HSP70 Stress Proteins, Nitric Oxide, and Resistance of August and Wistar Rats to Myocardial Infarction

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Accumulation of HSP70 stress proteins in the myocardium and blood content of nitrite/nitrate in August rats with modeled myocardial infarction surpassed these parameters in Wistar rats less resistant to cardiovascular disorders by 2-2.5 and 1.8 times, respectively. Our results suggest that various resistance of August and Wistar rats to myocardial infarction is related to genetically determined differences in the activity of HSP70 and nitric oxide systems.

Key Words: *August rats; Wistar rats; myocardial infarction; HSP70; nitric oxide*

Our previous studies showed that August rats are more resistant to myocardial infarction (MI) than Wistar rats (by the criteria of early mortality and contractile activity of the heart) [1]. The mechanism of these interstrain differences is unknown. It is well known that MI is always accompanied by pain and emotional stress reactions. The key element of this reaction is hyperactivation of sympathetic regulation of cardiac function. This factor plays an important role in the pathogenesis of arrhythmias [11,14] and damages to nonischemic heart tissues [11,12]. An important role in cardiomyocyte protection from ischemic and stress damage is played by stress proteins HSP70 synthesized in the myocardium [2,10]. On the other hand, MI is characterized by enhanced production of nitric oxide (NO) [5]. NO not only inhibits stress reaction [13], but also activates HSP70 synthesis [2]. In August rats the basal and stress-induced production of NO and reactivity of the NO system are higher than in Wistar rats [3,4]. These data suggest that the high resistance of August rats to MI can be related to high activity of the HSP70 and NO systems. For verification of this assumption we compared HSP70 content in the myocardium and intensity of NO production in August and Wistar rats with MI.

MATERIALS AND METHODS

Experiments were performed on male August and Wistar rats weighing 200.0±1.6 and 289.0±5.8 g, respectively. Acute MI was modeled by the method of H. Selye (ligation of the descending branch of the left coronary artery under ether anesthesia). Sham-operated (SO, surgery without ligation of the coronary artery) and intact rats served as the control. The animals were decapitated 2 days after ligation of the coronary artery or sham operation. The heart was immediately removed, the right ventricle, interventricular septum, and free left ventricular wall were dissected and placed in liquid nitrogen. Myocardial HSP70 content was measured in the cytosolic fraction by Western blot analysis. Heart samples were crushed in liquid nitrogen, placed in lysis buffer modified for HSP measurements, and homogenized in this buffer (1:3 tissue:buffer ratio). The homogenate was filtered through 8-layer gauze and centrifuged at 12,000*g* and 4°C for 20 min. The protein-containing supernatant was used for electrophoresis and blotting. The samples containing 200 and 125 μg total protein were subjected to electrophoresis in 12% polyacrylamide gel (PAAG) for 1 h [9]. Proteins were eluted from PAAG to a nitrocellulose mem-

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brane for 1 h [15]. The membrane was incubated in 5% delipidated dry milk in Tris-borate buffer for 1 h to block the remaining binding sites. The blots were incubated with mouse monoclonal antibodies against inducible HSP70 for 15 h (Amersham, dilution 1:500), washed, incubated with horseradish peroxidase-conjugated antimouse antibodies for 1 h (Amersham, dilution 1:6000), and developed using ECL RPN 2108 Batch 76 kits (Amersham). Myocardial HSP70 content was determined by the width of the corresponding band and intensity of its staining. NO system activity was estimated by the total content of stable NO metabolites (nitrates and nitrites) in the plasma, which reflects NO production [6]. The blood was taken after decapitation, stabilized with heparin, and centrifuged at 3000 rpm for 15 min. Plasma proteins were precipitated with 30% $ZnSO_4$ (1:20 v/v) and the samples were centrifuged at 3000 rpm for 15 min. Nitrates were reduced to nitrites in the presence of 0.5 M NH_4OH buffer (pH 9.0, 9:1 plasma:buffer ratio, v/v) in Nitralyzer reactors (World Precision Instruments). After reduction a plasma aliquot was mixed with the same volume of Griess reagent, incubated for 10 min at room temperature, and absorption was measured spectrophotometrically at 540 nm. Nitrite concentration was estimated by the calibration curve (5-50 μmol $NaNO_2$). The results were analyzed by Student's *t* test.

RESULTS

Over the first 48 h of coronary occlusion the mortality rate in August rats was much lower than in Wistar rats (22 and 69%, respectively), which is consistent with published data [1].

Myocardial content of HSP70 in intact, SO, and experimental (with MI) August rats 2-2.5-fold surpassed that in Wistar rats (Fig. 1, *a*, *b*). Western blot analysis of heart samples from Wistar rats revealed bands with various staining intensities, which allowed to estimate HSP70 accumulation in the myocardium in different experimental series (Fig. 1, *a*), while samples from August rats yielded only dark bands, which made impossible further evaluation of HSP70 accumulation in the myocardium in different experimental series (Fig. 1, *b*). In additional series, samples containing 125 μg total protein were used for electrophoresis (Fig. 1, *c*). These samples yielded narrower bands with lower staining intensity, which allowed us to estimate HSP70 accumulation in the myocardium in August rats in different experimental series. Accumulation of HSP70 in the myocardium of August and Wistar rats was most pronounced during MI. It should be emphasized that during MI the content of HSP70 in the left ventricle was higher than in the right ventricle and interventricular septum (particularly in Wistar rats). In

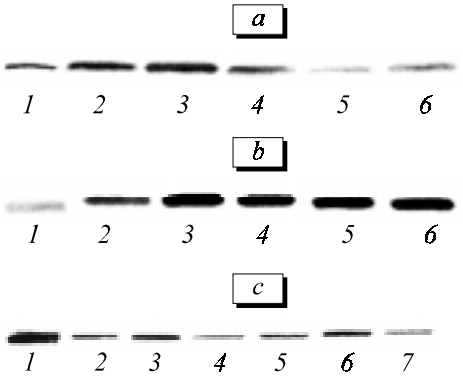

Fig. 1. Effect of acute myocardial infarction on myocardial HSP70 content in Wistar (*a*) and August rats (*b*, *c*). Western blot analysis of samples containing 200 (*a*, *b*) and 125 μg total protein (*c*). *a*, *b*) Left ventricle (*1*, *3*, *5*) and right ventricle with interventricular septum (*2*, *4*, *6*) in the control (*1*, *2*), after modeling of myocardial infarction (*3*, *4*), and after sham operation (*5*, *6*). *c*) Positive control (heat shock, *1*); left ventricle (*2-4*) and right ventricle with interventricular septum (*5-7*) in the control (*2*, *5*), after modeling of myocardial infarction (*3*, *6*), and after sham operation (*4*, *7*).

intact and SO animals HSP70 accumulation was less pronounced than in rats with MI. In these animals HSP70 content in the right ventricle and interventricular septum was higher than in the left ventricle.

Thus, HSP70 accumulation in the myocardium of August rats is much more pronounced than in control Wistar rats and Wistar rats with MI. It should be noted that activation of HSP synthesis during MI was more pronounced in the left ventricle, where 50-60% free wall area was occupied by necrosis. HSP70 synthesis was also activated in nonischemic myocardium, which probably represents a response to stress-induced damages. Our results are consistent with published data on HSP70 accumulation in ischemic and nonischemic rat myocardium [8]. Cardiac activity during MI depends not only on the area of necrosis, but also on the functional state of nonischemic working myocardium. It can be hypothesized that contractile activity of the myocardium in August rats is preserved due to more intensive production of HSP70 in all myocardial tissues.

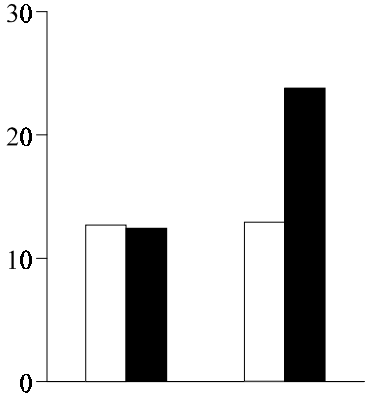

Fig. 2. Effect of acute myocardial infarction on plasma nitrate/nitrite content in Wistar (light bars) and August rats (dark bars): sham operation (*1*) and myocardial infarction (*2*).

Contractile activity of the heart in SO August and Wistar rats did not differ from the control [1]. Plasma nitrate/nitrite content was similar in SO animals of these strains. However, in August rats with MI the content of NO metabolites was 80% higher than in Wistar rats ($p<0.001$, Fig. 2). Electron paramagnetic resonance showed that cardiac NO content in Wistar rats decreases over the first 3 h after coronary occlusion, 2-fold increases 5 h after surgery, and than remains unchanged for 24 h [5]. In our experiments NO production in Wistar rats 2 days after modeling of MI did not differ from that in SO animals. However, the intensity of NO production in August rats with MI 2-fold surpassed that in SO animals and Wistar rats. These data suggest that high resistance of August rats to MI is associated with long-term stimulation of NO production. The protective effect of NO in August rats can be realized though inhibition of adrenergic mechanisms mediating the development of stress reactions and fatal arrhythmias [13] or activation of HSP70 synthesis [2]. The latter hypothesis is confirmed by published data that NO-induced activation of HSP70 synthesis reduces the severity of ischemic and reperfusion damages to rat cardiomyocytes [7].

Our findings suggest that different resistance of August and Wistar rats to MI is related to genetically determined differences in activity of stress-protecting HSP70 and NO systems in the myocardium.

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