Concerted All-or-none Subunit Interactions Mediate Slow Deactivation of Human ether-à-go-go-related Gene K⁺ Channels*

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Background: The N terminus of hERG1 subunits modulates the rate of channel deactivation.
Results: A single mutant subunit in a concatenated hERG1 tetramer accelerates channel deactivation.
Conclusion: All four subunits cooperate fully to slow the rate of hERG1 channel deactivation.
Significance: A single subunit harboring a mutation in the PAS domain or S6 segment can alter the gating properties of a tetrameric hERG1 channel.

During the repolarization phase of a cardiac action potential, hERG1 K⁺ channels rapidly recover from an inactivated state then slowly deactivate to a closed state. The resulting resurgence of outward current terminates the plateau phase and is thus a key regulator of action potential duration of cardiomyocytes. The intracellular N-terminal domain of the hERG1 subunit is required for slowing the rate of deactivation (7–10). Single-channel studies have shown that both N-terminal truncations and mutations of the N terminus can alter the rate of deactivation of the resulting heterologously expressed channel (8). The full-length subunit (hERG1a) can coassemble with hERG1b, an alternatively spliced subunit with a shorter N terminus. Western blot analysis indicates that both forms are expressed in sarcolemmal N-terminal “eag” domain deactivates very slowly, whereas erg1b homotetramers deactivate ∼10× faster (12–14).

This article has been withdrawn by M. C. Sanguinetti and A. Hansen. With the exception of R56Q1/wt3, wt2/R56Q1/wt1, and wt3/R4AR5A1, the tetramers studied in Figs. 2-5 were not properly constructed.
hERG1b cRNA (15). This finding suggests that full-length and N-terminal-truncated subunits cooperate during channel deactivation, but the nature of cooperative interactions (i.e., sequential versus fully concerted) has not been determined.

The N terminus of hERG1 is composed of 355 residues. Truncation of the entire N terminus (16–18) or just the first 26 residues (9) accelerates the rate of deactivation to an extent similar to that observed for hERG1b homotetramers. Fast deactivation of homotetrameric hERG1 channels can also be achieved by mutations that neutralize the charge of just two basic residues, Arg-4 and Arg-5 located in the PAS-cap region (9). The initial 135 residues of the N terminus form the ether-a-go-go (eag) domain, present in all members of the eag family of K⁺ channels. Residues 26–135 form a PAS (Per-Arnt-Sim) domain (9), and residues 1–26 form the PAS-cap. The structure of the hERG1 PAS domain was solved years ago and was proposed to have an important regulatory function (9). More recent studies have revealed that the PAS domain interacts with the cytoplasmic C-terminal domain in hERG1 channels (10, 20–22). Coexpression of eag domains together with N-terminal truncated channels can restore normal slow deactivation (22). With the exception of erg2, the other members of the eag K⁺ channel superfamily (Kᵥ10–Kᵥ12), including eag1 (23), eag2 (24), eag3 (25), eag-like (elk1) (26), and elk2 (27) deactivate rapidly compared with mammalian erg1a. Here we ask how many WT or mutant (e.g., R56Q) subunits are required for the homotypic hERG1a channel to deactivate with its characteristic slow kinetics.

**EXPERIMENTAL PROCEDURES**

**Construction of hERG1 Constructs**—The two WT and several mutant (e.g., R56Q, F656I) cDNAs were designed to allow for oocyte expression vector (Promega) mediated expression of individual types of tetramers engineered to contain a variable combination of WT or mutant KCNH2 cDNAs. Processing of the cyttoplasmic termini as previously described (28). Two single mutations (R56Q, F656I) and one double mutation (R4A/R5A) known to accelerate deactivation were studied. The R4A/R5A double mutation is located in the PAS-cap region, and R56Q is located in the PAS domain. The mutation F656I is located in a region of the S6 segment that contributes to formation of the “S6 bundle crossing,” the intracellular activation gate (29). In this study, channels formed from individual subunits expressed and assembled naturally in oocytes are called Xmonomer, where X is either a WT or mutant subunit. Homotypic concatenated tetramers formed by four WT or mutant (e.g., R56Q) subunits are designated WT₄ channels and R56Q₄, respectively. Heterotypic concatenated tetramers indicate the relative positioning of the single WT and mutant subunits. For example, the R56Q₄/WT₄/R56Q₄/WT₄ channel was engineered to contain WT subunits in the second and fourth positions together with subunits harboring the R56Q mutation in the first and third positions of the tetramer. Mutations were introduced into KCNH2 using the QuikChange site-directed mutagenesis kit (Agilent Technologies). Construction of dimers and fully concatenated tetramers was the same as previously described (28). Three types (1–3) of monomer constructs were used to make dimers. For all monomer constructs, a HindIII site was added just before the start codon. A KpnI site was added at the 3’ end just before the stop codon in Type 2 monomers or 3’ to the HindIII site in Type 3 monomers. Type 1 and 3 monomers were cut with HindIII then ligated together to form a Type I dimer (having a single KpnI site). Type 2 and 3 monomers were cut with HindIII then ligated together to form a Type II dimer (having two KpnI sites). Dimers were sequenced with forward and reverse primers based on vector sequences. Type I and II dimers were cut with KpnI then ligated together to create a tetrameric concatenated channel construct. Tetrameric constructs were verified by DNA sequence analyses using forward and reverse primers based on vector sequences, and the correct size of inserts was checked by digest with KpnI. Tetrameric KCNH2 plasmids were linearized with EcoRI before in vitro transcription using the mMessage mMachine SP6 kit (Ambion).

**Oocyte Isolation and cRNA Injection**—Procedures used for harvesting oocytes from Xenopus laevis were approved by the University of Utah Institutional Animal Care and Use Committee. Ovarian lobes were surgically removed from adult female X. laevis anesthetized in 0.2% tricaine solution. Ovarian lobes were dissected into clumps of 10–20 oocytes, which were injected with 1.1 ng/ml each of type I and II (28) hERG1a (KCNH2) cRNA (Promega) in Ca²⁺-free ND96 solution (96 mM NaCl, 1 mM MgCl₂, 5 mM HEPES and 1 mM pyruvate plus gentamycin (50 mg/liter) and ciprofloxacin (50 mg/liter); pH was adjusted to 7.4 with NaOH. Stage IV and V oocytes were injected with 46 nl (0.5–2 ng) of cRNA of either homotypic hERG1 constructs. Oocytes were incubated in ND96 solution for 1–7 days at 17 °C. Barth’s contained 88 mM NaCl, 2 mM KCl, 0.41 mM CaCl₂, 0.33 mM Ca(NO₃)₂, 1 mM MgSO₄, 2.4 mM NaHCO₃, 10 mM HEPES and 1 mM pyruvate with 46 nl (0.5–2 ng) of cRNA of either homotypic hERG1 constructs. Oocytes were incubated in ND96 solution for 1–7 days at 17 °C. Barth’s contained 88 mM NaCl, 2 mM KCl, 0.41 mM CaCl₂, 0.33 mM Ca(NO₃)₂, 1 mM MgSO₄, 2.4 mM NaHCO₃, 10 mM HEPES and 1 mM pyruvate plus gentamycin (50 mg/liter) and ciprofloxacin (50 mg/liter); pH was adjusted to 7.4 with NaOH.

**Electrophysiology**—Whole cell currents were recorded from oocytes by using standard two-electrode voltage clamp techniques (30, 31). Oocytes were bathed in ND96 extracellular solution that contained 96 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES; pH was adjusted to 7.6 with NaOH. Agarose cushion microelectrodes were prepared as described (32) using thin walled borosilicate glass (TW100F-4, World Precision Instruments, Inc.) and had a resistance of 0.5–1.5 megaohms when filled with 3 M KCl. A personal computer, GeneClamp 500 amplifier, Digidata 1322A, and pCLAMP 8.2 software (Molecular Devices) were used to acquire and digitize data.

The voltage dependence of current activation was determined by using a current-voltage (I–V) pulse protocol. From a holding potential of −90 mV, 5-s depolarizing pulses were applied to a test voltage (Vₜ) that ranged from −90 to +60 mV. After each test pulse, the membrane potential was returned to −70 mV to elicit a tail current, Iₜtail. The tail current amplitude (Aₜtail) for each Vₜ was measured as the difference between the peak outward Iₜtail and the current at −70 mV elicited during a short prepulse applied immediately before each test pulse. The voltage dependence of hERG1 channel activation was obtained by plotting Aₜtail measured after each 5-s test pulse as a function of Vₜ. Kinetics of current deactivation were evaluated using a
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two-step voltage pulse protocol. From a holding potential of −80 mV, a 1-s pulse to +40 mV was used to activate channels and was followed by a repolarizing pulse to a variable return potential ($V_{ret}$) that ranged from +40 to −140 mV and was applied in 10-mV increments.

Data Analysis—Voltage clamp data were off-line-analyzed with either pClamp 8.2 or 10.2 (Molecular Devices) and Prism 6 (Graph Pad) software packages. To estimate the voltage dependence of hERG1 channel activation, $A_{tail}$ was normalized to its maximum fitted value for each oocyte, plotted as a function of $V_t$, and the resulting conductance-voltage (G-V) relationship was fitted with a Boltzmann function,

$$G = \frac{1}{1 + e^{\frac{V_t - V_{0.5}}{\sigma}}}$$ \hspace{1cm} (Eq. 1)

where $G/G_{max}$ is relative conductance, $V_{0.5}$ is the half-point of the G-V relationship, $z$ is the equivalent charge for activation, $F$ is Faraday’s constant, $R$ is the gas constant, and $T$ is the absolute temperature.

To derive the kinetics and amplitudes of the fast and slow components of deactivation, $I_{tail}$ elicited at each $V_{ret}$ was fitted with a double exponential function,

$$I_{tail}(t) = A_fe^{-\frac{t}{\tau_{deact-f}}} + A_se^{-\frac{t}{\tau_{deact-s}}} + C$$ \hspace{1cm} (Eq. 2)

where $A_f$ and $A_s$ are the amplitudes of the fast and slow components of $I_{tail}$, $\tau_{deact-f}$ and $\tau_{deact-s}$ are the fast and slow time constants of tail current decay, and $C$ is steady state current. All data are presented as the mean ± S.E., where currents conducted by concatenated tetramers were plotted to a Boltzmann function (smooth curves). For WT4, $V_{0.5} = 28.1 \pm 0.47$ (n = 16); for WTmonomer, $V_{0.5} = 28.2 \pm 0.17$ (n = 17).

RESULTS

Concatenated hERG1 Tetramers (WT4) Formed Naturally from monomers Have Similar Properties of Activation and Deactivation—In our previous study of hERG1 channel activators, we reported similar biophysical properties for WT monomer channels and concatenated WT hERG1 (WT4) channels (28). These findings are confirmed for the present study in Fig. 1. Representative currents for WTmonomer and WT4 hERG1 channels are illustrated in Fig. 1A. Strong rectification for both channel types is indicated by the small size of the outward currents recorded at the $V_t$ of +40 mV. The rate of tail current decay (deactivation) is very slow at −40 mV and is progressively increased at more negative levels of $V_{ret}$. In Fig. 1B and C, the values of $\tau_{deact-f}$ and $\tau_{deact-s}$ and their relative amplitudes for WT monomer and WT4 channels are plotted as a function of $V_{ret}$ ranging from −80 to −40 mV. There was no significant difference between the channel types for the average values of either $\tau_{deact-f}$ or $\tau_{deact-s}$. The voltage dependence of activation determined with 5-s depolarizing pulses for WT4 and WT monomer hERG1 channels are compared in Fig. 1D. There was no significant difference between the $V_{0.5}$ values determined for the two channel types. Thus, the activation and deactivation properties of hERG1 channels are not altered by covalent linkage of four WT subunits.
The LQT2-associated point mutation R56Q, located in the PAS domain of hERG1, was previously reported to increase the rate of channel deactivation when expressed in Xenopus oocytes (8) and to increase the rate of activation and deactivation but not inactivation when channels were heterologously expressed in HEK-293 cells (34). To further explore how a mutation in the PAS domain affects the rate of deactivation, R56Q subunits were placed in either position 1 (R56Q₁/WT₃) or position 3 (WT₂/R56Q₂/WT₁) of a concatenated tetramer, and the current conducted by these channels was compared with R56Qₙmonomer and R56Qₙ channels. The mutant channels exhibited similar deactivation kinetics (Fig. 3A) and voltage dependence of activation (Fig. 3B, Table 1). The average values of deact-s and τ_deact-s and their relative amplitudes at potentials ranging from −40 to −80 mV for WT₄ and channels containing R56Q mutant subunits are summarized in Fig. 3, C and D. There was no significant difference (ANOVA) between the time constants at any voltage for R56Qₙmonomer and concatenated channels containing one or four R56Q subunits. Thus, similar to R4A/R5A subunits, the presence of a single R56Q subunit fully disrupted deactivation of hERG1 channels. More importantly, these findings indicate that all four WT subunits are required for hERG1 to deactivate with its characteristic slow kinetics.

A Single F656I Subunit Is Sufficient to Accelerate Deactivation of hERG1 Channels—In the closed state, the S6 segments of Kv channels converge near their cytoplasmic ends (the S6 bundle crossing) to form a narrow aperture that prevents entry of intracellular ions into the central cavity (35, 36). Kv channel opening induced by membrane depolarization is mediated by a final, voltage-independent step that involves fully cooperative interactions between the S6 segments of all four subunits (37–40). An outward splaying of the cytoplasmic ends of all four S6 segments widens this aperture (opens the pore) to permit K⁺ ion permeation. In hERG1a, Phe-656 is located in the S6 segment near the narrowest region of the pore (41), and mutation of this residue to Ile (F656I) causes fast deactivation of homomeric channels formed from monomers (F656Iₙmonomer; Fig. 4A).
FIGURE 3. A single R56Q mutant subunit is sufficient to accelerate rate of hERG1 channel deactivation. A, voltage clamp pulse protocol and currents conducted by R56Q\textsubscript{monomer} and concatenated tetrameric channels as indicated. B, voltage dependence of activation for indicated channels. Normalized tail currents were plotted as a function of $V_t$ and fitted to a Boltzmann function (smooth curves) to estimate $V_{0.5}$ and $z$ (summarized in Table 1). C and D, $\tau_{\text{deact-f}}$ and $\tau_{\text{deact-s}}$ and relative current amplitudes for both components plotted as a function of $V_{\text{ret}}$ for the indicated channels.

FIGURE 4. A single F656I mutant subunit is sufficient to accelerate rate of hERG1 channel deactivation. A, voltage clamp pulse protocol and currents conducted by F656I\textsubscript{monomer} and concatenated tetrameric channels as indicated. B, voltage dependence of activation for indicated channels. Normalized tail currents were plotted as a function of $V_t$ and fitted to a Boltzmann function (smooth curves) to estimate $V_{0.5}$ and $z$ (summarized in Table 1). C and D, $\tau_{\text{deact-f}}$ and $\tau_{\text{deact-s}}$ and relative current amplitudes for both components plotted as a function of $V_{\text{ret}}$ for the indicated channels.
To determine if the number or position of F656I subunits in a tetramer differentially alters the rate of channel deactivation, several concatenated tetramers were constructed. Tetramers that were formed from a single F656I subunit placed in either position 1 (F656IWT3) or position 3 (WT2F656IWT1), from two mutant and two WT subunits positioned in a diagonal orientation (WT2F656IWT3F656I), and from four F656I subunits (F656I4) deactivated with fast kinetics similar to F656I monomer channels (Fig. 4A). F656I monomer channels and all the mutant tetrameric channels, except F656IWT3, were activated at more positive potentials than WT4 channels (Fig. 4B). There was no significant difference in \( \tau_{\text{deact-f}} \) and \( \tau_{\text{deact-s}} \) between channels containing one or more F656I subunit (two-way ANOVA). However, compared with WT4 channels, the values of \( \tau_{\text{deact-f}} \) and \( \tau_{\text{deact-s}} \) were dramatically reduced at all voltages for concatenated channels containing one or more F656I mutant subunits. For example, at −60 mV \( \tau_{\text{deact-f}} \) and \( \tau_{\text{deact-s}} \) were 17 and 14 times faster for F656IWT3 channels compared with WT4 channels.

The time constants for the fast and slow components of deactivation measured at a \( V_{\text{rec}} \) of −70 mV for WT4 and all the heterotypic concatenated tetramers analyzed in this study are presented in Fig. 5. At this potential the rate constants for closed to open state transitions are very slow compared with the reverse transition that mediates channel deactivation. For all three mutations examined, a single mutant subunit accelerated deactivation in a dominant manner, confirming the importance of the N-terminal eag domain for all four WT subunits to achieve slow deactivation.

**DISCUSSION**

Early studies of hERG1 channel gating established the importance of the N-terminal eag domain (12–14,42) of the N terminus or specific point mutations of residues within the eag domain (9–11) to greatly accelerate the rate of channel closing (42). However, further investigation led to the current view that slow deactivation of hERG1 channels is dependent on an interaction between the N terminus and the C-terminal domain (composed of a C-linker and a cyclic nucleotide binding homology domain (CNBHD)) of adjacent subunits (45). A 2 Å resolution crystal structure of the mouse EAG1 eag domain and the CNBHD complex (46) has provided insight into the likely extensive structural interface of these two domains in the hERG1 channel. Specifically, an amphipathic \( \alpha \)-helix of the PAS-cap domain lies between a hydrophobic patch of the PAS domain and the \( \beta_4-\beta_5 \) strands and \( \beta_8-\beta_9 \) loop of the CNBHD. Despite the presence of a CNBHD in the C-terminal domain of hERG1, these channels are not directly modulated by cyclic nucleotides. The C-linker/ CNBHD of a mosquito erg channel was recently solved (47) and provided a structural explanation for this apparent discrepancy. A short \( \beta \)-strand (an “intrinsic ligand”) occupies the pocket where a cyclic nucleotide is bound in other (e.g. HCN) channels. Mutations in the intrinsic ligand speed the rate of hERG1a channel deactivation (47). Alignment with the homologous HCN2 channel indicates that the C-linker/CNBHD of eag or erg channels forms a concentrically arranged tetramer with the eag domains located at the periphery (46), and the PAS-cap of one subunit probably interacts with the CNBHD of an adjacent subunit (48).

Kinetic analysis of currents was used to distinguish between different models of subunit interactions that mediate inactivation (49). A similar approach can be used to analyze deactivation gating. If one assumes a two-state model for hERG1 channel deactivation (O to C), then \( \tau_{\text{deact}} \) is equal to the inverse of the sum of the forward and reverse rate constants: \( 1/(k_f + k_b) \). At sufficiently negative voltages, \( k_b \) is negligible, and the deactivation rate constant \( k \) can be estimated by \( 1/\tau_{\text{deact}} \). If an independent conformational change in any one of the subunits is sufficient to accelerate deactivation, then \( k \) would be the sum of the individual rate constants contributed by each of the subunits within a tetramer. If deactivation is a cooperative process
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involving sequential subunit transitions (39), then the deactivation rate of a heterotypic channel would be exponentially related to the sum of the energetic contributions of each of the WT and mutant subunits (49), and log k would be a linear function of the number of mutant subunits contained with a tetramer. Finally, if the rate constant of deactivation (i.e. 1/τ_deact) is the same for channels that contained one or more of the mutant subunits, then WT subunits must interact cooperatively in an all-or-none, concerted fashion. Because hERG1 deactivation was biexponential rather than monoexponential, we were unable to use the above analysis that requires a two state model (and a single k). Nonetheless, our finding that there was no difference in either the fast or slow time constants for deactivation of any tetramer containing a mutant subunit greatly simplifies the analysis, as it satisfies the requirements for the extreme case of cooperativity where normal gating (i.e. slow deactivation) requires the concerted interaction of all four WT subunits.

The nature of subunit interactions during activation of Kv channels has been extensively studied. Activation is initiated by outward movement of the voltage sensors in each of the four subunits. The final step in the activation pathway involves highly cooperative subunit interactions (37–40, 50). Thus, it is perhaps not surprising that the reverse process, channel deactivation, is also a highly cooperative process involving concerted subunit interactions. However, in most studies, mutant subunits were used to characterize channel findings have been consistent with a sequential, cooperative subunit interactions; i.e. equal contributions from each subunit to the tetrameric rate constant (38, 40, 50). In contrast, the concerted model of cooperativity assumes that a simultaneous rearrangement of the selectivity filter (52–54). Similar to slow deactivation in WT hERG1a, C-type inactivation of Kv1 channels involves highly cooperative subunit interactions (49, 55, 56). N-type inactivation is mediated by blocking the channel pore by an N-terminal “ball” domain (57, 58) or by the N terminus of an accessory β-subunit (59, 60). A single N-terminal domain acts independently of others to plug the pore and prevent ion permeation. Thus, opposite to N-terminal-mediated slow deactivation, N-type inactivation is considered a fully independent process. As discussed above, hERG1 channels deactivate fast in the absence of an N terminus, partial truncation of the N terminus, or as a consequence of specific mutations in the PAS-cap or PAS domain. Other mammalian members of the eag K channel family, including eag1, eag2, erg3, elk1, and elk2 deactivate faster than mammalian erg1a. A recent comparison of the biophysical properties of erg channels from different species suggests that slow deactivation may be the ancestral phenotype for these channels (61). Erg1 of the sea anemone Nematostella vectensis deactivates slowly, similar to mammalian erg1a channels. In contrast, erg4 from this sea anemone, erg (unc103) from the nematode Caenorhabditis elegans and Drosophila erg channels lack the N-terminal eag domain, and these channels deactivate rapidly. Together with sequence analysis, these findings suggest that the ancestral eag channel deactivates slowly, and that fast deactivation evolved later and independently in Nematoda and Anthozoa by loss or disruption of the N terminus.

Potential interactions between subunits containing either full-length or altered N termini have been studied before by injecting Xenopus oocytes with variable quantities of cRNA encoding hERG1a and hERG1b subunit isoforms (15). In this study the authors found that the fold changes in both τ_deact-t and τ_deact-s for deactivation at a V_m of −60 mV were a linear function of the ratio of hERG1a/hERG1b cRNA injected into the oocyte. Assuming that both proteins were expressed with similar efficiency and that subunits co-assembled in a random, non-biased fashion, then this result strongly suggests that hERG1a and hERG1b subunits interact in a cooperative manner to determine the rate of deactivation of heteromeric channels. However, although this approach mimics the physiological method of channel assembly, the resulting mix of heteromultimeric assemblies makes it impossible to quantify...
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the specific effects of a variable number (1–4) of hERG1b N termini on channel gating, a condition needed to fully characterize the nature of subunit cooperativity. We characterized mutations located in either the N terminus (R4A/R5A or R56Q) or S6 segment (F656L). In each case the presence of a single mutant subunit within a concatenated tetramer was sufficient to disrupt the normal gating process and increase the rate of channel deactivation.

In summary, our findings indicate that the slow rate of deactivation characteristic of a WT hERG1a homomeric channel involves an all-or-none or fully concerted, cooperative interaction between subunits. The PAS-cap of one hERG1a subunit involves an all-or-none or fully concerted, cooperative interaction characteristic of a WT hERG1a homomeric channel

REFERENCES
1. Sanguinetti, M. C., and Jurkiewicz, N. K. (1990) Two components of cardiac delayed rectifier K⁺ current: differential sensitivity to block by class III antiarrhythmic agents. J. Gen. Physiol. 96, 195–215
2. Sanguinetti, M. C., Jiang, C., Curran, M. E., and Keating, M. T. (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I₅₄ potassium channel. Cell 81, 299–307
3. Trudeau, M. C., Warmke, J. W., Ganetzky, B., and Robertson, G. A. (1995) A human inward rectifier in the voltage-gated potassium channel family. Science 269, 92–95
4. Sanguinetti, M. C., and Tristani-Firouzi, M. (2006) Models and cardiac arrhythmia. Nature 440, 463–466
5. Curran, M. E., Splawski, I., Timothy, K., Wray, D., and Keating, M. T. (1995) A molecule linking mutations causing long QT syndrome. Science 269, 92–95
6. Sanguinetti, M. C., Curran, M. E., and Keating, M. T. (1996) Spectrum of HERG K⁺ channel dysfunction. Proc. Natl. Acad. Sci. U.S.A. 93, 5704–5709
7. Anderson, C. L., Delsiède, B. P., Anson, P. A., Wray, D., Gekhtman, D. J., and Tester, D. I., Gong, Q., Zhou, Z., Ackerman, M. L., and Keating, M. T. (2006) Most LQT2 mutations reduce Kv11.1 (hERG) current: a class 2 (trafficking-deficient) mechanism. Circulation 113, 367–373
8. Chen, J., Zou, A., Splawski, I., Keating, M. T., and Sanguinetti, M. C. (1999) Long QT syndrome-associated mutations in the Prep-1 domain of HERG potassium channels accelerate channel deactivation. J. Biol. Chem. 274, 10113–10118
9. Morais Cabral, J. H., Lee, A., Cohen, S. L., Chait, B. T., Li, M., and Mackinnon, R. (1998) Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. Cell 95, 649–655
10. Al-Owais, M., Bracey, K., and Wray, D. (2009) Role of intracellular domains in the function of the herg potassium channel. Eur. Biophys. J. 38, 569–576
11. Jones, E. M., Roti Roti, E. C., Wang, J., Delfosse, S. A., and Robertson, G. A. (2004) Cardiac I₅₄ channels minimally comprise HERG I and 2 subunits. J. Biol. Chem. 279, 44690–44694
12. London, B., Trudeau, M. C., Newton, K. P., Beyer, A. K., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Satler, C. A., and Robertson, G. A. (1997) Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K⁺ current. Circ. Res. 81, 870–878
13. Lees-Miller, S. P., Kondo, C., Wang, L., and Huff, H. J. (1997) Electrophysiological characterization of an alternatively processed ERG K⁺ channel in mouse and human hearts. Circ. Res. 81, 719–726
14. Larsen, A. P., Olesen, S. P., Grunnet, M., and Jespersen, T. (2008) Characterization of hERG1a and hERG1b potassium channels – a possible role for hERG1b in the I₅₄ current. Pflugers Arch. 456, 1137–1148
15. Larsen, A. P., and Olesen, S. P. (2010) Differential expression of hERG1 channel isoforms reproduces properties of native I₅₄ and modulates cardiac action potential characteristics. PLoS ONE 5, e9021
16. Schönberg, R., and Heinemann, S. H. (1996) Molecular determinants for activation and inactivation of HERG, a human inward rectifier potassium channel. J. Physiol. 493, 635–642
17. Spector, P. S., Curran, M. E., Zou, A., Keating, M. T., and Sanguinetti, M. C. (1996) Fast inactivation causes rectification of the I₅₄ channel. J. Gen. Physiol. 107, 611–619
18. Terlau, H., Heinemann, S. H., Stühmer, W., Pongs, O., and Ludwig, J. (1997) Amino terminal-dependent gating of the potassium channel rat eag is compensated by a mutation in the S4 segment. J. Physiol. 502, 537–543
19. Muskett, F. W., Thoua, S., Thomson, S. J., Bowen, A. S., Stansfeld, P. J., and Mitcheson, J. S. (2011) Mechanistic insight into human ether-a-go-go-related gene (hERG) K⁺ channel deactivation gating from the solution structure of the EAG domain. J. Biol. Chem. 286, 6184–6191
20. Fernández-Trillo, J., Barros, F., Machín, A., Carretero, L., Domínguez, P., and de la Peña, P. (2011) Molecular determinants of interactions between the N-terminal domain and the transmembrane core that modulate hERG K⁺ channel gating. PLoS One 6, e24674
21. Gustina, A. S., and Trudeau, M. C. (2011) HERG potassium channel gating is mediated by N- and C-terminal region interactions. J. Gen. Physiol. 137, 315–325
22. Gustina, A. S., and Trudeau, M. C. (2013) The endog domain regulates hERG channel gating: an indirect interaction. J. Gen. Physiol. 141, 295–309
23. Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Chait, B. T., and MacKinnon, R. (1998) Crystal structure and functional analysis of the HERG potassium channel. Science 289, 666–676
24. Ju, M., and Wray, D. (2002) Molecular identification and characterization of HERG1a, HERG1b, HERG2 and HERG3 genes and cardiac arrhythmia. Circ. Res. 90, 870–878
25. Al-Owais, M., Bracey, K., and Wray, D. (2009) Role of intracellular domains in the function of the herg potassium channel. Eur. Biophys. J. 38, 569–576
26. Zou, A., Lin, Z., Humble, M., Creech, C. D., Wagoner, P. K., Krafte, D., Jegla, T. J., and Wickenden, A. D. (2003) Distribution and functional properties of human KCNHH (Elk1) potassium channels. Am. J. Physiol. Cell Physiol. 285, C1356–C1366
27. Trudeau, M. C., Titus, S. A., Branchaw, J. L., Ganetzky, B., and Robertson, G. A. (1999) Functional analysis of a mouse brain Elk-type K⁺ channel. J. Neurosci. 19, 2906–2918
28. Wu, W., Sachse, F. B., Gardner, A., and Sanguinetti, M. C. (2014) Stoichiometry of altered hERG1 channel gating by small molecule activators. J. Gen. Physiol. 143, 499–512
29. Wynia-Smith, S. L., Gillian-Daniel, A. L., Satyshur, K. A., and Robertson, G. A. (2008) hERG gating microdomains defined by S6 mutagenesis and molecular modeling. J. Gen. Physiol. 132, 507–520
30. Goldin, A. L. (1991) Expression of ion channels by injection of mRNA into Xenopus oocytes. Methods Cell Biol. 36, 487–509
31. Stühmer, W. (1992) Electrophysiological recording from Xenopus oocytes. Methods Enzymol. 207, 319–339
32. Schreibmayer, W., Lester, H. A., and Dascal, N. (1994) Voltage clamping of Xenopus laevis oocytes utilizing agarose-cushion electrodes. Pflugers Arch. 426, 453–458
33. Ng, C. A., Hunter, M. J., Perry, M. D., Mohi, M., Ke, Y., Kuchel, P. W., King, G. F., Stock, D., and Vandenberg, J. I. (2011) The N-terminal tail of hERG contains an amphipathic α-helix that regulates channel deactivation. PLoS ONE 6, e16191
34. Berecki, G., Zegers, J. G., Verkerk, A. O., Bhuiyan, Z. A., de Jonge, B., Veldkamp, M. W., Wilders, R., and van Ginneken, A. C. (2005) HERG channel dysfunction in an inherited cardiac arrhythmia revealed by dynamic action potential clamp technique. Biophys. J. 88, 566–578
35. Holmgren, M., Shin, K. S., and Yellen, G. (1998) The activation gate of a voltage-gated K⁺ channel can be trapped in the open state by an intersubunit metal bridge. Neuron 21, 617–621
36. Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M.,
N-terminal Regulation of hERG1 Deactivation

Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity. Science 280, 69–77

Hurst, R. S., Kavanaugh, M. P., Yael, J., Adelman, J. P., and North, R. A. (1992) Cooperative interactions among subunits of a voltage-dependent potassium channel. Evidence from expression of concatenated cDNAs. J. Biol. Chem. 267, 23742–23745

Tytgat, J., and Hess, P. (1992) Evidence for cooperative interactions in potassium channel gating. Nature 359, 420–423

Zandany, N., Ovadia, M., Orr, I., and Yifrach, O. (2008) Direct analysis of N-terminal Regulation of hERG1 Deactivation.

Wang, J., Trudeau, M. C., Zappia, A. M., and Robertson, G. A. (1998) Functional implications of C-terminal region of an ERG channel and its interaction with the S4-S5 linker. Biochim. Biophys. Res. Commun. 403, 126–132

Fernandez, D., Ghanta, A., Kauffman, G. W., and Sanguinetti, M. C. (2004) Physicochemical features of the hERG channel drug binding site. J. Biol. Chem. 279, 10120–10127

Wang, J., Trudeau, M. C., Zappia, A. M., and Robertson, G. A. (1998) Regulation of deactivation by an amino terminal domain in human ether-a-go-go-related gene potassium channels. J. Gen. Physiol. 112, 637–647

Li, Q., Gayen, S., Chen, A. S., Huang, Q., Raida, M., and Kang, C. (2010) NMR solution structure of the N-terminal domain of hERG and its interaction with the S4-S5 linker. Biochem. Biophys. Res. Commun. 403, 126–132

de la Peña, P., Alonso-Ron, C., Machín, A., Fernández-Trillo, J., Carretero, L., Domínguez, P., and Barros, F. (2011) Demonstration of physical proximity between the N terminus and the S4-S5 linker of the human ether-a-go-go-related gene (hERG) potassium channel. J. Biol. Chem. 286, 19065–19075

Gianulis, E. C., Liu, Q., and Trudeau, M. C. (2013) Direct interactions between C-terminal domains and cyclic nucleotide-binding homology domain of C-terminal region of an ERG channel and its interaction with the S4-S5 linker. Biochim. Biophys. Res. Commun. 403, 126–132

Haitin, Y., Carlson, A. E., and Zagotta, W. N. (2013) Mechanism of KCNH-channel regulation by C-terminal region of an ERG channel and its interaction with the S4-S5 linker. Biochim. Biophys. Res. Commun. 403, 126–132

Brelidze, T. I., Gianulis, E. C., Liu, Q., and Trudeau, M. C. (2013) Structure of the C-terminal region of an ERG channel and its interaction with the S4-S5 linker. J. Biol. Chem. 288, 444–448

Gustina, A. S., and Trudeau, M. C. (2014) Mechanism of KCNH-channel regulation by the C-terminal region of an ERG channel and its interaction with the S4-S5 linker. J. Biol. Chem. 288, 444–448

Ogiekwa, E. M., Zagotta, W. N., Hoshi, T., Zawadski, M. A., Haab, J., and Aldrich, R. W. (1995) Cooperative subunit interactions in C-terminal inactivation of K⁺ channels. Biophys. J. 69, 2445–2457

Zagotta, W. N., Hoshi, T., Dittman, J., and Aldrich, R. W. (1994) Shaker potassium channel gating. II: Transitions in the activation pathway. J. Gen. Physiol. 103, 279–319

Sigworth, F. J. (1994) Voltage gating of ion channels. Q. Rev. Biophys. 27, 1–40

Liu, Y., Jurman, M. E., and Yellen, G. (1996) Dynamic rearrangement of the outer mouth of a K⁺ channel during gating. Neuron 16, 859–867

Yellen, G., Sodickson, D., Chen, T.-Y., and Jurman, M. E. (1994) An engineered cysteine in the external mouth of a K⁺ channel allows inactivation to be modulated by metal binding. Biophys. J. 66, 1068–1075

Hoshi, T., and Armstrong, C. M. (2013) C-terminal region of an ERG channel and its interaction with the S4-S5 linker. Biochim. Biophys. Res. Commun. 403, 126–132

Panyi, G., Sheng, Z., and Deutsch, C. (1995) C-terminal region of an ERG channel and its interaction with the S4-S5 linker. Biochim. Biophys. Res. Commun. 403, 126–132
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J. Biol. Chem. 2014, 289:23428-23436.
doi: 10.1074/jbc.M114.582437 originally published online July 9, 2014

Access the most updated version of this article at doi: 10.1074/jbc.M114.582437

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September 8, 2016