

2672-Pos Board B364**Inhibition of RyR2 from Failing and Non-Failing Human Hearts by Calmodulin**

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The RyR2 is a macromolecular complex comprising a Ca²⁺ channel and many other accessory proteins that regulate channel activity. The binding of intracellular calmodulin (CaM, ~45 nM in the cytoplasm) partially inhibits calcium release in cardiomyocytes due to its ability to inhibit RyR2 channel opening. Single channel studies of RyR2 in lipid bilayers find that CaM inhibits RyR2 with an IC₅₀ of 100 nM. During the process of RyR2 isolation from the heart and their incorporation into lipid bilayers, the macromolecular complex stays mostly intact except for CaM which can dissociate from the RyR2 complex in minutes.

Heart failure is a complex disorder involving changes in Ca²⁺ handling protein expression, Ca²⁺ homeostasis and remodelling of the RyR2 complex. We compare RyR2 activity from healthy and failing human hearts and study their regulation by Ca²⁺ in the presence and absence of CaM. RyR2 was isolated from healthy human hearts and hearts with ischemic cardiomyopathy with ethics approval (H-2009-0369) and incorporated into lipid bilayers. CaM (0.5 μM with 0.1 μM Ca²⁺) caused a reduction in RyR2 Po of 57% ± 6% from failing human heart but did not affect RyRs from healthy hearts. Thus, action of CaM in the RyR complex is influenced by its remodelling that occurs in the failing heart.

2673-Pos Board B365**Structure-Function Relationships of M124Q Calmodulin, a Mutant that Mimics Oxidative Insults**

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Calmodulin (CaM) is a calcium-sensitive regulatory protein that interacts with numerous target proteins, including the ryanodine receptor (RyR). The RyR is a calcium channel responsible for releasing Ca²⁺ from the sarcoplasmic reticulum to induce muscle contraction. Due to its high methionine content, CaM is highly susceptible to oxidation by reactive oxygen species produced by normal metabolism. It has been shown that the oxidation level of CaM increases with aging, and contributes to muscle degeneration. Oxidized forms of CaM also have a decreased ability to regulate RyR function, thus disrupting the regulation of muscle contraction. The M124Q CaM mutant mimics the effects of a methionine sulfoxide at position 124, a key residue for CaM's interaction with the RyR. Here, we used dipolar electron-electron resonance (DEER) and NMR to characterize the structural dynamics of the M124Q-CaM. DEER analysis of M124Q-CaM suggests that the mutant exhibits open and closed conformations in micromolar Ca²⁺, but shifts to the more open conformation in nanomolar Ca²⁺. Differences in the population distributions were also observed for M124Q-CaM in the presence and absence of a RyR1 peptide that forms part of the CaM binding site. Finally, NMR analysis of wild-type and M124Q-CaM also indicated structural shifts due to the mutation and describe how both the wild-type and M124Q CaM respond to changes in Ca²⁺ concentration. These initial results give important insights on the structural effects of deleterious oxidative processes on the RyR regulation by CaM.

2674-Pos Board B366**Mechanisms Underlying Cooperativity in CaMKII Autophosphorylation and Substrate Phosphorylation**

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As a member of the calmodulin-activated kinases, Ca²⁺/calmodulin dependent protein kinase II (CaMKII) is a serine/threonine kinase coupled to calcium signaling. Unlike other multifunctional CaMK members, CaMKII has a unique dodecameric architecture potentially permitting cooperative forms of autoregulation and substrate phosphorylation. We observed that CaMKII phosphorylation of a peptide derived from the autoregulatory domain (AC-2) displays positive cooperativity (n_H=1.8). Another T-site binding peptide substrate derived from S1303 phosphorylation site on NR2B also displayed positive cooperativity (n_H=2.1). Surprisingly, a truncated form of CaMKII₁₋₃₁₇ monomer also shows this cooperativity towards NR2B and AC-2 peptides (n_H=1.6 vs 1.8, respectively). Syntide-2, a traditional substrate peptide lacking

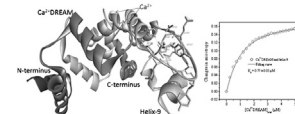
T-site interactions, does not exhibit cooperativity in substrate phosphorylation for either monomeric or multimeric CaMKII (n_H=1.0; n_H=1.1, respectively). These data suggest that the positive cooperativity seen with substrate phosphorylation is unique to T-site site binding substrates and may involve potential allosteric substrate interactions on the catalytic surface. Cooperativity within the holoenzyme occurring between subunits has been predicted based on the fact that Ca²⁺/CaM binding can induce Thr286 autophosphorylation, an inter-subunit intraholoenzyme reaction, whereby, neighboring subunits act as both kinase and substrate following coincident Ca²⁺/CaM binding. Using Ca²⁺/CaM-independent activity (i.e. autonomous) as a measure of Thr286 autophosphorylation, CaM titration experiments revealed that both Ca²⁺/CaM-dependent and autonomous forms of CaMKII activity were cooperative (n_H=2.1 for both) for Ca²⁺/CaM activation and autophosphorylation. Titrating CaM levels to ratios below 1 per holoenzyme (i.e. 12 CaMKII subunits per holoenzyme) generate submaximal autonomous activity, whereas, CaM levels at a ratio of ~2 per CaMKII holoenzyme generate maximal autonomous activity. Thus, CaM activation of CaMKII appears to follow a cooperative model whereby neighboring subunits within the holoenzyme preferentially obtain the activator to promote autophosphorylation even in the face of limiting CaM.

2675-Pos Board B367**Molecular Mechanism of Dream -Presenilin-1 Interactions**

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Downstream Regulatory Element Antagonist Modulator (DREAM), also known as calsenilin or potassium channel interacting protein 3 (KChIP3), interacts with presenilin 1 (PS1) and presenilin 2 (PS2) to promote accumulation of β-amyloid peptide in brain. To understand the molecular mechanism by which DREAM mediates β-amyloid formation, the interactions between calsenilin and presenilin were characterized using steady-state and time-resolved fluorescence anisotropy techniques. In addition, the structural changes in DREAM oligomerization state triggered by presenilin binding were studied. Here, we report the interaction between DREAM and a short segment of PS1 C-terminus (helix-9). The binding of DREAM to helix-9 is calcium dependent. The dissociation constant for Ca²⁺DREAM:helix-9 complexes was determined to be 0.77 ± 0.03 μM, whereas the Mg²⁺DREAM:complex helix-9 exhibits lower affinity, K_d = 19.3 ± 0.5 μM. Moreover, association of Ca²⁺DREAM to helix 9 results in slower depolarization with the rotational correlation time of Φ = 22.5 ± 0.1 ns, which matches with the estimated rotational correlation time for DREAM dimer bound to a helix-9. These results suggest that DREAM binds to presenilin in its dimeric form and the formation of the inter-protein complex is regulated by Ca²⁺.

**2676-Pos Board B368****Thermodynamic Impact of the Unstructured Linker Region on the Synaptotagmin I C2A Domain**

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Synaptotagmin I is a protein involved in the final steps of neurotransmitter release assisting in the fusion of vesicles with the plasma membrane of nerve cells. The synaptotagmin protein consists of a single transmembrane region connected to two C2 domains, C2A and C2B, through a long unstructured linker region. While much work has been done in order to understand how synaptotagmin functions in the fusion process, it has largely been focused solely on the C2 domains of the protein, with little work done examining the linker region. Recently it has been shown that these domains show remarkably low stabilities, and that the two C2 domains are energetically coupled. These results paint a picture of a protein that is exquisitely sensitive to its environment. As such these findings have led us to hypothesize that the unstructured linker region that tethers the C2 domains to the membrane may play an important role in the function of synaptotagmin. In order to gain a better understanding on how the presence of this unstructured region impacts synaptotagmin, we have investigated the stability of the C2A domain both with and without the linker region and have found dramatic differences between the two constructs. We have found that under all ligand conditions the stability of the domain decreases. For example, in the presence of Ca²⁺ the stability of the C2A domain with the linker region was found to be 3.61 ± 0.03 (kcal/mol) compared to a stability of 4.32 ± 0.05 (kcal/mol) for the domain without the linker. The decrease found in the stability of domain when connected with the linker region