Ca^{2+} Transport into Sarcoplasmic Reticulum and Immediate-Early Response Proteins in the Myocardium of Rats Resuscitated after Systemic Circulatory Arrest

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Activity of antioxidant defense enzymes and content of stress protein HSP70 in the heart increased in passive and, to a lesser extent, in active rats on day 7 of the postresuscitation period after systemic circulatory arrest. The resistance of membrane structures in the heart to endogenous damaging factors in passive rats was lower than in active animals. The degree of compensation in active rats was much higher than in passive animals at these terms of the postresuscitation period.

Key Words: *systemic circulatory arrest; antioxidants; HSP70; sarcoplasmic reticulum; Ca^{2+} transport*

Acute hypoxic and ischemic injury to the brain after systemic circulatory arrest is mediated by reactive oxygen species (ROS) that damage cell membrane structures [11,12,15].

Morphological study of rat brain after clinical death produced by cardiac arrest revealed changes in neurons in various CNS structures during the early posthypoxic period [13,14]. The general pathogenetic mechanisms of postresuscitation encephalopathies were elucidated [1]. In recent years much attention was paid to the progression of these processes not only immediately after acute exposure, but also at various terms of postresuscitation period (days 7, 30, *etc.*). Postresuscitation disease is a nonlinear and dynamic process [2]. Recent experiments revealed differences in the postresuscitation recovery of behaviorally active and passive animals [4]. These data explain the existence of different approaches to brain protection during the postresuscitation period.

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The mechanisms of cardiac dysfunction after resuscitation (particularly, in the delayed period) are poorly studied, despite the fact that the effectiveness of cardiovascular function largely determines the course of the postresuscitation period. Our previous experiments showed that acute ischemia and reperfusion are accompanied by a considerable suppression of antioxidant defense enzymes, accumulation of ROS, and changes in the membrane Ca^{2+} transporting system of the myocardial sarcoplasmic reticulum (SPR) [9]. This system maintains Ca^{2+} homeostasis and provides contractile function of the heart, which is impaired during ischemia and reperfusion.

The individual heart resistance to ischemia and reperfusion was studied in various animal species. Differences were revealed in the resistance to acute myocardial infarction in August and Wistar rats differing by parameters of stress reactivity [3]. Stability of myocardial antioxidant defense system during ischemic and reperfusion injury also differs in these animals [9], which correlated with the resistance of contractile function to these conditions. However, the mechanisms and degree of cardiac recovery in the delayed period after ischemia and reperfusion produced by systemic circulatory arrest remain unknown.

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Here we studied the resistance of cardiac membranes and intracellular protective system in behaviorally active and passive animals during the delayed postresuscitation period after systemic circulatory arrest (day 7).

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 200 g. Depending on behavioral activity in the elevated plus-maze the rats were divided into groups of behaviorally active and passive animals.

Circulatory arrest was modeled on the 10th minute of ether anesthesia by clamping of the vascular trunk [5]. The animals were resuscitated by cardiac massage, intratracheal administration of 0.1 mg/kg epinephrine, and jet ventilation of the lungs. The general state of animals, recovery of vital functions, and disappearance of neurological deficit were evaluated over 1 week after treatment [6].

External signs of neurological deficit disappeared 4-6 days after resuscitation. The animals were sacrificed with ether on day 7. The hearts were removed, frozen in liquid nitrogen, and homogenized in a medium containing 50 mM Tris-HCl and 100 mM NaCl (pH 7.2, 0°C, 1:6 tissue/medium ratio) using an Ultra-Turrox homogenizer for 40 sec. Activity of antioxidant defense enzymes was measured routinely. Catalase activity was estimated by the method of Luck. Superoxide dismutase (SOD) activity was determined spectrophotometrically by the difference between the rates of superoxide radical generation in the xanthine—xanthine oxidase system before and after addition of the sample.

Ca^{2+} transport in myocardial SPR was studied on an Orion EA 940 ionometer (Orion Research) equipped with a Ca^{2+}-selective electrode [8]. Incubation was performed in a medium containing 100 mM KCl, 20 mM HEPES (pH 7.0), 4 mM $MgCl_2$, 5 mM NaN_3, and 15 mM sodium oxalate at 37°C. The results were analyzed on-line using special computer software. The Ca^{2+} transport system was assayed by studying the resistance of myocardial SPR Ca^{2+} pump to Ca^{2+} in high concentration and autolysis. The sample incubated in the same medium with protease inhibitors (FMSF, leupeptin, and pepstatin) under similar conditions served as the control for autolysis. We compared Ca^{2+} pump function in the presence of 14 and 24 μM Ca^{2+}. The content of Ca^{2+} impurities did not exceed 10^{-7} M. The rate of Ca^{2+} transport in intact animals practically did not differ in the presence of 14 and 24 μM Ca^{2+}. However, Ca^{2+} transport in SPR was decelerated after exposure to stress, hypoxia, physical activity, ischemia, and reperfusion.

Cytosolic HSP70 concentration was measured by Western blotting in 10% polyacrylamide gel followed by transfer to a PVDF membrane. Primary monoclonal antibodies against HSP70 (Stressgen) and secondary peroxidase-labeled antibodies (Jackson Immuno Research) were used. The samples of rat thymus isolated after heat exposure (41.5°C, 30 min) served as a positive control. Detection was performed by measuring chemiluminescence with ECL reagents (Amersham) and Kodak roentgenographic film.

The results were analyzed by parametric tests (Instat2 software).

RESULTS

Basal activity of antioxidant defense enzymes, concentration of stress protein HSP70, and Ca^{2+} pump function in myocardial SPR practically did not differ in active and passive intact animals (Figs. 1 and 2, Table 1).

Active and passive animals did not differ in the time-to-recovery of cardiac activity and natural respiration after circulatory arrest. Body weight significantly decreased in active (by 16.8±3.8 and 13.2±3.3 g on days 1 and 2 postresuscitation, respectively) and passive rats (by 20.4±2.8, 15.9±2.4, and 10.4±2.8 g on days 1, 2, and 3 postresuscitation, respectively, $p<0.05$). External neurological status recovered in 91% active and passive animals by the 5th day after resuscitation.

Despite the absence of differences between intact animals with different behavioral characteristics by ac-

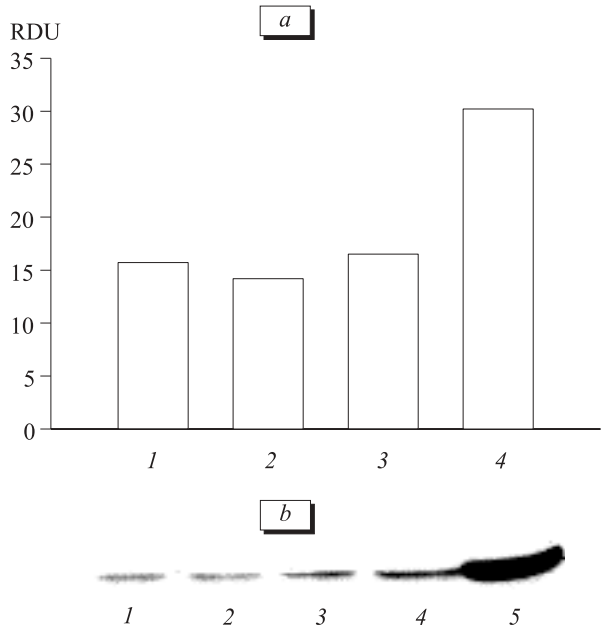

Fig. 1. Western blot analysis of stress protein HSP70 in the heart of resuscitated (RST) rats on day 7 after systemic circulatory arrest. Ordinate: relative densitometric units (RDU, *a*); proportional density and area of samples (standard treatment of scanned films with Photoshop software, *b*). Intact passive animals (*1*), intact active animals (*2*), RST active animals (*3*), RST passive animals (*4*), positive control for HSP70 (*5*). Thymus, 41.5°C, 30 min.

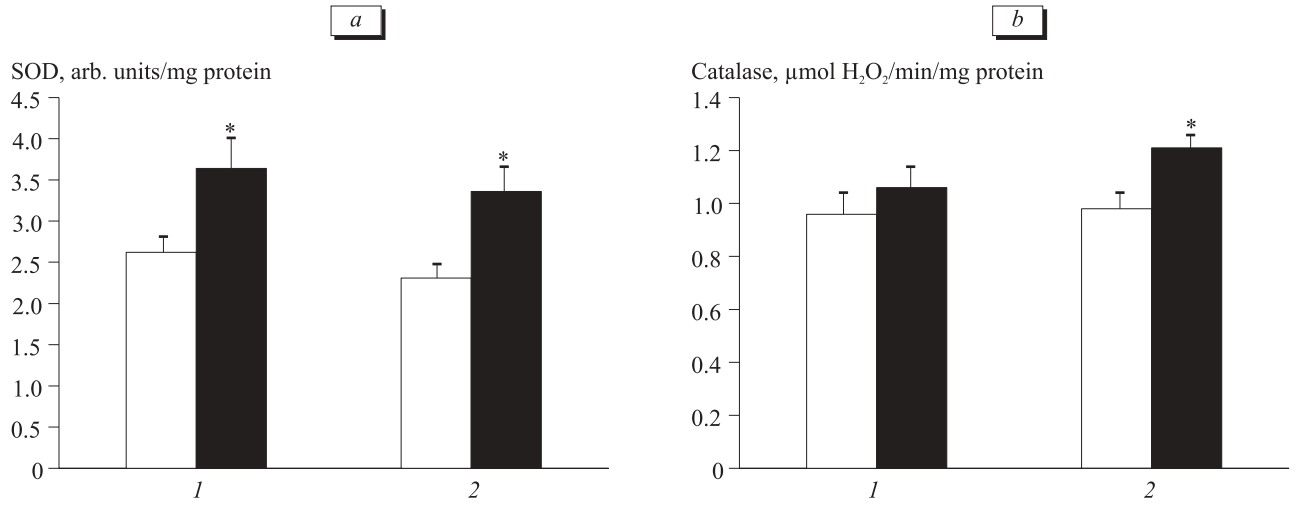

Fig. 2. Activity of antioxidant defense enzymes in the heart of resuscitated rats on day 7 after systemic circulatory arrest: SOD (*a*) and catalase (*b*). Active (*1*) and passive animals (*2*). Light bars: control. Dark bars: resuscitated animals. **p*<0.05 compared to the control.

tivity of the protective systems and resistance of myocardial membranes and also despite the fact that these indexes were recorded 7 days after resuscitation, which corresponded to the recovery of external neurological signs, active and passive animals differed by the degree of myocardial recovery.

TABLE 1. Resistance of Ca^{2+} Transport in Rat Myocardial SPR to High Concentration of Ca^{2+} (Ca^{2+} Transport Rate, -dmV/min, *M*±*m*)

Group	14 μM Ca^{2+}	24 μM Ca^{2+}
Active		
intact	2.15±0.25	1.84±0.25
resuscitated	2.27±0.30	1.77±0.19
Passive		
intact	1.99±0.25	1.52±0.12
resuscitated	1.76±0.16	1.57±0.13

TABLE 2. Resistance of Ca^{2+} Transport in Rat Myocardial SPR to Autolytic Damage (Ca^{2+} Transport Rate, -dmV/mg protein/min, 37°C, Protein Concentration 3 mg/ml, *M*±*m*)

Group	Before autolysis	After autolysis (20 min)
Active		
intact	2.20±0.21	1.58±0.06*
resuscitated	2.21±0.19	1.93±0.22
Passive		
intact	1.92±0.25	1.18±0.21*
resuscitated	2.34±0.18	1.58±0.18*

Note. **p*<0.05 compared to the corresponding parameter before autolysis.

Activation of intracellular protective systems in the myocardium differed in active and passive rats 7 days after 10-min circulatory arrest. In this period the concentration of immediate-early response proteins was estimated by the intensity of induced synthesis of heat shock protein HSP70 and activation of immediate-early proteins with antioxidant activity.

On day 7 after resuscitation the synthesis of stress proteins HSP70 in active and passive animals far surpassed that in intact animals (Fig. 1). Passive rats were characterized by a more significant increase in the expression of protective proteins, which agreed with our data on the postresuscitation induction of HSP70 synthesis in the brain. We showed that activation of protein synthesis in the heart differs in behaviorally active and passive animals. These data reflect the relationship between synthesis of protective stress proteins in the myocardium and individual behavioral characteristics of the animals.

The study of protective proteins with antioxidant activity produced similar results. Seven days after resuscitation activities of catalase and SOD exceeded the normal (Fig. 2). Antioxidant enzyme activity in the heart did not differ in intact active and passive animals. However, 7 days after circulatory arrest the increase in enzyme activity in the myocardium of passive rats was more pronounced than in active animals. Activities of SOD and catalase in resuscitated passive rats were higher than in intact animals by 45 and 21%, respectively. In resuscitated active rats we revealed a 27% increase in SOD activity, while catalase activity remained practically unchanged.

Thus, we observed intensive synthesis of protective proteins in the myocardium of passive animals even in the delayed period after resuscitation. The signal for protein synthesis was probably mediated by

ROS generation and persisted in passive rats during this period. These results are consistent with strong accumulation of lipid peroxidation products in passive rats after cerebral ischemia (as distinct from active animals) [7].

We evaluated whether activation of the myocardial protective system in behaviorally different animals can compensate for intensification of free radical processes and degradation of cell structures after ischemia and reperfusion produced by systemic circulatory arrest. The membrane Ca^{2+} transport system of myocardial SPR maintaining cell Ca^{2+} homeostasis was studied in intact and resuscitated rats with different behavioral characteristics. Our previous studies showed that function of this system significantly decreases upon activation of ROS-dependent processes (*e.g.*, after stress, ischemia, and reperfusion) [8,9]. It reflects the ratio between cell prooxidant and antioxidant systems that directly or indirectly (via modulation of the lipid bilayer) affect the sensitivity of Ca^{2+} transport in SPR to endogenous damaging agents.

Basal Ca^{2+} transport in myocardial SPR did not differ in intact and resuscitated rats with different behavioral characteristics (day 7 of the postresuscitation period, Table 1). Therefore, Ca^{2+} transport in SPR was completely recovered in behaviorally passive and active animals. However, the resistance of this membrane system to endogenous damaging agents differed in active and passive rats.

The resistance of myocardial SPR Ca^{2+} transporting system in active and passive animals returned to normal 7 days after resuscitation (Table 1).

However, the resistance of this membrane Ca^{2+} transporting system to autolysis differed in active and passive animals (Table 2). Ca^{2+} transport in intact active and passive animals decreased after autolysis over the specified period (by 29 and 39%, respectively). On day 7 after resuscitation, the resistance of SPR Ca^{2+} pump in passive rats was similar to that in intact animals. The resistance of this membrane system to autolysis not only returned to normal, but even increased in active rats. On day 7 of the postresuscitation period, the rate of Ca^{2+} transport in SPR of active rats did not differ from the basal level 20 min after autolysis. However, the rate of this process in passive rats decreased by 30% and was 22% lower compared to active animals.

Our findings suggest that synthesis of antioxidant and immediate-early response protective proteins in the heart decreased on day 7 of the postresuscitation period after systemic circulatory arrest. These changes in passive rats were more pronounced than in active animals. However, the resistance of cardiac membrane systems to endogenous damaging agents in passive rats was lower than in active animals. The degree of compensation in active rats was much higher than in passive animals at this stage of the postresuscitation period.

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