Effect of Emergent and Long-Term Adaptation to Physical Strength on the Resistance of Ca-Transporting System of Myocardial Sarcoplasmic Reticulum

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Experiments were carried out on rats adapted to physical exercise (on the next day after completion of 4, 11, 15, and 30 swimming sessions). Catalase and superoxide dismutase activities were similar in all rats. The resistance of Ca transport into sarcoplasmic reticulum to high Ca^{2+} concentrations and autooxidaton increased starting from 4 swimming sessions, and to thermal inactivation from 11 sessions; the maximum resistance was attained after 15 sessions 1.5- to 2-fold surpassing the initial level. Maximum initial rate of Ca^{2+}-transport (155% of the control) was observed after 30 swimming sessions. In acute physical strength and at the initial stages of adaptation (4 swimming sessions) functional properties of myocardial Ca-transporting system were preserved under optimal conditions, but can be readily disturbed by adverse factors.

Key Words: *Ca transport; sarcoplasmic reticulum; physical strength; dynamics*

Ca-transporting system of myocardial sarcoplasmic reticulum (SR) is involved into regulation of the contraction-relaxation processes through Ca^{2+} release and sequestration corresponding to certain stages of the contraction cycle. Free radical-induced damage of this system leads to accumulation of free Ca^{2+} in cardiomyocytes [1], thereby disturbing myofibril relaxation and leading to the development of cardiovascular pathologies [2]. Nondrug and adaptation approaches to the prevention of these diseases have being extensively explored, of special interest among them being adaptation to physical exercise (PE). There are two main stages in adaptive process: emergent and long-term adaptation. The latter results in a branched systemic structural trace [2].

Single strenuous PE leads to neurogenic activation of stress-realizing systems in nonadapted organism and triggers stress reaction which manifests itself as a pronounced and protracted release of tropic hormones, catecholamines, and corticosteroids [14]. Catecholamines activate lipolytic and phospholipolytic enzymes and lipid peroxidation (LPO) [3]. These processes modulate lipids adjacent to membrane-bound enzymes, ionic channels, and receptors [4]. When these processes become excessive, the positive effects of catecholamines (enhanced energy supply and increased functional capacity) change into negative and result in damage to cell membranes. Transition from emergent to long-term adaptation is accompanied by selective growth of some cell structures and potentiation of the stress-effector system, which leads to regression of damages typical of the emergent adaptation. Adaptation to PE induces the formation of a multicomponent structural footprint, which includes changes in the heart. It has been shown that adaptation to PE increases the capacity of SR [7] and ion-transporting systems in the myocardium

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[13], improves resistance of isolated heart to variation of Ca^{2+} concentration, and prevents stress-induced sensitization of the myocardium to these changes [2]. We previously found that long-term adaptation to PE improves the resistance of Ca pump of SR to adverse factors [16]. The aim of the present study was to investigate the dynamics of these defense mechanisms in the heart.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (250 g). Adaptation to physical exercise was effected via daily swimming sessions during 30 days (water temperature was 32°C). The duration of the sessions was prolonged from 15 to 30 min during the 1st week and to 60 min during the 2nd week, and all subsequent sessions lasted 60 min. The animals were decapitated on the next day after completion of 4, 11, 15, and 30 swimming sessions or one hour after a 60-min single acute PE. The heart was removed, dissected free of connective tissues, washed in ice-cold physiological saline, and stored in liquid nitrogen before use. Superoxide dismutase (SOD) and catalase activities were measured as described elsewhere [6,10]. The rate of Ca^{2+} transport in the SR was measured with an Orion 940 ionometer using a Ca^{2+} selective electrode [11] by the rate of Ca^{2+} uptake by SR vesicles at 37°C in a medium containing 100 mM KCl, 15 mM potassium oxalate, 20 mM HEPES (pH 7.0), 4 mM $MgCl_2$, and 5 mM NaN_3. ATP and Ca^{2+} in final concentrations of 4 mM and 10-30 μM, respectively, were added immediately before measurement. The resistance of Ca transport to *in vitro* autooxidation was measured at 37°C in the presence of 0.2 mM ascorbate, while the resistance to thermal inactivation was assessed at 40°C. Thermal inactivation was accelerated by preliminary partial autolysis at 4°C (the loss of 30% activity of Ca transport). Protein concentration was determined from the fourth derivative of the absorption spectrum at 240-320 nm in a medium containing 20 mM histidine (pH 7.2), 50 mM NaCl, 8.1% sodium dodecyl sulfate. Statistical processing of the results was performed using the Student t test.

RESULTS

As seen from Fig. 1, catalase and SOD activities in all experimental groups did not differ from the control. This is consistent with the absence of LPO activation after treadmill running both in adapted and nonadapted rats [8]. However, in other experiments, single physical strain (termed by authors swimming stress) caused accumulation of malonic dialdehyde and depletion of total phospholipids in heart microsomes, which attests to a damage to membrane structures [15]. Thus, strenuous PE leads to activation of LPO and antioxidant enzymes, while moderate PE used in our experiments did not induce such changes and probably produced specific effects on cell membranes.

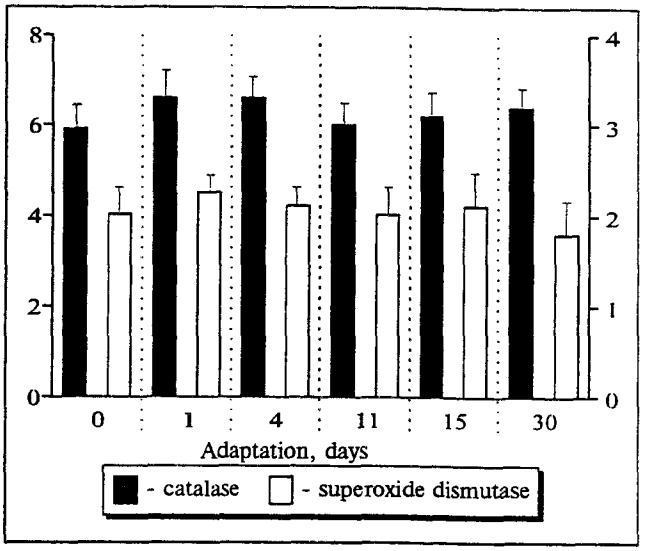

Fig. 1. Activities of the antioxidant enzymes catalase and superoxide dismutase in the control and during adaptation to physical exercise. Ordinate: activity of superoxide dismutase (activity units/mg protein, right axis) and catalase (μmolH_2O_2/min/mg protein, left axis).

Analysis of Ca pump in myocardial SR showed that the initial rate of Ca^{2+} transport undergoes considerable waveform changes throughout the adapta-

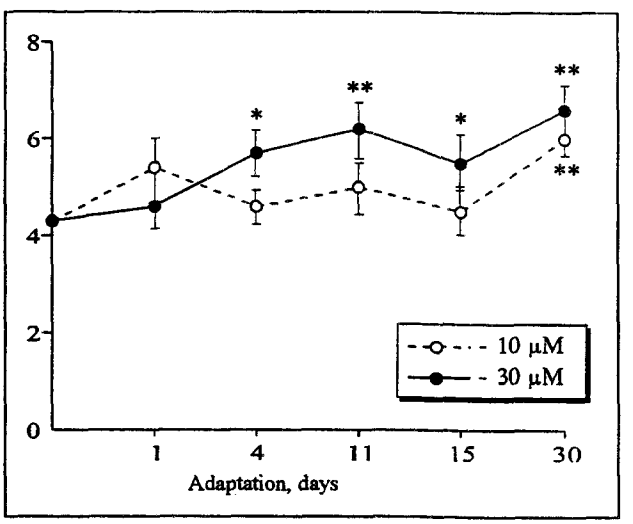

Fig. 2. Function of Ca pump of the sarcoplasmic reticulum at varied calcium concentrations in the incubation medium during adaptation. Ordinate: rate of Ca transport, nmol/min/mg protein. *p<0.05, **p<0.02 compared with the control.

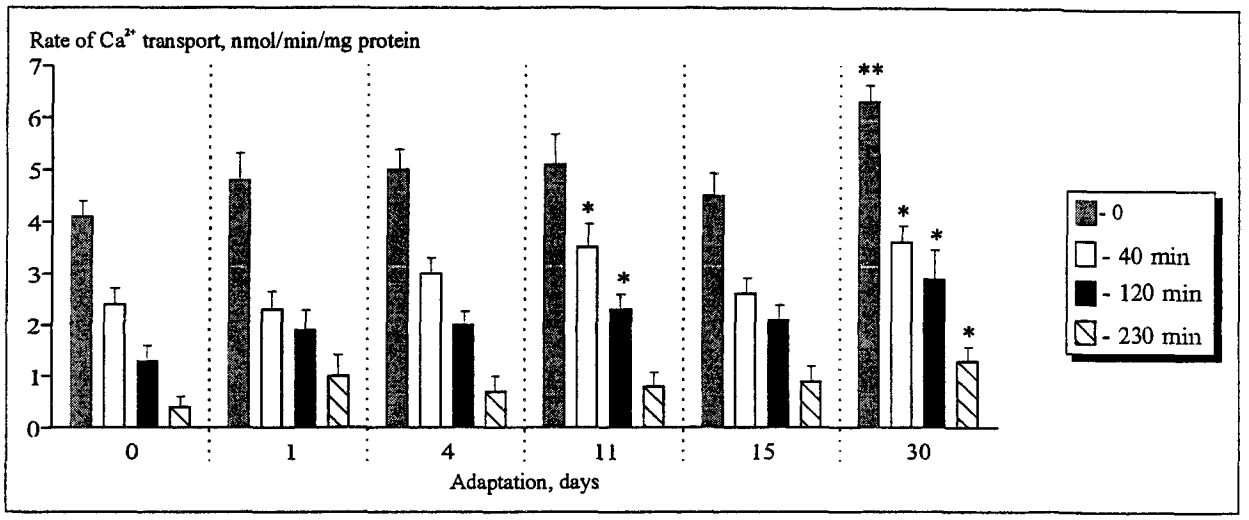

Fig. 3. Inhibition of Ca^{2+} transport in myocardial sarcoplasmic reticulum during autooxidation at 37°C in the control and after adaptation to physical exercise

Here and in Fig. 4.: *$p<0.05$, **$p<0.02$ compared with the controls corresponding to each term of adaptation.

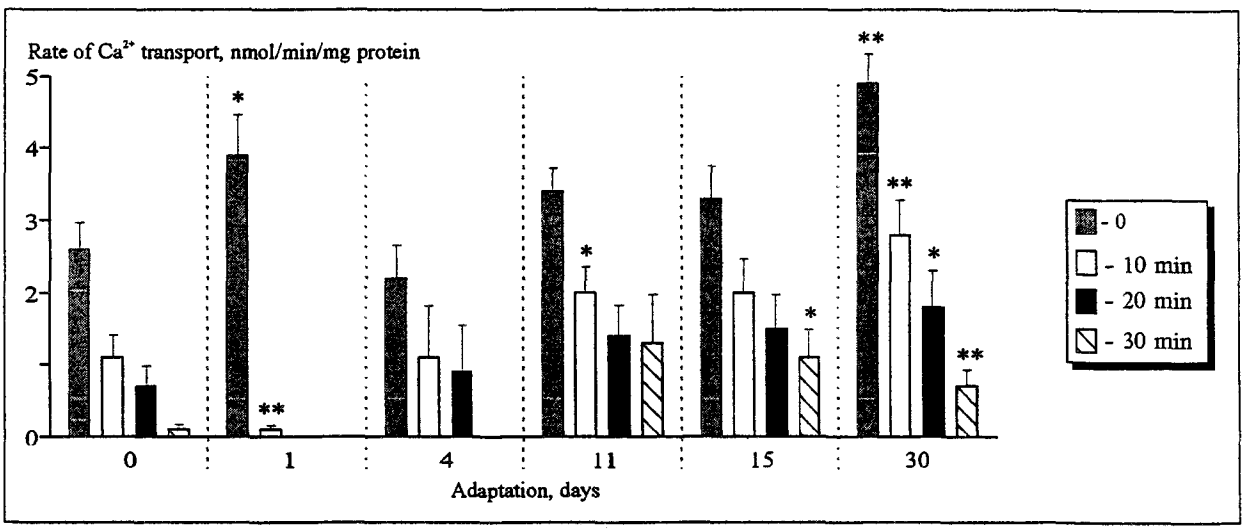

Fig. 4. Inhibition of Ca^{2+} transport in myocardial sarcoplasmic reticulum during thermal inactivation at 40°C after partial autolysis in the control and after adaptation to physical exercise

Rate of Ca transport, nmol/min/mg protein

tion (Fig. 2): it increases by 27% in acute PE, returns to control after 4 sessions, again increases on day 11 (by 12%), returns to normal on day 15, and finally increases by 43% to the end of adaptation (30 days).

Acute PE does not impair the resistance to autooxidation (Fig. 3), but against the background a higher initial rate of Ca^{2+} influx to SR in comparison with the control the resistance of the Ca-transporting system to high calcium concentration is decreased. Indeed, after Ca^{2+} concentration in the medium increased 3-fold (from 10 to 30 mM), the initial rate of Ca^{2+} influx in the control (without PE) remained unchanged, but after acute PE it decreased by 17% (Fig. 2). The resistance to thermal inactivation also decreased after acute PE: in the control, the initial rate of Ca^{2+} influx after 10- and 20-min thermal inactivation constituted 46% and 23% of the initial level, respectively, while after acute PE no Ca^{2+} transport was recorded in samples exposed to 10 min inactivation.

Other relationships were noted at the early stages of adaptation (4 sessions). First, the Ca-transporting system was considerably less sensitive to high Ca^{2+} concentrations. Figure 2 shows that in the presence of high Ca^{2+} concentration in the medium the initial rate of Ca^{2+} transport increased by 26%, whereas in the control is remained unchanged. Moreover, the sensitivity of Ca^{2+} transport to thermal inactivation

was considerably lower than after acute PE. Figure 4 shows that in SR vesicles obtained from rats after 4 swimming sessions, 10- and 20-min thermal inactivation reduced the initial rate of Ca^{2+} influx by 45 and 37%, and only 30-min inactivation completely inhibited Ca^{2+} transport, whereas after acute PE complete inhibition was observed after a 10-min inactivation. Thus, emergent adaptation modeled in our experiment by 4 swimming sessions is characterized by normal functioning of the Ca-transporting system under optimal conditions, but becomes more sensitive to damaging factors.

Transition from emergent to long-term adaptation occurs between 11 and 15 swimming sessions and is characterized, along with more efficient work of Ca pump under optimal conditions, by its improved resistance to adverse factors. After eleven PE sessions, the initial rate of Ca^{2+} transport did not differ from the control (Fig. 2), while its resistance to thermal inactivation (Fig. 4) and autooxidation (Fig. 4) 1.5-2-fold surpassed the control. A 3-fold increase in the external calcium concentration did not reduce the initial rate of Ca^{2+} transport in SR, as it did in SR vesicles from animals subjected to acute PE or completed 4 swimming sessions, but even increased this parameter by 30% (Fig. 2). After 15 PE sessions the initial rate of Ca^{2+} transport returned to normal (Fig. 2), its resistance to thermal inactivation (Fig. 4) and autooxidation (Fig. 3) did not differ from those observed after 11 swimming sessions and considerably surpassed the correspondent control values, and the initial rate of Ca^{2+} transport in the medium containing a 3-fold excess of calcium concentration increased by 27% and constituted 133% of the control. Thus, this stage of adaptation is characterized by pronounced protective changes in the Ca-transporting system, qualitative changes in the SR membrane (improved resistance to endogenous adverse factors) being more important than the quantitative rise of its Ca^{2+}-transporting capacity.

At the end of adaptation (30 days) the initial rate of Ca^{2+} transport considerably surpassed the control (143%) and the levels observed after 11 and 15 swimming sessions (Fig. 2). This is the maximum rise of the initial Ca^{2+} transport recorded by us in PE and other types of adaptations. This increased initial rate is resistant to adverse factors. As seen from Figs. 2-4, the rate of Ca^{2+} transport remains below the control level at all times of autooxidation and thermal inactivation as well as in the presence of a 3-fold excess of Ca^{2+} concentration in the incubation medium. Thus, the resistance of Ca transport is completely formed at the end of adaptation, while its capacity continues to increase. The absence of a compensatory activation of Ca-transporting system in response to high free Ca^{2+} concentration in the medium after 30 swimming sessions can be explained as follows. First, this probably characterizes the state of SR membrane: in the absence of Ca^{2+} leakage from SR vesicles there is no need to speed up its active transport. A similar rise of membrane integrity also accompanied long-term adaptation to stress, Ca^{2+} leakage from SR during autolysis being 1.7-fold decreased under these conditions in comparison with the control. Second, the number of Ca-ATPase can be increased due to resynthesis. Moreover, it should be noted that sharp rise of intracellular Ca^{2+} concentration can directly affect Ca-ATPase activity, since the SR proteins consisting mainly of Ca-pump molecules (90%) apart from high-affinity Ca^{2+} binding sites ($K_d = 10^{-7}$) include low-affinity binding sites ($K_d = 10^{-3}$) [5]. Since Ca^{2+} acts as a second messenger, variation of its tissue concentration modulate Ca-ATPase activity through regulatory proteins. This regulation has been demonstrated for phospholamban [9].

Our experiments show that judging from the queue of defense processes during adaptation, the rise of Ca-transport resistance to endogenous damaging factors dominates over quantitative increase in its capacity. A similar queue was observed during adaptation to stress. The resistance can increase due to modification of Ca-ATPase microenvironment determining its conformational mobility and transporting activity [4]. Adaptation to graduated PE (treadmill running) can be accompanied by accumulation of unsaturated phospholipids, which leads to a decrease in the plasma membrane viscosity and activation of membrane-bound enzymes. The rise of Ca-transporting capacity can be due to an increased number of Ca-ATPase molecules. During a long-term adaptation one pool of Ca-ATPase molecules is probably replaced with another (more rapid) pool. Such a replacement has been reported for some muscular proteins, both structural (myosin) and regulatory (phospholamban) [17]. Expression of these genes can be triggered by changes in intracellular Ca^{2+} concentration [12]. Ca-medicated metabolic processes are of particular importance for adaptive effects. Ca-ATPase isozyme replacement induced by changes in intracellular Ca^{2+} concentration have been previously observed in skeletal muscles during exercise or relief. This replacement is usually related to transformation of some rapid fibers to slow ones and vise versa. However, unlike metabolic shifts, the type of muscular fibers was altered by regular exercise only in few individuals. Hence, it can be hypothesized that metabolic shifts in the myocardium modulate the functioning of Ca-ATPase.

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