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Modulating Effect of Interleukin-4 on Free Radical Processes in the Brain of Rats during Emotional Stress

S. S. Pertsov, E. V. Koplik, and L. S. Kalinichenko

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We studied the effect of anti-inflammatory cytokine IL-4 on the intensity of free radical processes in emotiogenic brain structures (hypothalamus, sensorimotor cortex, and amygdala) in rats with different prognostic emotional resistance. One-hour immobilization of animals with simultaneous electrocutaneous stimulation was used as a model of acute stress. The stress was accompanied by accumulation of MDA (LPO end-product) in the sensorimotor cortex and amygdala of passive rats. Intraperitoneal administration of IL-4 (5 μg/kg) increased MDA content in the amygdala and hypothalamus of non-stressed rats with different behavioral activity. In the sensorimotor cortex of passive and, especially, active rats, a decrease in MDA level was observed after injection of this cytokine. Preliminary administration of IL-4 prevented LPO activation in the sensorimotor cortex of behaviorally passive animals observed after stress against the background of saline injection. Regional peculiarities of LPO under the influence of this cytokine can be determined by differences in both biochemical processes in the brain tissue and specific involvement of different emotiogenic structures in the formation of the stress response. The revealed differences in the effects of IL-4 on free radical processes in active and passive rats indicate peculiarities of immune mechanisms in animals with different resistance to the same type of stress.

Key Words: *interleukin-4; free radical processes; emotiogenic brain structures; rats with different resistance to emotional stress*

The problem of emotional stress is very important in modern medicine. Stress arises under conditions of acute or prolonged conflicts and leads to cardiovascular system disturbances, strokes, neuroses, cancer, and other pathological conditions [7,8].

In mammals, emotional stress is accompanied by an imbalance between oxidative and antioxidative reactions in the tissues. Excessive activation of free radical processes and, in particular, LPO in the cell structures is a universal mechanism of injury during stress [1]. The significance of these processes in CNS is related to an excess of free oxygen and deficiency of antioxidant enzymes in neurons and with high content of polyunsaturated fatty acids as the targets for the action of radicals, in brain tissue [8].

The pathogenesis of stress injuries is closely related to the development of the immune dysfunction. In mammals, changes in the cytokine profile in biological media during the formation of stress response were demonstrated [5]. In addition, emotional stress is accompanied by accumulation of cytokine mRNA in the brain [2].

P. K. Anokhin Institute of Normal Physiology of Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** s.pertsov@mail.ru. S. S. Pertsov

The data presented indicate a relationship between free radical processes and impaired immune status, in particular cytokine profile of the body, under conditions of emotional stress.

In our previous research, the pronounced modulating influence of key pro-inflammatory cytokine IL-1β on the intensity of free radical processes in the emotiogenic brain structures was revealed [4]. The impact of IL-1β on LPO differs in rats with various behavioral characteristics, which are considered as a prognostic criterion of animal resistance to emotional stress [3].

Published data suggests that anti-inflammatory cytokines are involved in the development of the stress response. In contrast to IL-1β, IL-4 reduces activity of the hypothalamic–pituitary–adrenal complex [15]. IL-4 inhibits production of inflammatory cytokines, *e.g.* IL-1β, and stimulates synthesis of its receptor antagonist [2,15].

The purpose of the work was to study the effects of IL-4 on free radical processes in the brain structures that play a decisive role in the development of stress response (hypothalamus, amygdala, and sensorimotor cortex). Particular attention was paid to peculiarities of the effects of this cytokine in rats with different prognostic resistance to emotional stress.

MATERIALS AND METHODS

The experiments were performed on 52 Wistar male rats weighing 249.6±4.1 g. The experiments were carried out in accordance with the Regulations of the work using experimental animals, approved by Ethic Committee of P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences (Protocol No. 1, September 3, 2005), Regulations of the World Society for Protection of Animals (WSPA), and European Convention for Protection of Experimental Animals.

Animals were kept in cages (6-7 animals in each group) in rooms with artificial illumination (8:00-20:00 light, 20:00-8:00 darkness) at 20-22°C under conditions of free access to food and water. The rats were adapted to laboratory conditions for 5 days.

Individual/typological characteristics of the rats were determined by open-field testing for 3 minutes. It is known that rat behavior in the open field test is a reliable indicator of their resistance to stress. Behaviorally active animals are more resistant to stressors compared to passive individuals [3]. To calculate the index of activity, the number of crossed peripheral and central sectors, peripheral and central rearing postures, and the number of explored objects were divided by the sum of latencies of the first movement and visit to the center of the open field.

Depending on the initial parameters of open-field behavior, the animals were divided into active (n=26) and passive (n=26). These animals differed by the mean activity index: 0.51±0.03 and 3.49±0.49 passive and active rats, respectively. Later, behaviorally active and passive rats were divided into 8 groups (6-7 animals in each group).

The animals received rat IL-4 (Sigma) in a dose of 5 mg/kg in 1 ml of sterile saline. IL-4 or saline (1 ml) was injected intraperitoneally 1 h before stress modeling. Control (non-stressed) rats received injections 2 h before decapitation.

Immobilization of animals in individual plastic boxes with simultaneous electrocutaneous stimulation at subthreshold intensity in the stochastic mode was used as the model of acute stress. The specified standard method of stress modeling was described earlier [5]. During this period, control (non-stressed) animals remained in home cages.

Rats subjected to emotional stress and control animals were decapitated immediately after the experiments. After decapitation, the brains were removed and the hypothalamus, sensorimotor cortex, and amygdala were isolated. These structures were frozen in liquid nitrogen and stored at -24-(-26)°C. The intensity of LPO was determined by the contents of MDA measured spectrophotometrically at λ=532 nm [11]. The content of MDA was expressed as nmol/mg protein measured by the method of Lowry.

The significance of differences between the groups was determined using nonparametric Mann–Whitney test. The data are presented as mean±SEM.

RESULTS

Initially, the content of MDA in the sensorimotor cortex and amygdala in behaviorally active rats was higher than in passive animals by 1.45 (p<0.05) and 1.35 times, respectively. MDA content in the hypothalamus was practically the same in animals with different behavioral parameters (Table 1).

Acute emotional stress was accompanied by an increase in MDA content in the sensorimotor cortex and amygdala of passive rats by 1.43 and 1.45 times, respectively (p<0.05), compared to non-stressed animals. MDA content in the hypothalamus of rats with different behavioral activity under these conditions was almost the same.

These findings are consistent with the results of our previous experiments demonstrating the presence of marked regional peculiarities of free radical processes in brain structures of rats [6].

Intraperitoneal injection of IL-4 was accompanied by MDA accumulation in the hypothalamus of active and passive rats by 3.27 (p<0.01) and 1.47 times, respectively (p<0.05), compared to animals receiving

TABLE 1. MDA Content in the Hypothalamus, Sensorimotor Cortex, and Amygdala in Control and Stressed Rats with Different Open-Field Activity Receiving Physiological Saline or IL-4 (nmol/mg protein, *M±m*)

Brain structure, preparation		Active rats (*n*=26)		Passive rats (*n*=26)	
		control	stress	control	stress
Hypothalamus	physiological saline	0.30±0.04	0.30±0.04	0.38±0.03	0.30±0.03
	IL-4	0.98±0.20^{++}	0.56±0.05^{++}	0.56±0.08^{+}	0.57±0.16
Sensorimotor cortex	Saline solution	2.22±0.25^{x}	2.05±0.18	1.53±0.19	2.19±0.27*
	IL-4	1.01±0.27^{+}	0.92±0.15^{++}	1.29±0.18	0.85±0.15^{++}*
Amygdala	Saline solution	3.19±0.56	3.68±0.41	2.36±0.26	3.42±0.36*
	IL-4	4.23±0.37	4.12±0.32	3.50±0.35^{+}	3.59±0.26

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

saline. MDA content in the amygdala of these animals significantly increased under these conditions by 1.33 and 1.48 times (p<0.05), respectively. Thus, administration of anti-inflammatory cytokine IL-4 led to activation of oxidative processes in the hypothalamus and amygdala of rats with different behavioral parameters. However, administration of IL-4 decreased the content of MDA in the sensorimotor cortex of passive and, especially, active rats by 1.19 and 2.20 times (p<0.05), respectively.

Later, we studied the effects of IL-4 on LPO in the emotiogenic brain structures of rats during stress. Under these conditions, MDA content in the amygdala did not change in animals with different prognostic stress resistance (Table 1). Preliminary administration of IL-4 prevented LPO activation in the sensorimotor cortex of passive rats detected after acute stress. MDA content in the sensorimotor cortex of passive rats subjected to immobilization with simultaneous electrocutaneous stimulation against the background of IL-4 treatment was by 1.52 times lower (p<0.05), than in stressed animals treated with saline. In contrast, stress exposure after injection of IL-4 was accompanied by an increase in the levels of MDA in the hypothalamus of active and passive rats compared to animals receiving saline. MDA accumulation after injection of IL-4 was observed in both controls and stressed animals with different behavioral activity (Table 1).

The revealed peculiarities of LPO in emotiogenic structures of the brain in rats treated with IL-4 can be due to the influence of this immunomodulator on the oxidative status. It was shown that IL-4 promotes the expression of lipoxygenase catalyzing oxidation of polyunsaturated fatty acids. This cytokine reduces activity of glutathione peroxidase catalyzing the formation of conjugated forms of glutathione [13]. These changes lead to intensification of LPO. It is also known that IL-4 activates glutathione-S-transferase protecting brain cells from reactive oxygen species [9].

The peculiarities of the action of IL-4 on LPO in different brain structures revealed in our experiments can be associated with topographical features of antioxidant defense in the CNS. In particular, maximum activity of glutathione-S-transferase in rats was noted in the hippocampus, brainstem, and cerebral cortex [12]. Maximum activity of lipoxygenase was detected in the hippocampus [10] and glutathione peroxidase in thalamic nuclei in mammals [14].

The results of our experiments demonstrate pronounced effect of IL-4 on free radical processes in the emotiogenic brain structures. The effects of IL-4 on LPO differ in behaviorally active and passive rats, which indicates peculiarities of immune mechanisms in animals with different prognostic resistance to emotional stress. Thus, our experimental data suggest that anti-inflammatory cytokine IL-4 is involved in the central organization of negative emotional states in mammals.

REFERENCES

1. V. A. Baraboy, *Lipid Peroxidation and Stress* [in Russian], St. Petersburg (1992).
2. C. A. Ketlinsky and A. S. Simbirtsev, *Cytokines* [in Russian], St. Petersburg (2008).
3. E. V. Koplik, *Vestn. Nov. Med. Tekhnol.*, **9**, No. 1, 16-18 (2002).
4. S. S. Pertsov, E. V. Koplik, L. S. Kalinichenko, and A. S. Simbirtsev, *Bull. Exp. Biol. Med.*, **150**, No. 7, 13-16 (2010).
5. S. S. Pertsov, E. V. Koplik, V. L. Stepaniuk, and A. S. Simbirtsev, *Ibid.*, **148**, No. 8, 161-165 (2009).
6. S. S. Pertsov and G. V. Pirogova, *Ibid.*, **138**, No. 7, 19-23 (2004).
7. K. V. Sudakov, *Emotional Stress: Theoretical and Clinical Aspects* [in Russian], Volgograd (1997).

8. R. M. Adibhatla and J. F. Hatcher, *Subcell. Biochem*, No. 49, 241-268 (2008).
9. S. Langouet, L. Corcos, Z. Abdel-Razzak, *et al.*, *Biochem. Biophys. Res. Commun*., **216**, No. 3, 793-800 (1995).
10. M. Nishiyama, T. Watanabe, N. Ueda, *et al.*, *J. Histochem. Cytochem*., **41**, No. 1, 111-117 (1993).
11. H. Ohkawa, N. Ohidhi, and K. Yagi, *Anal. Biochem*, **95**, No. 2, 351-358 (1979).
12. M. A. Otieno, R. B. Baggs, J. D. Hayes, and M. W. Anders, *Drug Metabol. Dispos*., **25**, No. 1, 12-20 (1997).
13. K. Schnurr, A. Borchert, and H. Kuhn, *FASEB J*., **13**, No. 1, 143-154 (1999).
14. G. Trepanier, D. Furling, J. Puymirat, and M. E. Mirault, *Neurosci*., **75**, No. 1, 231-243 (1996).
15. A. V. Turnbull and C.L. Rivier, *Physiol. Rev*., **79**, No. 1, 1-71 (1999).