SELECTIVE INHIBITION OF LIPID PEROXIDATION IN THE BRAIN DURING STRESS

F. Z. Meerson, Yu. V. Arkhipenko, and V. V. Didenko

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Recent investigations have shown that lesions of the internal organs, i.e., the stomach [7], heart [2], and liver [5], in severe emotional–painful stress, can be associated paradoxically with the more rapid formation and more complete preservation of defensive conditioned reflexes [4]. It has accordingly been suggested that the brain, as the organ controlling behavior, ought to play a particularly important role in stress situations, and the body should be able to inhibit processes in the brain responsible for the development of stress-induced injuries to other organs. One such process is stress-induced activation of lipid peroxidation (LPO) [3], which injures the lipid bilayer of biomembranes and disturbs activity of vitally important membrane-bound enzymes.

The aim of this investigation was accordingly to test the hypothesis of selective protection of the brain during stress, by comparing the dynamics of LPO in the brain, on the one hand, and in the heart and liver, on the other hand, during the response to stress.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-220 g. The animals were divided into four groups: 1) control, 2, 3, and 4) exposed to immobilization stress for periods of 1, 6, and 12 h respectively. Immobilization stress was induced by fixation of the animal by the limbs in the supine position. The rats were killed by decapitation 2 h after the end of exposure to stress. LPO in the brain, heart, and liver was compared, first, by determination of the concentration in vivo of malonic dialdehyde (MDA), an intermediate product of LPO, and second, by studying induction of LPO in tissue homogenates in vitro. For this purpose the heart, liver, and brain were isolated, washed, and kept in liquid nitrogen until required for use. The tissues were homogenized in the cold in medium containing 50 mM Tris-HCl, 100 mM NaCl (pH 7.4), and with the ratio of weight of tissue to volume of solution of 1:4. The resulting homogenate was filtered through gauze and used in the experiments. The protein concentration was determined by the biuret reaction. To induce LPO in vitro a system of ascorbic acid + Fe^{++} (50 μM + 5 μM) was used. Oxidation was carried out in homogenization medium in constant-temperature cuvettes at 37°C with continuous mixing. The protein concentration in the incubation medium was 5 mg/ml. After screening of the samples for their initial concentration of LPO products the reaction was started by the addition of the combined solution of ascorbate + Fe^{++}. LPO products were recorded spectrophotometrically by the reaction with 2-thiobarbituric acid [8]. The concentration of MDA formed was calculated by the use of a molar extinction coefficient, which was taken to be 1.56×10^5 $cm^{-1} \cdot M^{-1}$ liter. The results were subjected to statistical analysis by Student's test and to correlation analysis.

EXPERIMENTAL RESULTS

The data given in Table 1 can be used to estimate the time course of the concentration of LPO products after exposure to immobilization stress in the heart, liver, and brain, and to compare the process of LPO induction in vitro in homogenates of these organs prepared after exposure to stress of varied duration. Under the influence of immobilization stress the MDA concentration in the heart and liver increased, evidence of activation of LPO. This stress-induced activation of LPO in the internal organs, well known from previous investigations

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TABLE 1. Changes in MDA Concentration (in nmoles/mg protein) in Tissue Homogenates from Rats Exposed to Immobilization Stress (M ± m)

Duration of incubation, min	Control	Duration of stress, h		
		1	6	12
		Heart		
0	0,55±0,01	0,62±0,03*	0,59±0,02	0,55±0,05
10	0,74±0,04	1,27±0,11***	1,10±0,22	0,76±0,08
30	1,25±0,03	2,26±0,26***	2,00±0,23*	1,24±0,25
60	2,21±0,06	2,83±0,09***	2,25±0,55	2,16±0,09
		Liver		
0	0,50±0,03	0,77±0,10*	0,60±0,05	0,53±0,04
5	0,85±0,09	2,05±0,25***	1,40±0,17	0,74±0,13
10	1,65±0,17	4,08±0,57***	2,82±0,64	1,26±0,23
30	4,47±0,44	10,69±1,79**	6,53±0,68*	3,14±0,47*
		Brain		
0	0,62±0,04	0,30±0,03***	0,46±0,09	0,70±0,07
5	2,91±0,25	1,31±0,24***	1,48±0,17***	3,37±0,22
10	4,09±0,36	2,35±0,21***	2,79±0,19**	4,70±0,46
30	7,27±0,26	5,07±0,51***	5,53±0,26***	7,91±0,38

Legend. *p < 0,05, **p < 0,01, ***p < 0,001 compared with control. Number of experiments was 8.

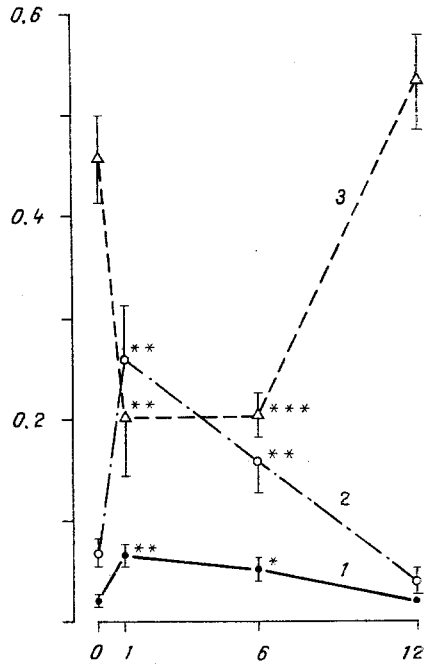

Fig. 1. Dynamics of initial velocity of MDA formation in rat tissue homogenates prepared after immobilization of varied duration. Abscissa, duration of immobilization of rats (in h); ordinate, initial rate of MDA formation (in nmoles/mg protein/min). 1) Heart, 2) liver, 3) brain. *p < 0.05, **p < 0.01, ***p < 0.001 compared with control.

[3, 5, 6], was combined with a reduction by half of the concentration of LPO products in the brain (Table 1). It is important to note that MDA accumulation in the internal organs and the fall in its level in the brain were observed in response to stress for 1 h. More prolonged stress abolished the differences between the tissues and brought the LPO level close to normal in all organs.

Incubation of heart, liver, and brain homogenates in the presence of an Fe^{++} + ascorbate system revealed differences in the resistance of the organs to free radical oxidation. It follows from the data given in Table 1 and Fig. 1 that opposite changes in the MDA concentration

in the test tissues during stress are not accidental, for a study of LPO induction in the homogenates showed that the initial velocity of this process was increased more than threefold under the influence of exposure to stress for 1 h, whereas in the brain, on the other hand, it was reduced by 2.3 times.

Thus in the early stage of the stress reaction, activation of LPO, adrenergic in its origin, and a fall of resistance to agents inducing LPO in the internal organs were combined with opposite changes in the brain, where the intensity of LPO fell simultaneously with substantial limitation of the possibility of chemical induction of LPO, under the conditions found in vitro. When this fact is assessed, attention must be paid to data (Table 1) indicating that, outside the stress situation, the concentration of LPO products in the brain did not differ significantly from their concentration in the internal organs, but the initial velocity of oxidation of the ascorbate + Fe^{++} system in brain homogenates was significantly higher (Fig. 1). Consequently, a characteristic feature of brain tissue is that the LPO process can be easily induced in it, and for that reason the MDA concentration in the control in vitro reached high values. The privileged position in which the brain finds itself during stress can hardly be explained by the presence of qualitatively more powerful antioxidant systems in the cells of this organ. The explanation is evidently that the first reaction in a situation of urgency activates a certain regulatory mechanism which not simply prevents activation of LPO in the brain, but which considerably depresses the intensity of LPO. During the evaluation of this mechanism, two circumstances are important: first, it is not identical with the blood–brain barrier, which could only limit the hormonal LPO-mediated damaging action of stress on the brain, but could not lower the level of LPO products during stress below the control level; second, the effect of the protective mechanism is long-lasting. In fact, the velocity of LPO induction in vitro in brain homogenate taken from the animals 2 h after exposure to stress for 1 h, is reduced in vitro by more than half.

It is important to note that reciprocal relations between the intensity of LPO processes in the brain and internal organs were found in the present experiment not only between control animals and animals exposed to stress, but also within the control group when individual data were compared. For instance, in rats with the highest concentration of LPO products in the liver, their concentration in the brain as a rule was lower ($r = -0.515$; $p < 0.05$). This fact, with a high degree of probability, can be explained by differences in the degree of short-term stresses to which the animals were exposed while being kept in the animal house and during the experiment. These reciprocal connections may also reflect differences in the emotional state of individual rats, reflected in their sensitivity to LPO inducers [1].

There is no doubt that this timely inhibition of LPO and protection of membrane structures of the brain, found in the present investigation, may have an important role in acute stress situations when the brain realizes its basic function of controlling the behavioral reactions of the body with a high level of intensity. It is important to discover how long the effect of this protective mechanism lasts when the duration of exposure to stress is increased, and also to determine what components of the stress reaction is responsible for inhibition of LPO in the brain.

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