Adenosine A₁ Receptor Activation Induces Delayed Preconditioning in Rats Mediated by Manganese Superoxide Dismutase

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Background—We have previously described a second window of protection against infarction in rabbits 24 to 72 hours after adenosine A₁ receptor (A₁Rs) activation. In this study, we examined the potential role of the mitochondrial antioxidant manganese superoxide dismutase (Mn-SOD) as a potential end effector in mediating this protection.

Methods and Results—Rats were treated with an intravenous bolus of the A₁R agonist 2-chloro-N⁶-cyclopentyladenosine (CCPA, 75 µg/kg) or saline vehicle. They were also given a 5 mg/kg IV infusion of a 22-mer phosphorothioate oligodeoxynucleotide (ODN) with sequence antisense to the initiation site of rat Mn-SOD mRNA. Sense ODN and scrambled ODN were used as controls. Twenty-four hours later, hearts were isolated and perfused with buffer at constant pressure and subjected to 35 minutes of regional ischemia and 2 hours of reperfusion. Treatment with CCPA compared with saline vehicle (control) significantly reduced infarct size, expressed as percentage of myocardium at risk (22.3±3.3% versus 42.1±3.8%, respectively; P=0.001). This protection was completely abolished by prior treatment with antisense ODN, which had no effect on its own. Neither sense ODN nor scrambled ODN had an effect on the CCPA-induced delayed cardioprotection. In separate animals, 24 hours after the same treatment, hearts were assayed for Mn-SOD content and activity. CCPA treatment induced a significant increase in myocardial Mn-SOD content and activity compared with the control condition; this increase was abolished by pretreatment with antisense ODN.

Conclusions—This is the first study to show that transient A₁R activation induces delayed cardioprotection in the rat. These results strongly suggest an important role for mitochondrial Mn-SOD as a potential end effector of this protection. (Circulation. 2000;101:2841-2848.)

Key Words: adenosine ■ myocardial infarction ■ superoxide dismutase

Brief periods of sublethal myocardial ischemia induce a biphasic pattern of protection against subsequent prolonged ischemic injury, which has been termed ischemic preconditioning.¹⁻³ In addition to the immediate infarct-limiting effect, ischemic preconditioning induces a delayed phase or “second window” of protection against myocardial infarction, which becomes apparent 12 to 24 hours after the preconditioning ischemia and persists up to 72 hours.²⁻⁷ Because of the remarkable potency and reproducibility of this phenomenon in all species studied, there is intense interest in exploiting ischemic preconditioning to develop therapeutic strategies that can enhance myocardial tolerance to ischemia/reperfusion injury in patients with coronary artery disease. However, the molecular and cellular mechanisms underlying this endogenous adaptive process have not been elucidated and need further clarification before clinical applications of ischemic and/or pharmacological preconditioning can be fully realized.

We have previously reported the important role of endogenous adenosine, released during the brief preconditioning ischemia and acting on adenosine A₁ receptors (A₁Rs), as a trigger of delayed preconditioning against infarction in rabbit myocardium.⁸⁻¹⁰ The cellular mechanisms downstream from A₁Rs mediating this delayed protection are not known. We have recently demonstrated the important roles of protein kinase C (PKC) and tyrosine kinases in mediating A₁R-induced delayed preconditioning against infarction in the rabbit heart.¹¹ Pharmacological inhibition of either PKC or tyrosine kinases abolished the delayed infarct-limiting effect of treatment with the selective A₁R agonist 2-chloro-N⁶-cyclopentyladenosine (CCPA) at 24 hours.¹¹ Another important issue regarding the delayed preconditioning against infarction conferred by transient A₁R activation is the nature of the distal effector or target protein(s) mediating this protection. Recent evidence suggests that ≥1 cellular antioxidant enzyme may be upregulated after heat stress or transient ischemia and contribute to the cytoprotection observed during a subsequent lethal ischemia/reperfusion. One such enzyme is the mitochondrial antioxidant manganese superoxide dis-
mutase (Mn-SOD). Kuzuya, Hoshida, and colleagues3,12 have shown that the infarct limitation observed in the canine myocardium 24 hours after ischemic preconditioning is accompanied by a significant increase in the activity of Mn-SOD. Yamashita and colleagues13,14 have recently reported the induction of Mn-SOD by heat stress13 or sublethal hypoxia14 in neonatal rat cardiomyocytes at a time point that paralleled the delayed cytoprotection conferred by these preconditioning strategies. Moreover, transgenic mice overexpressing Mn-SOD seem to be more resistant to myocardial ischemia/reperfusion injury.15 It has also been reported that treatment of a variety of tissues, including rat cardiac myocytes, with the A,R agonist N9-(phenyl-2R-isopropyl)-adenosine results in upregulation of endogenous antioxidant enzymes, including Mn-SOD, over a 90- to 120-minute period.16 Moreover, we have recently shown that the delayed limitation of infarction observed in rabbits 24 hours after treatment with CCPA is associated with enhanced myocardial Mn-SOD activity and that this increase is attenuated by prior inhibition of either PKC or tyrosine kinases; these strategies were also shown to abolish the cardioprotective effects of delayed preconditioning with CCPA.17

Therefore, we hypothesized that the subacute effect of A,R activation on tissue tolerance to prolonged ischemia may be mediated by upregulation of endogenous myocardial Mn-SOD. In the present study, we examined this hypothesis by evaluating the effect of suppression of increased myocardial Mn-SOD by intravenous injection with antisense (AS) oligodeoxynucleotides (ODNs) against rat Mn-SOD on the acquisition of delayed tolerance to myocardial ischemia 24 hours after CCPA treatment in the rat.

Methods

Male Wistar rats (body weight 250 to 350 g) were used in these experiments. All procedures were in accordance with UK Home Office Guidelines on the Animals (Scientific Procedures) Act 1986, published by Her Majesty’s Stationery Office. Animals were acclimatized in the institutional animal house for 5 to 7 days after delivery and had free access to a standard diet of pellets and water.

Experimental Protocol

Animals were lightly anesthetized with a combination of Hypnorm (Janssen Pharmaceuticals), containing 315 μg/mL fentanyl citrate and 10 mg/mL fluanisone, and midazolam (1 part midazolam +1 part Hypnorm+2 parts sterile water administered at a dose of 1 mL/kg SC). Under sterile conditions, after a lateral incision in the neck, the right jugular vein was cannulated with a Y-CAN 0.7-mm pediatric cannula (Simcare Ltd). Rats were assigned to 6 experimental groups (Figure 1). Group I (control) received an intravenous bolus of sterile 0.9% saline (0.5 mL). Group II (CCPA) was treated with an intravenous bolus of sterile 0.9% saline (0.5 mL). Group II (CCPA) was preconditioned with a single intravenous bolus of CCPA 5 minutes before the CCPA or saline bolus. In group I, the rats were subjected to 2-hour reperfusion (R) in vitro. Small arrow indicates timing of myocardial sampling in vivo. All animals were anesthetized, and hearts were isolated and subjected to 35-minute regional ischemia (I) and 2-hour reperfusion (R) in vitro. Twenty-four hours after various treatments, rats were anesthetized with pentobarbital sodium (60 mg/kg IP, Sagatal, Rhone Merieux) and anticoagulated with heparin (1000 U/kg IP). A median sternotomy was performed, and the heart was rapidly excised and immediately immersed in 4°C Krebs-Henseleit buffer solution containing (in mmol/L) NaCl 118, KCl 4.7, CaCl2 1.8, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25.2, and glucose 11.0. The aortic root was cannulated, and the heart was perfused retrogradely by the Langendorff technique at constant pressure (100 cm H2O). The Krebs-Henseleit buffer had a pH of 7.4. The medium for heart perfusion was replaced every 5 minutes to maintain pH at 7.4±0.05 (P02 60 to 75 kPa). A water-filled latex balloon, coupled to a pressure transducer (Lectromed UK Ltd), was inserted into the left ventricular cavity via the left atrial appendage for periodic pressure and heart rate (HR) recordings. Left ventricular end-diastolic pressure was adjusted between 8 and 12 mm Hg and maintained throughout the experiment. Myocardial temperature was measured by a thermocouple inserted into the right ventricle and maintained constant at 37.5±0.5°C. A 30 silk suture (Mersilk W546, Ethicon) was placed around the left coronary artery a few millimeters distal to the aortic root and threaded through a 10-mm polypropylene tube to form a snare. After 20 minutes of stabilization,
myocardial ischemia was induced by tightening the snare and clamping it onto the epicardial surface with a hemostat clamp for 35 minutes, followed by 120 minutes of reperfusion. The development of arrhythmias was monitored continuously from the pressure tracing. If ventricular tachycardia/fibrillation occurred during ischemia/reperfusion and did not resolve spontaneously within 5 seconds, manual cardioversion was attempted by gentle palpation of the nonischemic region of the heart. Coronary flow was measured periodically by timed collection of the coronary effluent. HR and left ventricular developed pressure (LVDP, which is left ventricular systolic pressure minus left ventricular end-diastolic pressure) were continuously recorded. The rate-pressure product was calculated as the product of HR and LVDP.

Assessment of Myocardial Infarct Size

At the end of the reperfusion, the coronary ligature was retied, and the aortic root was perfused with 2 to 4 mL of a 5 mg/mL suspension of 10 μm zinc cadmium sulfide microspheres (Duke Scientific) to define the risk zone. The hearts were then weighed, frozen, and cut into 2-mm slices from apex to base perpendicular to the long axis of the heart. After they were defrosted, the slices were incubated at 37°C in a 1% solution of triphenyltetrazolium chloride (Sigma Chemical Co) in phosphate buffer (pH 7.4) for 15 to 20 minutes and fixed for 24 to 48 hours in 4% (vol/vol) formalin solution. The slices were traced on acetate sheets, and under UV light, fluorescent (nonrisk) and nonfluorescent (risk) areas were distinguished. After appropriate magnification, the areas of infarcted tissue and myocardium at risk were determined by computerized planimetry. The ratio of infarcted tissue to myocardial volume at risk (I/R) was calculated.20

Determination of Myocardial Mn-SOD Content and Activity

In a different group of animals, 24 hours after the same treatments as outlined above, myocardial samples were obtained for assessment of Mn-SOD content and activity. As a positive control for the induction of Mn-SOD, group VII animals were treated with an intravenous injection of recombinant murine tumor necrosis factor-α (TNF-α, R&D Systems Europe Ltd) at a dose of 10 μg/kg. TNF-α is a known potent inducer of Mn-SOD.21,22 Twenty-four hours after the various treatments, animals were euthanized, their hearts were immediately excised and rinsed in PBS, and blood in the coronary arteries was washed out by retrograde perfusion of the ascending aorta with an adequate volume of PBS. The atriia and the right ventricle were dissected. Left ventricular myocardial samples were rapidly frozen by immersion in liquid nitrogen and stored at −80°C.

Myocardial Mn-SOD content was determined by SDS-PAGE. Myocardial tissue samples were prepared as described previously.20,23 Electrophoresis was performed with 100 μg of protein per sample on 12.5% polyacrylamide-SDS gels. Proteins were then transferred electrophoretically onto nitrocellulose membranes (Hybond-C, Amersham) overnight at 180 mA and 4°C. After transfer, filters were probed with rabbit polyclonal IgG against Mn-SOD (kind gift from Prof Taniguchi and Dr Kuzuya, University of Osaka, Osaka, Japan) at 1:200 dilution. After a rinse in the wash buffer, the filter was probed with horseradish peroxidase-conjugated swine anti-rabbit IgG (Dako Ltd, UK) at 1:2000 dilution. Antibodies were diluted in PBS, 0.05% Tween 20, and 5% dried milk powder (Marvel) at room temperature. Blots were developed by using an enhanced chemiluminescence detection system (Amersham) and exposed to Kodak X-Omat AR film. Autoradiographs were scanned with a Sharp JX-330 scanner, and the band density was analyzed by laser densitometry.

Mn-SOD activity in myocardial samples was determined by the nitro blue tetrazolium method.13-14 Myocardium was homogenized in 20 mMol/L PBS and 1 mMol/L EDTA and centrifuged at 900g for 15 minutes. The supernatant was sonicated and incubated with nitro blue tetrazolium, xanthine–xanthine oxidase, and 1 mMol/L potassium cyanide to inhibit the activity of the cytosolic copper-zinc SOD, and the Mn-SOD activity in the supernatant was measured colorimetrically. The measurements of Mn-SOD activity in each sample were performed in duplicate. The activity of Mn-SOD is expressed relative to the protein concentration in the supernatant determined by use of a bicinchoninic acid kit.

Statistical Analysis

The data are presented throughout as mean±SEM. The significance of the differences in mean values for the area of infarcted tissue, the area of myocardium at risk, I/R, and Mn-SOD content and activity between the treatment groups was evaluated by 1-way ANOVA, followed by the Fisher protected least significant difference test. Any differences between hemodynamic or coronary flow measurements at different time points were assessed by 2-way ANOVA with repeated measures, followed by the Fisher protected least significant difference test used post hoc for individual differences. The null hypothesis was rejected at P<0.05.

Results

A total of 87 rats were used for these studies: 59 rats were used in the infarction studies, and 28 (4 per group) were used in the analysis of myocardial Mn-SOD content and activity. The hearts of 9 rats were excluded from the final analysis of infarct size for technical reasons: 2 hearts were excluded because of failure of the triphenyltetrazolium chloride stain (1 each in the CCPA and the AS-ODN+CCPA groups); 3 hearts developed intractable ventricular fibrillation during ischemia/reperfusion (1 each in the CCPA, the AS-ODN+CCPA, and the Scr-ODN+CCPA groups); 2 hearts developed severe hypotension (LVDP <50 mm Hg) after insertion of the left ventricular balloon (1 in the control group and 1 in the S-ODN+CCPA group); 1 heart was excluded because of myocardial rupture at the time of coronary occlusion (Scr-ODN+CCPA group); and 1 heart was excluded because of air embolization at the start of reperfusion (CCPA group). Therefore, we report data on 50 successfully completed experiments.

Coronary Flow and Hemodynamic Measurements

Coronary flow measurements during the ischemia/reperfusion protocol are presented in Figure 2. There were no differences in baseline coronary flow values between the various groups, which averaged 11.5 to 13.0 mL/min. During regional ischemia, the coronary flow was significantly reduced (P<0.01) in all groups to mean values of 5.5 to 7.0 mL/min, with recovery to preischemic values during early reperfusion. There were no significant differences in coronary...
flow between the various groups at any time point during ischemia/reperfusion.

Table 1 summarizes HR, LVDP, and rate-pressure product data recorded in the 6 experimental groups at baseline and during ischemia/reperfusion. There were no significant differences in hemodynamic performance at baseline or at any time point during ischemia/reperfusion between the different groups.

**Myocardial Risk and Infarct Size**

Table 2 presents the volumes of risk and infarct zones in the 6 experimental groups. In these groups, the mean volume of myocardial tissue at risk during coronary artery occlusion ranged from 450 to 520 mm³, representing 45% to 50% of total left ventricular tissue volume. There were no significant differences in ischemic risk zone among the experimental groups. The absolute infarct size was significantly smaller in the CCPA and S-ODN+CCPA groups compared with the control group. Infarct size expressed as a percentage of the area at risk (I/R) in the 6 experimental groups is presented in Figure 3. Pretreatment with CCPA 24 hours before myocardial infarction resulted in a significant reduction in I/R compared with saline treatment (22.3±3.3% versus 42.1±3.8%, respectively; P=0.001). The effect of suppres-

<table>
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Values are mean±SEM. R indicates myocardial area at risk; I, absolute infarct size.

*P=0.05 vs control group (1-way ANOVA).
tion of Mn-SOD was evaluated by using AS-ODN administered intravenously before CCPA or saline injections. Prior treatment with AS-ODN (5 mg/kg) completely abolished the infarct-limiting effect of delayed pharmacological preconditioning with CCPA, whereas it did not affect infarct size in saline-treated animals (I/R 39.4 ± 2.8% and 46.5 ± 4.8%, respectively; P < 0.05 versus control group). Importantly, administration of S-ODN or Scr-ODN (5 mg/kg) 5 minutes before CCPA preconditioning did not affect the cardioprotection observed at 24 hours (I/R 24.7 ± 3.9% and 29.3 ± 1.8%, respectively; P = 0.001 versus control).

Mn-SOD Content and Activity
Figure 4 shows the myocardial content of Mn-SOD protein. Mn-SOD activity assayed in myocardial samples obtained 24 hours after various treatments is presented in Figure 5. Prior treatment with CCPA 24 hours earlier enhanced myocardial induction and resulted in a significant 56% increase in myocardial Mn-SOD activity compared with saline treatment (207 ± 25 versus 132 ± 16 U/mg, P < 0.001). The enhanced Mn-SOD content and activity were abolished by pretreatment with AS-ODN (120 ± 11 U/mg), whereas treatment with S-ODN or Scr-ODN significantly increased Mn-SOD protein and activity (200 ± 32 and 205 ± 20 U/mg, respectively; P < 0.001 versus control). TNF-α is a known potent inducer of Mn-SOD, and the TNF-α–treated animals had the highest level of myocardial Mn-SOD content and activity (243 ± 30 U/mg, P < 0.001 versus control), although these values were not statistically significant compared with values in the CCPA-treated group.

Discussion
The present study provides new insight into the cellular mechanisms responsible for conferring increased myocardial tolerance to lethal ischemic injury 24 hours after transient activation of A1Rs. We have shown that pretreatment with CCPA, a selective adenosine A1 agonist, in the rat produced significant protection against infarction 24 hours later, similar to the delayed CCPA-induced preconditioning previously reported in the rabbit.8–10 This delayed protection was associated with a significant increase in myocardial Mn-SOD content and activity. Pretreatment with AS-ODN to Mn-SOD completely abolished the cardioprotective effects of CCPA and the associated induction of Mn-SOD. Importantly, treatment with S-ODN or Scr-ODN did not affect either the cardioprotection or the enhanced Mn-SOD expression induced by CCPA, thereby ruling out nonspecific effects of treatment with ODNs in abolishing protection. Taken together, these data strongly point to an important role for the induction of Mn-SOD in mediating the delayed infarct-limiting effects observed 24 hours after A1R activation.

A1Rs and Preconditioning
Liu, Thornton, and colleagues24,25 were the first to demonstrate a role for adenosine, which is released during the brief periods of preconditioning ischemia and acts on A1Rs, as an important trigger of early or “classic” preconditioning in rabbit myocardium. These original findings were later confirmed in further studies in rabbit, dog, and pig models of ischemic preconditioning.26–28 In the rat heart, on the other hand, the role of adenosine in mediating cardioprotection has been controversial, with most studies failing to show a critical role for adenosine in mediating early preconditioning.29–31 However, this discrepancy could have resulted from the fact that after a brief period of ischemia, the interstitial concentration of adenosine released is 3- to 4-fold higher in the rat...
heart than in the rabbit heart, and higher concentrations of selective agonists and antagonists are required to mimic or abolish the protective effects of early preconditioning.32

Several studies from our laboratory have demonstrated the role of A1,R as a trigger of delayed (second-window) myocardial protection against infarction in the rabbit.8–11,13 Furthermore, we were able to maintain rabbits in a preconditioned state against myocardial infarction by repeated activation of A1,R by intermittent dosing with CCPA over a 10-day period.16 However, all the above studies have been performed in rabbit models. To the best of our knowledge, the present study is the first evidence that transient activation of A1,R in the rat induces similar delayed cardioprotective effects against lethal ischemic injury 24 hours later.

Cardioprotective Role of Mn-SOD

The mitochondrial Mn-SOD belongs to a class of enzymes that catalyze the dismutation of 2 superoxide radicals to form hydrogen peroxide and molecular oxygen.34 The demonstration that reactive oxygen species (ROS) contribute to ischemia/reperfusion injury suggests that increasing the content or activity of endogenous cellular antioxidant enzymes should protect tissues from the deleterious effects of ischemia/reperfusion injury. However, the addition of SOD alone or in conjunction with other antioxidants, such as catalase, to the perfusion solution after myocardial ischemia has provided conflicting results (reviewed in Reference 35). In addition to the variation in doses and kinetics under different experimental conditions, one of the major problems with these studies has been that the exogenous antioxidant enzymes cannot permeate the cells to the sites where free radicals are generated. On the other hand, strategies that have enhanced the activity of endogenous Mn-SOD have proven to be consistently protective. For example, it has been shown that pretreatment of rats with cytokines, such as interleukin-1β, leukemia inhibitory factor, and TNF-α, results in an increase in the activity of endogenous Mn-SOD and protects against subsequent myocardial ischemia/reperfusion injury.22–36–38 Moreover, Yamashita and colleagues6,13,14,18,39 have demonstrated that the subacute myocardial adaptation observed 24 to 48 hours after heat stress,13,39 sublethal hypoxia/ischemia,6,14 or exercise18 is mediated by the induction and enhanced activity of endogenous Mn-SOD. Similar results have been reported by Zhou et al40 in rat myocytes preconditioned with brief periods of anoxia. Furthermore, it has recently been demonstrated that overexpression of Mn-SOD in transgenic mice results in reduced infarct size and improved functional recovery after ischemia/reperfusion.15 Taken together, these results indicate an important protective role for Mn-SOD in reducing oxygen-derived free radical–induced injury during reperfusion of the ischemic myocardium, and they also indicate that the induction of endogenous Mn-SOD may play an important role in mediating delayed cardioprotection after a number of stressful stimuli, such as heat stress, ischemia, or exercise.

The results of the present study show for the first time that the delayed cardioprotection induced by transient activation of A1,R in the rat is also associated with significantly enhanced myocardial Mn-SOD content and activity and that pretreatment with AS-ODN, which inhibited the induction and activation of Mn-SOD, abolished the delayed infarct-limiting effects induced by CCPA. The ODNs used in the present study have previously been shown to abolish the induction of Mn-SOD after sublethal hypoxia in rat cultured cardiomyocytes14 and after exercise in an in vivo rat model.18 The suppression of induction of Mn-SOD in these studies also abrogated the delayed cardioprotective effects of sublethal hypoxia and exercise. In the present study, we did not determine the localization of ODNs after intravenous injection. Yamashita et al18 have recently characterized the time course of accumulation of ODNs within the myocardium after in vivo delivery with intraperitoneal injection in the rat with the use of 5′ FITC-labeled AS-ODNs to Mn-SOD. They reported prominent labeling of cardiomyocytes at 8 hours after systemic administration of ODNs.

The signaling pathways that regulate the induction of Mn-SOD many hours after transient activation of A1,R are not known. We have previously shown in rabbits that CCPA-induced enhanced Mn-SOD activity at 24 hours is mediated via a PKC-dependent and tyrosine kinase–dependent pathway, because pharmacological inhibition of either enzyme attenuated the increase in Mn-SOD activity and abolished the delayed cardioprotective effects of CCPA.17 This is in concordance with other reports of a role for protein kinases in the regulation of induction of Mn-SOD in endothelial cells,41,42 human lung adenocarcinoma cells,43 and human leukocytes.44 On the other hand, an important role has been demonstrated for ROS, cytokines, and nuclear factor-κB, an oxidant-sensitive transcription factor, in modulating Mn-SOD gene expression,18,22,45–47 all of which have also been implicated in mediating delayed cardioprotective effects after ischemic preconditioning.5,18,40,48–50 The interaction between the transient activation of A1,R and potential generation of ROS or the activation of cytokines or nuclear factor-κB was not addressed in the present study and warrants further investigation.

Other Mediators of A1,R-Induced Delayed Preconditioning

Other cytoprotective proteins have been implicated as potential end effectors mediating delayed cardioprotection after A1,R activation in the rabbit heart. For example, we51 and others52 have demonstrated that A1,R-induced delayed preconditioning is dependent on the opening of the ATP-sensitive K+ (KATP) channels during the index ischemic insult, because inhibition of KATP channels with glibenclamide or 5-hydroxydecanoate abrogated the infarct-limiting effect of treatment with CCPA 24 hours earlier. Furthermore, on the basis of the relative selectivity of 5-hydroxydecanoate for the mitochondrial rather than the sarcosomal KATP channels, it has been proposed that opening of the former may mediate the delayed cardioprotective effects of A1,R agonists.31 If so, it is currently unknown whether opening of the mitochondrial KATP channels and enhanced activity of mitochondrial Mn-SOD are related or whether they act independently to protect the myocardium from ischemia/reperfusion injury. Further
studies are currently under way in our laboratory to elucidate any potential relation between Mn-SOD and mitochondrial K<sub>ATP</sub> channels.

In conclusion, we have shown that transient activation of A<sub>1</sub>R induces delayed myocardial protection in rats, similar to that previously reported in rabbits. This protection is associated with enhanced Mn-SOD expression and activity and is abolished by prior treatment with AS-ODNs to rat Mn-SOD. These results provide the first direct evidence that induction and activation of Mn-SOD play a crucial role in mediating delayed myocardial adaptation after A<sub>1</sub>R activation. Our results point to a potential therapeutic role for adenosine or its analogues in protecting the myocardium against not only ischemia/reperfusion injury but also cardiotoxicity induced by ROS in other circumstances, such as that seen after treatment with anticancer chemotherapeutic agents.

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References


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