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Brief Communication

CONTRADICTORY EFFECTS OF SUPEROXIDE DISMUTASE AFTER GLOBAL OR REGIONAL ISCHEMIA IN THE ISOLATED RAT HEART

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Abstract—The effect of superoxide dismutase was investigated in two different models of ischemia and reperfusion in the isolated rat heart: global and regional ischemia. The results of this comparison show that reperfusion arrhythmias after 10 and 15 min of regional ischemia, induced by occlusion of the left coronary artery, can be prevented by SOD confirming the results of other investigators. Paradoxically SOD was without effect after 10 min of global ischemia, obtained by stopping coronary flow completely. After 15 min of global ischemia, SOD induced ventricle fibrillation. Apparently the effect of SOD depends on the model of ischemia and reperfusion that is used.

Keywords—Superoxide dismutase, Ischemia, Reperfusion, Ventricle fibrillation, Rat heart, Free radicals

INTRODUCTION

Reperfusion of ischemic tissue with normoxic medium is accompanied by the formation of oxygen-derived free radicals (ODFR) causing additional damage to the postischemic tissue. The phenomenon is known as the oxygen paradox or the reperfusion syndrome.[1,2] Recently ODFR were shown to occur in perfusate of reperfused rabbit[3] and rat[4] hearts using the spin trap agent 5,5-dimethyl-l-pyrroline-*N*-oxide (DMPO). In these studies the enzymatic activity of superoxide dismutase (SOD, EC 1.15.1.1) was shown to diminish the DMPO–OH signal. This predicts a protective effect of SOD in ischemia reperfusion experiments. Effects of the enzyme have been measured in a variety of models of ischemia and reperfusion with ambiguous results. Unambiguous protection has been found in the isolated rat heart after occlusion of the left coronary artery.[5] In this model a ligature is placed around the left coronary artery so that regional ischemia can be induced. This induces ventricle fibrillation upon reperfusion in control hearts.[6] A protective effect in the treatment group is measured as the decrease in the incidence and/or duration of the arrhythmias. Measurement of other parameters to assess protection may lead to biased conclusions because ventricle fibrillation as such may cause tissue damage.

In the global ischemia model the heart is made ischemic by stopping coronary flow completely. Usually no severe arrhythmias are induced in control hearts upon reperfusion which allows reproducible measurements of contractility and biochemical parameters. This model has been used in our laboratory extensively for other studies.[7] When it was used to study ischemia/reperfusion induced free radical generation in the isolated rat heart a deleterious effect of SOD was found.

In order to determine if the effect of SOD depends on the way ischemia is applied, these two models of myocardial infarction were compared in the Langendorff rat heart.

MATERIALS AND METHODS

Animals and perfusion protocols

Twelve-week-old male Wistar rats were used and the hearts were perfused according to Langendorff. The hearts were perfused with tyrode buffer (pH = 7.4 and 37 °C) containing 128 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 20.2 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 1 mM $MgCl_2$, and 11 mM glucose. The buffer was saturated with 95% O_2 and 5% CO_2. Bovine erythrocyte superoxide dismutase (EC 1.15.1.1) was obtained from Boehringer Mannheim and dissolved (30 mg/L, 9×10^4 units/L) in tyrode buffer.

Global ischemia

After stabilization by perfusion with tyrode for 15 min, explanted hearts were perfused for 10 min with

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tyrode (controls, $n=6$) or with tyrode containing 30 mg/L SOD (treated, $n=6$). The hearts were then subjected to global ischemia by closing the valve for 10 or 15 min. During the ischemic period the hearts were submerged in warm tyrode which was bubbled through with 95% N_2 and 5% CO_2. Control hearts were reperfused with tyrode only while treated hearts were reperfused with tyrode containing 30 mg/L SOD. Ten or 15 min of global ischemia is known to induce damage that is severe enough to allow reproducible detection a protective effect.[7]

Regional ischemia

Occlusion of the left coronary artery was performed as described by Bernier et al.[5] After a stabilization period of 15 min the hearts were perfused with tyrode only (controls, $n=7$) or with tyrode containing SOD (treated, $n=7$) for 10 min. Then the ligature was closed and released 10 or 15 min later. These ischemic periods have been shown to induce maximal incidence of ventricle fibrillation in control hearts.[8]

Functional parameters

Lactate dehydrogenase (LDH) release, as a measure of tissue damage, and coronary flow were measured as described earlier.[7] Apex displacement was detected with a smooth muscle transducer. Cardiac work, expressed as contractility, was calculated as the product of amplitude and frequency, which were recorded every 30 sec. Ventricle fibrillation (VF) was scored visually at time points indicated. A heart was considered to show VF when apex amplitude was less than 5% of the preischemic value and when the ventricle trembled at the same time. A time point was considered positive when the heart fibrillated during the entire period since the previous point. Sustained VF is defined as VF that does not stop within 5 min after reperfusion.[5]

Statistics

All data are presented as means ± SEM. To evaluate differences between groups, n-way Analysis of variance was performed on the data using the Stata release 2.0. (Computing Resource Center, LA, California). To analyze the effect of fibrillation on LDH release fibrillation score was entered in the data and assigned the value of "O" for prefibrillation, "1" for fibrillating, and "2" for postfibrillation.

RESULTS

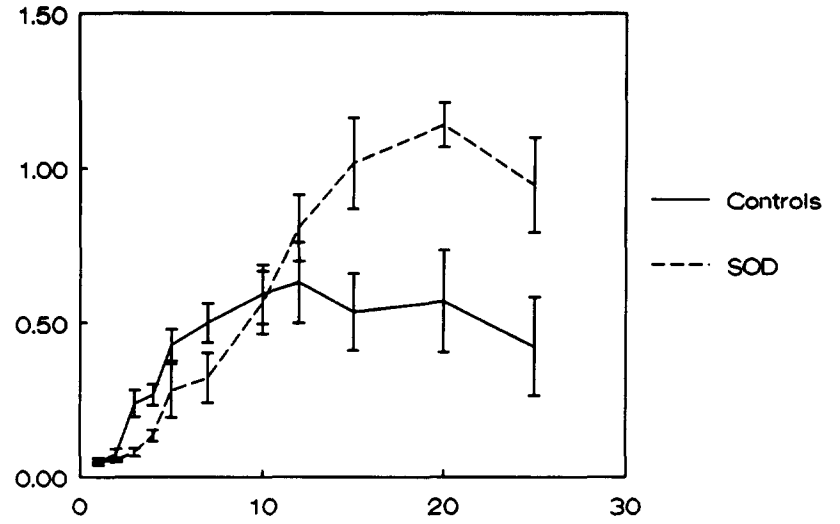
To examine whether SOD has a different effect in two models of ischemia reperfusion, treatment with the antioxidant enzyme was first tested in isolated rat hearts undergoing global ischemia. After 10 min ischemia the control and the protected hearts return to, respectively, 32.1 ± 6.8 and 33.2 ± 8.1% of preischemic contractility at 20 min of reperfusion. No ventricle fibrillation was observed in these groups. SOD infusion had no effect on LDH release or coronary flow after 10 min of ischemia. The peak in LDH release, expressed as units per minute per gram wet weight (U/min/gr ww) fell at 12 min after reperfusion in controls and SOD hearts and reached 0.196 ± 0.012 and 0.182 ± 0.019 U/min/gr ww, respectively (not shown).

After a period of 15 min of global, warm, ischemia transient VF occurs in three hearts in the control group (Table 1). In the SOD group VF occurs in all hearts in

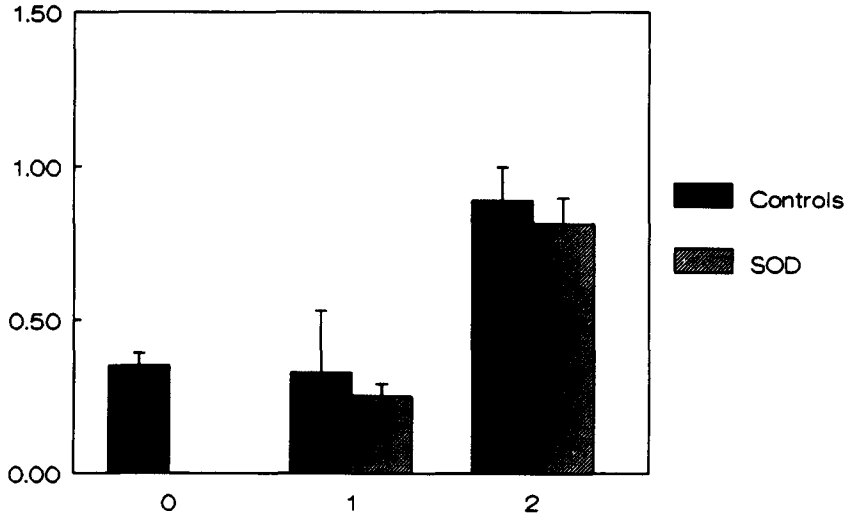
Table 1. Fibrillation Score of Individual Hearts After 15 Minutes Global Ischemia

	Minute After Reperfusion										
	1	2	3	4	5	7	10	12	15	20	25
Controls											
1	0	0	0	0	0	1	2	2	2	2	2
2	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	1	1	1	1	2	2	2
10	0	0	0	0	0	1	2	2	2	2	2
13	0	0	0	0	0	0	0	0	0	0	0
SOD											
3	1	1	1	1	1	1	1	2	2	2	2
4	1	1	1	1	1	1	1	1	1	2	2
7	1	1	1	1	1	2	2	2	2	2	2
11	1	1	1	1	2	2	2	2	2	2	2
12	1	1	1	1	1	1	2	2	2	2	2
14	1	1	1	1	1	1	1	1	2	2	2

A heart scores "0" when it does not show VF, "1" when it does, and "2" when it has stopped.



(a)



(b)

Fig. 1. LDH release after 15 min of global ischemia. LDH is expressed in units per minute per gram wet weight. panel a. Time course (min) of LDH release (mean ± SEM, $n=6$) after reperfusion. panel b. LDH release (mean ± SEM) by fibrilation score (see Table 1) after 15 min of global ischemia. Differences between controls and SOD treated hearts were not significant.

the first 4 min of reperfusion. The hearts were reperfused for 25 min and at that point all had stopped fibrillating. Contractility measured at 25 min after reperfusion was significantly higher in the control group (20.7 ± 5.50% in controls and 8.0 ± 3.0% in SOD group). The incidence of VF precludes a comparison of contractility values of both groups before this point, since fibrillating hearts do not contract.

The amount of LDH released after 15 min global ischemia (Fig. 1, panel a) was the same in both groups during the first 12 min of reperfusion. Thereafter, LDH release in the SOD group was higher than in the control group. Analysis of variance, performed to find out which parameters contribute to the LDH release, shows that LDH release is significant only on time ($p < 0.01$) and on the fibrillation score ($p < 0.01$) of the individual hearts but not on SOD treatment ($p < 0.966$). In Fig. 1, panel b, it is shown that LDH release is equal for fi-

Table 2. Incidence of Ventricle Fibrillation After Regional Ischemia

	Minute After Reperfusion						
	1	2	3	4	5	7	10
10-min Regional Ischemia (n = 7)							
Controls	7	7	7	6	5	5	4
SOD protected	4	3	3	2	1	1	1
15-min Regional Ischemia (n = 7)							
Controls	7	6	5	5	5	5	5
SOD protected	5	6	3	3	3	3	3

Presented are the number of hearts that showed VF during the entire period from the previous to the indicated time point, n = 7 in all groups.

brillation scores "0" and "1." The hearts that have reverted to a normal rhythm, score "2," release twice as much LDH. The differences between the controls and the SOD-treated hearts are not significant.

To study the effect of SOD on the incidence of ventricle fibrillation induced by regional ischemia, control and SOD hearts were subjected to either 10 or 15 min occlusion of the left coronary artery. The number of hearts showing ventricle fibrillation at the time points after reperfusion is presented in Table 2. After a 10-min occlusion of the coronary artery sustained VF, defined as continuous fibrillation in the first 5 min of reperfusion, occurred in five out of seven control hearts while only one out of seven SOD-treated hearts showed sustained VF. The incidence of sustained VF after 15 min coronary artery occlusion was five out of seven in the control group and three out of seven in the SOD group.

DISCUSSION

The results of the present study show that SOD decreases the incidence of ventricle fibrillation after a 10 or 15-min occlusion of the left coronary artery. This is in agreement with the results of other investigators.[5,8,9] In contrast, after 10 min global ischemia SOD has no effect, while the enzyme induces ventricle fibrillation after 15 min global ischemia.

Regional ischemia, which causes heterogeneous damage to the heart, is thought to induce arrhythmias because conduction is impaired to a different extent along the myocardium.[6] Therefore, the protective effect of SOD in the regional ischemia model is explained by scavenging superoxide that is generated during reperfusion.

In global ischemia conduction is probably diminished equally along the myocardium and generally no arrhythmias are induced by reperfusion in the control situation. The question arises by which mechanism SOD induces ventricle fibrillation in these hearts. The result of the present study shows that SOD treatment does not cause tissue damage as measured by LDH release. Therefore, it is likely that the enzyme causes the VF by a physiologic mechanism such as the release of arrhythmogenic substances. Histamine has been shown to enhance hypoxia-induced ventricle fibrillation in isolated rat hearts.[11] It is conceivable that the hydrogen peroxide that is generated from superoxide by SOD in the treated hearts triggers histamine release[12] from the resident cardiac mast cells.[13] There may be two explanations why no VF is observed after 10 min of global ischemia. One is that histamine did not induce arrhythmias in normoxic control hearts in the study of Dai,[11] but histamine did significantly shorten the time to onset of VF during anoxic perfusion by 30 min compared to controls. Although the time course of anoxic perfusions cannot simply be compared with global ischemia, this does show that a minimum period of oxygen deprivation is essential to make the heart susceptible to this effect. Another is that the amount of superoxide generated upon reperfusion depends on the duration of the preceding ischemia.[10] Therefore, in the present experiments the 10-min period could be too short because the hearts are not yet susceptible or too little superoxide is generated upon reperfusion.

Multiple interacting triggers, among these oxygen free radical-dependent and calcium-dependent, for reperfusion arrhythmias have been proposed.[8] These authors suggested that the dominance of one factor over another is determined by the duration of the regional ischemia. The results of the present study suggest that there is also a balance between several superoxide-dependent mechanisms contributing to the arrhythmias. The way the preceding ischemia was induced apparently tips the scales of this balance. Further experiments concerning mediator release and the effect of other experimental conditions will be necessary to elucidate the mechanisms behind the different role of the superoxide radical in these two models.

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