The stimulatory effect of negative air ions and hydrogen peroxide on the activity of superoxide dismutase

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Abstract The activity of erythrocyte cytosolic superoxide dismutase from rat, bovine, man and duck was considerably increased when measured after preparation or incubation in media pretreated with negative air ions (mostly superoxide) from electroeffluvial ion generator. 0.5–1.0 μM H_2O_2 was found in incubation medium after treatment with air ions. The stimulatory effect of air ions on superoxide dismutase activity was mimicked by addition of 0.5–6 μM H_2O_2. The primary physicochemical mechanism of beneficial biological action of negative air ions is suggested to be related to the stimulation of superoxide dismutase activity by micromolar concentrations of H_2O_2.

Key words: Negative air ions; Superoxide radical; Superoxide dismutase; Hydrogen peroxide

1. Introduction

The negatively charged air ions (NAI) are natural components of air and breath [1–4]. The exhaustion of NAI in polluted air, in closed and conditioned rooms, near television and computer monitors leads to disturbance of health. Inhalation of NAI generated by air ionizers by patients with certain pathologies was shown to render a therapeutic effect [1–6]. However, the primary physicochemical and biochemical mechanisms of beneficial biological action of NAI are still obscure. Two main mechanisms were proposed, namely, an exchange of NAI for charged species in tissues and a decrease in serotonin level [2,6–10]. The probability of the first seems to be questionable and the second, in our opinion, is not the primary effect.

The superoxide radical ($O_2^{\bullet}$) serves as the main negatively charged species in air [3,11]. Oxidative damage to tissues based on an initiation of the chain peroxidative reactions is well known to be involved in injuring action of superoxide radical. On the other hand, free radical processes of low intensity seem to be of a physiological importance [12–16]. In this connection, we suggested that beneficial biological effects of NAI are provided by $O_2^{\bullet}$. Possible effect of NAI on SOD, the key enzyme of $O_2^{\bullet}$ transformation, was tested.

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Abbreviations: SOD, superoxide dismutase; NAI, negative air ions; ESOD, erythrocyte cytosolic superoxide dismutase; ESOD-C, ESOD prepared with control saline; ESOD-I, ESOD prepared with saline pretreated with NAI; NTB, *p*-nitrotetrazolium blue

2. Materials and methods

Saponin, *p*-nitrotetrazolium blue, xanthine, xanthine oxidase, catalase, and purified superoxide dismutase from bovine erythrocytes were purchased from Sigma Chemical Co. Other chemicals were of analytical grade. Bidistilled water was used.

Rat, duck and human erythrocytes were studied. The cells were obtained from heparinized blood, separated from plasma by centrifugation at 1000×*g* for 10 min and washed twice with saline (0.9% NaCl). Then 0.1 ml of packed erythrocytes were suspended in 0.5 ml of saline. Hemolysate was obtained by adding 0.25 ml of water, 0.175 ml of 50 mM triethanolamine buffer, Ph 7.4, and 0.075 ml of 0.2% saponin to 0.25 ml of the erythrocyte suspension, followed by standing the mixture at 4°C for 15 min. Non-soluble material was discarded by the centrifugation at 1000×*g* for 15 min, and soluble fraction was used as ESOD preparation.

Two preparations of ESOD were obtained from one blood sample: ESOD-C with control saline and ESOD-I with saline pretreated with NAI for 60 min. SOD activity of both ESOD preparations were measured in two incubation media: control, CM, and the same medium pretreated with NAI, IM.

SOD activity was estimated according to Beauchamp and Fridovich [17] using xanthine plus xanthine oxidase as $O_2^{\bullet}$ generating system and inhibition of NTB reduction to assay the enzyme. ESOD in the amount equivalent to 10 μl of packed erythrocytes was added to 3 ml of 50 mM sodium carbonate buffer, pH 10.2, containing 0.1 mM EDTA, 25 μM NTB, 0.3 mM xanthine and 160 nM xanthine oxidase, and the reaction was followed by absorbance at 560 nm, at 25°C. One unit of the enzyme activity was defined as the quantity of SOD required to produce 50% inhibition of the rate of NTB reduction and the enzyme activity was expressed in units per 1 ml of packed erythrocytes.

H_2O_2 was measured by chemiluminescence with luminol and horse radish peroxidase [18].

Electroeffluvial ion generator, 'the Tchijevsky lustre', ELION-131M (Diod Co., Moscow) producing negative ions by dark discharge (that prevents ozone formation) was used as a source of NAI. The device generated 1 200 000 light negative charges at the point of application 1.2 m acid lustre. Before treatment of solutions the room was aired, then the window was closed and air was cleaned by more intensive ionization, i.e. 8 000 000 negative charges. For the treatment, a solution was poured by thin layer into a glass Petri dish and left for 60 min under the ionizer. Then the dish was covered and solution was used in experiment. Control samples were kept in identical Petri dish in an other room without ionization.

3. Results

Table 1 shows the activities of rat ESOD-C and ESOD-I as measured in control and NAI-pretreated medium. Pretreatment of the medium with NAI proved to result in the 2-fold increase in activity of rat ESOD-C. Similar increase in activity was observed when the ESOD-I was analysed in the CM, i.e. when the ESOD preparation was obtained with saline treated with NAI and incubated in control medium. Joint action of both saline and medium pretreatment with NAI caused an additional small rise of SOD activity, but the effect was insignificant.

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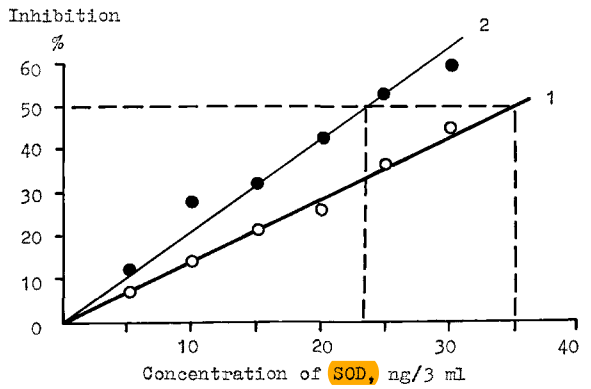

Fig. 1. Inhibition of NTB reduction in xanthine oxidase system by highly purified SOD from bovine erythrocytes. The commercial SOD was added to the control medium (curve 1) or to NAI-pretreated medium (curve 2), both containing xanthine oxidase system and NTB. Inhibition (%) of rate of NTB reduction as followed at 560 nm is presented as a function of SOD concentration. The data are $M \pm m$ for two–three measurements; m is in a range of 0.2–1.0% and lays within circles.

The stimulatory effect of the NAI pretreatment of the medium on SOD activity was also tested with the highly purified commercial SOD preparation from bovine erythrocytes. The SOD concentration providing the 50% inhibition of NTB reduction was determined using control and NAI-pretreated media. Results are illustrated in Fig. 1. 11.6 ng/ml of the purified SOD enzyme was required for 50% inhibition of NTB reduction in control medium and 7.7 ng/ml in NAI-pretreated medium.

The product of the SOD reaction, H_2O_2, can be a candidate for activator of the enzyme activity in solutions pretreated with NAI. It is known that H_2O_2 is formed from $O_2^{\cdot}$ non-enzymatically. Measurements showed the appearance of 0.5–1.0 μM H_2O_2 in incubation medium after 60 min treatment with NAI. On the other hand, H_2O_2 was reported to be a powerful inhibitor of SOD activity at high concentrations of 0.1–10 mM [19,20].

The effect of low concentrations of H_2O_2 on SOD activity was tested on human ESOD-C using catalase to eliminate H_2O_2 and sodium azide to inhibit the activity of endogenous catalase in ESOD. The effect of H_2O_2 was compared to that of NAI. Table 2 shows that the catalase addition to human ESOD-C leads to the dramatic decrease in SOD activity while sodium azide increases the activity considerably. Adding 1 μM H_2O_2 proves to increase SOD activity slightly while joint addition of H_2O_2 and azide provided pronounced stimulation close to that in the presence of azide. The increase in SOD activity under NAI influence in the same preparation is comparable to that after the H_2O_2 addition.

Catalase also decreases significantly the enhanced SOD activity as in the case of non-activated enzyme.

The inhibitory effect of catalase even in medium without H_2O_2 addition or without treatment with NAI suggests that H_2O_2 generated in the SOD reaction can activate SOD. The value of stimulation by added H_2O_2 and azide are considerably higher as compared with the sample in which endogenous H_2O_2 was eliminated by adding catalase. In particular, under the addition of 1 μM H_2O_2 stimulation is close to the 5-fold. This is comparable with the value of stimulation induced by NAI treatment.

The data clearly evidenced that H_2O_2 is responsible for SOD activation. However, the stimulatory effect of the H_2O_2 addition was less than that of azide or of H_2O_2 with azide. This may be due to the presence of some catalase activity in human and rat erythrocytes [21]. The first-order rate constant for the catalase reaction was 11 and 24 s^{-1}/ml of cells for rat and human ESOD, respectively.

To exclude the catalase effect, we used duck erythrocytes lacking catalase activity (< 0.1 s^{-1}/ml of cells; see also Ref. [21]). The results of the experiment on the action of H_2O_2 and NAI in duck ESOD-C are presented in Table 3. The addition of 0.5–6 μM H_2O_2 to duck ESOD-C induces its activation with a peak at 2–3 μM. Activation of ESOD by NAI-pretreated medium is between effects of 1 and 2 μM of added H_2O_2.

The results presented are an evidence for the stimulatory effect of added H_2O_2 on SOD activity and for operation of this mechanism under the NAI action.

4. Discussion

Experimental results obtained in the present study have shown for the first time that both NAI and H_2O_2 offer the stimulatory effects on the activity of SOD. The stimulation was revealed at lower concentrations of H_2O_2 than in previous investigations. It was shown earlier that H_2O_2 at high concentrations was the strong inhibitor of SOD activity. The inhibitory effect was increased with the H_2O_2 concentration. Thus, 50% inhibition of SOD activity occurred within 5 min with 0.76 mM H_2O_2 and within less than 1 min with 6 mM H_2O_2 [20], while the inhibitory effect of 42 μM H_2O_2 was much weaker, 22% in 30 min [19].

Stimulation of SOD activity in our experiments was observed under action of 0.5–6 μM H_2O_2 added or of 0.5–1 μM H_2O_2 formed during treatment with NAI. The values of stimulation by 1 μM H_2O_2 added and by NAI-treated medium containing similar amount of H_2O_2 are close. This quantitatively proves the suggestion that NAI effect is mediated by H_2O_2.

Table 1
SOD activity of rat erythrocytes as measured in the control medium and in a medium pretreated with NAI

SOD activity	ESOD-C		ESOD-I	
	Medium		Medium	
	Control	NAI	Control	NAI
U/ml of erythrocytes	1191 ± 39	2330 ± 212*	2490 ± 204*	2869 ± 523*

ESOD-C and ESOD-I in the amounts equivalent to 10 μl of rat erythrocytes were incubated in either the control medium or in a medium pretreated with NAI for 60 min.
Values are mean ± SD for nine measurements on three separate preparations.
*$p < 0.001$, significantly different from the ESS-C/B-C value by Student's t-test.

Table 2
Activity of cytosolic SOD of human erythrocytes in media with various levels of H_2O_2

Additions to control medium	Number of experiments	SOD activity (U/ml of cells)	%% to control
None	10	900 ± 50	100
Catalase 100 μg/ml	3	250 ± 90	27
100 μM NaN_3	4	1700 ± 100*	200
1 μM H_2O_2	4	1200 ± 100	130
100 μM NaN_3+1 μM H_2O_2	3	1500 ± 150	170
Additions to NAI-pretreated medium			
None	6	1400 ± 100*	160
Catalase, 50 μg/ml	1	550	60

ESOD-C in the amount equivalent to 10 μl of human erythrocytes was incubated in either control medium, or in the same medium pretreated with NAI for 60 min.
Means ± SD are given. *$p < 0.001$ as compared to the control medium by the Student's t-test.

Table 3
Activity of cytosolic SOD of duck erythrocytes in media with various levels of H_2O_2

Additions to control medium	Number of experiments	SOD activity (U/ml of cells)	%% to control
None	2	1300 ± 50	100
0.5 μM H_2O_2	3	1800 ± 100	140
1 μM H_2O_2	3	1800 ± 100	140
2 μM H_2O_2	4	2100 ± 100	160
3 μM H_2O_2	2	2100 ± 30	160
6 μM H_2O_2	1	1800	140
NAI-pretreated medium	3	2000 ± 50	150

ESOD-C in the amount equivalent to 10 μl of duck erythrocytes was incubated in either control medium, or in NAI-pretreated (60 min) medium. Where indicated, H_2O_2 was added.

The pronounced inhibitory effect of catalase on SOD activity in media without H_2O_2 addition or without NAI treatment shows that H_2O_2 generated in the superoxide dismutase reaction induces considerable activation of SOD. The total stimulation by generated and added H_2O_2 reaches a 5–8-fold rise with respect to the level of SOD activity in the presence of catalase. Our observations, together with earlier data [19,20], show bell-shaped effect of H_2O_2 on SOD activity: stimulation followed by inhibition with increase in the H_2O_2 concentration. Biphasic changes in SOD activity were also observed under addition of increasing concentrations of electron acceptors [22] and in patients under inhalation of different doses of NAI [23].

The key role of superoxide for elucidation of mechanism of NAI action was not considered earlier for the exception of sole hypothesis [11]. However, this view was argued only by data on lethal effect of NAI in microorganisms which was eliminated with SOD addition. In that case SOD was regarded only as an indicator of superoxide involvement. Any specific action of NAI on SOD activity was not treated and beneficial effects of NAI were not considered.

The stimulation of SOD activity by NAI found in our experiments allows the understanding of the broad beneficial effects of NAI. We suggest the activation of SOD by superoxide mediated by micromolar concentrations of H_2O_2 to be relevant to the primary physicochemical mechanism of beneficial biological action of NAI.

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