GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Heart Resistance to Oxidative Stress in Rats of Different Genetic Strains

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In August rats reperfusion after regional myocardial ischemia *in situ* or intracoronary administration of hydrogen peroxide less significantly suppressed contractile activity of the heart compared to Wistar rats. Activities of catalase and superoxide dismutase in the myocardium during reperfusion remained unchanged in August rats. In Wistar rats a profound inhibition of cardiac function was accompanied by a decrease in enzyme activity.

Key Words: *August and Wistar rats; myocardial ischemia and reperfusion; antioxidant enzymes; hydrogen peroxide*

August rats differ from Wistar rats by lower mortality rate during acute myocardial infarction [2]. Moreover, August rats are characterized by lower incidence and shorter duration of severe arrhythmias during acute ischemia and reperfusion compared to Wistar rats [1]. Activation of free radical processes plays a major role in the pathogenesis of ischemic and reperfusion injury. Reactive oxygen species (ROS) produce damage to cell membranes and ion transport system, which impairs contractile function of cardiomyocytes and electrical stability of the heart. These data suggest that August and Wistar rats have different genetically determined resistance to oxidative stress. Here we studied changes in contractile function of the heart in response to ischemia, myocardial reperfusion, and H_2O_2-produced oxidative stress [7,9] and measured activities of antioxidant enzymes in the myocardium of August and Wistar rats.

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MATERIALS AND METHODS

In series I we studied contractile function of the heart during local myocardial ischemia and reperfusion and measured activities of antioxidant enzymes in the myocardium.

Experiments were performed on male Wistar (n=11, 373±16 g) and August rats (n=9, 231±9 g) of the same age. Open-chest surgery was performed under urethane anesthesia (2 g/kg) and artificial ventilation with atmospheric air (VITA-1 device). A catheter was inserted into the left ventricle (LV) through the apex. Local myocardial ischemia was modeled by ligation of the left descending coronary artery. The ligature was removed in the follow-up period. LV pressure was recorded over 30-min ischemia and 15-min reperfusion using a Mingograf-34 device (Siemens). We measured the heart rate (HR), systolic pressure, diastolic pressure, developed pressure, and rate of pressure rise and drop. HR was multiplied by developed pressure to calculate the double product index. The structure function intensity (SFI) coefficient was estimated as a ratio between the double product and unit of LV weight.

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Antioxidant enzyme activity was measured in the ischemic area of the free LV wall. Similar measurements were performed in control rats. Tissue samples were washed with physiological saline and frozen in liquid nitrogen. The hearts were minced in an Ultra-Turrax homogenizer for 30 sec and filtered. The homogenization medium consisted of 20 mM Tris and 100 mM NaCl (pH 7.4). The tissue/medium ratio was 1:9. We measured activities of superoxide dismutase (SOD) and catalase. Protein content was estimated by the amplitude of the fourth derivative of absorption spectrum at 240-320 nm.

In series II the resistance of contractile function to H_2O_2 was studied on isolated hearts. Body weights of Wistar (n=9) and August rats (n=8) were 420±10 and 238±6 g, respectively. The hearts were removed under urethane anesthesia and perfused through the aorta using a peristaltic pump. Perfusion was performed with modified carbogen-saturated Krebs—Henseleit solution containing 11 mM glucose at 37°C. The initial flow rate was adjusted so that perfusion pressure in the aorta attained 70 mm Hg. A catheter equipped with a fluid-filled latex balloon was introduced into LV to measure pressure. The volume of fluid was sufficient to produce LV diastolic pressure of 10-15 mm Hg. The pressure in the balloon was measured using a Gould 2400S polygraph and Gould Statham P23Db transducer. Cardiac function was monitored under baseline conditions, after gradual increase in the perfusion rate from 10 to 22 ml/min, and during administration of H_2O_2 with a Sage infusion pump. The rate of H_2O_2 perfusion provided a constant concentration of 100 μM.

The results were analyzed by Student's t test and Mann—Whitney U test.

RESULTS

Series I showed that contractile activity of the heart practically does not differ in Wistar and August rats under physiological conditions. The following indexes in Wistar rats were insignificantly higher than in August rats: developed pressure (129±7 and 123±8 mm Hg, respectively), HR (396±20 and 350±17 bpm, respectively), and SFI (88±6 and 86±8 mm Hg/mg/min, respectively). Regional myocardial ischemia was

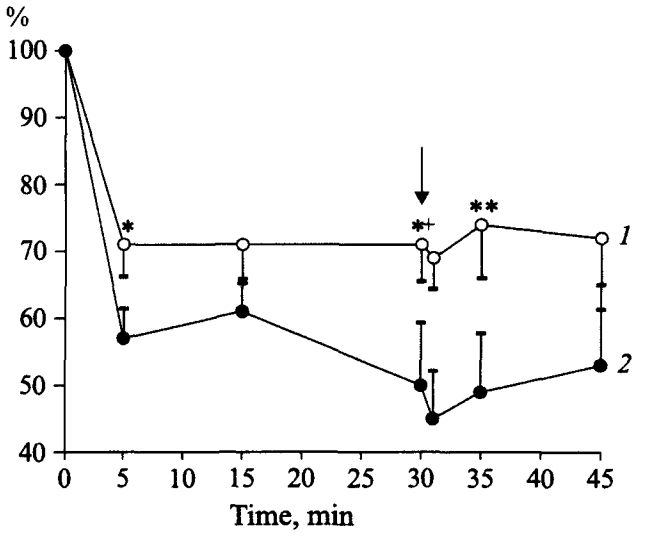

Fig. 1. Effect of myocardial ischemia and reperfusion on the structure function intensity coefficient in August (*1*) and Wistar rats (*2*). Arrow: start of perfusion. *p<0.05 and **p<0.01 compared to the control (Student's t test); +p<0.05 compared to Wistar rats (Mann—Whitney U test).

accompanied by a less significant decrease in cardiac function in August rats compared to Wistar rats (Fig. 1). By the end of ischemic period, SFI in August and Wistar rats decreased by 31 and 50%, respectively (p<0.05). In this period the absolute value of SFI in August rats was 32% higher than in Wistar rats (58.0±4.5 and 44.0±3.6 mm Hg/mg/min, respectively, p<0.05). After 5-min reperfusion the absolute value of SFI in August rats was 56% higher than in Wistar rats (64±4 and 41±7 mm Hg/mg/min, respectively, p<0.05). Greater efficiency of cardiac function in August rats was related to a lower degree of bradycardia. The weights of the heart and LV in August rats were higher than in Wistar rats by 14%. Our results are consistent with published data that August rats are more resistant to arrhythmia than Wistar rats [1]. Moreover, in August rats myocardial resistance to ischemia and reperfusion is higher than in Wistar rats.

These differences can be explained by 1) lower adrenoreactivity of the myocardium in August rats compared to Wistar rats [2] reducing the severity of adrenergic injury during acute myocardial ischemia; 2)

TABLE 1. Activities of Catalase and SOD in the Myocardium of Wistar and August Rats (M±m)

Enzymes	Rats (n=6)	Control	Ischemia	Reperfusion
Catalase, μmol H_2O_2/mg protein/min	Wistar	1.41±0.09	0.79±0.09**	0.94±0.09*
	August	1.06±0.17+	0.91±0.16	1.18±0.11
SOD, arb. U/mg protein	Wistar	6.03±0.64	6.49±0.86	4.72±0.53°
	August	4.89±0.45+	4.78±0.56+	4.56±0.59

Note. *p<0.01 and **p<0.001 compared to the control (Student's t test); °p<0.05 compared to the control (Mann—Whitney U test); +p<0.05 compared to Wistar rats (U test).

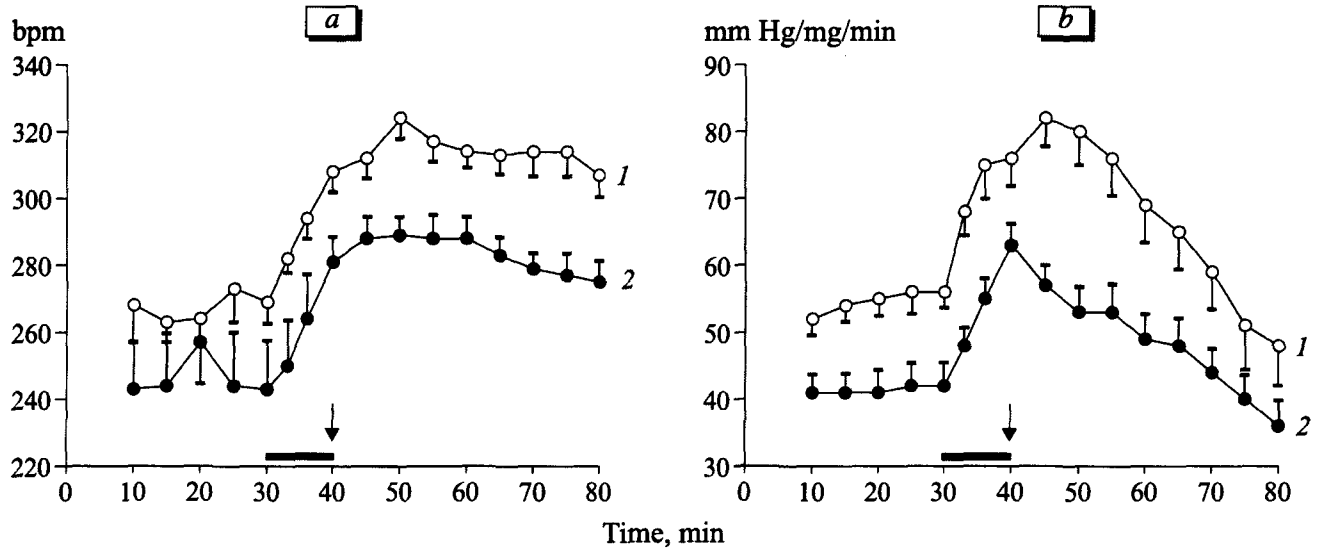

Fig. 2. Effect of H_2O_2 on HR (*a*) and structure function intensity coefficient (*b*) of the isolated heart in August (*1*) and Wistar rats (*2*). Line: increase in the coronary flow rate. Arrow: administration of H_2O_2.

high activity of the NO-generating system August rats [5], which suppresses the release of catecholamines from sympathetic terminals [10]; 3) different activity of antioxidant enzymes August and Wistar rats.

To test the latter hypothesis, we compared activities of SOD and catalase in August and Wistar rats after ischemia and reperfusion (Table 1). Under baseline conditions, enzyme activities in August rats were lower than in Wistar rats by 41 and 20%, respectively. Catalase activity in Wistar rats decreased by 44% after ischemia and was 33% below the baseline level by the end of reperfusion. SOD activity in Wistar rats decreased by 22% after reperfusion. It should be emphasized that in August rats activities of both enzymes remained unchanged. These data indicate that antioxidant enzymes in August rats are more resistant to myocardial ischemia and reperfusion compared to Wistar rats. High activity of catalase, which suppresses H_2O_2 generation from the toxic hydroxyl radical, is of particular importance in this respect.

We compared contractile function of isolated hearts. August rats differed from Wistar rats by a higher SFI coefficient under baseline conditions (56±4 and 42±3 mm Hg/mg/min, respectively, +33%, $p<0.01$) and during perfusion at the maximum rate (76 and 61 mm Hg/mg/min, respectively, +21%, $p<0.03$, Fig. 2). It was associated with higher HR in August rats compared to Wistar rats. Similar differences were observed in intact animals [1].

Addition of H_2O_2 to the perfusate significantly suppressed contractile activity of isolated heart in Wistar rats (Fig. 2), which manifested in a 2-fold decrease in SFI [4,7]. In August rats, SFI increased over the fist minutes after H_2O_2 application and remained high for more than 10 min (Fig. 2). Significant intergroup differences existed over 30 min after H_2O_2 administration (Fig. 2). In August rats this effect was related to a less significant decrease in developed pressure and greater increase in HR compared to Wistar rats. A similar but less pronounced positive chronotropic effect was observed in experiments on isolated heart [4] and isolated cardiomyocytes from Wistar rats. Treatment with catalase blocked this effect [9].

Series II showed that myocardial resistance to the toxic effect of ROS in August rats is greater than in Wistar rats. The observed differences are primarily related to the increase in HR in August rats produced by H_2O_2. This phenomenon should be studied in details to understand the mechanism of antioxidant protection of the myocardium. The SFI coefficient remained practically unchanged for at least 15 min (Fig. 2). Therefore, it is unlikely that treatment with H_2O_2 causes energy deficiency [7]. As differentiated from other mammals, contractile activity of the myocardium in rats depends on Ca^{2+} stores in the sarcoplasmic reticulum (SPR). It is important that H_2O_2 and caffeine cause similar effects [3]. Both compounds initially produce a positive effect, but then have a negative effect. Superoxide and H_2O_2 differently modulate ion transport. In low concentrations these compounds potentiate Ca^{2+} release from SPR, which is realized via the calmodulin-dependent mechanism [8]. In high concentrations superoxide and H_2O_2 inhibit sarcoplasmic Ca^{2+}-ATPase, decrease the volume of caffeine-mobilized Ca^{2+} stores in SPR [6], and activate Na^+/H^+ exchange [9]. It can be hypothesized that a constant activity of antioxidant enzymes in August rats prevents accumulation of excess ROS over the first minutes after H_2O_2 administration. These peculiarities contribute to the positive chronotropic effect

of H_2O_2. It should be emphasized that high resistance of the myocardium to oxidative stress in August rats is related to activity of other antioxidant components in cells, including glutathione peroxidase, ubiquinone, α-tocopherol, and glutathione. This problem requires further investigations.

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