1828-Pos Board B598
Cardiomyopathy - Associated W4R Variant of Muscle LIM Protein affects Skeletal Muscle Passive Mechanics
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Muscle LIM protein (MLP) is located at the Z-disc and the M-line of the sarcomere of striated muscle. It has multiple protein interaction partners. The MLP-W4R variant was shown to be associated with cardiomyopathy in humans. MLP-W4R knock-in mice showed myopathic changes in skeletal muscle and other mutations of MLP were associated with a skeletal myopathy in affected human individuals. Therefore, we investigated the influence of MLP-W4R on skeletal muscle function and ultrastructure.
Skinned single muscle fibers of M. vastus lat. of wildtype and MLP-W4R knock-in mice were used to characterize passive and active parameters like relaxed fiber stiffness, passive force-sarcomere length curve, isometric force generation, rate constant of force redevelopment and maximal shortening velocity. Thin sections of the muscle fibers were analyzed by transmission electron microscopy.
In comparison to wildtype, MLP-W4R muscle fibers showed a significant decrease in relaxed fiber stiffness and passive force-sarcomere length curve, whereas no significant changes occurred in isometric force generation, force redevelopment, and shortening velocity. Ultrastructurally, the alignment of myofilaments within the sarcomere was normal with MLP-W4R, however, Z-discs and M-lines were broadened.
MLP-W4R knock-in mice exhibit discrete morphological changes of skeletal myofibers consistent with a skeletal myopathy. The disturbed passive properties of the muscle fibers support the hypothesis that the MLP-W4R variant contributes to the disorder.

1829-Pos Board B599
Elastic Proteins in the Flight Muscle of Manduca Sexta
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Unlike the asynchronous flight muscles of *Lethocerus* or *Drosophila*, the flight muscles of the Hawkmoth *Manduca* sexta are synchronous, requiring a neural spike for each contraction. While the asynchronous muscles can only extend a few percent, *Manduca* flight muscle can reversibly extend 50% or more. Together with the observation that length-tension curves of *Manduca* flight muscles resemble mammalian cardiac muscle, these observations suggest that Manduca muscle might be a useful model system to study some aspects of cardiac muscle contractility. The detailed protein composition of *Manduca* flight muscle is not known. Here we aimed to identify proteins which might be responsible for the unique properties of *Manduca* muscle. We used 1% vertical SDS-agarose gel electrophoresis (VAGE) to separate the high molecular weight proteins in Maduca flight muscle, Lethocerus flight muscle and bovine ventricular myocardium. The *Manduca* sample showed two bands around 2MDa and 1.6MDa, smaller than the two titin isoforms in bovine cardiac muscle, but larger than the largest *Lethocerus* proteins. Projectin and Kettin are elastic proteins found in *Lethocerus* and *Drosophila* with sequence homologies to vertebrate titin. Using western blots, the Manduca sample showed two bands cross-reacting with projectin antibodies at ~800 kDa and ~1030 kDa. Kettin antibodies also cross-reacted to bands at the same position in both *Lethocerus* and *Manduca*. We also used western blots from 10% PAGE gels to detect a flightin -crossreacting band at around 23kDa in *Lethocerus* and 30 kDa in *Manduca*. Flightin is a thick filament associated protein that presumably helps filament assembly and stability. Thus, *Manduca* flight muscle has not only proteinshomologous to *Lethocerus* projectin, kettin, flightin, but also several unknown high molecular weight proteins which might play a role in stabilizing sarcomere structure. Supported by NSF IOS 1022058 and NIH RR08630.

1830-Pos Board B600
Role of Electrostatics in the Interactions of Muscle Thick Filament Proteins
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Electrostatic interactions play a primary role in the assembly and stability of muscle thick filaments. This is illustrated by the solubility properties of the filament and its periodic structure, which correlates with the periodic distribution of positively and negatively charged residues in the sequence of light meromyosin (LMM), the main component of the filament core. In this work, we analysed the electrostatic fields of proteins associated with LMM in thick filaments, titin and MyBP-C, to explore further their role in filament assembly. The thick filament bound part of titin consists mainly of immunoglobulin (Ig) and fibronectin (Fn3) domains arranged in repeated patterns, or super-repeats, which size correlates with the periodic structure of the filament. Analysis of the domain electrostatics suggests periodic oscillations of the field along the super-repeats, with a major interval of ~43 nm and a weaker (less consistent) interval of ~14 nm. These oscillations result from the mostly opposing electrostatic fields from the Ig and Fn3 domains, respectively, and correlate with the periodic structure of the filament.
Analysis of the electrostatic fields of the Ig and Fn3 domains of cardiac MyBP-C reveals three distinct regions: the N- (C0-C2) and C-terminal (C8-C10) groups of domains are positive or neutral, whereas the central domains (C3-C7) are mainly negative. Further analysis of binding partners of MyBP-C, including titin and LMM, illustrates general complementarity of the overall electrostatic fields of the interacting proteins, thus directly supporting the involvement of electrostatics in the interactions between all the major thick filament proteins.

1831-Pos Board B601
Structure of Titin PEVK Explored with FRET Spectroscopy
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The PEVK domain of the giant muscle protein titin is largely responsible for molecular extensibility within the physiological sarcomere-length range. Although the domain is thought to be an intrinsically unstructured random-coil structure, several observations suggest that it may not be completely devoid of internal interactions and structural features. To test the validity of random polymer models for PEVK, here we measured the contour-length scaling of the equilibrium mean end-to-end distance of synthetic PEVK peptides with FRET (Förster's Resonance Energy Transfer) spectroscopy. Mean end-to-end distances of an 11- and a 21-residue PEVK peptide were calculated from the energy transfer efficiency between an intrinsic tryptophan donor and a synthetically added IAEDANS acceptor positioned on the N- and C-termini, respectively. We find that the contour-length scaling of mean end-to-end distance deviates from the square-root law predicted for a purely statistical polymer chain. Addition of guanidine-HCl increased, whereas the addition of salt decreased the mean end-to-end distance, indicating that both H-bonding and electrostatic interactions play role in stabilizing PEVK structure. Increasing temperature between 5-50 °C resulted in monotonous increase in FRET efficiency, suggesting that PEVK may pass through multiple conformational states separated by small energy barriers. Simulations suggest that the residual structures are loose helical configurations. Varying the distribution across these states may tune the apparent local elasticity along the PEVK domain and modulate the dynamics of passive muscle force generation.
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1832-Pos Board B602
Stretching of Twitchin Kinase
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The giant proteins from the titin family, that form cytoskeletal filaments, have emerged as key mechanotransducers in the sarcomere. These proteins contain a conserved kinase region, which is auto-inhibited by a C-terminal tail domain. The inhibitory tail domain occludes the active sites of the kinases, thus preventing ATP from binding. It was proposed that through application of a force, such as that arising during muscle contraction, the inhibitory tail becomes detached,

lifting inhibition. The force-sensing ability of titin kinase was demonstrated in AFM experiments and simulations [Puchner, et al., 2008, PNAS:105, 13385], which showed indeed that mechanical forces can remove the autoinhibitory tail of titin kinase. We report here steered molecular dynamics simulations (SMD) of the very recently resolved crystal structure of twitchin kinase, containing the kinase region and flanking fibronectin and immunoglobulin domains, that show a variant mechanism. Despite the significant structural and sequence similarity to titin kinase, the autoinhibitory tail of twitchin kinase remains in place upon stretching, while the N-terminal lobe of the kinase unfolds. The SMD simulations also show that the detachment and stretching of the linker between fibronectin and kinase regions, and the partial extension of the autoinhibitory tail, are the primary force-response. We postulate that this stretched state, where all structural elements are still intact, may represent the physiologically active state.

Excitation Contraction Coupling I

1833-Pos Board B603

FRET Reveals Substantial Reorientation of the Cytoplasmic Interface of the Skeletal Muscle DHPR in the Presence of RyR1

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In skeletal muscle, the dihydropyridine receptor (DHPR) in the t-tubular membrane serves as Ca2+channel and as voltage sensor for excitation-contraction (EC) coupling, triggering Ca2+release via a physical/conformational coupling to the type 1 ryanodine receptor (RyR1) in the sarcoplasmic reticulum (SR) membrane. The particulars of the structural and functional links between these two proteins are widely unknown. The putative intracellular portions of the DHPR a1S subunit, the N-terminus, C-terminus, and the loops connecting the four homologous repeats (I-IV), play important roles in the communication with the RyR1. Examples are the β-subunit recruiting function of the I-II loop, the bi-directional signaling function of the II-III loop with the RyR1 during EC-coupling, the influence of the III-IV loop on RyR1 mediated Ca2+delivery, and the a1S C-terminus. These channel parts are believed to either directly or indirectly interact with the RyR1, and the close spatial proximity between the two channels at t-tubule/SR 'junctions' constitutes the structural prerequisite for this linkage. The present work provides for the first time a structural insight into the arrangement of the crucial molecular components of the DHPR-RyR1 interaction, by using measurements of fluorescence resonance energy transfer (FRET), conducted within the cellular environment of living myotubes. Upon expression, the degree of FRET was determined for different combinations of labeled cytoplasmic a1S domains, using a sensitized emission FRET variant. Confocal fluorescence microscopy was applied to check for correct expression and function of the constructs upon expression in dyspedic (RyR1 null) and dysgenic (a1S null) myotubes. The presence of RyR1 significantly altered the intramolecular energy transfer for almost every double tagged a1S construct. These measurements reveal that virtually the complete cytoplasmic a1S architecture is significantly rearranged by the presence of the RyR1.

1834-Pos Board B604

19-Residue Peptide from C-Terminal Tail of DHPR β1A Subunit Potentiates Voltage-Dependent Calcium Release in Adult Skeletal Muscle Fibers

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The skeletal muscle isoforms of the membrane-spanning α_{1s} subunit and the cytoplasmic β_{1a} subunit are essential during EC coupling. Recent evidence suggests that the activating effect of the full-length β_{1a} subunit on isolated ryanodine receptor (RyR1) Ca^{2+} channels in lipid bilayer can be reproduced by a peptide (β_{1a} 490-524) corresponding to the 35-residue C-terminal tail of the β_{1a} subunit and also confirmed a high-affinity interaction between the C-terminal tail of the β_{1a} and RyR1. We now tested the hypothesis that a 19 amino acid residue peptide (β_{1a} 490-508) may be sufficient to reproduce activating effects already observed for β_{1a} 490-524 as well as that of the full-length peptide. The hypothesis is based on existing results using overlapping peptides tested on isolated RyR1 in phospholipid bilayer (1). Here we examined the effects of β_{1a} 490-508 on Ca^{2+} release during whole cell voltage-clamp depolarization of adult mouse FDB muscle fibers. 25 nM or 100 nM of β_{1a} 490-508 peptide in a patch pipette caused a 25% increment in the SR Ca^{2+} release flux in single voltage clamped muscle fibers but with no significant shift in the voltage dependence of the maximum peak Ca^{2+} release flux. Considerably less activating effect was observed using 400 nM peptide. A scrambled form of the 19-residue peptide (100nM) was used as a negative control for the wild-type peptide and produced a negligible effect on the peak amplitude of Ca^{2+} release flux. Taken together, we have shown that the β_{1a} 490-508 peptide contains molecular components sufficient to modulate EC coupling between DHPR and RyR1 in adult functioning muscle fibers. Supported by R01-AR055099 and T32-AR007592.

1. Rebbeck, R.T. et. al. March (2011) Biophysical Society Abstract 3195-Pos/B300.

1835-Pos Board B605

Role of STIM1 in Skeletal Excitation-Contraction Coupling

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STIM1, a Ca^{2+}-sensing protein on ER/SR membrane, mediates a store-operated Ca^{2+}-entry (SOCE) by activating Orai1 Ca^{2+}-entry channel on plasma membrane. E136X mutant of STIM1, truncated STIM1 missing binding abilities to Orai1, has been found in patients with immunodeficiency accompanying muscular hypotonia. To identify causes of the muscular hypotonia, E136X was expressed in mouse skeletal myotubes and dominant-negative effects of E136X were examined. Myotubes expressing E136X showed increases in both KCl (a membrane depolarizer resulting excitation-contraction coupling) and caffeine (a direct RyR1 agonist) responses. On the other hand, SOCE, resting cytosolic Ca^{2+} level, SR Ca^{2+} level were not significantly changed by the expression of E136X. In addition, E136X did not interfere with puncta formations by endogenous STIM1 and Orai1. These data mean that muscular hypotonia found in patients with E136X is due to changes in excitation-contraction coupling. Additionally, we suggest that C-terminus of STIM1 that is missing in E136X participates in the regulation of skeletal EC coupling.

1836-Pos Board B606

Direct Quantification of Calsequestrin-Dependent Buffering in the Calcium Store of Skeletal Muscle

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In mouse FDB cells releasing Ca^{2+} under patch clamp we measured luminal $[Ca^{2+}]_{SR}$ with the novel sensor D4cpv-calsequestrin (Sztretye *et al.* JGP 2011a) in parallel with SR Ca^{2+} release flux (derived from the fluorescence of cytosolic X-rhod 1). These simultaneous measurements allowed the dynamic monitoring of Ca^{2+} buffering power of the SR ($BP \equiv \Delta[Ca]_{total,\ SR} / \Delta[Ca^{2+}]_{SR}$). *BP* started at 180 (SEM 30, 27 cells) and decreased to 89 (SEM 14) as the SR lost Ca^{2+} upon SR-depleting membrane depolarization (400 ms, +30 mV). The stage of high *BP* is also characterized by a ''hump'' in the release flux waveform. Recovery of SR Ca^{2+} content after the end of depolarization proceeded at the lower *BP* value (a mismatch that we call ''buffer hysteresis''). *BP* regained slowly its initial value, as demonstrated by persistent low power and absence of hump in the second depolarizing pulse of pairs separated by 600 ms. Full restoration of hump and *BP* was observed after 2 min rest. In calsequestrin1-null cells *BP* was a constant 40 during the pulse (3.6, 29 cells), there was neither buffer hysteresis nor flux hump. Therefore, calsequestrin 1 is the time-dependent, hysteretic buffer, and contributes approximately 75% of the SR buffering power. Total releasable Ca^{2+}, however, only decayed by 30% in CSQ-null cells, a paradox explained by a more thorough depletion in the null (Sztretye *et al*. JGP 2011b). The time dependence and hysteresis of the calsequestrin contribution to Ca^{2+} buffering is consistent with aggregation changes proposed to accompany Ca^{2+} binding (e.g. Park *et al*. JBC 2004). In this view, the hysteresis reflects the time required for the Ca^{2+} dissociation-induced structural changes in calsequestrin to reverse upon Ca^{2+} reuptake into the SR.
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1837-Pos Board B607

Two-Edged Sword: The Ca^{2+} Biosensor D4cpv-Calsequestrin Restores Functionality to Calsequestrin-Null Muscle

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SR Ca^{2+} buffering power, *BP*, decreases during Ca^{2+}-depleting depolarizations of mouse skeletal muscle. During Ca^{2+} release the stage of high *BP* is characterized by a ''hump'' in the release flux waveform. After the depolarization *BP* returns slowly to its initial value, as demonstrated by the absence of a hump in the flux induced by the second pulse of pairs separated by 600 ms. These time-dependent features were described as ''buffer hysteresis'' and shown to be contributed by calsequestrin in the Sztretye *et al.*, companion poster. SR release flux and *BP* were measured in calsequestrin 1-null cells expressing the biosensor D4cpv-calsequestrin. Null cells had lower *BP* and *generally* lacked the hump in the flux. In some regions of these cells, however, [biosensor] reached very