Allosteric Interaction of Rapid Delayed Rectifier Protein and Its Role in Cardiac Repolarization

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Abstract

The α-subunit of the rapid delayed rectifier I_{kr} has been identified to be composed of multiple function domains. However, much less is known about the electrophysiological consequences of the interaction properties in the assembled channel protein. In this paper, we present a detailed conformational kinetic model through characterizing allosteric interactions between the voltage sensing domain and the cytoplasmic activation gate. The correlation of kinetic properties to action potential (AP) dynamics was investigated at different basic cycle lengths. We found that in response to driving forces ranging from -40 mV to 60 mV, two open states were populated. A significant elevation of I_{kr} at the early AP was attributable to an available reserve and the open-state accumulation, leading to shortening of AP duration at rapid rates. In contrast, the development of a dominant late peak Ikr with a steep slope morphology observed at slow rates arose from the allosteric coupled activation pathway. The existence of available reserve suggests Ikr has a repolarization reserve which facilitates its rate adaptation.

1. Introduction

The malfunction of rapid delayed rectifier (I_{kr}) impairs the normal repolarization, leading to long QT syndrome, a pathological condition in which the affected individual can be predisposed to severe ventricular tachyarrhythmias. Over the last decade, cloning and mutagenesis studies have provided us a bunch of information about the voltage-gated K^+ channel family H, to which I_{kr} and other *Shaker*-like channels belong. The protein responsible for I_{kr} has been identified as a tetrameric complex with four identical α-subunits encoded by the HERG gene. Each of these subunits consists of six putative function segments, namely S1-S6, with a loop between S5 and S6.

Along with the progress in structure studies, the identification of protein interactions of cardiac ion channels are drawing more and more attention in the exploration of molecular mechanisms of ion channel diseases. To date, two types of interactions have been identified in the voltage-gated K^+ channels, i.e. subunit cooperativity and intrasubunit interaction. The molecular basis of cooperativity is believed to be a form of electrostatic interaction and hydrogen bonds across subunits in a protein complex [1]. The cooperativity has been well characterized in wild-type (WT) and mutant *Shaker* potassium channels such as ShBΔ6-46 [2] and L382V [3] expressed in *Xenopus* oocytes. In contrast, a breakthrough in understanding the intrasubunit interaction was not achieved until a few proeukaryotic and eukaryotic channel proteins had been crystallized. The solved crystal structures such as KcsA in closed state and Kv1.2 in open state provide the molecular bases for the build-up of new chimeric constructs used in the mechanistic investigation of the protein machinery. For example, two short, complementary sequences, one in S4-S5 linker and another at the COOH-terminal end of S6, have been found to be essential for the coupling between the voltage sensing domain and the activation gate located at the bottom of S6. More recently, a proximal domain has been reported to modulate the channel activation through interacting with the channel core [4]. Currently, the functional consequences of these interaction properties in an assembled I_{kr} protein complex are not well understood.

The purpose of this study was to examine the functional implication of subunit interactions in I_{kr}. With a focus on physical composition changes, we developed a kinetic model for the I_{kr} complex by characterizing allosteric inertaction between the S4 voltage sensor and the S6 gate. Our results showed that the conformation changes in the allosteric interaction pathway are functionally decisive for the development of available reserves as well as for the participation of I_{kr} in the repolarizaion process.

2. Methods

2.1. Electrophysiological data

Electrophysiological data were based on WT HERG proteins stably expressed in HEK293 cells at 35 °C [5]. A

potential range of -60 mV to 40 mV was used for steady-state activation, -40 mV to 60 mV for activation, -100 mV to -70 mV for deactivation, and -100 mV to 60 mV for inactivation and recovery from inactivation. The deactivation kinetics at potentials positive to -70 mV were excluded because of the recovery of some inactivated channels. The holding potential was set to -80 mV considering the Cole-Moore shift in activation sigmoidicity. The inactivation properties of single channel proteins were extended to accommodate some results from the study of Kiehn et. al.[6] using single HERG channels expressed in *Xenopus* oocytes.

2.2. Electromechanical coupling

The conformational activity for a single subunit was characterized by allosteric coupling (Figure 1). In response to voltage changes across the cell membrane, the voltage sensor transits between a resting and an active state. The conformation changes of S6 activation gate was invoked through electrostatic interactions between residues residing in the S4-S5 linker and the S6 segment. Because the S4-S5 linker is physically tethered to S4 segment, it can function as a transduction element. Thus, the translocation of S4 was electromechanically coupled to the motion of S6, giving rise to the widening or narrowing of S6 helical bundles near the cytoplasmic side, the base of the criss-crossed aperture of the channel proteins.

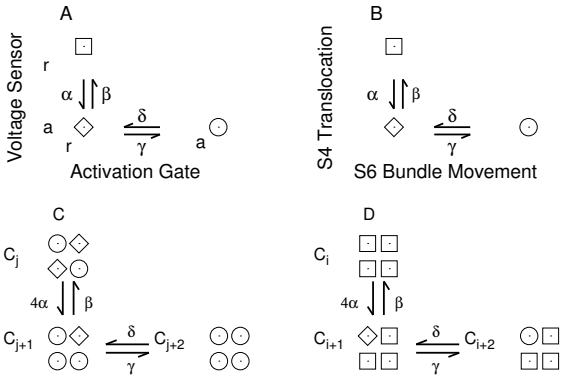

Figure 1. Subunit conformation changes. (A) Electrical activity, (r)-resting and (a)-activating. (B) Coupled mechanical motion. (C) Homomeric to heteromeric state. (D) Heteromeric to homomeric state.

2.3. Data fitting

The functional model for the tetrameric complex was constructed by extending the single subunit coupling model to the permutations of four subunits. The kinetics was described as time-homogeneous Markov process based on the stochastic property of single ion channels. Parameter estimation was performed by fitting the model to kinetic data. The merit function using a least-square criterion was derived in terms of the first direction derivatives [7] of the matrix exponential, which was solved by *scaling and squaring* [7] of an augmented block matrix.

3. Results

3.1. Conformational kinetics

For symmetrical molecular assembly of HERG subunits, there were 15-state conformation changes in the primray activation pathway before all the subunits activated. Two subsequent concerted transitions were required to characterize the properties of activation, steady-state activation, and deactivation, as shown in Figure 2. The fitting results for inactivation and recovery from inactivation were also illustrated in Figure 3. In the setting of similar transition probabilities for the preliminary open (O_1) to inactivation and to the open state O_2, our model generated a sigmoidal delay of $51.25 \pm 8.27(4)$ ms in the channel activation.

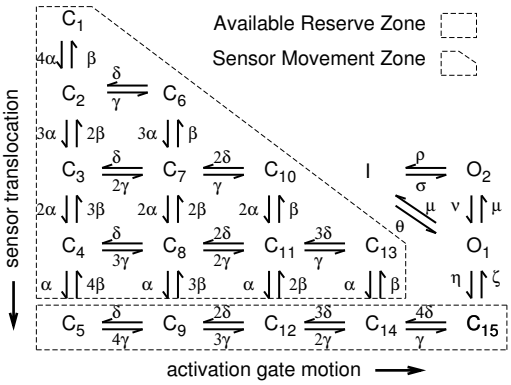

Figure 2. Electromechanical coupling model.

3.2. Repolarization reserve

Basic cycle lengths (BCL) ranging from 300 ms to 2000 ms were used to analyze the AP dynamics in response to I_{kr} conformational changes. The upstrokes of normalized I_{kr} at the early stage of AP were 0.019 ± 0.0015 at 1000 ms, 0.05 ± 0.0039 at 800 ms and 0.34 ± 0.028, as shown in Figure 4. In contrast, no significant difference was observed at the late peak of the normalized current density. The underlying conformation changes were revealed in terms of state occupation probability along with the time course, as shown in Figure 5. The available reserve formed by intermediate heteromeric states was increased from $20.9 \pm 0.09\%$ at 1000 ms to $24.1 \pm 0.08\%$

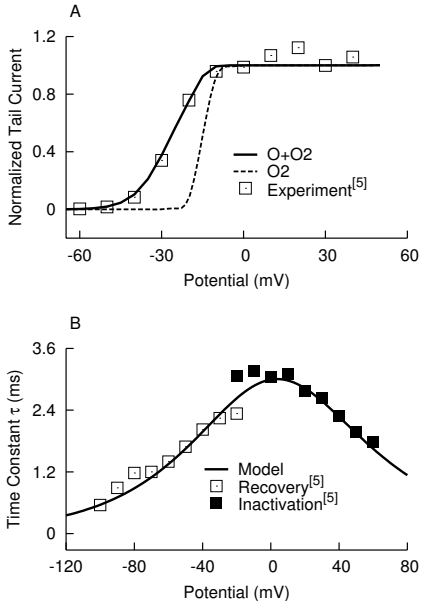

Figure 3. Fitting of I_{Kr} kinetics. (A) Normalized steady-state activation. (B) Inactivation and recovery from inactivation.

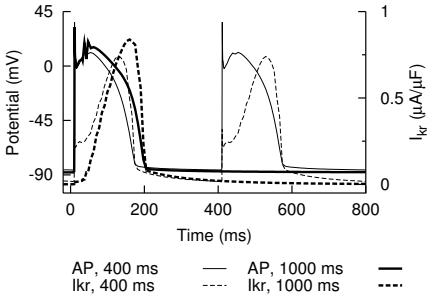

Figure 4. Action potential and I_{Kr} current.

at 400 ms. A more significant change was detected in the open-state accumulation due to incomplete deactivation, i.e., an augmentation from $1.3 \pm 0.07\%$ at 1000 ms to $3.5 \pm 0.2\%$ at 800 ms and to $22.5 \pm 1.31\%$ at 400 ms.

3.3. Abnormal AP duration

The effect of mutated I_{kr} channel on AP dynamics was examined by inducing a haploinsufficient mutation. The increased intracellular Ca^{2+} due to the malfunction of I_{kr} caused changes in several ionic pathways. Two calcium-sensitive ion channels were illustrated in Figure 7 and the results at a BCL of 400 ms are superimposed on that at 1000 ms for comparison. The delayed inactivation of I_{lca} accounted for its role in the prolongation of cardiac repolarization, whereas the sodium-calcium exchanger (I_{naca}) demonstrated a delayed inward current course (18.1 ± 7.79 ms at 400 ms and 37.29 ± 2.88 ms at 1000 ms), contributing to the delayed AP repolarization (Figure 6).

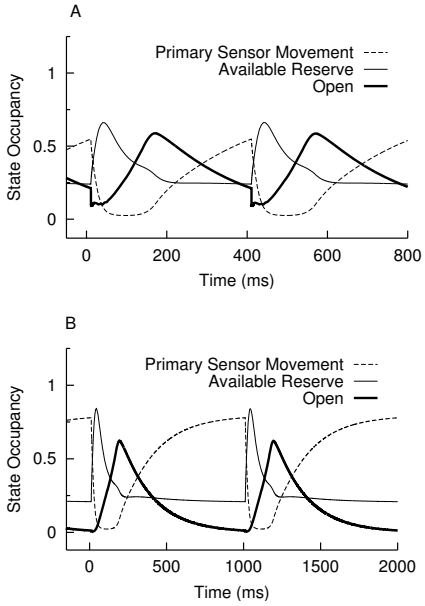

Figure 5. Occupancy of conformation states.

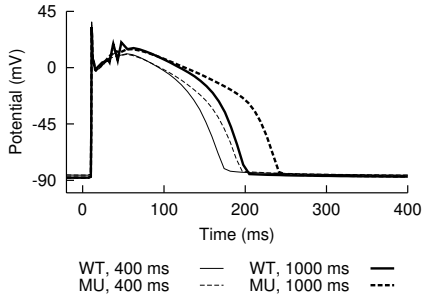

Figure 6. Mutation-induced AP prolongation.

4. Discussion and conclusions

4.1. Subunit basis of conformation changes

Through charactetrizing physical composition changes, we have established the subunit-based conformational model for the I_{kr} channel. The distinct property of this new interaction model is the participation of the voltage sensor in an allosteric interaction and its modulation in concerted channel opening transition. The direct electrostatic interaction between individual residues in the S4-S5 linker (D540K) and the C-S6 (Arg-665) has been found to stabilize the closed state in the KcsA-*Shaker* chimera, strengthening the role of S4-S5 linker in the electrocmechanical coupling. In contrary to the models for *Shaker* and recently published I_{ks} model, in which two transi-

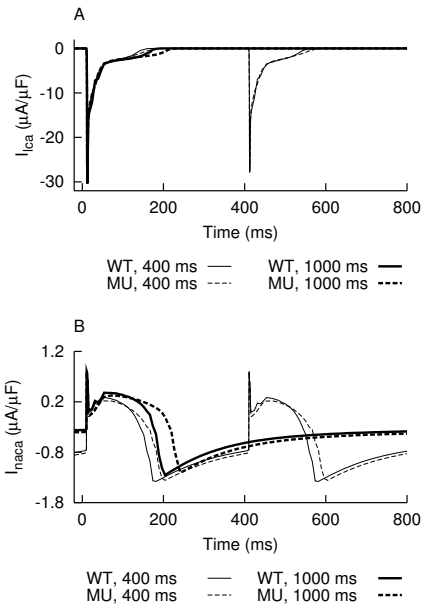

Figure 7. Mutation-induced changes in I_{lca} and I_{nana} pathways.

tions of sensors are required before channels open, our model demonstrated one-stage sensor transition was able to invoke the S6 transition allosterically, and further movement of S4 was invloved in the tightly cooperative process leading to channel opening, The similar finding has been reported in spHCN channels [8] in which an additional conformation change of the S4 C-terminal region has been proved to be essential for the cooperative channel opening. Additionally, the evaluation of time delay in the activation confirmed the sigmoidicity of activation with a $51.25 \pm 8.27(4)$ delay at 0 mV, being very similar to the result of Saenen et al. [4], i.e., 53 ± 8 ms at 10 mV.

4.2. Available reserve in rate adaptation

Available reserve is the intrinsic ability of some ion channels to adapt AP duration in response to the acceleration of heart rates [9]. In addition to commonly accepted open-state accumulation as an underlying mechanism, the closed-state accumulation in I_{ks}, the slow delayed rectifier, has been recently found to be responsible for its formation of available reserve between APs during rapid pacing. In our study, an early upstoke of I_{kr} during the phase 1 of the AP exhibited more than a 6-fold increment at a BCL of 400 ms compared with a BCL of 800 ms. The analysis of the conformation occupation probability revealed that an available reserve was developed from larger occupancy in transient heteromeric pore conformations, contributing to shortening of the AP duration at rapid rates. Secondary to the available reserve, the open-state accumulation of I_{kr} was also important for its adaptation to rate changes. In contrast, the development of a dominant late peak I_{kr} observed at slow rates resulted from an increased propensity to visit energetically favored conformation states in the allosteric coupled activation pathway, allowing for its participating in the repolarizing process during the plateau phase of AP duration.

In conclusion to our study, we have found that the existence of available reserve suggests I_{kr} has a repolarization reserve, facilitating its rate adaptation under physiological conditions. The allosteric coupling of activating and opening explains why I_{Kr} is a major repolarizing force during the plateau and late phases of the AP. Using a haploinsufficient mutation, we demonstrated that the malfunction of I_{kr} due to genetic mutations can cause instable ventricular repolarization, predisposing the affected individuals to long QT syndrome.

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