longitudinal section is reduced (54 ± 7 vs. 43 ± 6), suggesting a remodelling/fusion of these organelles. Finally, we have assessed the positioning of mitochondria in respect to myofibrils and triads: a) the number of mitochondria at the A band (misplaced) slightly increases with age (9% vs 3%), whereas the number of triads-mitochondria couples is significantly reduced: 39 ± 5 vs. 26 ± 4. Our observations indicates: a) a age-related partial disarrangement and spatial re-organization of EC coupling/mitochondrial apparatuses; and b) a decreased percentage of mitochondria functionally tethered to calcium release sites. This could in part explain the decline of muscle performance associated to increasing age.

2826-Pos

Knockdown of TRIC-B from *tric-a*$^{-/-}$mice Alters Intracellular Ca^{2+} Signaling in Skeletal and Cardiac Muscles

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Trimeric intracellular cation (TRIC) channel subtypes are present in the endo/sarcoplasmic reticulum (SR) and nuclear membranes of muscle cells and other tissues. Knockout mice lacking both TRIC-A and TRIC-B channels suffer lethal embryonic cardiac failure due to dysfunctional intracellular Ca^{2+} signaling in the mutant cardiomyocytes (Yazawa et al., Nature 448, 78-82). The lethality associated with double knockout of *tric-a* and *tric-b* prevents physiological assessment of TRIC channels in adult tissues. Here we took advantage of the viable *tric-a*$^{-/-}$ mice and employed RNAi-mediated knockdown of *tric-b*, in order to examine the physiological function of TRIC channels in adult muscle cells. We used electroporation-mediated delivery of shRNA against *tric-b* into the flexor digitorum brevis (FDB) muscles of living *tric-a*$^{-/-}$ mice. Individual FDB fibers with knockdown of TRIC-B were used to examine the Ca^{2+} sparks properties in response to osmotic stress, and voltage-induced Ca^{2+} release under voltage clamp. Compared with the *tric-a*$^{-/-}$ muscle treated with control shRNA, acute knockdown of TRIC-B leads to significant reduction of the amplitude of Ca^{2+} sparks accompanied with prolongation of the duration of Ca^{2+} sparks. In neonatal cardiomyocytes isolated from the *tric-a*$^{-/-}$ mice, knockdown of TRIC-B led to significant perturbation of Ca^{2+} signaling from the SR, evidenced by irregular intracellular Ca^{2+} signaling and reduced frequency of spontaneous Ca^{2+} oscillations. These results indicate that disruption of TRIC function can alter intracellular Ca^{2+} signaling in skeletal and cardiac muscles and this may underlie an increased susceptibility of these tissues to various physiological stresses.

2827-Pos

Local Ca^{2+} Releases Enable Rapid Heart Rates in Developing Cardiomyocytes

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Homogeneous intracellular Ca^{2+} release repeated with high frequency is the basis of the rhythmic contractions of cardiac myocytes. In adult ventricular myocytes, the t-tubular system enables transient homogeneous Ca^{2+} signals. Interestingly, the developing cardiomyocytes do not have t-tubuli and Ca^{2+} signal propagation in the cytosol is based on the relatively slow diffusion of Ca^{2+} ions. This is likely to result in spatiotemporal heterogeneity of Ca^{2+}, which limits the maximal frequency of the Ca^{2+} signals. We observed that intracellular Ca^{2+} signals of 12.5 days old mouse embryonic ventricular myocytes are more homogeneous than expected if the Ca^{2+} signals would propagate by pure diffusion. To study the propagation more accurately, we injected a small amount of Ca^{2+} to a single point in the cytosol via patch-clamp pipette while performing the line-scan imaging of the intracellular Ca^{2+}. With this method we found that inhibition of the sarcoplasmic reticulum (SR) Ca^{2+} release channels results in 3-fold slowing of Ca^{2+} signal propagation (control: 10.1 ± 2.7 ms/μm vs. ryanodine (50 μM): 33.6 ± 9.2 ms/μm, $P < 0.05$). This suggested that the propagation of Ca^{2+} signals is amplified with local SR Ca^{2+} releases. Immunolabeling of SR Ca^{2+} release and uptake proteins revealed a regular structure throughout the cytosol at ~2 μm intervals. These extensions of SR were equally functional in all parts of the cytosol. To further study the role of these local Ca^{2+} release sites in developing cardiomyocytes, we implemented a model of them into the previously published mathematical model of an embryonic cardiomyocyte. The computer simulations showed that the local Ca^{2+} releases are prerequisite for synchronizing the global intracellular Ca^{2+} releases upon electrical excitation and maintaining the capability of developing cardiomyocytes to generate spontaneous pacemaking at a sufficiently high frequency.

2828-Pos

Ca^{2+} Transients and Myosin Heavy Chain (MHC) Composition in Murine Enzymatically Dissociated Fibers

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Single and tetanic Ca^{2+} transients reported with MagFluo4-AM were obtained together with MHC electrophoretic patterns in enzymatically dissociated fibres from adult mice soleus and extensor digitorum longus (EDL) muscles. Kinetics of transient rise (Ca^{2+} release) and decay (Ca^{2+} clearance) of both twitch and tetanic responses showed a continuum from the slowest records obtained in fibers type I, to the fastest obtained in fibers IIX/D and IIB. Fibers IIA were fast regarding Ca^{2+} release but slow regarding Ca^{2+} clearance. Single transients decay was described by a double exponential function with time constants (τ_1 and τ_2, ms) of 3.2 and 49.5 in soleus (types I and IIA, n=23) and 1.6 and 10.5 in EDL fibres (types IIX/D and IIB, n=16). These time constants were associated with components A1 and A2 (%) of 28.1 and 71.9 for soleus, and 35.8 and 64.2 for EDL. For all fiber types, after few repetitive stimuli at 100 Hz there was a big change of decay kinetics compared to single transients and then mild changes were seen in records lasting from 50 to 350 ms. In EDL tetanic transients, the fast component A1 almost disappeared, leaving the A2 and a much slower third one (A3) with τ_2 and τ_3 of 14.6 and 1259.7 (n=6). In soleus the A1 disappeared, while A2 increased with a τ_2 of 74.6 (n=5). Preliminary experiments using CPA (1-2 μM) and FCCP (2-4 μM) have shed some light into the mechanisms involved in relaxation of tetanic transients in different fiber types. In conclusion, we show for the first time the diversity of Ca^{2+} transients in the whole spectrum of fibre types and correlate it with the structural and biochemical diversity of mammalian skeletal muscle fibres. (FONACIT G-2001000637).

2829-Pos

Effects of γ-Ketoaldehydes on Ca^{2+} Cuttrent Induced SR Ca^{2+} Release in Ventricular Myocytes

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• Oxidation increases RyR2 channel activity, enhances cardiac SR Ca^{2+} release and causes spontaneous SR Ca^{2+} waves. Isoprostanes have become a recognized marker of oxidative stress in rodents and humans. γ-ketoaldehydes(γ-KAs) are the most reactive product of the isoprostane pathway. Recently, we found that lipophilic pyridoxamine analogues, salicylamine(SA) scavenge γ-KAs and thereby prevent formation of γ-KA protein adducts in response to oxidative stress. We hypothesized that γ-KAs are potential mediators of oxidant-induced RyR2 channel dysfunction and spontaneous SR Ca^{2+} waves, and that SA would prevent oxidant-induced spontaneous SR Ca^{2+} waves(SCW) in the ventricular myocytes.

• We compared the effect of γ-KAs(1uM) or H_2O_2(10uM) and the effect of SA on Ca-current induced Ca release(CICR) in murine ventricular myocytes loaded with Fura-2AM or Fluo-4. All data are expressed relative to vehicle (Mean ± SEM, n=15-50 per group).

• Acute exposure(3 min) to γ-KAs(1 uM) or H_2O_2(10 uM) increased the amplitude of Ca^{2+} transients, and the fraction of Ca^{2+} released from the SR(γ-KAs130 ± 10%*, $H_2O_2$120 ± 10%, *p<0.05) during each beat. Furthermore, the rate of SCW was significantly increased(γ-KAs 42%*, $H_2O_2$33%*, *p<0.05) and SR Ca^{2+} content was reduced. In voltage-clamped myocytes, dialysis with γ-KAs enhanced Ca^{2+} release without changing L-type Ca^{2+} current, demonstrating that the effect of γ-KAs is the result of RyR2 modification. However, after chronic exposure(30 min) to γ-KAs(1 uM) or H_2O_2(10 uM), Ca^{2+} transients(γ-KAs 0.53 ± 0.1*, H_2O_2 0.7 ± 0.1*, *p<0.05) and SR Ca^{2+} contents decreased, and SCW remained elevated. Pre-treatment(3 days) of salicylamine reduced H_2O_2-induced spontaneous Ca^{2+} waves(SCWs/sec, $H_2O_2$1.2 ± 0.3*, SA-$H_2O_2$0.4 ± 0.2*, *p<0.05) preserved with SR Ca^{2+} content in ventricular myocytes.

• We found that H_2O_2 and γ-KAs have analogous biphasic effects on SR Ca^{2+} release in ventricular myocytes. The protective effect of γ-KA scavengers suggests that γ-KAs are possible mediators of oxidant-induced RyR2 channel dysfunction.

2830-Pos

CamkII Phosphorylation of RyRs: a Mechanistic Mathematical Model

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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