PHYSIOLOGY

Intensity of Oxidative and Antioxidant Processes in the Brain of Rats with Various Behavioral Characteristics during Acute Stress

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We studied the effect of acute emotional stress (1-h immobilization with simultaneous electrocutaneous stimulation) on the prooxidant-antioxidant balance in emotiogenic structures of the brain in rats with various behavioral characteristics. TBA-reactive substance content in the hypothalamus of rats remained practically unchanged after stress exposure. Opposite changes in activity of antioxidant defense enzymes in this structure of the brain in behaviorally active specimens probably compensate for the possible variations in LPO during emotional stress. Activities of glutathione reductase and Cu/Zn-containing SOD in the hypothalamus of passive animals decreased under these conditions. As differentiated from active rats, emotional stress in passive specimens was accompanied by the accumulation of TBA-reactive substances in the sensorimotor cortex and amygdala. The observed increase in glutathione peroxidase activity in passive animals probably serves as a secondary compensatory reaction to LPO activation. Our results illustrate specific changes in free radical processes and antioxidant defense in emotiogenic structures of the brain in rats with various behavioral characteristics after acute stress. These changes were more pronounced in behaviorally passive specimens than in active animals. It was probably related to differences in the oxidative status of CNS in rats with various prognostic resistance to similar stress factors.

Key Words: *prooxidant-antioxidant balance; emotional stress; brain; rats with various behavioral characteristics*

Modern life is characterized by strengthening of the tempo of life and associated with hypodynamia, informational overload, and increase in the incidence of social conflicts. These factors contribute to stress conditions. Emotional stress is accompanied by the development of severe psychosomatic diseases, including cardiovascular disorders, cerebral ischemia and stroke, neuroses, depressions, and malignant neoplasms [8].

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A change in the prooxidant-antioxidant balance of mammalian tissues serves as one of the pathogenetic mechanisms for stress injury [1]. The increased production of reactive oxygen metabolites and impairment of the prooxidant/antioxidant ratio in tissues are followed by hyperactivation of free radical LPO [9,12].

Variations in oxidative processes and antioxidant defense of brain structures are of considerable pathogenetic importance [7]. It is related to the excess of free oxygen and deficiency of antioxidant enzymes in nerve cells. These cells have the increased content of

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polyunsaturated fatty acids that serve as a target for radicals [9].

Much attention was paid to studying the effect of emotional stress on the prooxidant-antioxidant balance of the brain. However, specific features of the oxidative status in CNS tissues of mammals with various behavioral characteristics (*i.e.*, different prognostic resistance to stress) [3,8] are poorly understood.

Here we studied the effect of acute emotional stress on the ratio between oxidative processes and activity of the antioxidant system in CNS of rats with various behavioral characteristics.

MATERIALS AND METHODS

Experiments were performed on 52 male Wistar rats weighing 249.6±4.1 g. The experiment was conducted in accordance with the "Rules of Studies on Experimental Animals" (approved by the Ethics Committee of the P. K. Anokhin Institute of Normal Physiology; protocol No. 1, 3.09.2005), requirements of the World Society for the Protection of Animals (WSPA), and European Convention for the Protection of Experimental Animals.

The animals were housed in cages (6-7 specimens per cage) at 20-22°C and normal light/dark cycle (8.00-20.00, lightness; 20.00-8.00, darkness). They had free access to water and food. The animals were adapted to laboratory conditions for 5 days after delivery to the laboratory.

Individual and typological characteristics of rats were evaluated in the open-field test for 3 min. The method for behavioral testing of animals was described previously [3]. To calculate the index of activity, the sum of crossed peripheral and central squares, peripheral and central rearing postures, and explored objects was divided by the sum of latencies of the first movement and entry into the center of the open field.

Depending on the initial behavior in the open-field test, the animals were divided into active (n=26) and passive (n=26) specimens. They were characterized by high and low prognostic resistance to stress, respectively. These rats differed by mean index of activity (passive rats, 0.51±0.03; active rats, 3.49±0.49). In the follow-up period, the animals were divided into 8 groups of 6-7 specimens each.

Immobilization of rats in individual plastic cages and simultaneous delivery of subthreshold stochastic electrocutaneous stimulation (1 h) served as a model of acute emotional stress. This standard method of stress exposure was described previously [5]. Control (non-stressed) animals were maintained in home cages during this period.

Stressed and control rats were decapitated immediately after the experiment. The brain was removed rapidly after decapitation. The hypothalamus, sensorimotor cortex, and amygdala were isolated, frozen in liquid nitrogen, and stored in a freezing chamber at -24-26°C. The content of TBA-reactive substances (final products of LPO) and activity of antioxidant enzymes glutathione reductase, glutathione peroxidase, and Cu/Zn-containing SOD were measured in emotiogenic structures of the brain that play a key role in the stress response (hypothalamus, amygdala, and sensorimotor cortex). The amount of TBA-reactive substances in samples was estimated spectrophotometrically at 532 nm [14]. Activities of Cu/Zn-containing SOD, glutathione peroxidase, and glutathione reductase were measured spectrophotometrically [4,10,11,15]. The content of TBA-reactive substances was expressed in nmol/mg protein. Enzyme activity was expressed in U/mg protein. Protein concentration was measured by the method of Lowry [13].

The significance of between-group differences was evaluated by nonparametric Mann–Whitney test. The data are presented as the means and standard errors of the means.

RESULTS

In the initial state, the rats with various behavioral characteristics practically did not differ by MDA content in the hypothalamus (Table 1). The absence of significant differences in the intensity of LPO in the hypothalamus of active and passive rats is probably related to the same functional activity of the antioxidant system in these animals. It should be emphasized that antioxidant defense of the hypothalamus in specimens with these behavioral patterns was provided by various antioxidant enzymes. Behaviorally active rats were characterized by higher activity of hypothalamic glutathione peroxidase than passive specimens (by 1.52 times, p<0.01). By contrast, Cu/Zn-containing SOD activity in the hypothalamus of passive animals was 1.23 times higher than that in active rats (p<0.01).

The content of TBA-reactive substance in the sensorimotor cortex of behaviorally active rats (as distinct from the hypothalamus) was 1.45-fold higher than in passive animals (p<0.05). These differences were partially associated with lower activity of Cu/Zn-containing SOD in active specimens than in passive animals (by 1.17 times, p<0.01).

No statistically significant differences were found in the intensity of LPO and antioxidant enzyme activity in the amygdala of rats with various behavioral characteristics.

The ratio between oxidative and antioxidant processes in brain structures of animals was studied under acute stress conditions (Table 1). The content of

TBA-reactive substances in the hypothalamus of rats with different behavioral activity remained practically unchanged after acute stress. Opposite changes in activity of antioxidant defense enzymes in this structure of the brain in behaviorally active specimens probably compensate for the possible variations in LPO during emotional stress. Glutathione reductase activity decreased (by 1.76 times compared to non-stressed specimens, $p<0.05$), while Cu/Zn-containing SOD activity increased (by 1.43 times, $p<0.05$) in the hypothalamus of these animals after stress exposure. The development of a negative emotional state in passive rats was accompanied by a decrease in activities of glutathione reductase (by 1.45 times compared to non-stressed specimens) and Cu/Zn-containing SOD in the hypothalamus (by 1.27 times, $p<0.01$). A decrease in activity of these enzymes was not accompanied by variations in the intensity of free radical processes in the hypothalamus of passive animals. Our results suggest that the regulation of oxidative processes in the hypothalamus of passive rats under these conditions involves other compounds with antioxidant properties (not studied in the present work).

Acute stress was accompanied by similar changes in the intensity of LPO in the sensorimotor cortex and amygdala of rats with various behavioral characteristics. The content of TBA-reactive substances in the sensorimotor cortex of active specimens remained practically unchanged during immobilization with simultaneous electrocutaneous stimulation. Activities of Cu/Zn-containing SOD and glutathione peroxidase in the sensorimotor cortex of active animals tended to increase after stress exposure (by 1.24 and 1.17 times, respectively, compared to non-stressed specimens), which probably prevented activation of LPO under these conditions. No significant changes were revealed in the prooxidant-antioxidant balance of the amygdala in behaviorally active rats after acute stress.

Emotional stress was accompanied by the accumulation of TBA-reactive substances in the sensorimotor cortex and amygdala of passive animals (by 1.43 and 1.45 times, respectively, compared to non-stressed rats; $p<0.05$). The observed increase in glutathione peroxidase activity (by 1.29 [$p<0.05$] and 1.17 times in the sensorimotor cortex and amygdala, respectively) under these conditions probably serves as a secondary compensatory reaction to LPO activation. No significant changes were found in activities of glutathione reductase and Cu/Zn-containing SOD in these structures of the brain in passive rats.

We revealed significant changes in the prooxidant-antioxidant balance in the brain tissues from animals after acute emotional stress. It should be emphasized that the oxidative status of the hypothalamus, sensorimotor cortex, and amygdala of rats differs in the initial state and after stress exposure. These data show that emotiogenic structures of the brain are specifically involved in systemic organization of physiological

TABLE 1. TBA-Reactive Substances Content (nmol/mg protein) and Antioxidant Enzyme Activity (U/mg protein) in the Hypothalamus, Sensorimotor Cortex, and Amygdala of Rats with Various Behavioral Characteristics ($M\pm m$)

Brain structure	Parameter	Active rats (n=26)		Passive rats (n=26)	
		control	stress	control	stress
Hypothalamus	TBA-reactive substances	0.30±0.04	0.30±0.04	0.38±0.03	0.30±0.03
	Glutathione peroxidase	34.9±4.4^{++}	31.1±3.7	23.0±3.8	29.6±5.3
	Glutathione reductase	30.0±3.3	17.0±1.6*	35.2±6.8	24.2±3.9
	Cu/Zn SOD	388.2±30.0^{++}	554.3±30.5*	478.0±28.8	377.7±26.9**
Sensorimotor cortex	TBA-reactive substances	2.22±0.25^{+}	2.05±0.18	1.53±0.19	2.19±0.27*
	Glutathione peroxidase	24.1±1.6	28.2±3.5	22.0±1.3	28.4±1.8*
	Glutathione reductase	25.2±2.7	23.1±2.2	28.9±2.1	24.6±3.2
	Cu/Zn SOD	274.4±11.1^{++}	340.5±34.5	320.4±13.3	331.0±24.0
Amygdala	TBA-reactive substances	3.19±0.56	3.68±0.41	2.36±0.26	3.42±0.36*
	Glutathione peroxidase	33.7±3.3	32.7±2.7	33.5±5.2	39.1±4.0
	Glutathione reductase	27.1±2.1	24.5±1.0	29.2±2.5	24.5±2.4
	Cu/Zn SOD	406.7±20.8	380.8±12.4	350.1±10.5	352.6±25.2

Note. *$p<0.05$ and **$p<0.01$ compared to non-stressed rats; $^{+}p<0.05$ and $^{++}p<0.01$ compared to passive rats.

functions in mammals under normal conditions and during the development of a negative emotional state. The existence of regional differences in the oxidative status of brain structures is consistent with the results of previous experiments [6,7].

The content of TBA-reactive substances in the hypothalamus of rats (as distinct from the amygdala and sensorimotor cortex) remains practically unchanged after stress exposure. However, antioxidant enzyme activity in the hypothalamus varies significantly under these conditions. High functional activity of the hypothalamic antioxidant system under extreme conditions probably provides homeostasis of this pacemaker structure in the brain.

We showed that acute emotional stress is accompanied by accumulation of TBA-reactive substances in the sensorimotor cortex and amygdala of passive animals. These changes are followed by the increase in glutathione peroxidase activity. It can be suggested that the antioxidant enzyme glutathione peroxidase is most sensitive to stress-induced activation of LPO. Activation of glutathione peroxidase contributes to a rapid inhibition of oxidative processes in the mammalian brain during negative emotiogenic stimulation.

Our results illustrate specific changes in free radical processes and antioxidant defense in emotiogenic structures of the brain in rats with various behavioral characteristics during acute stress. These changes are more pronounced in behaviorally passive specimens than in active animals. It is probably related to differences in the oxidative status of CNS in rats with various prognostic resistance to similar stress factors.

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