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 ethanolamine, 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, 1.2 NaHCO₃, 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 achievement of steady state cardiac function, baseline functional parameters were recorded and coronary effluent samples were collected for biochemical analyses. 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 mmol/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 described previously. At the end of this period, hearts (except group I) were subjected to global ischemia for 30 minutes followed by 2 hours of reperfusion. The first 10 minutes of reperfusion was in the retrograde mode to allow for postischemic stabilization and thereafter in the antegrade working mode to allow for assessment of functional parameters. A schematic diagram of the protocol is shown 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 while creatine kinase (CK) release and malonaldehyde (MDA) formation were estimated in the coronary effluent as described below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Stabilization</th>
<th>Ischemia</th>
<th>Reperfusion</th>
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<tr>
<td>I</td>
<td>S</td>
<td>210 min P</td>
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<td>II</td>
<td>S</td>
<td>60 P</td>
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<td>III</td>
<td>KHB</td>
<td>1 I</td>
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<td>IV</td>
<td>DMTU</td>
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<tr>
<td>V</td>
<td>SN50</td>
<td>1 I</td>
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S = Stabilization  P = Perfusion  I = Ischemia  R = Reperfusion

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, derived 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-GGACTTTCCAGG) (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 100ng/µL, and 7 µL extract containing 10 µg protein. Composition of Buffer D and Buffer F were described in detail. Inclusion of 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, 200 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.
Bel-2

Total RNA was extracted from the heart tissues by the acid- 
guanidinium thiocyanate-phenol-chloroform method as described 
previously.19 For Northern blot analysis, total RNA was electropho-
resed in 1% agarose formaldehyde-formamide gel and transferred 
to Gene Screen Plus hybridization transfer membrane (Biotech Sys-
tems). The membrane was then baked under vacuum at 80°C for 1 
hour. Each hybridization was repeated at least 3 times with labeled 
Bel-2 cDNA probe using different membranes. After each hybrid-
ization, 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 com-
puterized 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 TdT enzyme which actually 
catalyzes a template independent addition of nucleotide triphosphate 
to the 3'-OH ends. The anti-digoxigenin fragment carries a fluores-
cecin 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 apo-
ptotic 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 cardio-
myocytes by standard techniques.19,10 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 
at least 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 
etidium bromide. DNA ladder 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 reperfu-
sion 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 38±2.8% in ischemic control) 
and decrease in CK release compared with ischemic reper-
fused myocardium (75±5.1IU/L compared with 135±4.5 
IUL 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/reper-
fused 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.

Consistent with the results of gel shift, immunoblot analysis of the nuclear extract obtained from preconditioned hearts pretreated with SN50 peptide did not show detectable P50 subunit protein (results not shown).

Northern blot analysis revealed Bcl-2 gene upregulation in ischemically adapted hearts (Figure 6, lane C). To the contrary, prolonged reperfusion (2 hours) after short-term ischemia (30 minutes) downregulated Bcl-2 gene significantly as shown in Figure 6, lane B. Perfusion of the hearts with DMTU increased Bcl-2 mRNA activities (Figure 6, lane D) compared with the ischemic control. Bcl-2 mRNA expression was reduced by preperfusing the heart with SN50 (Figure 6, lane E) compared with lane C.

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-
duction myocardial infarction, tissue injury, arrhythmias, and by short durations of reperfusion. Such adaptation, generally subjecting it to short-term reversible ischemia followed in the literature as described in Methods. First lane is the DNA marker. Lane A represents nonischemic control; lane B, ischemia/reperfusion; lane C, PC; lane D, DMTU+PC; and lane E, SN50+PC.

column C). Myocardial adaptation to ischemia reduced the number of apoptotic cells to ~5.2% compared with 24% in the ischemic control. DNA fragmentation was clearly visualized 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 reports 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 subsequent episode of ischemia/reperfusion. The amount of oxidative 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 different 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, Ca^2+ 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 in conjunction with apoptotic cell death 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 NFkβ 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. Our recent study demonstrated that the ischemic stress translocates and activates p38 MAP kinase, which directly activates MAPKAP kinase.2,31,32 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 antideath gene Bcl-2 in the preconditioned heart which regulates NFκB binding activity may be one of the many reasons for this paradoxical observation.

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References


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