

Lack of the effect of superoxide dismutase and catalase on Na⁺,K⁺-ATPase activity in stunned rabbit hearts.

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Summary

Reactive oxygen species (ROS) have been implicated in the mechanism of postischemic contractile dysfunction, known as myocardial stunning. In this study, we examined protective effects of antioxidant enzymes, superoxide dismutase (SOD) and catalase, against ischemia/reperfusion-induced cardiac dysfunction and inhibition of Na^+, K^+ -ATPase activity. Isolated Langendorff-perfused rabbit hearts were subjected to 15 min of global normothermic ischemia followed by 10 min reperfusion. The hearts treated with SOD plus catalase did not show significant recovery of left ventricular (LV) end diastolic pressure compared with untreated ischemic reperfused hearts. Treatment with antioxidants had no protective effects on developed LV pressure or its maximal positive and negative first derivatives ($\pm \text{LVdP/dt}$). Myocardial stunning was accompanied by significant loss in sarcolemmal Na^+, K^+ -ATPase activity and thiol group content. Inhibition of enzyme activity and oxidation of SH groups were not prevented by antioxidant enzymes. These results suggest that administration of SOD and catalase in perfusate do not protect significantly against cardiac dysfunction in stunned rabbit myocardium.

Key words: free radicals; sodium pump; stunning; reperfusion, superoxide dismutase, catalase

Introduction

It is generally acknowledged that reactive oxygen species (ROS) such as superoxide radical ($\cdot\text{O}_2^-$), hydroxyl radical ($\cdot\text{OH}$) or singlet oxygen ($^1\text{O}_2$) play an important role in tissue injury occurring during postischemic reperfusion (for review see Zweier and Talukder 2006). Production of ROS in ischemic reperfused myocardium was shown either directly, using electron spin resonance (EPR) spectroscopy (for review see Zweier and Talukder 2006) and chemiluminiscence (Toufektsian *et al.* 2001) or indirectly, demonstrating the ability of various free radical scavengers to reduce reperfusion injury (for review see Bolli and Marbán 1999, Dhalla *et al.* 2000, Paulis and Šimko, 2007). Numerous studies have demonstrated that administration of antioxidant enzymes, superoxide dismutase (SOD) and catalase, $\cdot\text{OH}$ radical scavengers or $^1\text{O}_2$ quenchers improves the recovery of heart contractile function, ATP content and ion transport activities and reduces the infarct size induced by ischemia and reperfusion (Dixon *et al.* 1990, Ambrosio G, Flaherty 1992, Li *et al.* 2000, Kaplan *et al.* 2005, Sahna *et al.* 2005). In contrast, other studies did not demonstrate protective effects of antioxidant therapy or some endogenous antioxidant enzymes against postischemic injury (Jeroudi *et al.* 1990, Voogd *et al.* 1991, Jones *et al.* 2003, for review see Dhalla *et al.* 2000).

Because of their high reactivity, ROS modify various cellular components and may contribute to postischemic contractile dysfunction termed myocardial stunning (Tatarková *et al.* 2005). Although the mechanism by which ROS may contribute to stunning is not fully understood, there is accumulating evidence that disturbance in the intracellular ion homeostasis is a potential mediator of reperfusion injury (Takeo and Tanonaka 2004, Wang *et al.* 2007). Several investigations suggest that sarcolemmal Na^+, K^+ -ATPase, which plays a key role in generating transmembrane Na^+ gradients, might be a critical site for damaging effects of ROS generated during reperfusion (Ravingerová *et al.* 1999, Inserte *et al.* 2005, Ostadal *et al.* 2004).

Previously, we examined the protective effects of mannitol and histidine on stunned myocardium and sarcolemmal Na^+, K^+ -ATPase (Kaplan *et al.* 2005). Our results showed that contractile function and the enzyme activity were significantly protected by $^1\text{O}_2$ scavenger histidine, but not by $\cdot\text{OH}$ radical scavenger mannitol. These results indicate that $^1\text{O}_2$ plays more important role in myocardial stunning than $\cdot\text{OH}$ radical, however, the role of other ROS, including $\cdot\text{O}_2^-$ and H_2O_2 , was not tested. Treatment with SOD and catalase, the $\cdot\text{O}_2^-$ and H_2O_2 scavengers, was investigated in the large number of studies, but their beneficial action remains unclear. These controversial results may arise from various experimental differences, including species variation and models of stunning. We performed the present study to determine whether SOD and catalase reduce myocardial stunning after total ischemia of isolated Langendorff-perfused rabbit heart.

Methods

Isolated heart preparation and perfusion protocol

New Zealand White rabbits of weight 2-3 kg were used in the present study and the investigation was performed in accordance with the guidelines of the National Institute of Health for the care and use of laboratory animals. The hearts were isolated and perfused according to the Langendorff method as described previously (Kaplan *et al.* 2005). Briefly, after complete anesthesia the hearts were excised via a midline thoracotomy and perfused immediately with Krebs-Henseleit (K-H) solution at 37 °C at a constant pressure of 65 mmHg. The left ventricular pressure (LVP), temperature and coronary flow were recorded continuously.

The hearts were divided into three experimental groups as follows:

1. Control group: perfusion for 35 min with K-H solution without ischemia.

2. Reperfusion group: normal perfusion with K-H solution lasting 10 min followed by 15 min ischemia plus 10 min reperfusion.
3. Reperfusion group treated with SOD plus catalase: perfusion protocol as in group 2 with K-H solution supplemented with SOD (10 000 U/l) and catalase (10 000 U/l). The antioxidant enzymes were added into perfusion solution before ischemia and were present in K-H solution during reperfusion.

At the end of the perfusion experiment the left ventricle was freeze-clamped in liquid nitrogen and stored at -80 °C for preparation of homogenates.

Preparation of homogenates and measurement of Na^+, K^+ -ATPase activity

Frozen powdered tissues of the left ventricles (approximately 1 g) were thawed in 10 volumes of homogenizing buffer containing 30 mM imidazol, 60 mM KCl and 2 mM MgCl_2 , pH=7.0 and homogenized 3x10s at 14 000 rpm using a Ultra-Turrax T25 homogenizer (Janke & Kunkel, Staufen, FRG) with a T25 probe. The homogenate was filtered through cheesecloth, quickly frozen in liquid nitrogen and stored at -80 °C until use.

Na^+, K^+ -ATPase activity in ventricular homogenates was assayed using the linked enzyme system of pyruvate kinase–lactate dehydrogenase by continuously monitoring NADH oxidation at 340 nm (Kaplan *et al.* 2005). The assay was performed at 37 °C after addition of homogenate (50µg protein/ml) into medium containing 25 mM imidazole (pH 7.4), 10 mM KCl, 95 mM NaCl, 5 mM MgCl_2 , 5 mM sodium azide, 0.5 mM EGTA, 3 mM ATP, 40 µg/ml alamethicin, 350 µM NADH, 1 mM phosphoenolpyruvate, 10 U/ml pyruvate kinase and 25 U/ml lactate dehydrogenase. Na^+, K^+ -ATPase activity is reported as the total activity minus the activity obtained in the presence of 1 mM ouabain.

Measurement of total thiol group content

Total -SH group content in tissue homogenates was determined spectrophotometrically by the 2,2-dithiobisnitrobenzoic acid (DTNB) assay (Jocelyn 1987) as described by Sivoňová *et al.* (2007). Aliquots of homogenates (0.15 mg proteins) were incubated in medium containing 30 mM imidazole (pH 7.4), 5 mM EDTA, 0.8% SDS and 0.4 mM DTNB. After incubation for 10 min at room temperature the sample absorbance was measured at 412 nm. Thiol group content was calculated using molar absorption coefficient of $13\,600\text{ M}^{-1}\text{cm}^{-1}$ after subtraction of blank absorbance from absorbance of sample.

Statistical analysis.

All data are presented as mean \pm SD. One-way analysis of variance was first carried out to test for differences between all groups. Between individual groups comparisons were made using an unpaired t-test with the Bonferroni correction. A value of $p < 0.05$ was considered to be statistically significant.

Results

Effects of ischemia and reperfusion on contractile function

Myocardial contractile parameters for control and ischemic reperfused hearts are shown in Table 1. There were no significant changes between pre- and postischemic values of heart rate and coronary flow in ischemic reperfused hearts treated with or without SOD plus catalase (data not shown). The recovery of sLVP in antioxidant-treated group was similar to that observed in untreated reperfused hearts. Ischemia and reperfusion markedly affected left ventricular end-diastolic pressure (LVEDP). By the end of 10 min reperfusion without antioxidants, LVEDP exhibited more than twofold increase over the baseline preischemic

value. In hearts treated with SOD and catalase, LVEDP also increased from the baseline, but the change was not significant when compared to preischemic or control value (Fig. 1).

Both, positive and negative values of LVdP/dt were significantly depressed in ischemic reperfused myocardium. Addition of SOD and catalase to the perfusion medium had no protective effect on the recovery of these parameters (Table 1). Similarly, reperfusion with antioxidants was not accompanied with significant improvement in the recovery of left ventricular developed pressure (devLVP) (Fig. 2). At the end of reperfusion period the devLVP values were 68 ± 5 and 74 ± 2 mmHg in untreated hearts and ischemic reperfused hearts treated with SOD plus catalase, respectively.

Effect of SOD plus catalase on Na^+ , K^+ -ATPase activity and thiol group content in stunned hearts

Figure 3 presents the activities of Na^+ , K^+ -ATPase in homogenates from control and stunned hearts treated with or without SOD plus catalase. Na^+ , K^+ -ATPase activity was significantly depressed in untreated stunned hearts as compared to control group. Addition of antioxidants to the perfusion medium prior to ischemia slightly improved the activity, but the effect was not significant.

Oxidative injury was assessed by determining thiol group content (Fig. 4). The concentration of SH groups in untreated stunned hearts was significantly lower than that in the control hearts ($80.5 \pm 6.5\%$, $p < 0.01$ vs. control). SOD plus catalase increased the SH level to $86.9 \pm 8.9\%$, but it was still significantly lower ($p < 0.05$ vs control).

Discussion

The present study suggest that treatment of ischemic reperfused rabbit hearts with SOD plus catalase had little protective effect on contractile parameters, Na^+, K^+ -ATPase activity and oxidative injury as detected by thiol group content.

Many studies have investigated the effect of SOD and/or catalase during cardiac ischemia and reperfusion, however, there is controversy regarding the efficacy of this treatment. Protection against reperfusion injury has been shown in studies using open-chest and conscious animals (Triana *et al.* 1991), as well as isolated heart preparations (Zweier *et al.*, 1989, Ambrosio and Flaherty 1992). In contrast, several studies have failed to show protective effect of these antioxidant enzymes against reperfusion injury (Richard *et al.* 1988, Jeroudi *et al.* 1990, Voogd *et al.* 1991, Euler 1995). Several factors may account for the discrepancy between the studies including experimental conditions, animal models of ischemia-reperfusion injury, ability of antioxidant enzyme to reach regions attacked by ROS and the dose or isoform of the enzyme tested. Voogd *et al.* (1991) have demonstrated protective effects of SOD in Langendorff perfused rat heart subjected to regional ischemia, but SOD was not protective in hearts subjected to 10 or 15 min of global ischemia, indicating important role of the experimental model. Omar *et al.* (1990) have shown that cardioprotective effect of SOD in Langendorf perfused rabbit hearts decreases at high doses (>5 mg/l of perfusate) and, at very high doses (>50 mg/l) SOD exacerbates the injury. Overdosing with SOD cannot explain the negative findings regarding the SOD effect, since estimated dose of SOD used in our experiments was about 5.5 mg/l and was much lower than that used in studies showing cardioprotection (Ambrosio and Flaherty 1992). Lack of protection by SOD and catalase could be explained by their inability to access structures attacked by ROS. Critical role of the antioxidant localization is supported by recent studies showing that overexpression of extracellular SOD or mitochondrial MnSOD attenuated

reperfusion injury, while overexpression or deficiency of cytosolic Cu/ZnSOD did not affect the degree of injury (Li *et al.* 1998, Asimakis *et al.* 2002, Jones *et al.* 2003). It is possible that the freely soluble SOD and catalase are distributed mainly across the extracellular space and their concentration in the intracellular and interstitial space or on the cell surface is too low to prevent toxic effects of ROS.

Using the same model of myocardial stunning, we have found (Kaplan *et al.* 2005) that treatment with $\cdot\text{OH}$ radical scavenger mannitol also afforded little protective effects. On the other hand, treatment with $^1\text{O}_2$ scavenger histidine significantly improved contractile function and prevented loss in Na^+, K^+ -ATPase activity. It remains to be elucidated whether considerable difference in protective effects of tested antioxidants results from different ability to reach sites of ROS production or from different importance of $\cdot\text{OH}$, $\cdot\text{O}_2^-$, H_2O_2 and $^1\text{O}_2$ in postischemic injury.

Conflict of interest

There is no conflict of interest.

Acknowledgement

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Table 1. Effects of SOD plus catalase (CAT) on cardiac contractile function after 15 min ischemia and 10 min reperfusion

	Control	Ischemia and reperfusion	
		Untreated	SOD+CAT
sLVP			
before I	134±11	120±24	124±11
after I	127±21	92±8*	92±5***
LVEDP			
before I	9±2	10±2	12±3
after I	11±3	24±10*	19±8
+LVdP/dt			
before I	1900±216	1588±536	1700±283
after I	1763±411	900±183**	1010±96**
-LVdP/dt			
before I	1275±166	1125±263	1090±143
after I	1200±268	750±92**	800±50**

sLVP, systolic left ventricular pressure; LVEDP, left ventricular end diastolic pressure; devLVP, developed LVP; +LV dP/dt, maximum rate of pressure development and -LV dP, maximum rate of relaxation are expressed in mmHg.. Values are means±SD of 5 hearts. *p<0.05, **p<0.01, ***p<0.001; significantly different as compared to control.

Figure legends

Fig. 1 Effect of SOD plus catalase on left ventricular end diastolic pressure in stunned myocardium. Control (○), untreated stunned hearts (●), stunned hearts treated with SOD+catalase (▲). Values are given as means±SD of 5 hearts.

Fig. 2 Effect of SOD plus catalase on developed LVP in stunned myocardium. Control (○), untreated stunned hearts (●), stunned hearts treated with SOD plus catalase (▲). Values are given as means±SD of 5 hearts.

Fig. 3 Effect of SOD plus catalase on Na^+, K^+ -ATPase activity in stunned myocardium. Values are given as means±SD of 5 hearts. * $p < 0.05$; ** $p < 0.01$; significantly different as compared to control.

Fig. 4 Effect of SOD plus catalase on total thiol group content in stunned myocardium. Values are given as means±SD of 5 hearts. * $p < 0.05$; ** $p < 0.01$; significantly different as compared to control.

Fig. 1

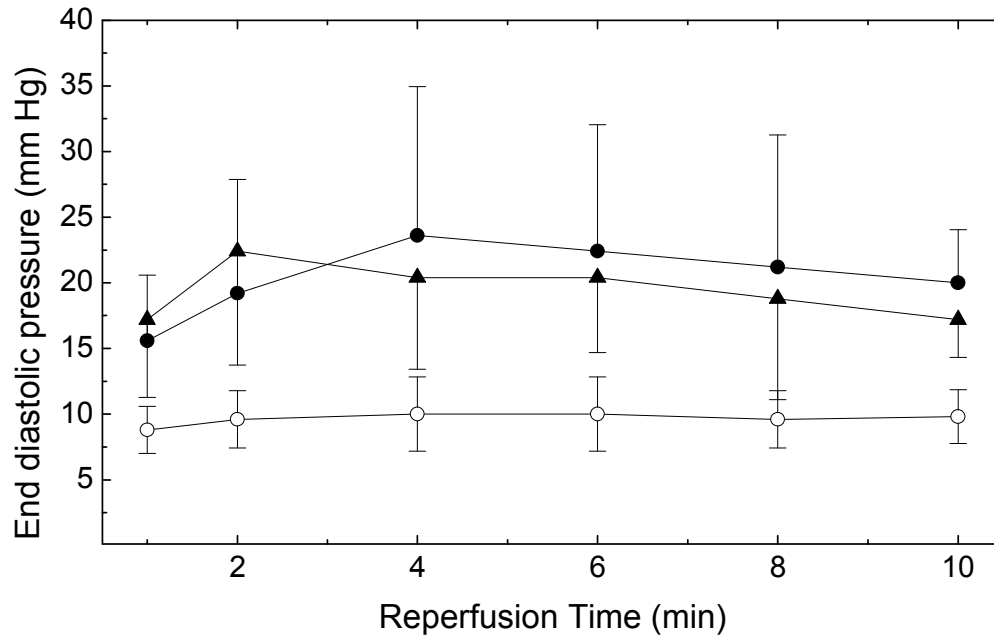


Fig. 2

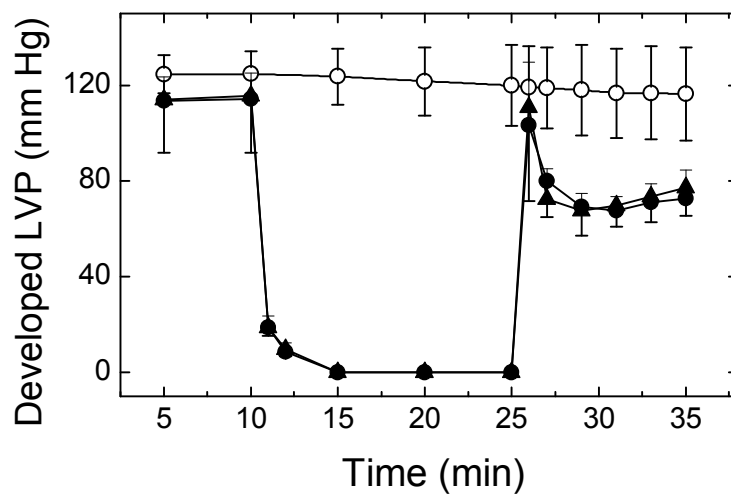


Fig. 3

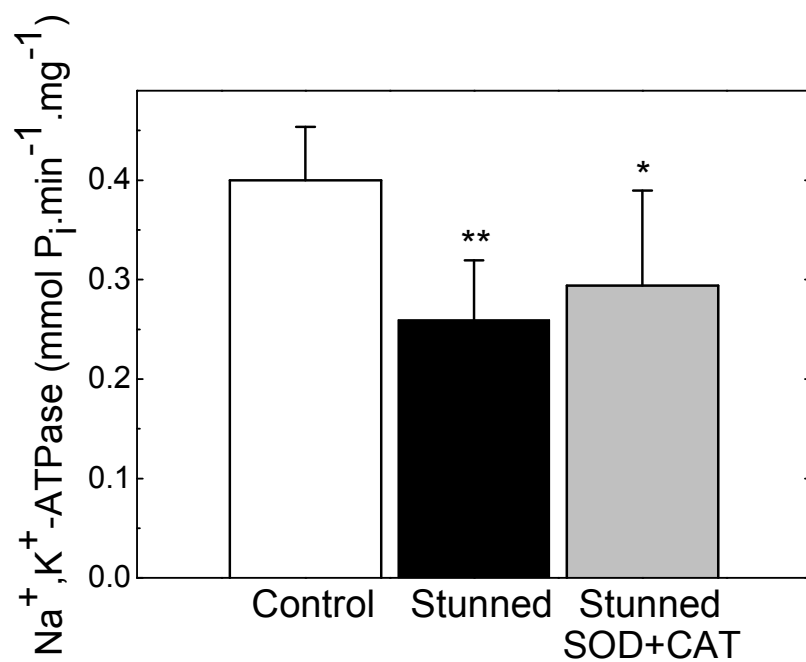


Fig. 4

