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**1182-Pos Board B26**
**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)].

**1183-Pos Board B27**
**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.

**1184-Pos Board B28**
**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.

**1185-Pos Board B29**
**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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[1] Protsenko et al., AJP 2005/289: H2733-H2746.
[2] Markhasin et al., Prog Biophys Mol Biol 2003/82: 207-220.
[3] Solovyova et al., Phil Trans R Soc A 2006/364: 1367-1383.

## 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

S-transferase-2 is stably associated with them (Clayton et al., 1998). Then an alternative explanation is that these isoforms also exist in *Ctenacroscelis*, and they are alternately arranged and only one of them binds GST-2. The scattering object remains to be identified, but the alternating arrangement of tropomyosin isoforms is the most conceivable mechanism to provide the periodicity needed to create the peculiar meridional reflection.

**1187-Pos Board B31**

**Effect Of Tropomyosin On The Binding Force Of Unphosphorylated Myosin To Actin**

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Smooth muscle (SM) is unique in its ability to maintain force for long periods of time at low energy cost. This property is called the latch-state. One of the assumptions of the latch state model of Hai and Murphy is that myosin must first be phosphorylated in order to attach to the thin filament. However, we previously demonstrated that unphosphorylated (unPHOS) myosin can attach to unregulated actin filaments. The goal of this study was to measure the binding force of unPHOS SM myosin to tropomyosin-regulated actin filaments. A microsphere captured in a laser trap was attached to an actin filament decorated with SM tropomyosin-$\alpha$ and $\beta$. The filament was brought in contact with a pedestal coated with unPHOS pig antrum myosin. The pedestal was then moved away from the trap at constant velocity (0.5 $\mu m.s^{-1}$). Despite pulling the pedestal away, the microsphere did not move until the force exerted by the trap on the microsphere was sufficient to overcome the binding force of myosin on the actin/tropomyosin filament. At this point, the microsphere sprang back to its unloaded position. The force of unbinding was calculated as the product of the trap stiffness and the maximal displacement of the trapped microsphere, as assessed by displacement of its center of mass. The average force of unbinding per myosin molecule ($F_{unb}$) was obtained by dividing the measured force of unbinding by the number of myosin molecules estimated per actin filament length. We found that $F_{unb}$ was greater in presence (0.222 pN $\pm$ 0.018; mean $\pm$ SE) than in absence (0.142 pN $\pm$ 0.019; $p<0.001$) of tropomyosin. These results demonstrate that tropomyosin strengthens the bond between unPHOS myosin and actin. Future studies will investigate the role of other regulatory proteins of the thin filament.

**1188-Pos Board B32**

**Tropomyosin Flexibility Evaluated by Electron Microscopy Image Analysis**

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Movement of tropomyosin (Tm) on thin filaments in response to $Ca^{2+}$-binding to troponin and myosin binding to actin is an inherent feature of muscle regulation. As part of this process, the cable-like mechanical properties of the Tm coiled-coil are thought to underlie cooperative on and off switching of contraction. In principle, movement of Tm over the flat surface of actin may not require significant molecular flexibility. However, local perturbations caused, for example, by myosin binding on actin may necessitate some plasticity of the Tm molecule. In contrast, any large-scale Tm flexibility might dampen its cooperative movement. In the current study, we directly assessed the flexibility of Tm by examining EM images of both rotary shadowed and negatively stained molecules. Single Tm molecules in both image sets showed no obvious signs of sharp bending or kinks, and displayed contours close to those predicted from a high-resolution 3D model of Tm (Lorenz *et al.*, 1995). Short multimeric strings of end-to-end bonded Tm were commonly observed in the rotary shadowed images. These showed no pronounced bending or joints at the intermolecular junctions. The persistence length of Tm was calculated to be over twice the length of the molecule based on these EM images. The data as a whole imply that Tm molecules exhibit an intrinsic stiffness sufficient to contribute to cooperativity on thin filaments and thus are consistent with models of muscle regulation (e.g. Lehrer & Geeves, 1998; Lehman *et al.*, 2000). A tropomyosin mutant in which the coiled-coil interface was destabilized by introduction of Ala clusters in the 2nd and 3rd periodic repeats (Y60A-L64A-L106A; Singh & Hitchcock-DeGregori, unpublished) was also examined by EM. No obvious extra bending was observed, although the calculated persistence length was significantly shortened, showing the sensitivity of the technique.

**1189-Pos Board B33**

**The Fast Skeletal Troponin Activator, CK-1909178, Increases Skeletal Muscle Force *in-vitro* and *in-situ***

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Previously, we have discovered small molecules that increase cardiac contractility by directly activating the cardiac sarcomere; this mechanism is now being investigated as a therapy for treating systolic heart failure. Using this precedent, we have focused on the identification of compounds that directly increase skeletal muscle contractility for the potential therapy of diseases that result in muscle weakness and fatigue.

CK-1909178 is a member of a class of fast skeletal troponin activators that were identified by high throughput screening of skeletal sarcomere preparations. We sought to understand how this compound altered force development in isometric skinned and live muscle fibers. Treatment of skinned rabbit psoas fibers with 0.1 $\mu$M CK-1909178 caused a dose-dependent left-shift of the force-pCa relationship without altering the Hill slope or maximum force, consistent with a calcium sensitizing effect on force production. In living rat flexor digitorum brevis (FDB) preparations, CK-1909178 (10 $\mu$M) caused significant increases in subtetanic force (150% of control at 10 Hz) without altering maximum force. Similar experiments were then performed using a rat extensor digitorum longus (EDL) preparation *in-situ*, where nervous and vascular connections were left intact and the muscle was stimulated via the peroneal nerve. Infusions of CK-1909178 up to 10 mg/kg rapidly increased sub-tetanic isometric force (190% of control at 30 Hz). In summary, we have identified a skeletal troponin activator that sensitizes the sarcomere to calcium and results in increased submaximal muscle force development *in-vitro* and *in-situ.* We believe that this mechanism may translate to improved physical power and strength in diseases where muscle function is compromised due to injury, disease or age.

**1190-Pos Board B34**

**The Small Molecule Skeletal Sarcomere Activator, CK-1909178, is a Calcium Sensitizer that Binds Selectively to the Fast Skeletal Troponin Complex**

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Striated muscle contraction is tightly coupled to the release of $Ca^{2+}$ from the sarcoplasmic reticulum by the sarcomeric calcium sensor, troponin. This complex of three proteins (troponins T, I, and C) undergoes calcium-sensitive conformational changes that regulate the accessibility of myosin binding sites along the actin filament. We used a high throughput screen to identify compounds that activate the ATPase activity of skinned fast skeletal myofibrils; optimization of the initial hit compounds has resulted in compounds with sub-micromolar affinity. A potent representative of this chemical series, CK-1909178, shifts the calcium sensitivity of detergent skinned skeletal myofibrils by >10-fold in a concentration dependent manner. This compound specifically activates fast skeletal myofibrils, with no effect on either slow skeletal or cardiac myofibrils. A reconstituted sarcomere assay using combinations of fast skeletal, slow skeletal, and cardiac components demonstrates that the activity of CK-1909178 requires the presence of fast skeletal troponin. Isothermal titration calorimetry indicates the compound binds directly to fast skeletal troponin with a sub-micromolar dissociation constant. Consistent with its calcium sensitization effects, CK-1909178 slows the dissociation of calcium from troponin as measured by Quin-2 fluorescence. Consistent with its mechanism of action, CK-1909178 sensitizes muscle *in vitro* and *in vivo*, suggesting this mechanism may increase power or strength in diseases where muscle function is compromised due to injury, disease or age.

**1191-Pos Board B35**

**Modulation Of Human Cardiac Troponin C-troponin I Interaction By An Analogue Of Levosimendan, (2′,4′-difluoro(1,1′-biphenyl)-4-yl) Acetic Acid (dfbp)**

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The binding of Ca2+ to cardiac troponin C (cTnC) triggers contraction in heart muscle. In diseased heart, the myocardium is often desensitized to Ca2+, leading to weak cardiac contractility. Compounds that can sensitize cardiac muscle to Ca2+ have therapeutic value in treating heart failure. Of the known drugs that are proposed to accomplish this, levosimendan is the best characterized and currently in clinical use. Levosimendan interacts with the regulatory domain of cTnC and promotes association of cTnC with troponin I (cTnI). Detailed understanding of the mechanism of levosimendan has been concealed by its unstable nature; however, the use of analogues that are more stable would