Nuclear Factor κB and Anesthetic Preconditioning during Myocardial Ischemia–Reperfusion

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Background: Volatile anesthetic preconditioning (APC) protects against myocardial ischemia–reperfusion (IR) injury, but the precise mechanisms underlying this phenomenon remain undefined. To investigate the molecular mechanism of APC in myocardial protection, the activation of nuclear factor (NF) κB and its regulated inflammatory mediators expression were examined in the current study.

Methods: Hearts from male rats were isolated, Langendorff perfused, and randomly assigned to one of three groups: (1) the control group: hearts were continuously perfused for 130 min; (2) the IR group: 30 min of equilibration, 15 min of baseline, 25 min of ischemia, 60 min of reperfusion; and (3) the APC + IR group: 30 min of equilibration, 10 min of sevoflurane exposure and a 5-min washout, 25 min of global ischemia, 60 min of reperfusion. Tissue samples were acquired at the end of reperfusion. NF-κB activity was determined by electrophoretic mobility shift assay. The NF-κB inhibitor, IκB-α, was determined by Western blot analysis. Myocardial inflammatory mediators, including tumor necrosis factor α, interleukin 1, intercellular adhesion molecule 1, and inducible nitric oxide synthase, were also assessed by Western blot analysis.

Results: Nuclear factor κB–DNA binding activity was significantly increased at the end of reperfusion in rat myocardium, and cytosolic IκB-α was decreased. Supershift assay revealed the involvement of NF-κB p65 and p50 subunits. APC with sevoflurane attenuated NF-κB activation and reduced the expression of tumor necrosis factor α, interleukin 1, intercellular adhesion molecule 1, and inducible nitric oxide synthase. APC also reduced infarct size and creatine kinase release and improved myocardial left ventricular developed pressure during IR.

Conclusions: The results of this study indicate that attenuation of NF-κB activation and subsequent down-regulation of NF-κB–dependent inflammatory gene expression plays an important role in the protective mechanism of APC against acute myocardial IR injury.

VOLATILE anesthetic preconditioning (APC) has been shown to promote protection from myocardial ischemia–reperfusion (IR) injury in a fashion similar to that of ischemic preconditioning (IPC).[1,2] Several signaling pathways are activated in response to myocardial IR injury. Nuclear factor (NF) κB is a pivotal transcription factor that has been shown to play a key role in oxidative stress and inflammatory response and is activated during IR. Activation of NF-κB induces expression of a variety of gene products of which cytokines, chemokines, and adhesion molecules are implemented in IR injury.[3,4] The increased concentrations of these inflammatory mediators will contribute to myocardial IR injury.[5]

Nuclear factor κB is typically composed of p50 and p65 heterodimers. Under normal physiologic conditions, NF-κB is maintained in an inactive form in the cytoplasm by its inhibitors, IκB-α and other members of the IκB family. On activation of IκB kinase, IκB is phosphorylated, leading to a dissociation of NF-κB and IκB, as well as ubiquitination of IκB and consequent degradation of IκB by proteasomes. IκB kinase can be activated by numerous factors, such as reoxygenation, reactive oxygen species (ROS), cytokines (tumor necrosis factor [TNF] α, interleukin [IL] 1), and ischemia. When dissociated, NF-κB can translocate to the nucleus and bind to consensus sites in promotor or enhancer regions of target genes to initiate transcription.[6–8] It has been reported that myocardial IPC attenuates NF-κB activation and reduces the expression of inflammatory mediators, which decreases myocardial IR injury.[5] Because APC also protects myocardium from IR injury[2] and no data on the relation between APC and NF-κB are currently available, we tested the hypothesis that APC attenuates the activation of NF-κB and subsequently suppresses the expression of NF-κB–regulated inflammatory genes during reperfusion, such as TNF-α, IL-1, intercellular adhesion molecule (ICAM) 1, and inducible nitric oxide synthase (iNOS), all of which contribute to decreased myocardial IR injury.

Materials and Methods

Preparation of Isolated Hearts

The study protocol was approved by the Animal Care Committee of the University of California, Davis (Davis, California), and all experiments were conducted in accordance with guidelines of animal care from the National Institutes of Health.

Hearts were obtained from male Sprague-Dawley rats (weight, 250–300 g). Anesthesia was first induced with an intraperitoneal injection of sodium thiopental (50–75 mg/kg) along with 1,000 U heparin. Sodium thiopental was chosen for initial anesthesia because this drug has been shown not to influence preconditioning.[9] The heart was excised and placed in an ice-cold solution of Krebs-Henseleit buffer. It was then cannulated and Langendorff perfused with Krebs-Henseleit buffer (127 mM

NaCl, 4.7 mM KCl, 1.25 mM MgCl$_2$, 2.5 mM CaCl$_2$, 25 mM NaHCO$_3$, 10 mM glucose) at a perfusion pressure of 140 ± 10 cm H$_2$O at 37 ± 0.5°C. The perfusate was continuously oxygenated with 95% O$_2$–5% CO$_2$. Pacing wires were placed in the right atrium after cannulation.

Experimental Design

Nine rats were randomly assigned to each of the three groups: (1) the control group: 130 min of continuous perfusion; (2) the IR group: 30 min of equilibration, 15 min of baseline, 25 min of global ischemia, 60 min of reperfusion; and (3) the APC + IR group: same as the IR group except 10 min of sevoflurane exposure followed by a 5-min washout was included immediately before 25 min of global ischemia. Sevoflurane was delivered at 2.5% to the gas mixture *via* a standard Sevotec5 variable bypass vaporizer (Datex-Ohmeda, Milwaukee, WI) to reach a final concentration of 0.4 ± 0.02 mM. Global ischemia was induced by stopping all flow to the heart. Atrial pacing at 5 Hz was used during all phases of the experiment except global ischemia. Any episodes of ventricular fibrillation were mechanically converted when they occurred.

Preparation of Heart Homogenates and Microsomal and Nuclear Extracts

Immediately after reperfusion, three hearts from each group were frozen in liquid nitrogen and subsequently homogenized in ice-cold Tris-HCl buffer (25 mM Tris, 1 mM EDTA, 10% glycerol, 1 mM DTT; pH 7.4) with a glass homogenizer. The homogenate was centrifuged at 10,000g for 20 min at 4°C. The supernatant and sediment fractions were separated. The supernatant obtained as described above was further centrifuged at 105,000g for 75 min. The microsomal pellet was suspended in 0.05 M Tris-HCl buffer, pH 7.4, with 0.25 M sucrose. The supernatant and microsomal extracts were placed in aliquots and stored at −80°C. The crude nuclear fraction in the low-speed centrifugation was collected and washed three times with homogenate buffer containing Triton X-100, followed by washing one time without Triton X-100. Nuclear protein was extracted with buffer C (20 mM HEPES, 25% glycerol, 0.42 M NaCl, 1 mM EDTA) by centrifuging at 50,000g for 30 min. Protease inhibitor cocktail was added into the homogenizing and extract buffer. Protein concentrations of the extracts were measured by means of a modified Bradford assay according to the manufacturer's instructions (Bio-Rad, Hercules, CA) using bovine serum albumin as a standard.

NF-κB–DNA Binding Activity

Electrophoretic mobility shift assay was performed to determine NF-κB–DNA binding activity. The oligonucleotide used as a probe (Promega, Madison, WI) was double-stranded DNA containing the NF-κB consensus sequence (5'-CCTGTGCTCCGGGAATTTCCCTGGCC-3') labeled with [Γ-^{32}P]dATP using T4 polynucleotide kinase. The binding reaction of nuclear proteins to the probe was assessed by incubation of mixtures containing 5 μg nuclear protein, 0.5 μg poly (dI · DC) and 40,000-cpm ^{32}P-labeled probe in the binding buffer (7.5 mM HEPES, pH 7.6, 35 mM NaCl, 1.5 mM MgCl$_2$, 0.05 mM EDTA, 1 mM DTT, 7.5% glycerol) for 30 min at 25°C. For the competitive assay, excessive unlabeled oligonucleotides were incubated with proteins before the addition of radiolabeled probe. Protein–DNA binding complex was separated by 5% polyacrylamide gel electrophoresis and autoradiographed overnight at −80°C. Supershift assays were used to identify NF-κB subunits. One microgram of the appropriate antibody was added (Santa Cruz Biotechnology, Santa Cruz, CA) after 20 min of preincubation of nuclear extract with labeled oligonucleotide. Specificity was confirmed using excess unlabeled NF-κB oligonucleotide and mutant oligonucleotide.

Western Blot Analysis

Western blot analyses were used to measure the levels of IκB-α, ICAM-1, and iNOS in the hearts. Heart cytosolic proteins (for IκB-α and iNOS) and microsomal proteins (for ICAM-1) were loaded and separated on 7.5–10% SDS-PAGE, followed by transblotting to an ImmunBlot PVDF membrane (Bio-Rad, Hercules, CA). The membrane was subsequently probed with primary IκBα, ICAM-1, and iNOS antibodies (Santa Cruz Biotechnology) at a dilution of 1:1,000. Horseradish peroxidase–conjugated secondary antibody was added at 1:3,000 dilution. The blots were subsequently developed using an enhanced chemiluminescence detection kit (AmerSham Pharmacia Biotech, Inc., Piscataway, NJ). After exposure on autoradiography film, immunoreactive protein bands were quantified by densitometry.

Proinflammatory Cytokines

The levels of proinflammatory cytokines TNF-α and IL-1β were assessed in heart homogenates by rat enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (R&D System, Minneapolis, MN). A 1:2 dilution of samples in calibrator diluent was used for cytokine determination. Quantitation of cytokines was normalized to wet tissue weight.

Hemodynamic Measurements

To measure left ventricular pressures, a latex balloon was filled with water and connected to a pressure transducer (Medex, Dublin, CA). The balloon was inserted into the left ventricle *via* the left atrial appendage through the mitral valve. The balloon volume was adjusted during the equilibration period to yield a left ventricular end-diastolic pressure of 5–10 cm H$_2$O. Pressures were recorded using Powerlab 4/20 hardware with an amplifier (ADInstruments, Colorado Springs, CO) and

Chart for Windows version 4.0.4 software (ADInstruments). Left ventricular developed pressure was used as the indication of left ventricular function.

CK Analysis

The runoff from the coronary sinus was collected for the first 10 min of reperfusion. This was placed in aliquots and stored at $-80°C$ until analysis. The amount of creatine kinase (CK) was determined using a CK-10 kit (Sigma Diagnostics, St. Louis, MO) and a Shimadzu UV-VIS recording photospectrometer (Shimadzu, Columbia, MD). Units are expressed as units per gram of dry weight.[10]

Determination of Infarct Size

At the end of reperfusion, six hearts from each group were quickly taken down from the Langendorff apparatus and sliced into 2 mm in cross sections. The sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride staining solution and placed in a 37°C incubator for 20 min. Noninfarcted myocardium stains a bright red that is caused by reduction of 2,3,5-triphenyltetrazolium chloride by dehydrogenases present in viable tissue.[11] After 20 min, slices were scanned into a computer using Adobe Photoshop 5.0LE software (Adobe, San Jose, CA). Standard computer planometric analysis, using NIH image 1.62 (public domain), was used to determine infarct area. Because the entire ventricles were at risk from global ischemia, infarct size was expressed by dividing the necrotic area by the total slice area to obtain the percent necrosis.

Analysis of variance and Student two-tailed, unpaired *t* test were used to determine the differences between groups. Data are presented as mean $\pm$ SEM. Statistical significance was set at $P < 0.05$.

Results

NF-κB Activation in Myocardial IR

The effect of myocardial IR and APC on the activation of NF-κB was examined by electrophoretic mobility shift assay. Compared with control, NF-κB–DNA binding activity was significantly increased at the end of reperfusion in the IR group (fig. 1A). Supershift assays showed that both p65 and p50 subunits were involved in the activation (fig. 1B). APC significantly attenuated NF-κB activation during reperfusion. The cytosolic level of IκB-α, the major NF-κB inhibitor, was also detected by Western blot analysis. As shown in figure 2, the IκB-α level was markedly decreased in the IR group compared with the control group ($P < 0.05$), whereas in the APC + IR group, the IκB-α level was similar to that of the control group.

Expression of Myocardial Cytokines

To investigate whether APC-induced suppression of NF-κB was associated with reduced inflammatory cytokine
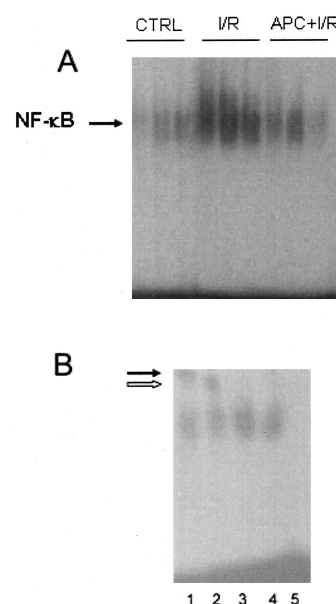

Fig. 1. Nuclear factor (NF) κB activation in rat hearts at the end of reperfusion (n = 3/group). (*A*) Electrophoretic mobility shift assay was performed to determine NF-κB–DNA binding activity. The *last lane* shows the competition assay with ×100 excess molar of unlabeled oligonucleotide. (*B*) Specificity of the NF-κB–DNA complex was confirmed by supershift assay. *Lanes 1* and *2* show the supershift assay using antibodies of p65 (*lane 1*) and p50 (*lane 2*). *Lane 3* shows electrophoretic mobility shift assay after reperfusion. *Lanes 4* and *5* show the competition assay with ×100 excess molar of activator protein 1 oligonucleotide (*lane 4*) and unlabeled NF-κB oligonucleotide (*lane 5*). The *open arrow* indicates a supershift band of p50, and the *closed arrow* indicates a supershift band of p65. APC = volatile anesthetic preconditioning; CTRL = control; I/R = ischemia–reperfusion.

expression, the levels of myocardial TNF-α and IL-1β were measured. The results show that myocardial TNF-α was greatly increased at the end of reperfusion in IR group compared with the control group ($P < 0.01$). APC significantly decreased the production of TNF-α ($P < 0.05$; fig. 3A). IR also significantly increased the IL-1β level, and it, too, was attenuated by APC ($P < 0.01$; fig. 3B).
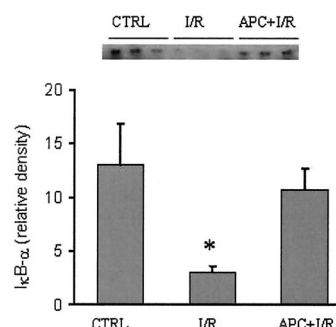

Fig. 2. Western blot analysis of the IκB-α protein levels in the cytoplasm of rat hearts. (*Top*) Autoradiograms highlighting IκB-α protein levels. (*Bottom*) Quantitative data indicating the relative density of IκB-α protein levels. Data are presented as mean ± SEM; n = 3/group. * $P < 0.05$ *versus* control (CTRL). APC = volatile anesthetic preconditioning; I/R = ischemia–reperfusion.
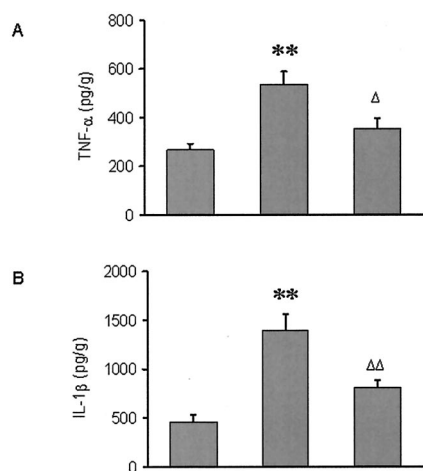
Fig. 3. Effect of volatile anesthetic preconditioning (APC) with sevoflurane on the myocardial levels of tumor necrosis factor (TNF) α (A) and interleukin (IL) 1β (B) measured by enzyme-linked immunosorbent assay. Data are presented as mean ± SEM; n = 3/group. ** $P < 0.01$ *versus* control (CTRL). Δ $P < 0.05$ *versus* ischemia–reperfusion (I/R). $\Delta\Delta$ $P < 0.01$ *versus* I/R.

Expression of ICAM-1 and iNOS

Compared with the control group, the expression of ICAM-1 protein in rat myocardium of the IR group was significantly increased as determined by Western blot ($P < 0.05$). APC markedly decreased ICAM-1 protein expression ($P < 0.05$; fig. 4A). The level of myocardial iNOS in the IR group was also increased. APC significantly decreased the expression of iNOS compared with
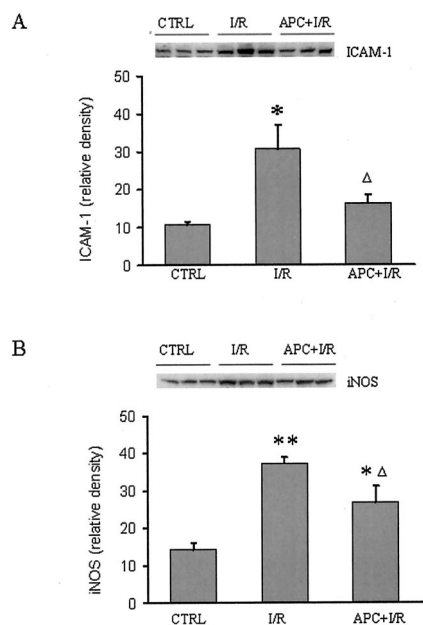
Fig. 4. Effect of volatile anesthetic preconditioning (APC) with sevoflurane on the myocardial levels of intercellular adhesion molecule (ICAM) 1 (A) and inducible nitric oxide synthase (iNOS) (B) measured by Western blot analysis. Band density is represented graphically beneath the Western blot. Data are presented as mean ± SEM; n = 3/group. * $P < 0.05$ *versus* control (CTRL). ** $P < 0.01$ *versus* control. Δ $P < 0.05$ *versus* ischemia–reperfusion (I/R).
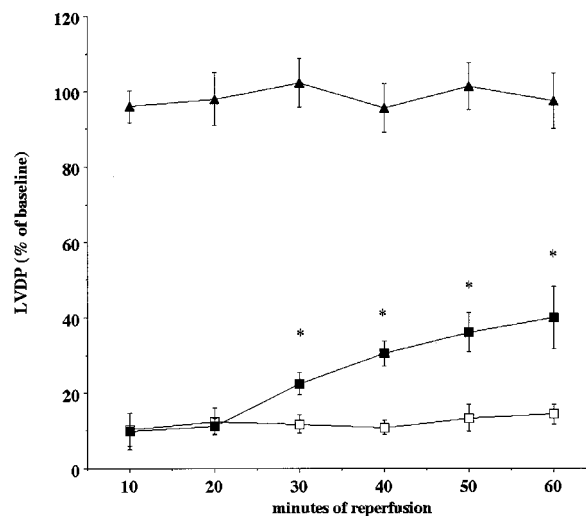
Fig. 5. Left ventricular developed pressure (LVDP) in the control (▲), ischemia–reperfusion (I/R; □), and volatile anesthetic preconditioning (APC + I/R; ■) groups during reperfusion. This result shows a better recovery of LVDP in the APC + I/R group than in the I/R group. It also shows that there is no significant change in the control group. Data are presented as mean ± SEM; n = 6/group. * $P < 0.05$ *versus* I/R.

that of the IR group ($P < 0.05$), although it remained higher than that of the control group ($P < 0.05$; fig. 4B).

Myocardial CK Releases, Infarct Size, and Function

The result also demonstrated that APC improved myocardial functional recovery during reperfusion. At the end of 60 min of reperfusion, left ventricular developed pressure recovered to 40.1 ± 8.3% of baseline in the APC + IR group *versus* 14.4 ± 2.7% in the IR group, compared with 97.5 ± 7.4 in the control group (fig. 5). CK release (units per gram dry weight) was significantly reduced by pretreatment with sevoflurane (24.63 ± 8.08 *vs.* 312.14 ± 28.57). CK released in control group was 2.37 ± 0.55 (fig. 6). The infarct size, expressed as percent necrotic area, was also reduced by APC (14.2 ± 2.7% in the APC + IR group *vs.* 30.90 ± 2.4% in the IR group; fig. 7), and there was no necrosis detected in the control hearts.
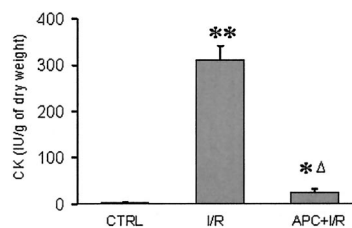
Fig. 6. Results of myocardial creatine kinase (CK) releases in the control (CTRL), ischemia–reperfusion (I/R), and volatile anesthetic preconditioning (APC) + I/R groups. CK is an indicator for myocardial injury. The CK release is much higher in the I/R group than in the APC + I/R group. It also shows that there is minimal CK release in the control group. Data are presented as mean ± SEM; n = 6/group. * $P < 0.05$ *versus* control. ** $P < 0.01$ *versus* control. $\Delta\Delta$ $P < 0.01$ *versus* I/R.
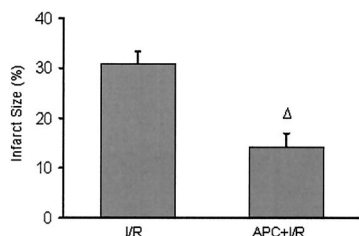
Fig. 7. Myocardial infarct size of the ischemia–reperfusion (I/R) and volatile anesthetic preconditioning (APC) + I/R groups. The results are presented as the percent necrotic area ventricles. Data are presented as mean ± SEM; n = 6/group. Δ *P* < 0.05 *versus* I/R.

Discussion

The benefits of APC have been shown by improvements in myocardial functional recovery and reduced infarct size after global ischemia.[12,13] Since the first description of a protective effect of APC against myocardial IR injury,[2] numerous studies have attempted to characterize the mechanisms responsible for the response. These studies have implicated sarcolemmal and mitochondrial adenosine triphosphate–sensitive potassium channels, protein kinase C, mitochondrial Ca, intracellular pH, and ROS,[14-16] but the precise mechanism responsible for APC remains undefined.

Nuclear factor κB has been shown to play an important role in many important physiologic and pathophysiologic processes, including myocardial IR injury and IPC.[17-19] It has been shown that NF-κB is activated during IR. NF-κB is a ubiquitous inducible transcription factor that regulates the expression of numerous cellular genes, particularly those involved in the inflammatory response, which is activated during IR. More than 150 NF-κB target genes have been identified, including a number of inflammatory mediators, such as cytokines (*e.g.*, TNF-α, IL-1), chemokines (*e.g.*, IL-8), adhesion molecules (*e.g.*, ICAM-1, vascular cell adhesion molecule-1, E-selectin), and proinflammatory enzymes (*e.g.*, iNOS, cyclooxygenase 2).[20,21] Myocardial reperfusion injury is thought to be partially caused by postischemic inflammation resulting from NF-κB activation.[17,20,21]

In myocardial IR and IPC, ROS constitute one of the most potent stimulators of NF-κB activation.[21-23] Several studies support the hypothesis that APC causes a reduced ROS formation and sevoflurane and isoflurane cause direct ROS formation in cardiac tissue[14,24] On the other hand, the inhibition of ROS formation not only blocks NF-κB activation, but also abolishes the cardioprotective effects of IPC in both classic and delayed models.[19,25] Those results indicate that ROS formation and NF-κB activation are essential for the development of preconditioning.

During the trigger phase of APC, there is an initial increase in ROS, which turns on a cardioprotective program, and ROS release after prolonged ischemia is decreased, which is a beneficial effect of the early small

ROS burst that occurred during the preconditioning stage of the experiment. It is postulated that reduced activation of NF-κB before the sustained IR injury and subsequent attenuation of its activation and further down-regulation of NF-κB–dependent inflammatory response during reperfusion all contribute to the myocardial protective effects of preconditioning. The attenuated NF-κB activation during IR in the preconditioned heart is thought to be caused by an increase of the inhibitor of NF-κB, IκB-α, which is also a transcriptional product of NF-κB during the preconditioning period.[19,21,26] That is, negative feedback on NF-κB activation is mediated by IκB-α, which itself is induced by NF-κB.

Tumor necrosis factor α and IL-1 are major proinflammatory cytokines that exhibit similar functional properties in their contribution to IR injury. The expression of these cytokines is regulated by NF-κB. Myocardial TNF-α is produced by resident cardiac macrophages, infiltrating leukocytes, and cardiomyocytes themselves.[27] The mechanisms by which TNF-α causes myocardial dysfunction include calcium dyshomeostasis, direct cytotoxicity, oxidant stress, and cell apoptosis as well as induction of other inflammatory cytokines (*e.g.*, IL-1), chemokines, and adhesion molecules. IL-1 can synergistically enhance TNF-α–induced myocardial depression and cytotoxicity.[27] Previous studies have shown that IR causes an increase in cardiac TNF-α that impairs myocardial function. The latter conclusion is supported by evidence that protection provided by IPC against IR is related to inhibition of TNF-α production.[28-30] Similarly, treatment with anti–TNF-α antibody has been shown to improve myocardial recovery after IR.[31,32] Our results are consistent with other observations that IR causes a significant increase in the production of proinflammatory cytokines. IR-induced increase of TNF-α and IL-1 is suppressed by APC, which is associated with reduced activation of NF-κB.

Adhesion molecules also play an important role in the pathogenesis of myocardial IR injury. These molecules are expressed on cell surfaces of leukocytes and several other cell types, including endothelial cells and myocardial cells. Adhesion molecules cause leukocytes to adhere to the ischemic tissue, where they release toxic mediators, which results in considerable damage to adjacent tissues, and amplify tissue injury. ICAM-1 mediates the firm binding of leukocytes to endothelial cells through interactions with its integrin counterreceptors on leukocytes, such as CD11/18. This process is central to the trapping and accumulation of activated leukocytes in IR myocardium.[33] Monoclonal antibodies for ICAM-1 reduce IR-induced myocardial injury.[34,35] Mutant mice deficient in ICAM-1 are less susceptible to injury after transient IR.[36] The gene expression of ICAM-1 is thought to be under the regulation of NF-κB. Activation of NF-κB during IR results in up-regulation of ICAM-1, whereas inhibition of NF-κB suppresses ICAM-1 gene expression

and reduces myocardial IR injury.[23,31,33,37] The results of this study reconfirm that the expression of ICAM-1 is increased during IR and show that APC significantly attenuates the expression during reperfusion.

The role of nitric oxide in IR remains controversial.[38,39] It has been suggested that nitric oxide has a dual role and can attenuate myocardial injury during IR as well as mediate reperfusion injury. Nitric oxide supplement or mimicry has been suggested to be protective. However, inhibition of nitric oxide synthase by the nitric oxide synthase inhibitor L-NAME has been shown to reduce myocardial infarct size during IR.[40–42] Furthermore, selective knock-down of iNOS with antisense oligodeoxynucleotides protects against ischemic injury.[43] It is thought that the cytotoxic effect of nitric oxide results from the formation of nitric oxide–derived free radical species, such as peroxynitrite.[44,45] Nitric oxide combines with superoxide to generate peroxynitrite, which then decomposes to yield cytotoxic hydroxyl radical and other radicals, which can cause myocardial injury. In addition, nitric oxide acts as a potent vasodilator. It is well documented that reestablishment of blood flow to ischemic tissues may exacerbate the myocardial injury, *i.e.*, causing reperfusion-induced injury. Inhibition of nitric oxide synthase might be expected to impair recovery of coronary blood flow by inhibiting nitric oxide–mediated coronary vasodilation and thus reduce reperfusion-induced injury. These mechanisms are consistent with our observations that IR increases the expression of iNOS, whereas APC attenuates iNOS expression, which might contribute to the protective effects of APC.

Ischemia-reperfusion–induced myocardial injury develops as a result of damage to cardiac myocytes and endothelial cells. Proinflammatory cytokines, ROS, and nitrogen species produced by myocardial tissue during IR can directly cause cell damage.[24,27,42,46] Furthermore, the infiltrated leukocytes, which are recruited by complex interaction of multiple cytokines, chemokines, and adhesion molecules, release toxic substances in the ischemic area and thus augment tissue injury.[33,34] Again, these noxious mediators are NF-κB dependent. To reiterate, our results show that IR results in NF-κB activation, as evidenced by the diminished cytosolic level of IκB-α and increased NF-κB–DNA binding activity. Furthermore, both p50 and p65 subunits are involved in the activation of NF-κB. With NF-κB activation by IR, proinflammatory mediators are up-regulated, contribute to increased infarct size and CK release, and lead to myocardial dysfunction. Consistent with others' findings, APC significantly decreases myocardial infarct size and improves cardiac function, which we believe is at least in part the result of reduced activation of NF-κB and down-regulation of proinflammatory responses.

The myocardial protective effects of APC may involve many different signaling pathways and mediators, such as protein kinase C, adenosine receptor, and adenosine triphosphate–sensitive K channels. This study only addresses phenomena related to NF-κB activation and the effects of APC that we observed during IR and the effect of APC in this specific model. Although NF-κB activation during preconditioning periods was not measured, the subsequent attenuation of NF-κB and the down-regulation of inflammatory gene expression are shown to be important mechanisms involved in the cardioprotection of APC with sevoflurane.

In conclusion, APC with sevoflurane decreases infarct size and CK release and improves function during reperfusion of isolated rat hearts exposed to global ischemia. NF-κB–DNA binding activity was significantly increased and cytosolic IκB was significantly decreased at the end of reperfusion. Both p50 and p65 subunits were involved in this response. Increased expressions of TNF-α, IL-1, ICAM-1, and iNOS were also observed at the end of reperfusion after untreated ischemia. APC attenuated NF-κB activation and subsequent expression of NF-κB–dependent inflammatory mediators. This attenuation of NF-κB activation could play an important role in the protective effect of APC during myocardial IR injury.

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