The Role of Cytoplasmic Factors in Stabilization of Ca^{2+}-Transporting Function of Myocardial Sarcoplasmic Reticulum in Rats During Adaptation to Stress

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Acute stress reduces and adaptation to stress enhances thermal resistance of Ca^{2+} pump of the sarcoplasmic reticulum fraction. Soluble cytoplasmic factors increase the rate of Ca^{2+} transport into myocardial sarcoplasmic reticulum and its thermal resistance in the stressed, stress-adapted, and control rats, the activating effect being most pronounced during acute stress. Structural and functional mechanisms underlying the protective effect of soluble cytoplasmic factors on membrane-bound enzymes are discussed.

Key words: *Ca transport; cytoplasm; stress; adaptation*

Stress and harmful exogenous factors induce a variety of adaptive changes occurring not only at the organ and tissue level [4], but also in cell membranes. The concept of membrane adaptation was helpful in studying the adaptive defense mechanisms and kinetics of the reactions associated with maintenance of Ca^{2+} homeostasis in myocytes accomplished by Ca^{2+} pump of the sarcoplasmic reticulum (SR) [1,6]. Apart from increased quantitative characteristics of Ca^{2+} pump function, higher resistance of SR Ca^{2+}-transporting system to various damaging factors has been observed during adaptation. The qualitative changes play a leading role in the dynamics of adaptation [2,8]. However, it is not clear whether such changes are restricted by membrane-bound enzymes, or other cellular components, in particular, soluble cytoplasmic factors (SCF) are also involved in these processes.

The aim of this work was to study the effect of SCF on Ca^{2+}-pumping function in myocardial SR during stress and adaptation.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-220 g. The animals were divided into four experimental groups (n=7 in each): 1) control group; 2) long-term immobilization stress (with fixed limbs in the supine position, 3 h); 3) adaptation to short-term stress (on day 1 — 15 min, 2 — 30 min, 3 — 45 min, for subsequent 11 days — during 60 min every other day); 4) long-term immobilization (3 h) 1 day after the course of adaptation to short-term stress. The animals were decapitated 2 h after the stress or 24 h after completion of the preadaptation course. The heart was removed, washed in ice-cold physiological saline, and stored in liquid nitrogen before use.

On the day of experiment, the heart was minced in a blade Ultra-Turrax homogenizer during 30 sec in a medium containing 100 mM KCl, 10 mM histidine, pH 7.5 (1:4 tissue/medium ratio). The homogenate was then centrifuged for 20 min at 10,000g. The supernatant was filtered through 4 layers of gauze and centrifuged for 60 min at 105,000g. The final supernatant was stored and used in further experiments as a soluble fraction, while the sediment was suspended in a small volume of saline containing 0.6 M KCl and 5 mM histidine (pH 7) using a Teflon-glass homogenizer. The suspension was centrifuged for 60 min at 105,000g and the sediment containing SR membrane vesicles was resuspended in the storage medium consisting of 20% glycerin, 5 mM imidazole (pH 7), and 50 mM KCl. All procedures were carried out at 3-4°C in order

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to protect SR Ca^{2+} pump from endogenous factors. The preparations of SR were stored in liquid nitrogen.

The rate of Ca^{2+} transport in SR was measured on an Orion EA 940 ionometer (Orion Research) with a Ca^{2+}-selective electrode [13] by the rate of Ca^{2+} uptake by SR vesicles at 37°C in a medium (7 ml) containing 100 mM KCl, 15 mM potassium oxalate, 20 mM HEPES (pH 7.1), 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 reaction was initiated by addition of SR membranes (40 μg protein) suspended in the homogenization medium (120 μl) or SCF (120 μl). The data were processed statistically using Student's *t* test.

Thermal resistance of the Ca^{2+}-transporting system was assessed as described elsewhere [5,6]. To this end, SR preparations (40 μg of protein) with 120 μl homogenization medium or SCF were incubated at 41°C under constant stirring. Aliquots for measuring the rate of Ca^{2+} transport were taken after 5, 10, and 20 min of incubation. Phenylmethylsulfonyl fluoride (5 μg/ml) was added for preventing protease activation.

RESULTS

The initial rate of Ca^{2+} transport measured under optimal conditions in SR preparations isolated from animals exposed either to stress or adaptation was close to the control. However, the resistance of Ca^{2+}-transporting system to damaging factors varied significantly in these groups.

In the control, thermal denaturation (41°C) in the absence of SCF reduced Ca^{2+}-ATPase transporting activity of the SR membrane fraction almost linearly to 66% of the baseline after 10-min and to 35% after 20-min incubation (Fig. 1). Immobilization stress promoted inactivation of Ca^{2+} transport 1.4-fold in comparison with the control. After adaptation to repeated stress the rate of Ca^{2+} transport during the first 5 min of thermal denaturation remained virtually unchanged and then declined 1.3-fold slower than in the control group: 56% of the baseline Ca^{2+}-transporting activity was preserved after 20-min incubation *vs.* 37% and 23% in control and stressed animals, respectively. In adapted rats subjected to stress (group *4*), Ca^{2+}-ATPase transporting activity decreased most slowly, and the thermal stability in this group was 1.3 and 4-fold higher than in rats subjected to adaptation (group *3*) or stress (group *2*), respectively. After 20-min incubation the rate of Ca^{2+} transport in SR in this group was 1.5- and 2-fold higher than in groups 1 and 2, respectively. Thus, adaptation to stress considerably increased the thermal resistance of SR membranes, and activity of the Ca^{2+} pump after a long-term immobilization stress remained as stable as in the adapted rats not subjected to stress.

The addition of SCF to control SR samples had no effect on the initial rate of Ca^{2+} transport (Fig. 2, *a*), but considerably improved thermostability of this membrane-bound system. After stress, the addition of SCF stabilized the initial activity during first 10 minutes of thermal denaturation. After 20-min incubation the rate of Ca^{2+} transport was 42% of the baseline (Fig. 2, *b*), which was 1.2-fold higher than in the control and 2-fold higher than in the stressed rats in the absence of SCF. In stress-adapted animals, the rate of Ca^{2+} transport in the presence of SCF increased to 121% of the baseline at the 5th min, returned to the baseline at the 10th min (93%), and then decreased more slowly than in the control (at the 20th min the rate of Ca^{2+} transport 1.5-fold surpassed the control level, Fig. 2, *c*).

We have previously shown that the thermal resistance is an indicator of structural damage of the enzyme and its susceptibility to denaturation. For instance, sarcolemmal Na,K-ATPase loses its native conformation upon acute stress and its thermal resistance significantly decreases [5]. In our experiments stress drastically disturbed the native structure of membrane-bound Ca^{2+} pump of SR, thus accelerating its denaturation. However, SCF released in response to stress can almost completely protect the protein complex and restore its thermal stability. Adaptation-induced conformational changes in the Ca^{2+} pump protein complex may account for nearly maximum protective effect, and further addition of SCF only slightly improved the thermal resistance in contrast to their considerable effect in stressed rats. The protective effect of adaptation was so potent that stress had no effect on the Ca^{2+} pump thermal stability.

Thus, SCF do not change significantly the baseline rate of Ca^{2+} transport in the myocardial SR under

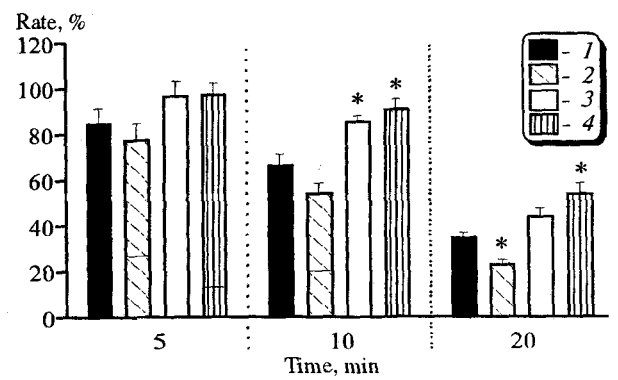

Fig. 1. Inhibition of Ca^{2+} transport into myocardial sarcoplasmic reticulum (%) during thermal inactivation at 41°C in the absence of soluble cytoplasmic factors in the control (*1*), after stress (*2*), after adaptation to immobilization stress (*3*), and after adaptation with subsequent stress (*4*). Here and on Fig. 2: *$p<0.05$ compared with the control.

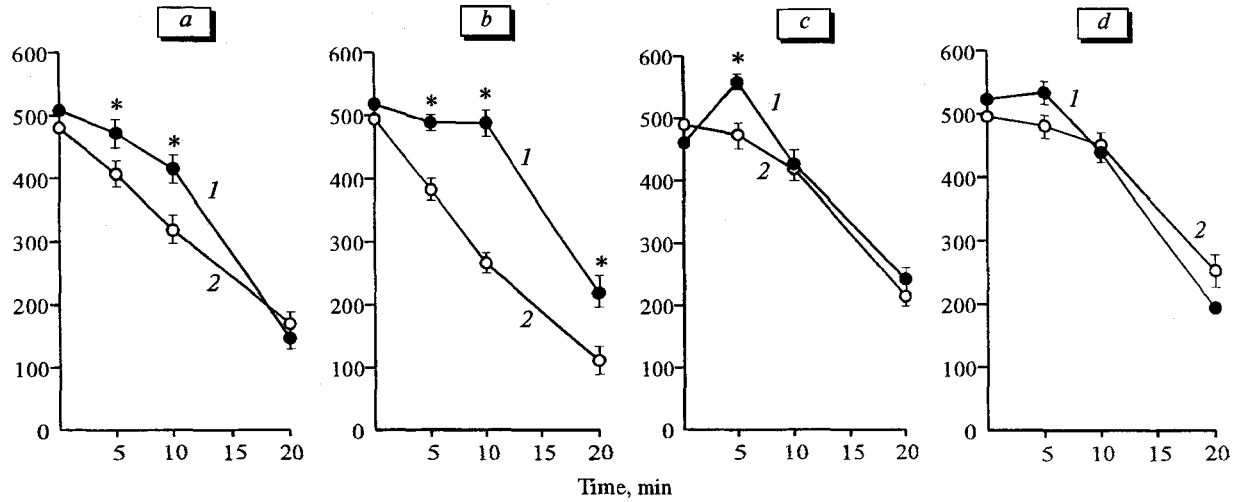

Fig. 2. Inhibition of Ca^{2+} transport into myocardial sarcoplasmic reticulum (nM/min/mg of protein) during thermal inactivation at 41°C in the control (*a*), after stress (*b*), after adaptation to immobilization stress (*c*), and after adaptation with subsequent stress (*d*) in the presence (*1*) and in the absence (*2*) of soluble cytoplasmic factors.

optimal conditions, but considerably improved the resistance of Ca^{2+} pump to damaging factors, in particular, thermal denaturation in all experimental groups. The protective effect of SCF on the Ca^{2+} transport depends on the initial state of this system. The maximum protective effect was observed during stress, when thermal stability of Ca^{2+} pump in SR was minimal. The minimal effect was observed in adaptation to stress (maximum thermostability). In the control, the effect was intermediate.

In the control, SCF-induced activation was most pronounced at the beginning of thermal inactivation (5-10 min) and became negligible at the later stages (Fig. 2, *a*). During stress, SCF produced a considerable activating effect (2-fold surpassing that in the control) throughout the period of thermal denaturation (Fig. 2, *b*). In adapted rats, SCF significantly activated Ca^{2+} transport only at the early stages (5 min). However, due to the initial (in the absence of SCF) increase of Ca^{2+} pump thermostability, the high rate of Ca^{2+} transport in SR in the presence of SCF was preserved for a longer period (by 62% higher than in the control, Fig. 2, *c*).

In group 4 (adaptation+stress) the SCF-induced activation was also significant during the first 5 min, while at the later stages SCF did not produce any protective effect (Fig. 2, *d*). This can be attributed to the fact that apart from induction of defense factors, adaptation is accompanied by inactivation of some systems ("payment for adaptation"). Therefore, in adapted rats stress can reduce the efficiency of the cell antioxidant enzyme system [16] and activate some Ca^{2+}-ATPase damaging factors (Ca^{2+}-dependent neutral proteases, multikinase complex) [14].

Thus, adaptation to immobilization stress improves the resistance of membrane-bound enzymes in SR, in particular, their thermal stability. Our previous experiments demonstrated similar effects of adaptation to stress and hypoxia on the resistance of these systems to high Ca^{2+} concentrations and activation of free radical oxidation [1,13]. In adapted animals, the thermal resistance of Ca^{2+} pump of myocardial SR increases in the presence of SCF. Moreover, stress adversely affects the SR membrane by decreasing the resistance of Ca^{2+} pump to damaging factors (which can serve as a marker of stress) rather then changing the baseline rate of Ca^{2+} transport. SCF produce a considerable protective effect on the membrane-bound enzymes during stress. It is noteworthy that SCF can produce both the positive and negative effects on membrane enzymes, and we can measure only their integral effect. Since both effects take place in a real cell, our model approximates to *in vivo* situation. It was most clearly seen when the effect of SCF on activation of Ca^{2+} pump of SR during stress and adaptation was assessed, which is presumably related to interactions between the enzyme molecule and different SCF components reactivating thermally denaturated Ca^{2+} pump protein complex.

Acute or repetitive oxidation stress (ischemia, stress or hypoxia) is accompanied by accumulation of protective SCF in the cytosole, in particular, antioxidant enzymes [7], specific modulator proteins similar to recently identified 12 kDa Ca-ATPase shaperon [9], nonspecific HSP protein, and other immediate response proteins [11,15]. The effect of SCF depends on the nature of damaging factor. For instance, unlike antioxidant enzymes, vitamin E, and specific shaperons, HSP do not protect Ca^{2+} pump of myocardial SR from ischemia-reperfusion-induced damage [15]. Taking into consideration a short duration of acute stress

stimulus, we can hypothesize that immediate response proteins, in particular, *c-fos* and *c-mys* oncogenes are involved in the protective response of SCF. The concentrations of these proteins increased after 1-4 sessions of short-term (5-10 min) ischemia and reperfusion [10]. Expression of *c-fos* and *c-mys* oncogenes in the myocardium increase during stress and in the early period of adaptation, while Ca^{2+}-ATPase gene expression and the rate of Ca^{2+}-transport in SR remain unchanged [3,8].

Recent studies have shown that cardiomyocyte cytoplasm contained up to 5% of a-crystallin stabilizing the conformation of membrane structures [17]. This protein can play a role in the studied protective effect probably due to homology of crystallin monomer with low-molecular weight HSP proteins [12].

In conclusion, membrane adaptation of Ca^{2+} pump of SR is not only formed at the level of the membrane enzyme complex, but is modulated by cell SCF.

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