Endogenous κ-Opioid Peptide Mediates the Cardioprotection Induced by Ischemic Postconditioning

Hai-Tao Guo, MD, PhD,*† Rong-Huai Zhang, MD, PhD,‡ Yan Zhang, MD,§ Li-Jun Zhang, MD,† Juan Li, MD,† Quang-Xing Shi, MD,† Yue-Min Wang, MD,† Rong Fan, MD,† Hui Bi, MD, PhD,† Wen Yin, MD, PhD,* and Jian-Ming Pei, MD, PhD*†

Abstract: The aim of this study was to investigate the underlying mechanism that dynorphin, an endogenous kappa opioid receptor (κ-OR) agonist, triggers antiapoptotic effect of postconditioning (Postcon). In addition to vehicle treatment, Sprague Dawley rats (n = 6) underwent a 30-minute left anterior descending occlusion followed by 2 hours of reperfusion with or without a Postcon stimulus. The selective κ-OR antagonist nor-binaltorphimine (Nor-BNI) was administered intravenously 5 minutes before reperfusion. Infarct size was determined by using 2,3,5-triphenyltetrazolium chloride staining. Blood plasma concentrations of creatine kinase (CK) and lactate dehydrogenase (LDH) and myocardial caspase-3 activity were analyzed spectrophotometrically. Myocardial apoptosis was analyzed by the detection of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end labeling. Immunoreactive dynorphin in blood serum and myocardium was measured by means of an antigen-competitive enzyme-linked immunosorbent assay. Infarct size, caspase-3 activity, apoptotic index, and CK and LDH levels were significantly higher in the ischemic/reperfusion group than in the vehicle group (P < 0.01). Postcon significantly reduced infarct size, caspase-3 activity, apoptotic index, CK and LDH levels (P < 0.01 vs. ischemic/reperfusion). Dynorphin content significantly increased after Postcon (P < 0.01). All the effects described above were abolished by Nor-BNI, with the exception of dynorphin content. We found that cardiac protection and antiapoptotic effect of Postcon is mediated by the activation of κ-OR. Effect of Postcon is mediated, at least partially, by enhanced dynorphin expression.

Key Words: postconditioning, apoptosis, heart, κ-opioid receptor, dynorphin

(J Cardiovasc Pharmacol™ 2011;58:207–215)

INTRODUCTION

Brief cycles of reperfusion and ischemia during the early phase of reperfusion after a prolonged ischemic insult protect the heart from infarction. Zhao et al. 1 referred to this cardioprotective phenomenon as “postconditioning” (Postcon), which is as powerful as ischemic preconditioning (IPC). 2 Clinical cardioprotective strategy to attenuate the pathophysiological consequences of ischemia/reperfusion (I/R) injury is limited by the inability to predict the onset of ischemia. Postcon is an “after the fact” protection strategy and may have greater clinical application, in particular, in reperfusion after myocardial infarction or cardiac surgery. Postcon is clinically more relevant than IPC is, and much research has been conducted on the topic. Because the regenerative capacity of the myocardium is limited, there is intense interest in the prevention of cardiomyocyte loss during ischemia and reperfusion. In addition to necrosis, cardiomyocytes also undergo apoptosis. Apoptosis is a significant component of cell loss during reperfusion after myocardial infarction. 3 Although apoptosis in reperfusion injury is minor compared with necrosis, as indicated by the reduced markers of necrosis [enzyme release and infarct size (IS)], apoptosis is a highly regulated process, which is a good potential target for therapeutic intervention. Postcon may limit all forms of cell death (apoptosis, autophagy, and necrosis) induced by I/R. 4,5

As with cardioprotective mechanisms of preconditioning, Postcon also includes triggers, mediators, and end effectors. Classical ligand triggers have also been reported to be involved in Postcon including adenosine, bradykinin, opioids, erythropoietin, and adrenergic and muscarinic agonists. 6 For example, the G protein–coupled adenosine receptor has now been likewise implicated in the reduction in IS after Postcon. Postcon protects the heart from reducing IS by the activation of opioid receptor (OR). 7 Opioids are well-known endogenous triggers of preconditioning and were recently determined to be effective in reducing cardiomyocyte apoptosis when given before ischemia through interaction with their cognate G protein–coupled receptors. 8 Because Postcon shares the protective pathways with preconditioning, G protein–coupled receptor activation may serve
as an essential mechanism that triggers the antiapoptotic effect of Postcon. Therefore, it is intriguing to determine whether OR activations are also involved in the antiapoptosis of Postcon.

Cardioprotective effects of Postcon involve the intrinsic activation of ORs. Cardiac enkephalin was enhanced by Postcon, and cardioprotection of Postcon is functionally associated with OR activation by local opioids; therefore, maintaining adequate supplies of enkephalin may be an integral part of the process. Myocytes have been shown to be sites of endogenous opioid synthesis, storage, and release. There are 3 main classes of ORs (µ, δ, and κ) in the heart. Opioid peptides, which are endogenous ligands of ORs and include enkephalins and proenkephalin mRNA, β-endorphin and pro-opiomelanocortin mRNA, and prodynorphin genes, have been found in cardiac tissue and isolated cardiac myocytes. Receptor-binding studies showed that kappa opioid receptor (κ-OR) is a predominant OR in the heart. Based on the above observation, Postcon mediated the cardioprotection by reducing cardiomyocyte necrosis, which was triggered by ORs. Whether κ-OR triggers antiapoptosis of Postcon has not yet been elucidated. Therefore, we determined whether the endogenous agonist of κ-OR, dynorphin, triggers Postcon, and specifically reduces apoptosis of the I/R myocardium.

MATERIALS AND METHODS

Materials
Adult male Sprague Dawley (SD) rats weighing 300 ± 50 g were provided by the animal center of the Fourth Military Medical University (Xi’an, China) and used for all the experiments. This study conformed to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Nor-binaltorphimine (nor-BNI) was purchased from Cookson (Ellisville, MO). The other chemicals were of analytical grade and purchased from Sigma Chemical Co (St Louis, MO). Double distilled water was used in all biochemical assays. All compounds were dissolved in normal saline (0.85% NaCl solution) before use. The terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) assay kit was purchased from Roche Diagnostics (Mannheim, Germany). The capspase 3 activity assay kit was purchased from the Beyotime institute of biotechnology (Nanjing, China). The rat dynorphin, Dyn enzyme-linked immunosorbent assay (ELISA) kit, was purchased from Wuhan Uscn Sciences Co, Ltd (Wuhan, China). The creatine kinase (CK) and lactate dehydrogenase (LDH) activity assay kits were purchased from Jiancheng Reagent Company (Nanjing, China).

Methods

Surgical Procedure for Regional Ischemia and Reperfusion
SD rats were anesthetized by injection of sodium pentobarbital (60 mg/kg, intraperitoneally). Supplemental doses of sodium pentobarbital were given when needed to maintain a uniform level of anesthesia. The surgical procedure was performed as previously described. The trachea was intubated and connected to a rodent ventilator (Jiangwan I ventilator; the Second Military Medical University, China) for artificial ventilation with room air (stroke volume, 10 mL/kg; 60 strokes per minute). The temperature of the heating pad was adjusted to 37°C by a temperature controller. Arterial blood pressure was continuously monitored via a saline-filled catheter (PE50, Becton Dickinson, Franklin Lakes, NJ) inserted into the right femoral artery, which was connected to a pressure transducer (AB-621G, Nihon Kohden, Tokyo, Japan). Electrocardiogram data and heart rate (HR) were measured by standard limb lead II electrodes using an isolated Electrocardiogram bioamplifier (V75-04; Coulbourn Instruments, Allentown, PA). PE50 catheters were inserted into the left external jugular vein of each rat for drug administration and into the left ventricle (LV) from the right carotid artery for the measurement of LV pressure (LVP) with a pressure transducer (AB-621G, Nihon Kohden). All the signals were sent to a recording system (RM6200, Nihon Kohden). The chest was opened by a left thoracotomy in the fourth to fifth intercostal spaces, and the pericardium was incised. A ligature (5-0 silk) was placed around the left anterior descending coronary artery (LAD) for later initiation of coronary occlusion and reperfusion. Both the ends of the ligature were exteriorized and passed through a 2.5-cm-long segment of polyethylene tube (PE100, Becton Dickinson). After stabilization for 15 minutes, the animals were subjected to 30-minute ischemia by complete tightening of the ligature around the LAD with the help of the PE tube and a small hemostat. Reperfusion was allowed for 2 hours. Apoptosis and myocardial enzyme leakages were measured at the end of reperfusion. All the following determinations were carried out by other authors, and the groups were blinded.

Experimental Protocol
The experimental design is illustrated in Figure 1. Baseline hemodynamics were recorded 20 minutes after instrumentation was completed. In addition to the vehicle group, the other rats underwent a 30-minute LAD occlusion followed by 2 hours of

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>30 min + 2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/R</td>
<td>30 min 1</td>
</tr>
<tr>
<td>Postcon</td>
<td>30 min 1 R</td>
</tr>
<tr>
<td>I/R+BNI</td>
<td>30 min 1 R</td>
</tr>
<tr>
<td>BNI (2 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Postcon+BNI</td>
<td>30 min 1 R</td>
</tr>
<tr>
<td>BNI (2 mg/kg)</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1. Schematic illustration depicting the experimental protocol.
reperfusion with or without a Postcon stimulus (3 cycles of 10-second reperfusion and 10-second reocclusion) initiated at the onset of reperfusion. The selective κ-OR antagonist Nor-BNI (2 mg/kg) was administered intravenously 5 minutes before the reperfusion (as a bolus injection).

**Determination of Myocardial Infarct Size**

The AAR (I/R region) and the IS were measured using Evans blue and 2,3,5-triphenyltetrazolium chloride (TTC) double-staining method. In brief, LAD was reoccluded, and 1 mL of a 2% Evans blue solution was administered into the heart via a catheter at the end of the 2-hour reperfusion. The heart was then removed, weighed, and immediately frozen at −20°C. The heart was cut into 5 transverse slices from the apex with a thickness of about 2 mm. Heart slices were incubated with 1% solution of TTC in phosphate buffer (pH 7.4) at 37°C for 15 minutes and then fixed overnight in 10% formalin solution. The images of heart slices were captured by a high-resolution charge coupled device camera, and the area of infracted myocardium (IS) was digitally measured using SigmaScan. Normal myocardium (area not at risk) is stained blue by Evans blue, and I/R myocardium (AAR) is not stained by Evans blue. Within AAR, I/R but viable myocardium was stained brick red by TTC, and I/R but dead myocardium (infarct) was not stained by TTC. IS was calculated as IS/AAR × 100%.

**Assay of Myocardial Enzyme Leakage**

At the end of reperfusion, blood samples were collected to measure myocardial enzyme leakage, including CK and LDH. Blood samples were centrifuged (3000 rpm, 10 minutes), and the plasma obtained was analyzed spectrophotometrically (Spectrophotometer DU640, Beckman Coulter, Fullerton, CA) for the determination of CK and LDH levels.

**TDT-mediated dUTP Nick End Labeling Assay**

Myocardial apoptosis was quantitatively analyzed by the detection of DNA fragmentation using TUNEL with an apoptosis detection kit. Briefly, the formaldehyde-fixed LV was embedded in paraffin and cut into 4-μm-thick transverse sections and deparaffinized with a graded series of xylene and ethanol solutions. Then, 20 μL of TUNEL reaction mixture was added to samples, and the slides were incubated in a humidified atmosphere for 60 minutes at 37°C in the dark. Twenty microliters of 4,6-diamidino-2-phenylindole (DAPI) was added for 10 minutes at room temperature in a humidified atmosphere. Total cell counts and TUNEL-positive cells in the specimens were determined using a fluorescence microscope (Eclipse 55i, Nikon, Japan). For quantitative purposes, 6 fields from the perinfarct zone were analyzed, and the number of TUNEL-positive cardiomyocytes was counted on 400 high-power fields.

**Measurement of Caspase-3 Protease Activity**

The substrate Ac-DEVD-pNA was used to determine the caspase-3 protease activity according to the manufacturer’s instructions. Myocardial tissue from the AAR at the end of reperfusion was homogenized in ice-cold lysis buffer and then centrifuged at 16,000g for 10 minutes at 4°C. The supernatants were incubated at 37°C for 1.5 hours with 10 μL of caspase-3 substrate (Ac-DEVD-pNA, 2 nmol/L). Substrate cleavage was measured with a spectrophotometer at 405 nm, and the results were expressed as folds of the vehicle group.

**Measurement of Serum and Myocardium Dynorphin Levels**

Immunoreactive dynorphin in blood serum and myocardium was measured by using an antigen-competitive ELISA. Serum from each animal was divided into three 100-μL samples, added to ice-chilled Eppendorf tubes, and kept at −20°C until the day of the experiment. After being trimmed of fat and great vessels, ventricular myocardia were flash frozen in liquid nitrogen and then stored at −80°C until further analysis. Ventricular myocardia were homogenized in phosphate buffered saline with 1 μg/L of PIC (protease inhibitor, Sigma) and centrifuged for 15 minutes at 12,000 rpm at 4°C. Then, 0.1 mL of supernatant was subsequently removed for the determination of protein content using the Bicinchoninic acid assay kit (Pierce, Rockford, IL). The other supernatant was decanted and then stored at −80°C until the analysis of dynorphin content. Dynorphin in blood serum and myocardium was detected using specific ELISA kits. Measurements were conducted in duplicate.

**Statistical Analysis**

ORIGIN8 software was used to analyze the data. The values are expressed as mean ± SD. Student t-test was used to compare differences between groups, and 1-way analysis of variance was used to determine statistical significance of the different groups. The significance level was set at P < 0.05.

**RESULTS**

**Hemodynamic Data**

Hemodynamic parameters were continuously recorded. Table 1 summarizes HR, mean arterial blood pressure (MABP), LVP, left ventricular systolic function (+LVdP/dmax), and diastolic function (−LVdP/dmax) in all groups determined at baseline. 25 minutes of ischemia, and 2 hours of reperfusion. There were no significant differences in HR among all groups during ischemia or the reperfusion period. MABP, LVP, +LVdP/dmax, and −LVdP/dmax were markedly reduced after 25 minutes of ischemia (P < 0.01 vs. baseline) and significantly increased after 2 hours of reperfusion (P < 0.05 vs. ischemia 25 minutes) but did not recover to baseline (P < 0.05 vs. baseline). No significant differences in systemic hemodynamics were observed among all groups under baseline conditions. There were no significant differences between I/R and treatment groups.

**Infarct Size**

Myocardial infarction was clearly present in the I/R group. Postcon significantly reduced myocardial infarction compared with I/R group (P < 0.01). This protective effect was totally abolished by nor-BNI (2 mg/kg) (Figs. 2A, B).
Regional myocardial ischemia for 30 minutes followed by 2 hours of reperfusion markedly increased the release of LDH and CK in blood as compared with the vehicle group (P < 0.01, Figs. 3A, B). Postcon significantly reduced I/R-induced release of LDH and CK (P < 0.01). However, nor-BNI (2 mg/kg) abolished Postcon-induced reduction of LDH and CK. These data suggest that the cardioprotection of Postcon was mediated by activating -OR. We then focused on the antiapoptotic effect in the following studies.

**Effect of Postcon in Plasma Creatine Kinase and Lactate Dehydrogenase**

Cardiomyocyte apoptosis was assessed by TUNEL staining with fluorescence microscopy. The number of apoptotic cells was counted and analyzed on the basis of the results of TUNEL staining (Figs. 4A, B). Regional myocardial ischemia and 2 hours of reperfusion resulted in a significant increase in cardiomyocyte apoptosis (P < 0.01 vs. vehicle). Postcon exerted a significant antialiopoptotic effect (P < 0.01 vs. I/R). This protective effect was attenuated by pretreatment with -OR antagonist Nor-BNI (2 mg/kg, P < 0.01 vs. Postcon). These data suggest that the antialiopoptotic effect of Postcon was mediated by activating -OR. Therefore, we studied the mechanism of -OR–mediated pathways in the following studies.

**Antiapoptotic Effect of Postcon**

Antiapoptotic effect of Postcon

Dissection

Effect of Postcon on Caspase-3 Activity

Because caspase-3 plays an important role in I/R-induced apoptosis, we examined whether caspase-3 was involved in the antiapoptotic effect of Postcon. As shown in Figure 5, caspase-3 activity in the I/R group was significantly enhanced compared with that of the vehicle group (P < 0.01). Caspase-3 activity significantly reduced after Postcon (P < 0.01 vs. I/R). This effect was attenuated by pretreatment with -OR antagonist Nor-BNI (2 mg/kg, P < 0.01 vs. Postcon).

**Postcon Enhances Dynorphin Content in Rat Serum and Rat Heart**

Regional myocardial ischemia and reperfusion significantly reduced immunoreactive dynorphin content in serum (Fig. 6) and in myocardium (Fig. 7). Immunoreactive dynorphin content in serum and myocardium significantly increased after Postcon (P < 0.01 vs. I/R). However, increased dynorphin was not reduced by -OR antagonist Nor-BNI (2 mg/kg, P > 0.05 vs. Postcon).

**DISCUSSION**

In our experiment, we found protective effects of Postcon, which supports previous data obtained in rats, rabbits, and human. Most of the detrimental effects of reperfusion are triggered within the first minutes after the reopening of the occluded coronary artery. This suggests that Postcon strategies need to be applied at this crucial time. In a rat model study by Kin et al, the authors demonstrated that three 10/10-second cycles of Postcon reduced the levels of tissue necrosis factor alpha and interleukin-6, consistent with the attenuation in the number of apoptotic cells in area-at-risk (AAR) myocardium. You et al suggested that ischemic Postcon reduced I/R-induced cardiomyocyte apoptosis in
rabbits. In a clinical trial, Zhao et al found that three 60/60-second cycles of Postcon significantly reduced soluble plasma Fas/APO-1 and Fas ligand, the surrogate markers for myocardial apoptosis. These beneficial effects were accompanied by an improvement in left ventricular function 7 days after stenting.20

There are many endogenous ligands contributing to Postcon-mediated tissue protection, including adenosine, opioid, bradykinin, erythropoietin, etc. There is emerging evidence that endogenous ligand binding at the time of myocardial reperfusion may contribute to the protection elicited by Postcon. Reports showed that Postcon was involved in the endogenous activation of adenosine receptors.22,23 Penna et al24 suggested that Postcon triggering includes bradykinin receptor activation and its downstream pathway. The infarct-limiting protection of Postcon was attenuated in adenosine receptor and bradykinin receptor gene knockout mice. It is believed that Postcon-induced infarct-limiting cardioprotection may be triggered by the activation of multiple types of cell membrane receptors, which include adenosine and bradykinin receptors.25 Opioids have been shown to open the adenosine triphosphate sensitive potassium channels of mitochondria.26 Chien et al27 showed that the active stereoisomer of naloxone blocked IPC in anesthetized rabbit hearts, whereas the inactive stereoisomer of naloxone had no effect on IPC. IPC caused a significant decrease in TUNEL-positive cardiomyocytes and inhibited the fragmentation of genomic DNA in the ischemic zone compared with vehicle hearts. Naltrexone, a selective δ-opioid receptor (δ-OR) antagonist, reversed the antiapoptotic effect of IPC before treatment.8 Zattas et al9 confirmed the integral involvement of the endogenous opioid, enkephalin, in the induction of Postcon. In the rat in vivo study, Jang et al7 reported an abolished Postcon-induced infarct reduction with the nonselective OR antagonist naloxone. U50,488H (a selective κ-OR agonist) given 5 minutes before reperfusion significantly reduced IS in rat and mouse hearts.28 Chen et al29 implicated morphine-induced Postcon in the isolated rat hearts subjected to I/R by κ-OR activation but not by δ-OR activation. Thus, experimental evidence generally supports the fact that κ-OR activation is protective during early reperfusion when activated by exogenously administered agonists. Our previous experiments found that the pharmacological effect of Postcon was abrogated by the κ-OR antagonist nor-BNI in rat heart,30,31 this phenomenon implies that Postcon may protect the myocardium against I/R injury via activating κ-ORs. Wang et al32 abolished Postcon-induced cardiac protection with the selective κ-OR antagonist nor-BNI. But they did not elucidate whether antiapoptosis played a role in the cardiac protection of Postcon triggered by κ-OR.
It is well known that apoptosis plays an important role in myocyte cell death after I/R. The importance of apoptosis in cell death after reperfusion has been demonstrated in vivo in rodent models. Yaoita et al. evaluated the effect of caspase inhibitors on IS in a rat model of I/R, and they found that ZVAD-fmk (a broad-spectrum caspase inhibitor) demonstrated an almost 21% reduction in IS and 72% reduction in apoptotic cells. Preservation of left ventricular function may be a result of the inhibition of detrimental effects of caspases on contractile machinery. In our experiment, we also found that the intrinsic activation of \( \kappa \)-OR triggered the cardioprotective effects of Postcon. Postcon not only significantly decreased cardiac necrosis but it also reduced myocardial apoptosis. More interestingly, the antiapoptotic effect was also inhibited by Nor-BNI administered just before reperfusion, indicating that \( \kappa \)-OR triggered the antiapoptotic effect of Postcon. Our previous experiments found that U50,488H postconditioning reduced myocardial cell apoptosis, which supports our study that \( \kappa \)-OR triggered the antiapoptotic effect of Postcon.

It is well known that caspases are the central molecular initiators and executors of apoptosis. Caspase-3 is one of the effectors’ caspases, which is believed to be a final killer of apoptosis. There is a large amount of evidence indicating that cardiac I/R can induce the activation of caspases including caspase-3, which have been found to precede the death of cardiomyocytes. In our present study, we found that Postcon significantly inhibited caspase-3 and that the effect was abolished by nor-BNI. These results provide further evidence that the intrinsic activation of \( \kappa \)-OR trigger the antiapoptotic effect of Postcon.

**FIGURE 4.** Effect of postconditioning on cardiomyocyte apoptosis. A, Representative apoptotic staining from 6 experiments. TUNEL: cardiomyocyte apoptotic staining; DAPI: nuclei staining; Merge: TUNEL staining merges with DAPI staining. B, Bar chart shows the percentage of apoptotic cells as assessed by TUNEL staining in each group (n = 6 in each group). Values are the mean ± SD. \( P < 0.01 \) versus vehicle (**), versus I/R (##), and versus postconditioning ($$).
Postcon after long periods of ischemia may alter the production of endogenous autacoids such as adenosine and bradykinin. Kin et al\textsuperscript{22} suggested that endogenously released adenosine is involved in the cardioprotection of Postcon. Opioid receptors have not only been implicated in protecting the heart from ischemic events,\textsuperscript{28} but opioids have also been demonstrated to provide cardiac protection against I/R injury.\textsuperscript{35} Romano et al\textsuperscript{36} found that hearts, pretreated with U50,488H at reperfusion, demonstrated significantly improved functional recovery versus vehicles and significantly depressed recovery with nor-BNI pretreatment. Their study demonstrates that selective \(\kappa\)-OR agonists provide significant myocardial protection. In our previous study,\textsuperscript{32} we administered a selective \(\kappa\)-OR agonist, U50,488H, just before reperfusion, and we found that it markedly reduced myocardial apoptotic death in rats. This experiment provided indirect evidence that the increased levels of endogenous \(\kappa\)-OR agonist, dynorphin, may play a role in the cardioprotective effect. It is well known that opioid peptide levels increase and are ultimately released into the peripheral circulation during stressful conditions.\textsuperscript{37} Myocardial ischemia has been shown to induce the synthesis and release of opioid peptides.\textsuperscript{9} Dynorphin shows a greater increase than met-enkephalin, although this difference does not reach statistical significance.\textsuperscript{38} Why do endogenous opioid peptide (EOPs) levels increase in an ischemic organ? It has been speculated that the increased levels of EOPs in infarcted ventricular tissue may counteract the high amount of catecholamine released during ischemia. Therefore, the heart under stress (ie, ischemia) may involve an autocrine process in which EOPs are released from cardiomyocytes and interacts directly with myocardial ORs to limit cellular injury by protecting the heart from \(Ca^{2+}\) overload. However, dynorphin levels rapidly fall far below the baseline level at reperfusion, whereas met-enkephalin returns to the baseline level.\textsuperscript{38} Some studies showed that the signaling pathways activated by \(\beta\)-adrenoceptor stimulation\textsuperscript{39} may be significantly inhibited by \(\kappa\)-OR stimulation.\textsuperscript{40} Our previous studies found that \(\kappa\)-OR inhibited the effect of adrenoceptor stimulation.\textsuperscript{41,42} Postcon may facilitate this opioid effect in part by protecting the enzymes responsible for the synthesis and processing of proenkephalin.\textsuperscript{9} Zhang et al\textsuperscript{43} measured the plasma levels of dynorphin in rats subjected to remote preconditioning (RPC) and found that the plasma dynorphin level was significantly increased. Cardioprotection by RPC was mimicked by intravenously administered dynorphin and U50,488H. Cardioprotective effects induced by RPC and U50,488H were attenuated by nor-BNI. Our experiment found that Postcon increased the dynorphin level in both the serum and the myocardium. Although the cardioprotective effects of Postcon, such as reduced enzyme leakage, inhibited the effect of apoptosis and were inhibited by nor-BNI, dynorphin level was not significantly reduced by nor-BNI. These results suggest that Postcon induced the release of endogenous \(\kappa\)-OR agonist dynorphin and that dynorphin may participate in this form of cardioprotection. In our experimental study, there is evidence that increased dynorphin may play a role in the cardioprotective effect. Further, experimental evidence is
required to demonstrate that they play a role and that the circulating levels can actually have a biological effect on the cardiomyocytes.

Collectively, these data suggest that the protection induced by Postcon, including antiapoptosis, is functionally associated with κ-OR activation by local opioids and that maintaining adequate supplies of dynorphin may be an integral part of the process.

The dynorphin increase of Postcon may result from several possibilities including (1) improved prodynorphin mRNA resulting in relatively more translation of the dynorphin precursors; (2) increased processing of receptor-active dynorphin from larger prodynorphin precursors; (3) relatively decreased release of dynorphin into blood; or (4) decreased rate of degradation of dynorphin. According to the study of Zatta et al., the most possible reason for an increase in endogenous opioid is due to an increase in the synthesis of total protein. Further studies are needed to identify the signals required for increased dynorphin production, processing and release in the myocardium.

Recently, the clinical use of opioid peptide as a treatment for cardiovascular disease has been attracting increasing attention. The study of κ-OR agonists involved in I/R may provide a new insight into the treatment of ischemic heart disease.

CONCLUSIONS

This is the first report that elucidates the mechanism of the antiapoptotic effect of Postcon triggered by κ-opioid peptide. We find that cardiac protection and the antiapoptotic effect of Postcon are mediated by κ-OR activation. Cardioprotective and antiapoptotic effects of Postcon are mediated, at least in part, by enhanced dynorphin expression.

ACKNOWLEDGMENTS

We thank Sharon Morey, Scientific Editor, for assistance in writing of the manuscript.

REFERENCES