Contractile characteristics of single muscle fibres of *Xenopus laevis* in long term culture at low length

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Investigation of adaptation of number of sarcomeres in series to mechanical stimuli is limited by the fact that the local conditions of muscle fibres cannot be controlled sufficiently in whole muscle. We used a culture system (Lee-de Groot and van der Laarse, 1996, *J Mus Res Cell Mot* **17**: 439–448) to study force, number of sarcomeres in series and physiological cross sectional area (CSA) of single muscle fibres maintained at low length. Single fibres ($n = 5$) of m. iliofibularis of the *Xenopus laevis* were dissected under aseptic conditions and the number of sarcomeres was determined using laser diffraction. The fibre was mounted between a force transducer and an adjustable rod at sarcomere length of about 2.3 μm (Lref). Culture medium consisted of 66% DMEM/F12, supplemented with 100 U ml^{-1} penicillin/100 μg ml^{-1} streptomycin, 5 mM sodium phosphate, 1 mM creatine, 0.5 mM L-carnitine and 1 μg ml^{-1} insuline, flowed through the chamber at a rate of about 0.5 ml h^{-1} at 20°C. The medium was equilibrated with air, containing 2.4% CO_2. Final pH was 7.6 and osmolarity was 235–250 mOsm kg^{-1}. The PO_2 of the culture medium was about 120–140 mm Hg.

After stabilisation at Lref (up to 2 days), fibre length was reduced by 20%. The culture period at this new length ranged from 3 days to 97 days. Isometric twitch and tetanus (50 Hz, 260 ms, 0.4 ms pulse width) characteristics were determined once daily. Imposing the lower fibre length reduced twitch force to $4.1 \pm 2.4\%$ (mean ± SEM) of the original value at Lref. During the adaptation period, twitch force increased to $36.0 \pm 12.4\%$ of the original value at Lref. Tetanic force dropped to $39.7 \pm 7.1\%$ after imposing the lower fibre length, but increased to $72.2 \pm 4.5\%$. The recovery of force was not due to a change in CSA and number of sarcomeres in series. Even after 97 days at this very limited degree of activity we found no change in these parameters.

Effect of hypothyreosis on the structural state of 1,5-IAEDANS-labelled-F-actin in slow (SOL) and fast (EDL) rat skeletal muscle fibres

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It is well established, that thyroid hormones influence the functional properties of skeletal muscle. They accelerate the velocity of muscle contraction and enhance tension development. However, there is no sufficient evidence of the effect of thyroid hormones on actin filament structure and interaction between myosin and actin. We studied the effect of hypothyreosis (2 and 5 weeks) on the structure of 1,5-IAEDANS (N-iodoacetyl-N′-(5-sulpho-1-naphtyl)-ethylenediamine)-labelled actin in glycerinated fibers from slow (SOL) and fast (EDL) rat skeletal muscles by polarized fluorimetry techniques. As control corresponding muscles of the euthyroid rats were used. Female albini Wistar rats 3 months old were used. Hypothyreosis was generated by surgical thyrectomy and then propylthiouracyl was given in drinking water (0.04%) for 2 or 5 weeks. Subfragment 1 (S1) from rabbit m. psoas has been obtained according to (Okamoto, Sekine, 1985, *J Biochem* **98**: 1143–1145). The intensities of four components of polarized fluorescence were measured in parallel (I, $I\perp$) and perpendicular ($\perp I$, $\perp I\perp$) orientation of the fiber axis to the polarization plane of the exciting light. From these four components the angle of absorption (Φ_A), and emission (Φ_E) dipoles of the fluorophore relative to the long axis of F-actin, the number of randomly oriented fluorophores (N) were calculated. The changes in the values of Φ and N were interpreted in terms of structure alterations of F-actin (Szczepanowska *et al.*, 1987, *Eur J Cell Biol* **43**: 394–402). In agreement with the data, obtained previously the binding of S1 to 1,5-IAEDANS-labelled-F-actin of control SOL and EDL muscle fibers resulted in an increase of the values of Φ and a decrease of the values of N, indicating the conformational changes typical for the formation of strong binding between actin and myosin heads. This effect was significantly inhibited by hypothyreosis. It should be noted that in fast muscles the maximum changes in F-actin structure are observed after 14 days of the disease development. In contrast, in the slow muscle the maximal effect was shown after 34 days. The data obtained suggest that the changes in the conformation of the actin filament, induced by hypothyreosis, modulate the character of actin–myosin interaction in fast and slow skeletal muscle fibers.

Incorporation of myosin alkali light chain into thick filament

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In order to study how newly synthesized myosin alkali light chain (MLC) molecules are incorporated into myofibrils, cDNA of fast skeletal muscle type of MLC tagged with green fluorescence protein (LC3f-GFP) was transfected into cultured chicken cardiomyocytes, and the assembly of expressed LC3f-GFP was observed in living cells under a fluorescence microscope equipped with a cooled CCD camera. At 14–16 h after transfection, LC3f-GFP was diffusely distributed in the cytoplasm of cardiomyocytes. In some cells, however, intense fluorescence spots of LC3f-GFP were found along myofibrils with a periodicity of 1.2 μm. Confocal microscopy of such cells stained with rhodamine-labeled phalloidin revealed that the fluorescence spots of LC3f-GFP were localized at both ends of the A-band. When these cells were further incubated, LC3f-GFP came to be localized at all levels of the A-bands by 26 h after transfection. These results indicate that myosin filaments are not replaced with newly synthesized myosin molecules at once along their entire length, but molecules infilaments are replaced individually from their ends.

Modelling of cross-bridge binding to actin in skeletal muscle and calculated X-ray diffraction patterns

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Structures of F-actin, myosin subfragment-1, S1, and their complex, actin-S1, (Holmes *et al.*, 1990, *Nature* **347**: 44–49; Rayment *et al.*, 1993, *Science* **261**: 58–65) were used to simulate 3D patterns of myosin heads bound to actin in skeletal muscle and to calculate the X-ray diffraction intensity. A 214.5 nm long hexagonal unit cell of the actin–myosin super-lattice consisted of three myosin and six actin filaments and 270 myosin heads (Squire, Harford, 1988, *J Mus Res Cell Motil* **9**: 344–358). Rigor binding was simulated assuming that (1) the catalytic domain of a myosin head stereo-specifically binds actin with the same orientation as in the acto-S1 complex; (2) all heads are bound; (3) two heads of a myosin molecule bind adjacent actin sites on the same thin filament; (4) a head binds the actin site that requires the least energy of elastic distortion from its original position on the myosin helix. Non-stereo specifically attached heads were assumed to be able to rotate azimuthally and axially with respect to the N-terminal of actin