Ca^{2+} Transport of the Skeletal Muscle Sarcoplasmic Reticulum during Readaptation of Rats after a 40-Day Load Relief on the Hind Paws

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The rate of Ca^{2+} accumulation in the sarcoplasmic reticulum of the skeletal muscle (*m. gastrocnemius lateralis*, *m. vastus medialis*, and *m. soleus*) is studied in rats under conditions of functional off-loading of the hind paws (suspending animals by the tail). The rate of Ca^{2+} transport in the sarcoplasmic reticulum is shown to be stepped up in all these muscles. In the sarcoplasmic reticulum of *m. gastrocnemius lateralis* and *m. vastus medialis* the Ca^{2+} transport rate reliably drops, which does not occur in *m. soleus*. During a 2-week period of readaptation of animals suspended for 40 days, the Ca^{2+}-transporting function of the *m. soleus* sarcoplasmic reticulum gradually recovers to reach the control values, whereas the time course of recovery of Ca^{2+}-pump activity in the sarcoplasmic reticulum of *m. gastrocnemius lateralis* and *m. vastus medialis* has a phasic pattern.

Key Words: *Ca^{2+} transport; sarcoplasmic reticulum; hypokinesia; hypodynamia*

Numerous studies have been devoted to changes of metabolism during restricted mobility. Hypokinesia-hypodynamia unevitably results in a reduction of the muscle tissue weight. This is due to a decrease in the protein content in the muscle [14], notably a loss of myofibrillar proteins [18]. The contractile properties of the muscles [16] alter, as well as the fiber type distribution [17] and the enzyme activity [18]. Metabolic peculiarities during rehabilitation after hypokinesia-hypodynamia have been less studied. It should be borne in mind, however, that it is during this period that the harmfulness of the metabolic shifts observed for hypokinesia may fully manifest itself. The weight of the hind limb muscles in rats which had been off-loaded for 7, 14, and 18 days was reported to normalize after 1-2 weeks [10,11]. During this period focal lesions were observed in the muscle fibers, notably, in animals subjected to physical loads [4]. However, there are no published data about the state of Ca^{2+} transport in the muscle sarcoplasmic reticulum (SPR) during readaptation after hypokinesia-hypodynamia. This system plays a crucial role in the electromechanical interactions, which may be disturbed both when the muscles are off-loaded and when the loads are reapplied.

The aim of the present study was to investigate the state of the Ca-pump in the SPR of skeletal muscles during a 2-week readaptation of animals after a long-term load relief.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing about 250 g. The hind paws were relieved of load by suspending the animals by the tail, as was described previously [9], for 40 days.

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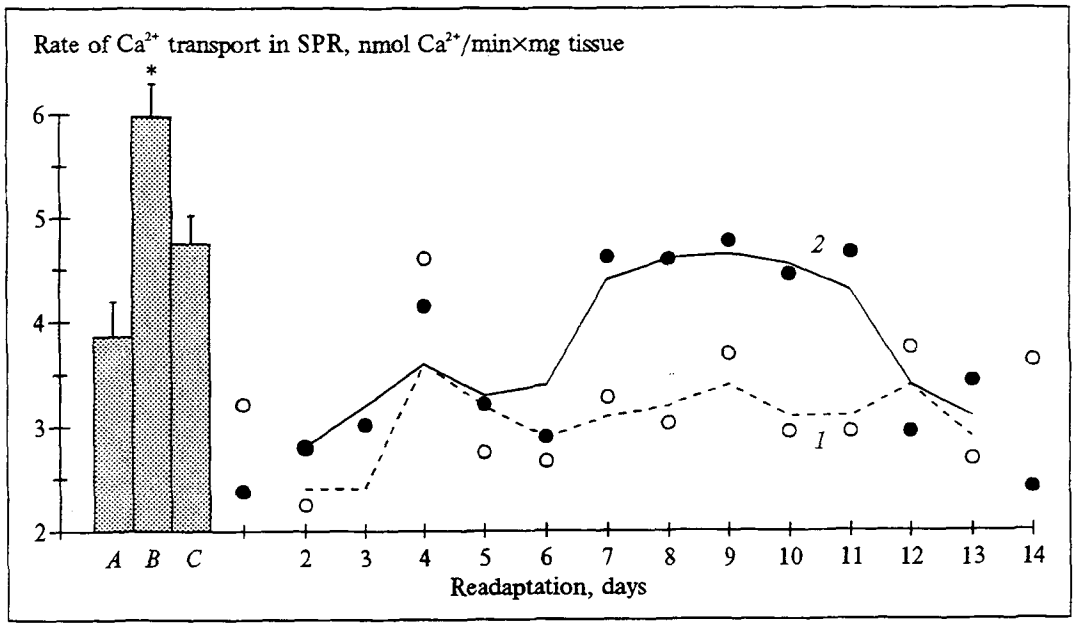

Fig. 1. Rate of Ca^{2+} transport in GL SPR in the control (*A*), on day 40 of suspension (*B*), and 4 hours after renewal of the motor load on the hind paws (*C*), and time course of Ca^{2+} transport rate in GL SPR during 14-day readaptation of rats suspended for 40 days. An asterisk denotes $p<0.05$. Here and in Figs. 2 and 3: the results for the control (*1*) and experimental (*2*) groups were processed using the sliding means method for the dynamic series. The differences between the control and experimental groups assessed using Wilcoxon's nonparametric test are reliable at $p<0.05$ (Figs. 1 and 2) and at $p<0.01$ (Fig. 3).

Rats were decapitated on day 40 of off-loading or 4 h after the load on the hind paws had been renewed on day 40 (6 rats from the control and 6 rats from the experimental group). We used the dynamic series observation method [1], decapitating rats (one animal from the experimental and one from the control group) daily on days 1 to 14 following a 40-day load relief (group of readaptation after suspension, SR). The skeletal muscles were isolated and rapidly frozen in liquid nitrogen.

The tissue was crushed using an Ultra-Turrax homogenizer with a 25N-10 blade for 60 sec at № 8 speed in a medium containing 100 mM KCl, 20 mM imidazole (pH 7.8), and 25% glycerol; the tissue to medium ratio was 1:5.

Transport of Ca^{2+} in the SPR was measured after Meerson *et al.* [8] with an Orion EA-940 ionometer using a Ca-selective electrode. The rate of Ca^{2+} transport in SPR was determined in *m. soleus* (SOL), *m. gastrocnemius lateralis* (GL), and *m. vastus medialis* (VM). The Ca^{2+} transport rate was assessed at 37°C by placing 25-100 μl of homogenate 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 (with continuous stirring). Directly before the measurements ATP and Ca^{2+} were added to attain a final concentration of 4 mM and 2-20 μM, respectively. Due to the nonlinear characteristics of the Ca-selective electrode, the Ca^{2+} transport rate was estimated from the slope of the experimental curve at a point corresponding to 2 min after the onset of changes in the Ca^{2+} transport. The Ca^{2+} transport rate was expressed in nmol Ca^{2+} accumulated by the vesicles during 1 min per milligram of tissue. The results were processed by the methods of variational statistics using the Student *t* test. The results obtained on days 1 to 14 of readaptation of animals which had been suspended for 40 days were processed using the sliding means method, and the reliability of differences was assessed by Wilcoxon's conjugate pair test.

RESULTS

On day 40 the rate of Ca^{2+} transport in the SPR of GL (Fig. 1), VM (Fig. 2), and SOL (Fig. 3) was 58%, 52%, and 31% higher, respectively, than in the control. Four hours after renewal of the load on the hind paws, the rate of Ca^{2+} transport in the GL SPR dropped 27% as compared to that on the 40th day of load relief, not differing from that in the control (Fig. 1). After 4 h of readaptation the rate of Ca^{2+} transport in the VM SPR was 31% lower than on the 40th day of off-loading and did not differ from the control (Fig. 2). The rate of Ca^{2+} transport in the SOL SPR after 4 h of readaptation did not differ from that in rats suspended for 40 days or from the control (Fig. 3).

Figure 1 shows the time course of Ca-pump activity in the GL SPR for a 1-14-day readaptation of rats which had been suspended for 40 days

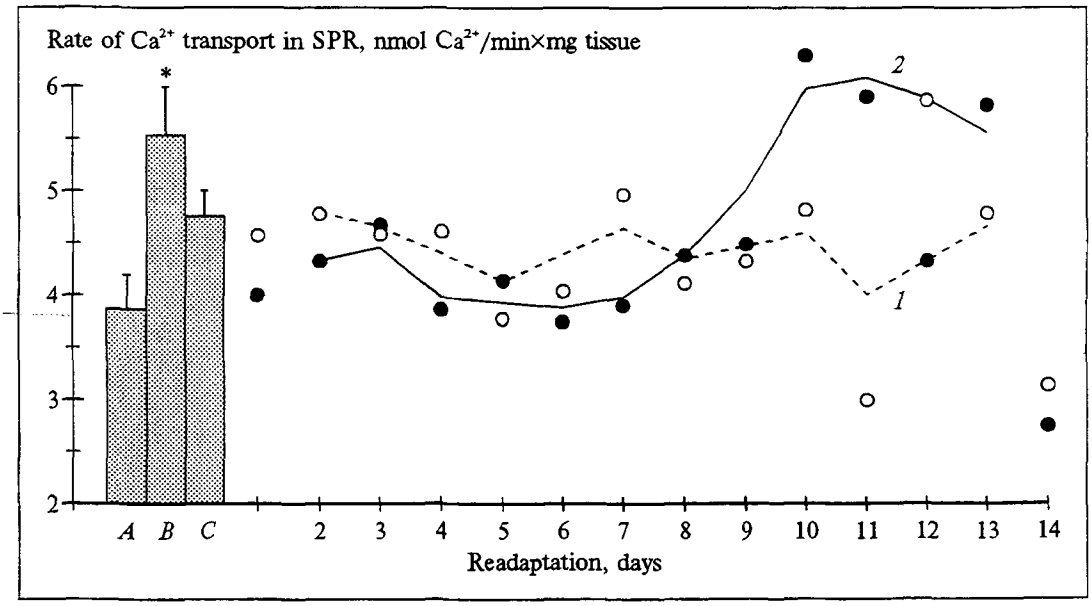

Fig. 2. Rate of Ca^{2+} transport in VM SPR and time course of Ca^{2+} transport rate in VM SPR during 14-day readaptation of rats suspended for 40 days. Designations as in Fig. 1.

(the SR group, curve *2*), and in the control for the same period (curve *1*). The values in the SR group reliably differed from the control ($p<0.05$). During the first week of readaptation the Ca^{2+} transport rate in the GL SPR remained approximately at the control level. During the second week this parameter increased, reaching its maximum on days 8-10 of readaptation and then gradually dropping to the control level. The pattern of changes in the state of the Ca-pump in the VM SPR is similar to that in the GL SPR, with a characteristic plateau at the control level during the first week of readaptation and an increase in the activity during the second week (Fig. 2). The values in the SR group (curve *2*) and in the controls (curve *1*) are virtually the same. Studies of the time course of Ca^{2+} transport activity in the SOL SPR demonstrated that on days 1-14 of readaptation this parameter gradually decreased from the level observed in suspended animals to the control level (Fig. 3); the values in the SR group (curve *2*) reliably differed from the control (curve *1*, $p<0.01$).

In some studies [12,15] the reliable increase in the rate of Ca^{2+} absorption and release in the SOL SPR after 14-15 days of suspension was at-

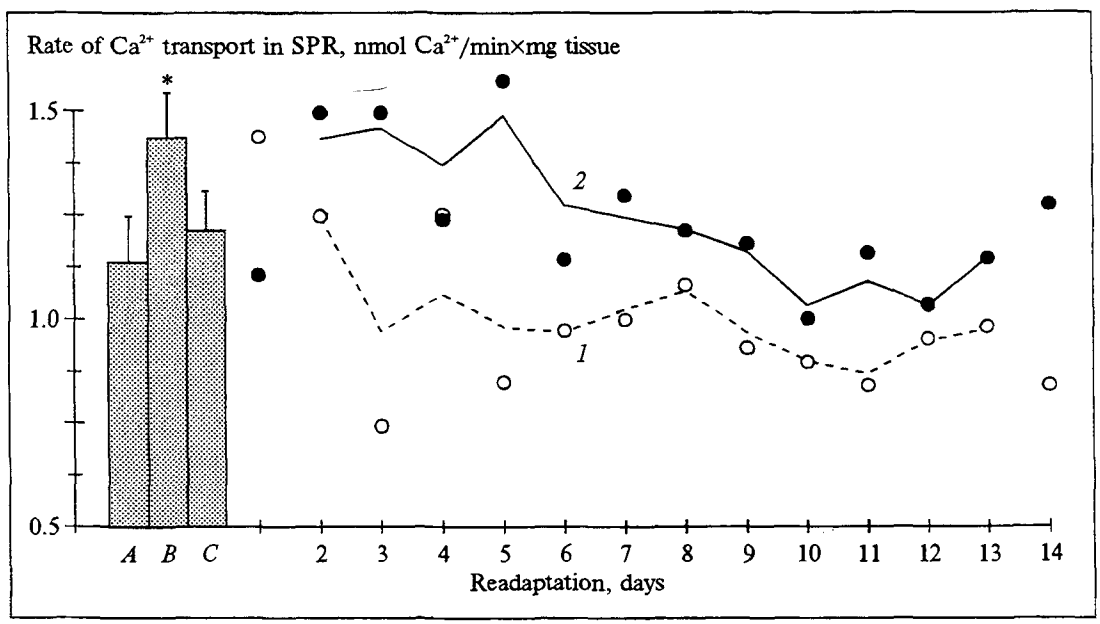

Fig. 3. Rate of Ca^{2+} transport in SOL SPR and time course of Ca^{2+} transport rate in SOL SPR during 14-day readaptation of rats suspended for 40 days. Designations as in Fig. 1.

tributed to load relief-induced transformation of slow fibers into fast fibers. Since different isoforms of Ca-ATPase that are specific for the fast or slow type of fibers in the skeletal muscle have been discovered in rabbits [6], and Ca^{2+}-transporting capacity and Ca-ATPase activity have been shown to be higher in fast than in slow fibers [13], we may speculate that during suspension, in slow fibers the "fast" Ca-ATPase is synthesized, whereas the synthesis of the "slow" isoform is suppressed.

During the very first hours of readaptation the Ca^{2+}-transporting ability of the SPR of muscles containing both fast and slow fibers (GL and VM) markedly dropped as compared with that on the 40th day of suspension of animals (Figs. 1 and 2). In the SPR of muscle with the predominantly slow type of fibers the rate of Ca^{2+} transport did not change reliably during the first 4 h of readaptation after a long-term load relief (Fig. 3).

The reduced activity of the SPR Ca-pump during the very first hours of readaptation, which is observed in the mixed muscles (GL and VM) and is not observed in SOL, may be attributed to the presence of fast fibers in the mixed muscles. Reportedly, a 2-h physical load on predominantly fast muscles results in a decrease of both the Ca-pump transporting function and the Ca-ATPase activity [2]. Continuous stimulation of rabbit fast skeletal muscles during 2 days reduces by 50% the initial rate of Ca^{2+} accumulation in SPR and the maximum Ca^{2+}-absorbing capacity of SPR vesicles [5]. Further stimulation of the muscles (longer than 2 days) does not further reduce the Ca-pump activity; moreover, the decrease in the Ca^{2+} transport rate has a reversible pattern, since after stimulation is discontinued, Ca-ATPase resumes its activity. In SPR of fast muscles stimulation-induced inhibition of Ca-ATPase is due to inactivation of some molecules of the enzyme, this loss of activity resulting from the inability of these molecules to bind ATP and, accordingly, to participate in phosphorylation [7]. It has also been shown that Ca-ATPase isolated from SPR of the stimulated muscles is not trypsinized at the first lysing site (Arg-505) [3]. Presumably, chronic stimulation causes some molecules of Ca-ATPase of fast fiber SPR to be conformationally changed in the region of the active center where the nucleotide binding site and the phosphorylation site are situated.

When loads are resumed in muscles off-loaded for a long time, this may be compared with continuous stimulation or physical loading of muscles in animals which are kept under normal conditions. Basing ourselves on the fiber type composition of the muscles, we may assume that after a long-term load relief SOL contains slow fibers and fibers with *de novo* synthesized fast isoforms of the enzymes, while in GL and VM there are fast fibers, slow fibers, and fibers transformed from slow into fast. Thus, the different responses of the SPR Ca-pump to reloading in SOL and in VM and GL may be attributed to the fact that Ca-ATPase in fast fiber SPR is more labile, i.e., it more readily responds to increased muscular activity, and this manifests itself as a phasic pattern of changes in the Ca-pump activity. The time course of the Ca-pump activity in the SOL SPR has a smooth pattern, the activity gradually decreasing to the control level (Fig. 3). Evidently, during the period of adaptation to the renewed motor load, a gradual degradation of the fast isoforms of Ca-ATPase in slow fibers of SOL is attended by synthesis of slow Ca-ATPase, typical of this type of fibers under normal conditions. The time course of readaptation for VM (Fig. 2) and GL (Fig. 1) is characterized by a marked decrease in Ca-pump activity during the first 7 days of readaptation, which is followed by a "surge" in the second week. A comparison of the two types of muscles (slow and mixed) suggests that the wavelike pattern of the time course of readaptation for the mixed muscles is due to the presence of initially fast fibers in them. After one week of readaptation the muscles adapt to the motor load, and the organism does not perceive this load as an excessive one. Thus, the conditions comparable to stimulation or physical load disappear in the fast muscles. Stimulation-induced inactivation of Ca-ATPase is reversible when stimulation is discontinued [5]. Hence, after one week of readaptation the transporting activity of Ca-ATPase in fast fibers may revert to the initial values, while in transformed fibers there is a residual increase in the activity of the Ca-pump, manifesting itself in the above-mentioned "surge" of the curves for VM and GL (Fig. 1 and 2). A comparison of the responses of different muscle types to renewal of the load and the assumption that during off-loading slow fibers become transformed into fast ones suggest that the properties of fibers transformed from slow into fast and those of intrinsically fast fibers are different. The state of muscle fibers for both relief of the motor load and reloading must be discussed taking into account that synthesis and degradation of the protein structures may become superimposed, yielding a complex picture, as was observed in the present series of experiments.

Thus, along with an increase in the Ca^{2+} transport rate in the skeletal muscle SPR of rats suspended for 40 days, we observed different responses

of the Ca-pump of SPR in the slow (SOL) and mixed (GL and VM) skeletal muscle to the resumption of the load after a long-term off-loading. Just 4 hours after the load was resumed, the rate of Ca^{2+} transport in the mixed type muscle markedly dropped, whereas in the slow muscle SPR it did not change markedly. During 14 days of readaptation the mixed type muscles exhibited a phasic pattern of changes in the transporting activity. In the muscles with the predominantly slow type of fibers during 2 weeks of readaptation, the transport rate gradually decreased to the control level.

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