from rats. Fluvastatin (Flv) for 3 days decreased caffeine- and ionomycin-induced contraction of myofibers and Ca(2+) release from sarcoplasmic reticulum. Ca(2+)-shortening curves measured in skinned myofibers indicated that myofibrillar Ca(2+) sensitivity was unaffected by Flv. A luciferin-luciferase assay revealed less ATP contents in Flv-treated myofibers. Among mevalonate metabolites, including geranylgeranylpyrophosphate (GGPP), farnesylpyrophosphate (FPP), coenzyme Q9, and coenzyme Q10, only GGPP prevented Flv-induced ATP reduction. A selective Rab geranylgeranyltransferase (GG transferase) inhibitor, perillyl alcohol (POH), and a specific GG transferase-I inhibitor, GGTI-298, both mimicked Flv in decreasing ATP and contraction. Mitochondrial membrane potential was decreased by Flv, and this effect was rescued by GGPP and mimicked by POH and GGTI-298. An endoplasmic reticulum (ER)-to-Golgi traffic inhibitor, brefeldin A, and a Rho inhibitor, membrane permeable exoenzyme C3 transferase, both decreased ATP. We conclude that statin-induced contractile dysfunction is due to reduced Ca(2+) release from SR and reduced ATP levels in myofibers with damaged mitochondria. GGPP depletion and subsequent inactivation of Rab1, possibly along with Rho, may underlie the mitochondrial damage by Flv.

1853-Pos Board B623

PTRF Anchors MG53 to Cell Injury Site for Initiation of Membrane Repair

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Membrane resealing is an elemental process in cell biology and disruption of this process can lead to degenerative human diseases. Early work from Bansal et al (*Nature 423: 168-72*) showed that dysferlin contributes to membrane resealing, as knockout mice for dysferlin display membrane repair defects in both skeletal and cardiac muscle (Han et al. *JCI 117: 1805-13*). Our recent study showed that MG53 can interact with dysferlin to facilitate muscle membrane repair, and defects in MG53-mediated membrane repair are linked to muscular dystrophy and cardiac dysfunction. Here, we report PTRF (polymerase I and transcript release factor), a gene previously identified in regulation of caveolae membrane structure, is an indispensible component of the membrane repair machinery. PTRF acts as a docking protein for MG53-mediated membrane repair through binding exposed membrane cholesterol at the injury site. Cells lacking endogenous expression of PTRF show defective membrane resealing. Mutation in PTRF associated with human disease alters PTRF localization in the nucleus and disrupts MG53 function in membrane resealing. While RNAi-silencing of PTRF leads to defective muscle membrane repair, overexpression of PTRF can rescue membrane repair defects in *dysferlin-/-* muscles, but not in *mg53-/-* muscles. Our data suggest that membrane-delimited interaction between MG53 and PTRF contributes to initiation of the cell membrane repair response. Since overexpression of PTRF can rescue the membrane repair defects in dystrophic muscle, targeting the functional interaction between MG53 and PTRF represents a potential therapeutic means for treatment or prevention of tissue injury in human diseases.

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Searching for MG53-Interacting Proteins in Skeletal Muscle

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Mitsugumin 53 (MG53, a muscle-specific tripartite motif family protein) contributes to cell membrane repair in skeletal muscle by facilitating vesicle trafficking. To examine possible involvement of MG53 in skeletal excitation-contraction coupling, GST-fused MG53 proteins purified using GST pull-down assay were incubated with solubilized triad preparation from rabbit skeletal muscle. The protein complexes with GST-fused MG53 obtained from the incubation were separated on SDS-PAGE. Major bands on the gel were subjected to in-gel digestions with trypsin for tandem mass spectrometric characterization and database searches for the identification of proteins on the bands. SERCA (sarco/endoplasmic reticulum Ca^{2+}-ATPase) was found as a MG53-interacting protein, and the interaction between SERCA and MG53 was confirmed by immunoprecipitation assays with anti-MG53 antibody in samples intrinsically expressing SERCA and MG53.

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Simulation of Myoplasmic Calcium Transients in Mouse Slow-Twitch Muscle Fibers

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ABSTRACT
Fiber bundles were dissected from soleus muscles of adult mice and individual slow-twitch fibers were micro-injected with furaptra, a low-affinity rapidly-responding fluorescent Ca indicator. Fiber activity was elicited by action potential stimulation at 16 °C and dfCaD, the fraction of the indicator in the Ca-bound form, was measured. The dfCaD waveform was simulated with a multi-compartment reaction-diffusion model that provides estimates of the amount and time course of Ca release from the sarcoplasmic reticulum (SR), the binding and diffusion of Ca in the myoplasm, the re-uptake of Ca by the SR Ca pump, and the myoplasmic free Ca transient itself (d[Ca]). In response to one action potential (AP), the following spatially-averaged estimates were obtained (concentration units are referred to the myoplasmic water volume): 107 micro-molar for the amount of Ca release; 57 micro-molar/ms and 1.7 ms for the peak and full-duration at half maximum (FDHM) of the release flux; 7.6 micro-molar and 4.9 ms for the peak and FDHM of d[Ca]. In response to five APs at 67 Hz, d[Ca] summated somewhat in response to later action potentials in the train, while the second and subsequent releases declined progressively, from 0.3 to 0.1 times that of the first release. Two important parameters of the model are the on- and off-rate constants of the reaction between Ca and the regulatory sites on troponin. Values of 0.4×10^8 M^{-1} s^{-1} and 26 s^{-1}, respectively, were found to be consistent with the measurements of dfCaD. The peak troponin occupancy is estimated to be 79% and 93% in response to one and five APs, respectively.

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Measurement of Myoplasmic Calcium Transients in Mouse Slow-Twitch Muscle Fibers

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Bundles of intact fibers from soleus muscles of adult mice were isolated by dissection and one fiber within a bundle was micro-injected with either furaptra or mag-fluo-4, two low-affinity rapidly-responding Ca^{2+} indicators. Fibers were activated by action potentials to elicit changes in indicator fluorescence (dF), a monitor of the myoplasmic free Ca^{2+} transient, and changes in fiber tension. All injected fibers appeared to be slow-twitch (type I) fibers as inferred from the time course of their twitch tension response, which was markedly slower than that in fast-twitch fibers from extensor digitorum longus muscle. The time of peak and full-duration at half maximum (FDHM) of dF were found to be essentially identical with the two indicators: 4.5 ± 0.2 and 8.4 ± 0.5 ms, respectively, with furaptra (n = 6) and 4.8 ± 0.4 and 8.3 ± 0.3 ms with mag-fluo-4 (n = 8) (16 °C). Mag-fluo-4's dF was also measured at 22 °C; its FDHM, 5.1 ± 0.3 ms (n = 6), is about one-third that reported previously in enzyme-dissociated slow-twitch fibers that had been AM-loaded with mag-fluo-4: 12.4 ± 0.8 ms (n = 20) and 17.2 ± 1.7 ms (n = 23). We attribute the larger FDHM in enzyme-dissociated fibers either to an alteration of fiber properties due to the enzyme treatment or to some error in the measurement of dF associated with AM-loading.
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High Levels of Calsequestrin in Some Snake Muscles. Why?

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Calsequestrin (CSQ) is the most abundant Ca2+ binding protein in sarcoplasmic reticulum (SR) of skeletal and cardiac muscle. It is characterized by a high capacity and a moderate affinity for Ca2+ and thus it maintains luminal free Ca2+ in the junctional SR cisternae (jSR) at the physiological concentration of 1mM, while storing up to 20mM total calcium. Thin-section TEMs of superficial epaxial muscles from Nerodia sipedon and Boa constrictor show at least two structurally distinguishable types of fibers, probably twitch and tonic. Twitch fibers have unusually wide jSR cisternae completely filled with a finely granular matrix of CSQ polymers. The visible luminal content also extends far from the triads into the longitudinal SR. This is also the case in anterior intermandibular muscles from the same snakes. The percentage of fiber volume occupied by SR in twitch fibers from superficial epaxial muscles was calculated, from morphometry of thin sections, to be 9.5% in Nerodia, 10.6% in Boa and 7.5% in leg muscles from the lizard Anolis carolinensis, another lepidosaur. Comparable data from the literature for mouse indicate 5.5% in EDL and 2.5% in soleus. So, overall, the snake muscles have considerably higher Ca2+ storage capacity than lizard and mouse, two species that move frequently. We suggest that this may be related to prolonged periods of inactivity of these snakes, interrupted by the very infrequent use of muscles during prey capture and locomotion.
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