EFFECT OF STRESS DURING PREGNANCY ON THE RATE OF LIPID PEROXIDATION IN ERYTHROCYTES AND BRAIN TISSUE OF NEWBORN RATS

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Stress at different stages of pregnancy in rats, and also in the period immediately before mating, causes an increase in postnatal mortality and the appearance of a physiologically immature progeny with smaller body weight, delayed ossification, delayed eye opening, and defective orientation, which as has been suggested [1, 8, 11, 14], are associated with disturbance of the hormonal status and metabolism in the mother. One of the basic mechanisms determining the rate of metabolic conversions at the cell membrane level is activity of lipid peroxidation (LPO), which is linked with adaptation of the animal to changing conditions of the external and internal environment [2, 5].

The aim of this investigation was to study LPO activity and the lipid composition of the erythrocytes and brain tissue in newborn rats whose mothers had been exposed to emotional stress in the second half of pregnancy.

EXPERIMENTAL METHOD

Experiments were carried out in the winter on 15 pregnant rats, eight of which had been exposed twice or three times to weak painful electrical stimulation for 20 min during the second half of pregnancy. Emotional stress consisted of aggressive interaction between two pregnant rats in an inescapable conflict situation, provoked by nociceptive stimulation [6]. Altogether 57 newborn rats aged 1 h and 1 and 15 days (experimental and control groups) and aged 20 and 30 days (control group) were studied.

The concentration of malonic dialdehyde (MDA) was determined in erythrocytes and brain homogenate after incubation for 10 min without and after ascorbate-dependent initiation of LPO [3], and the concentrations of total lipids and of total and lipid phosphorus were measured using kits from Boehringer (West Germany) and Lachema (Czechoslovakia). The degree of autohemolysis of the erythrocytes during incubation for 10 min [9], the H_2O_2 concentration [12], and catalase activity, based on utilization of H_2O_2 in samples of erythrocytes before and after boiling for 2 min, also were determined. The end result was expressed as the percentage of peroxide destroyed by catalase.

The physical development of the young rats was assessed by their body weight, time of eye opening, and behavioral response level.

EXPERIMENTAL RESULTS

The first fact to be noted was age changes taking place both in the erythrocytes and in the brain tissue of the young rats, which were particularly marked in the critical periods of life: the first hour — the action of birth stress, the 15th day — the beginning of rapid myelination of the brain and the time of eye opening, and the 30th day — the beginning of the pubertal period and reorganization of the hormonal status of the animal. The greatest changes in lipid metabolism and in the rate of LPO were observed on the 15th day after birth (Fig. 1). These were expressed as a marked increase in the concentration of MDA, total lipids, total (inorganic and organic) and lipid phosphorus, an increased degree of autohemolysis of the erythrocytes, and a low MDA/hemolysis ratio. The low value of the MDA/hemolysis

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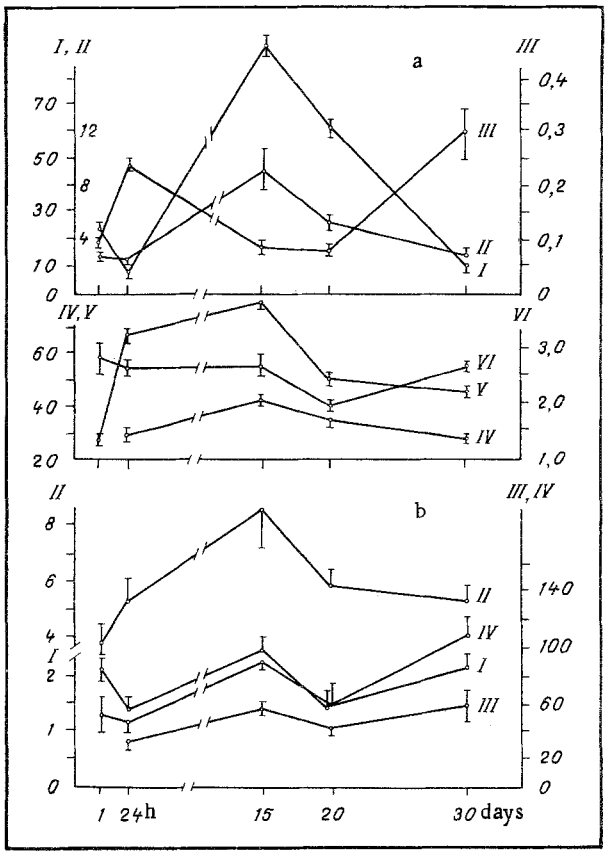

Fig. 1. LPO activity and lipid composition of erythrocytes and brain homogenate in rats of different ages. a) Erythrocytes. Ordinate: I) autohemolysis of erythrocytes (in %), II) MDA concentration (in nmoles/10^6 erythrocytes), III) MDA/hemolysis ratio, IV) lipid phosphorus level (in μg/10^6 erythrocytes), V) concentration of total phosphorus (in μg/10^6 erythrocytes), VI) total lipid level (in μg/10^6 erythrocytes). b) Brain homogenate. Ordinate: I) MDA concentration (in nmoles/mg total lipids), II) MDA concentration after ascorbate-dependent initiation of LPO (in nmoles/mg total lipids), III) lipid phosphorus level (in μg/mg total lipids), IV) total phosphorus concentration (in μg/mg total lipids). Abscissa, age of animal.

ratio, together with a high MDA concentration and a high degree of autohemolysis, indicates an increase in the rate of LPO and other processes, notably the rate of replacement of oxidized lipids by fresh, protecting the cell against destruction in the body [4]. In vitro, when oxidized lipids are not replaced by fresh, the highly metabolizing membranes are destroyed, which leads to intensified autohemolysis (up to 95%), and to a low value of the MDA/hemolysis ratio.

In rats aged 15 days the H_2O_2 concentration was lower than in rats aged 1 h: 1.7 ± 0.24 and 4.5 ± 0.45 mmoles/ml erythrocytes respectively ($p < 0.01$), and catalase activity also was reduced: 83.5 ± 7.49 and 99.4 ± 0.1% ($p < 0.05$), which also points to activation of LPO, during which hydrogen peroxide is utilized, in rats aged 15 days compared with those just born.

Rats whose mothers were exposed to stress were born at term; their body weight did not differ significantly from that of the control animals; the relative weight of the adrenal medulla compared with body weight showed a tendency to fall. The eye opening times of the young experimental rats as a rule coincided with the age norm, but in rats aged 15 days a higher level of emotional behavior and investigative activity was observed compared with rats of the control group.

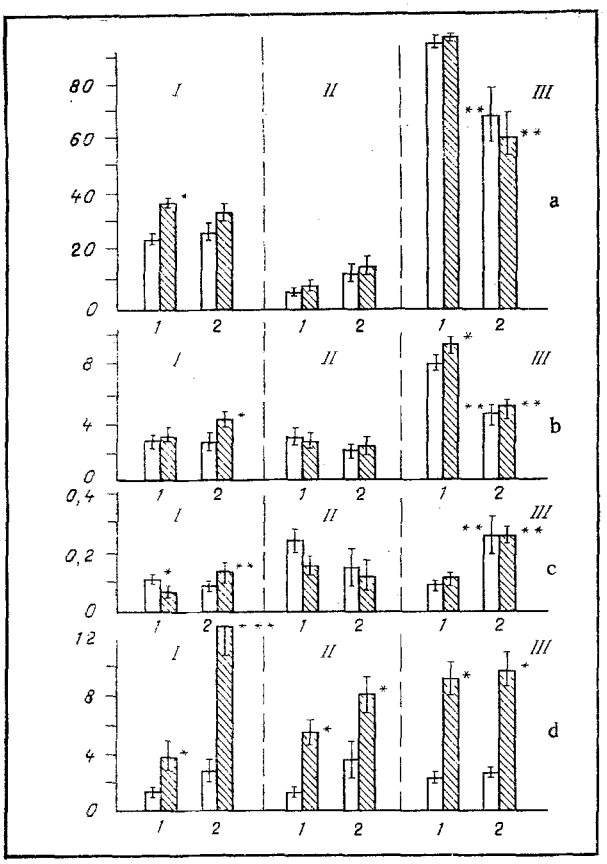

Fig. 2. Effect of emotional stress during pregnancy on rate of LPO in erythrocytes and brain homogenate of rats. a) Autohemolysis of erythrocytes (in %); b) MDA concentration in erythrocytes (in nmoles/10^6 erythrocytes; c) MDA/hemolysis ratio; d) MDA concentration in brain homogenate (in nmoles/mg total lipids). 1) Control; 2) stress; I, II, III) age of rats: 1 h, 1 and 15 days respectively. Unshaded columns represent endogenous LPO, shaded columns LPO initiated by Fe^{++} + ascorbate. *$p < 0.05$ indicates significant differences between endogenous and induced LPO, **$p < 0.05$ — differences significant compared with control.

Judging from the degree of autohemolysis and the MDA/hemolysis ratio, LPO activity in the erythrocytes of young rats in the 1st hour of life, whose mothers have been exposed to stress, was higher than in rats of the control groups (Fig. 2). On the 1st day of life in rats of the experimental group the degree of autohemolysis was higher and the MDA/hemolysis ratio was lower than in rats of the control group, indicating some increase in the rate of LPO and of metabolic processes in the membrane. However, on the 15th day after birth autohemolysis in the experimental rats and the MDA concentration in their erythrocytes were lower than in animals of the control group, whereas the MDA/hemolysis ratio was 2-3 times higher. It can be tentatively suggested that in the experimental animals, despite their lower rate of MDA formation, the red blood cells retained this substance to a greater degree than in the control rats, as shown by the increased MDA/hemolysis ratio. Retention of MDA in the cell, in turn, is the cause of reduced activity of many membrane enzymes, lowering of the metabolic activity of the cell membrane, and more rapid aging of the cell [7, 10, 13].

The MDA concentration in brain homogenate of the experimental rats at the 1st hour after birth was a little higher than in the control, but initiation of LPO, just as in the erythrocytes, led to a greater increase in the MDA concentration than in the control group. On the 1st day of life the MDA concentration in brain tissue before and after initiation of LPO remained high in rats of the experimental group, whereas by the 15th day after birth no differences were found in the MDA concentration in the brain homogenate from rats of the experimental and control groups.

The content of total lipids and total phosphorus in the erythrocytes was higher in rats of the experimental group throughout the period of observation than in the control, whereas the content of total lipids in their brain homogenate was lower and the concentration of total phosphorus was higher than in the control. The increase in the total phosphorus concentration recorded on the 15th day, moreover, took place on account of inorganic, and not lipid, phosphorus.

The H_2O_2 content in the erythrocytes at the 1st hour after birth was lower, but on the 15th day it was higher in the experimental rats, whereas there was no difference in their catalase activity. Changes in the H_2O_2 content in rats of the experimental group confirm the view that LPO is intensified during the 1st hour of life and inhibited on the 15th day.

Emotional stress in pregnant rats thus had no effect on the body weight or organs of the newborn rats, but led to changes in their behavioral response and their LPO activity, both in the erythrocytes and in brain tissue, starting with the 1st hour of life and continuing until the 15th day.

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