Int J Clin Exp Med 2016;9(8):15884-15891
www.ijcem.com /ISSN:1940-5901/IJCEM0029329

Original Article

Morphine postconditioning protection against reperfusion injury: PKC epsilon and extracellular signal-regulated kinase 1 and 2

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Received March 28, 2016; Accepted June 21, 2016; Epub August 15, 2016; Published August 30, 2016

Abstract: The purpose of this study was to determine whether protein kinase C-epsilon (PKCε) and extracellular signal-regulated kinase (ERK) 1/2 were involved in morphine postconditioning (MpostC) in isolated rat hearts. The isolated rat hearts were randomly assigned into one of the following groups. Hearts in the time control (TC) group were constantly perfused with K-H buffer for 105 min. Hearts in the ischemia-reperfusion (I/R) group were subjected to 45 min of ischemia followed by 1 h of reperfusion. MpostC was induced by 10 min of morphine administration at the onset of reperfusion. In the other four groups, $\varepsilon V_{1\text{-}2}$ and PD98059 were administered with or without morphine during the first 10 min of reperfusion following the 45 min of ischemia. I/R injury was assessed by functional parameters, creatine kinase-MB (CK-MB) release and infarct size (IS/AAR). Additional hearts were excised at 20 min following reperfusion in all groups to detect PKCε activation and ERK1/2 phosphorylation. MpostC markedly reduced CK-MB release and IS/AAR, and improved cardiac functional recovery. However, these protective effects were partly abolished in the presence of either $\varepsilon V_{1\text{-}2}$ or PD98059. The membrane translocation of PKCε and the phosphorylation of ERK1/2 were increased in the I/R hearts, and further enhanced by MpostC. $\varepsilon V_{1\text{-}2}$, whether used alone or together with morphine, eliminated translocation of PKCε. The phosphorylation ERK1/2 was also abolished in the presence of $\varepsilon V_{1\text{-}2}$ or PD98059, which was also administered either individually or together with morphine. These findings suggested that morphine postconditioning protected isolated rat hearts against ischemia-reperfusion injury via the recruitment of the PKCε-ERK1/2 signaling pathway.

Keywords: Morphine, postconditioning, reperfusion injury, protein kinases cepsilon, extracellular signal-regulated kinase

Introduction

In a previously study, we demonstrated that administration of morphine immediately at the onset of reperfusion, namely morphine postconditioning (MpostC), reduced the infarct size to an extent similar to morphine preconditioning (MPC) *via* activating κ-opioid receptor (OR) in isolated rat hearts [1]. However, the post-receptor signaling mechanisms involved in MpostC remained poorly defined.

One family of signaling proteins commonly linked to the modulation of ischemia-reperfusion injury is protein kinase C (PKC), particularly the PKC isozyme PKCε [2]. Previous research demonstrated that membrane translocation of PKCε was critical to triggering cardioprotective effects in both ischemic preconditioning (preC) [3] and ischemic postconditioning (postC) [4, 5]. Furthermore, activation of PKCε is also involved in the cardioprotective effect produced by opioid receptor agonist induced PC [6, 7]. Conversely, few studies have yet to verify whether or not PKCε is also involved in opioid receptor agonist induced posC, or identify the potential downstream targets of PKCε activation.

Extracellular signal-regulated kinase (ERK) 1/2 is one important component of reperfusion injury survival kinases (RISK), which are activated at the time of reperfusion and afforded opportunity for protecting the heart against

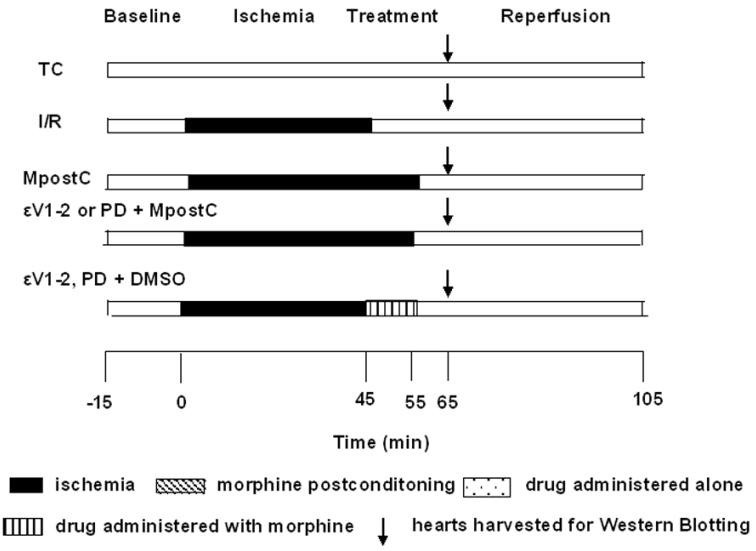

Figure 1. Scheme of treatment protocols. Hearts in the time control (TC) group were constantly perfused with K-H buffer for 105 min. Hearts in other groups were subjected to 45 min of ischemia and 60 min of reperfusion. Morphine postconditioning (MpostC) was performed by a 10-min perfusion of morphine (3×10^{-6} M) immediately at the onset of reperfusion. $\varepsilon V_{1\text{-}2}$ (1×10^{-6} M), PD (PD98059, 1×10^{-5} M), and DMSO (dimethyl sulfoxide, < 0.1%) were administered alone or together with morphine. Left ventricular tissue samples were collected for western blotting 20 min after reperfusion, and infarct size and CK-MB releases were measured at the end of reperfusion.

lethal reperfusion-induced injury [8]. Evidence shows that the activation of ERK1/2 was implicated in both preC [9] and postC [10], indicating that preC and postC possibly recruit a common signal pathway during myocardial reperfusion. Furthermore, recent studies suggested that the phosphorylation of ERK1/2 was involved in opioid receptor agonist induced postC [11, 12], though the exact mechanism through which ERK1/2 was activated remains unclear.

The activation of ERK1/2 is regulated by phosphorylation, and this process was shown to be PKC-dependent [13]. Additionally, the PKCε-ERK1/2 signaling pathway was involved in the protective effects of ischemic PC [9]. However, whether MpostC also shares the same RISK pathway is still unclear. Therefore, the objective of the present study was to determine (1) whether the membrane translocation of PKCε and ERK1/2 phosphorylation is involved in MpostC, and (2) whether the two events are linked.

Material and methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the Affiliated Hospital of Qingdao University and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* from the Institute of Laboratory Animals Resources. All reagents, unless specified, were obtained from Sigma Chemicals (St. Louis, MO).

Isolated heart preparation

These methods were similar to those used in previous experiments [1]. Briefly, male Sprague-Dawley rats weighing 180-200 g were anesthetized with an intraperitoneal injection of 40 mg/kg sodium pentobarbital and decapitated. The hearts were removed rapidly and mounted on a non-circulating Langendorff apparatus and underwent retrograde perfusion at 100 cm H_2O with Krebs-Ringer's solution (115 Mm NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose) gassed with 95% O_2-5% CO_2 (pH 7.4, temperature 37°C). An incision was made in the left atrium, and a fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon was connected to a pressure transducer for continuous monitoring of left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and heart rate (HR) via PowerLab Systems (PowerLab/8sp, AD Instruments, Australia). In the first 15 min of perfusion, the heart was allowed to stabilize, and any heart showing intractable arrhythmia or low left ventricular systolic pressure (LVSP) < 50 mmHg was excluded from the study.

Experimental protocols

The study consisted of 2 experimental protocols (**Figure 1**). All hearts were subjected to 45 min global ischemia and 60 min (protocol 1) or 20 min (protocol 2) of reperfusion after a 15-min stabilization period. MpostC was produced by a 10-min perfusion of morphine immediately at the onset of reperfusion.

Protocol 1: Survival kinases in MpostC-induced cardioprotection: To assess the potential roles of PKCε and ERK1/2 in MpostC-induced cardioprotection, hearts were randomly assigned to 1 of 8 groups (n = 8, respectively): (1) TC group (time control); (2) I/R group (ischemic control); (3) MpostC group (morphine, 3.0×10^{-6} mM); (4) I/R + $\varepsilon V_{1\text{-}2}$ group (PKCε inhibitor, 1×10^{-6} mM); (5)$\varepsilon V_{1\text{-}2}$ + MpostC group; (6) I/R + PD98059 group (ERK1/2 inhibitor, 1×10^{-5} mM); (7) PD98059 + MpostC group; (8) I/R + DMSO group (dimethyl sulfoxide, < 0.1%).

Protocol 2: Assessment of survival kinases: To assess the membrane translocation of PKCε and ERK1/2 phosphorylation in MpostC by standard Western blotting, additional hearts were used 20 min after reperfusion of the above 8 groups (n = 5, respectively). Left ventricular tissue samples were immediately collected, frozen in liquid nitrogen, and stored at -80°C until processing. Preliminary experiments in our model showed that PKCε membrane translocation and ERK1/2 phosphorylation consistently increased after 20 min of reperfusion and that differences between experimental groups were most pronounced at this time point.

The chemicals were perfused with or without morphine for a period of 10 min at the onset of reperfusion. Morphine, $\varepsilon V_{1\text{-}2}$, PD98059 and DMSO were all purchased from Sigma-Aldrich (Sigma-Aldrich, CA, USA), and the concentrations used in this study were based on previous studies [1, 10, 14].

Myocardial infarct size and myocardial injury

Myocardial infarct size and myocardial injury were measured in accordance to previous research [1]. In short, infarct size was determined by 2,3,5-triphenyltetrazolium chloride (TTC) staining, and then determined by dividing the total necrotic area of the left ventricle by the total left ventricular slice area (IS/AAR). Additionally, the release of creatine kinase-MB (CK-MB) was measured by collecting total coronary effluent over the 60 min reperfusion period, and expressed as U/h/g.

Cytosolic and particulate fraction preparation

To analyze cytosolic protein contents and particulate fraction of the myocardium, frozen samples were homogenized with a pestle motor at 0°C-4°C in buffer A consisting of 50 mM Tris-Cl, pH 7.5, 2 mM EDTA, 2 mM EGTA, 1 mM dithiothreitol, and inhibitors for protease and phosphatase (5 mg/mL each of leupeptin, aprotinin, pepstatin A, chymostatin, 50 mM KF, 50 mM okadaic acid, 5 mM sodium pyrophosphate) (Kontes; Sigma). These homogenates were centrifuged at 300,000 g for 30 minutes at 4°C in an Optima TLX Ultracentrifuge (Beckman Coulter CO.) to yield the cytosolic fraction. Resuspended in buffer B (buffer A + 0.5% Nonidet P-40), the crude membrane fraction pellet was sonicated and then centrifuged at 300,000 g for 30 minutes at 4°C. The final supernatant was the particulate fraction. To analyze protein expression in whole tissue homogenate, myocardium was homogenized and sonicated in buffer C (buffer A + 2% sodium dodecyl sulfate). The protein concentrations were measured using a BCA protein assay kit (Pierce, Rockford, IL) and bovine serum albumin as standards.

SDS polyacrylamide gel electrophoresis and western blot

To determine changes in cytosolic PKCε contents, the particulate fraction, and whole tissue homogenate, 40 μg of protein from each sample was loaded per lane in 10% SDS polyacrylamide gel electrophoresis (PAGE) gels. After being electrotransferred and blocked, the membrane was incubated overnight at 4°C with the primary antibody anti-PKCε (1:1000) and a secondary antibody conjugated with horseradish peroxidase (1:1000; Santa Cruz Biotechnology Inc., CA, USA) at room temperature. After washing three times, bands were detected using ECL-plus reagents (Pharmacia Biotech, Piscataway, NJ). To check the slight variation in protein loading between samples, western blot for β-actin was performed as an internal control. The relative optical density of bands from each sample was normalized against that of β-actin, and results were expressed as a percentage of total PKCε, which were then translated into a percentage of TC.

To determine the changes of ERK1/2 phosphorylation, 80 μg of protein from the whole tissue homogenate was loaded per lane in 8% SDS-PAGE gel. The primary antibodies specific toERK1/2 and phosphorylated ER $K1/2^{Thr\text{-}202/Tyro\text{-}204}$ were purchased from Santa Cruz (Santa Cruz Biotechnology Inc., CA, USA). The phos-

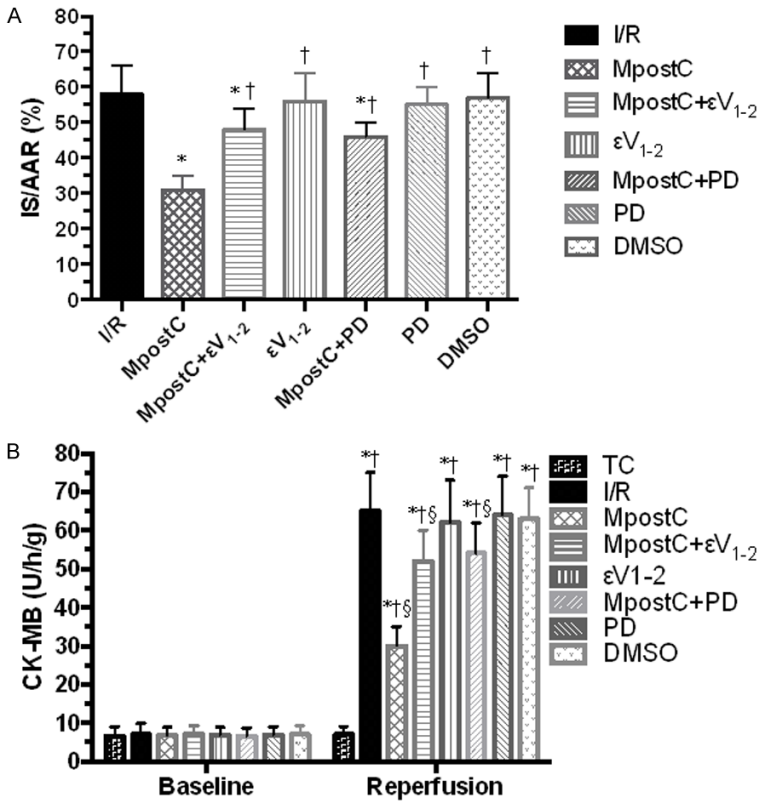

Figure 2. A. Effects of various treatments on infarct size, expressed as a percentage of the area at risk (IS/AAR). The time control (TC) group was not included because no obvious necrotic area was measured. Values are presented as mean ± SD. $^{*}P < 0.05$ versus I/R, $^{\dagger}P < 0.05$ versus MpostC. B. Effects of various treatments on CK-MB release. Values are presented as mean ± SD. $^{*}P < 0.05$ versus baseline, $^{\dagger}P < 0.05$ versus time control (TC), $^{\S}P < 0.05$ versus I/R. I/R, ischemia reperfusion; MpostC, morphine postcondtioning (3×10^{-6} M); $\varepsilon V_{1\text{-}2}$, PKCε inhibitor ($1\times10^{-6}$ M); PD, ERK1/2 inhibitor (1×10^{-5} M); DMSO, dimethyl sulfoxide.

phorylation levels of ERK1/2 were expressed as a percentage of ERK1/2.

Statistical analysis

Statistical analysis of infarct size and PKCε or ERK1/2 measurements was performed by Student's *t* test with Bonferroni's correction for multiple comparisons. Hemodynamics was analyzed using two-way repeated measures analysis of variance for time and treatment effects. If an overall significance was found, comparisons between groups were done for each time point using one-way analysis of variance, followed by Tukey *post hoc* testing. Time effects within each group were analyzed using repeated measures analysis of variance, followed by Dunnett *post hoc* testing, with the baseline value as the reference time point. Statistical differences were considered significant when $P < 0.05$. Data was expressed as means ± SD.

Results

PKCε and ERK1/2 in MpostC

Compared to the I/R group, MpostC markedly reduced IS/AAR ($P < 0.05$) (**Figure 2A**) and CK-MB ($P < 0.05$) (**Figure 2B**). However, the protective effects offered by morphine were partly reversed by co-administration of either $\varepsilon V_{1\text{-}2}$ or PD98059 ($P < 0.05$), which, when given alone, showed no influence ($P > 0.05$) (**Figure 2**).

The baseline hemodynamics was similar in all experimental groups ($P > 0.05$), and all variables remained constant in the TC group during the experiment (**Table 1**). Compared to baseline, the cardiac function in the other seven groups deteriorated, showing an obvious reduction in CF, HR, and LVDP, and a significant increase in LVEDP at 10, 30, and 60 min of reperfusion ($P < 0.05$). All hemodynamic variables were better in the MpostC group than in the I/R group ($P < 0.05$). However, the functional improvements elicited by MpostC were partly reversed by co-administration of $\varepsilon V_{1\text{-}2}$ and PD98059, each of which did not influence cardiac recovery when used alone ($P > 0.05$) (**Table 1**).

Membrane translocation of PKCε

Compared with TC hearts, the membrane translocation of PKCε from the cytosolic fraction to the particulate increased ($P < 0.05$) (**Figure 3**). However, MpostC further enhanced membrane translocation of PKCε when compared to the I/R hearts ($P < 0.05$) (**Figure 3**). $\varepsilon V_{1\text{-}2}$, administered alone or together with morphine significantly inhibited membrane translocation of PKCε ($P < 0.05$) (**Figure 3**), suggesting that the activation of PKCε might be involved in MpostC.

Table 1. Hemodynamic parameter

	Baseline	Reperfusion		
		10 min	30 min	60 min
CF, ml/min				
TC	11.0 ± 1.3	10.8 ± 1.1	10.5 ± 1.2	10.2 ± 1.3
I/R	10.7 ± 1.5	4.5 ± 0.5*,†	4.6 ± 0.4*,†	4.5 ± 0.8*,†
MpostC	11.2 ± 1.2	9.3 ± 1.4*,†,§	9.2 ± 1.0*,†,§	9.0 ± 0.9*,†,§
MpostC + $\varepsilon V_{1\text{-}2}$	10.8 ± 0.6	6.5 ± 0.5*,†,§	6.8 ± 0.8*,†,§	6.5 ± 0.6*,†,§
$\varepsilon V_{1\text{-}2}$	10.8 ± 1.2	4.8 ± 0.5*,†	4.9 ± 0.5*,†	4.8 ± 0.8*,†
MpostC + PD	10.6 ± 0.6	6.8 ± 0.5*,†,§	6.8 ± 0.8*,†,§	6.6 ± 0.6*,†,§
PD	10.9 ± 1.0	4.6 ± 0.5*,†	4.5 ± 0.6*,†	4.6 ± 0.2*,†
DMSO	10.8 ± 1.0	4.8 ± 0.5*,†	4.8 ± 0.7*,†	4.5 ± 0.4*,†
HR, beats/min				
TC	295 ± 12	290 ± 18	292 ± 15	288 ± 13
I/R	298 ± 15	165 ± 15*,†	168 ± 18*,†	160 ± 12*,†
MpostC	298 ± 11	238 ± 16*†§	236 ± 18*†§	230 ± 13*†§
MpostC + $\varepsilon V_{1\text{-}2}$	300 ± 14	185 ± 20*,†,§	188 ± 20*,†,§	182 ± 12*,†,§
$\varepsilon V_{1\text{-}2}$	296 ± 15	169 ± 17*,†	165 ± 16*,†	162 ± 13*,†
MpostC + PD	300 ± 14	187 ± 18*,†,§	190 ± 20*,†,§	185 ± 15*,†,§
PD	301 ± 15	166 ± 16*,†	165 ± 12*,†	166 ± 15*,†
DMSO	295 ± 16	170 ± 18*,†	160 ± 14*,†	162 ± 13*,†
LVDP, mmHg				
TC	120 ± 10	118 ± 15	116 ± 12	115 ± 10
I/R	118 ± 12	54 ± 10*,†	60 ±10*†	58 ± 8*,†
MpostC	120 ± 8	90 ± 10*,†,§	88 ± 8*,†,§	86 ± 5*,†,§
MpostC + $\varepsilon V_{1\text{-}2}$	119 ± 13	68 ± 10*,†,§	67 ± 8*,†,§	62 ± 5*,†,§
$\varepsilon V_{1\text{-}2}$	121 ± 11	55 ± 9*,†	60 ± 8*,†	60 ± 8*,†
MpostC + PD	116 ± 12	68 ± 8*,†,§	69 ± 10*,†,§	65 ± 5*,†,§
PD	117 ± 10	54 ± 10*,†	61 ± 11*,†	56 ± 6*,†
DMSO	118 ± 7	59 ± 11*,†	60 ± 7*,†	58 ± 7*,†
LVDEP, mmHg				
TC	4.1 ± 0.5	4.5 ± 0.5	4.2 ± 0.6	4.6 ± 0.3
I/R	4.2 ± 0.6	42.8 ± 7.0*,†	45.1 ± 6.8*,†	45.5 ± 6.5*,†
MpostC	4.1 ± 0.5	18.6 ± 5.0*,†,§	18.5 ± 6.5*,†,§	19.0 ± 5.8*,†,§
MpostC + $\varepsilon V_{1\text{-}2}$	4.5 ± 0.6	31.4 ± 6.0*,†,§	32.0 ± 5.0*,†,§	32.2 ± 7.2*,†,§
$\varepsilon V_{1\text{-}2}$	4.0 ± 0.2	42.3 ± 6.0*,†	44.7 ± 5.8*,†	45.0 ± 6.1*,†
MpostC + PD	4.6 ± 0.6	32.0.4 ± 6.5*,†,§	32.0 ± 5.0*,†,§	33.2 ±4.7*,†,§
PD	4.4 ± 0.5	41.3 ± 5.8*,†	42.4 ± 5.0*,†	43.0 ± 4.9*,†
DMSO	4.3 ± 0.4	42.5 ± 5.5*,†	42.7 ± 5.7*,†	43.0 ± 6.0*,†

Baseline = 15 min after stabilization; CF = coronary flow; $\varepsilon V_{1\text{-}2}$ = PKCε inhibitor; I/R = control; DMSO = dimethyl sulfoxide (vehicle; < 0.1%); HR = heart rate; LVDP = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; MpostC = morphine postconditioning; PD = PD98059. Data are presented as mean ± SD (n = 8/group). $^{*}P < 0.05$ versus baseline (intragroup comparison), $^{\dagger}P < 0.05$ versus respective value in the TC group (intergroup comparison), $^{\S}P < 0.05$ versus respective value in the I/R group (intergroup comparison).

ERK1/2 phosphorylation

Compared to the TC group, the results from western blotting showed increased ERK1/2 phosphorylation in I/R hearts ($P < 0.05$) (**Figure 4**). However, MpostC further enhanced ERK1/2 phosphorylation when compared to the I/R hearts ($P < 0.05$) (**Figure 4**). Both $\varepsilon V_{1\text{-}2}$ and PD98059, administered alone or together with morphine, reduced ERK1/2 phosphorylation ($P < 0.05$) (**Figure 4**), suggesting that PKCε might play a critical role in stimulating ERK1/2 activation. Lastly, DMSO did not influence ERK1/2 phosphorylation ($P > 0.05$) (**Figure 4**), and the total ERK1/2 did not differ among groups.

Discussion

In the present study, we investigated the role of PKCε and ERK1/2 in morphine-induced postC in the rat heart *in vitro*. Findings were summarized as follows: (1) Both PKCε and ERK1/2 were involved in MpostC; (2) Subsequent western blot analysis showed that MpostC significantly enhanced PKCε membrane translocation and ERK1/2 phosphorylation, and these effects were completely blocked by $\varepsilon V_{1\text{-}2}$, a selective PKCε inhibitor, demonstrating that PKCε might be an upstream regulator of ERK1/2. Taken together, the present study illustrated that the PKCε-ERK1/2 signaling pathway was involved in MpostC, and provided new insight into opioid receptor agonist-induced postC in myocardium.

Considering most ischemic events are unpredictable in clinical practice, ischemic postC is of great clinical interest because it is performed during the onset of the reperfusion period.

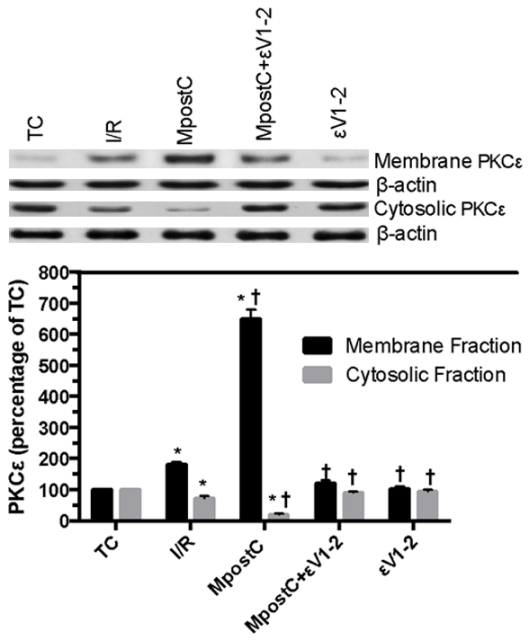

Figure 3. Effects of various treatments on membrane translocation of PKCε. The upper panel shows the representative membrane and cytosolic PKCε in each of the five groups. The densities of PKCε were normalized against that of β-actin, and results were expressed as a percentage of the time control group, as shown in the lower pane. TC, time control; I/R, reperfusion injury; MpostC, morphine postcondtioning (3×10^{-6} M); $\varepsilon V_{1\text{-}2}$, PKCε inhibitor ($1\times10^{-6}$ M). Values are presented as mean ± SD (n = 5). $^{*}P < 0.05$ *versus* TC, $^{\dagger}P < 0.05$ versus I/R.

However, it is difficult to routinely apply intermittent episodes of myocardial ischemia-reperfusion during the first moments of reperfusion in clinical practice. Using a pharmacological postC strategy, we previously showed that morphine-induced postC significantly reduced myocardial reperfusion injury [1].

The activation of PKC is associated with membrane translocation from the cytosol to the particulate fraction of the cell [15], and the activation of PKC, in particular, the PKCε isoform, is pivotal in protecting hearts from ischemia reperfusion injury in ischemic preC [3]. It was suggested that the infarct-sparing effect of ischemic postC is also dependent on the activation of PKCε [4, 5]; moreover, this protective effect was associated with endogenous opioid peptides [16-18]. The present study provides new evidence that the activation of PKCε is also involved in exogenous opioid receptor agonist-induced (morphine)postC in myocardium.

As an important reperfusion injury salvage kinase (RISK), ERK1/2 plays a pivotal role in protecting the heart against ischemia reperfusion injury [19]. Evidence indicates that the protective effects of ischemic and anesthetic postC were conferred by ERK1/2 phosphorylation [10, 20]. The present study demonstrated that MpostC protected the hearts against reperfusion injury through the phosphorylation of ERK1/2, and our findings were consistent with what Ha et al. [11] and Kim et al. [12] suggested using a RpostC (remifentanil, an exogenous opioid receptor agonist) strategy.

However, the mechanisms through which ERK1/2 is activated in postconditioned hearts are still unclear. Increasing evidence indicates that PC and postC appear to recruit a common signaling pathway during myocardial reperfusion [21]. The activation of ERK1/2 is regulated by phosphorylation, and this process was shown to be PKC-dependent [13]. Furthermore, the PKCε-ERK1/2 signaling cascade was shown to be involved in the protective effects of ischemic PC [9, 22]. Using a MpostC strategy, the present study reinforced these findings by illustrating that $\varepsilon V_{1\text{-}2}$ selectively blocked PKCε translocation and inhibited the phosphorylation of ERK1/2. This offered new insights into the post-receptor signaling pathway in opioid receptor agonist-induced postC.

On the contrary, there are experimental findings different from ours. It was demonstrated that ERK1/2-dependent activation of PKCε was involved indesfluranepreC induced protective effects in in vivo rat hearts [23]. Furthermore, Penna et al. suggested that acid postC rotected against myocardial I/R injury by activating ERK1/2-PKCε pathways in isolated rat hearts [24]. Though the differences of treatments (preC versus postC), dugs (morphine versus desflurane or acid perfusion), and animal models (in vitro versus in vivo) might account for the inconsistent observations, we cannot exclude the presumption that there might be an ERK1/2-PKCε positive feedback regulation. Namely, that PKCε dependently phosphorylates ERK1/2 while the phosphorylated ERK1/2 in turn activates PKCε. More evidence is necessary to further verify this presumption.

The current results must be interpreted within the constraints of potential limitations: (1) In contrast to an *in vivo* model, isolated hearts

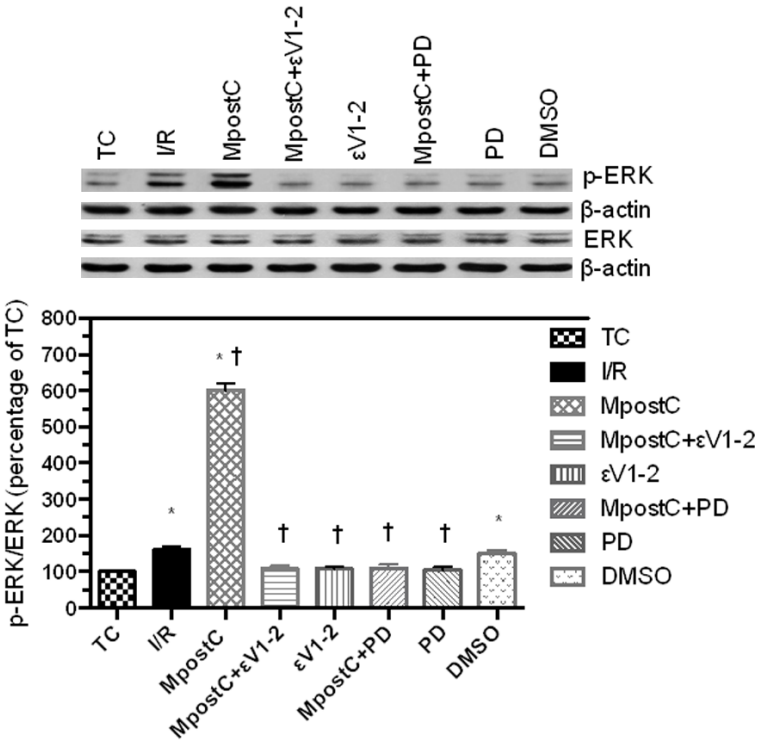

Figure 4. Effects of various treatments on ERK1/2 phosphorylation. The upper panel shows representative ERK1/2 phosphorylation of each of the eight groups. The densities of phosphorylated ERK1/2 were normalized against that of the total ERK1/2, as shown in the lower panel. TC, time control; I/R, reperfusion injury; MpostC, morphine postcondtioning (3×10^{-6} M); $\varepsilon V_{1\text{-}2}$, PKCε inhibitor ($1\times10^{-6}$ M); PD, ERK1/2 inhibitor (1×10^{-5} M); DMSO, dimethyl sulfoxide. Values are presented as mean ± SD (n = 5). $^{*}P < 0.05$ *versus* TC, $^{\dagger}P < 0.05$ versus I/R.

have limited long-term biologic stability and may undergo short confounding ischemic periods during the surgical procedure, which could potentially affect observations. Therefore, it is inappropriate to directly extrapolate our results to *in vivo* conditions. (2) The chemicals ($V_{1\text{-}2}$, and PD98059) were co-administered with morphine, and considering their differences in pharmacodynamics, it is possible that the chemicals may not have completely responsible for the pharmacological effects on MpostC, and this must be considered in the analysis.

In conclusion, the present study shows that morphine postconditioning protects isolated rat hearts against ischemia-reperfusion injury via the recruitment of the PKCε-ERK1/2 signaling pathway, and provides new insight into the post-receptor signal transduction mechanism involved in opioid receptor agonist-induced postC in myocardium.

Acknowledgements

This work is supported by funds from grants from Shandong Province science and technology plan (2010GSF-1023).

Disclosure of conflict of interest

None.

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