ENHANCEMENT OF LIPID PEROXIDATION IN THE RAT HYPOTHALAMUS AFTER SHORT-TERM EMOTIONAL STRESS

A. S. Sosnovskii and A. V. Kozlov

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During the last two decades much information has been obtained to show that processes of free-radical lipid peroxidation (LPO) are closely connected with lesions arising in the tissues of man and animals during emotional stress (ES) [1, 2, 4, 13].

Since free-radical processes play an important role in the development of many neurological and mental diseases [12, 14, 18], changes in brain LPO are of great interest.

The velocity of LPO processes in the brain varies at different stages of ES. In many investigations, including our own [6], activation of LPO was found during ES. Suppression of LPO in the initial phases of chronic [2] and acute [3, 5] ES also has been described. However, the dynamics of LPO in the brain during ES still remains incompletely clear. In particular, the regional distribution of LPO products has not been the subject of investigators' attention, with rare exceptions [3, 8].

The aim of the present investigation was to study the content of intermediate products of LPO in different brain structures during ES induced by short-term isolation of animals in constraining cages.

EXPERIMENTAL METHOD

Experiments were carried out on 23 adult male Wistar rats (356 9) and 27 male August rats (242 g).

Emotional isolation stress was induced by placing the animals for 1 h in plastic cages limiting their movements. Immediately after the end of isolation the animals were decapitated. Concentrations of LPO products were determined in the hypothalamus (HPT), parieto-occipital cortex (POC), sensomotor cortex (SMC), and limbic cortex (gyrus cingulus, LC), liver, lateral wall of the left ventricle of the heart, and the suprarenals, in samples weighing 8-30 mg.

The content of TBA-reactive LPO products (TBARP) was measured by a modified method in [15]. Tissue samples were weighed and homogenized with 1 ml of 1.5% H_3PO_4 solution. The homogenate (250 μg) was incubated with 1 ml of 1.5% H_3PO_4 solution and 0.5 ml of 0.5% TBA solution (Sigma) at 100°C. The stained products were extracted with butanol. Their concentration was measured or, a "Beckman DB-7" spectrophotometer (USA), using optical density at 535 nm and two basic wavelengths of 515 and 550 nm, and was calculated in nanomoles/g wet weight of tissue, taking the molar extinction coefficient of malonic dialdehyde to be $1.56 \cdot 10^5$.

The coefficient of variation of the TBARP concentrations in a series of 10 samples (17.8 ± 2.7 mg) of the heart of one animal was 23%, which does not exceed the variability of determination of TBARP in blood plasma, a more homogeneous material [11].

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TABLE 1. Changes in TBARP Content and Relative Mass of Rat Organs After Isolation for 1 h (M ± m)

Parameter, organ	August strain		Wistar strain	
	control (16)	isolation (11)	control (14)	isolation (9)
TBARP (nanomoles/g tissue)				
HPT	148,3±14,2	188,3±18,5*	143,9±12,6	181,8±22,8
POC	169,7±16,0	171,2±14,3	181,6±10,7	190,6±16,6
LC	199,2±19,7	225,5±19,8	168,4±16,8	207,9±28,9
SMC	168,4±25,0	—	166,9±12,7	129,3±16,3
Adrenals	75,2±6,3	85,9±5,9	55,0±4,2	66,8±4,2
Heart	49,4±2,7	54,7±4,5	44,2±2,6	39,6±2,6
Liver	160,3±31,2	305,3±50,5**	112,9±33,1	146,3±48,1
Relative weight of organs (mg/100 g body weight)				
Adrenals	11,8±0,9	12,0±0,8	7,5±0,5	8,8±0,7
Heart	410,7±9,2	404,4±8,2	329,3±12,8	333,1±9,7
Spleen	1,99±0,06	1,93±0,06	3,15±0,25	3,19±0,70
Testicle	1028,5±17,5	992,4±39,7	866,3±35,3	878,6±35,4
Thymus	82,8±5,6	93,4±6,6	74,4±8,4	93,2±10,4*

Legend. *$p < 0.05$ indicates significant differences compared with control in each strain (Mann–Whitney test); numbers in parentheses indicate numbers of rats.

The data were analyzed by nonparametric statistical methods not involving the shape of distribution of the data: Kendall's correlation, the two-way Mann–Whitney test, two-factor dispersion analysis after [17], followed by comparison of groups by Quade's method.

EXPERIMENTAL RESULTS

As our experiments showed, during isolation of August rats in a constraining cage more marked changes in concentrations of TBARP were observed (Table 1) than in Wistar rats, which are genetically more resistant to ES [7].

In the control August rats the relative mass of the thymus correlated with the TBARP content in the liver and HPT ($p < 0.005$), and also in LC ($p < 0.05$). The TBARP concentrations in all tissues tested except the heart and POC ($p < 0.005$) correlated with the relative mass of the adrenals. After isolation of the August rats, strong correlation ($p < 0.015$) was found between the TBARP content in POC and the relative mass of the thymus, and weak correlation ($p = 0.07$) was found between the relative mass of the thymus and TBARP in HPT, and the relative mass of the adrenals with TBARP in the liver and LC.

In the control Wistar rats the relative mass of the thymus correlated only with the TBARP concentration in LC. After ES, this correlation was not found.

In rats of both strains in the control group, but not after ES correlation was observed between the relative mass of the testicles and the TBARP content in HPT ($p < 0.025$ for August and $p < 0.02$ for Wistar rats) and in LC ($p < 0.025$ for August and $p < 0.05$ for Wistar rats).

Quade's test (nonparametric two-factor dispersion analysis) revealed significant differences in TBARP levels in the brain structures in the control ($p < 0.01$) for August rats and $p < 0.02$ for Wistar rats. In both strains of rats the TBARP level was lower in HPT than in both POC ($p < 0.01$) and LC ($p < 0.05$). The TBARP concentrations in POC and LC were identical. This pattern of distribution of TBARP may be due to the fact that catalase activity in rats is highest in the hypothalamus [10]. However, in groups subjected to 1 h of isolation the TBARP concentration in the brain structures did not differ significantly. Analysis of the mean values (Table 1) shows that "equalization" of the TBARP concentrations in the brain structures after isolation took place on account of accumulation of TBARP in the hypothalamus.

These experiments thus showed that, compared with cerebral cortical structures, the hypothalamus is the site of strongest activation of LPO in the early stages of isolation stress. The selective elevation of the TBARP level of HPT in ES may be due to the role which this structure plays in the organization of negative emotions [7], and also to differences in the character of local neurochemical processes [9, 16].

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