BIOCHEMISTRY AND BIOPHYSICS

Ca^{2+} Transport by Rat Skeletal Muscle Sarcoplasmic Reticulum during Load Relief of the Hind Paws

Yu. V. Arkhipenko, I. A. Popova, V. V. Stepanova, T. G. Sazontova, and F. Z. Meerson

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A weightless state involves bone tissue demineralization, skeletal musculature atrophy, and hemodynamic changes. Ground-based experiments simulating physiological effects of weightlessness (restricted mobility in narrow cases, suspending rats to relieve weight on the hind paws) lead to changes similar to those observed under microgravitation conditions [3-5,10,13]. Suspending animals by the tail is also known to cause significant changes in histochemical, enzymatic, and contractile characteristics of the muscles [6]. The status of the muscle tissue Ca-transporting system under conditions of weightlessness and hypokinesia - hypodynamia is still little known. The characteristics of the sarcoplasmic reticulum membranes have been shown to change markedly for dystrophic processes in the muscle [18,19]. Moreover, a study of Ca^{2+} transport in sarcoplasmic reticulum of rat *m. soleus* classified as the slow type has revealed that suspending an animal is associated with increased Ca^{2+} accumulation and release, as well as with an increase of passive calcium release in caffeine-induced contracture [17].

The present study was aimed at, first, elucidation of the effect of rat hind paw off-loading on calcium transport efficacy in *m. gastrocnemius lateralis* and, second, at detection of the time course of changes in the sarcoplasmic reticulum transporting function during suspension for 1 to 40 days.

MATERIALS AND METHODS

Male Wistar rats weighing about 250 g were used in the experiment. The hind paws were relieved of load by suspending the animals by the tail, as described previously [21]. Rats were decapitated from the first to the fourteenth day of the experiment, using the observation dynamic series method [1], one animal from the experimental and one from the control group daily, and then on days 25 and 40 of the experiment six animals from each group were killed. The gastrocnemius muscles were isolated and rapidly frozen in liquid nitrogen. The tissue was crushed with an Ultra-Turrax homogenizer with a 25N-10 blade for 60 sec at a rate corresponding to position 8 in a medium with 100 mM KCl, 20 mM imidazole, and 25% glycerol, the tissue to medium ratio being 1:5. Transport of Ca^{2+} in the sarcoplasmic reticulum was measured with an Orion EA-940 ionometer with a Ca-selective electrode, as described previously [14]. The rate of Ca^{2+} transport was assessed at

Institute of Biomedical Problems, Ministry of Health, Moscow; Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

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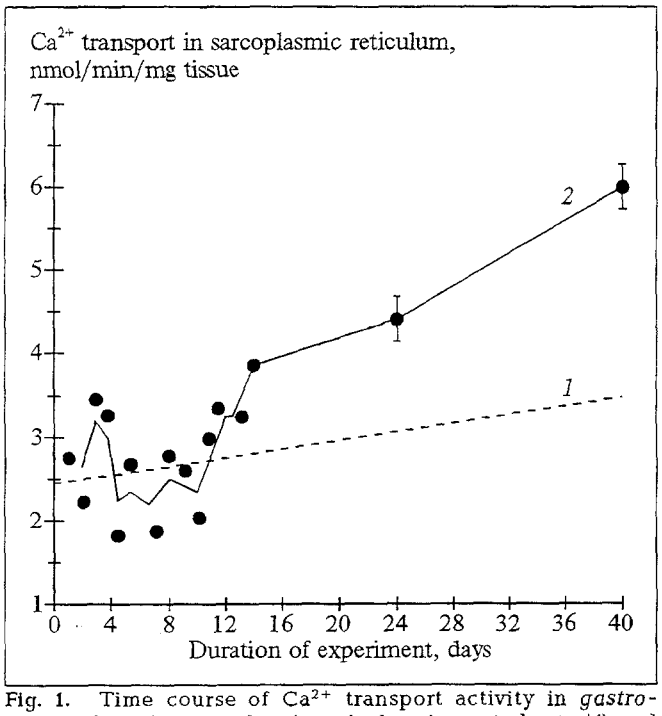

Fig. 1. Time course of Ca^{2+} transport activity in *gastrocnemius lateralis* sarcoplasmic reticulum in control rats (*1*) and in rats with relieved hind paws (*2*) from day 1 to day 40. Data of control experiments (*1*) summarized using regression analysis. Results of experiments with animal off-loading by suspension on days 1-14 processed by sliding means method. Values of Ca^{2+} transport rate on days 25 and 40 averaged by methods of variational statistics using Student *t* test.

37°C with constant stirring of the mixture, for which purpose 25-100 ml of homogenate were placed in 5 ml of a medium containing 100 mM KCl, 15 mM HEPES (pH 7.0), 4 mM $MgCl_2$, 5 mM NaN_3, and 15 mM Na oxalate. Directly before the measurements ATP and Ca^{2+} were added to attain the final concentrations 4 mM and 2-20 μM, respectively. The rate of Ca^{2+} transport had to be estimated from the slope of the experimental curve because of the nonlinear characteristics of the Ca-selective electrode. Ca-pump thermoinactivation was carried out at 45°C. The data were processed by methods of variational statistics using the Student *t* test. The data concerning the animals left hanging for 1 to 14 days were processed using the sliding means method and the reliability of the differences was assessed by Wilcoxon's conjugate pair test [2]. The results on the control animals from day 1 to 40 of experiment were processed by regression analysis.

RESULTS

In control rats body mass increased by day 25 of the experiment from 195 to 293 g and was virtually unchanged by day 40. In experimental (suspended) animals body mass increased by day 25 from 195 to 236 g and to 265 g by day 40, thus constituting 80 and 90% of the control, respectively. The muscular mass of experimental animals was 64% of that of controls on day 25 and 67% on day 40 (Table 1). The relative muscular mass (muscle mass/body mass) of suspended rats also differed little for both periods and was approximately 80% of the control. Hence, hind paw relief results in a reduction of the relative mass of rat skeletal muscles.

A study of the transport activity time course showed no reliable differences between the experimental and control groups on days 1-14 of the experiment, although in the experimental animals the changes were of a fluctuating nature and a trend toward a reduction could be traced by day 5 of exposure and toward an increase by days 12-14 (Fig. 1), which may be clearly detected by the sliding means method [1]. The data obtained in controls were processed by regression analysis to detect trends in calcium transport rate changes in their muscular sarcoplasmic reticulum (Fig. 2).

Figure 2 presents the relationship between Ca^{2+} transport rate in the sarcoplasmic reticulum and the time of Ca-pump thermoinactivation by preliminary heating of muscle homogenate at 45°C in the control and after 25-day suspension. The transport activity of native muscle homogenate in the experimental animals is 41% higher than that in the controls. One can see that the drop of the Ca^{2+} transport rate in the muscle sarcoplasmic reticulum of animals with relieved limbs is less rapid than in the controls and by the twentieth min of thermal treatment constitutes 30% of the initial activity, whereas in the controls residual activity is only 10% (Fig. 2). After a 40-day suspension the initial rate of calcium transport in rat muscle sarcoplasmic reticulum was 58% higher than in the controls (Fig. 3). Moreover, the response of rat muscle sarcoplasmic reticulum to thermal exposure was found to be similar to that on day 25 of suspension: in the experimental group residual trans-

TABLE 1. Rat Body and Muscle Mass

Group	Body mass, g	Muscle mass, mg	Muscle/body mass
Control, day 25	293±18.78	820±30	2.79
Experiment, day 25	236±22.01	530±40	2.24
Control, day 40	296±11.45	740±30	2.5
Experiment, day 40	265±19.62	500±30	1.8

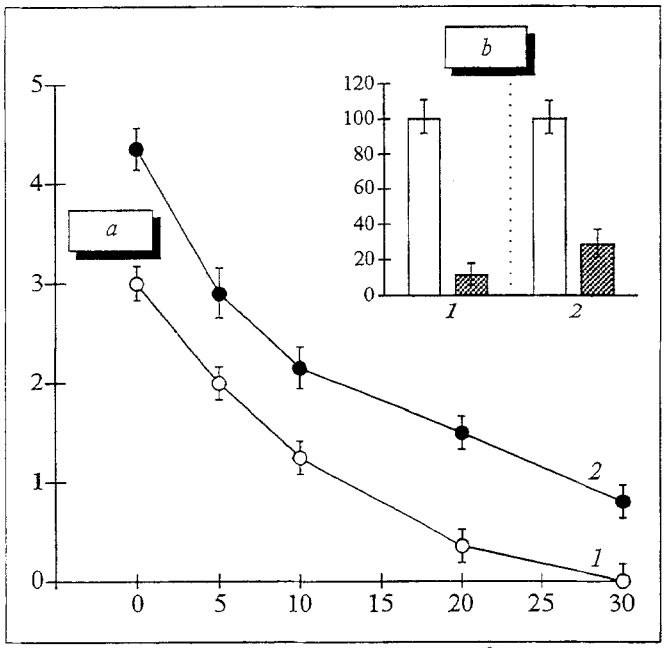

Fig. 2. Relationship between rate of Ca^{2+} transport in sarcoplasmic reticulum and time of incubation at 45°C of rat *gastrocnemius lateralis* homogenate in control (*1*) and after 25-day off-loading (*2*). *a*) abscissa: homogenate incubation time at 45°C, min; ordinate: Ca^{2+} transport rate in sarcoplasmic reticulum, nmol/min/mg tissue. *b*) degree of reduction of Ca^{2+} transport activity in sarcoplasmic reticulum vs. initial value (open bars), taken as 100% in control (*1*) and in suspended animals (*2*) after 20-min thermoinactivation (hatching bars).

port activity by the 20th incubation min at 45°C was 42% (Fig. 3) as against 28% in the control.

A reliable increase of Ca^{2+} absorption and release by *m. soleus* sarcoplasmic reticulum after 14-15 days of suspension, which has been observed by a number of authors [5,7], is explained by the transformation of slow fibers into fast ones during limb off-loading. In other words, slow fibers acquire properties similar to those of fast fibers. In our experiments no noticeable changes in the transport activity of gastrocnemius muscle sarcoplasmic reticulum are seen, just a trend toward an increase of this activity. Bearing in mind that *m. gastrocnemius* is a typical muscle with mixed fiber types, in contrast to *m. soleus*, a slow type muscle, we may understand why no reliable changes in the Ca-transporting system are observed in the mixed fibers of the gastrocnemius. But if the period of suspension is prolonged, a reliable increase of gastrocnemius sarcoplasmic reticulum transport activity in comparison with the control is recorded as soon as on day 25, and by day 40 this difference augments still more. It may be assumed that by day 25 and, especially, by day 40 the share of fibers transformed from type 1 (slow) into type 2 (fast) increases so much that their contribution to transport activity becomes noticeable.

In discussing the possible causes of the changes observed in *m. gastrocnemius* during off-loading, we may mention the following. First, sarcoplasmic reticulum of fast fibers are known to possess proteins absent in sarcoplasmic reticulum of slow fibers [7]. Moreover, various Ca-ATPase isoforms have been identified as specific to slow or fast types of rabbit skeletal muscle fibers [11]. Second, a higher Ca-transporting capacity and Ca-ATPase activity of fast fibers have been demonstrated as compared to slow fibers [16]. In addition, transformation of fast into slow fibers in experiments with chronic stimulation of rabbit fast skeletal muscles is paralleled by Ca-ATPase inhibition [12]. All these data permit us suppose that in our case skeletal muscle off-loading switches on a reverse mechanism leading to an increase of Ca-ATPase activity.

Hence, optimization of sarcoplasmic reticulum Ca-pump work during hind limb relief may be related both to enhanced activity of the enzyme itself at the expense of its changed kinetic parameters and to the appearance in slow fibers of a new Ca-ATPase isoform with increased transporting activity. One should not overlook here the real possibility of inducing these changes during the initial increase of the blood Ca^{2+} concentration as

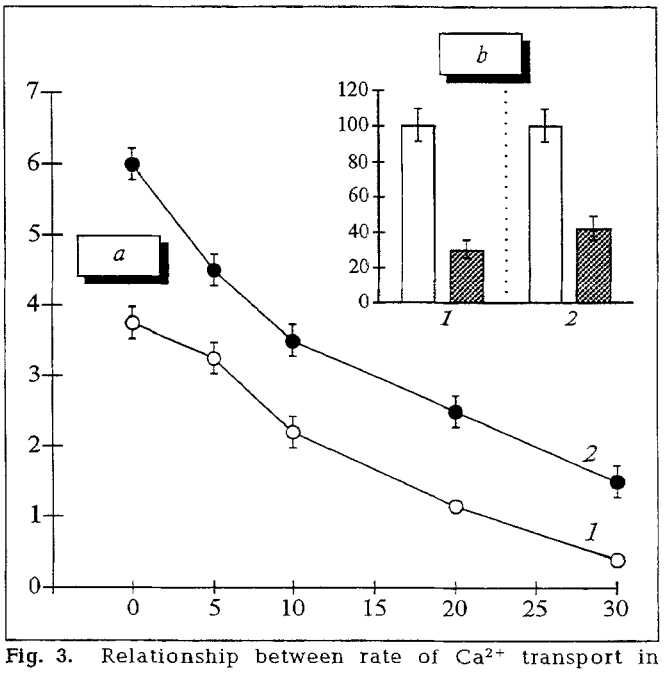

Fig. 3. Relationship between rate of Ca^{2+} transport in sarcoplasmic reticulum and time of incubation at 45°C of rat *gastrocnemius lateralis* homogenate in control (*1*) and after 25-day off-loading (*2*). *a*) abscissa: homogenate incubation time at 45°C, min; ordinate: Ca^{2+} transport rate in sarcoplasmic reticulum, nmol/min/mg tissue. *b*) degree of reduction of Ca^{2+} transport activity in sarcoplasmic reticulum vs. initial value (open bars), taken as 100% in control (*1*) and in relieved animals (*2*) after 20-min thermoinactivation (hatching bars).

a result of bone tissue demineralization and the overall change of the Ca^{2+} balance in the body during hypokinesia - hypodynamia.

The development of qualitative changes in the Ca-pump after muscle off-loading prompted us carry out a series of experiments aimed at studying the sensitivity of the Ca-transporting system to thermal exposure, which leads to dissociation of the transporting and hydrolytic functions of the enzyme. As was shown above, the resistance of the Ca^{2+} transport system to thermal exposure increases after 25 and even more so after 40 days of suspension (Figs. 2, *b* and 3, *b*). Presumably, the Ca-ATPase "fast" isoform is less labile, that is, it requires more stringent exposure for Ca^{2+} transport dissociation. A higher resistance to heating may be also explained by a lesser membrane permeability for passive Ca^{2+} release. A more active glycogen and ATP consumption and more active lactic acid output during *m. soleus* work are known to occur in the atrophied muscle after limb immobilization [20]; glycogen, ATP, and creatine phosphate depositions are known to increase after a two-week relief of rat hind paws [8,9]. It is possible that off-loading results in a shift toward a greater dependence on anaerobic metabolism in the muscles. This may involve lipid peroxidation inhibition, and thus the activation of natural factors of cell membrane destruction in the "hypokinetic" muscle may proceed more slowly.

Therefore, 25- and 40-day relief of rat hind paws by suspension results in an increase of Ca^{2+} transport in the gastrocnemius muscle sarcoplasmic reticulum and in an increase of the resistance of the sarcoplasmic reticulum transport function to injurious environmental factors, notably high temperature. These results confirm the hypothesis on the transformation of slow fibers into fast ones in relieved muscles during animal suspension in ground-based experiments simulating the physiological effects of weightlessness and under microgravitation conditions.

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