Effects of Long-Term Microgravitation Exposure on Cell Respiration of the Rat Musculus Soleus Fibers

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 3, pp. 292-294, March, 2011
Original article submitted March 15, 2010

Cell respiration of the *m. soleus* fibers was studied in Wistar rats treated with succinic acid and exposed to microgravitation for 35 days. The results indicated that respiration rates during utilization of endogenous and exogenous substrates and the maximum respiration rate decreased in animals subjected to microgravitation without succinate treatment. The respiration rate during utilization of exogenous substrate did not increase in comparison with that on endogenous substrates. Succinic acid prevented the decrease in respiration rate on endogenous substrates and the maximum respiration rate. On the other hand, the respiration rate on exogenous substrates was reduced in vivarium control rats receiving succinate in comparison with intact control group. That could indicate changed efficiency of complex I of the respiratory chain due to reciprocal regulation of the tricarbonic acid cycle.

Key Words: *microgravitation; musculus soleus; cell respiration*

Long-term microgravitation leads to reduction of the functional potential of postural muscles, thus complicating realization of long-term space missions and the course of rehabilitation period after them. In addition, functional unloading can determine longer rehabilitation period in traumatological and neurological patients. The negative consequences of long-lasting functional unloading are determined primarily by atrophic changes in muscle fibers, which can be observed already by day 7 of true or simulated microgravitation [7,8,14]. The development of atrophy is primarily caused by degeneration of the cytoskeleton proteins [5,11,12], induced, most likely, by elevated concentration of Ca^{2+} [4] and stimulation of Ca^{2+}-dependent proteases (calpains) [3]. The mechanism of Ca^{2+} accumulation under conditions of functional unloading remains unclear. This mechanism can be associated, among other things, with electromyographic (EMG) activity of the muscle. Changes in EMG activity of the muscle under conditions of its functional unloading, in turn, lead to modulation of its energy exchange. Modification of the type of energy exchange in myofibrils inevitably leads to changes in the cellular respiration rate as the terminal stage in high-energy phosphate (ATP) synthesis chain. It has been shown that the rate of ATP synthesis and the level of oxidation/phosphorylation conjugation in *m. gastrocnemius* decreased after 28 days of head-down tilt (hindlimb suspension) [13]. However, these parameters under conditions of microgravitation are poorly studied. Just few studies were aimed at the search for means preventing negative shifts in energy supply to the muscle under these conditions.

We studied parameters of cell respiration in *m. soleus* fibers under conditions of long-term exposure to microgravitation in animals treated with succinic acid (SA) as the additional substrate.

MATERIALS AND METHODS

Musculus soleus fibers were studied.

The study was carried out on 28 adult male Wistar rats (200-270 g), divided at random into 4 groups. Group 1 were controls, group 2 were exposed to micro-

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gravitation hindlimb suspension, group 3 control+SA, and group 4 hindlimb suspension+SA. The animals of groups 1 and 2 were kept in a vivarium on standard fodder and water ad libitum. The animals of groups 3 and 4 received, in addition to standard fodder and water, oral SA (Yantar-antitox preparation) in a dose of 50 mg SA/kg.

Microgravitation was simulated over 35 days by hindlimb suspension by the standard method of Ilyin–Novikov modified by Morey–Holton [9]. The experimental protocol and all manipulations on animals were approved by Biomedical Ethics Commission of Institute of Biomedical Problems.

The muscle was cut out from tendon to tendon and directly plunged in cold solution A (2.77 mM CaK_2 EGTA, 7.23 mM K_2 EGTA, 6.56 mM $MgCl_2 \times 6H_2O$, 0.5 mM DTT, 50 mM KMes, 20 mM imidazole, 20 mM taurine, 5.3 mM ATP, and 15 mM phosphocreatine; pH 7.1). The muscle was separated in solution into bundles of myofibrils 3-4 mm long and about 1 mm thick and incubated in solution A with saponin (50 μg/ml) for 30 min at 4°C with gentle stirring for partial removal of the membrane. The myofibril bundles were then washed from saponin in solution B for 10 min (2.77 mM CaK_2 EGTA, 7.23 mM K_2 EGTA, 1.38 mM $MgCl_2$, 0.5 mM DTT, 100 mM KMes, 20 mM imidazole, 20 mM taurine, 3 mM K_2HPO_4; pH 7.1).

Oxygen absorption rate was evaluated by polarography after Sax [10]. Bundles of membrane-free fibers were incubated in solution B with 2 mg/ml BSA free from fatty acids. A mixture of 5 mM glutamate with 2 mM malate served as exogenous substrate for the respiratory chain; 5 mM ADP was added for evaluating the maximum respiration rate. Oxygen concentration was measured using Clark electrode and YSI model 53 oxygen monitor (Yellow Spring Instrument Co.) at 22°C. Oxygen solubilization in 1 ml incubation medium at this temperature was assumed to be 460 ng-at.

The following respiratory parameters were evaluated: V_{endo}, oxygen consumption rate during utilization of endogenous substrate; V_{exo}, the same for exogenous substrates; V_{ADP}, maximum respiration rate. After measurements, the muscle fibers were removed from polarographic cells, dried at 95°C, and weighed, after which the rates were calculated and standardized by mg dry weight. The respiration control (RC) was calculated as the proportion of respiration rate in the presence of ADP to exogenous substrate respiration rate.

The results were statistically processed. The mean±standard deviation were calculated. The significance of differences between the groups was calculated by two-way ANOVA at $p<0.05$ significance.

RESULTS

In group 2, V_{endo} in *m. soleus* fibers decreased by 62% compared to that group 1 ($p<0.05$). In group 3, V_{endo} was 54% lower than in group 1. On the other hand, V_{endo} in group 4 did not change in comparison with group 3 (Table 1).

In group 2, V_{exo} decreased by 60% compared to that in group 1 ($p<0.05$). In group 4, V_{exo} was in fact similar to the parameter in group 3 and virtually did not differ from that in group 1.

In group 2, *m. soleus* fibers V_{ADP} decreased by 45% ($p<0.05$) compared to that in group 1. Despite the fact that V_{ADP} in group 3 was slightly lower (by 36%) in comparison with group 1, the parameter in group 4 did not differ from that in group 3.

Estimated indicator reflecting the efficiency of oxidation/phosphorylation conjugation in *m. soleus* fibers (RC) in group 2 virtually did not differ from that in group 1. The coefficient in group 4 also did not differ from that in group 3.

Our results indicate that oxygen consumption rate in *m. soleus* fibers after 35-day hindlimb suspension decreased significantly in comparison with the control level, but RC virtually did not change. These changes could be caused by a decrease in the number of mitochondria, concentrations of the respiratory chain complexes, and reduction and/or changes in the content of energy substrates. We showed that V_{exo} virtually did not differ from V_{endo}, which could be due to disorders in the work of the respiratory chain first complex (oxidoreductase Q NADH-coenzyme; NCCR complex), presumably caused by elevation of free radicals level

TABLE 1. Parameters of Cell Respiration in Rat Membrane-Free *M. Soleus* Myofibrils (*M*±*SD*)

Group	V_{endo}, ng-at O/sec×mg	V_{exo}, ng-at O/sec×mg	V_{ADP}, ng-at O/sec×mg	RC
1	1261±229	1407±336	3182±564	2.5±0.3
2	482±164*	557±164*	1753±639*	2.7±0.4
3	579±150*	975±289	2046±293	2.1±0.6
4	582±100*	1054±396	2057±429	2.0±0.3

Note. *$p<0.05$ compared to group 1.

[15]. On the other hand, early decrease in EMG activity in rat *m. soleus* [1] triggers the development of atrophy realized through the cytoskeleton protein degradation (*e.g.* the level of desmin decreased by more than 50% as early as by day 3 of microgravitation exposure [3]). The location of subsarcolemmal mitochondria is determined by primarily desmin content [2], while its disorders can lead to a decrease in their number under conditions of immobilization, which is in line with published data [6].

The results of experiment with succinate addition to the ration indicated that microgravitation did not lead to changes in the rate of oxygen consumption by muscle fibers in comparison with the control group of rats receiving the drug. This drug served as the substrate for the respiratory chain second complex (Q-oxidoreductase succinate coenzyme) and its effect was aimed at the maintenance and stimulation of the mitochondrial energy-producing function.

It was expected that specific improvement of availability of oxidative metabolism component (SA) would become an adequate stimulus for intensification of oxidative phosphorylation processes in muscle tissue. However, elevation of succinate content was paralleled by reduction of respiration rates in the control group of rats treated with SA in comparison with intact rats. This could be caused by changes in the Krebs cycle regulation. Presumably, the effect was caused by excessive availability of the tricarbonic acid cycle substrates and its disagreement with the content of the needed cofactors. However, it seemed that the protective effect of succinate played the key role in normalization of the function of complex I of the respiratory chain in rat *m. soleus* fiber mitochondria under conditions of microgravitation.

Hence, the results of this study attest to the key role of mitochondrial location and count in the energy metabolism level in *m. soleus* fibers under conditions of microgravitation and suggest its regulation by adding the Krebs cycle substrates to the ration.

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