2931-Pos Board B361

Convergent Modulations by Carboxyl-Termini across L-Type Calcium Channel Subtypes

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L-type calcium channels (LTCCs), also known as Ca_V1 family, are subject to diverse perturbations while acting as signaling hubs in cells, including autonomous modulations by the distal carboxyl terminus (DCT) of its own. Previous studies show that DCT of $Ca_V1.3$ and $Ca_V1.4$ (i.e., DCT_D and DCT_F respectively) competes with calmodulin (CaM) for IQ and IQ vicinity ($IQ\text{-}IQ_V$) of the channel, which results in attenuation of calcium dependent inactivation (CDI) (Liu *et al*. 2010). In parallel, DCT_C (i.e., DCT of $Ca_V1.2$) could autonomously inhibit voltage-gated activation (VGA) of the channel (Hulme *et al*. 2006). Here, our data demonstrate that distal carboxyl-terminus regulatory domain (DCRD) across Ca_V1 family could mediate both inhibition of CDI (I-CDI) and inhibition of VGA (I-VGA), suggesting a unified scheme of competitive autoregulation by DCRD across Ca_V1 family. Thus, the apparent discrepancy in phenotypes of I-CDI and/or I-VGA among Ca_V1 subtypes can be largely attributed to the different configurations of $DCT/IQ\text{-}IQ_V$ complex. Under such scheme, in addition to well-documented I-VGA, the proteolyzed carboxyl-terminus (CCT) of LTCC in neurons could also induce I-CDI. Both I-VGA and I-CDI play crucial roles in Ca_V1 signaling in neurons. Our results here provide converged mechanisms across Ca_V1 channel subtypes in their DCT modulations, highlighting the importance of comparative studies in the context of whole-family of Ca_V1 channels. Moreover, this study also reveals innovative features from $Ca_V1.1$ and $Ca_V1.2$, inviting further investigations into mechanism and physiology of DCTs.

2932-Pos Board B362

Modal Bifurcation of $Ca_V1.3$ Signaling in Cortical Neurons

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L-type Ca^{2+} channels, emerging as one of the central signaling hubs in healthy and diseased neurons, play prominent roles in diverse essential processes. However, it still remains largely as a puzzle how the same channel could simultaneously fulfill opposed tasks of signaling in neurons. Here we report that $Ca_V1.3$ channels are bifurcated into subpopulations with two distinct modes of signaling: *n*-mode signaling and *p*-mode signaling. This modal bifurcation is first demonstrated by genetically-encoded Ca^{2+} channel tuners (GECTs) of iCaMp and eCaMp, bioengineered to perturb autonomous competition between calmodulin and distal carboxyl tail of $Ca_V1.3$ channel. Channels driven into *p*-mode by eCaMp tend to produce ''sharp and large'' Ca^{2+} influx, which is kinetically distinguished from ''blunt and small'' Ca^{2+} influx via *n*-mode channels by iCaMp. Moreover, in cultured cortical neurons, modal bifurcation of $Ca_V1.3$ channels provides a pair of homeostatic opponents leading to constructive and destructive signaling events, eventually to morphological changes. In these neurons, kinase and phosphatase are preferentially activated by respective *p*-mode or *n*-mode channels and signals. Correspondingly, $Ca_V1.3$ signaling complex switches between its configurations to facilitate such signaling preference. Finally, the capabilities of GECTs, especially those of eCaMp in promoting *p*-mode channels and signals, highlight a new strategy to modulate L-type Ca^{2+} channels, as potential therapeutics for disorders with certain malfunctioned channels and/or dysregulated homeostasis.

2933-Pos Board B363

The Effect of Autism Candidate-Gene Mutations in the Voltage-Gated Calcium Channel β2 Subunit on Single Channel Kinetics

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The electrophysiological properties of voltage-gated calcium channel (VGCC) complexes are determined by the specific combination of subunit isoforms, *i. e.* of one pore-forming $Ca_V\alpha1$ subunit and the auxiliary subunits β, α2-δ (and γ). Therefore, VGCCs differentially modulate the cellular response to stimuli, which is relevant for neuronal function. Interestingly, the $Ca_V\alpha1C$ and $Ca_V\beta2$-subunit genes have been shown to be risk loci for five major psychiatric disorders including autism spectrum disease (ASD) (Lancet (2013) **381**:1371). Our group has recently found three missense mutations in the $Ca_V\beta2$ gene in ASD patients; while two mutants (G167S, S197F) resulted in a retardation of inactivation behavior, one mutant (F240L) accelerated the inactivation of whole-cell Ba^{2+} currents (PLOS One (2014) **9**(4): e95579). In the present study, we performed single channel patch clamp of HEK cells co-transfected with $Ca_V\beta2$ mutants and $Ca_V\alpha1C$. The gating parameters revealed a pronounced biophysical phenotype for all mutations: G167S and S197F persisted longer in an open state by elongating its mean open time ($p = 0.005$). F240L showed a trend for an increased open probability. Further, the transition rate constants obtained from Markov modelling of the single-channel data were consistent with the observed gating parameters. Here, the Markov model revealed significantly decelerated transition from open to closed state for G167S ($p = 0.008$) and S197F ($p = 0.03$). Both G167S and S197F showed slower transition rates suggesting a preference for deeper closed states (for S197F $p = 0.04$). That would explain why their open probabilities are not increased while mean open times are elongated. We conclude that the three mutations, each exhibiting different biophysical mechanisms, lead to the same outcome: more channel activity.

2934-Pos Board B364

Divergent Regulation of Cardiomyocyte Cav1.2 Currents by Calmodulin Mutants Associated with Human Sudden Death Syndromes

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Recent reports identified six calmodulin (CaM) missense mutations associated with three distinct human arrhythmia syndromes: N54I and N98S are associated with catecholaminergic polymorphic ventricular tachycardia (CPVT)-like clinical presentations, D96V, D130G and F142L with long QT syndrome (LQTS) and F90L with idiopathic ventricular fibrillation (IVF). We previously reported that all LQTS-CaMs exhibit reduced Ca affinity and normal or decreased (compared to WT CaM) ryanodine receptor (RyR2)-binding affinity, whereas CPVT-CaMs have normal or modestly lower Ca affinity but increase RyR2 single-channel open probability and show enhanced binding affinity to RyR2, causing significant increase in spontaneous Ca wave and spark activity in cardiac myocytes. Here, we investigate the effect of these mutant CaMs on another target potentially involved in arrhythmogenesis - Cav1.2 channels.

Using whole-cell patch clamp, Cav1.2 current (ICa) was measured in freshly isolated murine ventricular myocytes pre-treated with ryanodine (50 μM) and thapsigargin (10 μM) to prevent SR Ca release. Cells were dialyzed with either wild-type or mutant CaM (6 μM) via patch pipette. All LQTS-CaMs and IVF-CaM caused significant impairment of ICa inactivation: $\tau = 254 \pm 34$ ms (D96V), 251 ± 10 ms (F142L), 187 ± 13 ms (D130G) and 190 ± 8 ms (F90L) vs. 110 ± 5 ms for WT ($p<0.001$, n=4-7 myocytes/group), whereas CPVT-CaMs had no effect on ICa inactivation($\tau = 108 \pm 3$ ms (N54I), $\tau = 132 \pm 14$ ms (N98S) (NS, n=4-6 myocytes/group)). The effect of LQTS-CaMs and IVF-CaM was dominant, with a mixture of 25% mutant/75% WT CaM slowing ICa inactivation to a similar degree as 100% mutant CaM. Importantly, average peak current amplitude was unchanged in all groups. These results support the hypothesis that different targets are affected in arrhythmogenic disorders associated with LQTS-CaMs (impaired regulation of Cav1.2) vs. CPVT-CaMs (altered regulation of RyR2). The F90L CaM mutation shares characteristics with both CPVT and LQTS CaMs.

2935-Pos Board B365

A PQ-Channel Mutation Associated with Epilepsy Alters the Voltage Dependence of Channel Inactivation

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Epilepsy affects 1 in 140 people, or nearly 50 million people worldwide. It is characterized by seizures that often result from neuronal hyper-excitability. A recent genome-wide study uncovered many mutations associated with epilepsy in voltage-gated calcium channels (VGCC). However, the molecular and cellular consequences of these mutations, and hence, their epileptogenic mechanisms remain unknown. Here, we investigated two mutations that occur in PQ-type voltage-gated calcium channels, which are responsible for neurotransmitter release: R477H (in the I-II loop) and Q1957X (a truncation mutation). The mutations were introduced into a human PQ channel in the pGEMHE vector. The DNA for WT or mutant channels, together with the DNA for other VGCC subunits required for proper channel expression,