GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Opposite Effect of Adaptation to Physical Exercise on Myocardium and Skeletal Muscle. Ca-Transporting System of the Sarcoplasmic Reticulum and Antioxidant Defense Enzymes

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Adaptation to physical exercise was achieved via 60-min sessions of swimming at 32°C for 45 days, the duration of swimming being increased from 15 to 60 min during the first 14 days. Under these conditions, against the background of reduced catalase and superoxide dismutase activity the Ca-transporting system of the sarcoplasmic reticulum in the heart is shown to work more effectively: Ca^{2+} transport is characterized by a higher initial rate and is inactivated 1.5 times more slowly by *in vitro*-induced lipid peroxidation and not inhibited by high concentrations of free Ca^{2+}. In the skeletal muscle, on the other hand, catalase and superoxide dismutase activity rise, but this does not improve the functioning of the Ca pump: the initial rate of Ca^{2+} transport drops, its resistance to autooxidation is not increased in comparison with the control, and the resistance of the Ca^{2+}-transporting system to the inhibiting influence of free Ca^{2+} is lowered.

Key Words: *adaptation to physical exercise; Ca transport; sarcoplasmic reticulum; myocardium; skeletal muscle; catalase; superoxide dismutase*

Adaptation to physical exercise is known to boost the resistance of the animal and human organism to various damaging factors. Some protective effects of such adaptation have been demonstrated in the heart: the risk of cardiovascular disorders is lowered, the resistance of the myocardium to emotional pain stress and ischemia is elevated, the necrotic zone in subsequent myocardial infarction is restricted, and the electrical stability and its resistance to arrhythmogenic factors are heightened [4]. Analysis of the protective effect of adaptation to physical exercise revealed, along with a higher efficiency of the myocardial energy-accumulating system, activation of the antioxidant enzyme defense system, increased capacity of the sarcoplasmic reticulum (SPR), which is largely responsible for the relaxation process, and a more powerful Ca^{2+}-transporting system in the SPR [4,9,10]. Moreover, experiments of isolated rat papillary muscle have shown that adaptation to physical exercise lowers the sensitivity of the myocardium to variations of the Ca^{2+} level and prevents its stress-induced rise [4].

However, with respect to other organs no clear positive impact of adaptation to physical exercise has been found. Adaptation to physical exercise depletes the structural reserve of the kidneys, liver, and adrenal glands [8], and disturbs the function of the digestive organs [16]; high-intensity exercise reduced

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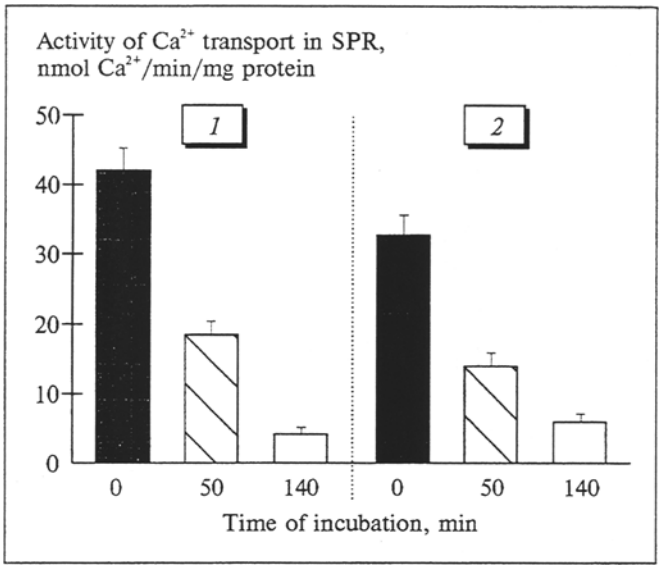

Fig. 1. Effect of autolysis at 37°C on the activity of the Ca^{2+} – transporting system of the sarcoplasmic reticulum (SPR) of skeletal muscles in the control (*1*) and after adaptation to physical exercise (*2*).

the efficiency of training [3] and lowers the resistance to cold stress [12]. The data concerning skeletal muscles are often ambiguous. Both an increase and a decrease in the relative number of mitochondria, intensity of oxidative phosphorylation, and activity of cell antioxidant enzymes in the course of adaptation have been reported [10,11,13,17]. Unlike that of the myocardium, Ca homeostasis of skeletal muscle has been little studied.

Hence, the aim of our study was to compare the functional parameters of the Ca pump of the SPR of skeletal muscles and myocardium, as well as its resistance to high Ca^{2+} concentrations and endogenous damaging factors, and to examine the activity of the antioxidant enzyme defense system in these tissues.

MATERIALS AND METHODS

The study was carried out on male Wistar rats weighing 250 g. Adaptation to physical exercise was effected via daily swimming sessions during 45 days. 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 water temperature was 32°C). One day after the adaptation was completed, the animals were decapitated and the heart and quadriceps femoris muscle were removed, dissected free of connective tissues, washed in ice-cold physiological saline, and frozen in liquid nitrogen.

The tissue was homogenized with the aid of an Ultra-Turrax TP-18/10 homogenizer with a 25N-10 knife (position 8) for 1 min in a medium containing 100 mM KCl and 20 mM Tris-HCl (pH 7.4 at 4°C) in a 1:7 tissue:medium ratio.

In the homogenates the rate of Ca^{2+} transport in the SPR was measured with an Orion 940 ionometer using a Ca^{2+} selective electrode as described elsewhere [15]. Incubation was carried out in thermocontrolled cells under constant stirring 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 with constant computer-assisted monitoring on line. ATP and Ca^{2+} in final concentrations of 4 mM and 7-28 μM, respectively, were added immediately before measurement.

Catalase activity was assayed as described previously [14]: H_2O_2 decomposition after the addition of homogenate aliquots was recorded on a Hitachi-557 spectrophotometer at 240 nm. Catalase activity was calculated from the initial rate of H_2O_2 decomposition (molar extinction coefficient $E=39.4$ $M^{-1}cm^{-1}$ per liter) and expressed in μmol H_2O_2/min/mg protein.

Total superoxide dismutase (SOD) activity was assayed as described elsewhere [7], by recording the rate of superoxide anion radical formation in the xanthine—xanthine oxidase system in the presence of tetranitroblue tetrazolium ($\lambda=560$ nm) before and after the addition of the homogenate. In the control the rate of the reaction was 0.024 optical density unit. Prior to measurement hemoglobin was elimi-

TABLE 1. Activity of the Antioxidant Defense Enzyme in the Heart and Skeletal Muscles in the Control and after Adaptation to Physical Exercise (*M±m*)

Enzymes	Control	Adaptation
Heart		
Catalase, μmol H_2O_2/min/mg protein	0.90±0.08	0.71±0.07
SOD, U/mg protein	3.06±0.20	2.55±0.17*
Skeletal muscle		
Catalase, μmol H_2O_2/min/mg protein	0.95±0.08	1.24±0.11*
SOD, U/mg protein	2.83±0.21	3.56±0.24**

Note. *$p<0.05$, **$p<0.01$ in comparison with the control.

nated from the supernatant by extraction with a chloroform:methanol (3:5 v/v) mixture in a ratio of 1:1. The mixture was then centrifuged for 10 min at 2300 g. SOD activity was measured in the top fraction by adding 300-500-μl aliquots to 3 ml of incubation medium containing 17 mM pyrophosphate buffer ($Na_4P_2O_7 \times 10\ H_2O$, pH 8.3 at 25°C), 0.1 mM xanthine, 0.1 mM EDTA, 0.05 mM tetranitro blue tetrazolium, 1% Triton X-100, and xanthine oxidase. The amount of enzyme necessary for 50% inhibition of the reduction reaction was taken as a unit of SOD activity.

Lipid peroxidation (LPO) was induced *in vitro* in homogenates of the myocardium using a system consisting of Fe^{2+} (10 μM)+ascorbate (0.57 mM) at 37°C in a medium containing 20 mM Tris-HCl (pH 7.4), 100 mM KCl, and 2 mg protein/ml (pH 7.4 at 37°C). Inhibition of Ca^{2+} transport in the SPR was recorded in parallel. Taking into account the high sensitivity of the Ca-transporting system of the SPR of skeletal muscles to LPO, we chose a milder system: autooxidation at 37°C without Fe^{2+} and ascorbate, the protein concentration and medium being the same as those used for LPO induction in the myocardium homogenate. The 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, and an aliquot of homogenate.

Statistical processing of the results was performed using the Student *t* test.

RESULTS

Experiments revealed that in skeletal muscle the two main enzymes of the antioxidant defense system, catalase and SOD, preventing activation of free radical processes in biological membranes (in particular, in SPR membranes), are activated after the course of adaptation to physical exercise by 31 and 26%, respectively. This activation of the enzymes evidently reflects the accumulation of reactive oxygen species in skeletal muscle due to the more intense work of mitochondria, increased myoglobin concentration, and enhanced oxygen supply in skeletal muscle during vigorous physical exercise [4,6]. However, it remains unclear, first, whether this activation of the antioxidant enzyme defense system is enough to completely compensate for the enhanced LPO and, second, whether the LPO process is inhibited due to the increased capacity of the antioxidant system, as has been shown for adaptation to physical exercise (jogging) [10,11].

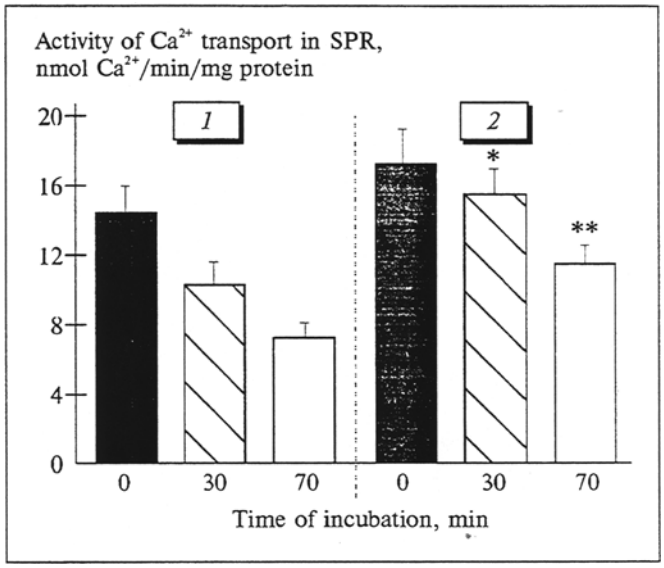

Fig. 2. Effect of *in vitro* induction of lipid peroxidation with the Fe^{2+} + ascorbate system on inhibition of the myocardial Ca^{2+} – transporting system in the control (*1*) and after adaptation to physical exercise (*2*).

*$p<0.02$, **$p<0.01$ in comparison with the control.

In order to elucidate this, we analyzed the state of the Ca-transporting system of the SPR of skeletal muscles, which is known to be very sensitive to free radical processes. Figure 1 shows that after a course of strenuous exercise (swimming) the initial rate of Ca^{2+} transport in the SPR of skeletal muscle does not increase but even drops by 23% in comparison with the control (32.7±2.3 *vs.* 42.0±2.6 nmol Ca^{2+}/

TABLE 2. Effect of an Increase in the Concentration of Free Ca^{2+} on the Activity of the Ca Pump (nmol Ca^{2+}/min/mg Protein) in the SPR of the Myocardium and Skeletal Muscles in the Control and after Adaptation to Physical Exercise

Concentration of free Ca^{2+} in medium, μM	Control	Adaptation
Heart		
7	14.55±11.15	17.55±1.50
28	10.25±1.5	17.10±1.85*
Skeletal muscle		
7	41.7±2.7	33.0±2.0*
28	26.8±1.9	11.2±1.1**

Note. *$p<0.01$; **$p<0.001$ in comparison with the control.

min/mg protein). The drop of Ca^{2+} transport in the SPR by itself does not attest to any damage to or impairment of the Ca^{2+}-transporting system, since it may be compensated by an increased number of SPR membranes and Ca pump units. This phenomenon along with muscle hypertrophy in physical exercise has been reported previously [6]. On the other hand, even with the increased initial rate of Ca^{2+} transport in the SPR after adaptation to hypobaric hypoxia [2] we observed a reduced resistance of the enzyme to autolytic damage. As is seen from Fig. 1, adaptation to physical exercise has no effect of the resistance on the Ca^{2+}-transporting system of the SPR to autolysis.

In the myocardium, the initial rate of Ca^{2+} transport in the SPR after adaptation to physical exercise is increased by 20% (17.25±1.60 *vs.* 14.45±1.35 nmol Ca^{2+}/min/mg protein in the control). A similar increase of the initial rate of Ca^{2+} transport in the SPR was demonstrated by us for adaptation to stress [15] and adaptation to periodic hypobaric hypoxia [2]. However, this parameter does not allow for the evaluation of many *in vivo* changes in the lipid-protein complex of the Ca^{2+} pump in the SPR induced by variations in the Ca^{2+} concentration, activation of endogenous damaging factors, etc. Therefore, a better approximation of the *in vivo* conditions may be derived from an analysis of not only the initial rate of Ca^{2+} transport but also its resistance to LPO and elevated concentrations of free Ca^{2+} occurring in the contraction-relaxation cycle. Such an analysis previously revealed a fundamental difference between the effect of two types of adaptation, to stress and periodic hypoxia, on the myocardial Ca^{2+}-transporting system [2,15]. Despite the fact that the initial rate of Ca^{2+} transport was increased in both types of adaptation, adaptation to stress enhanced, while adaptation to periodic hypoxia impaired, the resistance of the system to endogenous damaging factors.

Figure 2 shows the inhibiting effect of *in vitro*-induced LPO on Ca^{2+} transport in the SPR in the Fe^{2+}+ascorbate system. The adaptation to physical exercise is seen to enhance the resistance of the Ca-transporting system of the myocardial SPR to LPO. For instance, in controls 30 min after the activation of LPO the rate of Ca transport dropped to 71%, while in adapted animals this index did not differ reliably from the initial value. Further incubation reduced the rate of Ca^{2+} transport in the SPR in both the control and adapted animals but to varying degrees. After a 70-min oxidation this parameter was reduced by 50% in the control but only by 34% in adapted animals. It is important to note that the difference in the rate of Ca^{2+} transport between these series before oxidation was 20%, whereas after intensive oxidation it attained 58%.

Thus, adaptation to physical exercise greatly boosted the resistance of the myocardial Ca^{2+}-transporting system to *in vitro*-induced LPO. In view of the high sensitivity of the Ca-transporting system of the SPR of myocytes to LPO [1], it seems reasonable to assume that, similarly to the effect observed with adaptation to stress [5], the increased strength of the cell antioxidant system plays an important role in this process. The data in Table 1 indicate an inhibition rather than activation of the two main antioxidant defense enzymes of cardiomyocytes, catalase and SOD. Changes in the SPR membrane and structural and functional properties of the Ca pump arising from adaptation to physical exercise are apparently crucial factors improving the LPO resistance of the Ca pump.

Analysis of the resistance of the Ca^{2+}-transporting system of the myocardial SPR to another endogenous damaging factor, high concentrations of Ca^{2+}, showed that the rise of the level of Ca^{2+} from 7 to 28 μM led to a 30% drop of Ca^{2+} transport in the control animals but had no effect on this parameter in adapted rats (Table 2). Just as in the experiments with induction of LPO, as the damaging factor becomes stronger, the difference between the control and adapted animals increases. For instance, the difference in the rate of Ca^{2+} transport at a low Ca^{2+} concentration constituted 20%, while at a high concentration it amounted to 66%. Thus, adaptation to physical exercise improves the functioning of the Ca^{2+}-transporting system of the myocardial SPR at a high Ca^{2+} concentration and, thereby Ca^{2+} homeostasis can be controlled much more effectively than in nonadapted animals. This phenomenon is of particular importance in various states accompanied by activation of free radical processes such as ischemia-reperfusion and myocardial infarction, when membrane disturbances are attended by rapid Ca^{2+} entry followed by complex damage to the cell.

The Ca pump of skeletal muscles after adaptation to physical exercise turned out to be less resistant to a high level of exogenous Ca^{2+} (Table 1). Increasing the concentration of Ca^{2+} reduced the rate of Ca^{2+} transport in the SPR by 36% in the control, whereas after adaptation this inhibition constituted 66%, i.e., 1.8-fold higher. Thus, elevation of Ca^{2+} in skeletal muscle could lead to a pronounced inhibition of the Ca^{2+} transport in the SPR and its reabsorption, and, hence, a further rise of the Ca^{2+} level in the cell, which in turn would further reduce the rate of Ca^{2+} transport and interfere with the relaxation process. The effect of adaptation to physical exercise on the Ca^{2+}-transporting system of the SPR in skeletal muscles is similar to the effect of stress on the Ca pump of the myocardial SPR [15], albeit less pronounced, probably due to the high activity

of the antioxidant defense enzymes in skeletal muscles as opposed to the myocardium, where their activity is suppressed against the background of a marked accumulation of LPO products [5].

The antioxidant system of the cell consists of numerous components. An analogy may be drawn between our data and recent reports on the antioxidant system, in particular, the glutathione system of skeletal muscles in young animals adapted to physical exercise [13]. Catalase and SOD were shown to be activated, but the level of reduced glutathione and the ratio of reduced to oxidized glutathione were markedly lowered, the concentration of LPO products being unchanged. The authors conclude that despite the strengthening of some components of the cell antioxidant system, no additional protection against oxidative stress was found. Consequently, taking our finding into account, it may be assumed that the protection of the Ca^{2+}-transporting system of SPR is not increased either.

Thus, adaptation to physical exercise has an ambiguous effect on the functioning of muscle cells. In the adapted myocardium the resistance of the Ca^{2+}-transporting system to LPO induction and to high Ca^{2+} concentrations is increased, but no activation of the antioxidant defense enzymes occurs. In skeletal muscle adaptation to physical exercise does not enhance the resistance of the Ca pump to LPO induction and even impairs it for adaptation to high Ca^{2+} concentrations, while the cell antioxidant system is considerably activated.

It may be concluded that the "price" of adaptation to physical exercise is higher in skeletal muscle than in the myocardium, whose defense reserves allow it to maintain its function at the initial high level.

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