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Phosphocreatine and ATP concentrations increase during flow-stimulated metabolism in a non-contracting muscle

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Abstract. The gracilis muscle was excised from cold-acclimated rats, placed in vitro, and simultaneously perfused via its artery by high pO_2 medium and superfused by low pO_2 medium. With a doubling of the perfusion rate (from 50 to 100 µl/min) phosphocreatine and ATP increased by 39% and 44%, respectively.
Key words. Adenosine-triphosphate; phosphocreatine; nuclear magnetic resonance; muscle respiratory control.

The mechanisms by which cytosolic factors control cytochrome turnover and hence respiration in mitochondria remain controversial[1] at a cellular level. Even more at the organ level it remains unclear how some naturally-occurring factors stimulate the respiration of a skeletal muscle as a whole. One of these factors is blood flow, which under some conditions stimulates metabolism in a non-contracting skeletal muscle. A useful model for flow-stimulated metabolism is the rat gracilis muscle, simultaneously superfused and perfused through its nutrient artery with medium in vitro[2]. In this preparation, as the medium flow rate increases, the oxygen consumption and heat production rates increase in a flow-rate dependent manner. However, the metabolic energy flux (expressed as the product of oxygen consumption and the mean energetic equivalent for oxygen) is higher than the heat production[2]. These experimental results and a formulation of non-linear thermodynamics[3] offer a hypothesis that the difference between the two steady-state fluxes is due to an increase in steady phosphocreatine (PCr) and ATP concentration. Therefore the aim of this pilot study was to measure the concentration of both phosphates in the flow-dependent non-contracting rat gracilis.

Materials and methods

Gracilis cranialis muscles weighing 207 mg ± 31 SEM (n = 5) from male rats of the Sprague-Dawley strain were used for the preparation which was simultaneously perfused and superfused in vitro. The method used was published recently[2]. There were several differences in the present work compared with that described in the former paper. The rats were kept at 5.5 °C for 17 days prior to the experiment, which brought about a lesser metabolic response of the muscle to increased perfusion flow rate (about 70% of the previous response). The muscle was placed in a chamber with a total volume of 980 µl (the chamber volume within the radio frequency coil was 610 µl), and superfused and perfused with an inorganic phosphate (P_i) free MOPS-Ringer medium[4]. The medium was filter-sterilized, and contained 10 mg/l gentomycin and 5 mM glucose. The average superfusion flow rate was 1.1 ml/min; a low pO_2 of 5 kPa was achieved by equilibration of the medium with a gas mixture, consisting of 5% carbon dioxide and 4.9% oxygen, balanced by nitrogen. The superfusion medium was kept at a constant temperature of 28 °C by a glass heat exchanger placed in the close vicinity of the chamber containing the muscle preparation. Two flow rates (50 and 100 µl/min) were used for perfusion; a high pO_2 of 97 kPa was achieved by equilibration with a gas mixture (5% carbon dioxide, 95% oxygen). The perfusion medium reached the nutrient artery by a thin stainless steel tube which maintained gas content and partial pressures.

Oxygen consumption was monitored polarographically, using an oxygen electrode (Radiometer). Phosphates were measured using ^{31}P NMR spectroscopy. The preparation was fixed in the glass chamber, slipped into a solenoidal radiofrequency coil and placed in a superconducting magnet operating at a field of 2T. The rapid multi-pulsing technique was used with the following parameters: pre-delay 488 ms, mutation angle 47°, number of acquisitions 1024, total pulse sequence repetition time (τ) 1 s. The 47° pulse (width = 2 µs) was selected experimentally as the pulse giving an optimal signal-to-noise ratio for the PCr peak calculated from the Ernst

angle. It equalled 44° (assuming the average T_1 for the three observed phosphates: PCr, ATP, P_i = 3 s). The signal-to-noise ratio for PCr was 24. The problem of an absolute calibration was addressed by having an external standard solution, sealed in two capillaries and used for calibration of both multipulsing, as well as fully relaxed spectra. The external standard solution consisted of P_i (1 mM), ATP (5.4 mM), PCr (15 mM), acetate (38.77 mM), TRIS (68.28 mM), EGTA (4 mM), MOPS (250 mM). Signals were evaluated as the peak heights, as well as the appropriate intensities by integrated peak areas. The latter method was used for the main findings of this work.

Results

The time-course of a typical experiment is shown in the figure. Since acquisition of each spectrum took 17 min five spectra recorded at the low perfusion rate represent the metabolic pattern of an 85-min period. Then the high perfusion rate and recovery under the low perfusion rate are imposed. As regards the average height values for peaks of P_i, PCr and γATP, significant changes are seen in the peaks of P_i and PCr in this experiment.

A certain inertia of values, which carry over from one period of an experiment to the first spectrum of a following period (called a 'tail effect') is visible in the figure as in other experiments. A comparison of the values for P_i and γATP during periods of low and high perfusion rate gives an indication of an increase of the phosphorylation potential.

The intensities of PCr and γATP signals evaluated in arbitrary units from the integrated peak areas using low and high perfusion rates are presented in the table. Spectra (22) obtained from 5 experiments indicate the levels of PCr and ATP with the low perfusion rate of 50 µl/min. The values of 9487 AU ± 299 SEM for PCr and 5176 AU ± 225 SEM for γATP correspond, according to calibration by the external standard solution, to 14.3 µmols of PCr/g and 4.5 µmols of ATP/g. As the perfusion rate is increased up to 100 µl/min, no significant change can be observed in the concentrations of the two phosphates within the first 55 min, owing to the great variability of the values. After this period, however, the increase of both phosphates is significant, when an average of four spectra gives 13,143 AU and 8452 AU, which corresponds to 19.9 µmols of PCr/g and 6.5 µmols of ATP/g respectively. This means that in this experiment PCr concentration is enhanced by 39% (5.6 µmols/g) and ATP concentration is enhanced by 44% (2 µmols/g) as a result of the high perfusion rate.

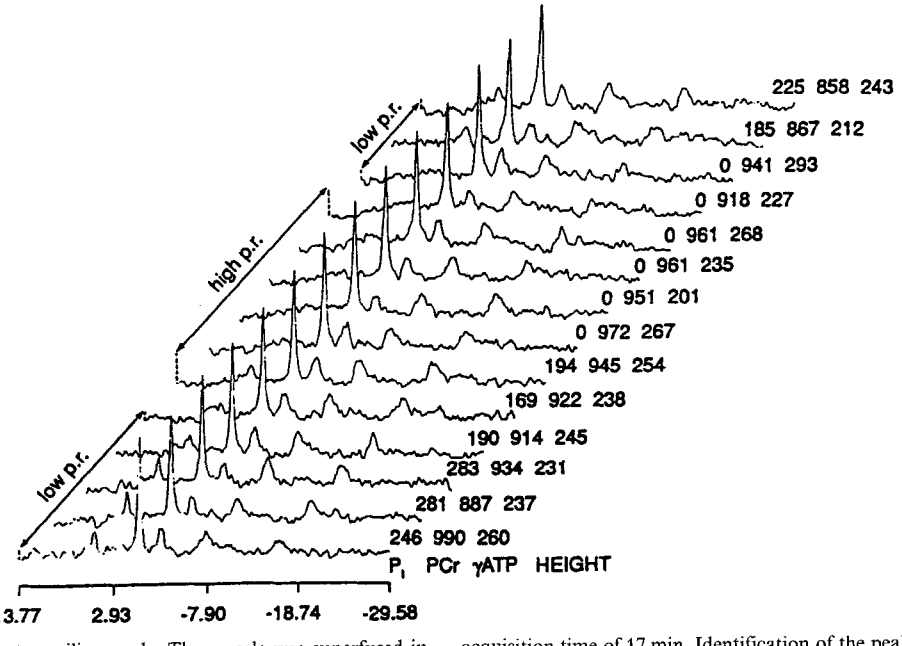
The intensities of PCr and γATP signals evaluated in arbitrary units (AU) of the integrated peak areas during low and high perfusion rates

Phosphate	Perfusion rate Low (initial)	High	Low (recovery)
PCr	9487 ± 299 (22)	13143 ± 634 (4)	10469 ± 2868 (6)
γATP	5176 ± 225 (22)	8452 ± 798 (4)	8061 ± 3321 (6)

The values are presented in average AU ± SEM. Number of spectra in parentheses.

Discussion

Even under optimal conditions for rapid multi-pulsing in the acquisition of NMR spectra, when the Ernst angle



^{31}P NMR spectra of rat gracilis muscle. The muscle was superfused in vitro with low pO_2 medium and perfused with high pO_2 medium at the rates 50 µl/min (low perfusion rate) and 100 µl/min (high perfusion rate). Each spectrum was obtained by averaging 1024 scans within a total acquisition time of 17 min. Identification of the peaks from left to right: P_i, PCr, γ-, α- and β-phosphorus of ATP. Numbers indicate the appropriate peak heights.

was used and τ/T_1 ratio (0.33) was within the range of 0.07 to 0.6[5], the spectra were relatively noisy, owing to the geometry of the solenoidal radiofrequency coil and a relatively weak B_0 magnetic field. It excluded the possibility of evaluating P_i. However, it showed a marked decrease during the periods with a high perfusion rate in all experiments, which suggested an increased phosphorylation potential during enhanced oxygen consumption. Parallel enhancements of PCr and ATP with increased perfusion rate, evaluated in arbitrary units, were highly significant (t test: $p < 0.001$ for PCr; $p < 0.02$ for ATP). A confirmation of the absolute values needs simultaneous biochemical analysis.

At the low perfusion rate, the mean specific O_2 delivery, due to the high pO_2 in the perfusate, was 4.4 pmols $\cdot$ $mg^{-1} \cdot s^{-1}$ during an O_2 consumption of 2.5 pmols $\cdot$ $mg^{-1} \cdot s^{-1}$. Intramitochondrial pO_2 is considered to be well above the critical level (when the rate of electron transport begins to be limited by O_2 availability), on the basis of a following speculation. The O_2 delivery is sufficient if the vascular bed of the microcirculation remains open. Under $pO_2 > 13$ kPa, the capillary functional density declines to zero[6]. At a $pO_2 = 20$ kPa the terminal arterioles are completely closed[7]. With 5 kPa in our superfusion medium we were well below this constrictive level. Under these conditions the blood vessels/myoglobin gradient is 15 Torr/μm[8]. 97 kPa in the perfusion medium means 727 Torr, which supplies this gradient for 48 μm on both sides, which is from 1/5 to 1/10 of the distance toward the muscle surface. Approximately the same additional distance brings the gradient down to the critical 0.5 Torr, which is a sufficient pO_2 value for a maximum cytochrome turnover during an O_2 consumption rate 47 times higher than in our case[8]. Thus for a microcirculation three open capillaries, distributed toward the surface of the muscle, could be enough to achieve this critical level.

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Enzymatic kinetic studies with the non-nucleoside HIV reverse transcriptase inhibitor U-9843

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Abstract. The polymer of ethylenesulfonic acid (U-9843) is a potent inhibitor of HIV-1 RT (reverse transcriptase) and the drug possesses excellent antiviral activity at nontoxic doses in HIV-infected lymphocytes grown in tissue culture. The drug also inhibits RTs isolated from other species such as AMV and MLV retroviruses. Enzymatic kinetic studies of the HIV-1 RT catalyzed RNA-directed DNA polymerase function, using synthetic template : primers, indicate that the drug acts generally noncompetitively with respect to the template : primer binding site but the specific inhibition patterns change somewhat depending on the drug concentration. The inhibitor acts noncompetitively with respect to the dNTP binding sites. Hence, the drug inhibits this RT polymerase function by interacting with a site distinct from the template : primer and dNTP binding sites. In addition, the inhibitor also impairs the DNA-dependent DNA polymerase activity of HIV-1 RT and the RNase H function. This indicates that the drug interacts with a target site essential for all three HIV RT functions addressed (RNA- and DNA-directed DNA polymerases, RNase H).
Key words. HIV RT; inhibitor; polysulfonate; inhibition kinetics.

Polysulfates and polysulfonates such as pentosan polysulfates, dextransulfates and suramin have been identified as potent inhibitors of the HIV RT (reverse transcriptase) enzyme and have shown antiviral activity in HIV-infected lymphocytes grown in tissue culture[1–8]. We have found that U-9843, the polymer of ethylenesulfonic acid, tested as its ammonium salt form, is a potent inhibitor of HIV RT and the drug showed excellent