Adaptation to Stress Enhances the Resistance of the Calcium Pump in Rat Myocardial Sarcoplasmic Reticulum

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The development of adaptation during stress is studied. At the early stages, adaptation exerts no protective effect: the activities of superoxide dismutase and catalase and the resistance and the Ca-transporting system of the myocardial sarcoplasmic reticulum to heat inactivation and high calcium concentrations decline. At the end of adaptation, superoxide dismutase and catalase activities increase, the function of the sarcoplasmic reticulum Ca-transporting system is improved, and the resistance of this system to high Ca concentrations increases compared with that in the control (1.4-fold) and during the early stages of adaptation (1.6-fold). The resistance to heat inactivation increased 1.5-fold compared with the control. Three days after the completion of adaptation, the activities of these enzymes and the resistance of Ca transport to heat inactivation and high Ca concentrations are lower than immediately after adaptation, but higher than in the control group and during the early adaptation period.

Key Words: *sarcoplasmic reticulum; calcium pump; catalase; superoxide dismutase; myocardium; adaptation to stress*

Adaptation to stress produces protective effects in a variety of cardiovascular diseases [3], and the antioxidant system contributes considerably to these effects [5,11]. Stimulation of this peripheral stress-limiting system, which prevents excessive activation of free-radical reactions at the tissue level, can increase tissue resistance to oxidative stress [2,7]. We showed that the antioxidant system is strongly inhibited in the heart during prolonged exposure to a stressor or ischemia, and the membrane structures, including those of the sarcoplasmic reticulum (SPR), are damaged [1,6]. It was unclear how protection is conferred by adaptation against short-term stresses, what alterations occur in the antioxidant and membrane systems during adaptation, and what minimal number of short exposures to a stressor is necessary for the protective effect of adaptation to develop at the membrane level. The aim of the present study was to examine changes in the Ca-transporting system of the myocardial SPR and in antioxidant enzymes during adaptation.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250 g. The animals were divided into four groups according to the time of their examination during or after a 15-day adaptation. Group 1 rats were examined on day 9 of adaptation, group 2 on day 11, group 3 on day 15, and group 4 three days after adaptation. Group 5 rats served as control.

Rats were adapted to stress by 15-min immobilization min on day 1, 30 min on day 2, 45 min on day 3 and for 60 min on alternate days until day 15. They were decapitated 24 h after 3 (group 1), 4

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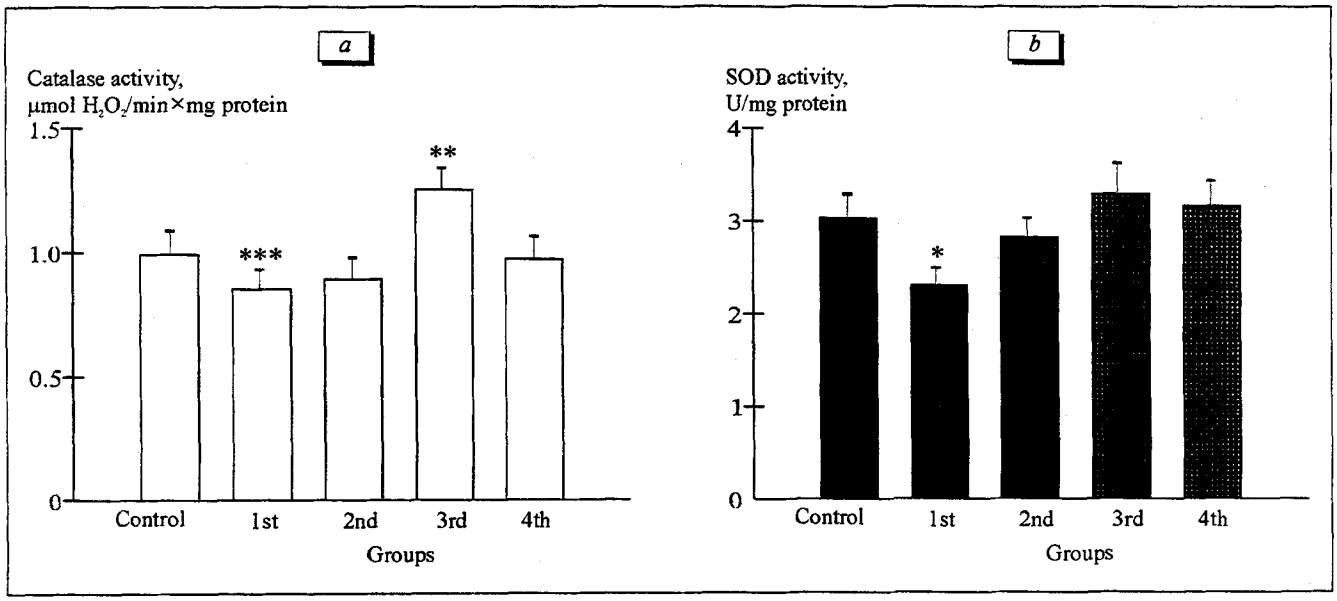

Fig. 1. Catalase (*a*) and superoxide dismutase (SOD, *b*) activities in the control and test groups (see Materials and Methods for designations of the groups). *$p<0.01$, **$p<0.02$, ***$p<0.05$ compared with the control group.

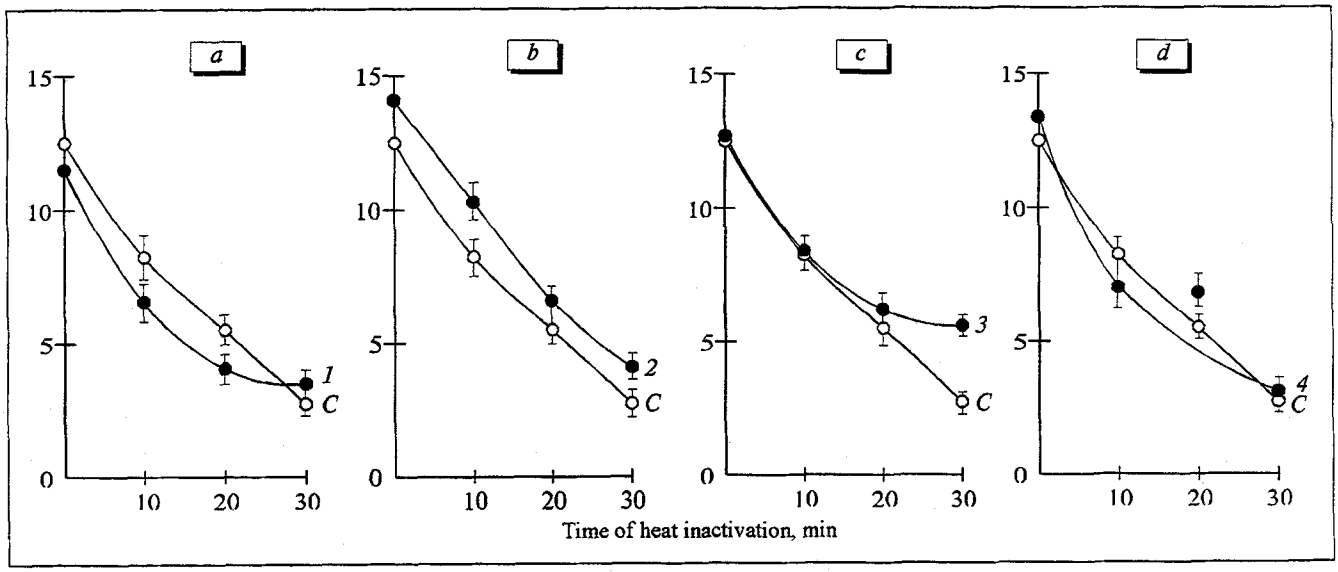

Fig. 2. Inhibition of the sarcoplasmic reticulum Ca-transporting system during heat inactivation (at 40°C) in control (*C*) and adaptation in groups 1 (*a*), 2 (*b*), 3 (*c*), and 4 (*d*) (curves *1*, *2*, *3*, and *4*, respectively). Ordinate: Ca transport rate, nmol/min×mg protein.

(group 2), and 6 (group 3) 60-min immobilization sessions and 3 days after completion of a 15-day adaptation (group 4). The hearts were excized, freed of connective tissue, washed in ice-cold physiological saline, and frozen in liquid nitrogen.

Catalase activity was assayed as previously [9] and expressed in μmol H_2O_2/mg protein×minute. The activity was calculated from the initial rate of H_2O_2 decomposition with the molar extinction coefficient equal to 39.4 $M^{-1}cm^{-1}$. The protein concentration was determined using the 4th derivative of the absorption spectrum in the 260-320 nm region in a medium containing 20 mM histidine, 50 mM NaCl, and 8.1% SDS (pH 7.2).

Superoxide dismutase (SOD) activity was measured spectrophotometrically [8] from the difference between the rate of superoxide radical formation in a xanthine-xanthine oxidase system before and after the addition of homogenate. Superoxide was detected at 560 nm by formation of formazan from tetranitro blue tetrazolium (TNBT). Before determination of SOD activity, hemoglobin was removed from the supernatant by extraction with a chloroform:methanol mixture (3:5 v/v) at a 1:1 ratio of the mixtures

(v/v) followed by centrifugation at 2300*g* for 10 min. A unit of SOD activity was defined as the amount of enzyme required for 50% inhibition of TNBT reduction to formazan.

The rate of Ca^{2+} transport in the SPR in the homogenates was determined in an Orion EA940 ionometer with an ion-selective electrode by measuring the rate of Ca^{2+} absorption by SPR vesicles in the presence of oxalate [4,10]. The reaction was conducted in thermostated cells with continuous stirring in a medium containing 100 mM KCl, 15 mM oxalate K, 20 mM HEPES (pH 7.0), 4 mM $MgCl_2$, and 5 mM NaN_3. ATP and Ca^{2+} were added to concentrations of 4 mM and 5-20 μM, respectively, immediately before the determination of Ca^{2+} transport. The results were statistically analyzed using Student's *t* test.

RESULTS

As shown in Fig. 1, at the early stages of adaptation (groups 1 and 2), the activities of the antioxidant enzymes catalase and SOD did not increase; on day 9 of adaptation they were even significantly lower than in the control group (by 24% and 15%, respectively). On day 15 of adaptation (group 3), catalase activity was significantly higher than in the control group (126% of control level), and both catalase and SOD activities were significantly higher than on day 9 (147% and 143%, respectively, of their levels on day 9). Three days after completion of adaptation (group 4), catalase activity was at the control level, while SOD activity was still significantly higher than on day 9 and only slightly lower than on day 15.

From these observations it can be concluded that considerable activation of free-radical processes occurred at the early stages of adaptation, which, on the one hand, resulted in inhibition of antioxidant enzymes and, on the other, could trigger the synthesis of catalase and SOD. By the end of adaptation period (day 15), the activities of both enzymes increased considerably, which probably led to stabilization of membrane structures and associated enzymes. In order to check this hypothesis, we examined the state of the SPR Ca-transporting system and its resistance to damaging factors. There were no significant intergroup differences in the initial rates of Ca transport in the myocardial SPR with the exception of group 2, where the rate of Ca transport was slightly increased.

Figure 2 shows a reduction in Ca transport rates in response to heat (40°C) inactivation which allows one to determine whether membrane enzyme complexes are damaged or stabilized. It can be seen that the resistance to heat inactivation on day 9 of adaptation (group 1) was lower than on days 11 (group 2) and 15 (group 3) when it was considerably higher compared with the control.

It is important to note that whereas on day 11 of adaptation (group 2) the rate of heat inactivation did not differ from that in the control group and the absolute rate of Ca transport was increased, these parameters had improved by day 15 (group 3): the initial transport rate increased while the heat inactivation rate decreased, particularly at the end of the inactivation period (30 min). Assuming the initial Ca transport rate in each group as 100%, after 10 min of heat inactivation, the transport rate in the control group was reduced to 66%, to 57% in group 1, and only to 70% in group 2 (Fig. 3). After 20 min of heat inactivation, in none experimental group did the reduction in Ca transport differ significantly from that in the control group, although the reduction in

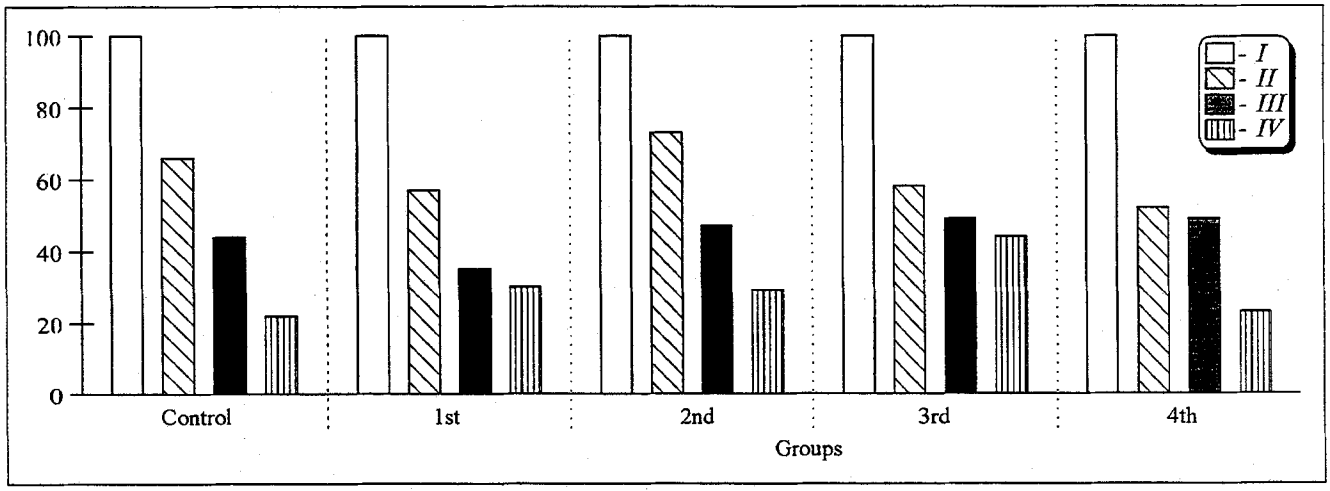

Fig. 3. Relative decreases in Ca transport rates in the myocardial sarcoplasmic reticulum during heat inactivation in control and experimental groups. Ordinate: percentage of retained activity after 0 (*I*), 10 (*II*), 20 (*III*), and 30 (*IV*) min of heat inactivation.

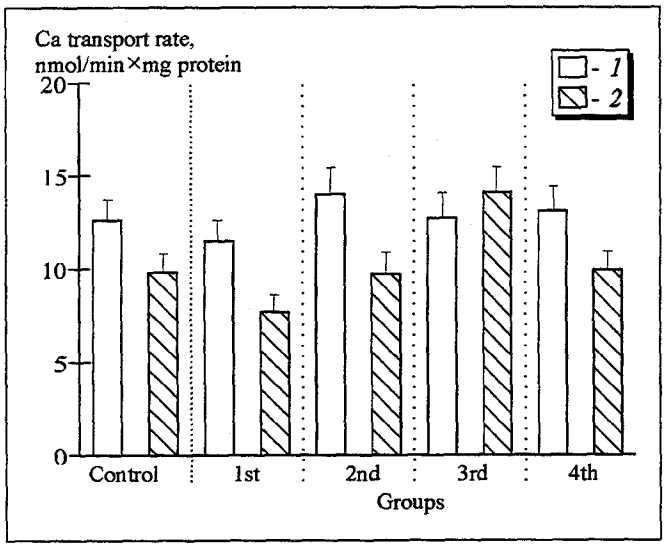

Fig. 4. Changes in the Ca transport rate after the concentration of free Ca was raised from 7 (*1*) to 20 (*2*) μM. Other designations are the same as in Fig. 3.

group 1 was greater than in groups 2-4. After 30 min of heat inactivation, no significant intergroup differences were observed with the exception of group 3, where the decrease in the rate of Ca transport was only half that in the control group. Thus, our data on heat inactivation attest to a complex nature of variations in thermal stability during adaptation. In group 1, thermal stability and Ca transport rates were reduced. In group 2, the initial Ca transport rate was slightly increased, thermal stability was comparable to that in the control group, while a substantial increase in the resistance to heat inactivation was recorded on day 15 (group 3), and the resistance was partially retained 3 days after completion of adaptation (group 4).

The resistance of the Ca-transporting system to elevated Ca concentrations depended to a greater extent on the duration of adaptation. Figure 4 shows changes in the Ca transport rate in response to an increase from 7 to 20 μM in free Ca. It can be seen that this rise of unbound Ca led to 22%, 33%, and 31% decreases in the Ca transport rates in the control and 1st and 2nd groups, respectively, whereas group 4 showed a 14% increase in this rate rather than its decline. If no differences between groups in the Ca transport rate were observed at low Ca concentration (7 μM), significant intergroup differences were recorded at high Ca concentration (20 μM): in comparison with the control, the rate was 78% in group 1 and 144% in group 3. Thus, during adaptation the resistance of the Ca-transporting system changed considerably from relatively low to relatively high.

From our results in can be concluded that: first, at the early stages adaptation exerts no protective effect on the membrane-associated system of Ca transport in myocardial SPR: the low activities of the antioxidant enzymes catalase and SOD as well as the resistance of this system to heat inactivation and to high Ca concentrations decrease. Second, at the late stages of adaptation, the activities of the enzymes increase, the function of the SPR Ca pump improves, and the system resistance to damaging factors increases.

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