Corticosterone and Lipid Peroxidation in Rats after Two Exposures to Cold

N. G. Kolosova, G. M. Petrakova, and M. A. Gilinskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 127, No. 3, pp. 261-264, March, 1999
Original article submitted May 12, 1998

The relationships between serum corticosterone content, intensity of lipid peroxidation (LPO) and the concentration of tocopherol in tissues, and the transmembrane potential in thymocytes were studied in rats exposed to two consecutive coolings. Both exposures increased serum corticosterone. The first exposure activated LPO in the serum, while the second stimulated LPO in thymocytes. The second cooling lowered body temperature to a lesser extent than the first one. Body temperature did not depend on the content of LPO products or corticosterone, but negatively correlated with the content of tocopherol in the brain hemispheres and adrenal glands. The rats exhibiting high-level thermoregulation after the first exposure to cold showed a higher thymocyte transmembrane potential after the second cooling. The second exposure potentiated the negative relationship between the brain and serum content of corticosterone and LPO products, which indicates that the content of LPO products cannot be used as an index of stress intensity.

Key Words: *cold; stress; corticosterone; lipid peroxidation; tocopherol*

Activation of lipid peroxidation (LPO) is the most important pathogenic event in stress causing disruption of biological membranes and uncoupling of oxidative phosphorylation. Along with the adrenal cortical hormones, LPO products are widely used as a measure of stress-response. It is currently accepted that the content of LPO products is directly proportional to the intensity of stress. However, the relationship between LPO activity and the duration and intensity of stress is not linear, and the content of LPO products can decrease during the initial phase of organism's response to stress. Such a decrease was observed at some periods of the emotional pain stress [2], during physical exercises [12] and cold exposure [5,10,11]. The data indicate that the periods during which the content of LPO products in diverse organs and tissues is decreased are not synchronized [10], but coincided with an increase in the serum content of corticosteroids. The total effect of steroids is probably determined by their antioxidant properties [9] and the modulating effects on the antioxidant system, the key stress-protecting system controlled by a variety of hormones, including corticosteroids [6,15]. Therefore, it remains unclear what is reflected by LPO changes at different stress-response periods and how these changes correlate with adrenal cortex activation.

To answer these questions we used the model of repeated cooling (two 1.5-h exposures with a 48-h interval). Correlation analysis was applied to assess the relations between serum concentration of corticosterone (CS) and the content of LPO products and tocopherol in tissues. Additionally, we measured the transmembrane potential of thymocytes ($\Delta\psi$), which is the sum of $\Delta\psi$ on the plasma and mitochondrial membrane. Thymocytes represent an adequate model for the assessment of the intensity of bioenergetic processes [1] which constitute the basis of thermoregulation under cold exposure. As shown previously, changes in thymocytes during low-temperature adaptation correlate with changes in bioenergetic processes in other tissues [10].

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

0007-4888/99/0003-0236$22.00

MATERIALS AND METHODS

Experiments were carried out on 58 male Wistar rats weighing 200-240 g. The rats were housed in plastic cages (8-10 per each) and maintained at room temperature and standard light/dark cycle (1:1) with free access to food and water. During the experiment the rats were exposed to cold (1-2°C for 1.5 h) in tight plastic boxes with numerous thermoregulatory holes. Group 1 rats were decapitated after the first exposure. Group 2 rats were returned to home cages, the cooling procedure was repeated after 48 h, and the rats were decapitated. A lesser decrease in body temperature after the second cooling was considered as the index of increased cold resistance. Rectal temperature was measured by a TPEM medical electrothermometer with a probe inserted to the depth of approximately 6 cm. Serum level of CS was determined by column chromatography (Nucleosil C18 250 mm, 5 μ) [13]. The intensity of lipid peroxidation was assessed in the anterior lobes of brain hemispheres, adrenal glands, and brown adipose tissue by measuring the TBA reactive products (MDA), conjugated dienes (CD, by UV spectroscopy), and Schiff bases (fluorescence of lipid extracts at 4°C in the presence of 0.001% ionol) [4]. The tocopherol concentration was determined fluorometrically [14]. Thymocytes were isolated by a standard technique, washed and resuspended in Hanks' solution. $\Delta\psi$ was measured using the fluorescent cationic probe DSM (4-(n-dimethylaminostyryl)-1-methylpyridine, Zonde) [3]. The data were analyzed statistically by Pearson's correlation test.

RESULTS

A decrease in body temperature caused by cooling was less pronounced during the second exposure, i.e., repeated cooling was accompanied by a lesser heat loss. After the first exposure the body temperature decreased by 3.5±0.62°C and after the second exposure only by 1.5±0.28°C ($p<0.001$). Serum CS content was elevated 5.7 times and 4.9 times after the first and second exposure, respectively (Table 1). Serum level of LPO products increased significantly (by 18%) only after the first cooling (Table 1). The thymocyte CD content tended to decrease after the first exposure but significantly increased after the second (Table 1). In the brain hemispheres, the content of CD remained unchanged, but the concentration of Schiff bases, the final LPO products, decreased after the second exposure. No changes in LPO products were observed in the adrenal glands and brown adipose tissue (Table

TABLE 1. Serum Content of CS and Tissue Contents of LPO Products and Tocopherol in Rats after Two Exposures to Cold (*M±m*)

Index	Control	Cold exposure	
		1st	2nd
Corticosterone, μg/ml	0.735±0.18	4.18±0.32*	3.59±0.29*
LPO products			
MDA, nmol/ ml serum	5.71±1.20	6.83±0.23*	6.69±0.83
Schiff bases, rel. units/mg lipids			
right hemisphere	12±2.5	10±1.9	5.9±1.2*
left hemisphere	13±3.5	9±2.1	6.8±1.9*
Conjugated dienes, A_{233}/mg lipids			
right hemisphere	2.0±0.23	1.9±0.19	1.5±0.26
left hemisphere	2.1±0.34	1.8±0.31	1.9±0.35
thymocytes	1.61±0.14	1.25±0.19	2.60±0.30*
brown adipose tissue	1.70±0.11	1.63±0.05	1.62±0.12
Tocopherol			
serum, μg/ml	6.8±0.9	8.6±1.9	15.2±3.2*
Tocopherol, μg/g			
brown adipose tissue	82±7	90±6	101±6*
adrenal glands	113±15	164±19*	146±21
right hemisphere	113±15	164±19*	146±21
left hemisphere	24±1.3	22±1.15	21±3.3

Note. *$p<0.05$ in comparison with the control.

TABLE 2. Correlations between Serum CS Content and LPO Activity in Serum and Brain Hemispheres after Exposure to Cold

Index	Control	Cold exposure	
		1st	2nd
Serum MDA	-0.336	-0.454	-0.961**
Conjugated dienes			
left hemisphere	0.230	-0.084	-0.478
right hemisphere	-0.133	-0.349	-0.714*
Shiff bases			
left hemisphere	0.041	-0.397	-0.563
right hemisphere	-0.391	0.022	-0.663*

Note. *$p<0.05$, **$p=0.001$ in comparison with the control.

1).The cooling procedure caused redistribution of tocopherol between different organs and tissues. The level of this lipid-soluble antioxidant increased 1.5 times in the adrenal glands after the first exposure and in serum and brown adipose tissue after the second exposure without changes in the brain (Table 1). Previously we showed that glucocorticoids play an important role in tocopherol mobilization under different stress conditions, including cooling [6]. Two cold exposures decreased $\Delta\psi$ from -210±3 to 191±4 and to 194±4 after the first and second exposure, respectively, which indicates uncoupling of oxidative phosphorylation.

Exposure to cold modified correlations between the studied parameters. In control rats, the weak negative correlation between serum content of CS and LPO products slightly increased after the first exposure and approached 1.0 (i.e., the level of a functional relation) after the second exposure (Table 2). Negative correlation between the level of serum CS and brain content of LPO products (CD and Schiff bases) appeared after the second exposure to cold. In thymocytes, the level of LPO products increased only after the second exposure with the emergence of a negative correlation with $\Delta\psi$ (r=-0.82, p=0.04).

The decrease in body temperature reflects the quality of adaptive changes and indirectly shows the level of heat loss. After the first exposure, its value correlated only with the body weight (r=-0.9, p=0.03). There were no significant correlations between this index and others (serum CS concentration, tissue content of LPO products, and $\Delta\psi$). However, after the second exposure $\Delta\psi$ was higher in animals with lower heat loss and, consequently, with better thermal homeostasis. The correlation between the indirect index of heat loss and body weight disappeared after the second exposure, but the temperature decrease turned out to be inversely proportional to the content of tocopherol in the brain hemispheres (r=-0.9, p=0.002 and r=-0.8, p=0.016 for the right and left hemisphere, respectively) and adrenal glands (r=-0.8, p=0.03).

Our data showed that after exposure to cold, changes in the content of LPO products are not co-directed with changes in the CS level and do not reflect the intensity of stress response. The stronger negative relations between these indices after repeated cooling and the emergence of new correlations can be considered as manifestations of adaptive changes aimed at the optimization of the organism's response to stress factors and reduction of heat loss. The inverse relations between the decrease in body temperature and tissue tocopherol content confirm its important role in the maintenance of organism's resistance to cold, which is realized not only through its free radial scavenger function, but also through systemic mechanisms including modulation of the state and reactivity of the adrenal cortex [7,8].

REFERENCES

1. Z. Z. Gizatullina, O. A. Sukacheva, and I. A. Gagel'gans, *Biokhimiya*, **61,** No. 3, 445-450 (1996).
2. N. V. Gulyaeva and M. Yu Stepanichev, *Zh. Vyssch. Nervn. Deyat.*, **27,** No. 4, 462-469 (1997).
3. G. E. Dobretsov, *Fluorescent Probes in Cell, Membrane, and Lipoprotein Research* [in Russian], Moscow (1989).
4. V. E. Kagan, O. N. Orlov, and L. L. Prilipko, *Advances in Science and Technology, Ser. Biophysics* [in Russian], **18.** (1986).
5. N. G. Kolosova, A. R. Kolpakov, and L. E. Panin, *Vopr. Med. Khim.*, No. 6, 31-37 (1995).
6. N. G. Kolosova and V. Yu. Kulikov, *Byull. Sib. Divis. Akad. Med. Nauk SSSR*, No. 4, 93-93 (1989).
7. Yu. P. Shorin, V. G. Selyatitskaya, N. G. Kolosova, and V. Yu. Kulikov, *Byull. Eksp. Biol. Med.*, **99,** No. 6, 69-71 (1985).
8. Yu. P. Shorin, V. G. Selyatitskaya, N. G. Kolosova, and V. Yu. Kulikov, *Fiziol. Zh.*, **32,** No. 2, 184-188 (1986).
9. Y. Goodman, A. J. Bruce, B. Cheng, and M. P. Mattson, *J. Neurochem.*, **66,** No. 5, 1836-1844 (1996).

10. N. G. Kolosova, A. R.Kolpakov, I. G. Shabalina, and L. E. Panin, *Membr. Cell Biol.*, **9,** No. 6, 641-648 (1996).
11. P. Kovacs, I. Juranek, T. Stankovicova, and P. Svec, *Pharmazie*, **51,** No. 1, 51-53 (1996).
12. T. Ohkuwa, Y. Sato, M. Naoi, *Acta Physiol. Scand.*, **159,** No. 3, 237-244 (1997).
13. A. Santos-Mentes, R. Gonzalo-Limbreras, R. Izquierdo-Hornillos, *J. Chromatogr. B. Biomed. Appl.*, **673**, No. 1, 27-33 (1995).
14. S. L. Teylor, M. Lamden, and A. L. Tappel, *Lipids*, **11,** No. 7, 530-538 (1976).
15. F. J. Walther, R. Daviv-Cu, E. I. Mehta, *et al.*, *Am. J. Physiol.*, **271,** No. 2, Pt. 1, L187-L191 (1996).