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BIOPHYSICS

Calmodulin acts as a state-dependent switch to control a cardiac potassium channel opening

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Calmodulin (CaM) and phosphatidylinositol 4,5-bisphosphate (PIP₂) are potent regulators of the voltage-gated potassium channel KCNQ1 (K_V7.1), which conducts the cardiac *I_{Ks}* current. Although cryo-electron microscopy structures revealed intricate interactions between the KCNQ1 voltage-sensing domain (VSD), CaM, and PIP₂, the functional consequences of these interactions remain unknown. Here, we show that CaM-VSD interactions act as a state-dependent switch to control KCNQ1 pore opening. Combined electrophysiology and molecular dynamics network analysis suggest that VSD transition into the fully activated state allows PIP₂ to compete with CaM for binding to VSD. This leads to conformational changes that alter VSD-pore coupling to stabilize open states. We identify a motif in the KCNQ1 cytosolic domain, which works downstream of CaM-VSD interactions to facilitate the conformational change. Our findings suggest a gating mechanism that integrates PIP₂ and CaM in KCNQ1 voltage-dependent activation, yielding insights into how KCNQ1 gains the phenotypes critical for its physiological function.

INTRODUCTION

The KCNQ (K_V7) voltage-gated potassium channel family consists of five members (KCNQ1 to KCNQ5), which provide K⁺ currents critical for physiological function of both excitable and nonexcitable tissues (1–4). All KCNQ channels require the association of calmodulin (CaM) (5, 6) and membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP₂) (7, 8) for voltage-dependent opening. In the heart, the predominant cardiac isoform KCNQ1 (K_V7.1) also associates with the auxiliary subunit KCNE1 (1, 2) to carry the slow delayed rectifier K⁺ current (*I_{Ks}*). *I_{Ks}* facilitates repolarization of the cardiac action potential, especially during β -adrenergic stimulation (9). Hundreds of mutations in KCNQ1 are linked to arrhythmias such as atrial fibrillation and long QT syndrome (LQTS), predisposing patients to sudden cardiac death (10, 11).

Structurally, KCNQ1 channels comprise four identical subunits, each with six transmembrane (S1 to S6) and four cytoplasmic [helix A (HA) to HD] α -helices, with S1 to S4 forming the voltage-sensing domain (VSD) and S5 and S6 the pore domain (Fig. 1, A and B) (12, 13). Upon membrane depolarization, the KCNQ1 VSDs activate with two experimentally resolvable steps: first from the resting state to an intermediate state and then to the fully activated state (14–19). The VSD conformational changes are communicated to the pore domain via a series of residue-residue interactions, leading to pore opening (14, 16, 19). KCNQ1 features a unique two-open-state gating mechanism (Fig. 1C). Previous work showed that KCNQ1 VSD can trigger pore opening when activated to both the intermediate and the fully activated states, yielding two open states: the intermediate-open (IO) and activated-open (AO) states (16, 18–22). The IO and AO states have been shown to give rise to biexponential current activation kinetics (16, 20, 21), with the ratio of the fast and slow components approximating the relative stability of IO and AO states,

respectively (Fig. 1C and fig. S1, A to C). The IO and AO states exhibit different functional properties important for KCNQ1 physiological function, with regulation of IO and AO by different auxiliary subunits leading to tissue-specific current phenotypes (18). For example, although KCNQ1 conducts predominantly at the IO state as seen by the large fraction of the fast-current component (Fig. 1C and fig. S1A), the auxiliary subunit KCNE1 eliminates the kinetically fast IO state and enhances the slow AO state, contributing to the kinetically slow *I_{Ks}* suitable for cardiac tissue needs (16, 18, 19).

KCNQ regulation by CaM (5, 6, 12, 13) is still not clearly understood. CaM features a two-lobe structural architecture with the N-terminal domain (N-lobe) and the C-terminal domain (C-lobe), and each lobe harbors two Ca²⁺ binding sites (23). CaM regulation of KCNQ channels is still debated, some studies suggested that CaM confers Ca²⁺-dependent inhibition of KCNQ2/3 and KCNQ4 currents but Ca²⁺-dependent enhancement of KCNQ1 currents (24–29). Other studies found that CaM C-lobe bound to KCNQ1 does not coordinate Ca²⁺ ions under 1 mM EGTA or 5 mM Ca²⁺ conditions (30). CaM binding by itself may therefore be sufficient to affect KCNQ1 function, similar to other voltage-gated channels (31). Structurally, CaM binds the KCNQ1 C-terminal domain (CTD) such that CaM N-lobe associates with KCNQ1 HB and CaM C-lobe interacts with KCNQ1 HA (Fig. 1A) (5, 6, 12, 13, 30). Recent cryo-electron microscopy (EM) structural studies of the full-length KCNQ1/CaM complex (12, 13) revealed intricate interactions between CaM, the KCNQ1 VSD, and the membrane lipid PIP₂. In the absence of PIP₂, the KCNQ1 CTD “bends” at the S6-HA linker to enable CaM interaction with the VSD S2-S3 linker (Fig. 1A) (12, 13). In the presence of PIP₂, the KCNQ1 CTD “straightens” to disengage CaM from the VSD, and the S2-S3 linker instead interacts with PIP₂ (Fig. 1B) (13). We will refer to these two cryo-EM structural states as the “CTD-bent” and the “CTD-straight” conformations (Fig. 1, A and B). The VSDs in both conformations adopt the fully activated state (12, 13, 18). The CTD-bent conformation features a closed pore, while the CTD-straight conformation exhibits a dilated pore (12, 13).

Although the KCNQ1/CaM structures have provided fresh insight into the conformational ensemble of KCNQ1, it is still unknown how

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Fig. 1. The S2-S3 linker within the KCNQ1 VSD modulates KCNQ1 currents. (A and B) Structural depiction of one monomer of the tetrameric KCNQ1/CaM complex when determined in the absence (A) and presence (B) of PIP₂ [i.e., CTD-bent (A) and CTD-straight (B) conformations]. (C) Left: A simplified KCNQ1 gating model (16, 18–21) with two open states: IO and AO. Horizontal transitions correspond to VSD activation, vertical transitions correspond to pore opening. RC, resting closed; IC, intermediate closed; AC, activated closed. Right: Exemplar biexponential activation kinetics fitting of a Q1-WT normalized current. Voltage was stepped from –80 to 0 mV. (D) Sequence alignment of the KCNQ family and KCNA2 at the S2-S3 linker region predicted to interact with CaM. (E) Mutagenesis screen of the KCNQ1 S2-S3 linker region shown in (D). Y axis: $\Delta V_{1/2}$ from WT channel [see (F) for example]. X axis: residue position. Stars indicate significant $\Delta V_{1/2}$ from WT (table S1). Error bars are SEM. (F) Left: Exemplar recordings from KCNQ1 WT and S2-S3 linker mutant channels. Voltage protocol is shown in inset. Currents were collected with 10-mV increments at the test pulse but shown in 20-mV increments to avoid crowding. Right: Average G-V relationship for the respective channels. An example of $\Delta V_{1/2}$ measurement is shown for mutant R181E. (G) Average normalized KCNQ1 channel activation kinetics, stepping from –80 to 0 mV, for Q1-WT (black, $n = 10$), Q1-S182D (blue, $n = 7$), and Q1-S182K (red, $n = 10$) channels. The light shadings show SEM. (H) Average biexponential kinetic fit parameters for Q1-WT (black), Q1-S182D (blue), and Q1-S182K (red) channels at different test voltages. Error bars are SEM. τ_{fast} and τ_{slow} are the time constants for the fast and slow components, respectively. f_{fast} is fraction of total current contributed by the fast component.

these structures correlate to the channel's voltage-dependent activation process. First, the CTD-straight structure likely corresponds to the AO state (Fig. 1C) based on its activated VSD and dilated pore (12, 13, 18), but functional data are lacking to support this. Moreover, the CTD-straight structure additionally features binding by the auxiliary subunit KCNE3 (13), which may lead to conformational differences compared to KCNQ1/CaM alone. Second, it is unknown whether the CTD-bent conformation corresponds to a functional state in the KCNQ1 activation pathway, since PIP₂ is present under

physiological conditions and the structure was solved without PIP₂. By extension, it is unclear whether the CaM-VSD interactions seen in the CTD-bent conformation occur during gating. No studies have shown simultaneous CaM and PIP₂ binding to the KCNQ1 VSD. The available studies thus suggest mutually exclusive CaM or PIP₂ binding to the S2-S3 linker (Fig. 1, A and B) (12, 13), with PIP₂ out-competing CaM for the activated VSD. Third, if the CTD-bent structure represents a functional state in the KCNQ1 activation pathway, what is the role of CaM-VSD interactions in KCNQ1 gating?

In summary, the relationship between KCNQ1/CaM structures and voltage-dependent gating, specifically the role of CaM-VSD interactions, remains unresolved.

Here, we combine electrophysiology experiments and network analysis of molecular dynamics (MD) simulations to show that CaM interacts with the KCNQ1 VSD during voltage-dependent activation to modulate ionic current, despite PIP₂ presence in the membrane. We demonstrate that both the CTD-straight and CTD-bent conformations are relevant in the KCNQ1 voltage-dependent gating process. We further provide evidence that the transition between the CTD-bent and CTD-straight conformations during gating is driven by VSD state-dependent interactions between the S2-S3 linker, CaM, and PIP₂. Functionally, we find that the loss of CaM-VSD interactions during gating controls channel occupancy of the AO state while having no effect on the properties of the IO state. Last, we map a CTD motif, formed by the S6-HA and HB-HC linkers, that works together with the CaM-VSD interface to affect pore opening to the AO state. On the basis of our results, together with the cryo-EM structural data, we suggest a gating model in which CaM acts as a VSD state-dependent switch to alter pore opening via the KCNQ1 CTD, thereby tuning the KCNQ1 current related to the cardiac repolarizing currents.

RESULTS

The cryo-EM studies showed two KCNQ1 structures with large conformational differences, with the CTD-straight structure solved in the more physiological conditions featuring lipid nanodiscs in the presence of PIP₂ and the CTD-bent structure solved in the absence of PIP₂ (12, 13). The CTD-straight structure is likely to reflect the AO state based on its activated VSD and dilated pore (13), but whether the CTD-bent structure corresponds to any gating state in voltage-dependent activation is not clear. We designed experiments and molecular dynamic simulations to address the following questions: (i) Is the CTD-bent structure part of the voltage-dependent activation process? (ii) Is there any functional evidence to support that the CTD-straight structure corresponds to the AO state? (iii) What is the mechanism and role of the conformational switch between the CTD-bent and CTD-straight in the gating process of KCNQ1?

The S2-S3 linker within the KCNQ1 VSD modulates KCNQ1 currents

The S2-S3 linker region is conserved among the KCNQ family (Fig. 1D) but not in the wider K_V superfamily. We began by examining the functional role of the KCNQ1 S2-S3 linker region close to either CaM or PIP₂ in the cryo-EM structures (12, 13). To this end, we undertook a dual alanine/tryptophan mutational scan of the S2-S3 linker (Fig. 1, A and B, maroon), designed to remove side chain properties and induce bulky steric effects, respectively. We assayed the mutant human KCNQ1 channels by two-electrode voltage clamp (TEVC) in *Xenopus* oocytes, in which the oocytes endogenously supplied PIP₂ and CaM, since endogenous *Xenopus* CaM shares 100% amino acid identity with human CaM. Each mutant channel was characterized by the half-activation voltage ($V_{1/2}$) of the measured conductance-voltage (G - V) relationship, which indicates the voltage dependence of channel open probability. The mutational scan revealed significant depolarizing shifts in the $V_{1/2}$ when mutating residues C180-K183 and V185 but not residues Y184 and G186-L187 (Fig. 1E and table S1). These results show the functional impact of

S2-S3 linker residues C180-K183 on KCNQ1 activation. As the S2-S3 linker may participate in electrostatic interactions with CaM or PIP₂, we further made charged mutations at two positions (R181 and S182) featuring clear effects. We found charge-dependent effects on KCNQ1 currents at positions R181 and S182: the mutants R181E and S182K induced ~20 mV depolarizing shifts in the $V_{1/2}$ (Fig. 1F, yellow and red). Conversely, the mutants R181Q and S182D caused little change from the wild-type (WT) G - V relationship (Fig. 1, E and F). As previous functional studies and the CTD-straight structure suggested that residue R181 interacts with PIP₂ (8, 13), the functional effects of the R181 mutants is likely due to altered PIP₂ binding. On the other hand, the CTD-bent structure showed that S182 interacts with CaM (12, 13); therefore, the S182 mutation effects may be due to disrupted CaM-VSD interactions.

We also noted that the S182K mutant exhibited a major slow-down in current activation kinetics compared to the WT channels, while the S182D mutant exhibited similar kinetics as the WT channel (Fig. 1, G and H). As the fast and slow components of the KCNQ1 current kinetics approximate the IO and AO states (Fig. 1C), we quantified current activation by fitting a biexponential function with fast and slow time constants (τ_{fast} and τ_{slow}). We also calculated the fraction of total current carried by the fast versus the slow component (f_{fast}), which approximates the relative contribution of the IO versus the AO states to the total current (fig. S1, A to C). The S182D mutant exhibited small changes in τ_{fast} and f_{fast} compared to WT (Fig. 1, G and H), which correlated with the minimal shift in $V_{1/2}$ induced by the S182D mutation. By contrast, the S182K mutant revealed a substantial slowing in current activation kinetics compared to WT channels (Fig. 1, G and H), which correlated with the large shift in $V_{1/2}$ caused by S182K. The slow time course of S182K currents resulted from specific increases in both the τ_{slow} and fraction of the slow component (Fig. 1H: τ_{slow} and reduction in f_{fast}) but not the fast kinetic component (Fig. 1H: τ_{fast}). The specific effect on the slow component of the current kinetics suggests that S182K may shift KCNQ1 to favor the AO state over the IO state (fig. S1, A to C), which may arise from destabilization of the IO state or stabilization of the AO state. The correlation between kinetics changes and significant $V_{1/2}$ shifts is preserved in other S2-S3 linker mutants (fig. S2A). In contrast to S182K, the R181E mutant did not affect the slow time constant or the fraction of the slow component but instead prolonged the fast time constant (fig. S3, A and B). These distinct kinetic effects are further suggestive that S182 and R181 might differentially affect KCNQ1 by interacting with CaM or PIP₂, respectively. Overall, the mutational scan provided functional evidence that the CaM- and PIP₂-interacting regions of the KCNQ1 S2-S3 linker, as seen in the cryo-EM structures, functionally affects KCNQ1 activation.

The S2-S3 linker interacts with CaM in KCNQ1 voltage-dependent gating

Is the current effect induced by the mutation S182K (Fig. 1, E and F) due to disruption of CaM-VSD interactions? We next examined whether these CaM-VSD interactions occur during physiological gating, when PIP₂ is available in the membrane. We undertook a second mutational scan of CaM residues close to the S2-S3 linker within the CTD-bent conformation (Fig. 2, A and B). We coexpressed each CaM mutant with human Q1-WT channels and compared their $V_{1/2}$ with that of Q1-WT coexpressed with CaM-WT. We note that KCNQ1 supplied with either endogenous or exogenous CaM WT yielded near identical phenotype (fig. S4). We found coexpression