Ischemic Preconditioning Reduces Apoptosis By Upregulating Anti-Death Gene Bcl-2

Nilanjana Maulik, PhD; Richard M. Engelman, MD; John A. Rousou, MD; Joseph E. Flack III, MD; David Deaton, MD; Dipak K. Das, PhD

Background—Reperfusion of ischemic myocardium causes cardiomyocyte apoptosis in concert with downregulation of Bcl-2 gene. Ischemic preconditioning (PC) mediated by cyclic episodes of short-term ischemia and reperfusion reduces apoptotic cell death. PC also triggers a signaling pathway by potentiating tyrosine kinase phosphorylation leading to the activation of p38 MAP kinase and MAPKAP kinase 2. The nuclear transcription factor, NFκB, plays a crucial role in this signaling process. Because NFκB is a target of oxygen free radicals and Bcl-2 is an antioxidant gene, we hypothesized that reactive oxygen species might play a role in the signaling process.

Methods and Results—Isolated rat hearts were perfused in the absence or presence of either dimethyl thiourea (DMTU), a hydroxyl radical scavenger, or SN50 peptide, a NFκB blocker. Hearts were then subjected to PC by 4 repeated episodes of 5-minute ischemia, each followed by 10 minutes reperfusion. All hearts were then made globally ischemic at normothermia for 30 minutes followed by 2 hours of normothermic reperfusion. Creatine kinase release and malonaldehyde formation were determined in the coronary effluent collected during reperfusion. At the end of each experiment, hearts were processed for infarct size determination and analyses of apoptosis, DNA fragmentation, NFκB, and Bcl-2. Myocardial infarction was reduced by PC. DMTU and SN50 abolished this cardioprotective effect of PC. PC resulted upregulation of Bcl-2 gene which was partially prevented by DMTU and SN50. Both ischemia/reperfusion and PC caused nuclear translocation and activation of NFκB, which was blocked by both DMTU and SN50. PC reduced cardiomyocyte apoptosis which was partially inhibited by DMTU and SN50. A substantial number of apoptotic cardiomyocytes were identified in the hearts subjected to 30 minutes ischemia and 2-hour reperfusion. PC significantly inhibited the extent of cardiomyocyte apoptosis and DMTU and SN50 reversed it only minimally.

Conclusions—The results demonstrate that reactive oxygen species play a crucial role in signal transduction mediated by PC. This signaling process appears to involve NFκB. NFκB becomes translocated and activated by both ischemia/reperfusion, which induces apoptosis and PC which reduces apoptosis. However, the amount of NFκB binding activity is significantly higher in the PC hearts compared with ischemic reperfused hearts. The upregulation of the antioxidant gene, Bcl-2, is inversely correlated with the reduction of cardiomyocyte apoptosis associated with PC. (Circulation. 1999;100[suppl II]:II-369–II-375.)

Key Words: oxygen  ■  apoptosis  ■  ischemia  ■  reperfusion  ■  signal transduction

Ischemic preconditioning (PC) is the manifestation of the earlier stress response that occurs during repeated episodes of brief ischemia and reperfusion and can render the myocardium more tolerant to a subsequent potential lethal ischemic injury.1 This transient adaptive response has been demonstrated to be associated with decreased reperfusion-induced arrhythmias,2 increased recovery of postischemic contractile functions,3,4 and reduction of infarct size.5,6 The adaptive protection has been found to be mediated by gene expression and their transcriptional regulation.7,8

Recent studies have demonstrated that myocardial ischemia and reperfusion result in apoptotic cell death, in addition to tissue necrosis.9–12 Studies from our laboratory indicated translocation of phosphatidyl serine and phosphatidyl ethanamine, a hallmark for apoptosis, occurs during ischemia, but apoptosis does not become apparent until hearts are reperfused following an ischemic insult.10 Myocardial adaptation to ischemia induced by repeated cyclic episodes of reversible short durations of ischemia each followed by short durations of reperfusion was found to be effective in reducing apoptotic cell death.11

Reactive oxygen species serve as trigger for apoptosis in a variety of cell types.13 A study from our laboratory demonstrated a role of oxygen free radicals in apoptotic cell death associated with ischemia and reperfusion.14 Another recent study documented that ischemic adaptation translocated and increased the binding of nuclear transcription factor NFκB in heart.15 NFκB is a member of Rel transcription factor family,
which is involved in the regulation of stress defense mechanisms.

Bcl-2 is an antioxidant oncogene which is inversely related to apoptosis. A preliminary study from our laboratory showed upregulation of Bcl-2 by ischemic adaptation. Induction of Bcl-2 was found to be associated with reduction of apoptotic cell death and DNA fragmentation. The present study was undertaken to further investigate the free radical signaling mechanism of preconditioning. Our results demonstrated that beneficial effects of preconditioning was abolished by preperfusing the hearts with dimethyl thiourea (DMTU, Sigma, St. Louis, Mo), a hydroxyl radical (OH-) scavenger, or with SN50 peptide, a NFκB blocker. In concert, preconditioning led to the induction of the expression of the antioxidant gene, Bcl-2, and reduced cardiomyocyte apoptosis and DNA fragmentation.

**Methods**

**Isolated Perfused Heart Preparation**

Sprague Dawley rats weighing about 300 g were anesthetized with pentobarbital (80 mg/kg IP). After intravenous administration of heparin (500 IU/kg), the chests were opened, hearts were rapidly excised and mounted on a nonrecirculating Langendorff perfusion apparatus. The perfusion buffer used in this study consisted of a modified Krebs-Henseleit bicarbonate buffer (KHB) (in mmol/L: 118 NaCl, 4.7 KCl, 1.29 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 10 Glucose and 1.7 CaCl2, gassed with 95% O2, 5% CO2, filtered through a 5-mm filter to remove any particulate contaminants, pH 7.4) which was maintained at a constant temperature of 37°C and was gassed continuously for the duration of the experiment. Left atrial cannulation was then performed and, after allowing for a stabilization period of 10 minutes in the retrograde perfusion mode, the circuit was switched to the antegrade working mode, which allows for the measurement of myocardial contractility as well as aortic and coronary flows, as described previously. Essentially, it is a left heart preparation in which the heart is perfused with a constant preload of 17cm H2O (being maintained by means of a Masterflex variable speed modular pump, Cole Parmer Instrument Company) and pumps against an afterload of 100 cm H2O. At the end of 10 minutes, after the attainment of steady state cardiac function, baseline functional parameters were recorded and coronary effluent samples were collected for biochemical assays. The circuit was then switched back to the retrograde mode. The hearts were divided into 5 groups: group I, hearts perfused with KHB buffer only for 210 minutes; group II, hearts perfused with KHB for 1 hour; groups III and IV, KHB for 15 minutes in the absence or presence, respectively, of 10 mmol/L DMTU or 18 μmol/L SN50 peptide (Cal Biochem, San Diego, Calif) (group V). After the hearts were preperfused with DMTU and SN50, they were washed for 5 minutes with fresh KHB buffer. All groups except I and II were then subjected to ischemic stress adaptation by repeated ischemia and reperfusion through induced global ischemia for 5 minutes followed by 10 minutes of reperfusion; the process was repeated 4 times as shown by the protocol in Figure 1. For Bcl-2 expression, NFκB binding activity and DNA fragmentation left ventricles from the control and experimental hearts were kept frozen at liquid nitrogen temperature. Myocardial infarct size and apoptosis were determined in the heart with creatine kinase (CK) release and malonaldehyde (MDA) formation were estimated in the coronary effluent as described below.

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<td>Group V</td>
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S = Stabilization  P = Perfusion  I = Ischemia  R = Reperfusion

**Figure 1.** Experimental protocol.

**Evaluation of Myocardial Infarct Size**

For infarct size determination, hearts were frozen and then sliced perpendicularly to the long axis from apex to base in 0.8-mm thick sections. Sections were then incubated for 20 minutes at 37°C in a 1% triphenyltetrazolium chloride in phosphate buffer (Na2HPO4 88 mmol/L, NaH2PO4 1.8 mmol/L). Thin heart cross-sections were digitally imaged by using a Mcintosh 7100/80 computer and a HP Scan Jet 5p scanner (Hewlett Packard). The image was adjusted with Adobe Photoshop 4.0 (Adobe System, Inc). The areas of infarct (tetrazolium negative) and risk (tetrazolium positive) were determined by using NIH Image 1.61 (National Institute of Health, Bethesda) in pixels. Infarct size was expressed as percentage of the risk zone infarcted.

**Estimation of CK Release**

CK was quantified from 0.5 mL of coronary effluent obtained before ischemic adaptation and at 1 minute, 3 minutes, 5 minutes, 30 minutes, and 120 minutes during reperfusion. CK was analyzed by the enzymatic assay method using a CK assay kit (Sigma Diagnostics). The absorbance was read at 340 nm using a Beckman DU-8 spectrophotometer.

**Measurement of MDA Formation in Heart**

MDA was estimated in the perfusate to determine the development of oxidative stress and free radical generation, as described previously. In short, about 0.5 mL of coronary effluent was mixed with 2 mL of 20% trichloroacetic acid, 5.3 mmol/L sodium bisulfite, kept on ice for 10 minutes, centrifuged at 3000g for 10 minutes, and then supernatants were collected, derivated with 2,4-dinitrophenylhydrazine (DNPH), and extracted with pentane. Aliquots of 25 μL in acetonitrile were injected onto a Beckman Ultrasphere C8 (3-mm) column. The products were eluted isocratically with a mobile phase containing acetonitrile-water-acetic acid (40:60:0.1, v/v/v) and measured at 3 different wavelengths (307 nm, 325 nm, and 356 nm) using a Waters M-490 multichannel UV detector. The peak for malonaldehyde was identified by cochromatography with DNPH derivative of the authentic standard, peak addition, UV pattern of absorption at the 3 wavelengths, and by GC-MS.

**Electrophoretic Mobility Assay (EMSA)**

Nuclear proteins were isolated from the heart according to the method described previously. The nuclear extracts were stored at −70°C. Protein concentration was estimated by using Pierce protein assay kit (Pierce Chemical Company). NFκB oligonucleotide (AGTTGAGG-GGACTTTCCCAGG) (2.5 μL [20 ng/μL]) was labeled using T4 polynucleotide kinase as previously described. The binding reaction mixture contained a total volume of 20.2 μL, 0.2 μL DTT (0.2 mol/L), 1 μL BSA (20 mg/mL), 4 μL Pdcl-dc (0.5 μg/μL), 2 μL Buffer D*, 4 μL Buffer F, 2 μL 3'P-oligo (0.5 ng/μL), and 7 μL extract containing 10 μg protein. Composition of Buffer D* was 20 mmol/L HEPES, pH 7.9, 20% glycerol, 100 mmol/L KCl, 0.5 mmol/L EDTA, 0.25% NP 40, whereas Buffer F contained 20% Ficoll 400, 100 mmol/L HEPES, pH 7.9, and 300 mmol/L KCl. Incubation was performed for 20 minutes at room temperature. Ten microliters of the solution was loaded onto a 4% acrylamide gel and separated at 80 V until the dye hit the bottom. After electrophoresis, gels were dried up and exposed to Kodak x-ray film at −70°C.
**Bcl-2**

Total RNA was extracted from the heart tissues by the acid-guanidinium thiocyanate-phenol-chloroform method as described previously. For Northern blot analysis, total RNA was electrophoresed in 1% agarose formaldehyde-formamide gel and transferred to Gene Screen Plus hybridization transfer membrane (Biotek Systems). The membrane was then baked under vacuum at 80°C for 1 hour. Each hybridization was repeated at least 3 times with labeled Bcl-2 cDNA probe using different membranes. After each hybridization, the residual cDNA was removed and rehybridized with GAPDH cDNA probe, the results of which served as a loading control. The autoradiograms were quantitatively evaluated by computerized beta scanner. The results of densitometric scanning were normalized relative to the signal obtained for GAPDH cDNA.

**Evaluation of Apoptosis**

Tissue sections were fixed in 10% formalin, dehydrated, and embedded in paraffin. The myocardium samples were cut into 4 μm in thickness and mounted on poly-L-lysine coated slides. The sections were stained by using ApopTag in situ fluorescein detection kit (Oncor). In this method, residues of digoxigenin-nucleotide are catalytically added to the DNA by DTd enzyme which actually catalyzes a template independent addition of nucleotide triphosphate to the 3′-OH ends. The anti-digoxigenin fragment carries a fluorescein to the reaction site. Apoptotic cells were visualized by direct fluorescence detection of digoxigenin-labeled genomic DNA by epifluorescence using standard fluorescein excitation and emission filters with an Axiovert 100 TV microscope. This method was based on the new 3′-OH DNA end generated by DNA fragmentation and typically localized in morphologically identifiable nuclei and apoptotic bodies. In contrast, normal nuclei, which had relatively insignificant numbers of DNA 3′-OH ends, was not stained with this reagent.

To further characterize apoptosis, DNA was isolated from cardiomyocytes by standard techniques. In brief, myocytes were pelleted in an Eppendorf tube using 1000g for 2 minutes. The supernatant was aspirated, 20 μL of lysis buffer (10 mmol/L EDTA, 0.5% sarkosyl, 50 mmol/L Tris-HCl, pH 8.0) was added, vortexed, and placed at 4°C for 15 minutes; 1 μL of proteinase K (stock solution 20 mg/mL) was added to each sample. The samples were vortexed and then incubated for 1 hour at 50°C. After incubation for 1 hour, 1 μL of RNase A (stock solution 10 mg/mL) was added and incubated for an additional hour at 37°C. Five microliters of gel loading buffer was added to the sample, and 10 μL of the DNA sample was electrophoresed on a 1.8% agarose gel with ethidium bromide. DNA laddering was visualized and photographed under ultraviolet transillumination.

**Statistical Analysis**

For statistical analysis, a 2-way ANOVA followed by Scheffe’s test was first performed using Primer Computer Program (McGraw-Hill, 1988) to test for any differences between groups. If differences were established, the values were compared using Student’s t test for paired data. Values were expressed as mean±SEM. P<0.05 was considered significant.

**Results**

**Effects of DMTU and SN50 on PC-Mediated Cardioprotection**

Thirty minutes of ischemia followed by 2 hours of reperfusion caused significant amount of infarct in the heart (Figure 2). Corroborating with the increase in infarct size, amount of CK release from the coronary effluent from the postischemic heart also increased (Figure 3). As expected, hearts subjected to ischemic PC demonstrated significant reduction in infarct size (15±2% compared with 60±7% in ischemic control) and decrease in CK release compared with ischemic reperfused myocardium (75±5.1U/L compared with 135±4.5 U/L in ischemic control) (Figures 2 and 3). Both DMTU and SN50 abolished this cardioprotective effect of PC, suggesting that reactive oxygen species and NFkB play roles in PC-mediated cardioprotection. Infarct size or CK release was not affected when hearts were perfused with SN50 peptide or DMTU only for 3.5 hours (results not shown).

**Reduction of Oxidative Stress by PC**

MDA formation truly reflects the development of oxidative stress in a biological system. MDA production was negligible and did not change even after 3.5 hours of perfusion with SN50 peptide or DMTU only (results not shown). In all groups, MDA increased significantly compared with baseline, except for DMTU during the early reperfusion (Figure 4). At 3 minutes of reperfusion, the MDA production (pmol/mL) in ischemic/reperfused group was 70±0.9; adapted, 64±1.4; DMTU+PC, 59±0.8; and SN50+PC, 70±0.9. At 5 minutes of reperfusion, the production of MDA in ischemic/reperfused group was 75±1.2; adapted, 60±1.2; DMTU+PC, 55±1; and SN50+PC, 72±1.2 pmol/mL, respectively. In the nonischemic/reperfused group, the MDA production was negligible. Preconditioning initially resulted in significant increase in the amount of oxidative stress, as evidenced by increased MDA formation (data not shown). However, the amount of MDA increased only slightly during subsequent ischemia and reperfusion compared with
control group. DMTU and SN50 had only a minimal effect on the MDA production in the preconditioned myocardium.

**Effects of PC on Bcl-2 and NFκB**

NFκB binding activity was found to be very low in nonischemic control hearts (Figure 5, lane A). Reperfusion of ischemic myocardium activated the NFκB binding activity (1.5-fold) significantly as shown in Figure 5, Lane B, compared with the perfused group (lane A). A dramatic increase in NFκB (4-fold) activity was found in the hearts that were preconditioned by 4 cyclic episodes of ischemia/reperfusion followed by ischemia and reperfusion (lane C). Immediately following PC, we also observed significant translocation of this transcription factor (results not shown). NFκB binding activity was significantly decreased in the hearts pretreated with either SN50 (lane E) or DMTU (lane D), suggesting that NFκB activation is regulated by the reactive oxygen species.

**Effects of PC on Cardiomyocyte Apoptosis**

The number of apoptotic cells was significantly higher (24%) in the ischemic/reperfused myocardium (Figure 7, column B) than in the nonischemic control hearts (Figure 7, column A). DMTU increased the number of apoptotic cells (10%) (Figure 7, column D) compared with the adapted hearts (Figure 7, column C).
ports exist in the literature to support a role of free radicals in to improve postischemic ventricular functions.1–6 Many re-
reduce myocardial infarction, tissue injury, arrhythmias, and 

The mammalian heart can be adapted to ischemia by repeat-
number of apoptotic cells to ~5.2% compared with 24% in 
the ischemic control. DNA fragmentation was clearly visual-
ized in the hearts subjected to 30 minutes of ischemia followed by 2 hours of reperfusion (Figure 5, lane C).

DNA fragmentation was not apparent in the nonischemic control hearts (Figure 8, lane A). Ischemic adaptation was associated with a significant decrease in DNA fragmentation (Figure 8, lane C) compared with the ischemic reperfused group (Figure 8, lane B). SN50 abolished the preconditioning mediated cardioprotection as evidenced by significant extent of DNA fragmentation in this group (Figure 8, lane E). In the DMTU group, the DNA fragmentation was negligible (Figure 8, lane D).

Discussion

The mammalian heart can be adapted to ischemia by repeat-
edly subjecting it to short-term reversible ischemia followed by short durations of reperfusion. Such adaptation, generally known as PC, is cardioprotective as evidenced by its ability to reduce myocardial infarction, tissue injury, arrhythmias, and to improve postischemic ventricular functions.1–6 Many re-
ports exist in the literature to support a role of free radicals in myocardial ischemic/reperfusion injury.23 Preconditioning consisting of one or more episodes of ischemia/reperfusion also causes the development of oxidative stress. However, the amount of oxidative stress is not cumulative for each subse-
quent episode of ischemia/reperfusion. The amount of oxida-
tive stress generated lessens during each subsequent episode of ischemia/reperfusion. During prolonged ischemia and reperfusion, the amount of oxidative stress is actually lower in the PC myocardium compared with non-PC hearts.24

The role of free radicals/oxidative stress in myocardial ischemia/reperfusion injury is further supported from the recent demonstration of apoptotic cell death during prolonged reperfusion of an ischemic myocardium. Studies from differ-
ent laboratories, including our own, demonstrated that reper-
fusion of ischemic myocardium results in cardiomyocyte apoptosis in addition to necrosis.10–12 We have shown that a hallmark of apoptosis, translocation of phosphatidyl serine and phosphatidyl ethanolamine, occurs during ischemia, but execution of apoptosis does not occur until the late phase of reperfusion.10 Oxidative stress developed in the ischemic reperfused myocardium was found to be instrumental for apoptotic cell death, because free radical scavengers were found to block apoptotic cell death simultaneously, providing myocardial protection.25 Another related study showed that PC provided cardioprotection by blocking apoptotic cell death.11 Prolonged reperfusion after ischemia caused down-
regulation of the antioxidant gene, Bcl-2, in concert with enhanced DNA fragmentation.26 The results of the present study support these previous findings and further demonstrate that an inverse correlation exists between cardiomyocyte apoptosis and induction of the antioxidant gene, Bcl-2.

Several oxidative stress-inducible genes become activated during PC.7,8 The results of our study demonstrated an induction of the expression of Bcl-2 after preconditioning. Bcl-2 appears to be the most important gene that inhibits apoptosis. This gene located at chromosome band 18q21 covers 230 kb, with a very large 225-kb intron separating 2 exons carrying the open reading frame.27 Bcl-2 may be regarded as an important cellular component that not only guards against apoptotic cell death but also impinges on multiple cellular events. In a recent study, Bcl-2 was found to be expressed following brain ischemia.28 The authors’ results indicated that Bcl-2 could play a role in determining cell survival in cerebral ischemia. Expression of Bcl-2 gene was found to be associated with the inhibition of apoptosis mediated by multiple agents, Ca2+ ionophore, glucose withdrawal, membrane peroxidation, and free radical injury,29 suggesting that this gene is likely to play a role in reperfusion injury. The results of the present study demonstrate down-regulation of Bcl-2 gene expression in the hearts subjected to 30 minutes ischemia and 2-hour reperfusion, whereas upregulation of Bcl-2 in concert with inhibition of cardiomyocyte apoptosis was observed in the preconditioned heart (suggesting an inverse correlation of Bcl-2 gene with apoptosis). De Moissac et al10 have recently demonstrated that Bcl-2 activates the transcription factor NFκB in the rat heart of neonatal ventricular myocytes. In the present study, the preconditioned hearts pretreated with SN50 showed downregulation of Bcl-2 gene expression. However,
our results do not provide any evidence for the involvement of SNS0 with Bcl-2.

Although the beneficial effects of ischemic stress adaptation are well recognized, controversies exist regarding the mechanism of signal transduction by which ischemic stress builds up the heart’s defense. Myocardial adaptation to ischemia has recently been shown to be mediated through the activation of tyrosine kinase receptor protein. The signal transduction process appears to involve tyrosine kinase, coupled phospholipase D, and MAP kinases which lead to the activation of MAPKAP kinase 2.31,32 Our recent study demonstrated that the ischemic stress translocates and activates p38 MAP kinase, which directly activates MAPKAP kinase 2,33 leading to the translocation and activation of the nuclear transcription factor NFκB.21 NFκB is activated in both ischemic/reperfused (group II) and adapted rat myocardium (group III). However, the binding activity of NFκB in the adapted group was found to be 4-fold higher, compared to only a 1.5-fold increase in NFκB binding activity in the ischemic reperfused group when compared with the perfused baseline control (group I). Interestingly enough, the presence of apoptotic cells becomes evident in the ischemic myocardium (group II), whereas apoptosis is completely blocked in the preconditioned heart (group III).

NFκB appears to be a critical regulator for gene expression induced by diverse stress signals, including mutagenic, oxidative, and hypoxic stresses. NFκB is a ubiquitous transcription factor which is translocated in response to oxidative stress from its inactive cytoplasmic form by releasing the inhibitory subunit IκB from NFκB.34 Activation of NFκB is likely to be involved in the induction of gene expression associated with the ischemic adaptation, because this transcription factor has recently been found to play a crucial role in the regulation of ischemia/reperfusion-mediated gene expression.21

Our study shows that the infarct size-limiting effect of ischemic PC was partially blocked by DMTU and almost completely abolished by SNS0 peptide, suggesting that both reactive oxygen species and nuclear transcription factor NFκB play crucial roles in preconditioning. DMTU is a hydroxyl radical scavenger, and DMTU may reduce myocardial ischemic reperfusion injury by directly scavenging OH-. Paradoxically, in the preconditioned myocardium, DMTU partially abolished the cardioprotective effects, presumably by scavenging the free radicals generated during ischemia reperfusion. This is only possible if the reactive oxygen species potentiate the signal transduction cascade leading to PC.

Mounting evidence exists to support the notion that oxygen-derived free radicals are generated during the reperfusion of ischemic myocardium resulting in the development of oxidative stress.23 Oxidative stress/free radicals have been shown to activate NFκB which in turn induces the expression of genes.35 Interestingly, H2O2 was found to activate DNA binding of NFκB in vivo, but not in vitro,36 suggesting that a byproduct of H2O2 and not H2O2 by itself may be responsible for the activation of NFκB. Another related study using transient catalase overexpression in cos-1 cells showed that H2O2 may not serve as a messenger for TNFα or phorbol ester–induced NFκB activation.37 It is possible that OH-

radical formed by transient metal-catalyzed Fenton reaction during the reperfusion of ischemic heart38 can induce NFκB activation. Inhibition of NFκB induction by antioxidants further supports a role of free radicals in NFκB activation.39 In this study, the adapted heart resulted in the nuclear translocation and activation of NFκB, which was completely blocked by both DMTU and SNS0. In conjunction, the beneficial effects of ischemic adaptation was blocked by pretreating the hearts with SNS0 peptide or DMTU. These results support our previous observation that NFκB, situating downstream of p38 MAP kinase, plays a crucial role in myocardial adaptation to ischemia and further suggest that it has minimal role in regulating cardiomyocyte apoptosis associated with ischemia/reperfusion and ischemic PC.

In summary, the results of our study provide evidence for the first time that the reactive oxygen species function as a second messenger for the signal transduction mediated by PC. The paradoxical role of DMTU in myocardial protection from ischemic/reperfusion injury and blocking the cardioprotective properties of PC support the role of oxygen free radicals in signal transduction. The nuclear transcription factor NFκB also play a crucial role in PC because an inhibitor of NFκB blocked the cardioprotective effects of PC. An inverse correlation exists between cardiomyocyte apoptosis and the induction of the antioxidant gene, Bcl-2, in PC hearts. The question still remains unanswered as to why NFκB, although upregulated (1.5-fold) in ischemic reperfused hearts, could not reduce the extent of apoptosis, whereas in the adapted group, where NFκB shows 4-fold activity, the number of apoptotic cells were significantly reduced. The upregulation of antiapoptic gene Bcl-2 in the preconditioned heart which regulates NFκB binding activity may be one of the many reasons for this paradoxical observation.

Acknowledgments

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References


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