Lipid Peroxidation and Antioxidant Enzymes in the Brain of Rats Exposed to Acute Emotional Stress: Effect of Interleukin-1β

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Acute emotional stress is shown to raise the level of malonic dialdehyde in the hypothalamus of August rats. After intraventricular administration of interleukin-1β, the malonic dialdehyde level and the activity of antioxidant enzymes tended to rise selectively in the hypothalamus (but not in the sensorimotor cortex) of August, Wistar, and WAG rats. In the presence of this interleukin, acute emotional stress did not cause increases in lipid peroxidation products in the hypothalamus of August rats.

Key Words: *emotional stress; interleukin-1β; hypothalamus; antioxidant enzymes; malonic dialdehyde*

Lipid peroxidation (LPO) is a universal nonspecific mechanism of tissue damage. The rate of LPO in brain structures has been shown to be influenced by emotional stress [9], and there are a variety of indications of LPO activation [3,7] or inhibition in the initial phases of chronic [1] and acute [2,5] emotional stress.

Previously, we described the regional distribution of LPO products and antioxidant enzymes in brain structures during emotional stress [8]. In rats immobilized for one hour, the accumulation of 2-thiobarbituric acid-reactive products was found to be maximal in the hypothalamus, where the greatest reduction in total antioxidant activity was also noted [19].

Interleukin-1β (IL-1β) is one of the mediators of acute-phase responses to stress [11]. In rats, it induces activation of the hypothalamic pituitary-adrenal axis [15] and boosts expression of the *c-fos* gene in the paraventricular and arcuate nuclei [18]. Immobilization stress induces, in turn, expression of the IL-1β mRNA in the hypothalamus of rats [17]. However, the influence of this interleukin on LPO processes in the brain during emotional stress has not been addressed.

Rats of different strains differ in their susceptibility to stress [10]. For example, August rats are more prone to develop disorders of cardiovascular functions than are Wistar or WAG rats. On the other hand, ulcerative gastric lesions can be induced in the Wistar strain more readily than in August rats [4,6].

The purpose of this work was to study the effect of IL-1β on the regional distribution of the LPO product malonic dialdehyde (MDA) and of the antioxidant enzymes superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GPR, EC 1.11.1.9), and glutathione reductase (GRE, EC 1.6.4.2) in brain structures of different rat populations during emotional stress.

MATERIALS AND METHODS

The study was conducted on male August (n=17), Wistar (n=15), and WAG (n=13) rats weighing 196.1±4.1, 180.4±11.2, and 268.5±13.9 g, respectively. The rats were kept in cages (4 animals in

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TABLE 1. Activity of Antioxidant Enzymes and MDA Levels in the Hypothalamus of August Rats ($M \pm m$)

Group	SOD	GPR	GRE	MDA
2: IL−1β, no stress	10.162±3.260	18.453±4.535	16.820±1.860	2.150±0.795
1: IL−1β+stress	6.390±1.030	8.203±0.688	15.105±1.650	1.887±0.335
4: saline, no stress	6.315±0.735	6.677±1.566	12.253±2.058	1.573±0.052
3: saline+stress	5.977±0.724	5.775±1.654	13.465±2.049	2.853±0.603

Note. Here and in Table 2: SOD = superoxide dismutase (U/mg protein); GPR = glutathione peroxidase (nmol/mg protein); GRE = glutathione reductase (nmol/mg protein); MDA = malonic dialdehyde (rel. units).

each) at 20-22°C under natural illumination and had free access to food and water.

Five days before the tests, a hollow steel guide 3 mm long and 0.8 mm in diameter was implanted into cranial bones (A-P=1 mm, L=1 mm) of each rat under Nembutal anesthesia (40 mg/kg intraperitoneally). The guide did not penetrate the brain tissue or the lateral ventricular cavity. Four days later the rats were tested for behavior in an open field and on the following day received an injection of IL-1β or physiological saline into the lateral ventricle from a microsyringe inserted via the guide 3 mm deep into the brain.

The rats were divided into four groups. Group 1 rats were injected with IL-1β in 10 μl of saline (10 μg/ml) and subjected to acute emotional stress; group 2 rats were injected with IL-1β as above and returned to their cages; groups 3 and 4 were treated like groups 1 and 2, respectively, except that only 10 μl of saline were injected. The IL-1β used was human recombinant IL-1β (activity 3×10^7 units/μl) obtained from the Institute of Immunology, Moscow.

The rats were deprived of food for 24 h before the tests, but continued to have free access to water. The acute emotional stress was produced as previously described [6]. Briefly, rats were each immobilized in a plastic case 16.5 cm long with an inner diameter of 5.5 cm, immersed for 2 h in water (23°C) up to the ensiform process, and then returned to their cages for 2 h. They were then immediately decapitated, their brains were removed, and microsamples (15-25 mg) of hypothalamus and sensorimotor cortex were prepared and examined. MDA levels were determined spectrophotometrically [21]. SOD activity was assayed by the degree to which the reaction of epinephrine conversion to adrenochrome was inhibited. GPR and GRE activities were estimated by recording changes in the optical density of NADPH with time. MDA levels and enzyme activities were calculated per mg of protein as determined by Lowry's method [19].

The results were subjected to analysis of variance taking into account three factors - stress, injected solution, and rat strain. The Mann-Whitney test was used for the comparison of independent groups.

RESULTS

As compared to the unstressed August rats of group 4 injected with saline, the hypothalamic level of MDA was 81.4% higher in the saline-injected stressed August rats of group 3 (2.853±0.603 *vs.* 1.573±0.052) and 36.7% higher in the IL-1β-injected group 2 rats of this strain (2.150±0.795 *vs.* 1.573±0.052); in contrast, the hypothalamic MDA level in the IL-1β-injected stressed August rats of group 1 was only slightly higher than in group 4 (1.887±0.035 *vs.* 1.573±0.052) (Table 1).

In the IL-1β-injected stressed August rats (group 1), SOD activity in the sensorimotor cortex was 67.8% higher than in the saline-injected unstressed August rats (group 2) (6.26±1.00 *vs.* 3.73±0.75; $p<0.05$), whereas GPR activity in the hypothalamus was, as shown in Table 1, 55.5% lower than in group 2 (8.20±0.69 *vs.* 18.45±4.54), but differed little from that in the unstressed August rats of group 4 given saline (8.20±0.69 *vs.* 6.68±1.57), probably because the hypothalamic activity of this

TABLE 2. Ratios between the Stressed and Control Groups of August, Wistar, and WAG Rats in Hypothalamic Antioxidant Enzymes Activities and MDA Levels after Intraventricular Administration of IL−1β or Physiological Saline

Rats	Solution	SOD	GPR	GRE	MDA
August	IL−1β	0.63	0.44	0.90	0.88
August	Saline	0.95	0.86	1.10	1.81
Wistar	IL−1β	0.83	0.52	0.84	0.49
Wistar	Saline	1.94	4.87	2.22	0.68
WAG	IL−1β	0.86	1.31	1.34	2.36
WAG	Saline	1.54	2.21	1.35	0.85

enzyme in group 3 was 2.8 times as high as in group 4. No changes in MDA levels or antioxidant enzyme activities were recorded in the emotionally stressed Wistar and WAG rats injected with IL-1β.

We also compared stress-induced changes in the mean antioxidant enzyme activities and MDA levels in the hypothalamus and sensorimotor cortex of rats preinjected with IL-1β or saline. In the IL-1β-treated groups, the stress/control ratios (group 1 *vs.* group 2) recorded for antioxidant enzyme activities and MDA levels in the hypothalamus proved to be lower (with the exception of MDA in WAG rats) than in the saline-treated animals (groups 3 and 4) (Table 2). No such differences were detected for antioxidant enzyme activities or MDA levels in the sensorimotor cortex. It may be tentatively suggested that IL-1β tends to raise hypothalamic levels of MDA and antioxidant enzymes in all three rat populations studied.

The results of this study do not allow any definite conclusions to be drawn about the mechanisms by which IL-1β influences the peroxidation status of hypothalamic cells. That IL-1β enhances free-radical reactions is indicated by its ability to inactivate redox-sensitive protein phosphatases whose SH groups are oxidized during the interaction of this interleukin with cells [14]. Studies by Carmankrzan *et al.* (1993) showed that accumulation of LPO products in the hypothalamus of animals may be associated with phospholipase A_2 activation and enhanced arachidonic acid oxidation by the lipoxygenase pathway. Our findings do not answer the question why the alterations in antioxidant enzyme activities and MDA levels caused by emotional stress in IL-1β-treated rats were less marked than in animals given physiological saline. Indeed, the combination of these two factors (stress+IL-1β), each of which activates LPO in the hypothalamus, could be expected to increase the total effect.

Data on how IL-1β affects the activity of antioxidant enzymes have been reported. In particular, exogenous IL-1β was found to induce expression of Mn-SOD by endothelial cells of human umbilical cord [20] and to activate Cu-Zn-SOD in pulmonary fibroblasts [13]. Oxidative stress is, in turn, an important regulator of gene expression in cytokines [12]. Brocomet *et al.* (1990) report that myocardial glucose-6-phosphate dehydrogenase activity is elevated by IL-1β; the activation of this enzyme is accompanied by a rise in the cell content of NADPH, which donates protons for the reduction of glutathione, reduced glutathione being required for the work of GPR. However, further assays for glucose-6-phosphate dehydrogenase activity in the hypothalamus are needed to confirm that such a mechanism of GPR activation operates in this structure.

The predominant influence of IL-1β on the hypothalamus rather than on the sensorimotor cortex may be attributed, in part, to the fact that the affinity of hypothalamic receptors for this interleukin is 4 times higher than that of cortical receptors [16].

In summary, the present study has shown that 1) acute emotional stress increases the hypothalamic content of MDA in August rats; 2) hypothalamic levels of LPO products are not elevated by acute emotional stress in August rats pretreated with IL-1β; 3) SOD activity rises in the sensorimotor cortex and GPR activity declines in the hypothalamus of IL-1β-pretreated August rats; and 4) intraventricular administration of this interleukin apparently tends to raise the MDA level and antioxidant enzyme activities in the hypothalamus (but not in the sensorimotor cortex) of Wistar, August, and WAG rats.

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