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Effect of Interleukin-1β on Lipid Peroxidation in Emotiogenic Structures of the Brain in Rats during Acute Stress

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We studied the effects of immunomodulatory cytokine interleukin-1β on lipid peroxidation in emotiogenic structures of the brain (hypothalamus, sensorimotor cortex, and amygdala) of behaviorally active and passive rats with different prognostic resistance to stress. Immobilization of animals with simultaneous electrocutaneous stimulation (1 h) served as the model of acute emotional stress. Intraperitoneal injection of IL-1β (5 μg/kg) was followed by accumulation of malonic dialdehyde (end-product of lipid peroxidation) in all structures of the brain in passive rats, as well as in the hypothalamus of active animals. As differentiated from active rats, stress exposure in passive specimens was accompanied by a selective increase in malonic dialdehyde content in the sensorimotor cortex and amygdala. Pretreatment with IL-1β prevented activation of lipid peroxidation in the studied structures of the brain in passive rats after stress exposure. Our results show the specific effect of IL-1β on free-radical processes in the hypothalamus, sensorimotor cortex, and amygdala in rats with various behavioral parameters. Regional features of lipid peroxidation in emotiogenic structures of the brain in animals with different emotional reactivity probably contribute to the existence of significant variations in the individual resistance to emotional stress.

Key Words: *interleukin-1β; emotional stress; lipid peroxidation; brain; active and passive rats*

Acute or chronic conflict situations due to the inability to satisfy the major biological and social needs are followed by the development of stress and related psychosomatic diseases. Stress causes cardiovascular disorders, brain ischemia and strokes, atherosclerosis, neuroses, malignant neoplasms, and other disturbances [7,8].

Significant differences were found in the genetic and individual resistance of mammals to negative consequences of emotional stress [7]. The possibility of predicting the resistance or predisposition of subjects to emotional stress before a conflict situation is of considerable importance. Our studies showed that behavioral activity of rats in the open-field test serves as a reliable prognostic criterion for their resistance to stress [3]. Specifically, behaviorally active animals with high orientation and exploratory activity in the open field are more resistant to stress than passive rats.

The pathogenesis of stress dysfunction is related to increased generation of reactive oxygen metabolites, imbalance between prooxidants and antioxidants in tissues, and resulting alteration of free radical lipid peroxidation (LPO) [8]. These processes are most significant in brain structures [6]. It is associated with

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the excess of free oxygen and deficiency of antioxidant enzymes in nerve cells. Moreover, these cells are characterized by high content of polyunsaturated fatty acids that serve as the target for free radicals [8]. Considerable variability of oxidative processes in the brain contribute to the development of severe disorders, including schizophrenia, Alzheimer's disease, Parkinson's disease [8], and Down's syndrome [11].

Immune dysfunction is one of the most serious disturbances in mammals during emotional stress. It is manifested in changes in the cytokine profile of biological tissues. Cytokines are polypeptide transmitters of the cell-cell interactions that play a regulatory role in normal physiological functions, progression of the defense response to foreign factors, and impairment of tissue integrity [2]. Much attention to proinflammatory cytokine IL-1β is due to its biological functions in mammals. IL-1β induces a cascade of cytokine secretion in the body and modulates functional activity of the hypothalamic-pituitary-adrenal axis [10]. Our previous studies revealed specific features of cytokine involvement in the stress response of specimens with different emotional reactivity. Injection of exogenous IL-1β is followed by specific changes in serum cytokine concentration [4] and functional state of lymphoid structures in the gastrointestinal tract of behaviorally active and passive rats [1].

The relationship between immune variations and LPO in tissues of mammals with different prognostic resistance to the same type of stress remains unknown. The possibility for directed modulation of oxidative processes in the body by affecting the immune state is poorly understood.

Here we studied the effect of an exogenous immunomodulator IL-1β on LPO in CNS tissues of rats with different behavioral activity under control conditions and during acute emotional stress. The content of malonic dialdehyde (MDA; end-product of LPO) was measured in emotiogenic structures of the brain, which play a crucial role in the stress response. They include the hypothalamus, amygdale, and sensorimotor cortex.

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 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.

Individual and typological characteristics of rats were evaluated in the open-field test over 3 min. The method of studying the behavioral parameters 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 the latency 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 groups of active (n=26) and passive (n=26) specimens. These animals differed in the average index of activity (passive rats, 0.51±0.03; active rats, 3.49±0.49). In the follow-up period, behaviorally active and passive rats were divided into 8 groups of 6-7 animals each.

Human recombinant IL-1β was obtained from the State 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 sterile physiological saline (PS). IL-1β or PS (1 ml) was injected intraperitoneally 1 h before stress exposure. Control (nonstressed) rats received these injections 2 h before decapitation.

Immobilization of rats in individual plastic cages and simultaneous delivery of stochastic electrocutaneous stimulation (1 h) served as the model of acute emotional stress. This standard method of stress exposure was described previously [4]. Control (nonstressed) rats were maintained in home cages during this period.

Stressed rats and control animals 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. MDA content was measured spectrophotometrically at λ=532 nm [15]. MDA content was expressed in nmol/mg protein. Protein concentration was measured by the Lowry method [13].

Between-group differences were evaluated by nonparametric Mann–Whitney test. The data are presented as means and standard errors.

RESULTS

In the initial state, behaviorally passive and active rats did not differ in hypothalamic MDA content (0.38±0.03 and 0.30±0.04, respectively; Table 1). However, MDA content in the sensorimotor cortex and amygdala in active animals was higher than in passive specimens by 1.45 (p<0.05) and 1.35 times, respectively.

TABLE 1. MDA Content in Various Emotiogenic Structures of the Brain in Control and Stressed Rats with Different Activity in the Open Field after Injection of PS or IL-1β (nmol/mg protein, *M±m*)

Brain structure		Active rats (*n*=26)		Passive rats (*n*=26)	
		control	stress	control	stress
Hypothalamus	PS	0.30±0.04	0.30±0.04	0.38±0.03	0.30±0.03
	IL-1β	0.50±0.09^{+}	0.53±0.08^{+}	0.56±0.10^{+}	0.55±0.05^{+}
Sensorimotor cortex	PS	2.22±0.25^{x}	2.05±0.18	1.53±0.19	2.19±0.27*
	IL-1β	1.38±0.21^{+xx}	1.88±0.22^{x}	2.36±0.20^{+}	1.27±0.16**$^{++}$
Amygdala	PS	3.19±0.56	3.68±0.41	2.36±0.26	3.42±0.36*
	IL-1β	2.60±0.16^{x}	2.38±0.15^{+x}	4.05±0.60^{+}	3.38±0.47

Note. *p<0.05 and **p<0.01 compared to nonstressed rats; ^{+}p<0.05 and ^{++}p<0.01 compared to PS-receiving rats; ^{x}p<0.05 and ^{xx}p<0.01 compared to passive rats.

MDA content in the hypothalamus of rats with various patterns of the behavior receiving physiological saline did not change after stress (as compared to nonstressed rats; Table 1). However, acute stress was accompanied by a significant increase in MDA content in the sensorimotor cortex and amygdala of passive rats (by 1.43 and 1.45 times, respectively, compared to nonstressed specimens; p<0.05). As distinct from passive animals, these changes were not found in behaviorally active rats.

Our results confirm the hypothesis about regional differences of LPO processes in CNS structures of rats with various behavioral characteristics. Some authors reported that the intensity of lipid metabolism differs in various structures of the brain in experimental animals [5,6]. The observed distribution of LPO products in the hypothalamus and sensorimotor cortex of passive rats after immobilization and simultaneous electrocutaneous stimulation is consistent with the results of our previous experiments on the model of water-immersion stress [5].

Intraperitoneal injection of IL-1β significantly increased MDA content in the studies brain structures of nonstressed passive rats (Table 1). After treatment with IL-1β, MDA content in the hypothalamus, sensorimotor cortex, and amygdala of these animals was higher than in PS-receiving rats (by 1.47, 1.54, and 1.72 times, respectively; p<0.05).

An increase in MDA content after treatment with IL-1β was also found in the hypothalamus of nonstressed active rats (by 1.67 times compared to PS-receiving animals; p<0.05).

MDA accumulation in emotiogenic structures of the brain is consistent with published data on increased generation of reactive oxygen metabolites and subsequent activation of LPO (*e.g.*, in mammalian CNS) after administration of IL-1β [14]. One of the mechanisms for intensification of lipid metabolism is probably associated with the cytokine-induced inhibition of some antioxidant enzymes in the brain [9].

It should be emphasized that the production of free radicals and activation of LPO are required for a variety of cell functions. Reactive oxygen metabolites play a role in cell-cell interactions, prevent infectious processes, and suppress tumor growth due to the induction of cell apoptosis. Moreover, LPO is involved in the regeneration of biological membrane lipids [12].

As differentiated from behaviorally passive animals, MDA content in the sensorimotor cortex and amygdala of active rats was reduced after IL-1β injection (by 1.61 [p<0.05] and 1.23 times, respectively; Table 1). Due to the opposite changes in LPO in brain structures of IL-1β-receiving rats, MDA content in the sensorimotor cortex and amygdala of passive animals was higher than in active specimens by 1.71 (p<0.01) and 1.56 times (p<0.05), respectively.

Similarly to PS-receiving rats, hypothalamic MDA concentration in behaviorally active and passive animals of the stress group did not change after pretreatment with IL-1β (as compared to nonstressed specimens; Table 1).

Exogenous IL-1β prevented activation of LPO in the sensorimotor cortex and amygdala of passive rats, which was found in stressed animals after PS injection. MDA content in the sensorimotor cortex of stressed passive animals receiving IL-1β was lower than in rats of the PS group (by 1.72 times, p<0.01).

It remains unclear why emotional stress after pretreatment with IL-1β does not cause a change in MDA content in study structures of the brain in passive rats (as differentiated from PS-receiving animals). It could be expected that a combination of the factors activa-

ting LPO in the sensorimotor cortex and amygdala will result in the potentiation of their effects.

Our results show the differences of free radical processes in the hypothalamus, sensorimotor cortex, and amygdala of rats with various behavioral parameters in the open field. Regional features of lipid peroxidation in emotiogenic structures of the brain in animals with different emotional reactivity probably contribute to the existence of significant variations in the individual resistance of rats to stress. The mechanisms for action of an immunomodulatory cytokine IL-1β on LPO in mammalian CNS tissues require further investigations.

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