BIOPHYSICS AND BIOCHEMISTRY

Serum Protein Peroxidation in Rats Selected by the Rate of Active Avoidance Conditioning under Normal Conditions and during Stress

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 3, pp. 286-289, March, 2002
Original article submitted October 22, 2001

Serum protein peroxidation was studied in rats with different strategy of adaptive behavior at rest and during short-term immobilization stress. Oxidative destruction of serum proteins was less intensive in KLA rats compared to that in KHA rats. Stress stimulated peroxidation of serum proteins in KHA rats.

Key Words: *lipid peroxidation; adaptive behavior; stress*

The relationship between human or animal behavior and reaction to external factors is a very interesting problem. Increased information flow, intensive intellectual work, and hence, increased incidence of central nervous system pathology require understanding of biochemical processes underlying adaptive behavior and factors inducing its disorders. Intensive fundamental studies in this field became possible after creation of mouse and rat strains selected by different behavioral signs [10]. KLA and KHA rat strains were selected at I. P. Pavlov Institute of Physiology. Apart from differences in the primary selection sign (rate of active avoidance conditioning) these strains represent a model of opposite strategies of adaptive behavior: Koltushi Low Avoidance (KLA) animals are characterized by passive behavior and Koltushi High Avoidance (KHA) animals by active behavior [6]. Study of biochemical reactions to stress in animals with genetically determined behavioral response helps to understand the mechanisms underlying animal resistance to emotiogenic factors and formation of pathological states.

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Free-radical oxidation of biomolecules by reactive oxygen species (ROS), by-products of reduction of molecular oxygen (superoxide ion, H_2O_2, hydroxyl radical, and organic radicals formed in the organism after reaction with these substances) [1,12], plays an important role in the development of pathological changes in the organism exposed to various factors [3,8,9]. It is believed that intensification of peroxidation processes indicates dysregulation of defense and adaptive reactions at the cellular level and in the whole organism [7]. Pathological effects of activation of peroxidation processes are explained by the formation of intermolecular cross-links modulating physicochemical properties of biomolecules [12].

Numerous publications describe the mechanisms of LPO and its role in normal and abnormal cell functioning, but active oxygen forms are also responsible for oxidative destruction of proteins. During oxidative stress cell proteins are first attacked by ROS [5,11,12]. On the other hand, posttranslation covalent modification of proteins by ROS is essential for physiological and biochemical processes, such as aging, tissue and energy metabolism [14]. Since metabolic processes in the blood reflect to a great extent processes in the whole organism, changes in blood protein peroxida-

0007-4888/02/1333-0243$27.00

tion (PPO) reflect changes in the general oxidant balance.

The purpose of this study was to elucidate the effect of immobilization stress on serum PPO in KHA and KLA rats. We analyzed spontaneous PPO reflecting realized oxidation potentials of the organism and stimulated PPO characterizing physiological reserve capacities.

MATERIALS AND METHODS

Adult male KLA and KHA rats (150-200 g) were exposed to 60-min immobilization stress in narrow plastic boxes. Immediately after stress the animals were decapitated, blood was collected into centrifuge tubes, and centrifuged at 200*g* for 10 min. The serum was collected and PPO products were analyzed as described previously [4] with some modifications. 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 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 (ketodinitrophenyl 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 $p \leq 0.05$.

TABLE 1. Spontaneous and Stimulated Serum Protein Peroxidation (optical density units/mg protein) in KLA and KHA Rats (*M*±*m*, *n*=5)

PPO; λ, nm	KLA	KHA
Spontaneous		
270	0.101±0.030	0.122±0.020
363	0.096±0.020*	0.146±0.008
370	0.176±0.010	0.164±0.008
Stimulated		
270	0.288±0.030	0.219±0.030
363	0.285±0.010	0.228±0.030
370	0.176±0.010**	0.241±0.020

Note. *$p<0.001$, **$p<0.05$ compared to KHA rats.

RESULTS

Control KLA rats were characterized by a lower content of neutral protein keto derivatives measured at λ=363 nm for spontaneous PPO ($p<0.01$) and at λ=370 nm for induced PPO ($p<0.05$) (Table 1). This indicates a lower level of oxidative destruction of serum proteins in KLA rats.

Immobilization stress induced considerable changes in plasma PPO, but these changes were opposite

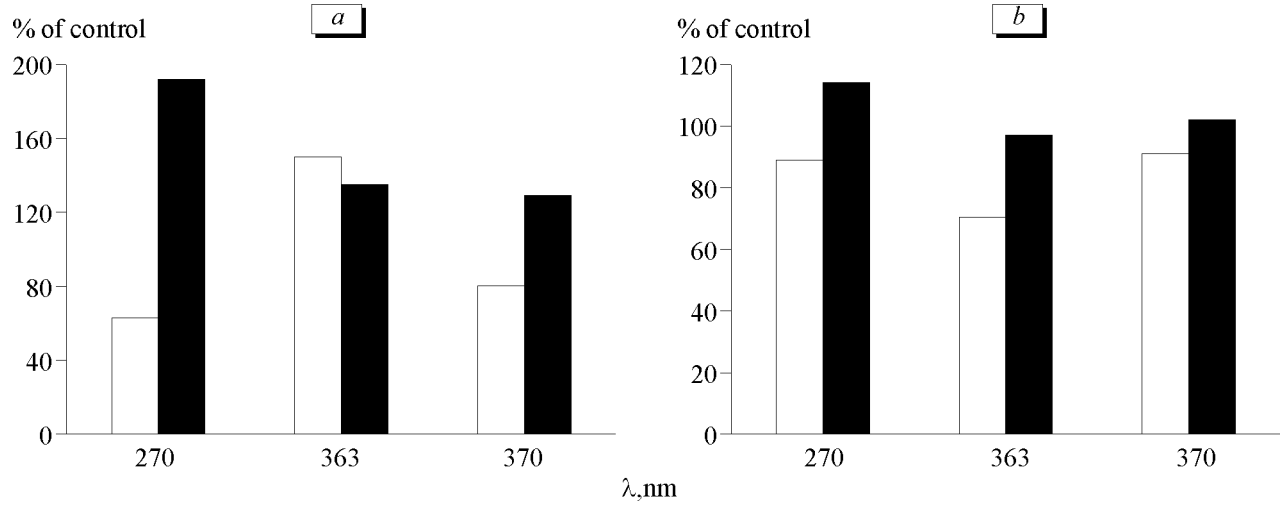

Fig. 1. Spontaneous (*a*) and stimulated (*b*) serum protein peroxidation in KLA (light bars) and KHA rats (dark bars) during short-term immobilization stress.

in KHA and KLA rats (Fig. 1, *a*). In KHA rats the content of all PPO products increased after stress ($p<0.01$. In KLA rats we observed accumulation of products measured at $\lambda=363$ nm ($p<0.01$), whereas the content of other PPO products tended to decrease.

Analysis of stimulated PPO showed that stress had no effect on maximum level of plasma PPO in KHA rats, while in KLA rats the levels of PPO products measured at $\lambda=363$ nm decreased ($p<0.01$, Fig. 1, *b*). It was previously shown that the differences in some physiological parameters between these rat strains manifested only under conditions of stress [6]. We obtained similar data for serum PPO, which can confirm the genetic difference in biochemical systems of protein metabolism in rats of these two strains.

Our experiments showed that stress intensified spontaneous PPO in rats with active behavior (KHA) and had no effect on these processes in rats with passive behavioral strategy (KLA). Parameters of stimulated PPO were similar in KLA and KHA rats and in rats exposed and not exposed to stress, which indicates similar physiological reserves in rats of each strain and the absence of pathological exhaustion in this model of stress. Intensification of spontaneous PPO in KHA rats during stress can be indicative of a more active course of oxidative metabolic processes during stress. It is quite possible that the processes inducing destruction of protein molecules will promote the development of depressive states in KHA rats after inevitable stress [6].

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