they have no sequence homology to apoA-I, the peptides bind to lipids in a manner similar to that of apoA-I (i.e. antiparallel double-belt on the edge of the lipid disc). Molecular dynamics simulations of the lipid-bound peptide mimetic 4F were performed for 30 ns in explicit water using CHARMM22/27 parameters. The peptides were arranged in a stacked and staggered conformation to determine if there was any difference in the stability of the belt structure of the peptides. In the initial model, 16 straight alpha-helical chains of 4F were placed around two leaflets of 108 dimyristoylphosphatidylcholines (DMPC). In both simulations all peptides remain in contact with the lipid. The staggered model gives a more circular shape while the stacked model distorts into an oval shape. The staggered model also has a lower conformational energy than the stacked model, indicating that peptide-lipid complexes in which the peptides are staggered may be the more stable form. Salt bridge analysis shows there are three additional salt bridge interactions formed that are not present in the stacked conformation. These interactions may be a contributing factor for the more stable form of the staggered conformation.

Platform AK: Voltage-gated K Channels - Gating: Gating Motions & Modulations

1953-Plat

The KCNE1 Subunit Modifies S2-S4 Interactions in the KCNQ1 Subunit of the I_{Ks} Channel Complex

Dick Wu, Kelli Delaloye, Jianmin Cui.
Washington University, St. Louis, MO, USA.

The I_{Ks} channel is formed by the coexpression of KCNE1 with KCNQ1. KCNE1 modifies KCNQ1 to bring about the characteristic I_{Ks} current that is essential for terminating ventricular action potentials. The objective of this study is to examine how KCNE1 modifies channel activation by altering the interactions between S2 and S4 in the voltage sensing domain (VSD) of KCNQ1. S2 and S4 contain a series of negatively (E1, E2) and positively (R1, R2, R4) charged residues conserved across all Kv channels, which are essential for voltage-dependent activation. We tested the accessibility of E1C by MTS reagents and found that E1C can be modified by $MTSES^-$ only when KCNE1 is present, suggesting that KCNE1 changes the packing of E1C. Likewise, E2 interactions are also altered by KCNE1. E2Q generates constitutively open channels with apparent partial inactivation, a phenotype distinctly different from WT KCNQ1. However, coexpressing E2Q with KCNE1 produces channels that are nearly identical to WT I_{Ks} in activation and deactivation, as if the drastic perturbations caused by E2Q in KCNQ1 were inconsequential to the function of I_{Ks}. Consistent with this view, in KCNQ1 a secondary mutation R2E can rescue the non-functional E2R. However, this double mutant remains non-functional in the presence of KCNE1. Therefore, E2 and R2 interact in KCNQ1 but not when the channel is coexpressed with KCNE1. Taken together, our data indicate that the association of KCNE1 either directly or allosterically changes the environment around E1 and breaks the interaction between E2 in S2 and R2 in S4. These findings offer new insight into the impact of KCNE1 on the structure of the VSD in KCNQ1, revealing a novel mechanism by which KCNE1 may modulate voltage-dependent activation in KCNQ1.

1954-Plat

Wild-Type KCNQ1 Modulates the Gating of the LQT1 Mutation R231C

Daniel C. Bartos[1], Jennifer L. Smith[1], Jennifer A. Kilby[2], Craig T. January[2], Brian P. Delisle[1].
[1]University of Kentucky, Lexington, KY, USA, [2]University of Wisconsin, Madison, WI, USA.

KCNQ1 mutations are linked to type 1 Long QT Syndrome (LQT1). *KCNQ1* encodes the voltage-gated K^+ channel α-subunit, Kv7.1, and LQT1 mutations typically reduce Kv7.1 current ($I_{Kv7.1}$). The missense mutation, R231C-Kv7.1, is associated with LQT1 and sinus bradycardia (Lupoglazoff et al., JACC 2004), but functional studies suggest that this mutation yields a constitutively activated large $I_{Kv7.1}$ (Rocheleau et al., JGP 2007). To better understand the molecular phenotype of R231C-Kv7.1, we transfected HEK293 cells with cDNA encoding the auxiliary K^+ channel subunit MinK1 and WT-Kv7.1, R231C-Kv7.1, or WT- and R231C-Kv7.1 (since LQT1 follows a dominant inheritance pattern). We measured $I_{Kv7.1}$ by prepulsing cells from -80 to 90mV in 10-mV increments for 5s, followed by a test-pulse to -50mV. $I_{Kv7.1}$ measured from cells expressing R231C-Kv7.1 was maximally activated at all potentials, and, compared to WT $I_{Kv7.1}$, increased the maximal peak tail $I_{Kv7.1}$ by ~350% (n=6-8 cells, $p<0.05$). In contrast, cells expressing WT- and R231C-Kv7.1 reduced the maximal peak tail $I_{Kv7.1}$ by ~50% compared to WT $I_{Kv7.1}$ (n=4-6 cells per group, $p<0.05$). We plotted the peak tail $I_{Kv7.1}$ measured during the test-pulse, as a function of the prepulse for cells expressing WT-Kv7.1 or WT- and R231C-Kv7.1, and described the data with the Boltzmann equation to calculate the midpoint potential ($V_{1/2}$) and slope factor (k) for peak tail $I_{Kv7.1}$ activation. Cells expressing WT-Kv7.1 had a $V_{1/2}$ of 19 ± 2mV and a k of 15 ± 2mV/e-fold change (n=6), and cells expressing WT- and R231C-Kv7.1 had a $V_{1/2}$ of -16 ± 5mV (n=5, $p<0.05$) and a k of 21 ± 2mV/e-fold change, ($p<0.05$). Cells expressing WT- and R231C-Kv7.1 also had a constitutively activated $I_{Kv7.1}$ that was ~22% of the maximal peak $I_{Kv7.1}$. These data demonstrate that WT-Kv7.1 dramatically alters the R231C-Kv7.1 phenotype and emphasize the importance of co-expressing WT-Kv7.1 and LQT1 mutations.

1955-Plat

Gated Motions and Interactions Between the Intra-Cellular Domains of the I_{KS} Channel Subunits

Yoni Haitin[1], Reuven Wiener[1], Dana Shaham[1], Enbal Ben-Tal[1], Asher Peretz[1], Liora Shamgar[1], Olaf Pongs[2], Joel Hirsch[1], Bernard Attali[1].
[1]Tel Aviv University, Tel Aviv, Israel, [2]Universitaet Hamburg, Hamburg, Germany.

Kv7.1 α-subunit assembles with the KCNE1 auxiliary subunit to form the cardiac I_{KS} K^+ channel. Mutations in these subunit genes produce the long QT syndrome, a life-threatening ventricular arrhythmia. Here we studied the static interactions and the voltage-dependent molecular rearrangements of the intra-cellular domains of the I_{KS} channel complex. The I_{KS} subunits were tagged with ECFP and/or EYFP and expressed in *Xenopus* oocytes. Simultaneous spectral analysis of the fluorescence resonance energy transfer (FRET) were combined with TEVC recordings of K^+ currents. In the channel closed state, a strong constitutive FRET signal between the C-termini of Kv7.1 and KCNE1 was observed. This static FRET signal was increased by 2-fold with a C-terminal truncation of Kv7.1 (Δ622-676). In addition, a marked FRET signal was observed between C-terminally CFP/YFP labeled Kv7.1 subunits, and between the N- and C-termini of double tagged α-subunits. Upon channel opening at +30 mV, concomitantly with I_{KS} K^+ currents recording, a voltage-dependent FRET elevation was detected between the C-termini of Kv7.1 and KCNE1 and between the N- and C-termini of the doubly-tagged Kv7.1. Notably, both K^+ currents and dynamic FRET changes were abolished by coexpressing the KCNE1 LQT5 mutant D76N along with Kv7.1. Direct interactions between the C-termini of Kv7.1 and KCNE1 were further explored by the use of purified recombinant peptides in a series of *in-vitro* pull-down experiments. These experiments indicated that the KCNE1 C-terminus physically interacts with the coiled-coil helix-C of the tetramerization domain. Thus, we suggest that the tetramerization domain of Kv7.1 possesses an additional function as an intra-cellular docking site for KCNE1. Moreover, we demonstrate that channel gating is propagated to the C-termini of both subunits, and accompanied by a spatial rearrangement of the channel complex.

1956-Plat

Using Voltage Clamp Fluorometry to Track Voltage Sensor Movement in a Mammalian Kv1.2 Channel in the Presence of the Kvbeta1.2 Subunit

Christian J. Peters, Moninder Vaid, Andrew Horne, David Fedida, Eric A. Accili.
University of British Columbia, Vancouver, BC, Canada.

The N-termini of Kv1 α-subunits bind co-translationally with cytosolic Kvβ-subunits, with 1:1 symmetry. Kvβ-subunits of three distinct families have been found in neural and cardiac tissue, and members of the Kvβ1 family confer fast inactivation and slowed deactivation when co-assembled with Kv1 α-subunits. These effects may be due to a blocking action by the Kvβ1 N-terminus. Kvβ1 subunits also cause an apparent hyperpolarizing shift in the activation curve of Kv1 channels, which may be a consequence of block by the Kvβ1 N-terminus, due to premature saturation of deactivating tail currents, or alternately may be due to an allosteric interaction between Kvβ1 and Kv1 α-subunits, modifying voltage sensor movement. Here, we use voltage clamp fluorometry to directly track the movement of the Kv1.2 voltage sensor in the absence or presence of the Kvβ1.2 subunit, or an N-terminally-truncated Kvβ1.2 subunit which does not produce fast inactivation. While Kvβ1.2 led to a spike-and-decay current waveform and a hyperpolarized shift in ionic current activation, the voltage dependence of ON gating charge movements were unaffected. Kvβ1.2 also slowed Kv1.2 fluorescence and current deactivation, implying that the return of the voltage sensor to its pre-activation position followed the closing of the activation gate. These findings suggest that the hyperpolarizing shift in channel activation is a consequence of pore block by the Kvβ1.2 N-terminus, and not an allosteric effect on the Kv1.2 voltage sensor, and that block prevents both closure of the activation gate and the return of the S4 helix upon repolarization.

1957-Plat

LRET Measurements In The Three Major Conformations Of The Shaker K Channel

Walter Sandtner, Jerome Lacroix, Janice Robertson, Ludivine Frezza, Clark Hyde, Francisco Bezanilla, Ana M. Correa.
University of Chicago, Chicago, IL, USA.