During heart failure (HF), the ability of the sarcoplasmic reticulum (SR) to store Ca^{2+} is severely impaired resulting in abnormal Ca^{2+} cycling and excitation-contraction (EC) coupling. While it has been demonstrated that SR Ca^{2+} ATPase function is reduced and Na^{+}/Ca^{2+} exchanger function is up-regulated in HF, recently it has been proposed that "leaky" ryanodine receptors (RyRs) also contribute to diminished Ca^{2+} levels in the SR. Various groups have experimentally investigated the effects of RyR phosphorylation mediated by Ca^{2+}/calmodulin dependent kinase II (CaMKII) and other kinases on RyR behavior. Some of these results are inconsistent, and are difficult to interpret since RyR gating is modulated by many external proteins and ions, including Ca^{2+}. Here, we present a mathematical model representing CaMKII-RyR interaction in the canine ventricular myocyte. This is an extension of our previous model which characterized CaMKII phosphorylation of L-type Ca^{2+} channels (LCCs) in the cardiac dyad. In this model, it is assumed that upon phosphorylation, RyR Ca^{2+}-sensitivity is increased. Individual RyR phosphorylation is modeled as a function of dyadic CaMKII activity, which is modulated by local Ca^{2+} levels. The model is constrained by experimental measurements of Ca^{2+} spark frequency and steady state RyR phosphorylation. It replicates steady state RyR (leak) fluxes in the range measured in experiments without the addition of a separate leak flux pathway. Interestingly, simulation results suggest that CaMKII phosphorylation of LCCs, but not RyRs, significantly increases RyR flux; i.e., increasing trigger Ca^{2+} has a stronger impact on RyR flux than phosphorylation-induced increases in RyR open probability under physiological conditions. We also show that phosphorylation of LCCs decreases EC coupling gain significantly. These results suggest that LCC phosphorylation sites may be a more effective target than RyR sites in modulating RyR flux and regulating abnormal Ca^{2+} cycling.

2831-Pos

Multi-Image Colocalization Applied to the Structure of the Cardiomyocyte

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The accurate localization of molecules within the cardiomyocyte is a hotly disputed area, and colocalization analysis one of its most often used tools. However, interpretation is often uncertain because colocalization between two or more images is rarely analyzed to determine whether the observed values could have occurred by chance. To address this, we have developed a robust methodology, based on the Monte-Carlo and bootstrap methods, to measure the statistical significance of a colocalization. The method works with voxel-based, intensity-based, object-based and nearest-neighbor metrics. We extend all of these metrics to measure colocalization in images with three colors and introduce a new metric, the cluster diameter, to measure the clustering of fluorophores in three or more images. In addition, we are able to determine not only whether the labeled molecules colocalize with a probability greater than chance, but also whether they are sequestrated into different compartments. The software, written in MatLab and C++, is freely available. We have applied this technique to examine the structure of the cardiomyocyte and the position of molecules essential for E-C coupling.

2832-Pos

Redox Modifications of Ca^{2+}-Release Events in Cardiomyocytes

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Several cardiac diseases (e.g. heart failure, muscle dystrophy) are known to be associated with cellular oxidative stress. It is established that SR Ca^{2+} release channels (a.k.a. ryanodine receptors, RyRs) are susceptible to oxidation. Furthermore, our recent studies suggest that CICR and EC-coupling are sensitized in cardiomyocytes isolated from dystrophic *mdx* mice due to elevated levels of reactive oxygen species. The aim of this study was to examine the Ca^{2+} spark activity (as an indicator of RyR Ca^{2+} sensitivity) in *mdx* and wild-type (WT) cardiomyocytes at relevant redox potentials. Ventricular myocytes were permeabilized and exposed to solutions containing the Ca^{2+} indicator fluo-3 (50 μM) and a Ca^{2+} concentration of 50 nM. Ca^{2+} sparks were recorded with a laser-scanning confocal microscope in the line-scan mode and analyzed using SparkMaster software. Solutions mimicked intracellular redox potentials ($E_{GSSG/GSH}$) determined in healthy hearts and in muscle dystrophy or heart failure, e.g. −226 mV and −217 mV. Under corresponding redox conditions the steady-state Ca^{2+} spark frequency did not show significant difference in *mdx* and WT cells (24 ± 0.4 vs. 22 ± 0.3 /100μms^{-1}). Therefore, we used stronger reducing and oxidizing conditions to derive a redox/response relationship of spark parameters over a wider range of $E_{GSSG/GSH}$ from −263 mV to −146 mV. Under very oxidative conditions ($E_{GSSG/GSH}$ −146 mV) the spark frequency gradually declined but long-lasting Ca^{2+} release events appeared (> 70 ms, up to 700 ms) that were more frequent in *mdx* compared to WT cardiomyocytes (5.6 vs. 0.6 /100μms^{-1}). Taken together, these results indicate that the average and modest change of the cytosolic redox potential may not significantly alter resting Ca^{2+} spark frequencies, but that stronger oxidative stress, as it has been reported to occur in subcellular regions as "superoxide flashes", can lead to dramatic alterations of elementary Ca^{2+} signaling events.

2833-Pos

The Ryanodine Receptor (RyR) Carries its Own Counter-Ion Current in Rabbit Permeabilized Myocytes

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Rapid sustained Ca release necessitates counter-ion fluxes across the SR membrane. Under physiological conditions, K is the most abundant cation in cytoplasm and the SR is highly K permeable, and thus K is thought to be the main counter-ion supporting Ca release. Three cationic channels could carry the counter-ion flux: 1) SR K channel, 2) TRIC channel which was newly identified as exclusively a monovalent cationic channel, 3) the RyR channel. Most counter-ion current studies to date have been done in skeletal muscle (either SR vesicles or skinned fibers) and there is limited information in cardiac cellular environment. Therefore, the purpose of this study is to determine which channel(s) carry the counter ion flux in saponin-permeabilized rabbit ventricular myocytes. Based on the known permeation properties of conventional SR K / TRIC / RyR channel, different monovalent cations were substituted for cytosolic K to differentiate the role of each candidate channel. Both local (Ca spark) and global Ca release (elicited by caffeine) were measured as indexes of SR Ca release efficiency. The effects of substituted ions on single RyR and SR K channels gating/permeation was defined. Preliminary spark and channel results indicate that the RyR channel mediates Ca release and carries most of the required counter current. Supported by NIH R01HL57832 & R01AR054098.

2834-Pos

Altered Mitochondrial Energetics and Increased ROS Generation Act Synergistically to Dampen β-Adrenergic Stimulated Contractility in the Diabetic Heart

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Background: Excitation-contraction coupling and β-adrenergic activation are altered in diabetic hearts, contributing to contractile dysfunction. We hypothesized that mitochondrial dysfunction in diabetic hearts contributes to altered β-adrenergic responses via increased oxidative stress and respiratory uncoupling. **Methods:** Basal and isoproterenol (ISO)-induced changes in sarcomere shortening and Ca^{2+} transients were assessed in cardiomyocytes from wild-type (WT) and *db/db* mice under euglycemia (5.5mM) or hyperglycemia (30mM). Reactive oxygen species (ROS; H_2O_2 and $O_2^{\cdot-}$), NADH, and $\Delta\Psi_m$ were monitored using two photon laser scanning fluorescence microscopy. **Results:** Basal fractional shortening (FS) and Ca^{2+} transients were not significantly different between WT and *db/db* myocytes, regardless of glucose concentration. Following ISO (10nM), FS increased by ≅150% and Ca^{2+} transients by ≅30%, in both WT and *db/db* myocytes under euglycemia. Under hyperglycemia, the WT ISO response was intact, but the increase in FS and Ca^{2+} transients was blunted in *db/db* cells (68 ± 2%, and 12 ± 3%, respectively, both $p<0.01$ vs euglycemia). Under euglycemia, *db/db* cardiomyocytes had H_2O_2 signals 31% higher than WT ($p<0.001$), but under hyperglycemia, they possessed higher H_2O_2 (+12%; $p<0.01$) and lower $O_2^{\cdot-}$ levels (−22%; $p<0.05$) vs WT. Isolated WT and *db/db* mitochondria showed impaired respiration for substrates of Complex I (16 ± 5 and 23 ± 8 nmol $O_2 min^{-1} mg^{-1}$, respectively), but normal activity for substrates of Complexes II or IV. Impaired energetics correlated with high levels of ROS generation from Complex I or II observed under similar conditions. State 3 mitochondrial respiration with succinate and total respiratory capacity were significantly lower in *db/db* cells compared to WT. **Conclusions:** The findings suggest that the reduced effectiveness of ISO in diabetic hearts under hyperglycemia is mediated by impaired mitochondrial energetics coupled to increased oxidative stress, leading to a deleterious synergistic effect on β-adrenergic response.

2835-Pos

β-Adrenergic Stimulation and SR Ca^{2+} Leak in Cardiomyocytes

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During β-adrenergic stimulation of cardiac muscle, excessive phosphorylation of Ca^{2+} release channels (ryanodine receptors, RyRs) by cAMP and