Antioxidant Protection of the Brain in Rats during Acute Stress and Administration of Interleukin-1β

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We studied the effect of IL-1β on antioxidant enzyme activity in emotiogenic structures of the brain (hypothalamus, sensorimotor cortex, and amygdala) in behaviorally passive and active rats with different sensitivity to stress. One-hour immobilization of animals with simultaneous electrocutaneous stimulation was used as a model of stress. An intraperitoneal injection of IL-1β (5 μg/kg) was followed by the decrease in glutathione reductase activity in the hypothalamus of rats. Behaviorally active animals of the IL-1β group were characterized by an increase in the activities of Cu/Zn superoxide dismutase and glutathione peroxidase in the sensorimotor cortex and amygdala, respectively. IL-1β administration was accompanied by activation of Cu/Zn superoxide dismutase and glutathione peroxidase in the amygdala of passive rats. Pretreatment with IL-1β abolished the poststress changes in enzyme activity in the hypothalamus and sensorimotor cortex of active and passive rats, respectively. These data illustrate the specific effects of IL-1β on antioxidant protection of CNS tissues in rats with various behavioral characteristics.

Key Words: *interleukin-1β; emotional stress; antioxidant enzymes; brain; rats with various behavioral characteristics*

Emotional stress is an urgent problem of modern medicine and biology. Stress is followed by the development of severe psychosomatic disorders, including cardiovascular diseases, ischemia and stroke of the brain, neuroses, depressions, malignant neoplasms, and other disturbances [13].

Much attention is paid to a pro-inflammatory cytokine IL-1β, which serves as one of the mediators of the acute stress response [15]. IL-1β induces a cascade of cytokine secretion in the body and activates the hypothalamic-pituitary-adrenal complex [10]. This cytokine is involved in the central mechanisms of the stress response. It is related to the modulatory effect of IL-1β on the expression of genes for neurotransmitters and neurohormones and changes in the production and metabolism of these substances in the brain [15].

We previously evaluated the effect of IL-1β on LPO in CNS structures of rats [5]. The pathogenesis of stress disorders is associated with an increased generation of free radicals and change in the prooxidant/antioxidant ratio in tissues. These processes are particularly significant in CNS tissues due to the excess of free oxygen and antioxidant enzyme deficiency in nerve cells [11]. We revealed that IL-1β activates LPO in the hypothalamus, sensorimotor cortex, and amygdala of behaviorally passive rats, as well as in the hypothalamus of active rats. Prior intraperitoneal injection of IL-1β abolished a poststress change in LPO in the sensorimotor cortex of behaviorally passive animals [5].

Here we studied the effect of IL-1β on antioxidant enzyme activities (glutathione peroxidase, GP; glutathione reductase, GR; and Cu/Zn SOD) in CNS tissues of rats with various behavioral characteristics under basal conditions and during stress.

MATERIALS AND METHODS

Experiments were performed on 56 male Wistar rats weighing 249.6±4.1 g. The experiment was conducted

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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 (7 rats per cage) at 20-22°C and artificial 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.

Initial behavioral characteristics of rats were evaluated in the open-field test for 3 min [1]. 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 the latency of the first movement and entry into the center of the open field [4].

Depending on the initial behavior in the open-field test, the rats were divided into active (n=28) and passive (n=28) rats. These animals differed in the average index of activity (passive rats, 0.44±0.02; active rats, 3.48±0.31). Behaviorally active animals with high rate of orientation-and-exploratory activity in the open field are more resistant to stress than passive rats [1]. In the follow-up period, the rats were divided into 8 groups of 7 rats each.

Human recombinant IL-1β was obtained from the Federal State Unitary Enterprise "Research Institute of Highly Pure Biopreparations" (Federal Medical and Biological Agency of Russia). IL-1β in a dose of 5 μg/kg (activity 10^8 U/mg) was dissolved in 1 ml 0.9% NaCl (physiological saline, PS). IL-1β or PS (1 ml) was injected intraperitoneally 1 h before stress exposure. Control (non-stressed) rats received these injections 2 h before decapitation.

Immobilization of rats in individual plastic cages with simultaneous delivery of subthreshold stochastic electrocutaneous stimulation (1 h) served as a model of acute emotional stress [6]. Control (non-stressed) animals were maintained in home cages during this period.

Stressed and non-stressed rats were decapitated immediately after the experiment. Enzyme activity was measured in emotiogenic structures of the brain that play a key role in the stress response (hypothalamus, amygdala, and sensorimotor cortex). The brain was removed rapidly after decapitation. The hypothalamus, sensorimotor cortex, and amygdala were rapidly isolated, frozen in liquid nitrogen, and stored in a freezing chamber at -24-26°C. The activities of GP, GR, and SOD were measured spectrophotometrically [8,9,13,14]. Enzyme activity was expressed in U/mg protein. Protein concentration was measured by the method of Lowry.

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, behaviorally active rats were characterized by a higher activity of hypothalamic GP than passive animals (by 1.52 times, p<0.01). SOD activity in the hypothalamus of passive animals was 1.23 times higher than in active rats (p<0.01). Similarly to the hypothalamus, SOD activity in the sensorimotor cortex of passive animals was 1.17-fold higher than in active rats (p<0.01). No significant differences were revealed in antioxidant enzyme activity in the amygdala of rats (Table 1).

These data explain the results of our previous experiments. We showed that the intensity of oxidative processes in the hypothalamus and amygdala does not differ in active and passive rats. By contrast, the concentration of LPO products in the sensorimotor cortex of active rats was higher than in passive animals [5].

Acute stress was accompanied by a decrease in GR activity in the hypothalamus of passive and, particularly, of active rats (by 1.45 and 1.76 times [p<0.05], respectively, in comparison with non-stressed rats). Opposite changes were found in SOD activity in the hypothalamus of animals with various behavioral characteristics. Enzyme activity decreased by 1.27 times in passive rats (p<0.01), but increased by 1.43 times in active rats (p<0.05).

Activities of GP and SOD in the sensorimotor cortex of active animals were shown to increase after stress exposure (by 1.7 [p<0.05] and 1.24 times, respectively, in comparison with nonstressed rats). The development of a negative emotional state in behaviorally passive rats was accompanied by the increase in GP activity in the sensorimotor cortex and amygdala (by 1.29 [p<0.05] and 1.17 times, respectively).

The observed features of antioxidant protection in the brain during restraint stress are consistent with the results of experiments on other models of stress. Previous studies showed that GP activity in the brain cortex of rats increases during acute water-immersion stress and alternating exposure to emotiogenic and physical factors [3,7].

Then we studied the effects of IL-1β on antioxidant enzyme activity in the brain of non-stressed rats. An intraperitoneal injection of IL-1β was accompanied by the decrease in GR activity in the hypothalamus of behaviorally active and passive rats (by 1.32 [p<0.01] and 1.22 times, respectively, in comparison with PS-

TABLE 1. Antioxidant Enzyme Activity in the Hypothalamus, Sensorimotor Cortex, and Amygdala of Control and Stressed Rats after Administration of PS or IL-1β (U/mg protein, *M±m*)

Group; brain structure		Active rats			Passive rats		
		GP	GR	SOD	GP	GR	SOD
PS	hypothalamus	34.9±4.4^{xx}	30.0±3.3	388.2±30.0^{xx}	23.0±3.8	35.2±6.8	478.0±28.8
	sensorimotor cortex	24.1±1.6	25.2±2.7	274.4±11.1^{xx}	22.0±1.3	28.9±2.1	320.4±13.3
	amygdala	33.7±3.3	27.1±2.1	406.7±20.8	33.5±5.2	29.2±2.5	350.1±10.5
PS+stress	hypothalamus	31.1±3.7	17.0±1.6*	554.3±30.5^{x}*	29.6±5.3	24.2±3.9	377.7±26.9**
	sensorimotor cortex	28.2±3.5	23.1±2.2	340.5±34.5	28.4±1.8*	24.6±3.2	331.0±24.0
	amygdala	32.7±2.7	24.5±1.0	380.8±12.4	39.1±4.0	24.5±2.4	352.6±25.2
IL-1β	hypothalamus	29.8±1.9	22.7±1.9 $^{++xx}$	447.1±29.0	30.5±4.0	28.9±1.9	427.9±34.9
	sensorimotor cortex	23.9±2.0	25.5±2.3	420.0±29.7^{xx+}	26.1±2.1	31.6±4.6	332.1±24.8
	amygdala	45.2±3.2^{++}	30.0±2.1	358.2±16.8	46.3±4.7^{++}	29.4±2.6	417.2±22.8^{++}
IL-1β+stress	hypothalamus	28.8±1.5	24.4±2.0^{+}	465.2±20.0^{++xx}	26.3±2.4	30.9±4.9	397.8±19.4
	sensorimotor cortex	22.6±1.6	34.2±4.7^{++}	316.8±17.5^{xx}**	19.3±0.8^{*+}	30.6±2.4	364.1±11.1
	amygdala	33.8±2.3^{x}*	34.5±2.2^{+}	260.8±13.8^{+}*	45.6±1.3	34.9±3.5^{++}	288.6±8.5^{++}*

Note. PS or IL-1β: non-stressed rats receiving PS or IL-1β, respectively. PS+stress or IL-1β+stress: stressed rats after pretreatment with PS or IL-1β, respectively. *p<0.05 and **p<0.01 in comparison with the PS group and IL-1β group, respectively; ^{+}p<0.05 and ^{++}p<0.01 in comparison with PS-treated rats; ^{x}p<0.05 and ^{xx}p<0.01 in comparison with passive rats.

treated animals). These data are consistent with the results of our previous experiments. A decrease in antioxidant protection in the hypothalamus of IL-1β-treated rats is accompanied by activation of LPO [5].

SOD activity in the sensorimotor cortex of IL-1β-treated active animals increased by 1.53 times (p<0.05). IL-1β injection was followed by an increase in GP activity in the amygdala of non-stressed active and passive rats (by 1.34 and 1.38 times, respectively; p< 0.01). Under these conditions, SOD activity in passive rats increased by 1.19 times (p<0.01 in comparison with PS-treated animals).

We evaluated the effect of acute stress on antioxidant enzyme activity in the brain of IL-1β-treated rats. Pretreatment with IL-1β attenuated the decrease in GR activity and increase in SOD activity, which were revealed in the hypothalamus of active rats after stress exposure. GR activity in the hypothalamus of these animals was 1.44 times higher (p<0.05), while SOD activity was 1.19 times lower (p<0.01) than in stressed rats of the PS group.

IL-1β administration to passive rats was shown to abolish a stress-induced increase in GP activity in the sensorimotor cortex. GP activity in stressed animals of the IL-1β group was 1.47-fold lower than in PS-treated rats (p<0.05).

GR activity in the sensorimotor cortex of stressed active rats from the IL-1β group was 1.48 times higher than in non-stressed rats receiving this cytokine (p<0.01).

The exposure to acute stress after IL-1β administration was accompanied a decrease in SOD activity in the amygdala of active and passive rats (by 1.46 [p<0.05] and 1.22 times [p<0.01], respectively, in comparison with PS-treated rats). Under these conditions the activity of GR in animals of these groups increased by 1.41 (p<0.05) and 1.42 times (p<0.01), respectively.

Our experiments illustrate the oxidative status of emotiogenic structures in the brain of rats after acute stress and pretreatment with IL-1β. We showed that IL-1β has a protective effect on brain tissues and prevents the poststress changes in antioxidant enzyme activity in the hypothalamus and sensorimotor cortex of active and passive animals, respectively. The observed features of antioxidant protection in the hypothalamus, sensorimotor cortex, and amygdala of rats with various behavioral characteristics probably contribute to differences in the individual resistance of rats to stress.

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