Differences in Protein Peroxidation in Pregnant Rats Selected by Nervous System Excitability Threshold

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Differences in the intensity of serum protein peroxidation were detected in pregnant rats with high and low peripheral nervous system excitability threshold in stress. Stress induced shifts in serum protein peroxidation, but these changes were opposite in rats of the two studied strains.

Key Words: *protein peroxidation; pregnancy; stress*

Free-radical oxidation of biomolecules with reactive oxygen species (ROS) plays an important role in the development of pathological changes during exposure to various factors. It is also known that pregnancy modifies many reactions of the organism at the level of nervous and endocrine systems [10]. Stress experienced by mothers during various terms of gestation can have a negative impact on physiological functions in their progeny [10], but biochemical mechanisms of these disorders are still unclear. On the one hand, we know the important role of the psychotype in the development of pathological reaction to stress [2,5-7]. Study of biochemical processes in animals with genetically determined differences in the CNS function will help to understand the mechanisms and regularities in the formation of pathological states. Rats with high and low excitability threshold (HET and LET, respectively) selected at I. P. Pavlov Institute of Physiology are models of opposite types of the nervous system functioning [6].

The study of biochemical mechanisms underlying the development of stress confirmed the primary role of oxidative reactions leading to accumulation of ROS and stimulation of free-radical oxidation processes [3]. Free-radical oxidation of proteins consists in their post-translation covalent modification essential for various physiological and biochemical processes, such as aging, tissue and energy metabolism, *etc*. [11]. Pathological effects of activated peroxidation processes are explained by the formation of intermolecular cross-links modifying physicochemical characteristics of cell membrane [8]. This process is a nonspecific reaction to stress in the course of cell adaptation to external factors.

Intensification of free-radical oxidation becomes more significant clinically manifested pathologies and during pregnancy. Changes in serum protein peroxidation (PPO) intensity reflect the intensity of free-radical processes in the whole body. We investigated the effect of emotional painful stress on serum PPO in HET and LET rats.

MATERIALS AND METHODS

Experiments were carried out on pregnant HET and LET rats, exposed to electrocutaneous stimulation during 15 min on day 16 of pregnancy; this term is critical for the formation of fetal neuroendocrine system, and hence, maternal neurohumoral status is in many aspects decisive for the differentiation and functioning of fetal brain [9]. One hour after stress the rats were decapitated, blood was centrifuged at 200*g* for 10 min. The serum was collected and PPO products were measured as described previously [4] with some modifications.

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For measurement of spontaneous PPO, 0.05 ml serum diluted 1:10 with normal saline was incubated with 0.95 ml 0.015 M potassium-phosphate buffer at 37°C for 15 min. The mixture for measurement of stimulated PPO contained 0.05 ml diluted serum, 0.75 ml buffer, 0.1 ml 10 mM Fe^{2+}, and 10 mM EDTA and 0.1 ml 1 M H_2O_2. The final volume of samples was 1 ml. After incubation proteins were precipitated with 1 ml 20% trichloroacetic acid, and PPO products were stained in the reaction with 1 ml 0.1 M 2,4-dinitrophenyl hydrazine diluted in 2 N HCl (1 h at 18-20°C). The samples were centrifuged at 200*g* for 10 min. The precipitate was washed twice in an ethanol-ethylacetate mixture of (1:1), dried, and dissolved in 3 ml 8 M urea with 1 drop of 2 N HCl.

Peroxidation products quantitatively reacted with 2,4-dinitrophenyl hydrazines with the formation of 2,4-dinitrophenyl hydrazones. The reaction products were measured at λ=270 nm (aldehydephenyl hydrazones), λ=363, and λ=370 nm (ketondinitrophenyl hydrazones); the degree of oxidative modification of proteins was expressed in optical density units/mg protein.

The data were processed using Excel 7 software, the arithmetic means and standard deviations were calculated. The significance of differences was evaluated using Student's *t* test. The differences between the means were considered significant at $p \leq 0.05$.

RESULTS

The content of PPO products measured at λ=270 nm tended to increase in HET rats, while the concentrations of PPO products measured at λ=363 and λ=370 nm were higher in LET rats (Table 1).

LET rats showed higher content of initial products of stimulated PPO measured at λ=270 nm compared to HET rats. The concentrations of PPO products measured at λ=363 and λ=370 nm was also higher in LET rats (insignificant), which probably indicates a more potent metabolic reserve.

TABLE 1. Serum Protein Peroxidation (optical density units/mg protein) in Pregnant HET and LET rats (*M*±*m*, *n*=6)

PPO; λ, nm	HET	LET
Spontaneous		
270	0.140±0.062	0.065±0.005
363	0.089±0.027	0.115±0.016
370	0.108±0.025	0.130±0.019
Stimulated		
270	0.091±0.043	0.179±0.024
363	0.144±0.022	0.216±0.029
370	0.179±0.017	0.227±0.021

Changes in the intensity of spontaneous PPO were similar in both rat strains (Fig. 1, *a*). The content of spontaneous PPO products detected at λ=270 nm decreased by 48 and 80% in LET and HET rats, respectively, that of PPO products detected at λ=363 and λ=370 nm by 70 and 50% in HET and LET rats, respectively. Changes in stimulated PPO in HET and LET rats were oppositely directed (Fig. 1, *b*). Significant changes in stimulated PPO were observed in HET rats: the content of PPO products measured at λ=279 and λ=363 nm increased by 116 and 74%, respectively. In LET rats the content of PPO products measured at λ=363 and λ=370 decreased by 24 and 29%, respectively.

Hence, the dynamics of changes in spontaneous PPO characterizing the total physiological status during stress was similar in both rat strains and attested to a decrease in the oxidation potential and, presum-

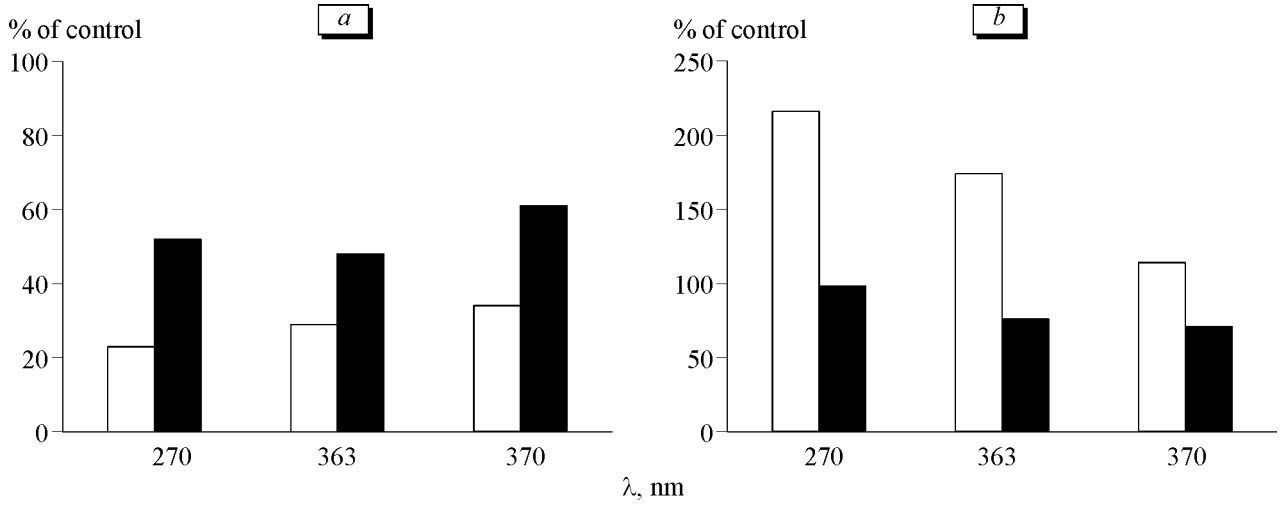

Fig. 1. Spontaneous (*a*) and stimulated (*b*) peroxidation of serum protein in pregnant rats with high (light bars) and low (dark bars) nervous system excitability thresholds after electrocutaneous stimulation.

ably, increased potency of the total antioxidant defense system. However changes in the intensity of stimulated PPO characterizing reserve physiological capacities of the organism were oppositely directed: the content of stimulated PPO products during stress increased in HET rats and decreased in LET rats. Hence, this type of stress induced opposite changes in reserve capacities of pregnant rats of the two studied strains. Evaluation of PPO intensity in stress can be used as a test for evaluation of the aftereffects of stress in pregnant animals with different excitability of the nervous system.

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