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Role of Adenosine in the Activation of Myocardial Catalase Induced by Brief Regional Ischemia

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The activities of myocardial antioxidant enzymes are known to increase in the hearts preconditioned with the brief episodes of ischemia. This study was undertaken to elucidate the possible involvement of adenosine in the stimulation of myocardial catalase induced by the brief regional ischemia in rabbit hearts. Coronary artery descending the middle anterior wall of left ventricle was occluded for 15 min, followed by 1 hr of reperfusion. Upon reperfusion after the brief ischemia, the activity of catalase increased significantly in both ischemic and non-ischemic parts of myocardium. Pretreatment of the heart with theophylline, a non-specific adenosine receptor blocker, completely abolished the increase of catalase activity in both the ischemic and non-ischemic regions of myocardium. On the other hand, the administration of exogenous adenosine instead of the ischemia failed to increase the catalase activity in *in vivo* hearts. Moreover, adenosine infusion did not affect the catalase activity in the isolated, perfused hearts either. These results suggest that the endogenous adenosine released from the ischemic myocardium is involved in the activation of catalase induced by brief ischemia, but that adenosine may not be a final direct activator of cellular catalase in the myocardium.

Key Words: Heart, Ischemia, Catalase, Adenosine

INTRODUCTION

Reactive oxygen species mediate tissue injuries in a number of disease states including ischemia-reperfusion injury, inflammatory diseases, etc. To face the hazardous attacks of reactive oxygen species, the cell is equipped with the endogenous antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. The regulation mechanisms for these antioxidant enzymes in eukaryotic cells are mostly unknown, and the basal levels of each antioxidant enzyme in mammalian cells are various (Halliwell & Gutteridge, 1989). The antioxidant enzymes in myocardial cells can be activated by different modalities of stress including oxidative stress (Hoshida et al, 1993; Das et al, 1995; Park et al, 1995), heat stress (Currie & Tanguay, 1991; Karmazyn et al, 1990), or inflammatory mediators (Brown et al, 1990). Studies on these phenomena have led to the conclusion that the myocardial cell possesses an efficient adaptation mechanism for a variety of cellular stresses and encouraged the trials to apply this adaptive phenomenon to the prevention of heart against cellular damages induced by oxidative stress (Park et al, 1995; Steare & Yellon, 1995).

Previously, we observed that a brief period (15 min) of a coronary artery occlusion, followed by reperfusion, led to a significant stimulation of catalase activity in both ischemic and non-ischemic regions of rabbit hearts. In that study, any evidence of altered redox state was not detected in the myocardial cells, suggesting that the oxidative stress incurred by ischemia-reperfusion may not be a direct factor involved in the activation of catalase in the non-iscchemic myocardium (Kim et al, 1996). Meanwhile, it has recently been reported that adenosine activates the antioxidant enzymes in the cell culture system (Maggirwar et al, 1994). As it is known that the accelerated production of intracellular adenosine in ischemic condition is accompanied with an increased level of interstitial adenosine in the non-ischemic as well as

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in the ischemic parts of myocardium (Dorheim et al, 1991), we hypothesized that the endogenous adenosine might play a role, as a direct mediator, in the myocardial catalase activation induced by brief regional ischemia.

METHODS

Experimental protocol and surgical procedures

Rabbits (New Zealand White, male, 1.5~2.5 kg) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). After tracheotomy, the rabbits were intubated and ventilated with room air. The hearts were exposed through a left thoracotomy. A silk snare was placed around the large branch of coronary artery descending the middle anterior wall of left ventricle. The artery was occluded for 15 minutes by pulling the snare and then reperfused by removing the snare. After the reperfusion for 1 hr, hearts were removed and washed with a cold saline perfusion. Myocardial samples were taken separately from the ischemic apical region and from the normally perfused, non-ischemic posterobasal region of the left ventricle. A sham-operation group was added to the protocol to exclude any influence of surgical and anesthetic procedures other than the ischemia and reperfusion. As for the sham operation group, all operation procedures were identically performed except the occlusion of coronary artery, and tissue samples were taken 1 hr after the sham operation. The basal activities of antioxidant enzymes were measured in tissue samples taken from the control group to which no operative procedures were given. To test the hypothesis that adenosine might act as a mediator in the stimulation of catalase, a non-specific adenosine receptor antagonist, theophylline (20 mg/kg), was administerd as a bolus 15 min before the coronary ligation. In other group of animals, exogenous adenosine (0.15 mg/kg/min) was infused through the ear vein for 15 min instead of coronary ligation. The hemodynamic parameters were monitored to confirm the delivery of adenosine.

Perfusion of isolated rabbit heart

Hearts were excised from the anesthetized rabbits and immediately connected to aortic cannulae. Isolated hearts were perfused in a constant pressure, non-recirculating Langendorff mode with Krebs-Henseleit (K-H) buffer containing: NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 1.25 mM, $MgSO_4$ 1.2 mM, glucose 10 mM, $NaHCO_3$ 25 mM, and KH_2PO_4 1.2 mM. The buffer solution was bubbled with 95% O_2-5% CO_2 mixture at 37°C, and perfusion pressure was maintained at 80 cm H_2O. Each preparation was equilibrated for 20 minutes with normal K-H solution, and then the perfusion solution was switched to the K-H solution containing adenosine (3 mg/l) for 15 min. After the adenosine treatment, followed by normal K-H perfusion without adenosine for 1 hr, myocardial samples were taken.

Measurement of catalase activity

Myocardial samples were homogenized in 4 vol of homogenation buffer (30 mM KCl, 1 mM EDTA, 10 mM potassium phosphate, pH 7.4), using a Polytron homogenizer (Brinkman Instruments, USA) and an ultrasonicator (Heat Systems-Ultrasonics, USA). The homogenate was centrifuged at 10,000 *g* for 15 min, and the supernatant was taken for enzyme assay. Catalase activity was measured by following the O_2 generation resulting from the decomposition of H_2O_2 (Karmazyn et al, 1990). H_2O_2 (final 4.6 mM) was added to a final 5 ml of reaction buffer (0.1 % EDTA, 10 mM potassium phosphate, pH 7.4) containing an aliquot of tissue homogenate equivalent to 80 micrograms of protein. The rate of O_2 generation was measured with a Clark-type oxygen electode at 30°C. Activity was calculated from the standard curve of purified catalase (Sigma, USA). To exclude the enzyme contamination from erythrocytes, hemoglobin content in tissue sample and the blood catalase activity were measured. The equivalent activity was subtracted from the tissue activity. Protein concentration was measured using BCA (bicinchoninic acid) method (Bio-Rad, USA) with bovine serum albumin as a standard.

Statistical analysis

Results are expressed as mean±SEM. Differences were compared by unpaired two-tailed *t*-test, with $p < 0.05$ considered significant.

RESULTS

Effect of adenosine receptor blockade on catalase activation induced by ischemia-reperfusion

Basal level of catalase activity in the normal control heart was 42.8±4.5 U/mg protein. Catalase activity of the sham-operated heart (apical region : posterobasal region=53.7±12.9 : 48.0±10.8 U/mg pro-

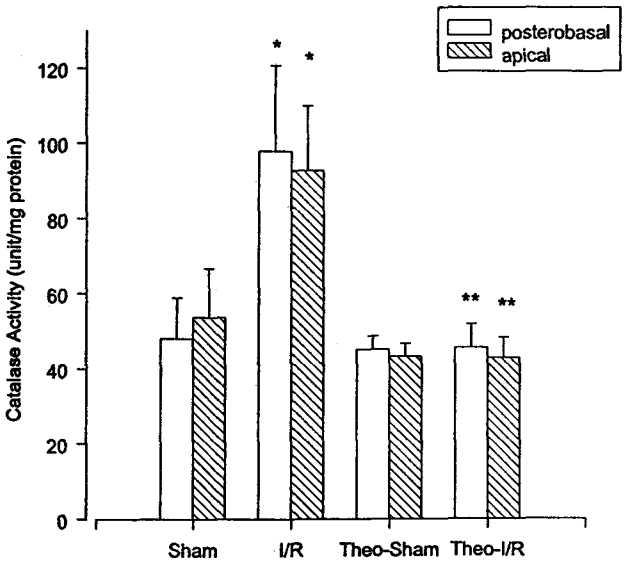

Fig. 1. Effect of an adenosine receptor blocker, theophylline, on catalase activation induced by ischemia-reperfusion. Left anterior descending coronary artery was occluded for 15 min and then reperfused for 1 hr (I/R). A non-specific adenosine receptor antagonist, theophylline (20 mg/kg) was administered at 15 min before the coronary artery ligation (Theo). Myocardial samples were taken from the ischemic apical region (▨) and the non-ischemic posterobasal region (□). Sham-operation was done without the coronary artery ligation (Sham). *: $p<0.05$ vs sham, **: $p<0.05$ vs I/R

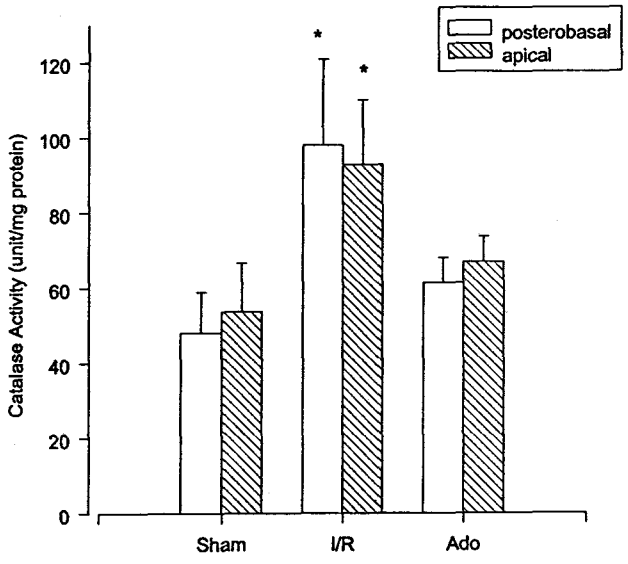

Fig. 2. Effects of ischemia-reperfusion and intravenous adenosine infusion on catalase activity in *in situ* rabbit hearts. Left anterior descending coronary artery was occluded for 15 min and then reperfused for 1 hr (I/R). Adenosine (0.15 mg/kg/min) was infused through the ear vein for 15 min instead of the coronary artery ligation (Ado). Myocardial samples were taken from the ischemic apical region (▨) and the non-ischemic posterobasal region (□). Sham-operation was done without the coronary artery ligation (Sham). *: $p<0.05$ vs sham

tein) was not statistically different from the normal control level. Fig. 1 shows the catalase activities in myocardium measured 1 hr after the 15 min of regional ischemia with or without the pretreatment with theophylline. Catalase activities in the myocardium were increased significantly by the brief regional ischemia in both the ischemic apical (92.8 ± 17.3 U/mg protein, $p<0.05$ vs sham-operated) and non-ischemic posterobasal (97.9 ± 22.9 U/mg protein, $p<0.05$ vs sham-operated) parts of myocardium, and the increased enzyme levels in both regions were comparable to each other. Pretreatment of the heart with theophylline almost completely abolished the increase of catalase activity by the regional ischemia in both the ischemic apical (42.9 ± 5.4 U/mg protein) and non-ischemic posterobasal (45.6 ± 6.3 U/mg protein) regions of myocardium. The administration of theophylline in sham-operated hearts had no apparent influence on myocardial catalase activities (Fig. 1).

Effect of intravenous adenosine on myocardial catalase and hemodynamics in vivo

Intravenous infusion of adenosine (0.15 mg/kg/min) instead of ischemia resulted in a slight, but not statistically significant increase of catalase activities (apical region : posterobasal region=66.9 ± 6.8 : 61.3 ± 6.7 U/mg protein) compared to those of sham-operated hearts (apical region : posterobasal region=53.7 ± 12.9 : 48.0 ± 10.8 U/mg protein) (Fig. 2). Systolic and diastolic blood pressures were markedly decreased by

Table 1. Hemodynamic changes induced by intravenous adenosine in rabbit

	Adenosine[a]		
	Before	During[b]	After
Blood pressure, mmHg			
Systolic	111.5 ± 6.5	69.0 ± 9.0*	95.0 ± 10.0
Diastolic	91.0 ± 4.0	27.5 ± 7.0*	85.0 ± 9.0
Heart rate, beat/min	297.0 ± 27.0	276.0 ± 6.0	309.0 ± 39.0

[a]: Adenosine (0.15 mg/kg/min) was infused for 15 min through the ear vein.
[b]: Parameters were measured at the end (15 min) of adenosine infusion
*: $p<0.05$ vs before adenosine

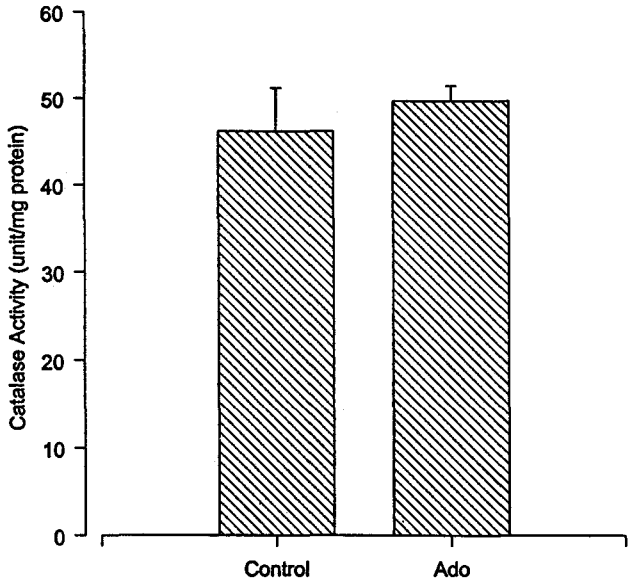

Fig. 3. Effect of adenosine perfusion on catalase activity in isolated rabbit hearts. Isolated rabbit hearts were perfused by Langendorff method with adenosine (3 mg/L) containing Krebs-Henseleit solution for 15 min. After the adenosine treatment (Ado), followed by 1 hr of normal perfusion, myocardial samples were taken.

adenosine infusion and recovered after the cessation of the 15 min infusion. Heart rate was also decreased by intravenous adenosine, though the negative-chronotropic effect was not so marked as the blood pressure changes (Table 1).

Effect of adenosine on catalase activity in isolated hearts

To investigate whether adenosine itself could directly activate myocardial catalase or not, we tried to evaluate the effect of adenosine in a controlled *in vitro* hearts. For this purpose, isolated rabbit hearts were perfused by Langendorff method with Krebs-Henseleit buffer solution containing adenosine (3 mg/l) for 15 minutes, and myocardial catalase activities were measured 1 hr thereafter. Compared with the control hearts (46.2 ± 4.9 U/mg protein) which were perfused with normal K-H solution for 75 min, the hearts treated with adenosine (49.7 ± 1.8 U/mg protein) did not show any significant change in catalase activity (Fig. 3).

DISCUSSION

In this study, brief regional ischemia of heart led to a significant stimulation of catalase activity in both the ischemic and non-ischemic parts of rabbit myocardium. This result implies that a signal generated by regional ischemia and/or reperfusion influences not only the affected ischemic-reperfused part of myocardium, but also the adjacent normally perfused myocardium to stimulate catalase. Periods of ischemia result in multiple physiological alterations in the hearts, which are represented by the deleterious changes in cellular metabolism and the simultaneous activation of defense mechanism to minimize these alterations resulted from oxygen depletion. Increased adenosine release is an important defense mechanism against the altered physiological changes, causing vasodilation and negative inotropism/chronotropism to oppose the oxygen demand-supply imbalance resulted from the reduced blood flow (Mullane & Bullough, 1995). In the present study, pretreatment of heart with a non-specific adenosine receptor antagonist, theophylline, completely abolished the increase of catalase activity in both the ischemic and non-ischemic myocardium, suggesting that adenosine released from the ischemic myocardial cells mediates an increase of the myocardial catalase activity in both regions. This is supported by a report which presented the adenosine-induced catalase activation in the cultured rat cardiomyocytes (Maggirwar et al, 1994). Contrarily, in this study, the administrations of exogenous adenosine did not increase the catalase activity either in normally perfused *in vivo* hearts or in isolated hearts. This result seems not to be consistent with that of the theophylline study. As the plasma half-life of adenosine is very short due to the rapid degradation by adenosine deaminase and uptake into blood cells (Olsson & Pearson, 1990), the failure of exogenous adenosine to increase the enzyme activity may result from an ineffective concentration reaching the myocardial tissue. However, considering the hemodynamic changes including the reductions of blood pressure and heart rate upon adenosine administration, this possibility could be excluded. It is, thus, postulated from these results that, though adenosine is involved in the catalase activation induced by brief regional ischemia, it may not be a final direct mediator which acts on the target cells.

Oxidative stress mediated by an increased generation of reactive oxygen species can trigger the induction of antioxidant defense system (Das et al, 1995, Park et al, 1995). So it is plausible that the increased oxidative stress incurred by the ischemia-reperfusion is involved in the catalase activation in the non-ischemic myocardium. However, in the previous study, we could not detect an altered cellular redox state in the non-ischemic part of the regionally ischemic hearts, suggesting that the factor (s) other than the

oxidavtive stress may be involved in the catalase activation in the non-ischemic myocardium (Kim et al, 1996). Systemic stress caused by operative procedures could be involved in the catalase activation. However, because of no observable changes in the enzyme activity in sham-operated rabbits, this possibility is not conceivable either. Mechanisms responsible for the catalase activation in the non-ischemic myocardium might include a signal transmission pathway involving sympathetic nervous system or hormonal factors. It has been reported that the endogenous norepinephrine released during a period of transient ischemia (Banerjee et al, 1993) and the exogenous adrenergic agonists (Kim et al, 1998) preconditioned the hearts to protect from ischemic cellular injuries. Considering that the adrenergic stimulation enhances the adenosine release from the ischemic myocardium (Richardt et al, 1994), it is assumed that sympathetic stimulation during the ischemia may participate in the stimulation of catalase, possibly by mediation of an increased adenosine release, to protect the ischemic-reperfused hearts. A hormonal factor like glucocorticoid could be also nominated, since glucocorticoids released in response to physiological stress or sympathetic stimulation has been known to induce antioxidant enzymes including catalase (Jose et al, 1997).

In summary, the present study suggests that the endogenous adenosine released from the ischemic myocardium is involved in the activation of catalase induced by brief ischemia. However, it is postulated also that adenosine may not be a final direct activator of cellular catalase in the myocardium.

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