Effect of Ceruloplasmin on the Number and Resistance of Erythrocytes during Acute Physical Exercise

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Acute physical exercise was followed by a decrease in the osmotic resistance of erythrocytes, shortening of the time-to-onset of erythrocyte hemolysis, and increase in the sorption capacity of the cell membrane. Administration of ceruloplasmin 24 h before physical exercise normalizes membrane resistance in red blood cells.

Key Words: *acute physical exercise; acid resistance of erythrocytes; osmotic resistance of erythrocytes; sorption resistance of erythrocytes*

Severe or prolonged physical exercise is accompanied by the adrenotoxic effect and development of oxidative stress [6]. Membrane of the peripheral erythron component is the main target for reactive oxygen metabolites [3].

Ceruloplasmin (CP) is a copper-containing glycoprotein of the α-2-globulin fraction from blood plasma. CP has a pleiotropic effect on blood cells [1,2,5,7]. Here we studied the effect of CP on quantitative composition and resistance of erythrocytes during acute physical exercise (APE)

MATERIALS AND METHODS

Experiments were performed on 22 male adult outbred rats weighing 240±7.5 g. Severe APE was produced as described elsewhere [3]. Experimental animals were divided into the following three groups: group 1, intact specimens; group 2, APE; group 3, administration of CP during APE. CP (10% of the normal physiological dose) was injected intraperitoneally 24 h before APE. Blood samples were taken immediately after exercise (according to the requirements for blood tests). The number of erythrocytes was estimated by routine method in a Goryaev chamber. Hemoglobin concentration was measured on a Linson Junior photometer according to manufacturer's instructions. Erythrocytes were assayed for the resistance to osmotic and acid treatment. Sorption capacity of cells was evaluated routinely.

RESULTS

Physical exercise was followed by an increase in the number of erythrocytes and concentration of hemoglobin in both experimental groups ($p<0.001$ compared to the control; Table 1). These changes are related to the release of red blood cells from blood-storage tissues. Although CP has a hematopoietic effect, 24-h exposure to this compound is insufficient for stimulation of erythropoiesis.

APE was accompanied by a decrease in the osmotic resistance of erythrocytes. The percentage of hemolysis in control animals was maximum after treatment with 0.45% NaCl. A leftward shift of this parameter was observed in animals of the treatment group (0.55% NaCl; Fig. 1).

Injection of CP 24 h before APE had a modulatory effect on the osmotic resistance of erythrocytes. The percentage of hemolyzed erythrocytes was reduced at a NaCl concentration of 0.55%. The base of the curve was shifted rightward.

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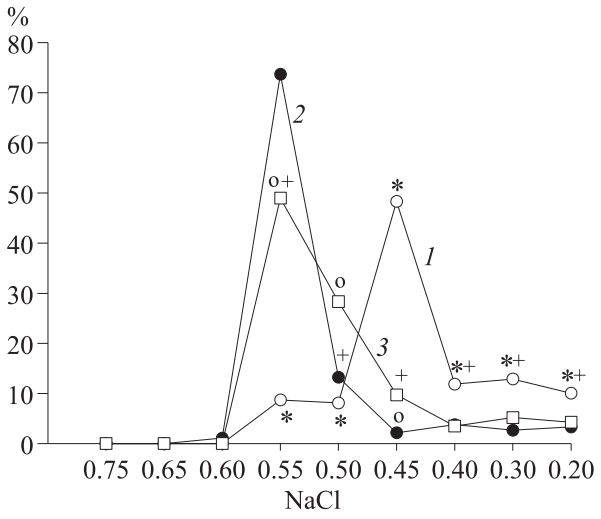

Fig. 1. Osmotic resistance of erythrocytes during acute physical exercise (the rule of three). Here and in Fig. 2: *1*, control (intact group); *2*, APE; *3*, APE+CP. $p<0.05$: *between the intact group and APE; +between the intact group and APE+CP; °between APE and APE+CP.

The acid resistance was also modified after APE. The base of the erythrogram was widened. This curve was shifted rightward (Fig. 2), which reflects the appearance of immature erythrocytes in the circulation. Older and less resistant erythrocytes were lysed 1 min earlier under conditions of severe metabolic acidosis (compared to the control). Younger erythrocytes were recruited into the circulation from blood-storage tissues and lysed in the later period. The acid resistance of circulating erythrocytes was increased by 3 times.

Injection of CP 24 h before the start of APE had a positive effect on the acid resistance of the erythrocyte membrane. The time-to-onset of hemolysis in these animals did not differ from the control. The erythrogram was widened to the right and had a higher amplitude (Fig. 2). These results illustrate the increase in erythrocyte membrane resistance.

The sorption capacity of erythrocytes is a criterion for membrane resistance to adverse factors. Severe APE was followed by a 3-fold increase in the sorption capacity of erythrocyte membranes in both experimental groups ($p<0.001$ compared to the control; Table 2). We believe that these changes are related to the increase in catecholamine concentration in the blood and activation of free radical oxidation. Injection of CP 24 h before APE produced a statistically significant decrease in the sorption capacity of erythrocytes, which was elevated under extreme conditions.

Our results indicate that CP maintains the integrity and functional properties of erythrocytes during APE, which is accompanied by severe hypoxia, hemodynamic stroke, blood concentration, and increased coagulation of the blood.

The CP-induced increase in the resistance of erythrocytes to osmotic and acid treatment and stabilization of erythrocyte membranes are probably related to

TABLE 1. Effect of CP on Erythrocyte Number and Hemoglobin Concentration during APE ($M\pm m$, σ)

Parameter	Intact group (n=7)	APE (n=14)	APE+CP (n=7)	Tc_1 Tc_2 Tc_3	χ_1 χ_2 χ_3
Erythrocytes, 10^{12} cells/liter	6.47±0.10 0.25	7.52±0.24 0.58	7.89±0.18 0.52	4.038 6.762 1.233	<0.001 <0.001 >0.05
Hemoglobin, g/liter	123.25±3.27 8.00	140.92±1.53 3.74	144.50±4.67 11.44	4.895 3.728 0.729	<0.001 <0.01 >0.05

Note. Here and in Table 2: χ_1, between the intact group and APE; χ_2 between the intact group and APE+CP; χ_3 between APE and APE+CP. Tc, calculated by Student's test.

TABLE 2. Effect of CP on the Sorption Resistance of Erythrocytes during APE ($M\pm m$, σ)

Parameter	Intact group (n=10)	APE (n=8)	APE+CP (n=7)	Tc_1 Tc_2 Tc_3	χ_1 χ_2 χ_3
Sorption capacity of erythrocytes (arb. units)	10.10±1.51 4.00	29.73±0.82 1.82	25.08±1.26 3.33	11.440 7.623 3.100	<0.001 <0.001 <0.01

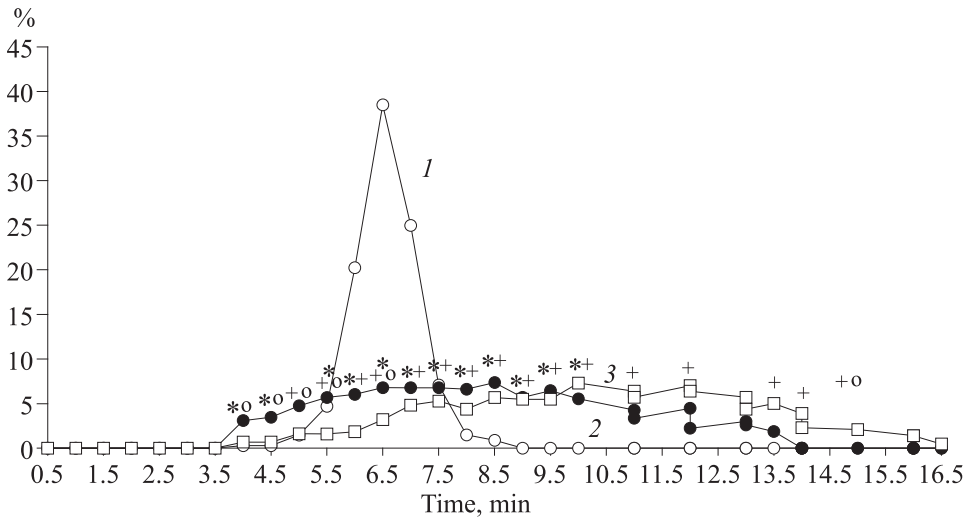

Fig. 2. Acid resistance of erythrocytes during acute physical exercise (the rule of three).

the pleiotropic properties of this compound. CP has a direct antioxidant effect, which normalizes the oxidation potential in the liquid part of the blood and cell membranes. Our findings are consistent with published data [1,4,5]. Moreover, specific receptors for CP were identified on the erythrocyte membrane [5].

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