

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 indicate: 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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ sparks properties in response to osmotic stress, and voltage-induced Ca²⁺ 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 Ca²⁺ sparks accompanied with prolongation of the duration of Ca²⁺ sparks. In neonatal cardiomyocytes isolated from the *tric-a*^{-/-} mice, knockdown of TRIC-B led to significant perturbation of Ca²⁺ signaling from the SR, evidenced by irregular intracellular Ca²⁺ signaling and reduced frequency of spontaneous Ca²⁺ oscillations. These results indicate that disruption of TRIC function can alter intracellular Ca²⁺ signaling in skeletal and cardiac muscles and this may underlie an increased susceptibility of these tissues to various physiological stresses.

2827-Pos

Local Ca²⁺ Releases Enable Rapid Heart Rates in Developing Cardiomyocytes

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Homogeneous intracellular Ca²⁺ 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 Ca²⁺ signals. Interestingly, the developing cardiomyocytes do not have t-tubuli and Ca²⁺ signal propagation in the cytosol is based on the relatively slow diffusion of Ca²⁺ ions. This is likely to result in spatiotemporal heterogeneity of Ca²⁺, which limits the maximal frequency of the Ca²⁺ signals. We observed that intracellular Ca²⁺ signals of 12.5 days old mouse embryonic ventricular myocytes are more homogeneous than expected if the Ca²⁺ signals would propagate by pure diffusion. To study the propagation more accurately, we injected a small amount of Ca²⁺ to a single point in the cytosol via patch-clamp pipette while performing the line-scan imaging of the intracellular Ca²⁺. With this method we found that inhibition of the sarcoplasmic reticulum (SR) Ca²⁺ release channels results in 3-fold slowing of Ca²⁺ signal propagation (control: 10.1 ± 2.7 ms/ μ m vs. ryanodine (50 μ M): 33.6 ± 9.2 ms/ μ m, $P < 0.05$). This suggested that the propagation of Ca²⁺ signals is amplified with local SR Ca²⁺ releases. Immunolabeling of SR Ca²⁺ release and uptake proteins revealed a regular structure throughout the cytosol at ~ 2 μ m intervals. These extensions of SR were equally functional in all parts of the cytosol. To further study the role of these local Ca²⁺ 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 lo-

cal Ca²⁺ releases are prerequisite for synchronizing the global intracellular Ca²⁺ releases upon electrical excitation and maintaining the capability of developing cardiomyocytes to generate spontaneous pacemaking at a sufficiently high frequency.

2828-Pos

Ca²⁺ Transients and Myosin Heavy Chain (MHC) Composition in Murine Enzymatically Dissociated Fibers

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Single and tetanic Ca²⁺ 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 (Ca²⁺ release) and decay (Ca²⁺ 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 Ca²⁺ release but slow regarding Ca²⁺ 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 μ M) and FCCP (2-4 μ M) 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 Ca²⁺ 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 Ca²⁺ Cuttrent Induced SR Ca²⁺ Release in Ventricular Myocytes

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● Oxidation increases RyR2 channel activity, enhances cardiac SR Ca²⁺ release and causes spontaneous SR Ca²⁺ 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 Ca²⁺ waves, and that SA would prevent oxidant-induced spontaneous SR Ca²⁺ waves (SCW) in the ventricular myocytes.

● We compared the effect of γ -KAs (1 μ M) or H₂O₂ (10 μ M) 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 $\pm$ SEM, n=15-50 per group).

● Acute exposure (3 min) to γ -KAs (1 μ M) or H₂O₂ (10 μ M) increased the amplitude of Ca²⁺ transients, and the fraction of Ca²⁺ released from the SR (γ -KAs $130 \pm 10\%$, H₂O₂ $120 \pm 10\%$, $p < 0.05$) during each beat. Furthermore, the rate of SCW was significantly increased (γ -KAs 42%, H₂O₂ 33%, $p < 0.05$) and SR Ca²⁺ content was reduced. In voltage-clamped myocytes, dialysis with γ -KAs enhanced Ca²⁺ release without changing L-type Ca²⁺ current, demonstrating that the effect of γ -KAs is the result of RyR2 modification. However, after chronic exposure (30 min) to γ -KAs (1 μ M) or H₂O₂ (10 μ M), Ca²⁺ transients (γ -KAs $0.53 \pm 0.1^*$, H₂O₂ $0.7 \pm 0.1^*$, $p < 0.05$) and SR Ca²⁺ contents decreased, and SCW remained elevated. Pre-treatment (3 days) of salicylamine reduced H₂O₂-induced spontaneous Ca²⁺ waves (SCWs/sec, H₂O₂ $1.2 \pm 0.3^*$, SA-H₂O₂ $0.4 \pm 0.2^*$, $p < 0.05$) preserved with SR Ca²⁺ content in ventricular myocytes.

● We found that H₂O₂ and γ -KAs have analogous biphasic effects on SR Ca²⁺ 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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