LIPID PEROXIDATION PRODUCT LEVELS IN INBRED MICE WITH DIFFERENT TYPES OF EMOTIONAL-STRESS RESPONSE

S. B. Seredenin, B. A. Badyshtov, and D. Yu. Egorov

UDC 613.863-07:616.153.915-039]-092.9

KEY WORDS: stress; lipid peroxidation; inbred mice

Considering data in the literature on the existence of correlation between lipid peroxidation (LPO) parameters and some characteristics of emotional-stress responses [2] it was decided to study the initial concentrations and time course of changes in levels of total lipids (TL), TBA-active products (TBA-AP), and diene conjugates (DC) in the liver and brain of C57BL/6 (B6) and BALB/c (C) mice, differing in their response to emotional-stress stimulation in the open field (OF) test, and in their B6 × C F_1-hybrids (F_1), similar in behavior to the parental line B6 [5].

EXPERIMENTAL METHOD

Experiments were carried out on male B6 and C male mice and their F_1-hybrids weighing 18-20 g, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, and kept on a standard diet with alternation of 12 h of daylight and 12 h of darkness, under normal animal house conditions.

The animals were decapitated 2, 6, 12, 24, and 48 h after the OF experiment (series OF + 2, ..., OF + 48). Intact mice, decapitated immediately after removal from the cage, served as the control (series IC).

Tissue samples were washed in cold physiological saline, dried on filter paper, weighed, and homogenized in 3 ml of Tris-HCl buffer, pH 7.4, for 30 sec at 3000 rpm. The homogenates were quickly frozen in liquid nitrogen and stored at −40°C before analysis.

Lipids from the brain and liver were extracted by the method of Folch et al. [9]. The lipid extract in chloroform was evaporated to dryness on a rotary evaporator and dissolved in 4 ml of hexane. To determine DC in the tissues the absorption spectrum of the solution obtained was recorded between wavelengths of 200 and 300 nm on a "Hitachi-624" spectrophotometer (Japan). The DC content was expressed as the ratio D_{232}/D_{215}, where D denotes optical density at 232 and 215 nm respectively.

The TBA-AP level in the samples was determined by the method of Michara and co-workers [10] and the level of total lipids by the sulfophosphovanillin method, using standard kits from "Lachema" (Czechoslovakia).

EXPERIMENTAL RESULTS

The initial interlinear differences in levels of LPO products were established in intact animals in both the brain and liver (Tables 1 and 2). The good reproducibility of the data obtained on mice of each line are evidence of genetic control over LPO processes.

After exposure to emotional stress in the OF test, fluctuations observed in levels of the recorded products in the brain did not exceed 65% of the control level. If these results are compared with those obtained by Prilipko et al. [4], who used stronger stimulation with a nociceptive component and observed marked activation of LPO, leads to the conclusion that the OF experiment does not induce gross disturbances of brain lipid metabolism in animals of the lines used. Deviations found were reversible (Table 1). Meanwhile, earlier investigations demonstrated

Research Laboratory of Pharmacogenetics, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 46-48, July, 1989. Original article submitted November 22, 1988.

0007-4888/89/0007-0957$12.50

TABLE 1. Dependence of Levels of LPO Products in Brain Tissue Homogenates on Time after OF Experiment (M ± m)

Experimental conditions	BALB/c			C57BL/6			F_1-hybrids		
	TBA-AP, conventional units*/mg	DC	TL, mg/g	TBA-AP, conventional units*/mg	DC	TL, mg/g	TBA-AP, conventional units*/mg	DC	TL, mg/g
IC	21,6±0,9 (9)	0,068±0,004 (9)	19,2±0,9 (8)	16,9±0,5 (10)	0,090±0,004 (10)	22,3±0,4 (10)	21,1±1,1 (10)	0,049±0,003 (10)	19,9±0,6 (10)
OF + 2	21,8±0,8 (10)	0,070±0,005 (9)	20,0±0,06 (10)	15,7±0,7 (9)	0,085±0,005 (9)	22,4±0,4 (10)	12,8±0,4 (10)	0,040±0,002 (10)	19,4±0,3 (10)
OF + 6	21,2±0,8 (9)	0,061±0,008 (7)	21,4±1,3 (8)	19,4±0,5 (10)	0,084±0,004 (10)	22,8±0,6 (10)	14,4±0,4 (10)	0,051±0,002 (10)	23,7±0,5 (10)
OF + 12	21,1±0,8 (10)	0,053±0,004 (10)	22,9±0,5 (10)	18,8±0,2 (9)	0,083±0,003 (9)	22,2±0,6 (9)	15,8±0,4 (10)	0,052±0,004 (10)	23,3±0,6 (9)
OF + 24	24,2±1,0 (9)	0,063±0,004 (10)	19,7±0,8 (10)	17,1±0,5 (9)	0,100±0,003 (10)	20,1±0,4 (10)	15,4±1,0 (9)	0,050±0,003 (10)	19,8±0,5 (10)
OF + 48	18,6±0,7 (10)	0,048±0,004 (10)	22,4±1,0 (10)	17,5±0,6 (9)	0,102±0,005 (8)	21,6±0,7 (9)	16,5±0,9 (10)	0,043±0,002 (10)	23,6±0,7 (10)

Legend. Here and in Table 2: b_3) $p < 0.001$ compared with OF + 2; c_2) $p < 0.01$, c_3) $p < 0.001$ compared with OF + 6; d_1) $p < 0.05$, d_2) $p < 0.01$, d_3) $p < 0.001$ compared with OF + 12; e_1) $p < 0.06$, e_2) $p < 0.01$, e_3) $p < 0.001$ compared with OF + 24; k_1) $p < 0.05$, k_2) $p < 0.01$, k_3) $p < 0.001$ compared with OF + 48; m_2^{II}) $p < 0.01$ compared with corresponding parameter in B6 mice; m_3^{II}) $p < 0.001$ compared with corresponding parameters in B6 mice; m_2^{III}) $p < 0.01$ compared with corresponding parameter in F_1 mice; m^{III}) $p < 0.001$ compared with corresponding parameter in F_1 mice. *) Optical density × 10^3. Number of experiments given in parentheses.

TABLE 2. Dependence of Levels of LPO Products in Liver Tissue Homogenates on Time after OF Experiment (M ± m)

Experimental conditions	BALB/c			C57BL/6			F_1-hybrids		
	TBA-AP, conventional units*/mg	DC	TL, mg/g	TBP-AP, conventional units*/mg	DC	TL, mg/g	DC	TBA-AP, conventional units*/mg	TL, mg/g
IC	11,6±0,8 (10)	0,130±0,007 (10)	19,6±0,7 (10)	10,7±1,7 (10)	0,078±0,003 (10)	21,3±0,9 (10)	8,5±0,8 (10)	0,090±0,004 (10)	19,7±1,2 (10)
OF + 2	17,1±0,7 (8)	0,152±0,004 (10)	24,7±1,2 (10)	14,1±1,1 (10)	0,107±0,006 (10)	28,9±1,2 (10)	10,1±1,1 (9)	0,113±0,007 (9)	25,7±1,0 (10)
OF + 6	75,9±5,7 (7)	0,211±0,017 (9)	31,8±1,8 (9)	21,1±5,3 (10)	0,104±0,008 (8)	32,5±2,5 (10)	37,0±5,6 (9)	0,110±0,005 (8)	28,2±2,3 (10)
OF + 12	8,9±0,8 (7)	0,101±0,005 (9)	22,5±0,9 (9)	106,4±6,0 (10)	0,118±0,008 (10)	41,6±2,6 (10)	89,9±10,4 (10)	0,164±0,010 (10)	49,0±4,3 (10)
OF + 24	5,5±0,3 (9)	0,166±0,009 (10)	39,7±0,9 (10)	10,0±1,3 (10)	0,086±0,005 (9)	22,7±1,2 (9)	9,1±0,9 (9)	0,091±0,007 (10)	25,9±1,2 (10)
OF + 48	10,1±1,1 (7)	0,096±0,008 (9)	26,0±1,4 (9)	9,1±1,1 (10)	0,076±0,004 (10)	19,8±0,6 (10)	4,7±0,3 (10)	0,093±0,007 (10)	32,3±1,2 (9)

that the OF test had some effect on the animals studied, as shown by changes in the Plasma concentrations of certain hormones and nucleotides characteristic of the stress response, differing in B6 and C mice during handling and the OF test [7, 8]. This conclusion is confirmed by the picture observed in the liver. In C mice that TBA-AP and DC levels rose sharply 6 h after the experiment and then fell. For OF a biphasic increase in concentration was observed after 6 and 24 h (Table 2). The character of the trend of LPO product levels in the liver of the B6 mice was similar, but the greatest rise of the TBA-AP and DC levels was observed 12 h after the experiment in OF (Table 2). As regards dependence of changes in LPO product levels on time, the F_1 hybrids were similar to the parental B6 strain, and the most marked increase in TBA-AP and DC levels of these animals also was observed after 12 h (Table 2).

It can be concluded from these results that exposure to emotional stress is accompanied by changes in levels of LPO products specific for animals of the selected genotypes. The fact that these changes were more marked in the liver than the brain is in agreement with data in the literature on the greater activity of the antioxidant systems of the brain than those of other organs [3]. As regards differences between strains, some agreement will be noted between the shorter time taken to reach maximal concentrations of TBA-AP and DC in animals of the

C line than in the B6 mice and the sharper changes in other parameters of the emotional-stress response in C mice, established previously, by contrast with the more inert responses in B6 mice [7]. Another characteristic feature is that the time course of changes in the level of LPO products in the liver of F_1 hybrids was similar to that in B6 mice, but not in C mice, for with respect to the behavioral type of response to the form of stress used and its hormonal and nucleotide parameters, the F_1 hybrids inherited the features of the parental B6 line [7, 8].

It can thus be concluded from the results described above that the time course of changes in levels of LPO products reflects certain characteristics of hereditarily different types of emotional-stress responses. Genetic differences in LPO processes may be very important for the understanding of the mechanisms of development of undesirable consequences of emotional-stress responses. It was shown previously, for instance, that the OF test led to a rise in the number of chromosomal aberrations in B6 mice but not in C mice [6]. Considering the ability of free-radical intermediates to induce DNA injuries [11], the possibility cannot be ruled out that differences in LPO processes in these animals, accompanying their response to stress, are important for the formation of established cytogenetic effects. If this hypothesis is true, the results as a whole will need to be taken into account in order to explain the link between emotional-stress responses and the appearance of malignant neoplasms [1].

LITERATURE CITED

1. K. P. Balitskii and Yu. P. Shmal'ko, Éksp. Onkol., No. 1, 7 (1983).
2. N. A. Bonarenko, T. A. Devyatkina, O. N. Voskresenskii, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 7, 12 (1985).
3. A. I. Zhuravlev, Bioantioxidants in the Regulation of Metabolism under Normal and Pathological Conditions [in Russian], Moscow (1982), pp. 3-37.
4. L. L. Prilipko, V. E. Kagan, and F. Z. Meerson, Byull. Eksp. Biol. Med., No. 11, 6 (1983).
5. S. B. Seredenin and A. A. Vedernikov, Byull. Éksp. Biol. Med., No. 7, 38 (1979).
6. S. B. Seredenin, A. D. Durnev, and A. A. Vedernikov, Byull. Éksp. Biol. Med., No. 7, 91 (1980).
7. S. B. Seredenin, B. A. Badyshtov, M. M. Nikitina, and V. B. Rozen, Byull. Éksp. Biol. Med., No. 8, 36 (1982).
8. S. B. Seredenin and B. A. Badyshtov, Byull. Éksp. Biol. Med., No. 11, 586 (1985).
9. J. Folch, M. Lees, and G. Sloane-Stanley, J. Biol. Chem., 226, No. 3, 497 (1957).
10. M. Michara and K. Ui, Biochem. Med., 23, 302 (1980)
11. T. E. Slater, J. Biochem., 282, No. 1, 1 (1984).