Effects of Electroactivated Solutions on Antioxidant Enzymes

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Electrochemically activated systems normalized activity of antioxidant enzymes (catalase, peroxidase, and superoxide dismutase). The baseline activity of antioxidant enzymes considerably varies in humans and animals. This effect of electrochemically activated systems having a negative oxidation-reduction potential was probably related to a training effect of excess electrons.

Key Words: *antioxidants; electroactivation of solutions*

Reactive oxygen species play an important role in various physiological processes regulated by specific enzyme systems. At the same time, free reactive oxygen species produce damaging effects either directly or after involvement in long-living chain reactions.

Experimental and clinical observations showed that pharmacological correction of the antioxidant system produces geroprotective, anticarcinogenic, radioprotective, and biostimulating effects. Such pharmacological preparations change the oxidation-reduction potential (ORP) of water and macromolecules. There are some methods for obtaining safe electrochemically activated systems (ECAS) with certain ORP from water [2-8,11,12].

The ECAS method is based on the use of water with standard ORP and pH and electrochemically enriched with OH^- or H^+ groups simultaneously. Water is dechlorinated, decontaminated, and detoxified by oxidation-reduction of toxic compounds and precipitation of heavy metal salts.

Here we studied the effect of ECAS on 3 major antioxidant enzymes.

MATERIALS AND METHODS

We studied the effects of ECAS on catalase (CT), peroxidase (PO), and superoxide dismutase (SOD) activities in peripheral blood erythrocytes from healthy Chinchilla rabbits. Erythrocytes were centrifuged and lysed with 5 mM Tris-HCl buffer (1:00, pH 7.8). For hemoglobin precipitation, 0.25 ml 96% C_2H_5OH and 0.15 ml chloroform were added to 1 ml lysate, mixed on ice for 15 min, and centrifuged at 4°C and 10,000*g* for 15 min. CT activity was measured by the reaction of H_2O_2 with ammonium molybdate [7]. PO activity was estimated by the reaction with indigo carmine [1]. SOD activity was determined by the inhibition of HCT reduction with superoxide anions generated in the reaction of NADPH with phenazine methosulfate [13]. The effects of ECAS on activity of extrapure human recombinant SOD (Tris) were studied.

ECAS was obtained by passing NaCl solution (1-2 g/liter) through an Izumrud device equipped with 2 reactor compartments. This procedure allowed us to vary ORP of ECAS from +200 mV to -70 mV. ORP was continuously measured on a pH meter with platinum electrodes under standard conditions.

RESULTS

To study the effect of ECAS on CT activity, we used 0.1 ml lysed and diluted rabbit erythrocytes (1:1000). Under standard conditions light absorption at 410 nm dropped by 50%, which was equivalent to a decrease in the content of ammonium molybdate substrate. Preincubation of erythrocyte lysate with various concentrations of ECAS for 5, 10, and 20 min caused a bi-

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phasic response: rapid activation of the enzyme followed by normalization or slight inhibition of its activity (Fig. 1, *a*). The time- and dose-dependent effects of ECAS were also observed at various ORP and constant exposure (10 min).

Therefore, preincubation required for enzyme activation decreased with increasing the concentration of ECAS and ORP.

ECAS produced similar effects on PO and SOD activities. ECAS modulated PO activity after 90-120-sec preincubation. Titers of H_2O_2 and indigo carmine were determined in the main reaction.

The higher was the concentration of ECAS during preincubation with erythrocytes, the shorter preincubation was required for maximum activation of rabbit PO and SOD (Fig. 1, *b*, *c*). ECAS produced similar effects on human recombinant SOD.

These changes were probably associated with the ability of ECAS to generate superoxide radicals producing moderate training effects on antioxidant enzymes. Negatively charged ECAS contained excess electrons formed after electrochemical activation, which contributes to superoxide anion generation. Components of the main reaction are inactivated at high negative values of ORP (-170 mV). After 5-min incubation with ECAS, the reaction between NADPH and phenazine methosulfate and HCT reduction were inhibited by 22-25%.

Thus, the effects of ECAS on antioxidant enzymes depend on ORP, ECAS concentration, and the time of preincubation with erythrocytes. After maximum activation of enzymes, the activating effect of ECAS is converted to the inhibitory influence. These changes are probably associated with the ability of ECAS to generate superoxide radicals, which in low concentrations activate antioxidant enzymes.

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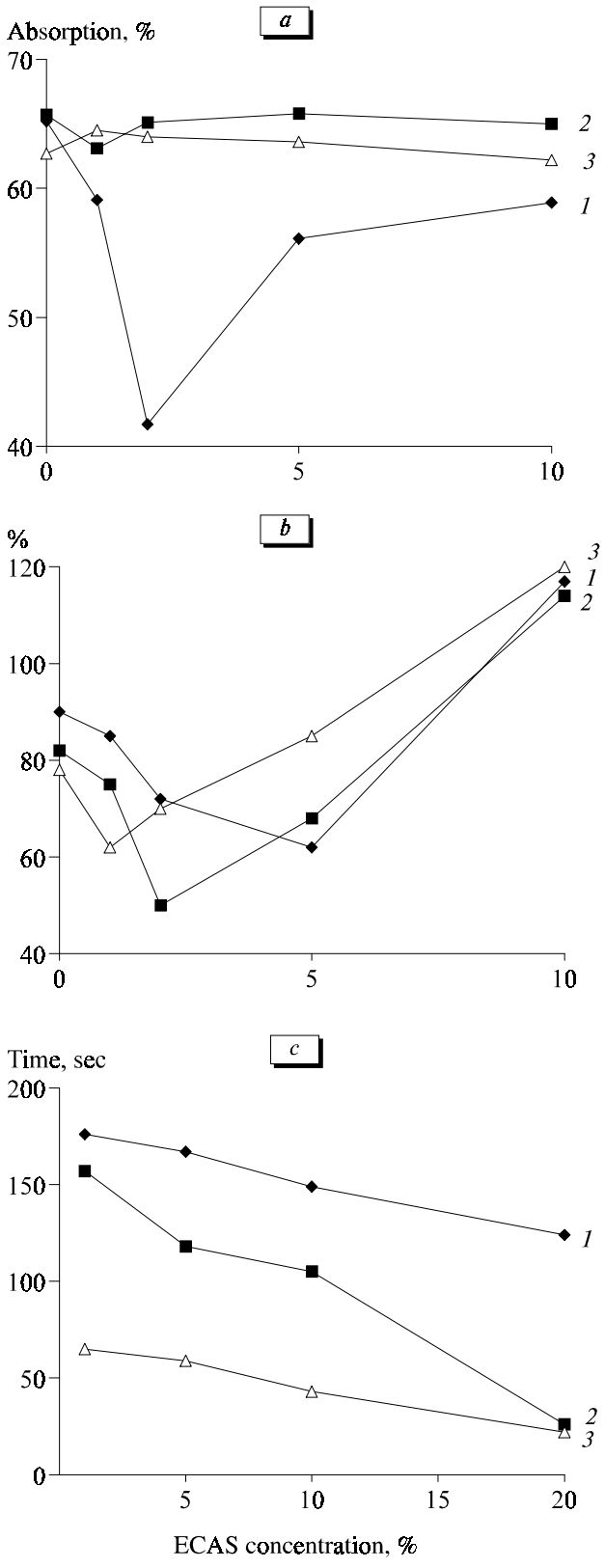

Fig. 1. Effects of electrochemically activated systems (ECAS) on catalase (*a*), peroxidase (*b*), and superoxide dismutase (*c*) activities in rabbit erythrocytes. Incubation for 5 (*1*), 10 (*2*), and 15 min (*3*).

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