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

Ca^{2+}/calmodulin dependent protein kinases, PKA and CaMKII respectively, has been linked to arrhythmogenic diastolic Ca^{2+} leak from intracellular Ca^{2+} stores (the sarcoplasmic reticulum, SR). Using confocal Ca^{2+} imaging, we have recently shown that β-adrenergic stimulation (1 μM isoproterenol, Iso) increases SR Ca^{2+} leak several fold in quiescent, whole-cell voltage-clamped guinea-pig ventricular myocytes without altering SR Ca^{2+} content (Ogrodnik & Niggli 2009, Biophys J 96:276a). Independent of extracellular Ca^{2+} and changes of diastolic intracellular Ca^{2+} concentration, this observation indicates a sensitization of the RyRs. Intriguingly, here we show that increasing cAMP production and PKA activity by direct stimulation of adenylate cyclase with forskolin (1 μM) does not significantly elevate SR Ca^{2+} leak under otherwise identical experimental conditions. As successful downstream activation of the cAMP/PKA pathway was confirmed by comparable stimulation of L-type Ca^{2+} current and SR Ca^{2+}-ATPase activity in both Iso and forskolin, these disparate results suggest a distinct signaling pathway by which β-adrenergic stimulation increases SR Ca^{2+} leak. Interestingly, we found that the increased SR Ca^{2+} leak observed in Iso was likely mediated by CaMKII, rather than PKA, as treatment with the CaMKII inhibitor KN-93 (5 μM) suppressed the increase without altering SR Ca^{2+} content, in contrast to inhibition of PKA with H-89 (5 μM). Taken together, we conclude that CaMKII activation during β-adrenergic stimulation may be rapid, may not require elevated cardiomyocyte Ca^{2+} cycling, and may increase SR Ca^{2+} leak independently of the cAMP/PKA signaling pathway, possibly via increased nitric oxide production (Curran et al. 2009, Biophys J 96:120-121a).

2836-Pos

Impaired Ca^{2+} Release Synchronization in RyR2-S2808a Mouse Cardiomyocytes During β-Adrenergic Stimulation

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Ca^{2+}-induced Ca^{2+} release via ryanodine receptors (RyR2) is crucial for cardiac contractile function. During periods of stress and exercise, the sympathetic nervous system stimulates cardiac contractility. β-Adrenergic receptor activation has been suggested to result in PKA-mediated phosphorylation of RyR2 at Ser2808. Hyperphosphorylation at Ser2808 has also been discussed as possible factor contributing to heart failure. However, the role of RyR2 phosphorylation in inotropic adaptations during β-adrenergic stimulation remains controversial. Previous reports on a mouse model with genetic ablation of this phosphorylation site (S2808A) did not confirm the putative involvement of RyR2 phosphorylation in EC-coupling changes during β-adrenergic stimulation. In the present study, we intensified the search for EC-coupling modifications in S2808A myocytes by challenging EC-coupling near threshold conditions. Single cardiomyocytes were patch-clamped in the whole-cell configuration to measure I_{CaL}, while Ca^{2+} transients were simultaneously recorded with confocal imaging of fluo-3. The EC-coupling gain, a measure for the effectiveness of I_{CaL} to trigger Ca^{2+} release from the SR, was determined from control and S2808A cardiomyocytes. Lowering the extracellular Ca^{2+} concentration, a maneuver often used to unmask latent EC-coupling problems, did not reveal significant differences in the EC-coupling gain in WT and S2808A myocytes before and during β-adrenergic stimulation with isoproterenol. However, comprehensive analysis of subcellular Ca^{2+} transient kinetics indicated subtle differences in coordination of RyR activation. Uncoupling of the EC-mechanism by reduced $[Ca^{2+}]_o$ resulted in a spatiotemporal de-synchronization of RyR openings. β-Adrenergic stimulation re-synchronized RyR openings under the same conditions less effectively in S2808A than in WT cardiomyocytes (time-to-peak of single Ca^{2+} release sites 181 ± 6 vs. 100 ± 3ms, respectively, $P<0.0001$). We conclude that although removal of the PKA phosphoepitope at Ser2808 does not critically derange EC-coupling, its ablation may interfere with synchronization of RyR2 activation during β-adrenergic stimulation.

2837-Pos

Ryanodine Receptors Outside of Couplons are Involved in Excitation-Contraction Coupling in Rabbit Ventricular Myocytes

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Current theories of excitation-contraction coupling (ECC) in ventricular myocytes assert that L-type Ca channels interact with clusters of ryanodine-sensitive Ca release channels (RyRs) within couplons. We hypothesized that RyR clusters exist also outside of couplons and contribute to ECC. We investigated this hypothesis by three-dimensional imaging of RyR clusters and sarcolemma of isolated myocytes lying flat (horizontal) and on end (vertical). We deconvolved the image stacks, created reconstructions of cell segments, and identified RyR cluster types. RyR clusters remote to sarcolemma were assumed to be outside of couplons. Similar studies were performed on intact ventricular tissue. Furthermore, we imaged evoked Ca transients and sarcolemma of horizontal cells labeled with fluo-4 and di-8-anepps. Image sequences were acquired using rapid two-dimensional scanning (Zeiss LSM5Live, rates up to 300HZ). The image sequences were corrected for bleaching and cross-talk. In horizontal and vertical isolated cells, RyR clusters appeared to be arranged in sheets in the vicinity of Z-disks. Some RyR clusters were associated with sarcolemma, in particular transverse tubules, and are presumably part of couplons. However, most RyR clusters were not. Examination of cells in intact tissue revealed a smaller number of RyR clusters not associated with sarcolemma than in isolated cells. The density of transverse tubules was higher than in isolated cells. This loss of transverse tubules might be caused by the isolation procedure. Analysis of the rapid image sequences indicated that both types of RyR clusters were activated during an action potential. However, the RyR clusters not associated with sarcolemma were activated with delays of up to 10ms. In conclusion, we demonstrated that RyR clusters outside of couplons are involved in ECC. We suggest that activation of RyR clusters outside of couplons occurs by a common pool mechanism.

2838-Pos

Remodelling of Calcium Handling, Ion Currents and Contraction in Rac1 Overexpressing Mouse Ventricle

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Increased production of oxygen radicals is involved in many cardiac deseases. In a cardiac-specific Rac1-overexpressing mouse line (RacET) NADPH oxidase activity is upregulated 6-7 fold. Here, we characterise ventricular remodelling processes with respect to calcium handling and ion currents in ventricular myocytes. We used 4-6 months old RacET and age-matched wt mice. In ventricular cells of RacET baseline calcium concentrations were significantly decreased. In post-rest behaviour the first amplitude was unchanged but the steady-state amplitude was down to almost 50% of the wt-value. Interestingly, RacET myocytes displayed significantly increased amplitudes of caffeine-induced calcium transients (up by 50%), while Na/Ca-exchange and SERCA-pump activity appeared unchanged. A similar behaviour was observed in cell-length experiments. Here, RacET myocytes displayed a significantly shorter resting cell length (down by 15%), in post-rest behaviour experiments the first twitch amplitude was unchanged while in steady-state their contraction was significantly reduced. When analysing calcium sparks we found that their amplitude was almost doubled in RacET cells while the recovery was speeded up 25%, their spatial spread was reduced by 25% when compared to wt. The membrane capacity of the RacET myocytes was significantly reduced (down by 40%) and action potentials were largely distorted, whereby both upstroke and repolarisation phase were altered. From these data we conclude that RacET overexpression and the accompanying increased oxygen radical load results in ventricular remodelling, even in the absence of hypertrophy.

Support by DFG (SFB530, GraKo1320, KliFor196), BfR, BMBF

2839-Pos

Mesenchymal Stem Cell Conditioned Tyrode is a Potent Activator of Akt in Cardiomyocytes

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Transplantation of bone marrow-derived mesenchymal stem cells (MSCs) in clinical trails has been reported to decrease infarct size and benefit ventricular ejection fraction of the heart. Differentiation of the MSCs into cardiac myocytes has been postulated, but stronger evidence points toward a paracrine mechanism. We tested the hypothesis that MSC conditioned tyrode (conT) results in improved cardiomyocyte survival through activation of the anti-apoptotic Akt protein kinase pathway. HEPES/ Bicarbonate buffered tyrode (pH 7.4) was placed on MSCs for 16 hrs at 37°C for conditioning. Isolated mouse ventricular cardiomyocytes (VMs) were treated with conT. Immunoblotting of VM lysates was used to examine the activation Akt, a downstream effector of the receptor-mediated PI3-Kinase pathway in conjunction with confocal imaging of intracellular Ca2+ (FLUO 4-AM). Superfusion of VMs with conT resulted in a progressive decrease of the Ca2+ transient duration (31 ± 3.4 %) and an