_0\) \(\geq 1\) kcal-mol\(^{-1}\) to denote a significant mutation-induced perturbation in inactivation gating.

**Molecular Dynamic Simulations**—The Kv1.2 pore domain system used for simulations was taken from (25). The WT Kv1.2 pore domain system was equilibrated and subjected to a 20-ns MD simulation as described (25). Using the equilibrated WT system, the A368T mutation was performed in silico on all four subunits. The mutant system was minimized and then run for a total of 8 ns of Langevin dynamics using the program NAMD (26) at 310°K and with periodic boundary conditions at constant pressure and surface area. The CHARMM PARAM27 force field was used for protein, lipids, and TIP3P water. The electrostatic interactions were calculated according to the particle mesh Ewald algorithm with real-space cutoff of 13.5 Å. 400 snapshots covering the last 4 ns were extracted from the mutant simulation and WT simulation. The van der Waals and electrostatic interaction energies between residue 368 and each of residues 340–346 were computed for each subunit using the Visual Molecular Dynamics program (27) and the NAMD Energy plug-in.

**RESULTS**

**Alanine Scan of the hERG1 Pore Helix and Selectivity Filter Reveal Residues Critical for Inactivation**—To determine the contribution of individual residues to inactivation gating, we sequentially mutated 20 pore helix residues to Ala (Lys-608-Gly-628, Fig. 1A) and assessed inactivation using a two-pulse protocol. An initial depolarizing voltage step was used to activate and inactivate channels, followed by a second voltage step to variable potentials to record peak current reflecting recovery from inactivation into the open state. In all cases, the voltage dependence of inactivation was determined in [K\(^+\)]\(_o\) = 96 mm, as several left-shifted inactivation mutants displayed extremely small currents in [K\(^+\)]\(_o\) = 4 mm, but robust tail currents in [K\(^+\)]\(_o\) = 96 mm (Fig. 1B). Four Ala mutants (T618A, T623A, V625A, and F627A) enhanced inactivation, as noted by a hyperpolarizing shift in the inactivation-voltage relationship (Fig. 1C). These mutants generated a ΔΔG\(_0\) of stabilization \(\geq 1\) kcal-mol\(^{-1}\) in regards to the inactivated state, with T618A causing the greatest change in free energy (-2.0 ± 0.1 kcal-mol\(^{-1}\), n = 8, Fig. 1D). Four mutants (Y611A, A614V, Y616A, and F617A) failed to traffic properly to the cell membrane as measured by Western blot analysis and the absence of detectable gating currents (Fig. 2). Aromatic residues at positions homologous to Tyr-616 and Phe-617 were recently shown to be critical for proper monomer folding during K\(^+\) channel biogenesis (28). Two mutants located within the K\(^+\) channel signature sequence G-F-G (G626A and G628A) expressed on the cell membrane (measured by Western blot analysis and gating currents) but did not conduct ionic currents (Fig. 2).

**Mutations in the Selectivity Filter That Perturb hERG1 Inactivation Gating**—The canonical sequence T-S(T)-V-G-F(Y)-G defines the selectivity filter in all voltage-gated K\(^+\) channels. Given the critical role of these residues in ion conduction and selectivity, we were not surprised that three of the four inactivation-perturbing residues in our Ala scan localized to the selectivity filter (Thr-623, Val-625, and Phe-627). However, we wished to define the side chain requirements for normal inactivation gating by mutating these positions to aromatic (Trp, Tyr, Phe), polar (Ser, Thr) and hydrophobic (Val, Leu) residues. Of the tested amino acids, only the conservative Ser substitution at position Thr-623 resulted in channels with essentially normal inactivation gating (Fig. 3A). Hydrophobic substitutions markedly enhanced inactivation, whereas aromatic substitutions were not tolerated at position Thr-623. For Val-625, the side chain requirements were even stricter, with the tested substitutions resulting in channels that failed to express functionally. Only the conservative Ala substitution resulted in functional channels, albeit with markedly perturbed inactivation gating. At position Phe-627, even the conservative Tyr substitution perturbed inactivation, despite the observation that Tyr occupies the equivalent position in the majority of K\(^+\) channels. Thus, the side chain requirements for maintaining stability of the selectivity filter are very strict, with most mutations resulting in nonfunctional channels or increased inactivation.

**Different Side Chains at Thr-618 Exert Opposite Effects on hERG1 Inactivation Gating**—We were more interested in Thr-618 as this inactivation-perturbing residue is located along the mid-portion of the pore helix and is predicted to face toward the S5 segment (Fig. 3B). To further define the role of this position in inactivation gating, Thr-618 was mutated to hydrophobic, uncharged polar and aromatic residues. T618V mutant hERG1 channels displayed a positive shift in the free energy of inactivation (ΔΔG\(_0\) 0.5 ± 0.1 kcal-mol\(^{-1}\), n = 4). Although this value is lower than our threshold of 1 kcal-mol\(^{-1}\), the rightward shift suggests that the mutation stabilizes, to some degree, the open state and/or destabilizes the inactivated state. Substitution of Thr-618 with a larger hydrophobic residue (Leu) or a conserved residue (Ser) caused a negative shift in inactivation gating (Fig. 4A). Aromatic amino acids were not tolerated at the 618 position. There were no appreciable differences in the values of the valence or the half point of activation between these two mutants (from 2-s depolarizing voltage steps), suggesting that these mutants did not alter activation gating (Fig. 4B).

Although the precise nature of the voltage-sensing mechanism for hERG1 inactivation is not known, gating charge displacement (Q-Inact) can be measured using a triple-pulse voltage step protocol similar to that used to determine the voltage-dependence of ionic current inactivation (I-Inact). We previously showed that an inactivation-deficient mutant (S631A...
hERG1) did not affect gating charge displacement (21), suggesting an alteration in coupling between the voltage-sensing mechanism and rearrangements in the pore helix that produce inactivation. We wondered whether the inactivation-perturbing T618V mutation affected gating charge displacement or coupling of voltage sensing to inactivation. The voltage dependence of \( I_{\text{Inact}} \) and \( Q_{\text{Inact}} \) of WT and T618V hERG1 channels were measured using identical triple-pulse voltage protocols in \( K^+/H_11001 \) and TEA-containing solutions, respectively (see “Experimental Procedures”). Representative gating current records for WT and T618V hERG1 channels are shown in Fig. 4C. The \( V_{1/2} \) and \( z \) for WT hERG1 Q-Inact was \(-127 \pm 1.4 \text{ mV} \) and \( 1.0 \pm 0.2 \text{ e}_p \) respectively (\( n = 4 \)). T618V shifted the \( V_{1/2} \) of Q-Inact +15 mV compared with WT (Fig. 4D). The \( V_{1/2} \) for WT I-Inact was 44 mV positive to that of WT Q-Inact, whereas the \( V_{1/2} \) for T618V I-Inact was 77 mV positive to that of T618V Q-Inact. Thus, our data suggest that T618V influences voltage sensor movement associated with inactivation and alters the coupling of voltage sensing to inactivation. We were unable to determine \( Q_{\text{Inact}} \) for T618A hERG1 channels, as the voltage dependence of inactivation was shifted too far in the hyperpolarized direction to allow for accurate measurements.

**A Point Mutation in the S5 Segment Abolishes hERG1 Inactivation and Alters Selectivity**—Because of its location along the midportion of the pore helix facing the S5 segment, Thr-618 might couple rearrangements in the S5 segment to the pore helix. This idea was tested by identifying and evaluating potential interacting residues between these two regions. Initially, we performed an alignment of the S5 domain of various \( K^+/H_11001 \) channels anchored by a highly conserved Glu that defines the end of the S5 (or outer helix) of the Kv1.2–2.1 chimera (37), KvAP (38), KcsA (39), and MthK (40) crystal structures. hERG1 residues in bold type represent key positions that perturb inactivation gating when mutated. The black bar demarcates the residues mutated in the Ala scan. For simplicity, the 32 amino acids comprising the S5-P linker turret are represented as a hatched box. B, WT and T618A hERG1 ionic currents recorded in solutions containing \( K^+/H_11001 \) o 4 mM (left panel) and 96 mM (right panel). Currents were elicited by a 2-s depolarization to +40 mV, followed by a voltage step to potentials between +140 mV and +40 mV in 10-mV increments from a HP = −80 mV. Displayed currents correspond to the time period shown as a dotted box surrounding the voltage protocol. The arrows indicate zero current. C, \( I_{\text{Inact}}-V \) relationships for WT ( ), T618A ( ), T623A ( ), V625A ( ) and F627A ( ) using a two-pulse protocol in 96 mM K. D, bar graph representing mean ± S.E. for \( \Delta G_{\text{inact}} \) (1 kcal-mol\(^{-1}\)) for residues in the pore-helix scan. The dashed line represents the arbitrary designation of a mutation-induced perturbation in inactivation (\( \Delta \Delta G_0 \approx (1 \text{ kcal-mol}^{-1}) \)). \( n = 6–10 \) cells. NE, no functional expression. Inactivation parameters for WT and all mutant constructs are listed in supplemental Table 1.
potential (Fig. 5, A and B). Similar to W568L, W568I hERG1 channels also failed to inactivate and displayed altered ion selectivity (Fig. 6). W568F mutant hERG1 channels expressed at low levels, but tail currents were measurable in \([\text{K}^+]/\text{H}^+\) 96 mM. The W568F mutation caused a negative shift in the free energy of hERG1 inactivation (\(\Delta G_0 = -0.6 \pm 0.1\) kcal-mol\(^{-1}\), \(n = 5\)). Other side chains at Trp-568 (Ala, Tyr, Thr, Asn, and Val) resulted in nonfunctional channels, and attempts at restoring the normal inactivation phenotype by various mutations at position Thr-618 (W568L-T618X, where \(X\) equals Ala, Val, Ser, Leu, Phe, or Trp) were unsuccessful (Fig. 6E). Taken together, these data highlight the importance of Trp-568 for normal hERG1 inactivation gating. Moreover, the observation that Trp-568 mutants alter ion selectivity supports the idea that Trp-568 faces toward the pore helix. The mutation of residues that neighbor Trp-568 (C566A, I567A, Y569A, and A570V) does not appreciably influence hERG1 inactivation gating (29).

Introducing the hERG1 Thr-618-equivalent Residue in Kv1.5 (A473T) Markedly Affects Inactivation—The equivalent positions of hERG1 Trp-568 and Thr-618 correspond to Ser and Ala, respectively, in the Kv family of K\(^+\) channels. To deter-

FIGURE 2. Characterization of hERG1 mutants that did not display ionic current. The inability to detect ionic current from six Ala mutants suggests a disruption in protein targeting to the cell surface or defective ion conduction. To distinguish between these two possibilities, we measured gating currents and cell surface expression by Western blot analysis. A, representative gating current traces elicited by 300-ms step depolarization to +40 mV from HP = -110 mV using a p/-8 leak subtraction protocol. T611A, A614V, Y616A, and F617A hERG1 channels failed to generate detectable gating currents. G626A and G628A hERG1 channels generated small but detectable gating currents qualitatively similar to WT hERG1a. The X-Y legend represents 100 ms and 0.2 \(\mu\)A, respectively. B, to further characterize cell surface targeting, we used Western blot analysis to compare the expression patterns of the immature core-glycosylated (cg) and mature fully glycosylated (fg) mature protein as described (41). For the four mutants that did not generate gating current, the fully glycosylated band was absent, confirming that these mutations disrupted cell surface expression. By contrast, the fully glycosylated protein was detected for the two mutants that generated gating current, confirming that these mutant subunits were targeted to the cell surface but failed to conduct ions.

FIGURE 3. The effects of selectivity filter mutations on hERG1 inactivation. A, selectivity filter residues identified in the pore helix Ala scan that perturbed inactivation were subjected to additional mutagenesis to determine the side chain requirements for normal inactivation gating. The bar graph represents mean \(\pm\) S.E. for \(\Delta G_0\) inactivation (kcal-mol\(^{-1}\)), \(n = 4-8\) cells. NE, no functional expression. B, homology model of the hERG1 pore domain, based on the Kv1.2 crystal structure, highlighting the residues that perturbed inactivation gating. The position of the Trp-568 side chain is shown in transparent gray.
mine whether these positions play a role in inactivation gating in channels other than hERG1, we introduced the Trp-568- and Thr-618-equivalent mutations into Kv1.5. S448W and S448W-A473T Kv1.5 channels failed to express functionally (on the basis of the absence of ionic and gating currents). Representative current traces for A473T Kv1.5 channels elicited by sequential step depolarizations to +40 mV are presented in Fig. 7A. The onset of A473T Kv1.5 channel inactivation during the depolarizing voltage step was qualitatively similar to WT Kv1.5 channels. However, the peak current magnitude was reduced with subsequent depolarizing voltage steps (Fig. 7, A and B). Shortening the interpulse duration (HP = -80 mV) from 15 s to 2.5 s accelerated the rate of current reduction, consistent with cumulative inactivation. Thus, introducing the hERG1 Thr-618-equivalent mutation in Kv1.5 stabilized the inactivated state, underscoring the importance of this pore helix residue for inactivation gating in both channels.

To explore potential molecular interactions between the pore helix and S5 domain, we performed molecular dynamic simulations using a full atom representation of the Kv1.2 pore domain embedded in lipid. Average interaction energies between Ala-368 in the pore helix (hERG1 618-equivalent) and side chains from residues in the S5 segment are depicted in Fig. 7C. Favorable nonpolar van der Waals interactions were measured between the Ala-368 side chain and...
both Phe-342 and Ser-343 (-0.8 ± 0.2 and -0.7 ± 0.3 kcalmol⁻¹, respectively, mean ± S.D.), residues that are equivalent to Ile-567 and Trp-568 in hERG1. These data indicate that the Ala-368 side chain interacts predominately with the side chains of residues 342 and 343 compared with the flanking residues. Replacing the native Ala with Thr at position 368 in silico markedly increased the average electrostatic interaction with Ser-343, the equivalent to Trp-568 in hERG1 (Fig. 7C). The A368T mutation did not alter the van der Waals interactions between 368 and residues in the S5 segment.

**DISCUSSION**

Identifying the mechanistic basis of hERG1 inactivation is important for understanding the role of this channel in mod-
ululating cardiac excitability in normal and disease states. Previous work in Kv channels implicated coupling between the activation gate at the bundle crossing and the inactivation gate in the pore region (30, 31). Recently, a direct link between residues in the inner helix and the base of the pore helix was established that couples channel opening to inactivation in KcsA (12). Here, we propose that coupling between activation and inactivation gates could be based on additional interaction contact points, such as between the S5 segment and pore helix.

Our Ala scan identified Thr-618 as an inactivation-perturbing residue, and sequence alignment predicted that Thr-618 faces toward the S5 segment near Trp-568. Trp-568 mutations eliminated inactivation and altered ion selectivity, suggesting that this residue faces toward the pore helix. We were unable to experimentally confirm direct contact between Trp-568 and Thr-618 in that Cys substitutions at these positions were not tolerated, precluding the use of cross-linking experiments (data not shown). Thus, we attempted to study a possible interaction between the equivalent positions in Kv channels. Our experimental data and molecular dynamic simulations suggest that the Thr-618-equivalent mutation in Kv channels stabilizes the inactivated state and markedly increases interaction-energies between hERG1 618- and 568-equivalent positions. In the absence of a high resolution hERG1 crystal structure, the precise molecular interactions that induce inactivation cannot be defined with certainty. Nevertheless, one could speculate that H-bonding between the hydroxyl group of Thr-618 and the N,H pyrrole ring of Trp-568 may participate in the coupling process. We also speculate that S5-pore helix interactions contribute to the later sequence of events, leading to C-type inactivation in hERG1. These later events could be initiated by voltage-dependent rearrangements in the voltage sensor, transmitted via the S4-S5 linker to the S5 segment and/or activation gate in S6. Indeed, the observation that T618V alters Q-Inact supports a long range interaction between the voltage sensor and the pore helix.

Our findings do not exclude the possibility that rearrangements in other regions of the channel participate in hERG1 inactivation, such as the linker between S5 and the pore helix (32–35). Indeed, recent evidence suggests that hERG1 inactivation involves a complex series of rearrangements involving multiple regions of the channel (36). Our hypothesis regarding coupling between the S5 segment and the pore helix could represent a component of the conformational changes, leading toward channel inactivation. Ultimately, insights into the atomic basis of hERG1 inactivation gating will be derived from the crystal structure of the hERG1 channel solved in the closed, open, and inactivated conformations.

Limitations—Several limitations inherent in this study are worthy of discussion. First, the equilibrium analysis is based on the assumption that complex gating processes can be simplified into transitions between two states (e.g. open versus inactivated). Although multiple channel states are known to exist, the equilibrium distributions measured here are well fit by single Boltzmann functions, supporting our use of a simplified two-state model. Second, our analysis identifies only residues that experience a change in local energetics in the transition from one state to another. Thus, we are unable to distinguish between residues that remain relatively static from those that move into a new but energetically similar environment. Third, molecular dynamic simulations were performed using a tetrameric Kv1.2 pore domain model in the absence of transmembrane voltage, whereas experimental data regarding inactivation properties was performed in Kv1.5 channels. Finally, the extrapolation of molecular dynamic simulation data to the hERG1 channel is complicated by the obvious differences in amino acid sequence.
REFERENCES

1. Warmke, J. W., and Ganetzky, B. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 3438–3442
2. Hufnäker, S. I., Chen, J., Niedenmuk, K. S., Sambataro, F., Yang, F., Mattay, V., Lipska, B. K., Hyde, T. M., Song, J., Rujescu, D., Giegling, I., Mayilvann, K., Proust, M. J., Soghoian, A., Caforio, G., Callicott, J. H., Bertolino, A., Mayer-Lindenberg, A., Chang, J., Ji, Yi, Egan, M. F., Goldberg, T. E., Kleinman, J. E., Lu, B., and Weinberger, D. R. (2009) Nat. Med. 15, 509–518
3. Sanguinetti, M. C., Jiang, C., Curran, M. E., and Keating, M. T. (1995) Cell 81, 299–307
4. Wang, H., Zhang, Y., Cao, L., Han, H., Wang, J., Yang, B., Nattel, S., and Wang, Z. (2002) Cancer Res. 62, 4843–4848
5. Trudeau, M. C., Warmke, J. W., Ganetzky, B., and Robertson, G. A. (1995) Science 269, 92–95
6. Sanguinetti, M. C., and Tristani-Firouzi, M. (2006) Nature 440, 463–469
7. Perrin, M. J., Kuchel, P. W., Campbell, T. J., and Vandenberg, J. I. (2008) Mol. Pharmacol. 74, 1433–1452
8. Hoshi, T., Zagotta, W. N., and Aldrich, R. W. (1991) Neuron 7, 547–556
9. Cordero-Morales, J. F., Cuello, L. G., Zhao, J., Jogini, V., Cortes, D. M., Roux, B., and Perozo, E. (2006) Nat. Struct. Mol. Biol. 13, 311–318
10. Cordero-Morales, J. F., Cuello, L. G., and Perozo, E. (2006) Nat. Struct. Mol. Biol. 13, 319–322
11. Cuello, L. G., Jogini, V., Cortes, D. M., and Perozo, E. (2010) Nature 466, 203–208
12. Cuello, L. G., Jogini, V., Cortes, D. M., Pan, A. C., Gagnon, D. G., Dalmas, O., Cordero-Morales, J. F., Chakrapani, S., Roux, B., and Perozo, E. (2010) Nature 466, 273–275
13. Schonherr, R., and Heinemann, S. H. (1996) J. Physiol. 493, 635–642
14. Smith, P. L., Kaufkowitz, T., and Yellen, G. (1996) Nature 379, 833–836
15. Rasmusson, R. L., Morales, M. J., Wang, S., Liu, S., Campbell, D. L., Brahmajothi, M. V., and Strauss, H. C. (1998) Circ. Res. 82, 739–750
16. Spector, P. S., Curran, M. E., Zou, A., Keating, M. T., and Sanguinetti, M. C. (1996) J. Gen. Physiol. 107, 611–619
17. Zhang, M., Liu, J., and Tseng, G. N. (2004) J. Gen. Physiol. 124, 703–718
18. Tristani-Firouzi, M., Chen, J., and Sanguinetti, M. C. (2002) J. Biol. Chem. 277, 18994–19000
19. Stühmer, W. (1992) Methods Enzymol. 207, 319–339
20. Stefani, E., and Bezanilla, F. (1998) in Methods in Enzymology (Conn, P. N., ed.) pp. 300–318, Academic Press, San Diego, CA
21. Piper, D. R., Varghese, A., Sanguinetti, M. C., and Tristani-Firouzi, M. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 10534–10539
22. Armstrong, C. M., and Bezanilla, F. (1977) J. Gen. Physiol. 70, 567–590
23. Piper, D. R., Hin, W. A., Tallurri, C. K., Sanguinetti, M. C., and Tristani-Firouzi, M. (2005) J. Biol. Chem. 280, 7206–7217
24. Piper, D. R., Rupp, J., Sachse, F. B., Sanguinetti, M. C., and Tristani-Firouzi, M. (2008) Cell Physiol. Biochem. 21, 37–46
25. Jogini, V., and Roux, B. (2007) Biophys. J. 93, 3070–3082
26. Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R. D., Kalé, L., and Schulten, K. (2005) J. Comput. Chem. 26, 1781–1802
27. Humphrey, W., Dalke, A., and Schulten, K. (1996) J. Mol. Graph. 14, 33–38, 27–38
28. Gajewski, C., Dagcan, A., Roux, B., and Deutsch, C. (2011) Proc. Natl. Acad. Sci. U.S.A. 108, 3240–3245
29. Lu, P., Pages, G., Riek, R. P., Chen, P. C., Torres, A. M., Bansal, P. S., Kuyucak, S., Kuchel, P. W., and Vandenberg, J. I. (2009) J. Biol. Chem. 284, 1000–1008
30. Panyi, G., and Deutsch, C. (2006) J. Gen. Physiol. 128, 547–559
31. Yifrach, O., and MacKinnon, R. (2002) Cell 111, 231–239
32. Clarke, C. E., Hill, A. P., Zhao, J., Kondo, M., Subbiah, R. N., Campbell, T. J., and Vandenberg, J. I. (2006) J. Physiol. 573, 291–304
33. Jiang, M., Zhang, M., Maslennikov, I. V., Liu, J., Wu, D. M., Korolkova, Y. V., Arseniev, A. S., Grishin, E. V., and Tseng, G. N. (2005) J. Physiol. 569, 75–89
34. Liu, J., Zhang, M., Jiang, M., and Tseng, G. N. (2002) J. Gen. Physiol. 120, 723–737
35. Torres, A. M., Bansal, P. S., Sunde, M., Clarke, C. E., Bursill, J. A., Smith, D. J., Bauskin, A., Breit, S. N., Campbell, T. J., Alewood, P. F., Kuchel, P. W., and Vandenberg, J. I. (2003) J. Biol. Chem. 278, 42136–42148
36. Wang, D. T., Hill, A. P., Mann, S. A., Tan, P. S., and Vandenberg, J. I. (2010) Nat. Struct. Mol. Biol. 18, 35–41
37. Long, S. B., Tao, X., Campbell, E. B., and MacKinnon, R. (2007) Nature 450, 376–382
38. Jiang, Y., Lee, A., Chen, J., Ruta, V., Cadene, M., Chait, B. T., and MacKinnon, R. (2003) Nature 423, 33–41
39. Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) Science 280, 69–77
40. Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R. (2002) Nature 417, 515–522
41. Zhou, Z., Gong, Q., Epstein, M. L., and January, C. T. (1998) J. Biol. Chem. 273, 21061–21066