Protein Peroxidation in the Plasma of Prenatally Stressed Rats

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We studied the effect of prenatal stress on protein peroxidation in the plasma of rats during postnatal ontogeny. Oxidative destruction of proteins in prenatally stressed rats differed from that in control animals. These differences were most pronounced in postnatal ontogeny, *i.e.* during the development and maturation of CNS.

Key Words: *protein peroxidation; prenatal stress; ontogeny*

Stress exposure of mothers in various periods of pregnancy has an adverse effect on intrauterine growth and further development of the organism until the period of maturity [8]. Stress during the late prenatal ontogeny is most hazardous. This period corresponds to the integration of all components of the neuroendocrine regulation system, which is responsible for adaptive function in adult animals [9]. Studies in this field would help to understand the mechanisms and pathogenesis of disorders in the organism exposed to stress during intrauterine development. Little is known about the effect of prenatal stress on protein peroxidation (PPO), which serves as a criterion of organism's reaction to adverse factors.

The development and progression of stress are closely related to oxidative reactions. This process leads to increased production of reactive oxygen species (ROS) and stimulates free radical oxidation of biological molecules [1,11,12]. Free radical oxidation of proteins is a posttranslational covalent modification, which plays an important role in various physiological and biochemical processes (*e.g.*, aging and tissue or energy metabolism) [12]. Intensification of peroxidation has an adverse effect, which is associated with the appearance of intermolecular cross-links and changes in the physicochemical state of cell membranes [7]. This process is a nonspecific reaction to stress that characterizes adaptation of cells to exogenous factors [3,5]. Changes in oxidative modification of plasma proteins reflect the directionality of free radical processes in the organism and brain tissue [5]. Here we studied the effect of prenatal stress on PPO in the plasma of prenatally stressed rats during various periods of ontogeny. Basal PPO (spontaneous PPO) characterizes realized oxidative capacity of the organism. PPO induced by Fenton's reagent reflects the reserve physiological capacity of the organism.

MATERIALS AND METHODS

Experiments were performed on Wistar rats. Pregnant females were exposed to psychoemotional stress on the 3rd week of pregnancy (days 16-19), which corresponds to the development of the neuroendocrine system in fetuses [9]. The neurohormonal state of mothers in this period determines differentiation and brain function in the offspring. The animals were immobilized in narrow plastic cages (20×7×6 cm) for 1 h. The offspring of prenatally stressed rats were decapitated on days 10, 20, 30, and 90 after birth. The blood was collected and centrifuged at 200*g* for 10 min. The content of PPO products in the plasma was measured by the method [4] with modifications.

The plasma was diluted 1:10 with physiological saline. To study basal PPO, the plasma (0.5 ml) was incubated with potassium phosphate buffer (0.015 M,

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0.95 ml) at 37°C for 15 min. For measurements of induced PPO the volume of potassium phosphate buffer was decreased to 0.75 ml. The mixture of 10 mM Fe^{2+} and 10 mM EDTA (0.1 ml) and 0.1 ml 1 M H_2O_2 were added to stimulate PPO. The final volume of samples was 1 ml. After incubation, 1 ml 20% trichloroacetic acid was added to the sample for protein precipitation. PPO products were stained with 1 ml 0.1 M 2,4-dinitrophenylhydrazine in 2 M HCl at room temperature for 1 h. After staining, the samples were centrifuged at 200*g* for 10 min. The pellet was washed 2 times with ethanol-ethyl acetate mixture (1:1), dried, and dissolved in 3 ml 8 M urea containing 1 drop of 2 M HCl.

Peroxidation products quantitatively reacted with 2,4-dinitrophenylhydrazines, which results in the formation of 2,4-dinitrophenylhydrazones. Reaction products were assayed at 270 (aldehyde phenylhydrazones), 363, and 370 nm (ketone dinitrophenylhydrazones). The content of these compounds was expressed in optical density units per 1 mg protein (U/mg protein). Statistical treatment involved methods of variational statistics [2]. The results were analyzed by Student's *t* test (Excel 7 software).

RESULTS

During the early postnatal period, the content of initial products formed after spontaneous PPO and detected at 270 nm significantly decreased in prenatally stressed rats (18% of the control, Table 1). The content of final products detected at 363 and 370 nm practically did not differ in 10-day-old prenatally stressed and control rats. In 20-day-old prenatally stressed rats the concentration of initial PPO products detected at 270 nm was 90% higher than that in 10-day-old prenatally stressed animals. Considerable accumulation of initial products of PPO can be related to intensive myelination and synthesis of myelin proteins during this period of postnatal ontogeny. Probably, prenatal stress activates PPO, which is accompanied by the release of oxidation products into the plasma. Functional development of the brain is completed by the 30th day of postnatal ontogeny. During this period the contents of initial (270 nm) and final products of spontaneous PPO (363 nm) in prenatally stressed rats decreased by 55 and 20%, respectively. The content of initial products of spontaneous PPO (270 nm) in the plasma from adult rats was 20% lower than in control animals. The concentration of final PPO products in prenatally stressed adult rats practically did not differ from the control.

The content of products of induced PPO underwent most pronounced changes in 30-day-old rat pups and 90-day-old adult animals. In 30-day-old prenatally

TABLE 1. Content of PPO Products in the Plasma of Control and Prenatally Stressed rats during Various Periods of Postnatal Ontogeny (*M*±*m*)

λ, nm	10 days		20 days		30 days		90 days	
	control	treatment	control	treatment	control	treatment	control	treatment
Spontaneous PPO								
270	0.062±0.024 (10)	0.011±0.005* (9)	0.055±0.008 (18)	0.061±0.007 (14)	0.064±0.007 (10)	0.026±0.008* (11)	0.125±0.014 (6)	0.115±0.008 (8)
363	0.082±0.009 (10)	0.081±0.002 (9)	0.111±0.005 (17)	0.125±0.005 (16)	0.159±0.002 (10)	0.127±0.005* (9)	0.211±0.014 (11)	0.209±0.006 (11)
370	0.101±0.011 (10)	0.097±0.006 (9)	0.113±0.005 (18)	0.122±0.005 (21)	0.152±0.002 (10)	0.148±0.007 (10)	0.210±0.015 (11)	0.224±0.008 (11)
Induced PPO								
270	0.095±0.013 (8)	0.113±0.011 (9)	0.215±0.029 (15)	0.141±0.009* (17)	0.066±0.009 (10)	0.124±0.009* (10)	0.140±0.009 (5)	0.212±0.015* (8)
363	0.075±0.006 (10)	0.069±0.011 (8)	0.103±0.014 (18)	0.085±0.007 (17)	0.059±0.005 (11)	0.102±0.005* (9)	0.173±0.013 (10)	0.104±0.004* (10)
370	0.069±0.005 (10)	0.075±0.009 (8)	0.107±0.011 (18)	0.095±0.007 (15)	0.076±0.004 (10)	0.112±0.008* (8)	0.200±0.018 (10)	0.138±0.009* (10)

Note. Number of animals is shown in parentheses. *$p<0.05$ compared to the control.

stressed rat pups the content of products of induced PPO surpassed normal by 50%. The concentration of initial products of induced PPO in prenatally stressed adult animals increased by 50% (270 nm). However, the content of final products of induced PPO in these rats decreased by 28% (363 and 370 nm).

Our results indicate that the intensity of spontaneous and induced PPO in prenatally stressed rats undergoes wave-like changes during postnatal ontogeny. These variations reflect an imbalance in the peroxidation—antioxidant system, which is especially pronounced during the development and maturation of CNS in early postnatal ontogeny. It can be hypothesized that prenatal stress modifies the dynamics of brain maturation. These changes produce an adverse effect in the follow-up period.

A significant decrease in the content of PPO products in the plasma from prenatally stressed rat pups aging 10 and 30 days is probably followed by inhibition of heat shock protein synthesis. Published data show that structurally modified proteins are the major inductors of heat shock proteins [10]. Probably, weak induction of heat shock proteins in this period has an adverse effect on adaptive behavior of prenatally stressed rats. Accumulation of initial and final products of induced PPO in this period reflects improvement of physiological reserves, which plays a compensatory role. No differences were revealed in the contents of products of spontaneous PPO in control and stressed rats aging 90 days. Probably, the negative consequences of prenatal stress are compensated in the absence of physiological stresses. However, we observed accumulation of initial products of induced PPO (270 nm) in prenatally stressed adult rats. These changes reflect prevalence of antioxidant components responsible for inactivation of primary ROS (superoxide and peroxide). It should be emphasized that they act as secondary messengers and receptor modulators [11]. The reduced content of final products of PPO illustrates deficiency of the system for quenching of free radical chain reactions and decrease in physiological reserves of the organism. The observed changes can contribute to impairment of higher nervous activity in prenatally stressed rats during the late postnatal ontogeny.

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