Activity of Antioxidant Enzymes in the Limbic Reticular Structures of Rat Brain After Short-Term Immobilization

A. S. Sosnovskii, T. S. Balashova, G. V. Pirogova, A. A. Kubatiev, and S. S. Pertsov

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During emotional stress (ES), both the generation of free radicals and lipid peroxidation (LPO) are intensified in many tissues [2-6], which suggests that the enzyme system of antioxidant protection plays an important part in preventing the pathogenic effects of ES. Altered catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSH) activities have been recorded in the heart, liver, and kidneys [3-5] and in the cerebral cortex of acutely or chronically stressed rats [2]. Although many researchers have paid special attention to LPO in the brain in view of its high sensitivity to this peroxidation [12], there is virtually no information on the regional distribution of antioxidant enzyme activities in the limbic reticular structures of the brain, which play a leading role in the genesis of ES [7].

LPO products are known to be distributed nonuniformly in the human [13] and animal [10] brain. We found [6] that in rats immobilized for 1 h, TBA-reactive LPO products first of all accumulated in the hypothalamus, probably because of the activation of neurotransmitter circulation in this emotiogenic structure during the early phases of ES [1,11,14]. However, such a finding could also be due to local weakening of antiradical protection, in particular to reduced activities of antioxidant enzymes. In the present study, we measured the activities of SOD, GSH, and glutathione reductase (GR) in individual brain structures of August rats (a strain highly sensitive to ES) after their short-term immobilization and of control rats of this strain.

MATERIALS AND METHODS

The study was carried out on 28 male August rats weighing 299.7±3.9 g. Immobilization of the test rats was produced by keeping them in very small plastic boxes for 60 min in the morning (from 09:00 to 11:00 h). Immediately after the immobilization, blood was collected from the rats for analysis of cellular elements (data not shown here). Their brains were then extracted and tissue specimens from the sensorimotor cortex, amygdala, hypothalamus, and midbrain were placed in liquid nitrogen and stored at -30°C prior to assays for enzyme activity. The entire procedure of obtaining these specimens, which weighed 12-30 mg, did not take more than 20 min after exsanguination of the animal.

The tissues were homogenized in the cold in a glass homogenizer for 3 min in 250 μl physiological saline to which 1% Triton X-100 had been added [13]. The homogenates were clarified by centrifugation at 1000 *g* for 10 min. For measuring SOD activity we used enzyme-dependent inhibition of epinephrine conversion to adrenochrome, the accumulation of which over time was followed by

P. K. Anokhin Research Institute for Normal Physiology, Russian Academy of Medical Sciences, Moscow; Department of Pathophysiology, Central Institute for Advanced Medical Studies, Moscow. (Presented by K. V. Sudakov, Member of the Russian Academy of Medical Sciences)

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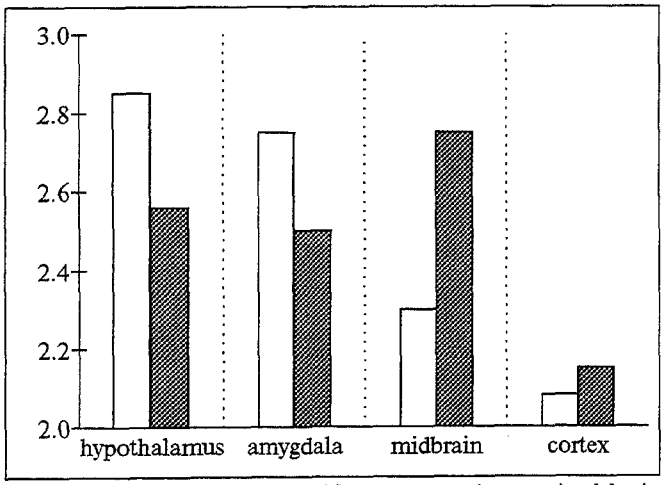

Fig. 1. Distribution of antioxidant enzymes in examined brain structures of control (white bars) and immobilized (dark bars) rats. Rank estimates were made by comparing the antioxidant activities of each enzyme in the sensorimotor cortex, hypothalamus, amygdala, and midbrain of each rat. The values are means for all antioxidant enzymes.

measuring light absorption at 480 nm in a DU-50 spectrophotometer (Beckman, USA). GSH activity was measured spectrophotometrically at 340 nm using H_2O_2 as the substrate for the reaction. GR activity was also measured spectrophotometrically at 340 nm in a continuous recording mode [8]. All enzyme activities were calculated per mg of protein, which was determined by Lowry's method. The differences between mean values were estimated by Student's *t* test, while those between the activities of each enzyme in the four brain structures were estimated by Friedman's test (nonparametric two-factor analysis of variance).

RESULTS

The four brain structures differed in GR levels in the samples from control rats (amygdala > hypothalamus > midbrain > cortex; $p<0.025$) and in SOD levels in those from stressed rats (midbrain = hypothalamus > amygdala > cortex; $p<0.01$).

In the brain structures from stressed rats, the activities of all three antioxidant enzymes were lower than in those from control animals (Fig. 1), although statistically significant differences were only found for SOD in the sensorimotor cortex and for GSH in the amygdala (Table 1). In addition, a tendency toward reduced SOD ($p<0.07$) and GSH ($p<0.073$) activities in the hypothalamus was noted following the immobilization.

The mean ranks of activities of the three enzymes (Fig. 1) give an indication of the overall antioxidant potential of each of the brain structures examined. As is evident from the figure, antioxidant protection in the control rats was most pronounced in the hypothalamus and amygdala, while the midbrain and cortex lagged behind considerably in this respect. In the test rats, the total activities of the three antioxidant enzymes were more or less equal in the hypothalamus, amygdala, and midbrain; in the cortex, however, their total activity was much lower than in the subcortical structures.

In the control rats, therefore, the brain structure best protected by antioxidant enzymes was the hypothalamus (Table 1). The latter has also been found to have a higher catalase activity than other brain structures in rats [8]. However, the hypothalamus proved also to be the structure where the short-term ES elicited the greatest reduction in the activity of antioxidant enzymes. The activities of the three enzymes in the hypothalamus, amygdala, and midbrain were reduced, on average, by 30.2%, 10.8%, and 16.5%, respectively, as compared to control specimens. These results can explain in part our previous finding of the selective accumulation of TBA-reactive products in the hypothalamus of rats after their 60-minute immobilization [6].

A correlation analysis according to Kendall revealed a significant association between GR and GSH activities in the amygdala ($p<0.025$ for control rats; $p<0.02$ for stressed rats). In addition, correlations were recorded between hypothalamic SOD and GSH activities for control rats ($p<0.05$) and between SOD and GR activities in the reticular formation for stressed rats ($p<0.004$).

TABLE 1. Activity of Antioxidant Enzymes in Brain Structures of Control and Immobilized (Stressed) Rats (mean±SEM)

Structure	Superoxide dismutase, units/mg protein		Glutathione peroxidase, nmol/mg protein		Glutathione reductase, nmol/mg protein	
	control rats	stressed rats	control rats	stressed rats	control rats	stressed rats
Sensorimotor cortex	22.97±5.14	14.00±1.32**	13.56±1.79	11.57±2.16	34.55±3.66	34.34±3.71
Amygdala	18.08±4.03	20.41±2.43	15.42±1.79	10.80±1.32*	49.68±7.02	38.79±3.13
Hypothalamus	36.59±8.10	24.78±3.30	18.77±4.39	11.89±1.55	43.49±6.52	34.02±3.10
Midbrain	26.95±5.75	24.66±2.55	21.46±5.88	13.59±2.00	37.94±6.49	36.36±3.75
Friedman's test (for nonuniformity of enzyme distribution)	NS	$p<0.01$	NS	NS	$p<0.025$	NS

Note. NS = not significant. *$p<0.05$, **$p<0.03$ relative to control by Student's *t* test.

Following the immobilization, highly significant positive correlations in GR activity were found between virtually all of the brain structures examined: amygdala-cortex ($p<0.003$), hypothalamus-midbrain ($p<0.015$), cortex-hypothalamus ($p<0.005$), and midbrain-cortex ($p<0.02$). None of these correlations was observed in the control. Uniform changes in all structures in the activity of an enzyme that restores the cellular reserves of glutathione may, on the one hand, be associated with the general mechanisms regulating the activity of that enzyme and, on the other hand, may reflect the enhanced detoxification, in the early phases of ES, of free-radical products of the circulation of neurotransmitter and other metabolites.

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Characteristics of Iron Metabolism and State of the Transferrin-Ceruloplasmin System in Rats with Varying Resistance to Hypoxia

M. K. Soboleva, M. A. Kolpakov, V. I. Sharapov, and O. R. Grek

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Studies of the functional differences between animals with high and low resistance to hypoxia (HR and LR) are ongoing. It is known that unadapted animals with a high natural resistance to hypoxia demonstrate a higher lability of succinate dehydrogenase, cytochrome oxidase, ATPases, as well as of glutamic acid-dependent enzymes catalyzing α-glutarate production [1,3].

The above specificity of the functional properties of enzymes in animals highly tolerant of hypoxia

Novosibirsk Medical Institute; Institute of Clinical and Experimental Lymphology, Siberian Branch of the Russian Academy of Medical Sciences. (Presented by Yu. I. Borodin, Member of the Russian Academy of Medical Sciences)

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