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Activity of 20S Proteosomes and Content of Oxidized Proteins in Rat Liver after Long-Term Cold Exposure

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We studied the effect of long-term (5 and 10 days) cold exposure (4°C) on oxidative damage to proteins and proteosomal activity in the liver of Wistar rats. It was shown that core temperature on the 10th day of cold exposure decreased by 1.1°C. The content of oxidized proteins increased at this term. Chymotrypsin-like and peptidylglutamyl peptide hydrolyzing activities increased, while trypsin-like activity decreased during cold exposure.

Key Words: *cold stress; liver; oxidative damage to proteins; proteosomal activities*

Exposure to various environmental factors activates cellular and molecular processes of homeostasis regulation. Adaptation of the organism to cold is based on stimulation of oxidative metabolism, which is coupled to enhanced generation of ROS and increased risk of oxidative damage to macromolecules. The result of adaptation under these conditions is determined by adequate reaction of systems preventing and/or correcting these shifts. We previously showed that accumulation of free-radical oxidation products during long-term exposure to moderate cold is confined to activation of antioxidant defense enzymes, mobilization of endogenous vitamin E stores, and intensive extraction of this vitamin from the food [2]. We found no published data on accumulation of oxidized proteins and changes in systems responsible for their elimination.

Intracellular proteolysis is effected by lysosomal and proteosomal systems [1,7]. Most oxidized intracellular proteins undergo selective proteolysis by proteosomes [6], multicatalytic multifunctional proteinases. The best studied 26S proteosome responsible for ATP-dependent degradation of ubiquitin-labeled proteins contains a 20S proteolytic core with 19S regulatory complexes. In parallel, 20S proteosomes realize ATP-independent degradation of oxidized intracellular proteins [6]. 20S proteosomes exhibit three types of endopeptidase activity (trypsin-like, chymotrypsin-like, and peptidylglutamyl peptide hydrolase activities) determining cleavage of the protein molecule in sites formed by basic, hydrophobic, and acidic amino acid residues, respectively.

Proteosomal activity and protein oxidation at low temperatures are actively studied on cell cultures. The aftereffects of cold exposure on protein oxidation and proteosomal activity in living organism were described earlier [12].

Here we studied the effects of long-term moderate cold exposure on the level of oxidative damage to proteins and on endopeptidase activities of 20S proteosomes in rat liver.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 150-200 g (Laboratory of Animal

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Breeding, Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk). The animals were kept in individual cages and had free access to standard fodder and water. The animals of the experimental groups were maintained at 4°C for 5 on 10 days, controls were kept at room temperature. Rectal temperature was daily measured. The rats were decapitated under ether narcosis.

Cytosol fraction of liver cells was isolated routinely by differential centrifugation.

Oxidative damage to proteins of the cytosol fraction was evaluated by the content of carbonyl group (measured spectrophotometrically after the reaction with (2,4-dinitrophenyl)hydrazine [11]. Three samples containing 0.5 mg cytosol protein were used in each case. Two samples were incubated in 4-fold volume of 10 mM (2,4-dinitrophenyl)hydrazine in 2.5 M HCl for 1 h at room temperature. The third sample (blank) was incubated in 2.5 M HCl. The reaction was stopped by adding 5-fold volume of 20% TCA. The pellet was twice washed with ethylacetate-ethanol 1:1 mixture, the protein was dissolved in 6 M guanidine hydrochloride, and optical density was measured at 370 nm. The protein yield was determined in each sample. The content of carbonyl groups was calculated using molar extinction coefficient for aliphatic hydrazones 22,000 $M^{-1}cm^{-1}$ and was expressed as 1 nmol carbonyl groups per 1 mg protein.

Three types of peptidase activity (peptidylglutamyl-peptide hydrolase, trypsin-like, and chymotrypsin-like activities) were determined in liver cytosol using fluorogenic substrates Leu-Leu-Glu-AMC (aminomethyl coumarin), Pro-Phe-Arg-AMC, and Leu-Leu-Val-Tyr-AMC, respectively [12]. The cytosol protein was incubated with 10 μl 2 mM substrate in a buffer containing 0.15 M sucrose, 25 mM HEPES (pH 7.8), 20 mM $MgCl_2$, 1 mM EDTA, and 1 mM dithiothreitol for 30 min at 37°C with constant shaking. The reaction was stopped by adding an equivalent volume of 96% ethanol. Fluorescence was measured at excitation and emission wavelengths of 380 and 440 nm, respectively, using animomethyl coumarin as the standard.

The results were standardized by protein content (measured after Lowry using BSA as the standard). The data were processed statistically using Statistica 6.0 software. Significance of differences was evaluated by Student *t* test.

RESULTS

The exposure at 4°C for 10 days led to a reliable decrease in core temperature by 1.1°C (p=0.003, Fig. 1, *a*). Body weight in rats decreased by day 5 of cold exposure, but returned to the control value by day 10 (Fig. 1, *b*).

The content of carbonyl groups reflects the intensity of oxidative damage to liver cytosol proteins (Fig. 2). This parameter started to increase on day 5 of cold exposure and 2.4-fold surpassed that in controls on day 10 (p<0.01). Similar results were reported previously [12]: the content of oxidized proteins in the liver of field voles increased after 10-h and 4.5-day exposures at 4-10°C. The increase in the content of carbonyl groups in field voles after 4.5-day cold exposure was comparable to that observed in our experiment on day 5 of cold exposure (~40%).

Changes in 20S proteosomal activities in rat liver after cold exposure varied (Fig. 3). Two pro-

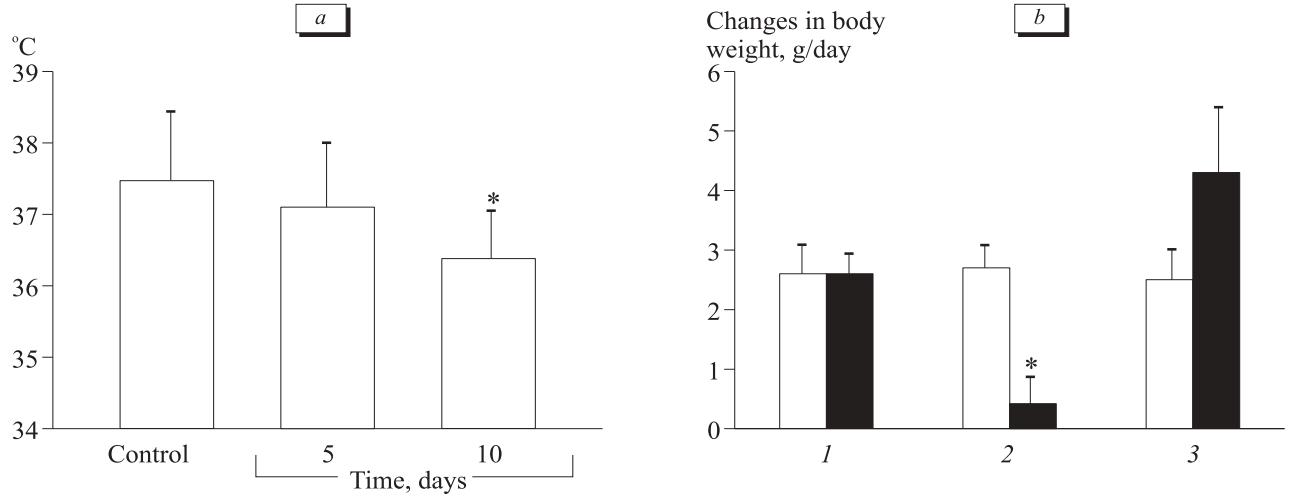

Fig. 1. Dynamics of core temperature (*a*) and mean daily weight gain (*b*) in rats. *1*) 5 days before the experiment, *2*) after 5-day cold exposure, *3*) cold exposure for 6-10 days. Light bars: control animals, dark bars: cold exposure. *p<0.005 compared to the control.

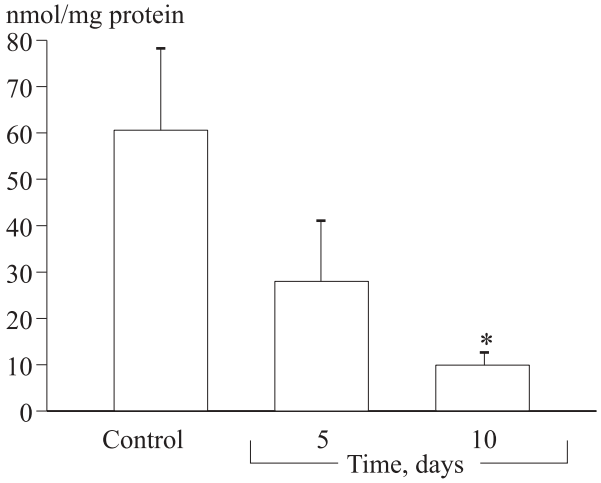

Fig. 2. Dynamics of the content of carbonyl groups in rat liver cytosol during cold exposure. Here and on Fig. 3: *1*) control, *2*) 5 days, *3*) 10 days. *$p<0.01$ compared to the control.

teosomal activities increased. Peptidylglutamyl-peptide hydrolase activities increased 2-fold ($p<0.01$) on day 10, while chymotrypsin-like activity increased more markedly and rapidly: 3.4-fold ($p<0.0005$) on day 5 of the experiment, and remained at this level to day 10. Trypsin-like activity decreased 2.7-fold ($p<0.01$) by day 10. When comparing our data on proteosomal activities in rat liver on day 5 with the results obtained on field voles after 4.5-day cold exposure [12], we found that chymotrypsin activity in voles (in contrast to rats) after 4.5 days did not differ from the control.

Proteosome activity depends on various factors (oxidative stress, age, malnutrition) [8,14]. Our findings suggest that cold exposure had different effects on proteosome activities in the liver. The content of oxidized proteins can both activate and inhibit 20S proteosomal activities, as it was demonstrated for chymotrypsin-like activity [10]. Positive regulation of peptidylglutamyl-peptide hydrolase and chymotrypsin-like activities after 10-day cold exposure probably reflects an adaptive response to accumulation of moderately oxidized proteins. At the same time, severe oxidative damage to proteins leads to inhibition of proteolysis by 20S proteosomes due to cross-links formation, aggregation, and increase in protein hydrophobicity [6]. The observed decrease in trypsin-like activity in rat liver on day 10 of cold exposure can be a result of this negative regulation. Moreover, 20S proteosomes also can be a target of oxidative stress [4]

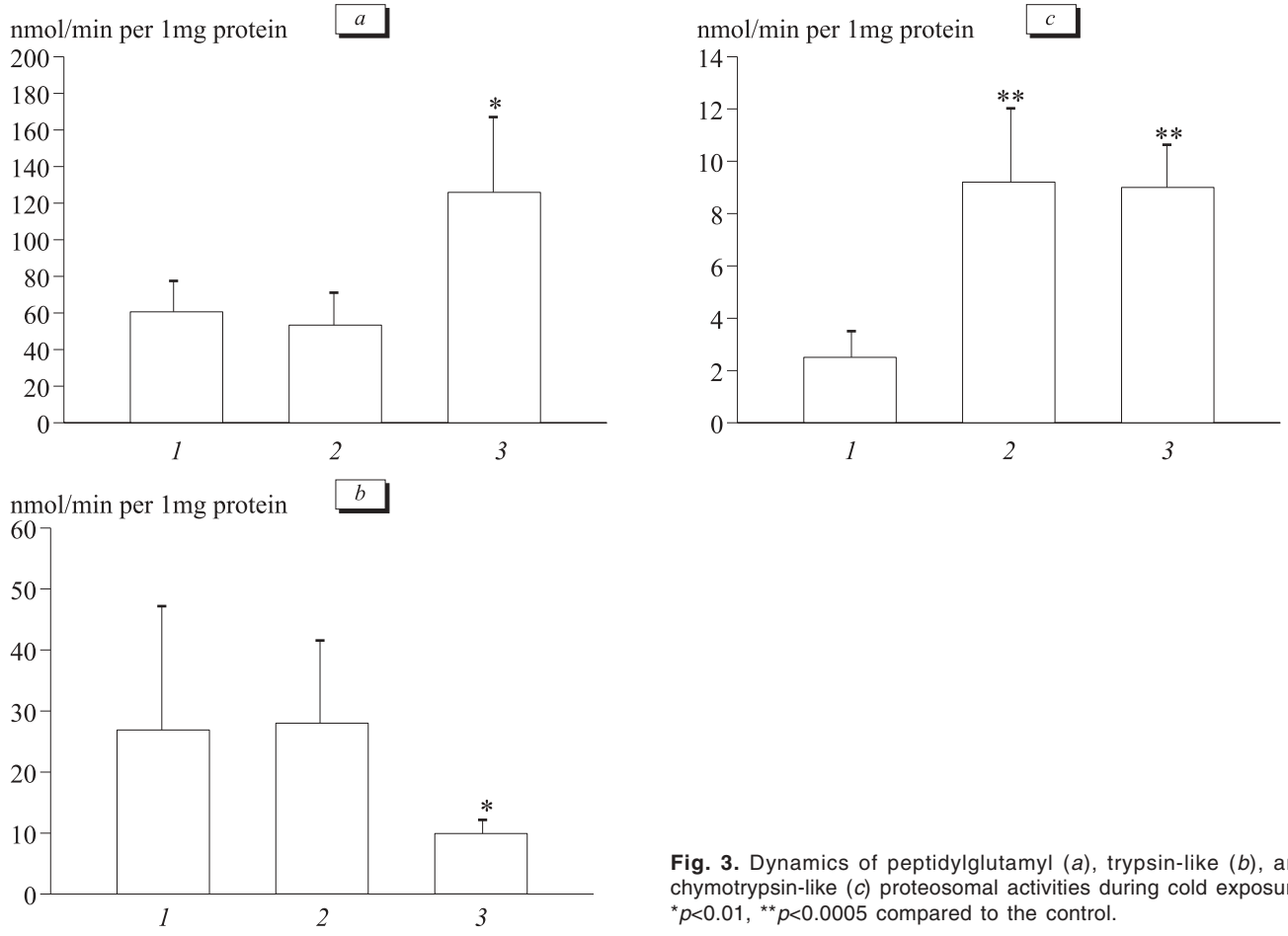

Fig. 3. Dynamics of peptidylglutamyl (*a*), trypsin-like (*b*), and chymotrypsin-like (*c*) proteosomal activities during cold exposure. *$p<0.01$, **$p<0.0005$ compared to the control.

and it cannot be excluded that different 20S proteosomal activities have different resistance to cold-induced oxidative damages.

The maintenance of core temperature is a measure of cold adaptation. Hence, the decrease in core temperature by 1°C on day 10 of cold exposure attests to insufficient adaptive reaction of animal organism. However, this term corresponded to body weight recovery after its transient decrease on day 5 of cold exposure. These changes were paralleled by accumulation of oxidized proteins in the liver and maximum changes in 20S proteosomal activities. It is well established that oxidative stress, *i.e.* imbalance in the systems of ROS generation and detoxification, induces accumulation of oxidized proteins [5]. At the same time, their accumulation can be associated with decreased proteosomal activity and impaired elimination of damaged proteins. This takes place during aging and some pathologies [15]. Intensification of metabolism during cold exposure and physical exercise also leads to oxidative stress, so called physiological oxidative stress [3,13], resulting in not only oxidative damages to macromolecules, but also activation of intracellular signal transduction responsible for triggering of adaptive response program in the cell aimed at fortification of the functional reserves [9].

We previously showed that activation of free-radical lipid oxidation in tissues is observed at the initial stages of moderate cold exposure, while then the content of LPO products returns to normal against the background of activation of antioxidant enzymes, mobilization of endogenous vitamin E stores, and intensive extraction of this antioxidant from the food [2]. Our experiments demonstrated activation of the system of elimination of oxidized proteins; maximum accumulation of these proteins coincides with the decrease in body temperature and body weight recovery, *i.e.* intensification of plastic processes after their suppression under the effect of cold exposure.

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