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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