Increased Stress Reactivity as a Possible Factor of Early Degenerative Changes in OXYS Rats

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After stress (immobilization in cages) plasma corticosterone level in prematurely aging OXYS rats was higher, while the content of NO metabolites was much lower than in Wistar rats. Stress increased blood pressure in OXYS and Wistar rats, the maximum values were observed in control and stressed OXYS rats. The concentration of reduced glutathione in the brain of OXYS rats was lower than in Wistar rats. After immobilization the concentration of reduced glutathione decreased in animals of both strains. The concentration of oxidized protein increased by 1.5 times only in OXYS rats. SOD activity remained unchanged, but in OXYS rats this parameter was higher than in Wistar rats. It can be hypothesized that high blood pressure, low NO content, high corticosterone concentration, and stress-induced deficiency of the antioxidant system (or combined effects of these factors) contribute to the development of neurodegenerative changes in the brain of OXYS rats.

Key Words: *stress; oxidative stress; premature aging; brain; OXYS rats*

Early changes in cognitive and emotional functions typical of elderly people and animals can be studied on the model of premature aging in OXYS rats [2,3]. These changes were believed to be associated with hereditary overproduction of free radicals. This assumption was based on the results of EPR studies demonstrated enhanced generation of oxygen radicals in the liver and myocardium of OXYS rats (compared to control animals) after addition of hydrogen peroxide [9]. No differences were revealed in the degree of oxidative damage to proteins and lipids in brain homogenates from 3-month-old Wistar and OXYS rats characterized by severe impairment of associative learning. Stress (especially chronic stress) is a risk factor for the development of early degenerative changes. Activation of the hypothalamic-pituitary-adrenal axis increases blood glucocorticoid concentration, promotes neurodegenerative processes in elderly people, and forms a basis for memory disorders [7]. The mechanisms of these disturbances include a direct damaging effect of hormones in high concentration on neurons, changes in energy metabolism, and oxidative stress. However, these mechanisms are poorly understood. Arterial hypertension can accelerate the development of cognitive dysfunction in the brain of OXYS rats. The increase in blood pressure in these animals is probably associated with NO deficiency, which alleviates the central and peripheral stress response [7]. Here we studied immobilization-produced changes in the adrenocortical and cardiovascular systems, NO concentration, and content of markers of oxidative stress in the brain of Wistar and OXYS rats.

MATERIALS AND METHODS

Experiments were performed on 74 OXYS and Wistar rats aging 3 months and obtained from the Laboratory of Animal Breeding (Institute of Cytology and Genetics). Blood pressure (BP) was measured in the caudal

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vein under light ether anesthesia 10 days before the start of the experiments. The animals were immobilized in tight metal-grid cages for 30 or 120 min. BP and concentration of corticosterone (CS) in the blood obtained from the caudal vein were measured in rats exposed to 30-min stress. Control Wistar and OXYS rats were exposed to 120-min stress and sacrificed by decapitation. The brain was removed, placed in liquid nitrogen, and stored at 70°C. Plasma CS concentration was measured by radioimmunoassay. The amount of carbonyl groups in the forebrain (parameter reflecting the severity of oxidative damage to proteins) was evaluated spectrophotometrically in the reaction with 2,4-dinitrophenylhydrazine [8]. Homogenates were prepared on cold phosphate buffer (25 mM, pH 7.4). SOD activity was determined by cytochrome *c* reduction in the xanthine—xanthine oxidase system. Reduced glutathione content was measured.

NO production was determined by measuring the content of stable metabolites in the plasma (NO_2^-/NO_3^-) with Griss reagent. Nitrates were preliminary reduced to nitrites using cadmium granules treated with $CuSO_4$ [6]. Data processing involved multifactor dispersion analysis (ANOVA/MANOVA) with post-hoc comparison of the means (Newman—Keuls test). The genotype and stress exposure were treated as independent variables.

RESULTS

Immobilization stress produced changes in the adrenocortical system, which manifested in increased plasma CS concentration ($F(2.48)=96$, $p=0$, Fig. 1). It should be emphasized that plasma CS concentration depended on the genotype of animals. Two-factor ANOVA showed that plasma CS concentration in OXYS rats is higher than in Wistar rats ($F(1.48)=4.54$, $p=0.038$). BP was elevated in OXYS rats ($F(2.56)= 62.9$, $p=0$, Fig. 2). Stress increased BP in Wistar and OXYS rats (by 29 and 25 units, respectively). The content of stable NO metabolites (NO_2^- and NO_3^-) in OXYS rats was lower than in Wistar rats ($p=0.025$, Fig. 3). Stress increased plasma concentration of NO_2^- and NO_3^- in both strains ($p=0.006$). After stress the content of these metabolites in OXYS rats was lower than in Wistar rats ($p=0.039$).

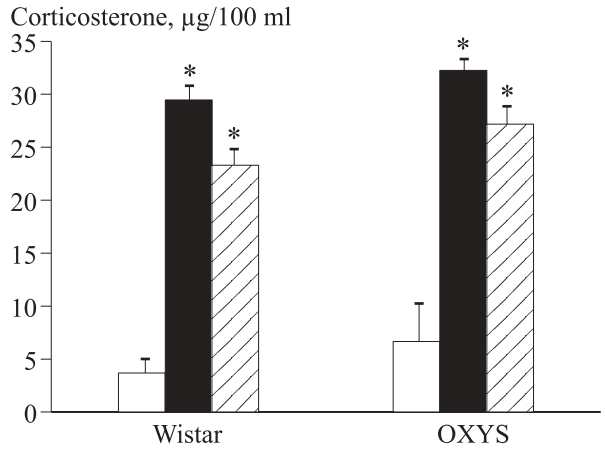

Fig. 1. Effect of stress on plasma corticosterone concentration in Wistar and OXYS rats. Light bars: control. Dark bars: 30-min stress. Shaded bars: 120-min stress. *$p<0.001$ compared to the control.

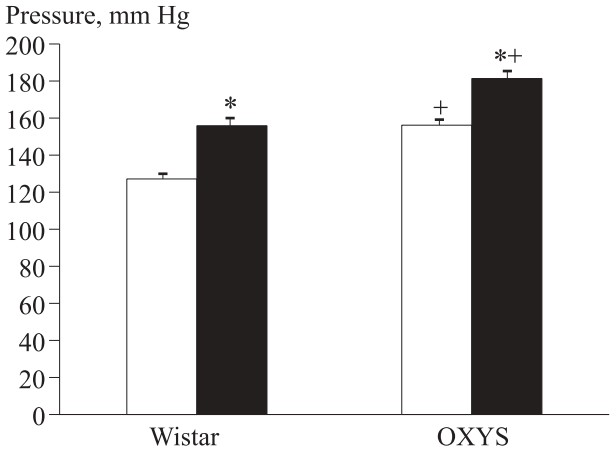

Fig. 2. Blood pressure in Wistar and OXYS rats under control conditions (light bars) and after 30-min stress (dark bars). $p<0.001$: *compared to intact animals; +compared to Wistar rats.

Two-hour immobilization activated free radical processes. The concentration of carbonyl compounds in the brain increased by 1.5 times in OXYS rats ($F(1.9)=10.5$, $p=0.01$), but remained unchanged in Wistar rats (Table 1). SOD activity in these animals remained unchanged after stress. Two-factor analysis showed that enzyme activity in OXYS rats was higher

TABLE 1. Effect of 2-h Immobilization on Brain Markers of Oxidative Stress in Wistar and OXYS Rats (*M*±*m*)

Parameter	Wistar		OXYS	
	control	stress	control	stress
Reduced glutathione, nmol/mg protein	218±17	177±6*	181±14+	150±6*+
SOD activity, U/mg protein	42.9±3.25	41.22±4.40	51.83±2.64	45.07±1.40
Carbonyl groups, nmol/mg protein	3.11±0.30	3.23±2.99	2.61±0.34	3.92±0.34*

Note. $p<0.01$: *compared to the control; +compared to Wistar rats.

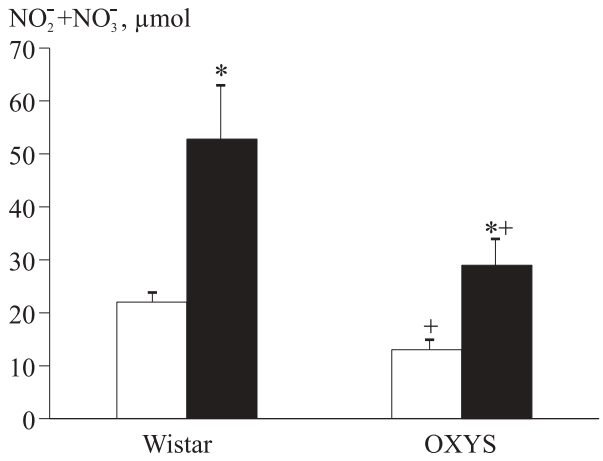

Fig. 3. Plasma concentration of stable NO metabolites in Wistar and OXYS rats under control conditions (light bars) and after 2-h immobilization (dark bars). *p<0.001 compared to intact animals; +p<0.01 compared to Wistar rats.

than in Wistar rats (p=0.028). Reduced glutathione content depended on stress exposure (F(1.18)=7.28, p=0.014) and animals genotype (F(1.18)=5.68, p=0.028). The content of reduced glutathione in OXYS rats was lower than in Wistar rats. Stress decreased the content of reduced glutathione in animals of both strains (Table 1).

Thus, we revealed increased activity of the adrenocortical system in OXYS rats. Only dispersion analysis revealed this minor, but sustained increase in CS concentration in different groups of animals. Low concentration of NO metabolites in the plasma of OXYS rats suggest that elevated BP in these animals can result from insufficient NO production. A negative correlation was found between NO concentration and BP in OXYS rats (r=-0.576, p<0.01). This relationship became more significant after stress (r=-0.74, p<0.024). NO is an important stress-limiting factor in the organism. NO deficiency affects animal resistance to environmental factors [5]. Chronic arterial hypertension of different genesis is accompanied by damage to arteries, circulatory disturbances, rapid development of atherosclerosis, and premature aging of vessels. The brain is the main target organ in this disorder. Long-lasting arterial hypertension is accompanied by structural and functional changes in cerebral arteries, which can lead to cognitive dysfunction.

A large body of evidence indicates that free radicals can produce changes in cells and tissues of living organisms, underlying their aging. The statement on hereditary overproduction of free radicals in tissues of OXYS rats [9] suggests that we know the source of this overproduction. According to modern notions, changes in mitochondria play a key role in aging of nondividing cells and are the main source of reactive oxygen species (ROS). Functional changes in mitochondria are revealed in young OXYS rats and become more pronounced in adult animals [1]. The data suggest that mitochondria play a role in intensive generation of ROS in tissues. ROS generation by liver mitochondria was studied in various metabolic states by chemiluminescence assay. The intensity of ROS generation in OXYS rats did not increase, but even decreased compared to Wistar rats. These differences in 12-month-old rats were more significant than in animals aging 4 months [4].

Our study showed that the degree of oxidative damage to proteins and lipids in the brain is similar in intact OXYS and Wistar rats. SOD expression increases in response to stimulation of ROS production. High activity of SOD and low content of glutathione can be considered as an indirect evidence for enhanced generation of oxygen radicals and strain of the antioxidant system in the brain of OXYS rats. Insufficiency of this system manifests under conditions of immobilization stress, which results in oxidative damage to proteins and lipids.

High BP, decreased NO level, high CS concentration in the blood, and stress-induced deficiency of the antioxidant system in the brain (or the combined effect of these factors) contribute to the development of neurodegenerative changes in OXYS rats. They manifest in activation of radical generation [10] and oxidative damage to lipids, proteins, and DNA in the brain of 14-18-month-old OXYS rats.

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