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Myosin SH1 Thiol as a Sensor of Rotation of the C-terminal Region of the Motor Domain, the "Converter"

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Actin-myosin interaction in smooth muscle is regulated by the phosphorylation of myosin regulatory light chains (RLCs), which is mediated by Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK). Therefore, contraction and regulation are controlled by the intracellular Ca^{2+} concentration. Previously we found that chemical modification of a reactive thiol SH1 of smooth muscle myosin leads to a complete loss of Ca^{2+}-regulation of the contractile system. Here we investigate why SH1 modification can functionally mimic the phosphorylation of RLCs, even though the position of SH1 is far away from the neck region, where phosphorylation sites reside. SH1 locates near the converter, which rotates by ~70° upon the transition from the ''nucleotide-free'' to ''pre-power stroke'' state. The modification rate of SH1 with a thiol reagent, IAEDANS, was dramatically inhibited by the formation of 10S myosin. Comparison between myosin structures in the pre-power stroke state and the nucleotide-free state explained why SH1 is especially sensitive to a conformational change around the converter, and thus can be used as a sensor of the converter rotation. Modeling of the myosin structure in the pre-power stroke state, in which SH1 was selectively modified with IAEDANS, revealed that this bulky probe buried in a deep cleft of myosin becomes an obstacle when the converter rotates toward its position in the pre-power stroke state. This result suggests that SH1-modified myosin cannot assume 10S myosin formation, because of an incomplete rotation of the converter in the pre-power stroke state. We propose that the loss of the phosphorylation-dependent regulation of the actin-activated ATPase activity of smooth muscle myosin by SH1 modification is due to the modification-induced inhibition of the head-head interaction proposed by Wendt et al. [*J. Cell Biol. 147,* 1385-1390 (1999)].

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Training Effects On Skeletal Muscle Calcium Handling In Chronic Heart Failure (CHF) Patients And Controls

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CHF patients typically complain about increased skeletal muscle fatigability. This is not due to reduced skeletal muscle blood flow and seems to persist even after cardiac transplantation. We have previously reported that in contrast to normal muscle, reduced intracellular calcium release was not related to fatigue development in CHF rats (Lunde et al. Circ Res 98:1514, 2006). Therefore we hypothesize that training might affect intracellular calcium cycling differently in muscles from patients with CHF as compared with healthy controls (HS). Before and after six weeks of ergometer training of one leg muscle biopsies were taken from vastus lateralis bilaterally and analyzed both for Ca^{2+} handling proteins (Serca1 and 2, PLB and RyR) and the capability of sarcoplasmic reticulum (SR) vesicles to take up, hold and release calcium. Endurance of the trained leg was 17 and 6% greater than in the untrained leg in CHF and HS respectively. For the HS group training resulted in a higher Ca^{2+} release rate and lower leak in the trained leg associated with a tendency of increased RyR content with reduced phosphorylation level. In the CHF patients Ca^{2+} uptake rate was higher in the untrained leg but Serca levels were unchanged and ser16 phosphorylation of the PLB monomer paradoxically reduced. In the trained leg of CHF patients RyR was down regulated, but without associated changes of either Ca^{2+} leak or release rate. No change in fiber type composition was seen in either group. We conclude that training in HS has effect foremost on SR Ca^{2+} leak and release, but that in CHF patients training is achieved without such changes of SR function. Thus, in line with experiments in rats, in human CHF SR is not the site of increased fatigability.

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The Role of the Frank-Starling Effect in the Transduction of Cellular Work into Whole Organ Pump Function: A Computational Modeling Analysis

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We have developed a multi-scale biophysical electromechanics model of the rat left ventricle at room temperature. This model has been used to investigate the role of length dependent regulators of tension in the transduction of cellular work into whole organ pump function. Specifically the role of the length dependent Ca^{2+} sensitivity of tension (Ca_{50}), filament overlap tension dependence, velocity dependence of tension and tension dependent binding of Ca^{2+} to Troponin C on metrics of effective transduction of work were predicted by performing simulations in the absence of each of these feedback mechanisms. The length dependent Ca_{50} and the filament overlap, which make up the Frank-Starling effect, were found to be the two dominant regulators of effective transduction of work. Analyzing the fiber velocity field in the absence of the Frank-Starling mechanisms showed that transduction of work from the cell to the whole organ in the absence of filament overlap effects was caused by increased post systolic shortening, whereas the decrease in efficiency observed in the absence of length dependent Ca_{50} was caused by an inversion in the regional distribution of strain.

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Slow Changes of Calcium Transient During Interaction of Inhomogeneous Cardiac Muscles

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To assess influence of mechanical interaction of cardiac cells to calcium handling we used hybrid duplex method [1]. The hybrid duplex consists of papillary muscle and computational model of electromechanical coupling in cardiac cell (virtual muscle) [2] coupled in-series to simulate mechanical interaction of cardiac fibers from different regions of the ventricular wall. We analyzed calcium transients of the both muscles during slow force change (SFC) appearing after either coupling muscles together or discoupling.

Rat right ventricular papillary muscles were washed in Tyrode with 1 mM extracellular calcium (25°C, 0.33 Hz) and loaded with fura-2/AM. We registered simultaneously force and free intracellular calcium transients in biological and virtual muscles during contraction in isolation and during interaction in duplex with different activation sequence and delay between the muscles. We have shown previously [3] that coupling of papillary and virtual muscles in-series or disconnection to isolation causes SFC in both muscles, together with slow changes in calcium handling in virtual muscle. The SFC was depended on sequence and delay of activation between the muscles. Present study experimentally confirms that the slow changes in peak calcium takes place in biological muscle during its interaction with counterpart.

In conclusion, slow mechanical responses of interacting inhomogeneous cardiac muscles accompanies with slow changes in intracellular calcium handling in their cells, for example, peak systolic calcium.

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Muscle Regulation II

1186-Pos Board B30

A Peculiar Meridional Reflection in the X-ray Diffraction Pattern from Dipteran Flight Muscle Suggests an Alternating Arrangement of Tropomyosin Isoforms

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The X-ray diffraction pattern from the flight muscle of a cranefly, *Ctenacroscelis mikado* (Diptera), exhibits a prominent meridional reflection not observed in *Lethocerus* at a spacing of 25.8 nm. Since this spacing is two thirds of the pseudo-repeat of the long-pitched actin helix (38.7 nm), the reflection is likely to be of thin filament-origin. Its occurrence is fully explained if the scattering objects have a basic axial repeat of 77.4 nm (= 2 x 38.7 nm), and the six thin filaments surrounding a thick filament are arranged with an axial stagger of 25.8 nm (= 77.4/3).

A possible mechanism to create the 77.4-nm repeat is the presence of two different tropomyosin isoforms. Dipteran flight muscle is known to express usual (~35 kDa) and heavy (~80 kDa) tropomyosin isoforms, and the extra mass of the latter is ascribed to the C-terminal extension of a pro- and ala-rich sequence. Tropomyosin is a uniform alpha-helical protein that forms a dimer with a typical coiled-coil structure, but the mass of the C-terminal extension would be localized. Thus, the reflection is most readily explained if the two isoforms produce an alternating array of homodimers. However, a cross-linking study suggests that the *Ctenacroscelis* isoforms produce heterodimers. In *Drosophila*, two heavy isoforms are known to exist (TmH-33 and TmH-34), and glutathione