conduction and QT prolongation observed in SUDEP and that TTX-sensitive channels play an important role in these changes. Our results suggest a new paradigm by which some of the arrhythmias observed during epilepsy are not centrally mediated but also occur as a consequence of electrical remodeling of the heart.

Voltage-gated Ca Channels II

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Profiling Mechanisms of RGK Inhibition Across the Family of High-Voltage-Activated Cav1/Cav2 Calcium Channels

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Rad/Rem/Rem2/Gem (RGK) proteins are Ras-like monomeric G-proteins that powerfully potently inhibit all high-voltage-activated Ca_V1 and Ca_V2 calcium channels. Since all RGKs bind auxiliary $Ca_V\beta$s it was generally assumed that the RGK-β interaction is essential for Ca_V channel inhibition. Recently, using a mutated β (β_{TM}) which), which selectively loses the ability to interact with RGKs, we reported that Rem inhibits $Ca_V1.2$ channels using both β-binding-dependent and direct α_1-binding-dependent mechanisms (Yang et al, 2013, PLoS One, 7:e37079). Our aims here were twofold: (1) to identify determinants and mechanisms underlying direct Rem binding to, and inhibition of, $Ca_V1.2$ pore-forming α_{1C} subunit;; (2) to profile the relative prevalence of β-binding-dependent and -independent mechanisms of inhibition across the RGK and Ca_V1/Ca_V2 channel families. Using a combination of FRET, co-immunoprecipitation assays, systematic truncations, and whole-cell electrophysiology we found that Rem C-terminus interacts with α_{1C} N-terminus to inhibit $Ca_V1.2$ current ($I_{Ca,L}$) and gating charge. For profiling, we compared the impact of the four RGKs on currents through recombinant channels ($Ca_V1.3$, $Ca_V2.1$, $Ca_V2.2$) reconstituted with either wt β_{2a} or $\beta_{2a,TM}$, respectively. When reconstituted with wt β_{2a}, all three channel types were strongly inhibited by each RGK. By contrast, when reconstituted with $\beta_{2a,TM}$, $Ca_V1.3$ and $Ca_V2.1$ were completely refractory to all four RGKs indicating these channels display only $Ca_V\beta$-binding-dependent mechanisms of inhibition. $Ca_V2.2$ channels reconstituted with $\beta_{2a,TM}$ displayed a strong inhibition solely to Rad, identifying a second example of $Ca_V\beta$-binding-independent regulation of a Ca_V channel by an RGK protein. The results reveal latent capabilities of distinct RGKs to selectively inhibit particular Ca_V1/Ca_V2 channels in an isoform-specific manner. These dormant capabilities may be exploitable to develop novel genetically-encoded isoform-selective Ca_V1/Ca_V2 channel inhibitors.

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Leucine-Rich Repeat Containing 10 (Lrrc10) Protein is a Novel Regulator of Cardiac Cav1.2 L-Type Calcium Channels

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Leucine-rich repeat containing 10 (LRRC10) is a cardiac-specific protein that plays a critical role in cardiac function. We have demonstrated that the *Lrrc10*-null (*Lrrc10*$^{-/-}$) mice develop dilated cardiomyopathy. Our recent data indicate that *Lrrc10*$^{-/-}$ cardiomyocytes exhibit reduced L-type Ca^{2+} channel (LTCC) current ($I_{Ca,L}$). However, it is unclear how LRRC10 regulates $I_{Ca,L}$ in the heart. To investigate the role of LRRC10 in the regulation of LTCCs, we co-expressed the Myc tagged LRRC10 (LRRC10-Myc), heamagglutinin tagged $Ca_v1.2$ ($Ca_v1.2$-HA) and the auxiliary $Ca_v\beta_{2C}$ subunit in HEK293 cells and performed co-immunoprecipitation (co-IP) on lysates using either anti-HA, anti-Myc antibody or control IgG. Western blot analysis demonstrated that $Ca_v1.2$ and LRRC10 associated with one another without the co-expression of $Ca_v\beta_{2C}$ subunit. Also, the $Ca_v\beta_{2C}$ and LRRC10 did not co-IP with one another suggesting that the LRRC10 may directly interact with $Ca_v1.2$ subunit. We then tested if a single point mutation H150A or triple point mutations Y104A, W127A and H150A would alter putative functional interaction sites in the LRRC10 and investigated if these mutations disrupt LRRC10 association with $Ca_v1.2$. Both LRRC10 mutants did not associate with $Ca_v1.2$. Additionally, co-IP analysis using mouse ventricular homogenates demonstrated that LRRC10 and $Ca_v1.2$ subunit are associated with one another. Finally, whole-cell patch clamp experiments performed in ventricular myocytes from *Lrrc10*$^{-/-}$ mice demonstrated a significant reduction in the $I_{Ca,L}$ density (−2.5 0.2 pA/pF) and delayed inactivation, compared to WT myocytes (−6 0.6 pA/pF). In summary, we demonstrate that the LRRC10 and $Ca_v1.2$ subunit of LTCC may directly interact with one another and that mutations in LRRC10 residues, likely important for protein-protein interactions, disrupts this association. We conclude that LRRC10 is a novel and essential regulator of the LTCC function in ventricular myocytes.

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A Unified Framework for Calcium Channel Modulation by Calcium Binding Proteins

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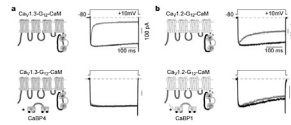
Distinguishing between allostery and competition among modulating ligands is challenging for large target molecules. One key example of such ambiguity concerns calcium-binding proteins (CaBPs) that tune signaling molecules regulated by calmodulin (CaM). In L-type Ca^{2+} channels, CaBPs can potently eliminate CaM-dependent inactivation (CDI). However, the mechanism for this modulation remains controversial. In past, using a live-cell holomolecule approach, we resolved a cyclical allosteric binding scheme for CaM and CaBP4 to $Ca_V1.3$ channels. In this scheme, both CaBP4 and CaM can simultaneously bind, resulting in strong inhibition of CDI despite the presence of a covalently attached CaM (**a**), supporting an allosteric regulatory mechanism. By contrast, Findeisen *et al* (*J Mol. Biol.* 425(17):3217-34) showed that fusion of CaM to $Ca_V1.2$ channels prevents CaBP1 modulation, thus arguing for a competitive regulatory mechanism. These results are confounded by limited delivery of CaBPs through pipet dialysis. Here, we show that the CDI of $Ca_V1.2$ channels with a fused CaM is robustly inhibited by recombinantly expressed CaBP1 and CaBP4 (**b**). Thus, it appears that the cyclical allosteric scheme first resolved in $Ca_V1.3$ now stands as a common framework for CaBP modulation of L-type calcium channels.



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C-Terminal Modulation of Cav1.3 L-Type Calcium Channels Modifies their Gating Properties in Cochlear Inner Hair Cells

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The Ca^{2+} currents in inner hair cells (IHCs) are crucial for synaptic transmission and flow through voltage-gated calcium channels (VGCCs) formed by the α1 subunit $Ca_v1.3$. VGCCs exhibit a calmodulin (CaM) mediated calcium-dependent inactivation (CDI), via binding CAM to the channel's C-terminus. In IHCs, $Ca_v1.3$ exhibits unusually weak CDI, probably caused by calcium binding proteins (CaBP) competing with CaM. IHCs express long and short $Ca_v1.3$ splice variants either including (long variant, $Ca_v1.3L$) or excluding (short variants) a C-terminal modulatory domain. In expression systems - lacking CaBPs - the C-terminal modulatory mechanism (CTM) functions via intramolecular interaction of a proximal (PCRD) and a distal C-terminal regulatory domain (DCRD) by inhibiting CaM binding near the PCRD, thereby inhibiting CaM-mediated CDI (Bock et al., JBC 2011). Here, the role of the CTM for IHC VGCCs was investigated in $Ca_v1.3L$-DCRDHA/HA mice in which CTM was disrupted by partial replacement of the DCRD with an HA tag.
Localization of HA-tagged $Ca_v1.3$ channels in IHCs was determined by immunohistochemistry. Channel properties were investigated by whole-cell patch-clamp recordings. Hearing was assessed using auditory brainstem responses (ABR) and distortion products of otoacoustic emissions (DPOAE).
Anti-HA immunolabeling was present at all IHC ribbons. Patch-clamp recordings revealed significantly reduced CDI and increased amplitudes of Ca^{2+} and Ba^{2+} currents in $Ca_v1.3L$-DCRDHA/HA IHCs. Non-stationary fluctuation analysis showed unchanged numbers of $Ca_v1.3$ channels and single channel currents. Voltage dependence and activation kinetics of I_{Ca} and I_{Ba}, ABR thresholds and DPOAEs were unaffected.
Our data demonstrate that the long $Ca_v1.3$ isoform is an intrinsic component of $Ca_v1.3$ clusters at *all* IHC ribbon synapses and that its DCRD is required for normal CDI and I_{Ca} amplitude.

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