STATE OF LIPID PEROXIDATION IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Injury to the membrane structures of the brain in experimental allergic encephalomyelitis (EAE), which is manifested in particular by the development of demyelination and disturbance of phospholipid metabolism in the brain, suggests a change in the intensity of lipid peroxidation (LPO) in the membranes.

In the investigation described below LPO parameters were determined in blood and brain tissue of guinea pigs in the course of EAE.

EXPERIMENTAL METHOD

Experiments were carried out on 105 guinea pigs weighing 250-300 g. EAE was produced by a single immunization with encephalitogenic material containing Freund's complete adjuvant (5 mg/ml of a killed culture of BCG) and 50% homogenate of homologous brain in physiological saline. The encephalitogenic material was injected into the hind-limb footpads in a dose of 0.2 ml each. On the 3rd, 5th, and 7th days of immunization and during the development of EAE the following parameters were determined: the blood and brain tissue concentrations of diene conjugates [10], the concentration of malonic dialdehyde (MDA) in the blood serum and brain tissue [4, 14], the intensity of formation of peroxides and of unsaturated fatty acids (USFA) in erythrocyte membranes [2], the peroxide resistance of the erythrocytes [9], activity of catalase [12] and of superoxide dismutase (SOD) [13] in blood and brain tissue, total peroxidase activity of the blood serum [5, 8], and the rate of spontaneous and NADPH-induced LPO in brain tissue [4].

EXPERIMENTAL RESULTS

All guinea pigs immunized with the encephalitogenic material developed clinical manifestations of EAE on the 10th-13th day of immunization, in the form of loss of body weight, apathy, and the development of paresis and paralysis of the limbs and sphincters. During the first week after the appearance of neurological symptoms all the animals died.

On the 3rd-5th day of immunization a marked increase was observed in the blood level of diene conjugates, and on the 7th day and during the development of EAE it fell, although it still remained above normal. A similar trend was observed in the brain, but the peak concentration of diene conjugates was recorded on the 5th-7th day of immunization (Fig. 1). The MDA concentration in the blood serum and brain rose progressively in the course of immunization, but in the blood it began to rise after the 3rd day of immunization, and in the brain not until the 5th day (Fig. 2). The maximal increase in catalase activity in the blood occurred on the 5th day and in the brain on the 7th day of immunization, followed by a fall below the background values (Table 1). SOD activity fell in the blood and brain, starting with the 7th day of immunization, and minimal values were reached during the development of EAE (Table 1). From the 3rd day of immunization the concentration of peroxides of USFA in the erythrocytes membranes began to rise progressively (Fig. 3) and the percentage of erythrocytes hemolyzed through the action of peroxide increased (up to 70% from a normal level of 8-9%). Total serum peroxidase activity reached its highest values on the 3rd day of immunization and this was followed by a fall below the back-

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TABLE 1. Changes in Catalase and SOD Activity in the Course of EAE in Guinea Pigs (M ± m)

Experimental conditions	Catalase (8)		SOD (7)	
	blood, millimoles H_2O_2/ml/min	brain, micromoles H_2O_2/g tissue/min	blood, activity units/ml	brain, activity units/mg protein
Background	0,10±0,01	2,1±0,3	133,4±4,4	9,8±0,1
Immunization				
3rd day	0,13±0,01	2,2±0,1	—	—
5th day	0,24±0,02*	3,1±0,2*	132,4±1,3	9,5±0,4
7th day	0,13±0,02*	6,5±1,5*	120,2±1,6**	6,3±0,2*
EAE	0,05±0,01*	1,8±0,1*	94,8±4,7*	3,8±0,1*

Legend. *P < 0.001, **P < 0.05 compared with background.

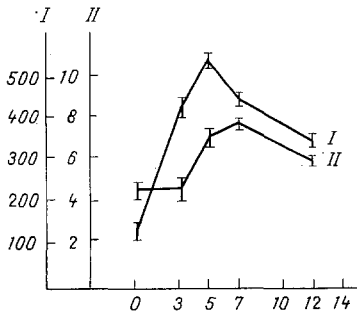

Fig. 1

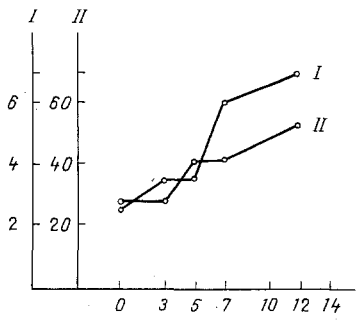

Fig. 2

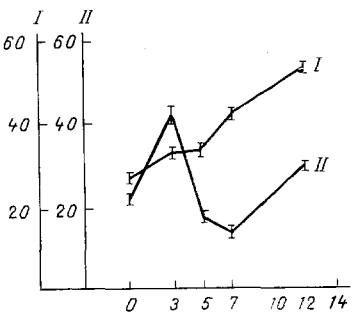

Fig. 3

Fig. 1. Concentration of diene conjugates in blood (I) and brain (II) of guinea pigs in the course of EAE. Abscissa, time after immunization (in days); ordinate, concentration of diene conjugates in blood (in nmole/ml) and brain (in nmole/g tissue).

Fig. 2. MDA concentration in blood serum (I) and brain (II) of guinea pigs in course of EAE. Abscissa, time after immunization (in days); ordinate, MDA concentration in blood (in nmole/ml) and brain (in nmole/g tissue).

Fig. 3. Peroxides of USFA in erythrocyte membranes (I) and total serum peroxidase activity (II) of guinea pigs in the course of EAE. Abscissa, time after immunization (in days); ordinate, concentration of peroxides of USFA in erythrocyte membranes (in nmole MDA/ml of erythrocyte suspension) and total serum peroxidase activity (in relative units of activity/ml serum).

ground values toward the 7th day, although during the development of EAE a further rise in peroxidase activity was observed (Fig. 3). The rate of spontaneous and NADPH-induced LPO decreased after the 5th day from the beginning of immunization.

The decrease in the concentration of antioxidants in the erythrocyte membranes and simultaneous accumulation of diene conjugates and MDA indicate activation of LPO processes in the blood as early as on the 3rd-5th days of immunization. The increase in catalase activity and total peroxidase activity of the blood serum confirms accumulation of peroxides in the blood. Coincidence of the temporal characteristics of the immunologic reaction, namely the appearance of antibrain antibodies and of circulating immune complexes on the 3rd-5th day after immunization with encephalitogenic material [7] with intensification of LPO suggests that activation of these processes is the result of an antigen–antibody reaction, beginning in the blood in the early stages of EAE. The increase in the concentration of diene conjugates and MDA in the animals' brain took place in the later stages of immunization. The increase in activity of LPO processes in the blood evidently affects the permeability of the blood-brain barrier and facilitates the passage of antibrain antibodies, potentiating LPO in the brain, through the barrier. The most marked increase in LPO in the brain occurred on the 7th day of immunization. These results are in agreement with earlier observations showing that this period, immediately preceding the development of clinical symptoms, is marked by a particular increase in severity of metabolic disturbances in the brain [11]. It has also been shown that disturbances of protein, nitrogen, and energy metabolism and changes in the glycolipid composition of the brain in EAE are similar in their trend to those arising after intracisternal injection of the γ-globulin fraction of blood serum containing antibodies against brain antigens [3, 6].

The decrease in the rate of spontaneous (from 1.6 ± 0.05 to 0.46 ± 0.02 nmole MDA/g tissue/min, $P < 0.001$) and of induced LPO (from 2.6 ± 0.08 to 1.6 ± 0.08 nmole MDA/g tissue/min, $P < 0.001$) in the brain during the development of EAE is a very interesting fact. Under these circumstances there was a change in the character of the curves of velocity of spontaneous LPO, with the appearance of a latent period. It has been shown that the latent period of formation of TBA-active products is observed after addition of Fe^{++} salts [4], but the mechanism of delay has not been finally explained. The decrease in the rate of spontaneous and NADPH-induced LPO in the brain on the 7th day of immunization and during the development of EAE can be explained by a decrease in the phospholipid content [1]. The fall in catalase and SOD activity evidently takes place on account of their inhibition by lipid peroxides.

The increased activity of LPO processes in EAE can be regarded as one of the initial stages of injury to the membrane structures of the various elements of the brain. Intensification of LPO is evidently the result of immunologic conflict, taking place at the initial stages of EAE in the blood, and subsequently in the brain. The initial character of the increase in LPO activity may be confirmed by the protective effect of antioxidant therapy. The further development of our ideas on the role of immunologic reactions in the disturbance of the normal level of activity of LPO processes is important for our understanding of the pathogenesis of the immunologic conflicts which lie at the basis of many diseases of the nervous system, and also for the improvement of diagnostic methods and the development of new approaches to the treatment of immunopathologic processes.

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