Involvement of adrenergic pathways in activation of catalase by myocardial ischemia-reperfusion

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Kim, Young-Hoon, Yang-Sook Chun, Jong-Wan Park, Chan-Hyung Kim, and Myung-Suk Kim. Involvement of adrenergic pathways in activation of catalase by myocardial ischemia-reperfusion. Am J Physiol Regulatory Integrative Comp Physiol 282: R1450–R1458, 2002; 10.1152/ajpregu.00278.2001.—In situ rabbit hearts were subjected to 15 min of regional myocardial ischemia, and at various time points of reperfusion, antioxidant enzyme activity and mRNA expression were measured in ischemic and nonischemic myocardium. Catalase activity increased significantly in both ischemic and nonischemic myocardium, peaking at 1 h after reperfusion and then gradually returning to the control level. Northern blot analysis showed enhanced expression of catalase mRNA in both areas. There were no changes in redox status, because glutathione levels were not altered by ischemia-reperfusion (I/R). We also tested whether catalase activation in the heart results from signaling pathways that might influence not only the heart but also other organs. We found that catalase activity in the brain was increased after myocardial I/R and ischemic stress to the intestine was equipotent to myocardial I/R in catalase activation. We next sought to elucidate the possible involvement of the adrenergic system in catalase stimulation induced by ischemic stimuli. After pretreatment with the α-adrenergic receptor antagonist prazosin, I/R failed to increase catalase activity in the heart and brain. Intravenous norepinephrine increased catalase activity in the heart, brain, and liver. This study shows that brief I/R activates a signaling mechanism to induce catalase activation in multiple organs and the α-adrenergic system is involved as an intermediate pathway in this signal transmission.

antioxidant; adrenergic system

REACTIVE OXYGEN METABOLITES, including superoxide anion, hydrogen peroxide, and hydroxyl radical, are continuously produced during metabolic processes of aerobic organisms, and sometimes the generation of reactive oxygen metabolites is accelerated by certain exogenous energy or chemicals, including radiation energy and some anticancer drugs. Reactive oxygen metabolites are highly reactive electrophiles, which may attack cellular constituents like protein, lipid, or nucleic acid, resulting in various abnormalities of structures and functions. In normal conditions, the small amount of reactive oxygen metabolites produced in metabolic processes is neutralized by the endogenous antioxidant defense system. The antioxidant system consists of antioxidant enzymes, including superoxide dismutase (SOD) and catalase, and nonenzymatic antioxidants, including glutathione, vitamin C, and vitamin E. However, when the balance between the production and the neutralization of reactive oxygen metabolites is deterred by certain causes, various patterns of oxidative cell injuries occur. It is generally accepted that these oxidative damages play an important role in some major pathophysiological situations, including inflammatory diseases, ischemia-reperfusion injury, aging, and cancer (13, 26).

Therefore, it is presumed possible to develop practical measures to prevent or control those diseases through inhibition of oxidative cell damages. In this regard, many investigators have tried experimental approaches to reduce the oxidative damages (14, 25). Some of these studies (14) adopted methods to suppress the generation of reactive oxygen metabolites or scavenge them by administration of exogenous antioxidant agents like SOD, catalase, or vitamin E. However, in many conditions, these exogenous agents hardly reach the target in active and stable forms, so that most of these studies have been far from satisfactory. There have been some attempts (29) to enhance endogenous antioxidant system to prevent oxidative damage. It has been reported (22, 25) that antioxidant enzymes can be stimulated by various modalities of cellular stress. For example, mild ischemia-reperfusion (7), heat stress (6), inflammatory mediators (3), and hyperbaric oxygenation (21) were reported to increase antioxidant enzyme activity in experimental animals. Among these, mild ischemic stress was shown to enhance mRNA expression of several genes, including the catalase gene, which was significantly increased as early as 30 min after the ischemic stress (7). On the
other hand, in some of these experiments (2, 16), it was also observed that the activation of antioxidant enzymes is accompanied by the suppression of ischemia-reperfusion injuries, showing that the increased antioxidant enzyme capacity actually induces the tolerance to oxidative insults. Therefore, defining the physiological conditions and underlying mechanism of antioxidant activation would lead to the development of effective measures to minimize oxidative injuries.

In the present study, we tried to characterize the changes in antioxidant enzyme activity after ischemic stress. In the ischemic and the nonischemic myocardium of rabbit hearts subjected to a brief regional ischemia, the activity and mRNA expression of catalase increased not only in the affected ischemic myocardium but also in the virgin nonischemic myocardium. Increased catalase activity was evident in the brain as well, indicating that a certain multiorgan signal transmission mechanism is involved. Moreover, ischemic stress to the intestine also evoked catalase activation in the heart and brain. We next sought to find the transmitter of this signaling pathway. Because it is well known that the sympathetic nervous system is activated by stress, including myocardial ischemia, and induces some physiological responses to compensate the stress, we speculated that this system may play a role in the defensive antioxidant activation and tested whether the sympathetic nervous system is involved.

**MATERIALS AND METHODS**

**Experimental Protocol and Sample Collection**

Rabbits (New Zealand White, male, 1.5-2.5 kg) were used for all procedures. Each experimental group included six animals.

**Experiment 1:** changes in SOD, glutathione peroxidase, and catalase activity in ischemic and nonischemic myocardium after regional myocardial ischemia. Rabbits were subjected to 15 min of regional myocardial ischemia. Tissue samples from ischemic and nonischemic myocardium were taken 0, 1, 3, and 8 h after the onset of reperfusion for assays of SOD, glutathione peroxidase, and catalase.

**Experiment 2:** changes in catalase activity in heart, brain, and liver after regional myocardial ischemia. Rabbits were subjected to 15 min of regional myocardial ischemia. Tissue samples from the heart, brain, and liver were taken after reperfusion for 1 h. Catalase activity in each organ was measured and compared with the values of the sham-operated group.

**Experiment 3:** changes in catalase activity in heart, brain, and liver after regional intestinal ischemia. Rabbits were subjected to 15 min of regional intestinal ischemia. Tissue samples from the heart, brain, and liver were taken after reperfusion for 1 h. Catalase activity in each organ was measured and compared with the values of sham-operated group.

**Experiment 4:** effect of adrenergic antagonists on catalase activation by regional myocardial ischemia. As a fundamental procedure to test the hypothesis that the adrenergic nervous system might act as a mediator in catalase stimulation, we studied whether catalase stimulation could be inhibited by blockade of the adrenergic receptors before ischemia and reperfusion. Prazosin (α-adrenergic receptor antagonist) or propranolol (β-adrenergic receptor antagonist) was adminis-tered before the myocardial ischemia, and catalase activity was measured in the heart, brain, and liver after 1 h reperfusion following 15 min of ischemia.

**Experiment 5:** effect of adrenergic agonist on catalase activity in heart, brain, and liver. To test whether an adrenergic agonist can exert an effect on catalase activity similar to that of regional ischemia, norepinephrine was administered intravenously for 10 min. Organ samples were taken 1 h after the infusion was finished.

**Operative Procedures**

For regional myocardial ischemia, rabbits were anesthetized with pentobarbital sodium (30 mg/kg, iv). After tracheotomy, rabbits were intubated and ventilated with room air. The hearts were exposed by a left thoracotomy through the fourth intercostal space. A silk snare was placed around the large marginal branch of the circumflex artery 2 cm from the atrioventricular groove. The artery was occluded for 15 min by pulling the snare and then reperfused by removing the snare. For intestinal ischemia, anesthetized rabbits were subjected to laparotomy and the superior mesenteric artery was occluded for 15 min. For the sham-operation groups, all operative procedures were performed identically, except for the occlusion of the coronary artery.

**Drug Treatments**

Prazosin was dissolved in saline at a concentration of 0.5 mg/ml, and 2 mg/kg were intraperitoneally injected 10 min before the onset of ischemia. Propranolol was also dissolved in saline at a concentration of 0.5 mg/ml, and 2 mg/kg were continuously injected via the ear vein for 10 min before the onset of ischemia. Norepinephrine was dissolved in normal saline at a concentration of 2.5 µg/ml, and 10 µg/kg were infused continuously for 10 min. The doses of antagonists or agonists were selected on the basis of previous experiments using rabbits (1, 27). All stock solutions were freshly prepared to avoid degradation.

**Preparation of Tissue Samples**

In experiment 1, hearts were removed and washed by perfusion with cold saline. Myocardial samples (1-2 g) were taken from the ischemic area and the normally perfused, nonischemic area of the left ventricle. To estimate the ischemic area, we performed separate preliminary experiments using Langendorff-perfused, isolated hearts. Isolated hearts were perfused and stained with Evans blue (0.1%) during occlusion of the coronary artery to demarcate the ischemic risk area and the normally perfused area. In situ experimental hearts, the region of pallor and akinesia during the coronary occlusion was visually inspected to confirm the demarcation estimated in the isolated Langendorff-perfused hearts. The myocardial samples were taken from the central zone of the estimated areas, frozen quickly in liquid nitrogen, and stored at −70°C until the enzyme assays.

In subsequent experiments, whole left ventricles were used for myocardial samples, and at the same time, samples were taken also from liver and brain. Liver samples were taken from the inferior medial portion of the left lobe, and the cerebrum was used for brain samples.

**Measurement of Antioxidant Enzyme Activity and Other Biochemical Assays**

Myocardial samples were homogenized in 4 vol of homogenization buffer (30 mM KCl, 1 mM EDTA, and 10 mM KH₂PO₄, pH 7.4) using a Polytron tissue disintegrator.
(Brinkman Instruments) and an ultrasonicicator (Heat Systems, Ultrasone). The homogenate was centrifuged at 10,000 g for 20 min, and the supernatant was taken for the enzyme assay. The protein concentration was measured by the bicinchoninic acid method (Bio-Rad) with BSA as a standard.

SOD activity was determined by the epinephrine autoxidation method according to Misra and Fridovich (18). The prepared sample was added to 3 ml of 50 mM carbonate buffer (pH 10.2, 30°C) containing 0.1 mM EDTA and 0.13 mM epinephrine. The absorbance change was monitored at 325 nm by ultraviolet spectrophotometer (Hewlett-Packard). One unit of SOD was defined as the enzyme amount that reduces the rate of epinephrine autoxidation by 50%.

Glutathione peroxidase activity was assayed according to the method described by Delmaestro and McDonald (8). The sample (20 μl) was added to 0.98 ml of 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.24 U/ml glutathione reductase, 1 mM GSH, 0.15 mM reduced NADP (NADPH), and 1.2 mM t-butyl hydroperoxide. The absorbance change was monitored at 37°C and 340 nm by ultraviolet spectrophotometer. One unit of glutathione peroxidase was defined as the enzyme amount to consume 0.5 μmol of NADPH for 1 min.

Catalase activity was measured by monitoring the rate of O₂ generation resulting from the decomposition of H₂O₂ (16). H₂O₂ (final, 4.6 mM) was added to 5 ml of reaction buffer (0.1% EDTA and 10 mM potassium phosphate, pH 7.4) containing an aliquot of the tissue sample. The rate of O₂ generation was measured with a Clark-type oxygen electrode at 30°C. Enzyme activity was calculated from the standard curve of purified catalase (Sigma).

**Northern Blot Analysis**

Total RNA was extracted by the acid-guanidinium thiocyanate-phenol-chloroform method (5). Human catalase (American Type Culture Collection) and human β-actin (Clontech) cDNAs were used as probes. Fifteen micrograms of total RNA were applied to electrophoresis and transferred to a nitrocellulose filter. Probes were labeled by using a random-primed DNA labeling kit (Life Technologies). Filters were prehybridized at 42°C in a solution containing 50% formamide, 5× SSPE (NaCl, NaH₂PO₄, and EDTA), 0.1% SDS, 2× Denhardt’s solution, 100 g/ml denatured salmon sperm DNA, and 2% dextran sulfate and then incubated with the cDNA probes overnight at 42°C. Filters were washed at 60°C successively in 3× SSC (NaCl and sodium citrate) for 1 h, 1× SSC for 1 h, and 0.2× SSC plus 0.1% SDS for 30 min and exposed to X-ray film at −70°C using intensifying screens. The intensity of blots was analyzed by densitometric scanning.

**Measurement of Glutathione Contents**

Cellular redox state was evaluated by measuring the glutathione contents. Total glutathione (GSH + GSSG) and GSSG contents were analyzed according to the method of Griffith (12). The myocardial samples were homogenized in 5 vol of 6% perchloric acid. The homogenates were centrifuged at 10,000 g for 2 min, and the supernatant was neutralized with 5 M K₂CO₃. The supernatant was buffered by 2 vol of NaHCO₃ (100 mM, pH 6.5). 2-Vinylpyridine (6 μl) was added to a 0.3-ml aliquot of the mixture and incubated for 1 h to inactivate GSH. The mixtures with and without 2-vinylpyridine were used for measurement of GSSG and total glutathione, respectively. Samples were added to the reaction mixture containing 1.8 ml KH₂PO₄ (120 mM, pH 7.5), 20 μl NADPH (17 mM), 100 μl DTNB (12 mM), and 10 μl glutathione reductase (200 U/ml). Changes in absorbance at 412 nm and 30°C were monitored and compared with the values of standards. The GSH level was calculated by subtracting the GSSG level from the total glutathione level.

**Statistical Analysis**

Results are expressed as means ± SE. Differences were compared by unpaired two-tailed t-test, with P < 0.05 considered significant.

**RESULTS**

**Responses to Regional Myocardial Ischemia**

The area supplied by the large marginal branch of the left circumflex coronary artery was ~40–50% of the total left ventricle, including about one-third of the apical portion, a part of the anterior wall, and the papillary muscles; this demarcation was of little variance among animals. We tried to ligate only the artery excluding vein branches, except for cases in which the two vessels were too close to isolate. The ST segment elevation on the electrocardiogram monitor was apparent right after the ligation.

In preliminary experiments, we compared the effect of 15-min ischemia and 30-min ischemia. By 30-min ischemia, ~40% of the risk area showed irreversible injury, which was indicated by triphenyltetrazolium chloride nonstaining. In addition, 30-min ischemia resulted in hemorrhagic lesions in most cases (data not shown). However, none of these signs were observed in hearts subjected to 15-min ischemia. Therefore, we speculated that ischemia of more than 15 min in duration is unsuitable for observing responses of viable cells to stressful stimulation.

**Changes in Antioxidant Enzyme Activity After Ischemia-Reperfusion**

Fifteen minutes of regional ischemia followed by reperfusion for 1–8 h failed to induce any significant changes in SOD and glutathione peroxidase activity in both ischemic and nonischemic myocardium (Fig. 1). However, catalase activity was significantly changed by this stimulus. Although the catalase activity in ischemic and nonischemic myocardium was not altered immediately after the ischemia, reperfusion evoked an increased catalase activity in both ischemic and nonischemic regions, with a peak observed at 1 h after reperfusion and a gradual return to the control level at 8 h. Compared with the control heart, the enzyme activity in the ischemic myocardium increased by 79.5% and 62.3% at 1 and 3 h after reperfusion, respectively. The extent of the increase in the nonischemic myocardium at each time point was comparable to that in the ischemic myocardium, and there were no statistical differences between them. The catalase activity of the sham-operated heart was not different from the control level (Fig. 2).
Expression of Catalase mRNA

Northern blot analysis revealed an enhanced expression of catalase mRNA in both the ischemic and nonischemic myocardium on reperfusion after 15 min of regional ischemia. The enhancement of mRNA expression paralleled the increase in the enzyme activity, showing a peak at 1 h after reperfusion and a gradual decrease thereafter. As determined by densitometric analysis, the amount of mRNA expressed at 1 h after reperfusion was more than twofold higher in both the ischemic and the nonischemic myocardium than in the sham-operated hearts (Fig. 3).

Changes in Myocardial Glutathione Content

To investigate the involvement of oxidative stress mediated by reactive oxygen species in the stimulation of antioxidant enzymes, we evaluated cellular redox state by measuring myocardial glutathione contents. As shown in Fig. 4, there was no apparent change in either the GSH or GSSG content in both ischemic and nonischemic myocardium after 15 min of ischemia and reperfusion of varying duration.

Changes in Catalase Activity in Heart, Brain, and Liver After Regional Myocardial Ischemia

In subsequent experiments, it was confirmed that the catalase activity of the heart after 1 h of reperfusion following 15 min of regional myocardial ischemia was significantly higher than that in the sham-operated animal. The catalase activity of the reperfused heart was 49 ± 8.54 U/mg protein, which was significantly higher than in the sham-operated animal (26.02 ± 1.23 U/mg protein). Measured in samples taken from the same animals, the catalase activity in the brain showed a similar pattern. Brain catalase activity increased to 7.61 ± 0.88 U/mg protein after myocardial ischemia-reperfusion, whereas that of the sham-operated animal was 4.84 ± 0.22 U/mg protein. On the other hand, the catalase activity in the liver was not altered by myocardial ischemia-reperfusion (Fig. 5).

Changes in Catalase Activity in Heart, Brain, and Liver After Regional Intestinal Ischemia

In another series of experiments, intestinal ischemia by occlusion of the superior mesenteric artery was employed. Rabbits were subjected to intestinal ischemia for 15 min followed by 1 h of reperfusion. As with myocardial ischemia, intestinal ischemia-reperfusion evoked increased catalase activity in the heart and brain. The degree of catalase activation was comparable to that in the myocardial ischemic stress group. Liver catalase activity was not affected by intestinal ischemia (Fig. 6).

Effect of Adrenergic Antagonists on Catalase Activation by Regional Myocardial Ischemia

It was tested whether stimulation of catalase in the heart and brain by myocardial ischemia could be in-
hibited by adrenergic receptor blockade. After pretreatment with prazosin, an α-adrenergic receptor antagonist, ischemia-reperfusion failed to increase catalase activity in the heart and brain. However, propranolol, a β-adrenergic receptor antagonist, did not exert any influence on catalase activity. The catalase activity in the liver was not altered by any intervention (Fig. 7).

**Effect of Adrenergic Agonist on Catalase Activity in Heart, Brain, and Liver**

Norepinephrine, administered for 10 min intravenously, increased catalase activity in the heart to 66.81 ± 16.69 U/mg protein and the brain to 8.34 ± 0.77 U/mg protein. The degree of catalase activation was higher than that observed in animals subjected to regional myocardial ischemia-reperfusion. Moreover, norepinephrine also increased the catalase activity in the liver from 237.36 ± 12.74 to 305.03 ± 17.79 U/mg protein (Fig. 8).

**DISCUSSION**

The antioxidant enzyme-mediated defense mechanism against reactive oxygen metabolites is essential to all cells under oxygenated environments. The regulatory machinery of the antioxidant system in prokaryotes is well documented (9, 10). Although the knowledge about the responsive regulation of mammalian antioxidant enzymes is limited, it has been reported (3, 6, 7, 21, 24) that the endogenous antioxidant defense system could be activated by various modalities of sublethal stresses. In the present study, we observed increased catalase activity and mRNA expression after 1-h reperfusion following brief ischemia, which is similar to the results of some previous reports (4, 7). Surprisingly, however, catalase activity and mRNA expression were increased not only in affected ischemic myocardium but also in nonischemic myocardium and were comparable in degree.

The simultaneous activation of catalase in the nonischemic and ischemic regions of the heart implies that a certain signal generated by regional ischemia and/or reperfusion influences not only the affected ischemic-reperfused part of the myocardium but also the adjacent normally perfused myocardium to stimulate catalase. An increased oxidative stress could trigger the antioxidant defense system to be induced in tissues. We thus hypothesized that myocardial catalase activity would increase in association with an increased oxidative stress mediated by increased production of reactive oxygen species during ischemia and reperfusion. However, we failed to detect any evidence of altered redox state as there were no changes in the GSH and GSSG contents in either the ischemic or the nonischemic myocardium. It was, therefore, suggested that some factor(s) other than the oxidative stress incurred by the ischemia-reperfusion might be involved in the activation of catalase in both areas. Involvement of systemic stress caused by operative procedures other than the coronary artery occlusion was excluded as there were no changes in either the enzyme activity or mRNA expression in the sham-operated animals.

The signal transmission responsible for the stimulation of catalase in the nonischemic and ischemic myo-
cardium would involve a local myocardial pathway or systemic pathway. For the local pathway, we proposed several hypotheses. First, a kind of chemical mediator produced in the ischemic area might diffuse to the nonischemic area through interstitial space or gap junctions to stimulate catalase simultaneously in both areas. In another possibility, alterations in myocardial contractility by ischemia-reperfusion might stimulate both areas mechanically to result in some changes in enzyme activity. However, these hypotheses were presumed less probable as the observed catalase stimulation was uniform while the effects of diffusing chemical mediators or mechanical stimuli were not presumed to be uniform in two distant areas.

Therefore, we supposed that the catalase activation in the nonischemic region of the heart is probably the result of a certain signal originating from the systemic pathways. To test this hypothesis, we observed whether catalase activity in other organs is also influenced by this signal in the same manner as the nonischemic myocardium. Catalase activity in the brain as well as in the heart was found to be increased, indicating that the signaling pathway involves a systemic transmission.

Moreover, ischemic stress to the intestine was equipotent to myocardial ischemia-reperfusion in catalase activation, showing that the hypothesized signaling pathway triggered by ischemic stimulus is not confined to the myocardium. Gho et al. (11) have quantitated the ischemic stress-induced protective effect against cardiac ischemia-reperfusion injury, in which they compared the ischemic stresses to the heart, intestine, and kidney in rabbits. It was reported (11) that 15-min coronary artery occlusion and 15-min mesenteric artery occlusion were equally effective to limit the infarct size after lethal myocardial ischemia-reperfusion.

For the next step, we sought to find the transmitter of this signaling pathway. Systemic catalase activation...
by ischemia is presumed to be a kind of stress adaptation mechanism. As the primary systems activated in response to stressful stimuli, sympathetic nervous system and several stress hormones are well known. Among these, we tested whether the adrenergic nervous system is involved. We found that pretreating animals with prazosin abolished the catalase activation, suggesting that the \( \alpha \)-adrenergic receptor pathway is involved. In addition, intravenous norepinephrine administration instead of ischemia-reperfusion induced catalase activation in the heart, brain, and liver. Transient hypertension induced by norepinephrine might have exerted mechanical stimuli on the myocardium, but this presumably did not contribute to the effect on catalase activity, as the simultaneous catalase activation in the brain and liver was not attributable to hemodynamic alterations. From these results, we concluded that the \( \alpha \)-adrenergic receptor is a part of the signal transmission.

However, we couldn’t clarify whether the \( \alpha \)-adrenergic agonist itself induces catalase activation of the target cells or plays a role in transmitting the signal to another signaling system. The assumption that the \( \alpha \)-adrenergic system is not the final effector is sup-

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**Fig. 6.** Changes in catalase activity in the heart (A), brain (B), and liver (C) after intestinal ischemia. Rabbits were subjected to 15 min of superior mesenteric artery (SMA) occlusion. Tissue samples from the heart, brain, and liver were taken 1 h after the onset of reperfusion for assays of catalase. For the sham-operation group, all operative procedures were performed identically, except for the occlusion of the artery. *\( P < 0.05 \).

**Fig. 7.** Effect of adrenergic antagonists on the catalase activation by regional myocardial ischemia. Rabbits were subjected to 15 min of regional myocardial ischemia. Tissue samples from the heart (A), brain (B), and liver (C) were taken 1 h after the onset of reperfusion for assays of catalase. For the sham-operation group, all operative procedures were performed identically, except for the occlusion of the coronary artery. PN-I/R, treatment with prazosin (2 mg/kg) before I/R; PL-I/R, treatment with propranolol (2 mg/kg) before I/R. *\( P < 0.05 \).
ported by the pharmacological properties of norepinephrine. Because of the low permeability to the blood-brain barrier, intravenous norepinephrine cannot reach the brain parenchymal cells in an effective concentration. Therefore, it is hardly possible that intravenous norepinephrine as was that of the heart and brain. These results imply that the regulation machinery of different organs might differ in sensitivity to the endogenous catalase-activating signal.

The present study suggests the involvement of the adrenergic system, but we still do not know the signal transmission steps between ischemia-reperfusion and adrenergic activation or between adrenergic activation and systemic catalase activation. For the mechanism of adrenergic activation by ischemia-reperfusion, several related phenomena have been reported. Numerous biological alterations might be induced by ischemia-reperfusion, but among these, the increased production of adenosine is most conspicuous. Endogenous adenosine production is increased in response to a decrease in oxygen supply-to-demand ratio as ATP degradation is accelerated (19). Adenosine is known to possess various biological activities to reduce the deleterious effects of ischemia-reperfusion (17), and at the same time, stimulate sympathetic afferents to increase systemic sympathetic activity. Therefore, it is probable that adenosine mediates the sympathetic activation following catalase activation.

Further research is required to understand the signal transmission step(s) between adrenergic activation and catalase activation. A hormonal factor such as glucocorticoid could participate in the enzyme activation. Glucocorticoid is known to be released in response to physiological stress or sympathetic stimulation, and it has also been known to induce antioxidant enzymes, including catalase (15).

Another facet of the phenomenon attracting interest is the relation with so-called “ischemic preconditioning.” It has been known for a long time that brief ischemia renders the heart resistant to subsequent severe ischemia-reperfusion. This phenomenon, known as ischemic preconditioning, is the most promising among the experimental methods currently known to reduce ischemia-reperfusion injury (20, 23, 24). However, many questions about the mechanism of ischemic preconditioning are still unknown. Although the protocol of the present experiment is different from that of conventional ischemic preconditioning, catalase activation is likely to contribute, at least in part, to cardioprotection against ischemia-reperfusion. However, in a study using an in situ regional ischemia model of rabbit hearts, Turrens et al. (28) reported that the activity of the major myocardial antioxidant enzymes, including catalase, was not changed in either the ischemic or nonischemic regions. This result is not consistent with our findings. The apparent discrepancy may be explained by the different experimental protocols. Turrens et al. (28) measured the antioxidant en-
zyme activity 5 min after reperfusion following 30 min of ischemia, whereas we did so after 1–8 h of reperfusion following 15 min of ischemia. Immediately on reperfusion after ischemia, we did not observe any significant changes in the antioxidant enzyme activity either. Hence, more detailed studies are required to clarify the issue.

In conclusion, brief regional myocardial ischemia induced catalase activation in the nonischemic myocardium and brain as well as the ischemic myocardium, and the α-adrenergic system was involved in the systemic signal transmission.

**Perspectives**

Numerous reports have indicated that antioxidant enzymes, including catalase, can be enhanced by sublethal experimental stimuli (25). However, as far as we know, this is the first report demonstrating that catalase activation can be achieved through endogenous systemic signaling pathways. This finding implies the existence of an inherent regulatory mechanism that maximizes the antioxidant capacity of multiple remote organs in response to oxidative stimuli.

Further research on the signal transmission pathway of systemic catalase activation may lead to the development of useful pharmacological methods to activate the endogenous antioxidant defense system in target organs. Through these medical interventions, we may be able to pretreat patients, who are to be subjected to inevitable oxidative injury like open heart surgery, organ transplantation, radiation therapy, and Adriamycin therapy, to minimize the problems of oxidative injury.

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