BIOPHYSICS AND BIOCHEMISTRY

Sex-Related Differences in the Degree of Lipid Peroxidation Activation and Resistance to Cardiovascular Damage Induced by Stress in Rats

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It is shown that during stress a rapid twofold increase of erythrocyte acid resistance in rats of both sexes was followed by a 1.5-fold decrease toward the 60th min in males and the 120th min in females. In males, in contrast to females, the level of malonic dialdehyde was raised not only during stress, but also 1 and 24 hours after its completion. Stress-induced dystrophic changes of cardiomyocytes were more marked in males. The area of myocardial damage in females was almost twice as small as in males. It is assumed that the better resistance of females to stress-induced cardiovascular damage may be due to increased efficacy of antioxidant mechanisms inhibiting lipid peroxidation.

Key Words: *stress; sex-related differences, lipid peroxidation; antioxidant system; cardiovascular damage*

It has been established that decompensated activation of lipid peroxidation (LPO) during severe and long-lasting stress affects the integrity of cell membranes, leading to the damage of various organs, including the cardiovascular system [3,4,8]. The antioxidant system (AOS) in the female organism is known to be more active than in males, owing to antioxidative enzymes [14], alpha-tocopherol [15], estrogens that possess antioxidant properties [11], and a higher stress-induced glucocorticoid level [1]. This suggests that stress-induced responses in females are associated with a lesser activation of LPO and an increased resistance of the cardiovascular system. To check this hypothesis, we carried out a study of the degree of LPO activation and resistance to cardiovascular damage in female and male albino rats subjected to severe stress.

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MATERIALS AND METHODS

Experiments were carried out on 200 rats of both sexes. As an extreme agent acting on the cardiovascular system we used a two-hour acoustic stress against the background of strict immobilization [10]. For estimation of the integral ratio of LPO to AOS activity we studied changes in erythrocyte membrane structural-functional properties, evaluating the erythrocyte acid resistance (EAR) by the method of erythrograms [5]. The erythrocyte content of malonic dialdehyde was assayed as described earlier [12]. Indexes of LPO intensity were estimated in rats decapitated 30 sec and 10, 60, and 120 min after the start of stress, and 1 and 24 hours after its completion. Hearts taken immedi-

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ately and 1 and 24 hours following the discontinuation of stress were fixed in 10% formalin solution. Deparaffinized sections were stained with hematoxylin-eosin and iron hematoxylin after Heidenhain, Li, and Mallory. The size of the damaged myocardial area and number of functioning capillaries were estimated by the morphometric method. The results were statistically evaluated using Student's test.

RESULTS

Analysis of erythrograms showed that as early as 30 sec after the start of the experiment the resistance of erythrocyte membranes drastically increased. This led to a delay of the maximal hemolysis time by 1.8 and 1.6 times in females and males, respectively (Fig. 1), thus reflecting an increased resistance of most erythrocytes. The fraction of highly resistant erythrocytes also increased, as judged by the 1.6-fold increase of the total hemolysis time in both females and males (Fig. 2). Moreover, the indexes of the maximal degree of hemolysis were reduced more than twofold. The EAR increase may be a consequence of LPO inhibition in the early stages of stress. This process is thought to be mediated via AOS activation caused by an emergency release of catecholamines and glucocorticoids [2,3,7]. A short-term LPO inhibition at the beginning of stress, similar in females and males, was replaced by LPO enhancement and EAR decrease. Towards the 10th minute of stress a dramatic shortening of the maximal as well as the total hemolysis time took place in both females and males, the magnitude being independent of sex (Figs. 1 and 2). As stress progressed, males showed a higher level of LPO activation as compared to females, which led to a more rapid fall of EAR in them. Thus, at the 60th min the resistance of the bulk of the erythrocyte population was reduced to 42% of the control level, whereas in females the resistance did not differ from the control level. Only after the completion of stress was the maximal hemolysis time lowered in both females and males (Fig. 1). Notably, the total hemolysis time was shortened in females by the 60th min, and in males by the 120th min of stress (Fig. 2). In the post-stress period the sex-associated differences in the dynamics of EAR changes assumed a qualitative nature. In females the completion of stress was accompanied by a rapid increase in both the resistance of the main erythrocyte fraction and the number of highly resistant erythrocytes. Thus, 60 min after stress completion the maximal hemolysis time increased

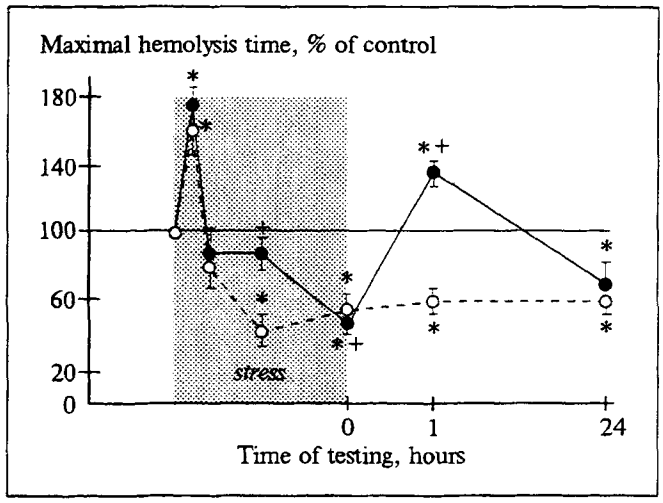

Fig. 1. Dynamics of maximal hemolysis time in female (unbroken line) and male (dotted line) rats subjected to experimental stress. Here and in Figs. 2 and 3: the difference is significant ($p<0.05-0.001$) as compared to the control (*) and males (+). Each point represents the mean value and standard deviation for 10–14 rats.

by 2.5 times as compared to the preceding level (under the stress conditions) and exceeded the control index by 40% (Fig. 1). The total hemolysis time showed a 1.7-fold increase, exceeding the control value by 53% (Fig. 2). Moreover, the maximal degree of hemolysis showed a 2.6-fold reduction. Increased cell membrane stability in the post-stress period in females reflected a shift in favor of AOS in the LPO/AOS balance. In males the EAR level of most erythrocytes remained at the stress level as long as 60 min after stress abolition (Fig. 1); the total hemolysis time rose only to the control level (Fig. 2), and the maximal degree of hemolysis showed a 1.5-fold reduction. The dynamics of the malonic dialdehyde content

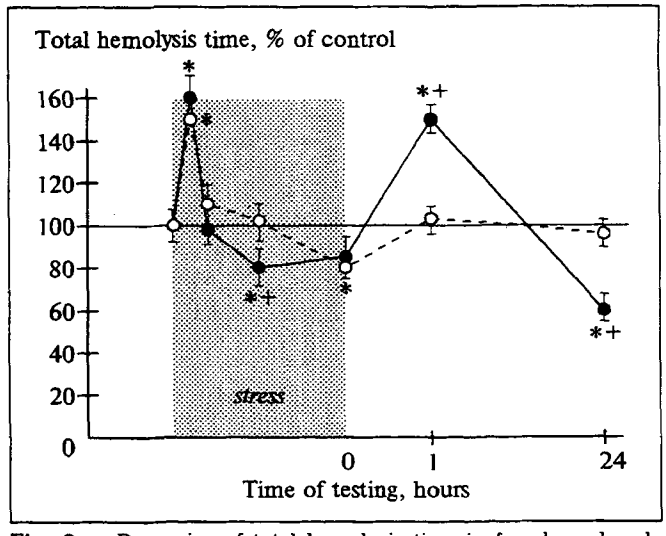

Fig. 2. Dynamics of total hemolysis time in female and male rats subjected to experimental stress.

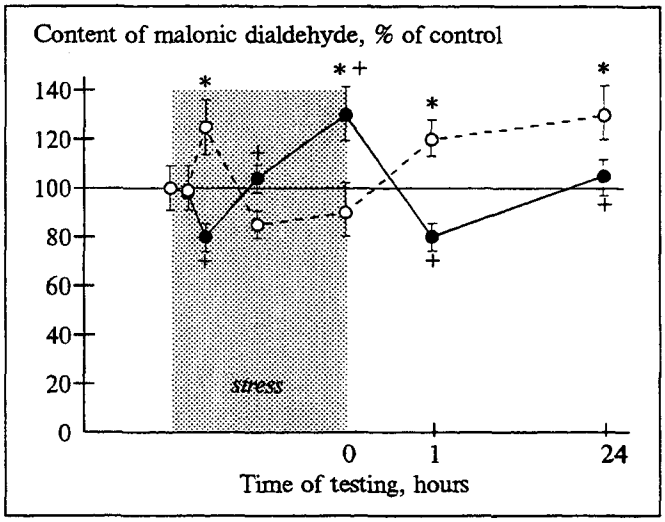

Fig. 3. Dynamics of erythrocyte content of malonic dialdehyde in female and male rats subjected to experimental stress.

in erythrocytes confirmed the sex-related differences in the activity of compensatory processes in the post-stress period. In distinction to females, males showed an increased content of malonic dialdehyde not only during stress, but also 1 hour and even 24 hours after its abolition, thereby providing evidence of long-lasting post-stress LPO activation (Fig. 3). This means that in females an increased AOS activity was expressed both during stress (in agreement with published data [9]) and after its end, creating more favorable conditions for restoration processes.

In both females and males stress was accompanied by disturbance of coronary blood flow and dystrophic alterations in cardiomyocytes. The deleterious effect of stress on coronary blood flow was comparable in females and males (Table 1) and was manifested in alterations of vascular permeability, leading to hemorrhages, and in dilatation of vessels with an increase in the number of functioning capillaries. Dystrophic cardiomyocyte changes in the middle layers of the left ventricle myocardium could be detected immediately after the removal of the stress factors. The myocardial damage was of a diffuse character and the areas of damage were similar in females and males (Table 1). During the first post-stress hour dystrophic processes in the myocardium progressed. Along with myofibril damage, the denaturation of sarcoplasm protein was observed as well as individual necrotized cells; in addition, altered cardiomyocytes were revealed in the subendocardial layer. However, in females the pathological processes were less marked, since the total area of damaged sites was 1.7 times less in females than in males (Table 1). Moreover, in two of 11 females (18%) focal myocardial damage did not exceed 3-4%. Twenty-four hours later, among the cardiomyocytes with segmental dystrophic alterations there appeared foci of necrotized cells which reflected further aggravation of the pathological process. However, in females, unlike males, this did not result in a substantial increase of the area of damaged myocardium. Therefore, one day after stress removal the sex-associated differences in the degree of myocardial damage became more pronounced. The area of diffuse myocardial damage in females was almost twice as small as in males (Table 1).

Thus, in the model of stress discussed, females proved to be more resistant not only to cerebrovascular damage, as shown by us earlier [1], but also to cardiovascular disorders. This is consistent with the data concerning the higher resistance of females to experimental myocardial infarction [6,13]. The inverse correlation between the level of LPO

Table 1. Development of Stress-Induced Cardiovascular Damage in Female and Male Rats

Time of testing	Sex	Number of functioning capillaries in visual field	Percentage of area of dispersed myocardial damage on heart section
	Control		
	Females (7)	10.8±0.7	–
	Males (10)	11.2±1.0	–
	Stress		
Immediately after stress abolition	Females (8)	15.8±1.5*	12.5±3.8
	Males (10)	18.2±0.8*	14.2±2.9
1 hour after abolition	Females (9)	16.8±1.0*	17.1±2.6**
	Males (10)	14.8±1.2*	28.6±3.4+
24 hours after abolition	Females (8)	16.1±0.7*	20.7±2.1**
	Males (10)	18.3±0.9*	38.2±3.6+

Note. Number of animals given in parentheses. The results differ reliably ($p<0.05-0.001$) from the control (*), from males (**), and from the preceding level (+).

activation and cardiomyocyte resistance to stress suggests that the increased resistance of females to cardiovascular diseases is largely due to increased AOS activity during and after stress.

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