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Modulation of superoxide dismutase by electron donors and acceptors

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The competition between superoxide dismutase (SOD) and nitroblue tetrazolium (NBT) for O_2^- radicals in the presence of a number of physiologically active compounds was studied. The Na^+ channel blockers, ajmaline, tetracaine, bipuvacaine, lidocaine and etmozine produced an increase in the amount of O_2^- reacting with SOD. Nitroprusside, ferricyanide, BAY K8644, levomycetin, cGMP, cAMP and GMP acted in the opposite way. All the SOD activtors had in common the property of being electron donors in the reactions with the light-induced free radicals of eosin whereas the SOD inhibitors behaved as electron acceptors. The electron activity of SOD modulators correlated qualitatively with their regulating efficacy.

Superoxide dismutase; Electron donor; Electron acceptor

1. INTRODUCTION

Superoxide dismutase (SOD) are the enzymes responsible for the catalytic scavenging of superoxide anion radicals (O_2^-) and play an important role in anti-radical protection of living systems [1]. Enzymes of this type, especially the copper,zinc-containing SOD from erythrocytes, are now attracting much interest as a result of their clinical use as effective anti-inflammatory agents [2]. The possible mechanisms of SOD regulation in living cells, however, remain obscure. The exposure of living organisms to oxygen at high partial pressures has been shown to induce SOD [3]. However, as yet data on the possible in vivo modulators of SOD activity are not available.

In vitro SOD is inhibited by nitroprusside [4] which is similar in structure to ferricyanide, known as a strong electron acceptor. Recently, it has been shown that electron acceptors and donors regulate in opposite ways the function of proteins responsible for potential-dependent transport of Ca^{2+} and Na^+ through excitable membranes [5,6]. Catecholamines and their antagonists also demonstrate opposed redox properties in reactions with free radicals [7]. If some general principles underlie the enzymes' functioning it can be expected that electron donors and acceptors should affect SOD activity differently.

The present results show that Cu,Zn-SOD is activated in vitro by compounds with electron donor properties and inhibited by electron acceptors. The possible biological implications of the phenomenon observed are discussed.

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2. MATERIALS AND METHODS

2.1. *Materials*

Cu,Zn-SOD (bovine erythrocyte, 3000 U/ml), nitroblue tetrazolium (NBT), xanthine, xanthine oxidase, NADH, phenazine methosulphate, cAMP, cGMP and GMP were from Serva. The Na^+-channel blocker ajmaline and its derivatives were from Giulini Pharma; tetracaine, bipuvacaine

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and lidocaine from Astra, the Ca^{2+}-channel agonist BAY K8644 from Bayer; and etmozine and levomycetin from the Pharmacological Institute (Moscow). Sodium nitroprusside and potassium ferricyanide were of the highest grade available from Reachem (USSR).

2.2. *Measurement of SOD activity*

The inhibition by SOD of the rate of NBT reduction by superoxide radicals was followed spectrophotometrically at 560 nm. Two systems for superoxide radical generation (xanthine/xanthine oxidase [8] and NADH/phenazine methosulphate [9]) were used. The concentration of SOD in the reaction mixture in all the control samples corresponded to 50% inhibition of the rate of NBT reduction.

2.3. *Evaluation of electron donors and acceptors*

The electron donor and acceptor properties of SOD modulators were investigated in reactions of these compounds with the light-excited dye eosin [5,6].

Ajmaline and its derivatives, tetracaine, bipuvacaine, lidocaine and etmozine, exhibit electron donor properties and nitroprusside, ferricyanide, BAY K8644, levomycetin, cAMP, cGMP and GMP were used as electron acceptors.

3. RESULTS

NBT reduction by superoxide radicals produced by both generating systems was not affected by the compounds under study in the absence of SOD (fig.1, curve 1). Added to the reaction mixture, SOD competed with NBT for O_2^- and slowed the rate of NBT reduction (fig.1, curve 2). SOD activity was enhanced by the anti-arrhythmic ajmaline (fig.1, curve 3) and its derivatives and also by the other Na^+-channel blockers bipuvacaine, etmozine, lidocaine and tetracaine. The concentrations of activators which cause 2-fold stimulation of SOD activity are listed in table 1. The data demonstrate that ajmalines are the most effective and lidocaine and tetracaine the weakest activators, although their efficacy differs less than 3-fold.

All the SOD activators tested showed electron-donor properties in reactions with dye free radicals [5,6], their electron donor activity decreasing in the order: ajmalines, bipuvacaine, etmozine, lidocaine, tetracaine (in aqueous solutions). The electron donor activity of the channel blockers correlates qualitatively with their blocking potency [5,6] and SOD activation as well.

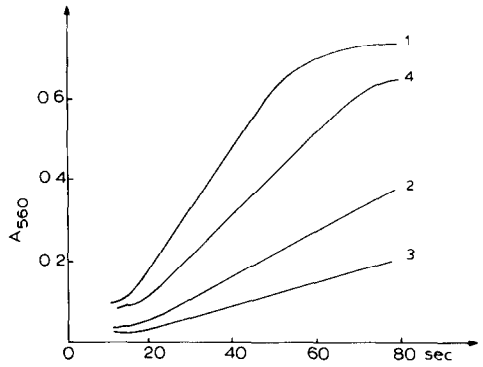

Fig.1. Time course of NBT reduction in the systems for superoxide generation. (1) Control sample without SOD; the kinetics of NBT reduction without SOD in the presence of ajmaline and ferricyanide coincide with curve 1. (2) Control with SOD (1 U); (3) with SOD (1 U) in the presence of 10 μM ajmaline; (4) with SOD in the presence of 20 μM ferricyanide. The reaction mixture contained an O_2^--generating system [NADH (0.35 mg/ml) and phenazine methosulphate (0.17 mg/ml) in phosphate buffer (pH 8.3) or xanthine (0.7 mg/ml) and xanthine oxidase (0.003 U/ml) in sodium carbonate buffer (pH 10.2) containing 0.15 mM EDTA] and NBT (0.12 mg/ml).

Table 1

Activation of Cu,Zn-SOD by compounds with electron donor properties

Compound	EC_{50} (concentration corresponding to 2-fold SOD activation (μM)
Ajmaline-C_3H_7	10
Ajmaline-C_6H_5	13
Ajmaline	13
Ajmaline-C_5H_{11}	14
Etmozine	15
Bipuvacaine	15
Lidocaine	18
Tetracaine	24

All concentrations shown are final reaction concentrations. Assay conditions are given in the legend to fig.1

Table 2

Inhibition of Cu,Zn-SOD by compounds with electron acceptor properties

Compound	EC_{50} (concentration corresponding to 2-fold SOD inhibition) (μM)
Nitroprusside	18
Ferricyanide	21
BAY K8644	24
cGMP	30
Levomycetin	41
GMP	>40
cAMP	>40

All concentrations shown are final reaction concentrations. Assay conditions are given in the legend to fig.1

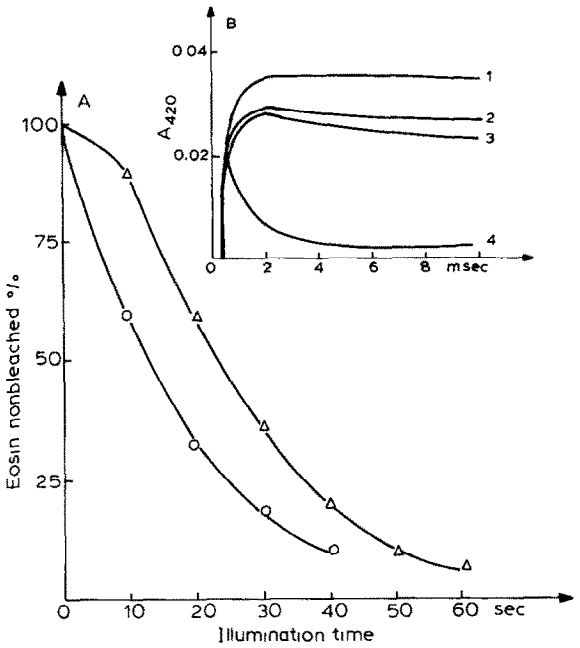

Fig.2. Time course of the reactions of electron acceptors with eosin anion radicals. (A) Reactions were carried out under continuous light illumination ($\lambda \geqslant 500$ nm) and followed at 517 nm. (1) Control sample containing eosin (10 μM) and NADH (100 μM). (2) Control sample with 3 μM levomycetin added. (B) Reactions after 50 μs flash illumination ($\lambda \geqslant 500$ nm) were followed at 420 nm. (1) Control sample containing eosin (10 μM) and NADH (100 μM). (2–4) Control samples with GMP (1 mM), cAMP (1 mM) and cGMP (1 mM), respectively. All samples were deaerated by pumping.

SOD activity was inhibited by ferricyanide, a potent electron acceptor (fig.1, curve 4), as well as by nitroprusside [4] which differs from ferricyanide by one NO group only. The dihydropyridine derivative BAY K8644, a novel Ca^{2+}-channel agonist [10], effectively inhibited SOD activity (table 2). In the reactions with dye free radicals BAY K8644 has shown strong electron acceptor properties [5].

The antibiotic levomycetin behaved as a strong electron acceptor slowing the initial rate of eosin photobleaching at micromolar concentrations (fig.2A).

The interception of electrons from dye anion radicals by cGMP, cAMP and GMP in flash experiments (fig.2B) shows cGMP to be the strongest electron acceptor among the nucleotides.

All the electron acceptors tested acted as SOD inhibitors (table 2), nitroprusside, ferricyanide and BAY K8644 being the most potent while GMP and cAMP were the weakest. The ID_{50} values of the electron acceptors correlate qualitatively with their electron activity.

4. DISCUSSION

Although SOD plays a crucial role in the protection of living cells from toxic oxygen radicals, little is known about the regulation of this antioxidant enzyme in vivo. In this work we succeeded in revealing a number of SOD inhibitors and for the first time in demonstrating the existence of SOD activators in vitro. Neither activators nor inhibitors interact with the systems for superoxide generation used, suggesting that these modulators affect the enzyme directly.

The activators tested are known as local anesthetics and anti-arrhythmics, these properties being due to their blockade of Na^+ channels. Though SOD activators differ significantly in chemical structure, electrical charge and hydrophobicity, all behave as electron donors in reactions with free radicals [6]. The electron donor properties of these drugs correlate qualitatively with their potency of increasing SOD activity. Ajmaline and its derivatives are the best electron donors among the compounds tested and activate SOD at micromolar concentrations. Tetracaine and lidocaine demonstrate the same effect at 2-fold

higher concentrations in agreement with their weaker electron donor activity.

The importance of the redox activity of SOD modulators is emphasized by the finding that all the SOD inhibitors tested showed electron acceptor properties. The strongest electron acceptors, nitroprusside, ferricyanide and the dihydropyridine derivative BAY K8644, effectively diminish SOD activity at micromolar concentrations.

It seems likely that the redox activity which SOD modulators demonstrate in reactions with free radicals is the most important feature of those resulting in enzyme modulation.

The acting concentrations of activators and inhibitors are much lower than the NBT concentration used in the assay (0.3 mM) and if SOD inhibitors acted as irreversible electron acceptors (or if SOD activators were irreversible electron donors) they would modulate SOD reaction only at the initial stage unless they were fully reduced. In fact, the substances tested modulate the reaction throughout the time of observation (i.e. for some minutes).

SOD inhibition and activation by compounds with definite redox activity towards free radicals raises the question as to whether free radical states are transiently involved in the enzyme functioning.

The observed activation of SOD by electron donors and inhibition by electron acceptors could be relevant to the processes in vivo. Thus, it has been demonstrated that the accumulation of semi-reduced metabolites and O_2^- radicals in mitochondria takes place as the oxygen tension in cells decreases [11]. In this case local SOD activation by increased concentrations of reduced metabolites – electron donors (or by decreased concentrations of oxidized products – electron acceptors) would be directed towards the elimination of the superoxide excess. This all holds true for conditions of superoxide generation during intensive energy expenditure (e.g. heavy physical exercise).

The finding that SOD is regulated in opposite ways by electron donors and acceptors, similarly to the regulation of the proteins forming Ca^{2+} and Na^+ channels as well as of the catecholamine receptors, indicates that such a type of enzyme regulation is rather widespread. The basis for this is formed by the possibility of the unpaired electrons being transferred through various proteins, as has been suggested in the model of transmembrane transport of solutes [12] and predicted theoretically as a common protein feature [13].

We believe that the approach used in the present work allows a deeper insight into the functioning of SOD as well as of some other enzymes.

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