ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY IN RATS SUBJECTED TO A TOXIC REGIME OF INTERMITTENT HYPERBARIC OXYGENATION

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The development of toxic manifestations in rats exposed to hyperoxia is accompanied by a significant decrease in the activity of erythrocytic superoxide dismutase (SOD). Incubation of hemolysates from control animals with H_2O_2 (10^{-3} M) or cumene peroxide ($1.6 \cdot 10^{-4}$ M) also led to a marked decrease in the initial SOD activity. The decrease in the initial SOD activity during hyperoxia is thus evidently connected with the formation of peroxidation products in the cells.

KEY WORDS: *Superoxide dismutase; hyperoxia; toxic action of oxygen.*

A leading factor in the mechanism of oxygen poisoning is stimulation of free-radical processes [13]. Resistance to the toxic action of oxygen may depend on the state of both enzymic and nonenzymic mechanisms for the regulation of these processes. One of the chief enzymes of antiradical protection of the cell is superoxide dismutase (SOD), an enzyme discovered by McCord and Fridovich in 1969 [9]. SOD renders superoxide anions (O_2^-) harmless by catalyzing their dismutation reaction

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

and so converting them into the less reactive molecules of H_2O_2 and triplet O_2. In the absence of the enzyme spontaneous dismutation of superoxide anions leads to the formation of H_2O_2 and singlet oxygen [8]. The substrate for this enzyme, superoxide anions, are formed during many enzymic and nonenzymic reactions in the body [1, 5].

SOD is found in all tissues of aerobic organisms but is absent in obligate anaerobes [10]. When grown in an atmosphere of oxygen, facultatively aerobic microorganisms have a higher level of activity of the enzyme and, at the same time, are more resistant to the action of oxygen under pressure [6]. These facts indicate that, first, SOD is an important cell component for protection against hyperoxia and, second, that induction of this enzyme may be an essential mechanism of adaptation to exposure to hyperoxia. Induction of SOD in animal cells has not yet been shown to be possible. The study of SOD activity in the tissues of mammals exposed to the single or chronic action of oxygen has not shown any statistically significant change of activity in most animal organs [3].

In this investigation erythrocytic SOD activity was studied during exposure to a toxic regime of hyperbaric oxygenation.

EXPERIMENTAL METHOD

Male Wistar albino rats weighing 150-200 g were used. The experimental animals were exposed to the action of hyperbaric oxygen at a pressure of 3 atm for 2 h daily until clear signs of oxygen poisoning appeared (convulsions, lateral position, disturbance of external respiration). Poisoning occurred at different times between the fifth and 10th sessions of exposure on account of differences in individual sensitivity of the animals to hyperoxia. Animals of the control group were not exposed to hyperoxia.

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TABLE 1. Decrease in SOD Activity in Blood of Rats Exposed to Hyperbaric Oxygenation

Group of animals	SOD activity, units	
	per ml blood	per million erythrocytes
Control	546±28 (11)	115±6 (11)
Experimental	456±17 (10)	90±4 (10)
	$P<0,05$	$P<0,01$

Legend. Number of experiments in parentheses

Blood was obtained from the animals by decapitation. The blood sample for determination of SOD activity was taken immediately after decompression on the first appearance of signs of oxygen poisoning in the rats. An equal number of rats of the control group was decapitated at the same time. Since a high hemoglobin concentration interferes with the determination of SOD and gives values for the enzyme activity that are too low, hemoglobin was removed from the hemolysate by chloroform–ethanol treatment [9]. The supernatant obtained after centrifugation, containing the whole of the initial SOD activity [9], was used for the tests.

SOD activity was determined from the inhibition of autooxidation of adrenalin [11]. Standard determinations were made in 3 ml of 0.05 M Na-carbonate buffer, pH 10.2, containing 10^{-4} M EDTA (VEB Berlin), in a thermostatically controlled cuvette at 25°C. The reaction mixture contained $3 \cdot 10^{-4}$ M L-adrenalin (Sigma) and 10^{-5} M adrenochrome (Calbiochem). The reaction was started by the addition of 0.4 ml of an acid solution of adrenalin (pH 2.5). Under these circumstances no change was observed in the pH of the reaction mixture. The initial velocity of adrenalin oxidation determined in this way, recorded spectrophotometrically as adrenochrome formation at 480 nm, was 0.025 optical units/min. The unit of SOD activity was taken to be that quantity of the enzyme required to inhibit the initial rate of autooxidation of adrenalin by 50% under the conditions described above. All the spectrophotometric determinations were carried out on an SP-8000 (Unicam, England) recording spectrophotometer. The erythrocyte count in the animals' blood was determined so that the specific activity of SOD could be calculated in units per million erythrocytes.

EXPERIMENTAL RESULTS AND DISCUSSION

The results (Table 1) show that the development of toxic manifestations of hyperoxia was accompanied by a marked decrease in SOD activity. This decrease was clearly revealed whatever method was used to calculate activity (per ml blood, per million erythrocytes), and it amounted to 18 and 22% of the mean level of SOD activity in the control rats respectively.

Anuclear erythrocytes were unable to replace the damaged enzymes by newly formed enzymes. The SOD activity fell during hyperoxia either as a result of inhibition of the enzyme or through the appearance of newly formed erythrocytes deficient in that enzyme in the blood stream. The first hypothesis seems likely to be true, for high concentrations of hydrogen peroxide are known to inhibit the purified enzyme [2]. The presence of catalase and glutathione-peroxidase in the cells ought to prevent the harmful action of peroxide on SOD and other enzymes. However, as the experiments described above showed, addition of H_2O_2 (10^{-3} M) to hemolysate of blood from the control rats followed by incubation for 60 min at 37°C led to a fall of the original SOD level by 44%. Cumene hydroperoxide had an even stronger inhibitory action. For instance, incubation of hemolysates of the control animals for 15 min at 37°C with cumene peroxide ($1.6 \cdot 10^{-4}$ M) led to a decrease in the initial SOD activity of the hemolysate by 70%. This fact suggests that one of the causes of inhibition of SOD activity in the erythrocytes may be the formation of peroxides of unsaturated fatty acids, which are not detoxicated by catalase. The results do not support the view that the action of peroxide is the only cause of the decrease in SOD activity of the erythrocytes. The possibility of inhibition of the enzyme by other products of free-radical oxidation formed in the cell during hyperoxia likewise cannot be ruled out [7]. Autooxidation of oxyhemoglobin into methemoglobin is known to be accompanied by generation of a superoxide anion and to be inhibited by SOD [12]. The accumulation of methemoglobin in the blood is one sign of oxygen

poisoning, and it is readily explained by the inhibition of SOD demonstrated in the present investigation. There is also every reason to suppose that SOD insufficiency is an important factor in the decreased resistance of erythrocytes to hemolysis in certain hyperoxic regimes [4].

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