RyR1 mutations, Y522S (YS) and I4898T (IT), which cause MH and CCD, respectively. The AD mode of inheritance and data indicating knock-out of one RyR1 allele is well-tolerated in mice led us to hypothesize that allele-specific gene silencing (ASGS) of the mutant allele would rescue RyR1 functional defects in skeletal muscle cells from YS and IT knock-in mice.
We evaluated the functional consequences of allele-specific silencing in YS and IT muscle cells using short interfering RNAs (siRNAs). To screen potential siRNAs for relative knockdown efficacy and allele specificity, we generated cDNAs encoding fusion proteins derived from wild type (WT) (Venus-Exons-3XFLAG) and either YS or IT mutation-containing (Cherry-Exons-3XHA) exons. Simultaneous transfection of these cDNAs and siRNAs into HEK293 cells and subsequent evaluation of mRNA (semi-quantitative RT-PCR) and protein levels (fluorescence microscopy and western blotting) was used to determine knockdown efficacy and allele-specificity prior to functional rescue experiments. Myotubes derived from heterozygous YS mice (YS/+) exhibit ~4-fold increase in caffeine sensitivity (EC_{50} values were 0.5mM and 2.3mM for YS/+ and WT, respectively). Treatment with a YS-selective siRNA, normalized caffeine sensitivity (EC_{50} = 2.5mM) without decreasing peak caffeine-induced release. Similarly, YS-selective siRNA treatment rescued the increased voltage sensitivity of Ca^{2+} release in YS/+ myotubes determined in perforated-patch clamp experiments ($V_{F1/2}$: WT = −18mV, YS/+ scrambled = −35mV, YS/+ YS-selective = −18mV). These results indicate that ASGS represents a promising approach for normalization of RyR1 function in MH and CCD. Similar functional rescue experiments in adult skeletal muscle fibers are currently underway.

3709-Pos

Increased Fatigue in Sarcoglycan Knock Out Mouse Skeletal Muscle Fibers

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Background: The δ-sarcoglycan (δ-SG) knockout (KO) mice develop skeletal muscle histopathological alterations similar to those seen in humans with limb muscular dystrophy. Membrane fragility and increased Ca^{2+} permeability have been linked to muscle degeneration. However, little is known about the mechanisms by which the genetic defects lead to disease.
Methods: Isolated skeletal muscle fibers of wild type and δ-SG KO mice were used to investigate whether the absence of δ-SG alters the increase in intracellular Ca^{2+} during single twitches and tetani or during repeated stimulation. Immunolabeling, electrical field stimulation and Ca^{2+} transients recording techniques with fluorescent indicators were used.
Results: Ca^{2+} transients during single twitches and tetani, generated by muscle fibers of δ-SG KO mice, are similar to those of wild type mice, but their amplitude is greatly decreased during protracted stimulation in KO compared to wild type fibers. This impairment is independent of extracellular Ca^{2+} and is mimicked in wild type fibers by blocking SOC channels with 2-Aminoethoxydiphenyl borate (2-APB). Also, immunolabeling indicates the localization of a δ-SG isoform in the sarcoplasmic reticulum of the isolated skeletal muscle fibers of wild type animals, which could be related to the functional differences between wild type and KO muscles.
Conclusions: δ-SG has a role on calcium homeostasis in skeletal muscle fibers. The alterations caused by the absence of δ-SG may be related to the pathogenesis of muscular dystrophy.
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3710-Pos

Nuclear Translocation and Possible Transcriptional Regulation in Skeletal Muscle by $Ca_V\beta_{1a}$

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Abstract: The classically described function of $Ca_V\beta$ subunits is to modulate the biophysical properties and enhance trafficking of voltage gated calcium channels (Ca_Vs), making $Ca_V\beta$ subunits important in a number of physiological processes. Most notably, the skeletal muscle-specific isoform $Ca_V\beta1a$ is critical for proper EC coupling. Recently, several other protein binding partners of various neuronal and cardiac $Ca_V\beta$ isoforms ($Ca_V\beta_{1b}$, $Ca_V\beta_{2a}$, $Ca_V\beta_3$, and $Ca_V\beta_{4c}$) have been described, which has revealed novel functions for these subunits beyond the aforementioned augmentation of Ca_Vs. One especially interesting finding is that these Ca_V subunits enter the nucleus of neuronal cells and may participate in the regulation of gene transcription. However, this novel role for $Ca_V\beta$ subunits has only been minimally explored in neurons, and to our knowledge has not been examined in skeletal muscle. We therefore investigated whether the $Ca_V\beta_{1a}$ subunit may also act as a transcription factor. Using an array of biochemical and molecular techniques, we examined the following: $Ca_V\beta_{1a}$'s subcellular localization during various stages of myogenic development; binding partners of $Ca_V\beta_{1a}$, which may facilitate its transcriptional regulation; and also the functional role of $Ca_V\beta_{1a}$ nuclear localization. Our results show strong evidence for $Ca_V\beta_{1a}$ nuclear localization in skeletal muscle and provide insight into the mechanism and function of this phenomenon.
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3711-Pos

Effects of Domain Peptide DP4 on Depolarization-Induced Calcium Transients and Calcium Currents in Voltage-Clamped Fibers from Mouse FDB Skeletal Muscle

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Skeletal muscle excitation-contraction coupling involves sequential activation of dihydropyridine receptors (DHPRs) and type-1 ryanodine receptors (RyR_1) to produce depolarization-dependent sarcoplasmic reticulum Ca^{2+} release via ''orthograde'' signaling. Another form of DHPRs-RyR_1 communication is ''retrograde'' signaling, in which RyRs modulate the gating of DHPR. Domain peptide 4 (DP4), a peptide corresponding to residues Leu^{2442}-Pro^{2477} of RyR_1, interferes with inter-domain interactions within RyR_1 that normally stabilize the closed state of the RyR_1 channel. DP4 has been shown to potentiate force in response to submaximal depolarization by ionic substitution and caffeine-induced Ca^{2+} release in peeled muscle fibers. In sarcoplasmic reticulum vesicles, DP4 directly potentiates RyR_1 opening as shown by increased ryanodine binding and sensitization of Ca^{2+} release. Here we explore possible effects of DP4 on excitation-contraction coupling in whole-cell voltage clamped adult FDB skeletal muscle fibers. Depolarization-induced fluo-4 fluorescence transients (F/F0) became detectable at smaller depolarizations, and were increased at larger depolarizations in fibers dialyzed with DP4 compared to those without DP4 in the patch pipette, as were the Ca^{2+} transients and Ca^{2+} release calculated from F/F0. The amplitude of peak Ca^{2+} release (R) depended on voltage according to a two-state Boltzmann function. DP4 increased the amplitude of maximum release rate (R_{max}) by *ca* 54% when compared to control fibers. DP4 also induced a negative 5 mV shift in the potential at which R = 0.5 of R_{max} and an augmentation of macroscopic DHPR Ca^{2+} current density at all voltages tested. Thus, DP4 potentiates both depolarization-dependent Ca^{2+} release via RyR_1 and Ca^{2+} influx via DHPR, the later possibly mediated by retrograde signaling between the RyR_1 and DHPR Ca^{2+} channels in adult mammalian muscle fibers. Supported by NIH-NIAMS Grants R01-AR055099 and T32-AR007592.

3712-Pos

Small Ankyrin 1 Organizes the Ca2+ Stores of the Sarcoplasmic Reticulum in Skeletal Muscle

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Excitation-contraction (EC) coupling in striated muscle requires the coordinated functions of the transverse (t-) tubules, sarcoplasmic reticulum (SR) and the sarcomeric contractile proteins. The transient rise in [Ca2+]i that activates contraction depends on Ca2+ release from the SR. Maintaining the structural and functional integrity of the SR is crucial to maintaining the efficiency of EC coupling in skeletal muscle. Small ankyrin 1 (sAnk1, Ank1.5), a small alternatively spliced product of the ANK1 gene, is an integral membrane protein of the network compartment of the SR (nSR). sAnk1 binds obscurin, a giant cytoskeletal protein that surrounds the sarcomere at the level of the M-band and Z-disc. Our earlier results show that reducing the expression of sAnk1 with siRNA decreases the protein level, but not the mRNA, of the SR Ca2+ pump, SERCA, and may lead to a decrease in SR [Ca2+] load. We used Fluo-5N, a low affinity Ca2+ indicator, which we specifically loaded into the SR lumen, as a reporter of SR Ca2+ stores in control and siRNA treated myofibers isolated from rat flexor digitorum brevis muscle. The intensity of Fluo-5N fluorescence and its distribution in the SR lumen were altered when sAnk1 expression was reduced, consistent with a loss of nSR. Further, fluorescence recovery after photobleaching (FRAP) experiments showed that even small changes in sAnk1 expression altered the interconnectivity of the SR. Functional measurements of SR Ca2+ dynamics also suggested an important role for sAnk1 in maintaining EC-coupling. Our results are consistent with the hypothesis that sAnk1 is essential for the integrity of the nSR compartment and its Ca2+ stores in skeletal myofibers.