Sex-Related Differences in Nitric Oxide Content in Healthy and Hypertensive Rats at Rest and under Stress Conditions

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Immobilization was followed by an increase in blood NO concentration in male and female rats. After renovascular hypertension modelingblood pressure was higher and the decrease in nitric oxide concentration was more pronounced in males than in females. The levels of nitric oxide in healthy and hypertensive females at rest and during stress were higher than in males. These specific features probably contribute to higher resistance of the cardiovascular system in females to pathological changes.

Key Words: *nitric oxide; stress; hypertension; cardiovascular system; sex differences*

Sharply increased stress load of modern life contributes to high cardiovascular mortality, particularly in men. Nitric oxide (NO) probably plays an important role in systemic mechanisms determining gender differences in the cardiovascular resistance. NO is involved in the regulation of cardiovascular function. This compound increases cholinergic [12], but inhibits sympathetic influences on the heart [14]. NO produces a regional vasodilatory effect [4] and possesses stress-limiting activity [3]. NO deficiency is accompanied by the development of hypertension [4]. *In vitro* experiments showed that NO production in the endothelium of various vessels (including coronary vessels) in females is higher than in males [11,13]. However, gender differences in activity of the NO-ergic system during cardiovascular dysfunction and stress remain little studied.

Here we measured NO concentration in blood plasma of healthy and hypertensive female and male albino rats under resting and stress conditions.

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MATERIALS AND METHODS

Experiments were performed on 172 healthy and hypertensive female and male rats. Goldblatt renal hypertension model was used [10]. A silver clip was applied under nembutal anesthesia (35-40 mg/kg). Persistent hypertension developed after 7 weeks. The animals were subjected to 60-min immobilization in a supine posture (immobilization stress, IS). On minutes 5 and 60 of IS and 5 and 60 min after it NO concentration was measured by the presence of nitrites in the blood using Griess reagent [2]. Normotensive females and males were exposed to the same stress. Our previous studies showed that blood corticosterone level returns to normal 1 day after surgery [1], therefore we used healthy, but not sham-operated rats as controls.

The differences from the initial level in the same group were evaluated by Wilcoxon test. Intergroup differences were evaluated using Mann—Whitney test and ANOVA-2 (post hoc analysis with

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Duncan's rank test). The differences were significant at $p<0.05$.

RESULTS

In female rats the basal blood level of NO was higher than in males. NO synthesis in stressed females increased more significantly than in males (Fig. 1, *a*). Blood NO concentration in females by the 5th and 60th minutes of IS increased by 1.7 ($p<0.05$) and 2.1 times ($p<0.05$), respectively. During these periods NO concentration in males increased by 1.3 ($p<0.05$) and 1.6 times ($p<0.05$), respectively. The stress-induced activation of NO production in females was more pronounced than in males, which increased the degree of sex differences in NO concentration. Under resting conditions blood NO concentration in females was 1.3-fold higher than in males, while by the 5th and 60th minute of IS this parameter in females was 1.7-fold higher than in males. Blood NO concentration rapidly returned to normal after the end of IS. In both females and males NO concentration did not differ from the basal level 5 minutes after the end of stress. During the recovery phase, blood NO concentration in females was higher than in males.

These data indicate that IS is accompanied by an increase in NO concentration in the blood, which is consistent with the results of previous studies obtained on other models of short-term stress [3]. The basal level of NO in females is higher than in males. Moreover, the stress-induced activation of NO production in females is more pronounced than in males. Therefore, activity of the NOergic system in females is higher than in males at rest [11,13] and during IS. We conclude that functional reserves of the NOergic system in females are greater than in males.

Impairment of renal blood flow with a clip was followed by the development of renovascular hypertension. This disorder in females was less severe than in males. Seven weeks after application of the clip the mean blood pressure in females and males increased to 118±2 ($p<0.05$) and 140±3 mm Hg ($p<0.05$), respectively. Blood pressure did not differ in normotensive females and males (96±3 and 99±2 mm Hg, respectively). The development of hypertension was accompanied by a decrease in blood NO concentration in females and, especially, in males. NO concentration in hypertensive females and males decreased by 1.2 and 1.9 times, respectively ($p<0.05$). Gender differences in NO concentration in the blood of hypertensive rats were more pronounced than in normotensive animals (females, 0.44±0.05 μg/ml; males, 0.22±0.03 μg/ml, $p<0.05$).

IS was accompanied by an increase in NO concentration in the blood of hypertensive rats with low basal activity of the NOergic system. However, NO concentration in these rats was lower than in stressed normotensive animals (Fig. 1, *b*). In contrast to healthy rats, in hypertensive animals blood NO concentration did not progressively increased by the 60th minute of IS. Taking into account low basal level of NO in hypertensive rats, we conclude that hypertension is accompanied by a decrease in reserve capacities of the NOergic system. Similarly to healthy animals, NO concentration in hypertensive females was much higher than in males. These differences were revealed during stress and post-stress period. Five minutes after the end of stress, blood NO concentration in females practically did not differ from the basal level. However, NO con-

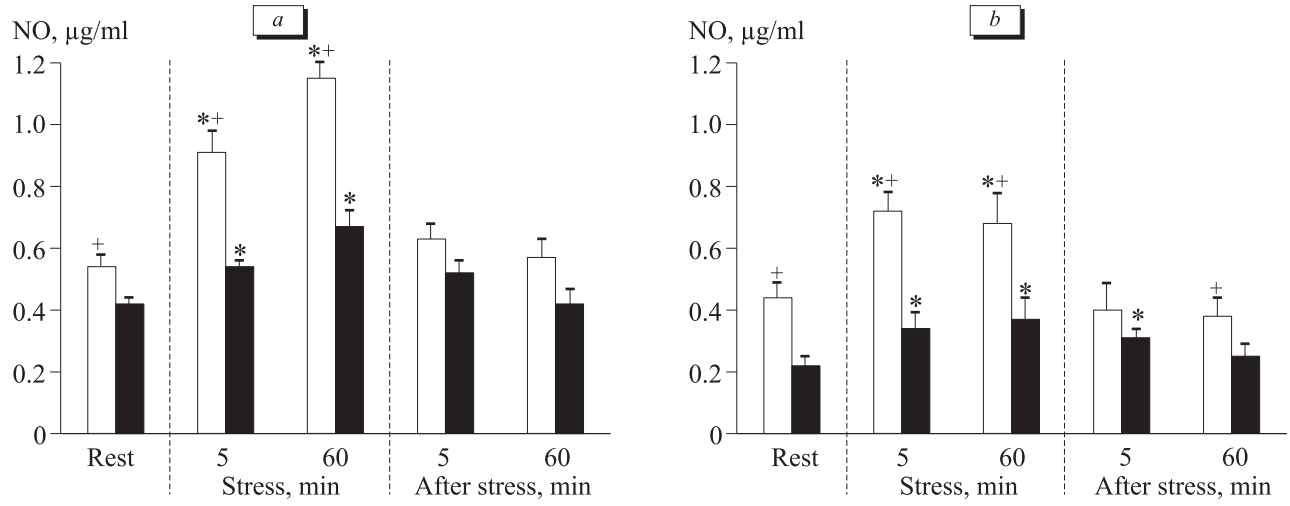

Fig. 1. Blood NO concentration in healthy (*a*) and hypertensive rats (*b*) at rest, during stress, and after stress. Light bars, females; dark bars, males. $p<0.05$: *compared to the basal level; +compared to males.

centration in the blood of males remained high during this period and returned to normal only by the 60th minute.

Therefore, the severity of hypertension associated with impairment of renal blood flow in females is lower than in males. These data are consistent with the results of previous studies on other models of hypertension [8]. NO concentration in the blood decreased insignificantly in hypertensive females, while in hypertensive males blood NO concentration was 2-fold lower than in normotensive rats. NO production in hypertensive females and males increased less significantly than in stressed normotensive rats. However, the poststress level of NO in the blood of hypertensive females was also higher compared to males.

The severity of hypertension in females was lower than in males. Moreover, NO concentration in the blood of females decreased less significantly than in males. Our results indicate that endothelial dysfunction underlies the pathogenesis of hypertension. These specific features probably contribute to high resistance of the female cardiovascular system to stress. The described mechanism includes function of the NOergic system. In the state of physiological rest and stress (*i.e.*, normal and pathological conditions), activity of the NOergic system in females is higher than in males. This conclusion is derived from data that the basal and stress level of NO in healthy and hypertensive females exceeds that in males.

High activity of the NOergic system in females is determined by estrogens that stimulate NO synthase formation and potentiate the endothelium-dependent effect of acetylcholine [5,6]. It should be emphasized that acetylcholine synthesis increases under the influence of NO [12]. The reciprocal stimulatory effects of NO and acetylcholine increasing NO production in females underlie more pronounced cholinergic influences on the cardiovascular system in males compared to males [7,9]. It reduces activity of this system and decreases the risk of cardiovascular dysfunction during stress.

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