pulsed-field-gradient nuclear magnetic resonance spectroscopy has been applied to probe their oligomeric state in solution and conformational changes of specific ligands due to Ca^{2+} binding were investigated using heteronuclear-labeled proteins with different 2D and 3D NMR techniques.

2774-Pos Board B744

CaMKII-Induced Shift in Modal Gating Explains L-type Ca2+ Current Facilitation: A Modeling Study

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Ca2+/calmodulin-dependent protein kinase II (CaMKII) plays an important role in L-type Ca2+ channel (LCC) facilitation, the Ca2+ -dependent augmentation of Ca2+ current (ICaL) that manifests itself during rapid repeated depolarizing stimuli. Multiple mechanisms may underlie facilitation, including increased LCC rate of recovery from Ca2+−dependent inactivation, and a shift in distribution of LCCs into high activity mode 2 gating, characterized by prolonged channel openings. To understand the mechanisms behind facilitation, a stochastic model was formulated which describes the dynamic interactions among CaMKII, LCCs and protein phosphatases in the cardiac dyad, as a function of subspace Ca2+ and calmodulin levels. This model faithfully reproduces single channel experimental results, and has been incorporated into an integrative computational model of the canine ventricular myocyte. Simulations demonstrate that the phosphorylation dependent shift in LCC modal gating distribution accounts for the hallmarks of ICaL facilitation, namely, ICaL amplitude augmentation, apparent macroscopic increase in rate of recovery from inactivation, and observed slowing of the inactivation rate of ICaL. A shift in LCC gating modes increases the probability of groups of LCCs re-opening during the late phase of the action potential, thus augmenting the risk of early-after depolarizations (EADs). EADs are believed to possibly trigger cardiac arrhythmias, therefore pharmacologic interventions which prevent EADs are likely to have therapeutic value. CaMKII inhibition has been proposed as a therapeutic agent for preventing arrhythmias. However, CaMKII has many phosphorylation targets, including phospholamban and Na+ channels. Our simulation findings suggest that facilitation and risk of EADs can be modulated by reducing mode 2 LCC gating directly. This solution is a favorable alternative to CaMKII inhibition because it will not disrupt the function of other CaMKII targets. This work is supported by National Institute of Health Grant R33HL87345.

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A Mechanistic, Minimal Model of Ca2+/Calmodulin Dependent Kinase II Signaling in the Cardiac Myocyte

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Numerous experiments have shown that Ca2+/calmodulin-dependent protein kinase II (CaMKII) expression level and activity are increased in human cardiac myocytes from patients with dilated cardiomyopathy. The role of CaMKII in heart failure remains unclear, in part because of an incomplete understanding of how the kinase helps regulate the normal cardiac environment. CaMKII modulates the behavior of many proteins involved in excitation-contraction coupling, including L-type Ca2+ channels (LCCs), phospholamban (PLB) and Na+ channels. In order to understand the combined effects of CaMKII's modulation of these targets, a stochastic model was built to investigate the molecular mechanisms behind ICaL facilitation. Simulations show that a CaMKII-driven shift in LCC modal gating distribution from mode 1 to mode 2 is sufficient to account for experimentally observed increases in ICaL amplitude, changes in ICaL inactivation kinetics, and alterations in recovery from inactivation. This CaMKII-LCC model was then minimized, reduced to a system of ordinary differential equations, and incorporated into an integrative model of the cardiac myocyte that accounts for graded calcium release. The effects of CaMKII phosphorylation of PLB and Na+ channels were also modeled, based on data from CaMKII-PLB kinase assays and in vitro experiments as well as CaMKII-Na channel patch clamp studies performed in healthy myocytes. Preliminary simulation results show that at high CaMKII levels, hyperphosphorylation of LCCs results in spontaneous early after depolarizations. In addition, increased CaMKII levels result in prolonged action potential duration, primarily because of increased persistent INa. The model therefore serves as an important tool to identify and study the mechanisms by which CaMKII activity modifies action potential shape and duration. This work is supported by National Institute of Health Grant R33HL87345.

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IP_3 Receptor-mediated Ca Release Facilitates RyR-Ca Release To Cause Inotropy And Arrhythmogenicity In Mouse Ventricular Myocytes

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Endothelin (ET-1)-induced IP_3R-dependent Ca release in atrial myocytes is well established. ET-1-induced positive inotropy and arrhythmogenicity have also been shown in ventricular myocytes from rat and rabbit. Here we used transgenic mice overexpressing IP_3R2 and IP_3 sponge to study ET-1-induced inotropy and arrhythmogenicity in ventricular myocytes. 100 nM ET-1 (14 min) increased ventricular myocyte Ca transients (ΔCa) in IP_3R2OX by 37% (0.5 Hz, fluo4 AM, n=11) vs WT and IP_3 sponge ventricle (-6 ± 10 %). ET-1 increased spontaneous calcium transient (SCT) frequency in IP_3R2OX ventricles compared with WT and IP_3 sponge (10 ± 4 vs 1 ± 1 SCT/min). Similar ΔCa results were obtained using the Ca indicator indo-1 (41 ± 9 vs 19 ± 12 % IP_3R2OX vs WT, n=6). We found no differences between IP_3R2OX (n=17) and WT (n=10) ventricular myocytes in control (0.5 Hz) ΔCa (350 ± 70 vs 330 ± 28 nM Ca_i) or SR Ca content as assessed with 10 mM caffeine (1368 ± 273 vs 1385 ± 78 nM Ca_i). Basal Ca spark frequency in saponin-permeabilized ventricular myocytes did not differ between IP_3R2OX and WT (19 ± 4 vs 16 ± 4 sparks x s^{-1} x (100 μm)$^{-1}$). However, direct application of 10 μM IP_3 produced higher increase in Ca spark frequency on IP_3R2OX (35 %, n=4) than WT (18 %). After 3 min of IP_3 application SR Ca content was depleted to 80 % of control. These data suggest that IP_3R-dependent inotropy is mediated via IP_3R-dependent Ca release that facilitates Ca release through ryanodine receptors. This IP_3-dependent inotropy is associated with spontaneous Ca release activity which may be arrhythmogenic (inducing action potentials). We conclude that IP_3R expression and IP_3-dependent Ca release play an important role in the generation of ventricular arrhythmias.

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IP_3-induced Ca^{2+} Signals at the Cytoplasm and Nucleus in HL-1 Atrial Cells: Possible Roles of IP_3 Receptor Subtypes

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HL-1 cells are the only adult cardiac cell line available that continuously divides while maintaining an atrial phenotype. We examined the expression and localization of inositol 1,4,5-trisphosphate receptor (IP_3R) subtypes and their functional roles in the local Ca^{2+} signaling of HL-1 cells. RT-PCR and western blot analyses of IP_3R revealed significant expression of type 1 (IP_3R1) and type 2 IP_3R (IP_3R2) in HL-1 and isolated atrial cells. IP_3R1 was more abundant in HL-1 cells than atrial cells, while IP_3R2 protein band was darker in intact atrial cells than HL-1 cells. Immunostaining of the IP_3R subtypes in HL-1 and intact atrial cells demonstrated that IP_3R1 localized to nuclear envelope and that IP_3R2 was distributed at the cytoplasm as a punctate form. Extracellular application of 1 mM ATP, known to generate IP_3, in intact HL-1 cells elicited Ca^{2+} rise with oscillation, while 10 mM caffeine produced a Ca^{2+} transient with no oscillation. Exposure of saponin-permeabilized cells to IP_3 in the presence of tetracaine (1 mM) elicited transient Ca^{2+} increases. The percentage of cells with the IP_3 response, the magnitude of IP_3-induced Ca^{2+} rise, and propensity of Ca^{2+} oscillations were dependent on the concentrations of IP_3. The IP_3-induced Ca^{2+} oscillations were more pronounced in the cytoplasm than the nucleus, such that they developed faster and lasted longer at the cytoplasm, and that their magnitudes were larger. In contrast, IP_3-induced nuclear Ca^{2+} signal showed more prolonged and larger increase in the basal level with less oscillation. The IP_3-induced Ca^{2+} changes were prevented by the blockers of IP_3Rs, heparin and 2-APB. These results suggest that specific subcellular localization of IP_3R subtypes may be responsible for distinct temporal properties of cytoplasmic and nuclear Ca^{2+} signaling.

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Dynamic Measurements of Luminal Ca2+ in the SR of Mammalian Skeletal Muscle

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Excitation-contraction (EC) coupling in skeletal muscle is the process by which an action potential (AP) activates a global increase in [Ca2+]i which then induces contraction. The global increase in [Ca2+]i is mediated by the release of Ca2+ from the lumen of the sarcoplasmic reticulum (SR) through the opening of ryanodine receptors (RyR1). We examined the structure and function of the SR Ca2+ store dynamically, using the low affinity Ca2+ indicator, fluo-5N, which when loaded as an -AM derivative concentrates in the lumen of the SR. Fluo-5N has been used extensively to measure SR Ca2+ and to characterize the Ca2+ stores in cardiac myocytes, with high spatial and temporal resolution (Brochet et al 2005; Wu and Bers 2006). Using dissociated flexor digitorum brevis (FDB) fibers in culture, we studied the dynamics of fluo-5N in