5. SUMMARY OF RESULTES

All results have been published or accepted in journals with IF. The list of publication is enclosed in the chapter 6.

1) Levels of energy-related metabolites in intact and isolated perfused-superfused rat skeletal muscles. (*Štefl et al. 1994*)

Adenosine 5'-triphosphate (ATP), phosphocreatine (PCr), creatine (Cr), inorganic phosphate (Pi), lactate (LAC), pyruvate (PYR) and glycogen as glucose (GLU) were determined and free adenosine 5'-diphosphate (ADP) was calculated from ATP:creatine phosphokinase (CPK) reaction in the gracilis muscle of cold-acclimated rats in vivo, and in completely isolated muscles under medium perfusion and superfusion in vitro, using the freeze-clamping method. The mean in vivo levels (μmol/g w.w.) were: ATP 4.8, PCr 12.0, Cr 7.8, Pi 16.1, LAC 1.6, PYR 0.09, GLU 22.9, ADP 0.62 x 10^{-3}. Isolation of the muscle (about 11 min of anoxia followed by perfusion in the air with a high pO_2 medium) decreased macroergic phosphate levels (ATP 3.0, PCr 8.3). In isolated muscles perfused with a high pO_2 medium (99 kPa O_2, perfusion rate 70 μl/min) and simultaneously superfused with a low pO_2 medium (6.2 kPa O_2, 2.3 ml/min) at 28° C in vitro the levels of metabolites were (μmol/g w.w.): ATP 3.1, PCr 8.5, Cr 5.6, Pi 9.2, LAC 2.1, PYR 0.19, GLU 6.6, ADP 0.44 x 10(-3). The mean steady oxygen uptake of the isolated muscle was 97 nmol O2 x min-1 x g-1 w.w. Thus, the levels of macroergic phosphates and their derivatives are lower after isolation and perfusion of the muscle, but the creatine charge [PCr]/([PCr]+[Cr]) remains stable (0.61 in vivo versus 0.60 in the isolated muscle). This indicates that the steady-state and high energy status of the isolated perfused-superfused gracilis muscle is maintained.

2) Creatine kinase reaction in skinned rat psoas muscle fibers and their myofibrils. *(Gregor et al. 1999)*

The aim of this study was to evaluate myofibrillar creatine kinase (EC 2.7.3.2) activity on the background of the effect of substrate channelling by myosin ATPase and to compare it with

creatine kinase (CK) activity of whole skinned fibers. In order to assess CK activity, skinned fibers were prepared from the rat psoas major muscles defined by light microscopy. The activity in permeabilized fibers after treatment with saponin, Triton X-100 and Ca^{2+}-free medium reached 2.80, 6.97 and 3.32 µmol ATP min^{-1} mg^{-1} protein, respectively, when a coupled enzyme assay system with external hexokinase and glucose-6-phosphate dehydrogenase was used. Transmission electron microscopy (TEM) revealed a possible interference among activities of sarcolemmal, sarcoplasmic, myofibrillar and mitochondrial CK from persisting structures. For evaluation of the myofibrillar CK itself, a pure myofibrillar fraction was prepared. Fraction purity was confirmed by TEM and by enzymatic assays for marker enzymes. Two procedures, i.e. the coupled enzyme assay and the evaluation of phosphocreatine (PCr) concentration before and after the CK reaction, were used for measurement of CK activity in this fraction. The procedures resulted in 3.2 nmol ATP min(-1) mg(-1) protein and 7.6 nmol PCr min^{-1} mg^{-1} protein, respectively. These alternative approaches revealed a discrepancy between the reacting portions of PCr by more than 50 %, which provides information about the size of the effect, generally described as substrate channelling.

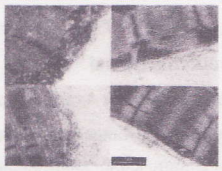

Fig.1 Photomicrograph of skinned fibers prepared from psoas muscle. In the upper left field is a fiber skinned by Ca++ free medium, the upper right field by saponin , the lower left field by triton TX100 and non-skinned fiber in the lower right field as a control.

3) Substrate channelling in a creatine kinase system of rat skeletal muscle under various pH conditions. *(Gregor et al. 2002)*

The aim of this study was to evaluate myofibrillar creatine kinase (CK) activity and to quantify the substrate channelling of ATP between CK and myosin ATPase under different pH conditions within the integrity of myofibrils. A pure myofibrillar fraction was prepared using differential centrifugation. The homogeneity of the preparation and the purity of the fraction were confirmed microscopically and by enzymatic assays for contaminant enzyme activities. The specific activity of myofibrillar CK reached 584 ± 33 nmol PCr min^{-1} mg^{-1} at pH 6.75. Two methods were used to detect CK activity: (1) measurement of direct ATP production, and (2) measurement of PCr consumption. This method of evaluation has been tested in experiments with isolated creatine kinase. No discrepancy in CK activity between the methods was observed in the pH range tested (6.0-7.5). However, the same procedures

resulted in a significant discrepancy between the amounts of reacted PCr and produced ATP within the pure myofibrillar fraction. This discrepancy represents the portion of ATP produced by the CK reaction, which is preferentially channelled to the myosin ATPase before diffusing into the bulk solution. The maximum evaluated difference reached 42.3 % at pH 6.95. The substrate channelling between myofibrillar-bound CK and myosin ATPase was evaluated under various pH levels within the physiological range and it reached a maximum value in a slightly acidic environment. These results suggest that ATP/ADP flux control by the CK system is more important at lower pH, corresponding to the physiological state of muscle fatigue.

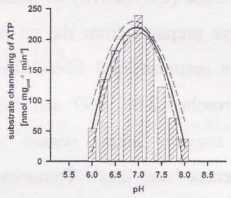

Fig 4 The effect of different pH levels on substrate channelling between myofibrillar CK and myosin ATPase. Substrate channelling was expressed as a difference in PCr consumption and apparent ATP production. Columns represent means (n = 4), continuous line represents weighted least squares polynomial fit (n = 36) and dashed lines represent the 95 % confidence interval of the corresponding function (quadratic function reaching maximum at pH 6.95).

4) **Creatine kinase binds more firmly to the A-band of rabbit skeletal muscle myofibrils in the presence of its substrates.** *(Žurmanová et al. 2007)*

Creatine kinase (CK) (E.C. 2.7.3.2) buffers cellular ATP concentration during fluctuating ATP turnover. Muscle cytosolic CK isoform interacts with various subcellular structures where it is functionally coupled with relevant ATPases. However, how this interaction, affects its activity is not known. We have therefore studied the interaction of CK with myofibrils and the role of different conformational states of CK molecule induced by ATP, phosphocreatine, ADP and the ATP-creatine pair. Purified rabbit psoas myofibrils with CK specific activity of 0.4±0.02 IU/mg were used. The exchange rates between the myofibrillar A-band and its

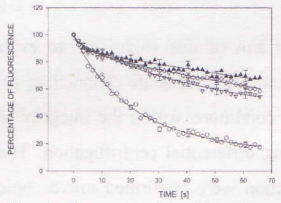

Fig 3 Fluorescent intensities decrease, which represents the rate of the exchange of fluorescently labelled CK molecules (CK-IAF) in the A- bands of myofibrils, evaluated in time by FLIP. The values expressed as means ±S.E.M. (n=6) The exchange rate of CK-IAF molecules with substrates (represents triangles) differ from molecules CK-IAF substrate free (open circles) ($p<0.05$).

surroundings were measured with fluorofore conjugated CK (IAF) by the Fluorescence Lost in Photobleaching (FLIP) method within a very narrow pH range 7.1-7.15. For CK-IAF without docked substrates, the time derivative of the initial loss of the fluorescent signal within the A-band equalled -3.26 at the fifth second and the decrease reached 82% by the 67th second. For CK-IAF with added substrates, the derivatives fell into the range of -0.95 to -1.30, with respective decreases from 16 to 46% at the 67th second. The results show that the substrates slowed down the exchange rate. This indicates that the strength of the bond between CK and the A-band of myofibrils increased.

6. LIST OF PUBLICATIONS

Publications with IF

Šteft B., J.A. Mejsnar, **J. Karasová**: Levels of energy-related metabolites in intact and isolated perfused-superfused rat skeletal muscles. Physiol. Res. 43: 175-180, 1994.

Gregor M., J. Mejsnar, A. Janovská, **J. Žurmanová**, O. Benada, B.Mejsnarová: Creatine kinase reaction in skinned rat psoas muscle fibres and their myofibrils. Physiol. Res. 48: 27-35, 1999.

Gregor M., A. Janovská, B. Štefl, **J. Žurmanová**, J. Mejsnar: Substrate channelling in a creatine kinase system of rat skeletal muscle under various pH conditions. Exp. Physiol. 88: 1-6, 2003.

Štefl B., **J. Žurmanová**: Effects of the perfusion flow rate on skeletal muscle energy metabolism and a possible role of second messengers in this process. Physiol. Res. 55: 79-88, 2006.

Štefl B., M. Vojtíšek, L. Synecká, **J. Žurmanová**: Whole body exposure to low frequency magnetic field: no provable effects on the cellular energetics of rat skeletal muscle. Mol. Cell. Biochem. 284: 111-115, 2006.

Žurmanová, J., F Difato, D. Maláčova, J. Mejsnar, B. Štefl, I. Zahradnik. Creatine kinase binds more firmly to the A-band of rabbit skeletal muscle myofibrils in the presence of its substrates (accepted Moll. Cell. Biochem. 2007)