BIOPHYSICS AND BIOCHEMISTRY

Short-term Cold Exposure Improves Antioxidant Status and General Resistance of Animals

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 1, pp. 31-34, January, 2006
Original article submitted April 1, 2005

We studied the effect of short-term swimming in cold water (13°C) on parameters of the blood antioxidant system (activities of superoxide dismutase and catalase, concentrations of ceruloplasmin and nonprotein thiols), heme oxygenase activity, and nonprotein thiol level in mouse liver. The test parameters of antioxidant protection increased 1 h after cold exposure and remained high 1 day after treatment. These changes were accompanied by an increase in the adaptive capacity. After swimming in cold water the resistance of animals to another stress factor (administration of epinephrine) was higher compared to controls.

Key Words: *cold stress; superoxide dismutase; ceruloplasmin; catalase; nonprotein thiols*

Intracellular factors determining the resistance to adverse environmental events probably play a role in the adaptive response. This reaction is required to counteract the effects of unfavorable factors. The study of adaptive effects would allow us to modify the resistance of living organisms. Moreover, this approach holds much promise for antistress protection. Any moderate adverse event activates defense reserves of the organism via stimulation of oxidation processes, which in turn activate antioxidant system and improve general organism's resistance to stress factors [3]. Cu/Zn-superoxide dismutase (SOD, EC 1.15.1.1) and ceruloplasmin (CP, EC 1.16.3.1) are the major antioxidant enzymes of blood plasma involved in utilization of $O_2^{\bullet}$ (precursor of other reactive oxygen species, ROS). SOD catalyzes dismutation of $O_2^{\bullet}$ with the formation of H_2O_2. CP is a polyfunctional enzyme. CP not only converts $O_2^{\bullet}$ into water without formation of H_2O_2, but also plays a role in the transport, distribution, and metabolism of copper and iron initiating generation of ROS [8,15]. H_2O_2 formed during $O_2^{\bullet}$ dismutation is neutralized by the glutathione peroxidase system. This reaction involves reduced glutathione, which constitutes a major part in the pool of nonprotein thiols (NPT), and catalase (EC 1.11.1.6) [7,10].

Here we studied the antioxidant status and measured the concentration of substances reacting with thiobarbituric acid (TBA-reactive products, one of the criteria for oxidative stress) in the blood/plasma of animals swimming in cold water. Activity of the antioxidant system in some tissues was determined by the concentration of NPT. Hepatic antioxidant status was also estimated by heme oxygenase activity (EC 1.14.99.3). This enzyme is induced under conditions of oxidative stress [6,9].

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice aging 7-9 weeks and obtained from the Andreevka nursery (Research Center of Biomedical Technologies, Russian Academy of Medical Sciences). The animals were kept in a vivarium (Biological Fa-

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0007-4888/06/1411-0026

culty, M. V. Lomonosov Moscow State University) under standard housing and feeding conditions. The mice were divided into 2 groups of control animals and animals exposed to cold water swimming at 13°C for 5 min. The animals were killed by cervical dislocation 1 h and 1 day after treatment. Tissue and blood samples were taken. The blood was incubated at room temperature for 2 h and centrifuged at 3000 rpm for 15 min; the plasma was used for biochemical studies. NPT concentration in the blood and tissues was measured as described elsewhere [14] and expressed in μmol/ml blood and μmol/g tissue, respectively. Catalase activity in hemolysed blood was expressed in rel. units [5]. The total activity of SOD in blood plasma was estimated as described previously [2]. This method is based on the inhibition of quercetizn oxidation in the present of a biological material. Changes in optical density were recorded at 406 nm. SOD activity was expressed in arb. units/mg protein. The amount of SOD that inhibited quercetin oxidation by 50% was taken as 1 arb. units. Plasma CP level was determined by oxidase activity relative to the substrate *o*-phenylenediamine at 492 nm [4]. It was expressed in μg/ml plasma using the calibration curve. The amount of TBA-reactive products in blood plasma was measured as described elsewhere [1] and expressed in nmol/ml plasma. Heme oxygenase activity in the liver was expressed in pmol/mg protein/h [12]. Protein concentration was estimated as described elsewhere [13]. The resistance of animals to adrenal shock was determined after subcutaneous injection of 0.1% epinephrine in LD_{50}. Previous studies by the method of probit analysis showed that LD_{50} of epinephrine is 2.2 mg/kg.

The results were analyzed by means of GraphPad Prism 4.0 software. The data are expressed as means and standard errors (6-10 mice for each experimental point). Inter-group differences were estimated by Student's *t* test or analysis of variance (ANOVA).

RESULTS

Plasma SOD activity increased by more than 1.5 times 1 h after cold water swimming and remained

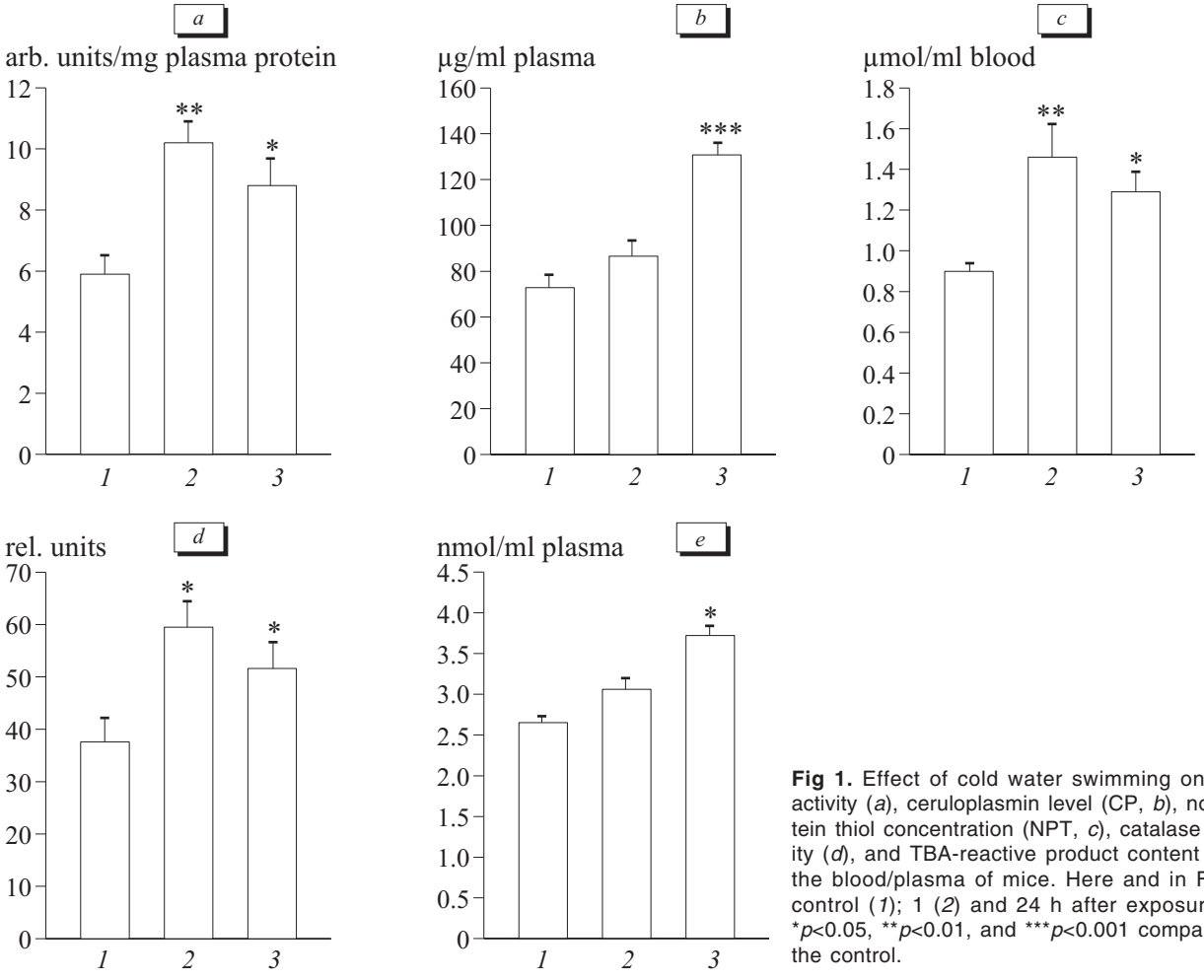

Fig 1. Effect of cold water swimming on SOD activity (*a*), ceruloplasmin level (CP, *b*), nonprotein thiol concentration (NPT, *c*), catalase activity (*d*), and TBA-reactive product content (*e*) in the blood/plasma of mice. Here and in Fig. 2: control (*1*); 1 (*2*) and 24 h after exposure (*3*). **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to the control.

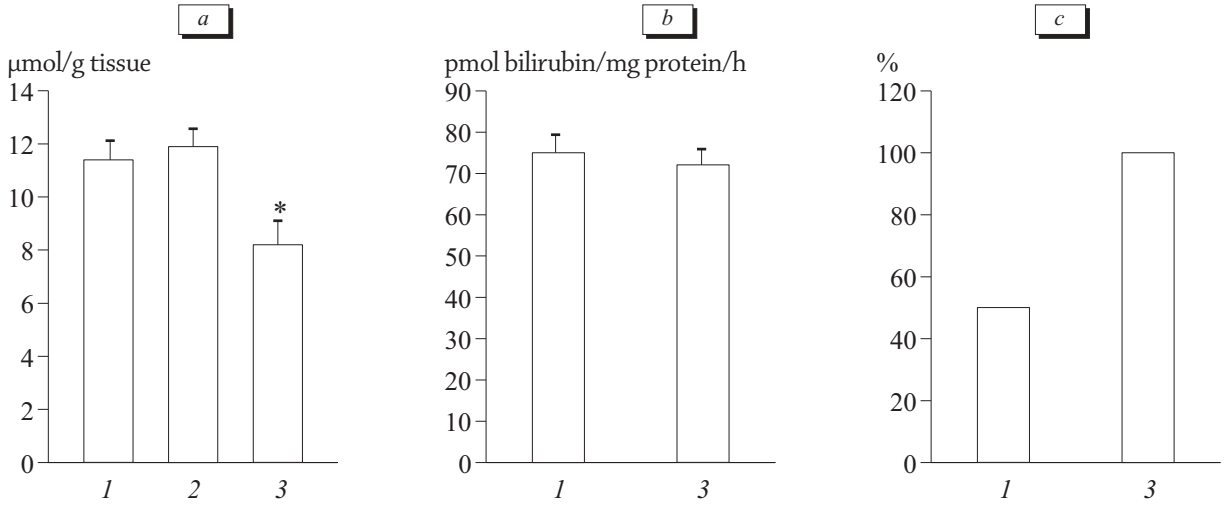

Fig. 2. Effect of swimming in cold water on NPT concentration (*a*), heme oxygenase activity in the liver (*b*), and survival of mice after adrenal shock (*c*). **p*<0.05 compared to the control.

high on day 1 after treatment (Fig. 1, *a*). CP is an acute phase protein synthesized in hepatocytes. CP concentration increased by 2 times on day 1 after swimming (Fig. 1, *b*). NPT concentration and catalase activity in the blood increased by 60% 1 h after cold water swimming and remained high on day 1 after treatment (Fig. 1, *c*, *d*). These changes reflect stimulation of the major antioxidant system, which maintains antioxidant activity of the blood and prevents activation of lipid peroxidation (increase in plasma TBA-reactive product, Fig. 1, *d*). The observed changes have a positive impact, which manifests in stimulation of the compensatory reaction to stress and activation of several protection systems (*e.g.*, immune response). We estimated the weight of immunocompetent organs. The weight of the thymus increased from 109.9±9.5 to 204.7±8.5 mg on day 1 after cold water swimming ($p<0.01$). The weight of the spleen tended to increase in this period (from 100.8±9.8 to 133.0± 25.0 mg). It was interesting to evaluate whether blood changes in stressed animals result from activation of oxidation processes. The concentration of NPT was measured in the brain, thymus, lungs, heart, spleen, and liver. It should be emphasized that a change in NPT concentration is one of the criteria for oxidation processes. On day 1 after swimming in cold water NPT concentration decreased by 30% in the liver (Fig. 2, *a*), but remained unchanged in other organs. The exposures accompanied by oxidative stress are followed by a decrease in NPT concentration and increase in heme oxygenase activity in tissues. The observed changes reflect intensification of oxidation processes [11]. However, in our experiments the decrease in NPT concentration was not accompanied by an increase in heme oxygenase activity in the liver (Fig. 2, *b*). It can be hypothesized that the decrease in liver NPT concentration is associated with increased release of NPT into the blood, but not with its consumption in oxidation reactions. NPT are mainly synthesized in hepatocytes. The increase in blood NPT concentration provides support for this hypothesis. These changes can be considered as a compensatory reaction of the organism to stress.

For evaluation of changes in the general resistance of animals upon variations in activity of the antioxidant system, the resistance to adrenal shock (generalized stress analysis model) was studied after cold water swimming. The increase in the antioxidant status on day 1 after swimming was accompanied by a significant decrease in the sensitivity of mice to adrenal shock. Epinephrine in LD_{50} caused death of control mice, but had no effect on treated animals (Fig. 2, *c*). Therefore, cold exposure increases general resistance to stress.

Our results indicate that short-term swimming in cold water at 13°C stimulates the blood antioxidant system, improves general resistance to extreme factors and increases adaptive capacity of mice.

REFERENCES

1. V. B. Gavrilov, A. P. Gavrilova, and L. M. Mazhul', *Vopr. Med. Khim.*, **33**, No. 1, 118-122 (1987).
2. V. A. Kostyuk, A. I. Potapovich, and Zh. B. Kovaleva, *Ibid.*, **36**, No. 2, 89-91 (1990).
3. T. G. Sazontova and Yu. V. Arkhipenko, *Fiziol. Zh.*, **91**, No. 6, 636-655 (2005).
4. O. B. Siverina, V. V. Basevich, R. V. Basova, *et al.*, *Lab. Delo*, No. 10, 612-618 (1986).
5. S. Chevari, T. Andyal, and Ya. Shprenger, *Ibid.*, No. 10, 9-13 (1991).

6. L. A. Applegate, P. Luscher, and R. M. Tyrrell, *Cancer Res*., **51**, No. 3, 974-978 (1991).
7. M. W. Fraaije, H. P. Roubroeks, W. R. Hagen, and W. J. Van Berkel, *Eur. J. Biochem*., **235**, 192-198 (1996).
8. B. Halliwell and J. M. Gutteridge, *Arch. Biochem. Biophys*., **280**, No. 1, 1-8 (1990).
9. S. M. Keyse and R. M. Tyrrell, *Proc. Natl. Acad. Sci. USA*, **86**, No. 1, 99-103 (1989).
10. L. K. Moran, J. M. Gutteridge, and G. J. Quinlan, *Curr. Med. Chem*., **8**, No. 7, 763-772 (2001).
11. I. Rahman and W. MacNee, *Free Radic. Biol. Med*., **28**, No. 9, 1405-1420 (2000).
12. S. W. Ryter, E. Kvam, and R. M. Tyrrell, *Methods Mol. Biol*., **99**, 369-391 (2000).
13. G. R. Schacterle and R. L. Pollack, *Anal. Biochem*., **51**, No. 2, 654-655 (1973).
14. J. Sedlak and R. H. Lindsay, *Ibid*., **25**, No. 1, 192-205 (1968).
15. K. Yoshida, K. Kaneko, H. Miyajima, *et al*., *J. Neurol. Sci*., **175**, 91-95 (2000).