O1.9 Contractile structure, Thu 11:35–11:50

Reduced right ventricular cardiomyocyte passive force due to hypophosphorylation at Ser-282 of cardiac myosin binding protein C in a rat model of post-ischemic heart failure

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Left ventricular (LV) remodeling in myocardial infarction (MI)-induced heart failure (HF) is well studied. Less is known about the concomitant right ventricular (RV) changes. In this rat model of MI-induced HF we aimed to: (1) characterize the contractile function of RV cardiomyocytes, (2) compare it with the LV, (3) investigate the underlying molecular mechanisms in the RV. Rat hearts in moderate HF were studied after 8 weeks of LV MI. Sham-operated animals served as controls. Maximal active force F(max), Ca-sensitivity (pCa_{50}) and passive force F(pass) were measured in skinned isolated RV and LV cardiomyocytes (n = 12–15). Titin, myosin binding protein C (MyBP-C) and Troponin I (TnI) phosphorylation (P) were tested by gel-electrophoresis-based and Western blot methods. RV weight was higher in HF (0.48 ± 0.04 g, mean ± SEM) than in the Sham group (0.31 ± 0.02 g) ($P \leq 0.05$). F(max) and pCa_{50} of RV cardiac cells were similar in the HF and in the Sham group (18.7 ± 1.1 kN/m^2; 5.88 ± 0.03 vs. 18.3 ± 2.1 kN/m^2; 5.87 ± 0.03, respectively). In HF, the cross section area of RV cells was comparable to Sham (876 ± 133 μm^2; 1007 ± 194 μm^2). However, in HF F(pass) of RV cardiomyocytes was less (2.1 ± 0.2 kN/m^2) than that in the Sham group (3.2 ± 0.4 kN/m^2). LV cells had higher F(max) and pCa_{50} values in HF than in the Sham group (34.9 ± 2.7 kN/m^2; 5.97 ± 0.03 vs. 26.9 ± 1.6 kN/m^2; 5.85 ± 0.03). LV cell size in HF was not higher than in the Sham (1104 ± 151 μm^2; 1029 ± 118 μm^2). LV F(pass) was not altered in HF (3.37 ± 0.37 kN/m^2) compared to Sham (2.83 ± 0.27 kN/m^2). In HF animals relative to Sham total P of titin and MyBP-C showed no difference in the RV or in the LV, but P at Ser-282 of MyBP-C was decreased in the RV. In HF both the RV and the LV TnI P (total and Ser-22/23) was lower than in the Sham. Our data suggest distinct changes in RV cardiomyocyte structure and function in HF due to LV MI. Reduced RV F(pass) seems to be independent of titin P, rather might be attributable to hypo P of protein kinase A sites of MyBP-C.

Keywords: Right ventricle, Passive force, Cardiac myosin binding protein C

O2.1 Contractile function, Thu 14:00–14:15

What do we learn by the rates of tension development k(act) and redevelopment k(TR) in activated myofibrils and muscle fibres

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When the length of active muscle fibres/myofibrils are released by ~20 % and restretched after ~50 ms, tension redevelops with an exponential time course with the rate constant k(TR) in rabbit psoas fibres [1]. A similar time course (rate const: k(act)) is observed on Ca^{2+} activation in single myofibrils [2]. These are generally interpreted to mean that k = f + g, where f is the attachment rate constant and g is the detachment rate constant in the two state model [1]. With this formulation, k is limited by a fast reaction. However, a problem has developed, because k has been generally 5–40/s in rabbit psoas, which is too slow for a fast cross-bridge reaction. Alternatively, we propose a model in which cross-bridges cycle many times by stretching series elastic elements to develop force, hence k is limited by a slow reaction: $k \approx (f + g)/fg$ (≈means proportional). To set up this model, we made an assumption that the stepping rate (v) decreases linearly with force (F) (Fenn effect, [3]). The distance traveled by a cross-bridge stretches series elastic elements with stiffness ρ, which is registered as force. The rate constant of resulting time course is: $k = \rho\eta_0 v_0(1 - \lambda)/F_1$, where $\lambda = v_1/v_0$, η = step size, the subscript 0 indicates unloaded, and the subscript 1 indicate isometric conditions. We demonstrate that the ATP hydrolysis rate is proportionate to k(TR) with $\lambda = 0.28$ as the temperature is changed. We conclude that k(TR) and k(act) are limited by the cross-bridge turnover rate; hence it represents the rate constant of the slowest reaction of the cross-bridge cycle. This model further explains why the time course of tension rise (stretch series elastic elements) is slower than that of tension decay (direct observation of cross-bridge detachment) when the P_i concentration is quickly changed with myofibril experiments [2].

Keywords: Turnover rate, Elementary steps, Cross-bridge

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O2.2 Contractile function, Thu 14:15–14:30

Mutations in the central part of α Tropomyosin molecule alter Ca^{2+} sensitivity and tension relaxation of skeletal muscle myofibrils after troponin–tropomyosin removal and reconstitution

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Tropomyosin (Tm) is a α-helical coiled-coil actin-binding protein regulating muscle contraction. Previous observations suggested that the highly conserved non canonical residues Asp-137 and Gly-126 in the central part of Tm confer instability. Replacement of these residues by canonical ones (Leu and Arg, respectively) decreases molecular flexibility and modulates the Ca^{2+} sensitivity of in vitro regulated actomyosin systems (Sumida et al. J Biol Chem 283:6728–6734, 2008; Nevzorov et al. J Biol Chem 286:15766–15772, 2011). Here, we investigated the functional impact of recombinant α Tm carrying one (D137L) or both (D137L/G126R) stabilizing substitutions on the mechanical behavior of skeletal myofibrils. Endogenous Tm and Tn were replaced into rabbit psoas myofibrils (Scellini et al. Adv Exp Med Biol 682:163–174, 2010) with purified Tn and recombinant α Tm (WT,D137L and D137L/G126R). Force recordings from myofibrils (15 °C) showed the increase in Ca^{2+} sensitivity expected from in vitro studies. At saturating [Ca^{2+}] maximal isometric tension and the rates of force activation (kACT) and redevelopment (kTR) were not significantly affected by Tm mutations. Interestingly, a clear effect was observed on force relaxation: D137L/G126R myofibrils showed

prolonged duration of the slow phase of relaxation and a decreased rate of the fast phase compared to WT. This effect was smaller in D137L myofibrils. Tm mutations also strongly decreased slack sarcomere length (SL) at sub-activating [Ca^{2+}] as well as they increased the steepness of the SL—passive tension relation. These effects were reversed by 10 mM BDM, suggesting that single and double Tm mutations compromise the full inhibition of acto-myosin interactions in the absence of Ca^{2+}. These data support the hypothesis that flexibility of the Tm coiled–coiled structure critically modulates the turning off of the thin filament system and muscle relaxation dynamics. Supported by PRIN 2010–2011 of the Ministero Università e Ricerca (MIUR).

Keywords: Tropomyosin, Skeletal muscle, Regulation

O2.3 Contractile function, Thu 14:30–14:45

Stiffness of the myosin head domain

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Elastic distortion of the myosin head is fundamental to the generation of contractile forces. Thus, stiffness of the myosin head, together with filament compliance, is a main determinant of forces generated by a myosin head. Differences in head stiffness among myosin isoforms, or changes in head stiffness with nucleotide would yield different forces generated by different myosin isoforms and in different states of the ATPase cycle, respectively. Studying effects of point mutations in the converter of the slow skeletal/β-cardiac myosin heavy chain that cause familial hypertrophic cardiomyopathy, we found some mutations to increase head stiffness and force generation by the myosin head. This implied that the converter is a main determinant of myosin head stiffness. These FHC-mutations were at positions in the converter sequence where fast skeletal myosin differs from the slow/β-cardiac isoform, suggesting that different heavy chain isoforms may have different head stiffness. Comparing in skinned fibers and by optical trapping fast (rabbit psoas) and slow (rabbit/human soleus) myosins we found head stiffness of the slow myosin to be $\leq$1/3rd of the fast isoform stiffness. The finding that changes in the converter strongly affect head stiffness implies that reorientation of the converter vs. the catalytic domain, e.g., during the myosin working stroke, may also change stiffness of the head domain. We tested this hypothesis by optical trapping of a single headed myosin-5a construct. We selected myosin-5a because a sub-step had been observed in optical trapping and was assigned to a reorientation of the converter in the myosin-5 working stroke. We found that after this substep, i.e., after converter reorientation, the stiffness of the myosin-5a head domain was $\geq$2-fold increased. In conclusion, stiffness and force generation of a myosin head (i) are different for fast and slow myosin-2 isoforms, and (ii) change as a myosin head progresses through its working stroke.

Keywords: Force per myosin head, Stiffness of myosin isoforms, Stiffness of cross-bridge states

O2.4 Contractile function, Thu 14:45–15:00

The conformation of myosin motors in relaxed skeletal muscle

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Electron microscopy studies of isolated thick filaments from a wide range of muscle types and species are consistent with an asymmetric arrangement of the two motor domains of each myosin molecule folded back against the filament backbone and called the J motif (Woodhead et al. Nature 436:1195–1199, 2005). This conformation is associated with the OFF state of myosin, although it is unclear how the molecule is switched on during contraction. In this study we investigated the in situ conformation of myosin motors in relaxed demembranated fibres from rabbit psoas muscle using fluorescence polarisation from bifunctional rhodamine (BSR) probes at four sites on the myosin regulatory light chain (RLC). Under relaxing conditions the order parameters of the orientation distribution of each probe, P_2 and P_4 respectively, had a sigmoidal dependence on temperature in the range 3–33 °C, with half-maximal change at 18 °C. Either lattice compression by 5 % dextran or addition of 25 μM Blebbistatin decreased the transition temperature to 13 °C. Maximum entropy analysis showed that at 5 °C the RLC domain of myosin is almost perpendicular to the filament axis, whereas above 20 °C two populations with more parallel orientations appear, and either dextran or Blebbistatin stabilize these two conformations above 16 °C. The angles of these two conformations match the RLC orientations of the blocked and free heads in the J motif observed in isolated filaments, suggesting that this fraction of motors occupy the J motif in relaxed muscle in situ. Slow ramp stretches applied in relaxing solution at 25 °C and at sarcomere lengths >2.6 μm produced large changes in RLC orientation towards the ON conformation seen during calcium activation, with partial reversal during force relaxation after the stretch. The correlation between RLC orientation and passive fibre tension provides evidence that the orientation of relaxed myosin motors is sensitive to thick filament strain. Supported by Wellcome Trust, UK.

Keywords: myosin, skeletal muscle, muscle fibres

O2.5 Contractile function, Thu 15:00–15:15

The increase in non cross-bridge force after stretch of activated striated muscles is related to titin isoforms

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Introduction: There is evidence that skeletal muscles present a cross-bridge independent increase the sarcomere stiffness upon Ca^{2+} activation. It has been hypothesized that the increase in stiffness is caused by Ca^{2+}-dependent changes in the properties of titin molecules. To test this hypothesis, we investigated the cross-bridge independent stiffness in muscles containing different titin isoforms.
Methods: Permeabilized myofibrils were isolated from the psoas, soleus and heart ventricle muscles of the rabbit. Intact trabeculae were isolated from the heart of the mouse. Myofibrils were tested in relaxing and activating conditions, before and after treatment with chemicals that inhibit myosin-actin interactions. Trabeculae were electrically stimulated in Krebs-Henseleit solution. The force produced by these preparations during and after stretches of different magnitudes was measured in sarcomere lengths between 1.6 and 2.4 μm for the heart, and between 2.2 and 3.0 μm for skeletal muscles. Titin isoforms in these muscles were identified with gel electrophoresis.