interacts with CaV1.2 in a similar domain as calmodulin (CaM). The purpose of this study is test the hypothesis that CaM and CCt compete for functional interaction with CaV1.2. ICa,L and barium current (IBa,L) was recorded from HEK 293 cells transfected with CaV1.2 + CaVbeta2a. This background was compared to cells additionally transfected with CaM and/or CCt. The CaV1.2 expressed was deleted at position 1733 (numbering based on rabbit sequence), and CCt corresponded to amino acids 1821-2171. ICa,L and IBa,L was recorded in each cell, and we compared the increase of current, the shift of activation midpoint, and current kinetics of ICa,L versus IBa,L within a given cell. Maximal conductance ratio Ca/Ba is ~0.4 for CaV1.2+CaVbeta2a expression. Addition of CaM co-expression does not alter Ca /Ba conductance. CCt co-expression significantly increases the relative Ca /Ba ratio 2-fold, and this effect is reversed by CCt+CaM co-expression. Examination of the peak I(V) curves suggests that midpoint of activation was not affected, and ICa,L density is not different in for all transfection conditions. We conclude that CCt attenuation of conductance occurs only with Ba, and is consistent with a Ca alleviation of CCt block. Thus, CaM and Ca functionally compete to limit CCt auto-inhibition of CaV1.2 current.

2685-Pos

Calmodulin Release from the IQ Domain of $Ca_v1.3$ Channels During Calcium Dependent Inactivation?

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Calmodulin (CaM) regulation of Ca_V channels has long fascinated biophysicists, with structure-function analysis mostly focused on an IQ domain in the carboxy-terminus of channels. It is clear that Ca^{2+}-free CaM (apoCaM) preassociates with the IQ domain before Ca^{2+} entry through channels (see Liu X *et al* this meeting), and that Ca^{2+}/CaM has the potential to bind the IQ. Hence, Ca^{2+}-dependent inactivation (CDI) of channels has been thought to result by transducing Ca^{2+}-dependent conformational changes of CaM, all while bound to the IQ. By contrast, functional analysis of our structure of Ca^{2+}/CaM complexed with the IQ domain of $Ca_V2.1$ (Structure **16**:607) hints that Ca^{2+}/CaM may *depart* from the IQ domain during channel regulation. To generalize this hypothesis, we here alanine scanned the entire IQ domain of $Ca_V1.3$, an exemplary prototype of Ca_V1 channels with high homology to long-studied $Ca_V1.2$. Importantly, alanine substitution likely preserves backbone fold, whereas prior studies often used more disruptive mutations targeting specific loci. As before, alanine substitution of the signature isoleucine strongly suppressed CDI. Suprisingly, however, CDI was strongly diminished only at one other residue, *upstream* of the central isoleucine. Altering many other sites, presumed important for Ca^{2+}/CaM-IQ binding in crystal structures, left CDI unscathed. Moreover, we homology modeled Ca^{2+}/CaM bound to the $Ca_V1.3$ IQ domain, based on x-ray structures of $Ca_V1.2$. We then computed (*Robetta*) the energetic cost of alanine mutations ($\Delta\Delta G$ for binding). If Ca^{2+}/CaM-IQ binding begets CDI, plots of CDI versus $\Delta\Delta G$ should define a Boltzmann function. Instead, a highly scattered relationship was produced. Thus, CDI may involve (partial) departure of Ca^{2+}/CaM from the IQ domain of $Ca_V1.3$, to interact with alternative sites (Yang, Ben Johny, & Yue, this meeting). This departure would fundamentally transform our understanding of CaM/channel regulation.

2686-Pos

Structural and Functional Comparison Between the Effects of CaBP1 and Calmodulin on the Voltage-Gated Calcium Channel Cav1.2

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Calcium-dependent feedback modulation is central to voltage-gated calcium channel (CaV) function. The bilobed EF-hand containing calcium-binding protein 1 (CaBP1) is thought to modulate CaV function by competing with the homologous calmodulin (CaM) for binding to the CaV C-terminal IQ-domain. In CaV1.2, CaBP1 inhibits calcium-dependent inactivation (CDI) of CaV1.2 and introduces calcium-dependent facilitation (CDF). We investigated the origins of functionally important differences between CaM and CaBP1 by creating a number of CaM/CaBP1 chimeras and found a clear division of function between the various elements. Determination of the CaBP1 X-ray structure revealed an interaction site between linker and one of the lobes that is required for inhibition of CaV1.2 CDI. Using titration calorimetry, we found that, similar to CaM, the CaBP1 C-terminal lobe is responsible for high-affinity interaction with the IQ-domain. CaM requires functional C-terminal lobe EF-hands for supporting CDI. In contrast, we found that CaBP1 does not require functional EF-hands for inhibition of CDI. CaBP1-mediated CDF however requires all EF-hands to be functional and also has different requirements from CaM-mediated CDF, suggesting that these are two distinct processes. Overall, the data reveal those parts of CaBP1 that set it functionally apart from CaM and provide a framework for understanding how CaBP1 and CaM regulate CDI and CDF on CaV1.2.

Voltage-gated K Channels-Gating III

2687-Pos

***In-Silico* Activation and Deactivation of the Pore Domains of Voltage- and Ligand-Gated Ion Channels**

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Several homotetrameric ion channels have been crystallized in either the open or closed state. Here we used Monte Carlo-energy minimizations and the program ZMM, which takes advantage of channel symmetry, to simulate activation/deactivation of the pore-forming domains of four channels and estimate their state-dependent energetics. Deactivation of the open-Kv1.2 x-ray structure by a counter-clockwise torque (intracellular view) applied to four S5 N-ends yielded a conformation with S5/S6 alpha-carbons RMS-deviating by 1.8 Å from the closed-channel model (Pathak et al., 2007). The data that cadmium binds to the closed *Shaker* with engineered cysteines in S6s (del Camino et al., 2005) is rationalized in our model with two cadmium ions neutralizing the four ionized cysteines (Bruhova and Zhorov, 2005). Reactivation of our deactivated-Kv1.2 model by the clockwise torque yielded a conformation that RMS-deviates from the open-Kv1.2 structure by 1.4 Å. Using the same approach we deactivated and reactivated the pore-domain of KvAP (truncated to match the KcsA-sequence length) and arrived at models with RMSDs of 2.0 and 1.9 Å from the KcsA and KvAP x-ray structures, respectively. Activation and deactivation of pH-gated KcsA with centrifugal and centripetal forces, respectively, applied to M2 C-ends (Tikhonov and Zhorov, 2004) yielded conformations that RMS-deviate from the x-ray structures of MthK and KcsA by $<$ 2.0 Å. Using the same forces, we activated and deactivated the cyclic-nucleotide gated NaK channel (truncated to match the open-NaK sequence length) and arrived at models with RMSDs $\leq$ 1.5 Å from the closed and open x-ray structures, respectively. In our models, the pore domains of the voltage- and ligand-gated channels are most stable in the open and closed states, respectively, suggesting an intrinsic instability of the pore domains in non-crystallized states. Supported by CIHR and NSERC.

2688-Pos

All-Atom Molecular Dynamics Simulations of the K+ Channel Chimera Kv1.2/Kv2.1

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Voltage-gated K^+ channels (Kv) are composed of four subunits, each of which contains six trans-membrane domains (TMs), S1 through S6. The S1-S4 segments comprise the voltage-sensing domain (VS), which senses membrane potential and controls the gating of the pore domain (PD). Although still controversial, the voltage-sensing domain undergoes conformational changes within the membrane electric field, upon membrane depolarization, that is mechanically transferred via the S4-S5 linker to the intracellular gate of the channel. MD simulations of portions of PD and VS regions highlighted the importance of their flexibility for proper channel function. Nevertheless, a comprehensive description of the dynamics of both domains at atomic level has not been provided yet. Here we report the analysis of all-atom multiple molecular dynamics simulations (~200 ns) of the entire Kv1.2/2.1 chimera, consisting of the α and β chain embedded in a 549 monomer POPC bilayer, and immersed in a box of 135K explicit SPC water molecules at 300 K. We used principal components analysis (PCA) of the Cα atomic fluctuations covariance matrix to analyze the essential subspace that characterizes the channel internal dynamics. Briefly, we observed an up to 4.5 Å conformational drift of VS from its starting position. The average RMSF of the S3b-S4 domain was between 1.6 and 3.0 Å. Relative to the pore region, i) the second principal component shows that all four VS domains fluctuate in a concerted manner and affect the flexibility of the intracellular gates; ii) the first principal component reveals that T1 domain moves approximately 3.5 Å downwards, influencing the local structure and dynamics of the neighboring intracellular gate. Protein-lipids interactions are crucial for channel structure/function. Thus, the contributions of H-bonds and salt-bridges between channel atoms and lipid head-groups on global channel dynamics will be illustrated.