CASQ2-knockout animals (Knollmann et al., 2006. *J Clin Invest* 116:2510). Lack of both CASQ isoforms was confirmed by western blot. The double-null mice are viable and breed normally, however the rate of spontaneous mortality of male animals is higher than CASQ1-null animals. Whereas the overall phenotype of mice is similar to that of CASQ1-null mice, significant differences are found in Soleus. From the structural point of view, in Soleus muscle we find many fibers (about 30%) with severe structural damage that were not found in CASQ1-null animals. Functional studies indicate significant prolongation in twitch time parameters, increased twitch tension and impaired tension generation during prolonged tetani both in EDL and Soleus, likely related to abnormal calcium release kinetics. These findings suggest that: a) expression of CASQ2 is essential for the maintenance of a subpopulation of Soleus fibers; and b) lack of both CASQ1 and 2 exacerbates the overall phenotype of CASQ1-null mice.

2821-Pos

Refractoriness of Sarcoplasmic Reticulum Calcium Release in Cardiac Muscle Due to Calsequestrin

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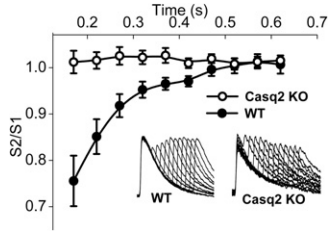
In cardiac excitation-contraction coupling, L-type Ca^{2+} current (I_{Ca}) triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) via ryanodine receptor (RyR) Ca^{2+} release channels. It is unclear why SR Ca^{2+} release cannot be elicited by premature stimuli, even though I_{Ca} is fully recovered. Here, we use calsequestrin null mice (Casq2 KO) and wild-type littermates (WT) to test the hypothesis that calsequestrin (Casq2) determines refractoriness of SR Ca^{2+} release. Ca^{2+} release refractoriness was measured in voltage-clamped myocytes dialyzed with Fluo-4 by applying premature extrastimuli (S2) at successively shorter S1-S2 coupling intervals following a 1 Hz train (S1 stimuli). To maintain constant trigger, Ca^{2+} release was activated with I_{Ca} tail currents that elicited maximal Ca^{2+} release during the S1 train. WT S2 Ca^{2+}release was significantly depressed with short coupling interval whereas Casq2 KO cardiomyocytes exhibit no refractoriness of Ca^{2+} release (Figure, n=11 WT, 12 KO, $p = 0.01$). At the same time, I_{Ca} current density, SR Ca^{2+} content, and steady-state Ca^{2+} transients (S1) were not significantly different from WT-myocytes. We conclude that calsequestrin is a critical determinant of SR Ca^{2+} release refractoriness in cardiac muscle (Supported by NIH-R01HL71670, R01HL88635).



2822-Pos

Effect of Triadin on Retrograde and Orthograde Signaling between RyR1 and DHPR in Cultured Myotubes

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Using pan triadin-null mice we previously showed that triadins ablation did not disrupt EC-coupling in muscle cells. However, calcium imaging studies in cultured myotubes did reveal that triadin-null myotubes had slightly smaller depolarization-induced Ca^{2+} transients than Wt cells. Here, using whole-cell voltage clamp, we analyze the effect of triadin ablation in skeletal EC-coupling by characterizing the retrograde and orthograde signaling between RyR1 and DHPR of triadin-null myotubes. Calcium currents elicited by 200ms depolarization steps in Wt and triadin-null cells showed slow kinetics of activation and peak current at approximately +30 mV. Although, the overall voltage dependence was preserved between Wt and triadin-null cells a leftward shift in the I/V curve was observed in triadin-null cells ($V_{1/2}$, 22.3 ± 0.8 mV in Wt vs 16.6 ± 1.1 mV in triadin-null cells, $p<0.05$). In addition, kinetic analysis of the DHPR Ca^{2+} current shows that the activation time constant of the slow component (τ_{slow}) was slightly decreased from 37 ± 2.4 ms in Wt to 26 ± 2.6 ms ($p<0.05$) in triadin-null cells.

The voltage-evoked Ca^{2+} transient, on the other hand, showed a small but significant reduction of the peak fluorescence amplitude of triadin-null cells ($\Delta F/F_{max}$, 0.72 ± 0.2 in Wt vs 0.61 ± 0.1 in triadin-null) with no differences in voltage dependence (V_m, -7.2 ± 1.1 mV in Wt vs -10.1 ± 1.9 mV in null cells). Our results suggest that the absence of triadin expression preserves the orthograde and retrograde signaling between DHPR and RyR1 nearly intact and that the effect of triadin ablation on $\Delta F/F_{max}$ would be secondary to the dysregulation of calcium homeostasis observed in triadin-null cells. These data give further support to the idea that skeletal triadins do not play a direct role in skeletal EC-coupling.

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2823-Pos

Altering Skeletal Muscle EC Coupling by Ablating the Sarcoplasmic Reticulum Protein JP45 Affects Both Metabolism and Muscle Performance in Old Mice

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JP45, a sarcoplasmic reticulum protein, appears to be mainly expressed in skeletal muscle. In mice, its expression is down-regulated during aging, and it interacts with the α1.1 subunit of the dihydropyridine receptor (Cav1.1) and calsequestrin, two key components of the excitation-contraction (EC) coupling machinery.

We examined 12- and 18-month-old JP45 knock-out mice and compared them with age-matched, wild-type littermates. The JP45 KO mice exhibit a phenotype consistent with impaired skeletal muscle EC coupling, confirming our previous results in young JP45 KO mice. Spontaneous motor activity assessed with a running wheel revealed that the older JP45 KO group runs less and much more slowly than age-matched WT and young JP45 KO mice. *In vitro* muscle contractile property analysis showed lower twitch and tetanic absolute and specific force, evident mostly in the EDL of aged JP45 KO mice compared to age-matched WT, which correlates with type-II fiber atrophy. Cav1.1 expression and SR Ca^{2+} release in voltage-clamped flexor digitorum brevis muscle fibers of aged JP45 KO mice were reduced compared to age-matched WT. Additionally, aged JP45 KO mice exhibited decreased food intake and body weight.

Our results show that JP45 plays a role in EC coupling and regulation of body metabolism. *Supported by NIH/NIA, Japanese Science Foundation, M.U.R.S.T., A.F.M., and Swiss Muscle Foundation.*

2824-Pos

Characterization of Calumenin-RyR2 Interaction in Murine Heart

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Calumenin is a multiple EF-hand Ca^{2+}-binding protein localized in the sarcoplasmic reticulum (SR). In our recent study, we showed that calumenin-knockdown (KD) of HL-1 cells led to enhanced Ca^{2+} release and Ca^{2+} uptake in the SR (*Sahoo et. al. J. Biol. Chem.*, 2009). To elucidate the underlying mechanisms responsible for the enhanced Ca^{2+} release from the SR in calumenin-KD samples, the possible interaction between calumenin and RyR2 was examined by various methods. GST pull-down assay showed a direct interaction between calumenin and RyR2. We have further found that the middle region of calumenin (aa 132-222) interacts with RyR2. GST pull-down assay also shows that RyR2 intra luminal loop-I region (aa 4519-4576) is the binding site for calumenin. Immunofluorescence study shows that RyR2 and calumenin are co-localized in the junctional region of SR in rat ventricular cardiomyocytes. The detailed amino acid residues involved in the interaction between calumenin and RyR2 are currently under investigation. (This work was supported by the Korean Ministry of Science and Technology grant, Systems Biology Research Grant, M1050301001-6N0301-0110, and the 2009 GIST Systems Biology Infrastructure Establishment Grant).

2825-Pos

Progressive Triad-Mitochondria Un-Coupling in Aging

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An impairment of the mechanisms controlling the release of calcium from internal stores (excitation-contraction (EC) coupling) has been proposed to contribute to the age-related decline of muscle performance that accompanies aging (EC un-coupling theory). EC coupling in muscle fibers occurs at specialized intracellular junctions called calcium release units, or triads, which are specifically placed at sarcomere's I-A band transition. In recent publications we have shown that: a) in human muscle, the frequency of triads decreases significantly with age (Boncompagni et al., 2006; J Gerontol 61:995); and b) in mice, triads are tethered to mitochondria placed at the I band (Boncompagni et al., 2009; MBC 20:1059). Here we have studied the frequency, sarcomeric-localization, ultrastructure, and coupling of triads/mitochondria in EDL from male WT mice using transmission electron microscopy (TEM).

Preliminary results indicates that the number of triads/100μm2 of longitudinal section in aging mice (n=4, 25-35 months of age) decreases compared to the adult mice (n=5, 3-12 months of age): 92 ± 9 vs. 79 ± 8. In addition, the percentage of abnormally positioned triads (longitudinal and/or oblique) increases. On the other hand, the total volume of mitochondria does not change significantly with age. However, the number of mitochondria-profiles/100μm2 of