(ACTC) were probed. Skinned papillary muscle fibers (strips) from 2- and 5-mo old E99K transgenic mice were subjected to sinusoidal length perturbations to study tension transients, and the results were compared with those from age-matched non-transgenic (WT) mice. (1) At the standard activation (5 mM MgATP, 8 mM Pi, 200 mM ionic strength with K acetate, pH 7.00, 25°C), fibers from 5-mo E99K mice produced tension, stiffness, the rate of delayed tension (2πb), and magnitudes B and C, which were significantly less than those from 5-mo WT mice. In 2-mo mice, these differences were either significant (tension, stiffness, C), slight (B), or none (2πb). The rate of fast exponential advance (2πc) was not much different among 4 groups. (2) Tension and stiffness at rigor state were not significantly different among 4 groups. (3) pCa-tension study showed increased Ca sensitivity with aging in both E99K and WT groups, and pCa50 was slightly larger (~0.07 units) in E99K than in WT. A significant decrease in cooperativity (nH) was observed only in 5-mo E99K. The difference in pCa was larger in solutions that did not contain Pi (ΔpCa50=0.09, P=0.047) than that contained 8 mM Pi (ΔpCa50=0.04, P=0.091). (4) The ATP association constant (K1) increased with aging, and it was significantly larger in E99K than in WT; there were no differences in the rates (k2, k-2) of the cross-bridge detachment step. (5) The Pi association constant (K5) increased with aging, but there was little difference between E99K and WT; there was no difference in the rate of the force generation step (k4), but it reversal step (k-4) was slightly larger in E99K than in WT. We conclude that AHCM-causing ACTC E99K mutant resulted in progressive alterations in biomechanical parameters: changes were small at 2 months, but larger at 5 months, which correlates well with the development of AHCM.

S02.O4-219/S02.P1-219

Functional properties of αβ-tropomyosin with mutations in the α-chain are different from those of αα-tropomyosin with mutations in both α-chains

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Tropomyosin (Tpm) molecule can exist either as αα-Tpm homodimers or as αβ-Tpm heterodimers. We studied the effects of stabilizing substitutions in the central part of Tpm molecule on functional properties of αα-Tpm homodimers and αβ-Tpm heterodimers where only α-chain carried these mutations. Previously we showed that substitutions of non-canonical aminoacid residues Gly126 and Asp137 in the middle part of α-Tpm molecule by canonical Arg126 and Leu137 appreciably affect its structure and properties (Matyushenko et al., FEBS J., 2014). With an optical trap we measured the parameters of single interactions of skeletal myosin with regulated thin filaments containing Tpm and troponin at saturating Ca^{2+}. Stabilizing of the middle part of Tpm molecule did not affect step size and unitary force of myosin but decreased the step duration. Besides D137L/C190A and G126R/D137L/C190A mutations increased the duration of force events. The extent of thin filament activation was evaluated by dependence of their sliding velocity over myosin surface in an in vitro motility assay at pCa 4 on the myosin concentration. The sliding velocity of the filaments containing Tpm with stabilizing mutations was by 20–50% higher than that with control Tpm C190A. Myosin concentration required to achieve half-maximal velocity for thin filaments with with single mutations Tpms was half, and with double mutation more than 20-fold less than that for the Tpm C190A. Surprisingly, the stabilizing mutations in α-chain of heterodimers decreased the sliding velocity by 25–30% as compare to that of control αβ-Tpm. Functional properties of αβ-Tpm heterodimers with mutations in the α-chain are quite different from those of αα-Tpm homodimers with mutations in both α-chains.

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Muscle performance in response to resistance training is mainly controlled by an increase in myosin ATPase activity independent of the MHC shift

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It is generally believed that contractile and energetic properties of skeletal muscle rest mainly on myosin heavy chain (MHC) isoform content, that is primarily responsible for speed of contraction and myosin ATPase activity. Nevertheless, experimental evidences suggest that adaptive processes could occur directly at the myosin ATPase level in response to different stimuli such as endurance training, hypoxia and aging. Since resistance training is known to determine large changes in contractile activity, the aim of the present study was to detail the adaptations of the myosin ATPase activity independently of MHC isoform content, in response to resistance training, in rats. In purified myofibrils, ADP production over time was measured by Rapid Flow Quench method and High Performance Liquid Chromatography. MHC was measured by RT-qPCR. Beyond the discrete phenotypic shift from MHC1, MHC2X/2D toward MHC2A and the moderate fiber hypertrophy, a mean increase of 123 ± 61% in myosin ATPase activity was observed with the trained compared to the control group in the three muscles studied. Kinetics simulations with a simple scheme of the cross-bridge cycle suggest that adaptations responsible of the enhancement of the myosin ATPase activity come mainly from an increase in the rate constant of ADP and Pi release that occurs during the powerstroke, and to a lesser extent, from the ATP cleavage rate constant.

Posters

S02.P3-285

C-Terminal Truncation of Troponin I and Substitutions of Non-Canonical Residues in the Central Part of Tropomyosin 1.1 Disrupt Thin Filament Switched Off State in Rabbit Psoas Myofibrils

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The Ca^{2+}-dependent and highly cooperative activation of striated muscle contraction critically depends on the factors affecting Tpm-Tn

position on actin filament. Based on previously developed methods to remove/reconstitute striated muscle myofibrils with exogenous regulatory proteins [1], we investigate here the functional impact of the exchange of: (i) native fast skeletal Tn for human recombinant cTn containing either full length cTnI (cTnIFL) or truncated cTnI (cTnI1-192); (ii) native Tpm-Tn with purified skeletal Tn and recombinant Tpm1.1 either wild type (WT) or carrying one (D137L) or two (D137L/G126R) stabilizing substitutions in the central part of the molecule, decreasing its flexibility [2]. In myofibrils replaced with cTnI1-192 or D137L/G126R Tpm (15°C) force relaxation from maximal activation (pCa 4.5) to relaxing solution (pCa 9.0), was similarly prolonged (increased duration of slow phase, decreased rate of fast phase). Effects on maximal isometric tension and on rates of force activation (kACT) and redevelopment (kTR) were small (cTnI1-192) or absent (D137L or D137L/G126R Tpm). Both cTnI1-192 and Tpm substitutions strongly and additively decreased slack sarcomere length (sl) at sub-maximal activating [Ca^{2+}] and increased the steepness of the sl-passive tension relation. These effects were reversed by 10 mM BDM, suggesting that both cTnI1-192 and Tpm substitutions compromise the full inhibition of acto-myosin interactions in the absence of Ca^{2+}. This hypothesis is further supported by the significant increase of ATPase activity in relaxing solution of D137L and D137L/G126R Tpm myofibrils compared to WT Tpm. Data support the hypothesis that flexibility of the Tpm coiled-coiled structure critically modulates the turning off of the thin filament system and muscle relaxation dynamics, likely in interaction with the C-term of TnI.

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S02.P4-249

Ratio of fast and slow muscle myosin in tissue samples identified by kinetic analysis

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Cardiac myosin II exists as two isoforms - α (fast type) isoform and β (slow type) isoform. The isoforms are localised in different areas and ratios within the heart. In large mammals the atria contains approximately 90 % α with 10 % β whilst the ventricle contains approximately 85 - 90 % β with < 15 % α. During a failing human heart, the fraction of α-myosin in the ventricle falls dramatically and drug treatments that result in re-expression of α-myosin give some recovery of function. Kinetically the α-S1 has a 3 fold faster ATPase cycle and contraction velocity than the β-S1. In detail the hydrolysis step of the cross-bridge cycle is up to 10 time faster in α compared to β; the α ADP affinity for actoS1 is 10 times weaker and actin affinity is also weaker (>4 fold). Similar results are reported for both human and mouse α & β-myosin's. Preparations of cardiac myosin from tissue contain mixed isoforms and the two are difficult to separate. We report here a simple effective methods to establish the ratio of α and β myosin's (or S1) in a mixture by measuring the displacement of ADP from actomyosin in a Stopped Flow assay. ADP is displaced from actin·β-myosin at 48 s-1 whereas ADP is released at > 200 s-1 from actin·α-myosin. Thus displacing ADP from a mixture of the two myosin by an excess of ATP results in a biphasic reaction. The slow phase represents the amount of β-myosin while the fast phase reports the amount of α-myosin. In current systems the assay requires 125 μg of myosin and by miniaturising the system the assay could be performed with as little as 10 μg of myosin.

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Effect of interchain disulfide cross-linking on the functional properties of tropomyosin

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Normally, the SH-groups of skeletal or cardiac α-tropomyosin (Tpm 1.1, onwards Tpm) are in a reduced state [Lehrer et al., J. Muscle Res. Cell Motil. 2011]. Disulfide cross-linking of the cardiac Tpm occurs upon human end-stage heart failure [Canton et al., J. Am. Coll. Cardiol. 2011]. It was shown with differential scanning calorimetry that this cross-linking increases the thermal stability of C-terminal part of Tpm [Kremneva et. al., Biophys. J. 2004]. We studied how disulfide bond affects the functional properties of Tpm and actin-myosin interaction using a number of approaches. With cosedimentation assay we found that the disulfide bond decreases affinity of Tpm for F-actin. Cross-linking decreased stability of Tpm–F-actin complexes as was shown by measuring the temperature dependence of dissociation of these complexes. In an in vitro motility assay the cross-linking increased the sliding velocity (Vmax) of regulated thin filaments containing F-actin, Tpm and troponin (Tn) at saturating Ca^{2+} concentration, with no appreciable effect on Ca^{2+}-sensitivity of pCa-velocity relationship. This effect highly depends on proteins, skeletal or cardiac, used in the motility assay. The most pronounced effect of the Tpm cross-linking on Vmax was observed with cardiac Tn, skeletal F-actin and cardiac myosin. This situation seems to be quite possible during cardiac hypertrophy where expression of α-skeletal actin is increased that leads to a partial replacement of cardiac α-actin isoform by skeletal one [Clement et al., Circ. Res. 1999]. Thus interchain cross-linking of SH-groups may have a significant effect on the functional properties of Tpm, and this can explain, at least partly, why this cross-linking is associated with human heart diseases.

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Alterations in actomyosin interaction and myofibrillar force under nitrosative stress conditions

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Aim: This study aimed to investigate the effect of peroxynitrite (ONOO)-derived nitrosative stress on actin and muscle contractile properties, and how it relates to muscle weakness.
Methods and Results: Nitration of myofibrils (M. psoas major, rabbit) was induced by addition of either ONOO or 3-Morpholino-sydnonimine chloride (SIN-1), and the myofibrillar forces were measured using an atomic force cantilever during activation of the myofibril (pCa 6.5 - pCa4.5). The maximal force was clearly lowered in myofibrils nitrated with either 150 uM ONOO or 10 mM SIN-1. Motility of actin was measured using an in vitro motility assay (IVMA). Nitration of isolated actin filaments were induced by addition of ONOO and were adsorbed to a heavy meromyosin coated nitrocellulose surface. The myosin-induced actin translocation was initiated by addition of 1 mM MgATP at different ionic strengths (IS). When tested at 40–130 mM IS, actin treated with