Ca^{2+} Transport in the Myocardial Sarcoplasmic Reticulum of Rats during Antiorthostatic Off-Loading and during Readaptation

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The rate of Ca^{2+} accumulation in the myocardial sarcoplasmic reticulum is studied in experiments on rats under conditions of hind paw functional off-loading by suspending animals by the tail for 1 to 40 days, as well as during a 2-week period of readaptation after a 40-day load relief. The rate of Ca^{2+} transport in the myocardial sarcoplasmic reticulum reliably drops (by 33%) after 40 days of suspension. At earlier stages of off-loading Ca-pump activity in the sarcoplasmic reticulum does not change reliably. After resumption of the loads in animals suspended for 40 days, the transporting function of the myocardial sarcoplasmic reticulum rapidly reverts to the control level, which is indicative of a reversible pattern of load relief-induced changes in the rate of Ca^{2+} transport.

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

A weightless state, as well as its physiological simulation in ground-based experiments, entrains a considerable decrease in the weight of the myocardium and a reduction in the activity of the cardiovascular system [3,5]. Marked ultrastructural changes are known to occur in the myocardial tissue under conditions of microgravitation and hypokinesia of various origin [6,14]; disturbances of Ca^{2+} metabolism and an increased Ca^{2+} release from the bone tissue are also typical.

When the physiological effects of microgravitation are simulated by functional off-loading of the hind paws (rats are suspended by the tail at an angle of 30°), this results in displacement of the fluids in the organism toward the head. There are few published data about the changes occurring in the cardiovascular system in suspended rats, and some of these data are contradictory [13,19]. The key functions of the main structures in cardiomyocytes during readaptation after long-term suspension have not yet been studied.

The sarcoplasmic reticulum (SPR), which is responsible for the electromechanical interactions in cardiomyocytes, is a key component in the maintenance of Ca homeostasis. Damage to this Ca^{2+}-transporting system in the myocardial tissue, notably against the background of reduced synthesis of ATP, which is observed for restrained mobility [8], and increased Ca^{2+} concentration in the fluid media of the organism at the initial stage of adaptation to hypokinesia, may severely interfere with cardiac activity and, primarily, the relaxation rate.

In view of the foregoing, we studied changes in the state of the Ca-pump of the myocardial SPR under conditions of fluid medium redistribution and hind paw off-loading in suspended rats and in rats during readaptation after long-term load relief.

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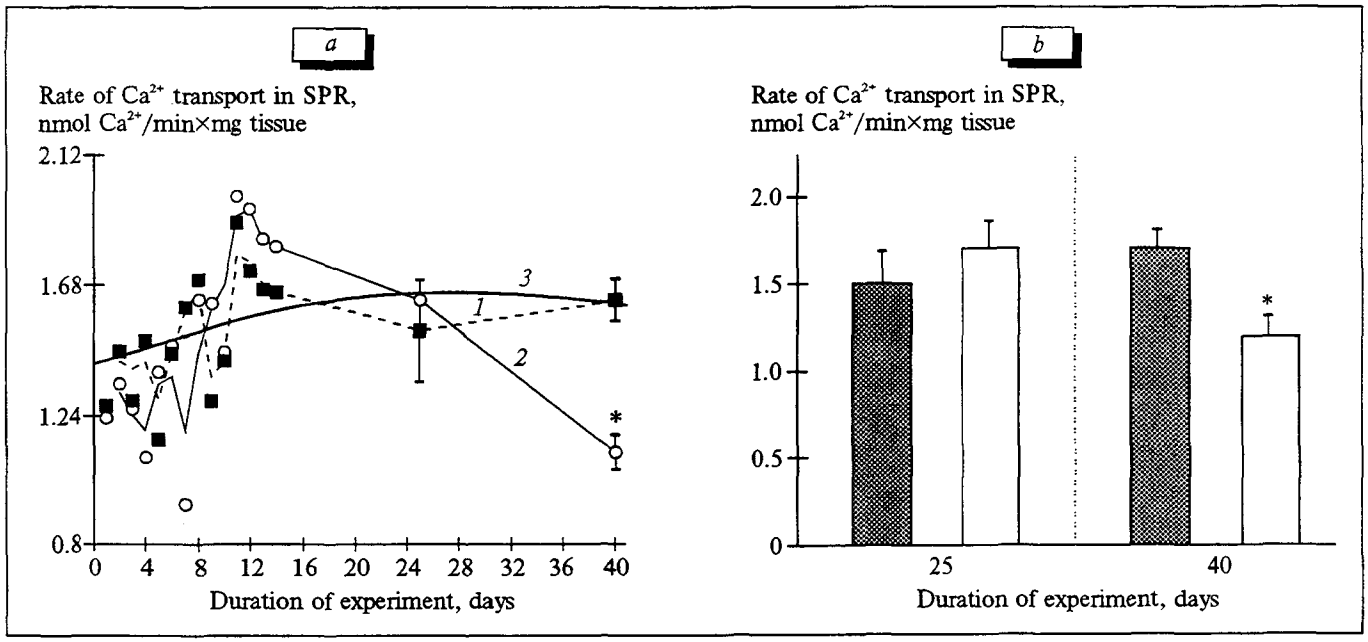

Fig. 1. Changes in the rate of Ca^{2+} transport in SPR of the rat myocardium during hind paw off–loading by suspension. *a*) time course of Ca^{2+} transport in the control (*1*) and for hind paw off–loading (*2*). The results obtained in the control were processed using regression analysis (*3*). *b*) rate of Ca^{2+} transport in the control (dark bars) and on days 25 and 40 of suspension (open bars). Here and in Fig. 3: an asterisk denotes $p<0.05$.

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 described previously [12], for 40 days. Rats were decapitated on day 40 of suspension or 4 h after renewal of the load on the hind paws on day 40 (6 rats from the control and 6 rats from the experimental group). We used the dynamic series observation method [4], decapitating rats suspended for 40 days (one animal from the experimental and one from the control group) daily from the first to the fourteenth day of the readaptation period. The hearts were isolated, washed in physiological saline, 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.* [11] with an Orion EA-940 ionometer using a Ca-selective electrode. The Ca^{2+} transport rate in the SPR was determined at 37°C by placing 25-100 μl of homogenate in 5 ml of 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 Ca^{2+} transport. The rate of Ca^{2+} transport was expressed in nmol Ca^{2+} accumulated by the vesicles during one minute per milligram of tissue. The results were processed by the methods of variational statistics using the Student *t* test. The results obtained in animals readapted over 1 to 14 days after a 40-day load relief were processed using the sliding means method, and the reliability of differences was assessed by Wilcoxon's conjugate pair test.

RESULTS

In rats suspended for 40 days the weight of the myocardium did not differ from the control values (860±30 mg), although a tendency was observed toward its decrease (by 10-14%) on days 25 (797±68 mg) and 40 (740±50 mg) of the experiment. The relative myocardial mass (myocardial mass/body mass) in suspended animals on days 25 (3.38) and 40 (3.3) of the experiment also did not reliably differ from the control values (2.9 and 3.04 on days 25 and 40, respectively).

During days 1-14 the rate of Ca^{2+} transport in the SPR of the myocardium was not reliably different from the control values (Fig. 1, *a*), although

there was a tendency toward its decrease during the first week of off-loading and toward its increase on days 11-13 (curve *2*), as compared with the mean value in the control (curve *1*). On day 25 the Ca^{2+} transport rate in the group of suspended animals also did not reliably differ from the control. On day 40 the rate of Ca^{2+} transport in SPR of the myocardium in suspended animals was 33% lower than in the control (Fig. 1, *b*).

The properties of the Ca-pump of the myocardial SPR were studied by heating tissue homogenate at 44°C and analyzing the rate of Ca^{2+} transport as a function of the time of thermal exposure of ATPase. Figure 2, *a* shows the Ca^{2+} transport rate in the myocardial SPR as a function of the time of thermal inactivation in the control group (curve *1*) and on day 40 of suspension (curve *2*). The heating-induced decrease in the transport rate in suspended rats was similar to that in controls (Fig. 2, *b*). On day 25 of load relief the heating-induced decrease in the Ca^{2+} transport rate in the myocardial SPR was also similar in the control and experimental groups.

Thus, in suspended rats the Ca^{2+} transport rate in the myocardial SPR reliably decreased only toward the 40th day of the experiment. The sensitivity of the Ca-pump to the higher temperature was similar in the experimental and control groups on days 25 and 40 of off-loading.

Four hours after rats were returned to the normal state, the Ca^{2+} transport rate in the myocardial SPR was 19% higher than the value obtained on day 40 of load relief; however, it remained 20% lower than in the control (Fig. 3).

A comparison of the time course of Ca-pump activity during a 2-week period of readaptation (Fig. 3, *2*) demonstrated no reliable differences from the control (Fig. 3, *1*), although the Ca^{2+} transport rate in the SPR tended to decrease during the first week of readaptation, while on the following 6-7 days its value reverted to the control level.

Thus, we found that the Ca^{2+} transport rate in the myocardial SPR did not reliably decrease over a 40-day period of suspension except for the 40th day of the experiment; 4 h after rats suspended for 40 days were returned to the normal state, this parameter increased, though without attaining the control values. Changing the orientation of the rat body with respect to the direction of the gravitation vector (when the hind paws were off-loaded by suspending the animals by the tail) did not result in marked disturbances in Ca-pump activity of the myocardial SPR during the first 1-14 days or on day 25 of the experiment, the time course of the Ca^{2+} transport rate exhibiting an oscillatory pattern from day 1 through 14. During the first week there was a tendency toward a decrease in the Ca^{2+} transport rate as compared with the control level.

Thus, the Ca^{2+} transport rate in the myocardial SPR dropped in suspended rats on day 40, while at earlier stages no reliable differences in the activity of the Ca-pump were observed. During readaptation after a 40-day off-loading the transport activity rapidly reverted to the control level,

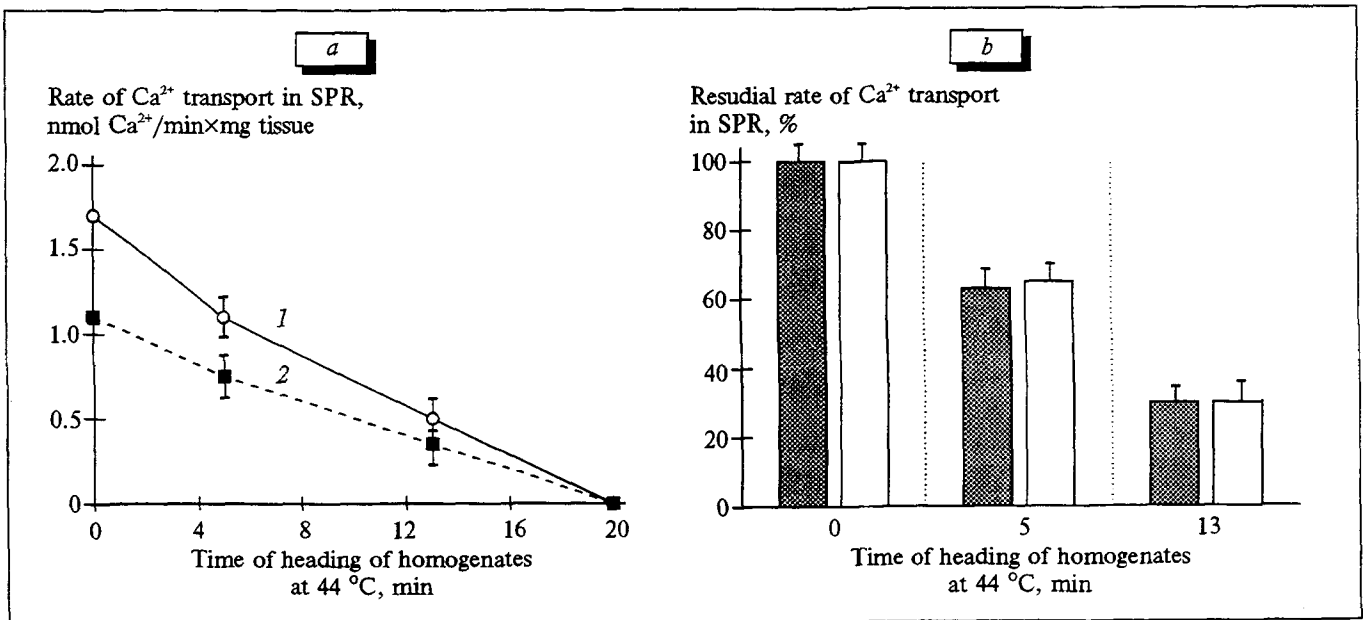

Fig. 2. Thermal inactivation of Ca^{2+} transport in myocardium of suspended rats. *a*) rate of Ca^{2+} transport in SPR as a function of time of incubation of myocardial homogenates at 44°C in the control (*1*) and on day 40 of off-loading (*2*); *b*) decrease in the rate of Ca^{2+} transport in SPR of rat myocardium as a function of time of thermal exposure of homogenates at 44°C in the control (dark bars) and on day 40 of off-loading (open bars).

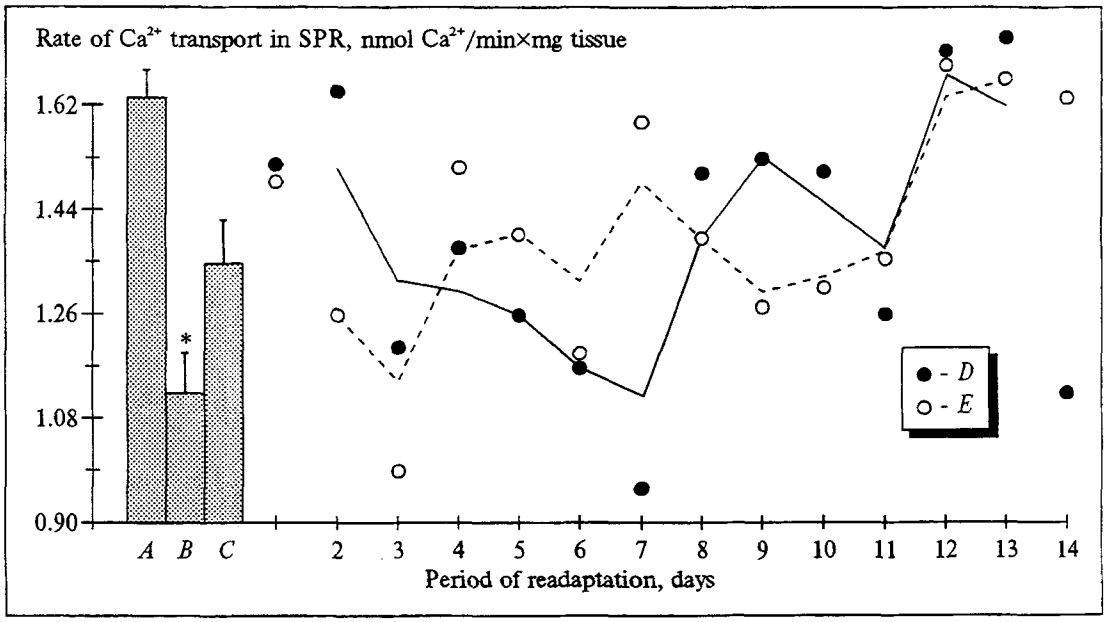

Fig. 3. Rate of Ca^{2+} transport in myocardial SPR in the control (*A*), on day 40 of suspension (*B*), and 4 hours after renewal of the load on the hind paws (*C*) and time course of Ca^{2+} transport rate in myocardial SPR during 14-day readaptation of rats suspended for 40 days. Symbols denote individual values of Ca^{2+} transport rate in SPR in the control (*1*, *D*) and experimental (*2*, *E*) groups.

exhibiting a reversible pattern of load relief-induced changes in the Ca^{2+} transport rate. It may be assumed that the reduced transporting activity of Ca-ATPase in the myocardial SPR is due to shifts in the hormonal and electrolyte balance in the organism, which on the cellular level lead to such regulatory changes in the activity of the SPR Ca-pump as inhibition of Ca-ATPase via associated enzymes in the membrane and suppression of the synthesis of Ca-ATPase per se [1,2,7,9-11,16-19].

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