Imbalance between Rat Blood Metalloproteins in the Early Stage of Hypokinesia

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Changes in the contents of blood metalloproteins with prooxidant (plasma cytochromes b_{558} I and b_{558} II, erythrocyte membrane cytochromes b_{558} III and b_{558} IV, superoxide-producing plasma lipoprotein suprol, and cytochrome b_5 from soluble erythrocyte fractions) and antioxidant activities (Cu,Zn-superoxide dismutase, catalase, ceruloplasmin, and transferrin) depended on the duration of hypokinesia (5, 10, and 15 days). The content of metalloproteins, particularly cytochrome b_5 and ceruloplasmin, increased at the initial stage, but decreased at later stages of hypokinesia (except for cytochrome b_5 concentration, which continued to increase).

Key Words: *hypokinesia; blood; metalloproteins; oxidative damage*

Hypokinesia produces various adverse effects. Short-term hypokinesia leads to congestion in parenchymal organs [7], impairment of the cerebral blood flow [1], cardiac dysfunction [12], and decrease in mouse bone mass due to stimulation of osteoclast growth [11]. These changes are associated with insufficient oxygen supply to cells, which is also characteristic of hyperkinesia, high physical activity, when oxygen consumption by cells increases by almost 10 times [9]. Oxygen deficiency during hypokinesia is related to circulatory disturbances [2]. Intensification of lipid peroxidation (LPO) in cell membranes and extracellular systems induced by reactive oxygen species (ROS) is the pathogenetic mechanism of damages to cells and blood components [10]. These processes are associated with insufficient supply of molecular oxygen, which is involved in oxidation-reduction metabolism in cells. This results in oxidative damages to biological systems. The molecular mechanisms of oxidative changes in rat blood during short-term hypokinesia can be associated with an imbalance between blood metalloproteins that produce and neutralize ROS [6]. However, these mechanisms are poorly understood. Here we evaluated changes in the content of rat blood metalloproteins in the early stage of hypokinesia.

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MATERIALS AND METHODS

Experiments were performed on adult albino rats weighing 150-170 g. To induce hypokinesia the animals were immobilized in individual tubes for 5, 10, and 15 days (groups EH-5, EH-10, and EH-15, respectively). Each group included 16 rats. Sixteen intact animals served as the control. The rats were decapitated under light ether anesthesia. The blood was collected into tubes with sodium acetate buffer. We obtained 4 blood samples from animals of each group (20 ml). Metalloproteins with prooxidant activity, including cytochrome b_5 from the soluble erythrocyte fraction, total fraction of plasma cytochromes b_{558} I and b_{558} II, total fraction of erythrocyte membrane cytochromes b_{558} III and b_{558} IV, and suprol, were isolated from blood samples. $O_2^{\cdot-}$-producing activity and content of phospholipid residues in suprol were estimated. We also isolated and purified metalloproteins with antioxidant activity, including superoxide dismutase (SOD) and catalase from soluble erythrocyte fraction and ceruloplasmin (CP) and transferrin (TF) from the plasma.

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The soluble erythrocyte fraction, plasma, and fraction of solubilized hemoproteins from erythrocyte membranes were dialyzed against distilled water. Supernatants were subjected to ion-exchange chromatography on DE-52 and CM-52 celluloses (Whatman) and Sephadex DEAE A-50 (Pharmacia). Gel filtration was performed on P-100 and P-150 biological gels (Reanal). To isolate metalloproteins we measured densities of their characteristic optical absorption maxima, which appeared at 610, 470, 430, 525, and 530 nm for CP, TF, suprol (weak arm), cytochrome b_5, and cytochromes b_{558} I-IV, respectively. SOD activity and O_2^--producing activity of suprol were estimated routinely using nitroblue tetrazolium (NBT). The decrease (SOD) or increase (suprol) in optical density of formazan formed after NBT reduction with superoxide radicals at 560 nm was calculated and expressed in percents [6]. Catalase activity was estimated by permanganatometry. We measured the amount of H_2O_2 degraded by test fractions at 20°C over 1 min. The specific activity of enzymes was calculated per 1 ml erythrocytes (SOD and catalase) or plasma (suprol).

The content of suprol phospholipids was estimated by thin-layer chromatography [13] and expressed in percents of the total lipid content per 1 mg dry fraction weight.

Absorption spectra were recorded on a Specord M-40 spectrophotometer at 20°C (optical path 1 cm).

The results were analyzed by Student's *t* test and exact Fischer's test.

RESULTS

Changes in the content of endogenous blood metalloproteins with antioxidant and prooxidant activities depended on the duration of hypokinesia (Table 1). In group EH-5 rats with unexhausted adaptive reserves the content of prooxidant metalloproteins most markedly increased in soluble erythrocyte fraction (cytochrome b_5) and to a lesser extent in the plasma (total fraction of cytochromes b_{558} I and b_{558} II), while in erythrocyte membranes these changes were negligible (total fraction of cytochromes b_{558} III and b_{558} IV). Metalloproteins with prooxidant activity are NADPH-dependent superoxide-producing systems. It should be emphasized that plasma cytochrome b_{558} II not only generates O_2^-, but also protects the blood system from H_2O_2-induced damages [5]. The increase in prooxidant metalloprotein content stimulates antiradical protective systems in the blood (plasma CP and, to a lesser degree, TF, SOD, and catalase). These metalloproteins with antioxidant activity utilize and neutralize ROS. Exhaustion of adaptive reserves in group EH-10 and, particularly, EH-15 rats manifested in a sharp increase in cytochrome b_5 content. TF concentration increased insignificantly, while the content of other antioxidant metalloproteins slightly decreased. O_2^- is an intermediate compound formed during oxidative metabolic processes. Our results indicate that cytochrome b_5 from the soluble erythrocyte fraction is the main source of O_2^- during short-term hypokinesia. The increase in

TABLE 1. Relative Changes (% of Control) in the Content of Rat Blood Metalloproteins at Various Stages of Hypokinesia (*M*±*m*, *n*=4)

Metalloproteins	EH-5	EH-10	EH-15
Cytochrome b_5	192.0±8.0	410.0±12.1	980.4±56.7
Total fraction of cytochromes b_{558} I and b_{558} II	30.1±0.9	-21.8±1.1	-31.1±1.3
Total fraction of cytochromes b_{558} III and b_{558} IV	2.3±0.3	-1.9±0.1	-21.0±0.9
Cu,Zn-SOD	4.1±0.2	-2.5±0.2	-21.1±1.1
Catalase	3.6±0.3	1.8±0.1	-20.3±0.8
CP	105.0±4.5	-31.1±1.4	-10.3±0.7
TF	2.9±0.4	5.8±0.2	6.1±0.2

TABLE 2. Relative Changes (% of Control) in the Content of Suprol, Its O_2^--Producing Activity, and Number of Phospholipid Residues in Rats in Various Stages of Hypokinesia (*M*±*m*, *n*=4)

Parameter	EH-5	EH-10	EH-15
Suprol content	42.0±1.8	-2.3±0.2	-34.2±1.6
O_2^--producing activity	3.1±0.2	2.5±0.2	-16.3±0.8
Phosphatidylcholine	28.3±4.2	4.1±0.1	-15.5±2.2
Phosphatidylethanolamine	10.3±2.1	2.2±0.3	-4.1±0.1
Phosphatidylserine	6.5±0.4	-3.2±0.2	-7.2±1.3

cytochrome b_5 content can be used as a diagnostic marker of experimental short-term hypokinesia.

In group EH-5 rats the content of suprol considerably increased, which was accompanied by a slight rise in the number of its phospholipid residues (particularly, phosphatidylcholine). O_2^--generating activity of suprol increased insignificantly (Table 2). Test parameters decreased with an increase in the duration of hypokinesia (EH-10 and EH-15 rats). These changes were probably associated with suprol consumption during energy metabolism, which involves LPO. LPO is induced by superoxide radicals *in vivo* generated by suprol in the presence of Cu^{2+} or Fe^{2+} in trace concentrations [6]. However, the metabolism of Cu^{2+} and Fe^{2+} is impaired during short-term hypokinesia (as indicated by variations in CP and TF levels).

Our results indicate that long-term hypokinesia disturbs the balance between antioxidant and prooxidant metalloproteins regulating ROS metabolism. This results in accumulation or deficiency of ROS and impaits function of blood components (plasma and erythrocytes). These changes can be used as quantitative criteria of oxidative blood damages during experimental short-term hypokinesia.

REFERENCES

1. V. P. Akopyan and A. G. Kocharyan, *Eksp. Klin. Med*., No. 1-2, 85-89 (1999).
2. E. Yu. Bychkova, D. D. Matsievskii, and V. P. Krotov, *Byull. Eksp. Biol. Med.*, **126**, No. 9, 289-293 (1998).
3. E. A. Kovalenko and N. N. Gurovskii, *Hypokinesia* [in Russian], Moscow (1980), p. 320.
4. G. M. Simonyan, M. A. Babayan, R. M. Simonyan, and M. A. Simonyan, *Biol. Zh. Armenii*, **52**, 18-21 (1999).
5. G. M. Simonyan, G. G. Grigoryan, R. M. Simonyan, and M. A. Simonyan, *Urgent Problems of Military Medicine* [in Russian], Erevan (1999), pp. 48-51.
6. M. A. Simonyan, A. V. Karapetyan, M. A. Babayan, and R. M. Simonyan, *Biokhimiya*, **61**, 932-938 (1996).
7. V. S. Bendenko, G. P. Stupakov, M. A. Nesterov, and V. A. Mukhin, *Aviakosm. Ekolog. Med*., **33**, No. 3, 25-31 (1999).
8. G. G. Corbucci, R. Sessero, C. Velluti, and M. Salvi, *Int. J. Tissue React*., **17**, No. 3, 125-127 (1995).
9. R. A. Fielden and M. Meydani, *Aging (Milano)*, **9**, 12-18 (1997).
10. J. M. C. Gutteridge, *FEBS Lett*., **172**, 245-249 (1984).
11. T. Kawata, T. Fujita, and C. Tokimasa, *J. Nutr. Sci. Vitaminol*., **44**, No. 5, 581-590 (1998).
12. V. I. Korolkov, V. P. Krotov, V. Konvertino, *et al*., *Aviakosm. Ekolog. Med*., **32**, No. 3, 30-37 (1998).
13. V. E. Vaskorsky and V. I. Svetashov, *J. Chromatogr*., **65**, 451-453 (1972).