properties of identified types of single muscle fibres, and the concentration of myosin in single fibres were studied in elderly subjects. Three groups of two subjects were enrolled in the study: 1) subjects (60–70 years old) who underwent to the replacement of a total knee prosthesis after a period of 3–4 months with the leg immobilized in extended position (el-imm); 2) control adult subjects sampled by needle biopsy (30–40 years old) (yo-ctrl); 3) control aged subjects (60–70 years old) who underwent to knee surgery for various reasons (el-ctrl). This represents a likely explanation of the decrease in Po/CSA concentration was found significantly lower in fibres from el-ctrl than for type 1 fibres. Myosin concentration in single fibres was similarly affected on the contrary, Vo of type 2A fibres was statistically higher following aging and immobilization. Differences in Po/CSA between el-imm and the other groups were smaller for 2A than for type 1 fibres. Myosin concentration in single fibres was determined by densitometry of myosin electrophoretic bands using samples of known concentration of myosin as standards. Myosin concentration was found significantly lower in fibres from elctrl than in fibres from yo-ctrl and in fibres from el-imm than in fibres from el-ctrl. This represents a likely explanation of the decrease in Po/CSA observed in aging and following immobilization. Consistently with the decrease in myosin concentration, preliminary analysis of longitudinal sections of muscle bundles by electron microscopy suggested a decrease in myofibrillar density in el-imm in relation to el-ctrl and yo-ctrl.

δ3-CaMKII: expression in dilated cardiomyopathy and establishing of a model for functional characterisation

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The Ca2+/calmodulin-dependent protein kinase II (CaMKII) contributes to the regulation of Ca2+-homeostasis in the heart. Cardiac tissues taken from patients with dilated cardiomyopathy show a significant increase in mRNA of the CaMKII isoform δ3 as well as in the CaMKII protein subclass to which δ3 belongs. Isoform δ3 is characterised by the presence of nuclear localisation signal. To gain insight in possible functions of δ3 in the myocardium, we stated analysis of this isoform in the myogenic cell line H9c2. This cell line is derived from embryonic ventricular tissue from rat and expresses potential cardiac CaMKII substrates as well as the CaMKII isoform δ2. Overexpression of isoform δ3 results in localisation of the protein in the nucleus. A similar localisation could also be demonstrated for endogenous expressed CaMKII in isolated cardiomyocytes of the adult rat. Stimulation of CaMKII leads to autophosphorylation and Ca2+-independent activity of the enzyme. Using an antibody directed against the δ-class specific autophosphorylation site, autonomous activation could be demonstrated in δ3 overexpressing H9c2 cells. As a control, CaMKII isoform δ2 which lacks the nuclear localisation signal of δ3 overexpressed. Therefore the H9c2 model system can now be used to compare the influence of the overexpression of distinct CaMKII isoforms on gene expression and phosphorylation state of potential cardiac CaMKII substrates in stimulated and unstimulated H9c2 cells.

Effects of stretch on mature single muscle fibres of Xenopus laevis in long term culture are not explained by the Feng effect

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Stretch of skeletal muscle in vivo stimulates hypertrophy and increases the number of sarcomeres in series. We developed a culture system in which twitch and tetanic force and number of sarcomeres in series of single fibres can be monitored. In this system fibres dissected from m. iliofibularis of Xenopus laevis can be studied for long periods (Lee de Groot and Van der Laarse, 1996, J Muscle Res Cell Motil 17: 436–448). For fibres cultured below passive slack length, tetanic force remained constant up to 50 days in 66% Dulbecco’s MEM/F12 equilibrated with 2.5% CO2 in air (Jaspers et al., 1999, J Muscle Res Cell Motil 20: 822). However, within the first 7 days after setting the fibres at a mean sarcomere length of 2.4–2.5 μm (i.e. 3–10% over fibre slack length), 70% of the fibres died or swelled substantially and became inexcitable within a week. For several species it has been shown that the resting rate of oxygen consumption (V0,2O2max) of skeletal muscle increases by stretch (Feng, 1932, J Physiol 74: 441–454). If the Feng effect is also present in stretched single Fibres of Xenopus laevis, this may cause metabolic exhaustion. Using the method described by Elzinga and Van der Laarse (1988, J Physiol 399: 405–418) we measured VO2 of two single fibres and a small bundle of fibres (type 2 and 3) at sarcomere lengths ranging form 2.3 to 3.7 μm (60% over fibre slack length). For this length range resting VO2, normalized for fibre volume, remained constant at a value smaller than 1% of V0,2O2max. In culture medium, V0,2O2max was similar to values determined previously in Ringer solution (0.12 ± 0.016 mmol mm−3 s−1 at 20°C). We conclude that the Feng effect in not present for single fibres of m. iliofibularis of Xenopus laevis up to at least a fibre mean sarcomere length of 3.7 μm and that the Feng effect per se is not a limiting factor in long term culture up to this length.

The response of muscle proteins and hormonal system to a overtraining syndrome

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The physical training is based on the overload principle and negative feedback theory, which means that the stimulus must be strong enough to induce disturbance of homeostasis so that the body has to initiate reaction to adapt to the training stimulus. This means that overloading is a natural part of training process. Imbalance in the training load-recovery relationship is the primary factor causing to overtraining syndrome. The damaging effect of skeletal muscle fibers depend on the muscle fiber type. Due to the destruction of myofibrils and atrophy of muscle fibers, exercise myopathy develops as a result of overtraining. It has been shown that most sensitive to the long-lasting exhaustive endurance type of exercise are fast-twitch muscle fibers. The present study was undertaken in order to investigate the changes in relative content of myosin heavy chain isoforms (MHC) in overtraining conditions caused by exhaustive endurance exercise and to follow the hormonal responses in overtraining conditions. Male rats of the Wistar