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Evidence of Protein Kinase C Translocation by Ischemic Preconditioning in Global Ischemia Model

We tested recent evidence that ischemic preconditioning (PC) involves in translocation of protein kinase C (PKC) from the cytosol to myocyte membrane. Isolated Langendorff-perfused rabbit hearts (n=96) were subjected to 60 or 45 min of ischemia (I) and 120 min of reperfusion (R) with or without PC (4 cycles of 5 min I and 5 min R; or single dose of 5 min I and 10 min R), respectively. Left ventricular function and infarct size (IS) were measured; myocardial cytosolic and membrane PKC activity were determined by ^{32}P-γ-ATP incorporation into PKC-specific peptide. PC enhanced improvement of functional recovery and reduced IS (26.9±1.4% versus 15.3±1.9%, p<0.01, in 60 min of I; 18.3±2.6% versus 8.6±2.5%, p<0.05, in 45 min of I); cytosolic PKC activity decreased 74% of total activity (p<0.05) both in 60 and 45 min of I; membrane PKC activity increased (1.7-fold of baseline, p<0.01, in 60 min of I; 1.8-fold, p<0.01, in 45 min of I; 1.5-fold, p<0.05, in 60 of min I and 120 min of R). From these results, it is concluded that translocation of PKC from the cytosol to myocyte membranes is an important mechanism responsible for PC effect.

Key Words : *Cardiac function; Infarction; Ischemic preconditioning; Rabbit; Heart; Protein kinase C*

Hyun Kim, Dae-Joong Kim, Hee-Soo Chung, Sang-Joon Shim,* Un-Ho Yoo,* Bong-Jin Rah, Ho-Dirk Kim

Departments of Histology and Internal Medicine*, College of Medicine, Chung-Ang University, Seoul, Korea

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Address for correspondence
Dr. Ho-dirk Kim
Department of Histology, College of Medicine, Chung-Ang University, Seoul 156-756, Korea
Tel : +82.2-820-5646, Fax : +82.2-815-4814
E-mail : cardio2@cau.ac.kr

INTRODUCTION

It is a well established fact that brief periods of ischemia and intervening reperfusion before a subsequent sustained ischemia render the heart more resistant to the injury instead of making the injury more serious. This phenomenon termed ischemic preconditioning (PC) has proved to be a powerful endogenous form of myocardial protection against infarction. Since the first description of PC effect (1), numerous studies have documented similar phenomenon in dogs (2), pigs (3), rabbits (4, 5), and rats (6, 7). Recent indirect evidence suggests that brief antecedent ischemia may also protect the human heart (8). The cardioprotective effects of PC include limitation of infarct size, enhancement of postischemic contractile function, and reduction of the frequency of reperfusion-induced arrhythmias. In the rat heart model, Cave and Hearse (6) reported that a single brief episode of ischemia and reperfusion could enhance postischemic contractile function even in a prolonged sustained ischemia for more than 1 hr, however, controversies still remain. Recent studies have reported that PC did not have any effect on recovery of cardiac function (4, 5, 9).

On the other hand, despite intensive investigation, the precise mechanisms responsible for PC also remain to be elucidated. Recent studies, derived largely from indirect evidence in the rabbit model (10, 11), suggest that PC triggers a sequence of events that initiate the activation and subsequent translocation of protein kinase C (PKC) from the cytosol to the myocyte membranes. PKC is proposed to remain in the cell membranes during the intervening brief reperfusion, where it would be strategically positioned during the early phase of the subsequent sustained ischemia to facilitate phosphorylation of unknown protein effectors, perhaps an ion channel or a cytoskeletal element (12), and thereby eliciting the ultimate reduction in infarct size. Thus, cardioprotection by PC has been proposed to be critically dependent on the presence of PKC in myocyte membranes early during the sustained ischemia. This hypothesis has been supported by numerous experiments using PKC activators and inhibitors in rats (13), rabbits (10), and human tissues (14). However, some controversy still exists (15-17), since the concept does not seem to apply consistently in all species and under all conditions.

Following this background, in global ischemia using the isolated Langendorff-perfused rabbit heart model, the present study was designed to test: (i) whether PC (by

a single or a multiple doses of ischemia and reperfusion) improves postischemic cardiac function and reduces infarct size, and (ii) if so, is the cardioprotective effect of PC related to activation and translocation of PKC from the cytosol to the myocyte membranes. For the purpose, we directly measured both cytosolic and membrane PKC activity.

MATERIALS AND METHODS

Experimental animals and chemicals

The present study follows the guidelines for the use of laboratory animals from the American Physiological Society. New Zealand White rabbits of either sex (n=96, 1.5-2.0 kg body weight) were used. They were kept in a constant condition and fed a balanced diet and water ad libitum. They were kept on water only 24 hrs before sacrifice.

All chemicals except protein kinase C assay kit (Amersham) were purchased from the Sigma Chemical, St. Louis, Mo., U.S.A.

Preparation of heart

The animal was stunned by a blow to the neck 30 min after heparinization (300 IU/kg) and the heart was isolated. The heart was immediately mounted on a perfusion apparatus (size 5, Hugo Sachs Elektronik, March-Hugstetten, Germany) and perfused with oxygenated Tyrode solution (containing in mmol/L: NaCl 140.0, KCl 4.4, $CaCl_2$ 1.0, $MgCl_2$ 1.0, HEPES buffer 3.0, and glucose 10.0), maintained at 37℃, by non-recirculating Langendorff technique. The pH of the Tyrode solution was adjusted at 7.6 to maintain a physiological range (7.3-7.4) during perfusion. The perfusate was delivered via an aortic cannula connected to the perfusion apparatus at a mean flow rate and pressure of 35 ml/min and 60 mmHg, respectively. A latex balloon connected to a pressure transducer (Isotec, Hugo Sachs Elektronik) was inserted into the left ventricle (LV) through pulmonary vein, and the LV end-diastolic pressure (LVEDP) was adjusted at 8-10 mmHg. The heart was constantly paced at 150 beats/min (4 volts, 0.5 msec interval) through the right atrium with an electrical stimulator (Advanced Stimulator, Harvard Apparus, Edenbridge, U.K.).

Experimental protocol

After stabilization of baseline hemodynamics (Table 1, 2), the hearts were treated as in the experimental protocol (Fig. 1).

Experiment 1

In this series of experiments, hearts were subjected to 60 min of global ischemia with PC (PC+I+R, n=19) or not (control, n=18), followed by 120 min reperfusion. PC was induced by four consecutive cycles of 5 min global ischemia and intervening 5 min of reperfusion. To determine changes in the PKC activity, supplementary experiments (n=5 each) were performed: experiments were terminated after stabilization of baseline hemodynamics (base), baseline and PC (PC) , baseline, PC and 60 min of global ischemia (PC+I), and after baseline and 60 min of global ischemia (I), respectively. As a result (see Results for detail), functional parameters were not significantly different, despite reduction in infarct size in

Table 1. Hemodynamic changes in the experiment 1 (mean±S.E.M.)

	LVDP (mmHg)	$+dP/dt_{max}$ (mmHg/sec)	LVEDP (mmHg)	CF (ml/min)
Baseline				
Control (n=18)	92.1±3.7	1,129±49	8.1±0.2	28.1±0.2
Preconditioned (n=19)	89.0±1.3	1,146±48	8.5±0.2	28.2±0.2
30 min on reperfusion (after 60 min of ischemia)				
Control	36.7±3.7	689±49	77.3±4.0	23.2±1.1
Preconditioned	47.4±4.8	788±50	60.0±5.0*	26.0±0.6*
60 min on reperfusion (after 60 min of ischemia)				
Control	44.6±4.2	781±55	71.2±4.0	23.0±0.7
Preconditioned	58.3±4.9	871±56	55.1±5.0*	25.3±0.8*
120 min on reperfusion (after 60 min of ischemia)				
Control	45.4±4.0	791±53	70.7±4.3	22.1±0.7
Preconditioned	53.0±4.2	841±57	55.1±4.4*	23.8±0.8

CF, coronary flow; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure.
*$p<0.05$, control vs preconditioned hearts.

Table 2. Hemodynamic changes in experiment 2 (mean±S.E.M.)

	LVDP (mmHg)	$+dP/dt_{max}$ (mmHg/sec)	LVEDP (mmHg)	CF (ml/min)
Baseline				
Control (n=12)	86.8±2.1	1,150±48	8.3±0.3	28.0±0.2
Preconditioned (n=13)	85.0±1.6	1,140±46	8.3±0.2	28.6±0.3
30 min on reperfusion (after 60 min of ischemia)				
Control	65.5±4.2	772±54	35.7±3.6	26.0±0.3
Preconditioned	74.7±2.2	857±36*	24.7±3.0*	27.8±0.3†
60 min on reperfusion (after 60 min of ischemia)				
Control	72.5±4.6	870±66	33.1±3.3	24.7±0.4
Preconditioned	83.5±2.2*	999±43*	22.8±2.8*	27.2±0.3†
120 min on reperfusion (after 60 min of ischemia)				
Control	65.5±5.8	833±74	31.2±3.7	23.6±0.4
Preconditioned	79.0±2.2*	941±49	22.0±2.4*	25.9±0.4†

CF, coronary flow; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure.
*$p<0.05$ and †$p<0.01$, control vs preconditioned hearts.

the preconditioned hearts. The preconditioned hearts also showed increased activity of membrane PKC, therefore, we concluded that the induction protocol of PC in experiment 1 might be inadequate and the duration of subsequent sustained ischemia was too long to improve postischemic functional recovery, although the PC reduced infarct size and increased membrane PKC activity. Therefore, we decided to perform another series of experiments (Experiment 2) to clarify whether a single dose of PC could improve postichemic functional recovery.

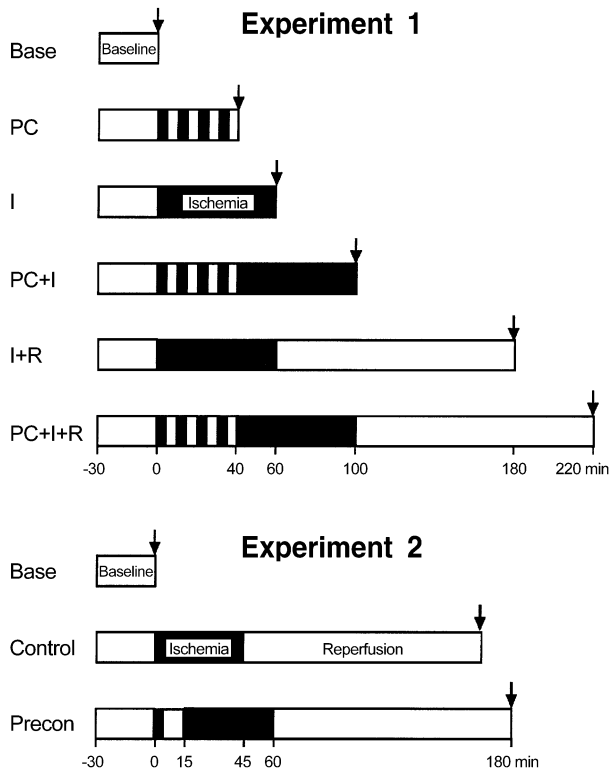

Fig. 1. Schematic illustration of the experimental protocol. Arrows indicate points of protein kinase C measurement. Base, baseline; PC, preconditioned (Precon); I, 60 min of sustained ischemia; R, 120 min of reperfusion.

Experiment 2

In this series of experiments, hearts were subjected to 45 min of global ischemia with PC (n=13) or not (control, n=12), followed by 120 min of repefusion. PC was induced by a single dose of 5 min global ischemia and 10 min of reperfusion. To determine changes in the PKC activity, supplementary experiments (n=5) were performed: they were terminated after stabilization of baseline hemodynamics (base).

Functional study

During all experiments, the left ventricular (LV) pressure, contractility (LV dP/dt), and perfusion pressure were recorded on a multi-channel polygraph (WR9000, Watanabe-Graphtech, Japan).

After initiation of coronary perfusion, the pulmonary artery and venae cavae were ligated and a cannula was inserted through the right atrium into the right ventricle to continually collect the coronary venous effluent and empty the right ventricle completely. Coronary perfusion flow was measured by timed collections of coronary venous effluent.

PKC assay

Tissue was processed as described by Takai et al. (18). Briefly, on completion of each experiment, the left ven-

tricle containing interventricular septum was homogenized using a Ultra-Turrax homogenizer (3 times for 30 seconds each, 10,000 rpm) in 3 vol of a buffer (containing in mmol/L: Tris-HCl 20, sucrose 250, iodoacetic acid 1.0, phenylmethylsulfonyl fluoride 1,0, EDTA 1.0, EGTA 1.0, and β-mercaptoethanol 10, pH 7.4, 4℃). Crude homogenates were centrifuged (360 *g* for 10 min at 4℃). Supernatant was centrifuged at 100,000 *g* for 1 hr at 4℃ and the resulting supernatant was used to measure cytosol PKC activity. Pellets were resuspended in 2 vol of the same buffer containing 0.3 vol% Triton X-100 and gently stirred for 1 hr at 4℃, centrifuged at 100,000 *g* for 1 hr, and the resulting supernatant was used to measure membrane PKC activity. The activity of PKC was measured with a commercial kit (Protein Kinase C Enzyme Assay System, RPN 77A, Amersham), which has been accepted as a reliable method of estimating PKC. Fractions were diluted with buffer containing all components described above, except for the ion chelators EDTA and EGTA. 10 μg of specimen was incubated with 0.2 μCi of ^{32}P-γ-ATP for 15 min. Radioactivity was determined using a Beckman liquid scintillation counter, and blanks for calcium phospholipid-independent PKC activity and endogenous phosphorylation were subtracted from the total counts for each sample. Triplicate aliquots were assayed for all samples. Results were expressed as pmol/mg protein/min and as percentage of total PKC activity. Protein concentrations were measured using DC Protein Assay (Bio-Rad) and the purity of cytosolic and membrane fractions was confirmed by microscopic examination and measurement of lactate dehydrogenase.

Determination of infarct size

After experiments, the hearts were perfused with 1% triphenyltetrazolium chloride (in phosphate buffer) at room temperature for 20 min and immersed overnight in 10% neutral buffered formalin. The heart slices (2 mm in thickness) were photographed and the outlines of cross sections were traced onto a transparent paper. The left ventricle area including the interventricular septum and the infarct area were measured 3 times by independent observers using computerized planimetry (Ushikata X-plan 360 dII, Tokyo, Japan).

Criteria for exclusion

Hearts (n=9) which did not establish baseline (Table 1, 2) even perfusion prolonged for more than 50 min and which showed persistent cardiac standstill on reperfusion after a subsequent sustained ischemia were excluded for functional, morphometric, and biochemical data analyses.

Statistics

Data were expressed as mean±S.E.M. Differences in the functional parameters between the control and PC hearts were analyzed using t-test for unpaired observation and those in the biochemical data and infarct size were done using t-test for paired observation. Differences in the PKC activity among groups were analyzed using one-way analysis of variance (ANOVA) and comparisons were made with Tukey's test if ANOVA detected significant differences. Values of $p<0.05$ were considered statistically significant.

RESULTS

Functional parameters

The hemodynamics changes are summarized in the Table 1 and 2.

In Experiment 1, recovery rate of the LV developed pressure (LVDP) and contractility (LV dP/dt) gradually increased until 1 hr on reperfusion and then remained stable both in the control and preconditioned hearts (Fig. 2). The coronary flow abruptly increased on reperfusion and then gradually decreased (Fig. 2). In comparison with the control, the LVDP and coronary flow were significantly different in the preconditioned hearts ($p<0.05$) during reperfusion; but they were not different at the end of reperfusion. The LV end-diastolic pressure (LVEDP) was significantly higher in the control during throughout the 120 min reperfusion ($p<0.05$, Fig. 2).

In Experiment 2, the recovery pattern of the functional parameters during reperfusion was similar to those in Experiment 1. The LVDP and contractility increased until 1 hr on reperfusion and then remained stable. The coronary flow was abruptly increased on reperfusion and then gradually decreased. These parameters were significantly different between the control and preconditioned hearts ($p<0.05$ in the LVDP and $p<0.01$ in the coronary flow, Fig. 3). In addition, the LVEDP was significantly higher (absolute values were lower than those in experiment 1) in the control during 120 min reperfusion ($p<0.05$, Fig. 3).

These results indicate that PC can provide improvement of post-ischemic recovery.

PKC assay

In Experiment 1, the baseline total PKC activity was 1731.2±55.9 pmol/mg protein/min, and the cytosolic and membrane PKC activity were 84.2±2.8% (1455.5 ±47.6 pmol/mg protein/min) and 15.8±0.5% (273.4±

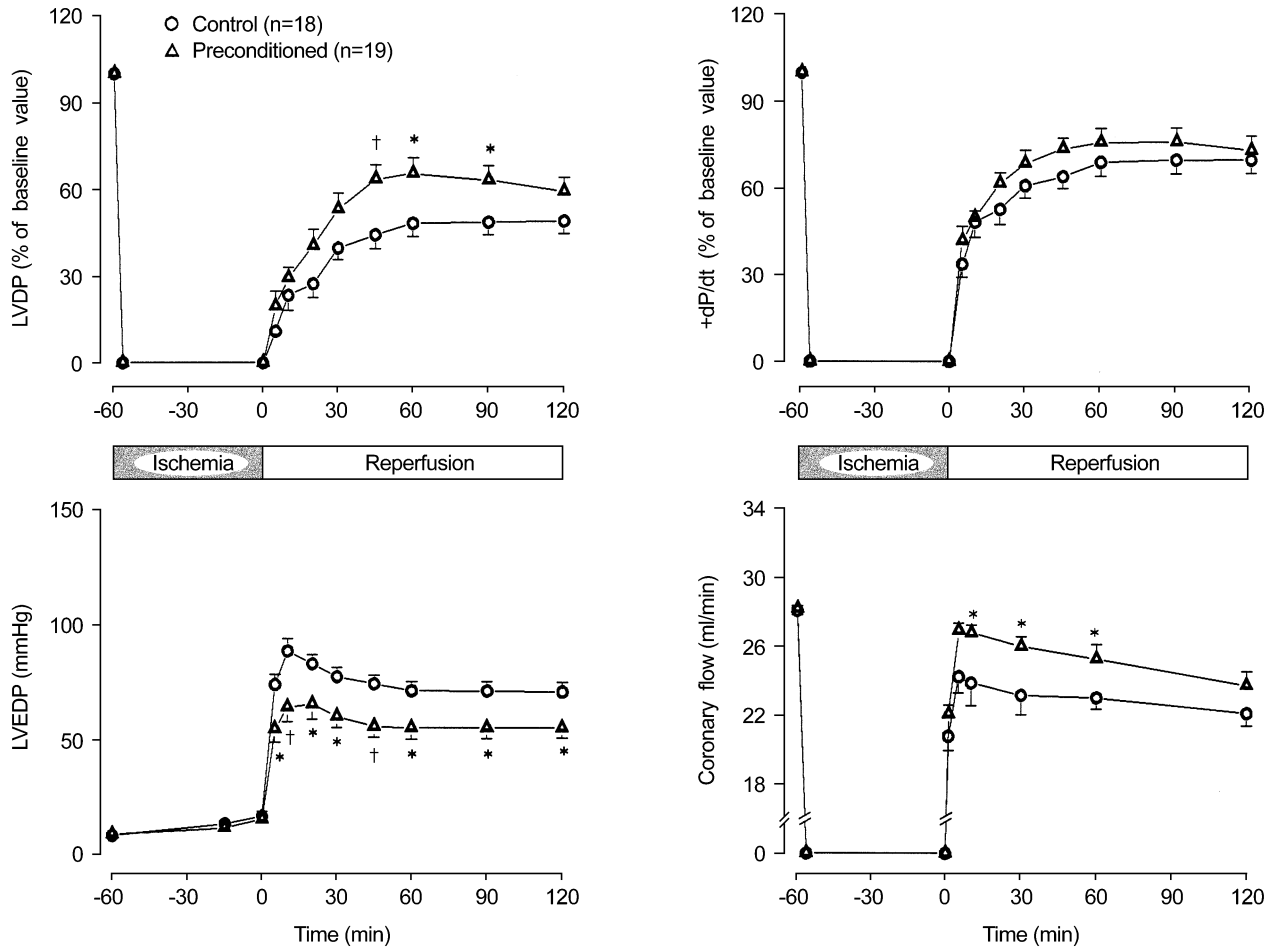

Fig. 2. Recovery of cardiac function and coronary flow on reperfusion after 60 min of sustained ischemia (Experiment 1). Data are expressed as mean±SEM. LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure. *$p<0.05$ and †$p<0.01$, control vs preconditioned hearts.

4.5 pmol/mg protein/min) of the total PKC activity, respectively. As expected the total PKC activity was not significantly different among groups, but the PKC activity was significantly higher in the cytosol versus the membrane fraction. The PKC activity both in the cytosolic and membrane fractions was not changed just after PC regimen. However, in the preconditioned hearts, the cytosolic PKC activity significantly decreased to 73.7±1.2% of the total PKC activity and the membrane PKC activity significantly increased to 26.3±1.4% of the total PKC activity after 60 min of subsequent sustained ischemia ($p<0.01$, Fig. 4); the cytosolic and membrane PKC activity were 76.2±1.2% and 23.8±1.9% of the total PKC activity after 60 min of subsequent sustained ischemia followed by 120 min of reperfusion ($p<0.01$, Fig. 4). These results indicate that activation of PKC may be initiated by PC, translocated from the cytosol to myocyte membrane during subsequent sustained ischemia, and gradually decreased on reperfusion.

In Experiment 2, the baseline total PKC activity was 1728.0±70.5 pmol/mg protein/min, and the cytosolic and membrane PKC activity were 84.3±3.5% (1456.18±60.9 pmol/mg protein/min) and 15.7±1.7% (271.9±28.8 pmol/mg protein/min) of the total PKC activity, respectively. As in Experiment 1, the total PKC activity was not significantly different among groups and the PKC activity was significantly higher in the cytosol versus the membrane fraction. Both of the cytosolic and membrane PKC activity was not significantly changed in the control which received 45 min of subsequent sustained ischemia (83.7±4.5% and 16.3±1.4% of total PKC activity in the cytosolic and membrane fractions, respectively); however, the cytosolic PKC activity decreased to 73.0±2.6% of the total PKC activity ($p<0.05$, Fig. 5) and the membrane fraction increased to 27.0±0.7% of the total PKC activity ($p<0.01$, Fig. 5) in the preconditioned hearts. These results indicate that activation and translocation of PKC from the cytosol to

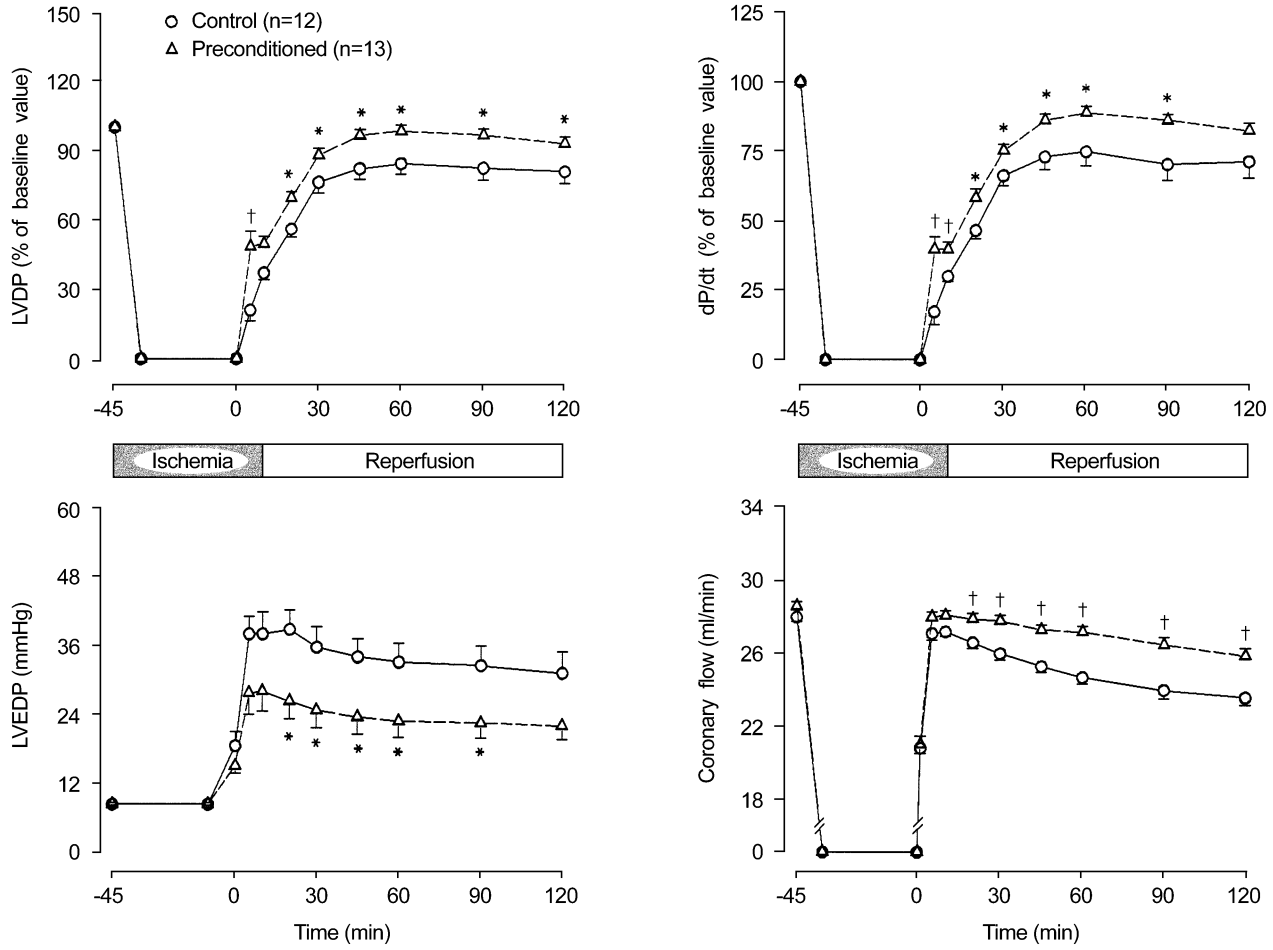

Fig. 3. Recovery of cardiac function and coronary flow on reperfusion after 45 min of sustained ischemia (Experiment 2). Abbreviations, see Fig. 2. *$p<0.05$ and †$p<0.01$, control vs preconditioned hearts.

myocyte membrane may be initiated by PC.

Infarct size

Both in Experiment 1 and 2, infarct size significantly reduced (from 26.9±1.4% to 15.3±1.9%, $p<0.01$, in Experiment 1; and from 18.3±2.6% to 8.6±2.5%, $p<0.05$, in Experiment 2, Fig. 6) in the preconditioned hearts.

DISCUSSION

In the present study, we provided direct evidence to support the hypothesis that translocation of PKC which has been implicated as an important mediator of the cardioprotective effect of PC.

Ischemic preconditioning and improvement of cardiac function

In Experiment 1 of the present study, PC was induced by a multiple doses (x4) of brief period of ischemia (5 min) and intervening reperfusion (5 min). It has been reported that this protocol was found to be optimal in rabbit hearts to produce PC (19). In the present study, a multiple doses of PC regimen resulted in approximately 40% reduction in infarct size and partial improvement of functional recovery. However, at the end of reperfusion (120 min), recovery of the LVDP, contractility (+dP/dt), and coronary flow in the preconditioned hearts reached only 60%, 74%, and 85% of the baseline, respectively. In addition, the increase in LVEDP was nearly 40 mmHg (indicates contracture). In our previous studies using isolated rabbit hearts with the same PC protocol as in Experiment 1, the cardiac function was not improved (5, 20), regardless the duration of sustained ischemia (for 10 to 120 min), and the recovery of the cardiac function tended to be lower in the preconditioned hearts than in the non-preconditioned hearts, when subsequent ischemia was extended for more than 60 min. Sandhu et al. (9)

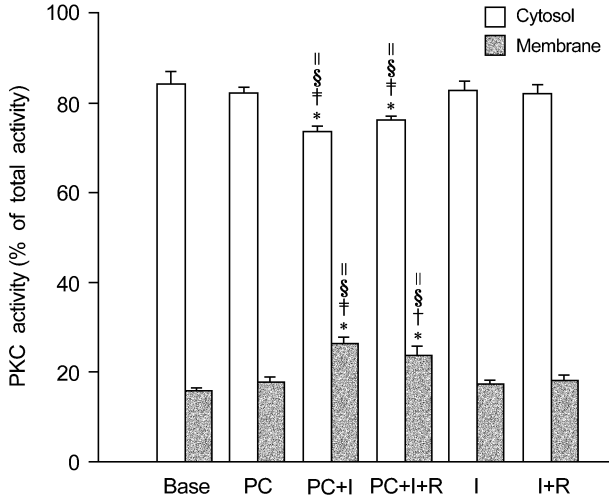

Fig. 4. PKC activity among groups in Experiment 1 (n=5 each). Base, baseline; PC, preconditioning regimen; I, 60 min of sustained ischemia; R, 120 min of reperfusion. Data are expressed as mean±SEM. *p<0.01, base vs PC+I or PC+I+R; †p<0.05, PC vs PC+I+R; ‡p<0.01, PC vs PC+I or PC+I+R; §p<0.01, I vs PC+I; ‖p<0.05, I vs PC+I.

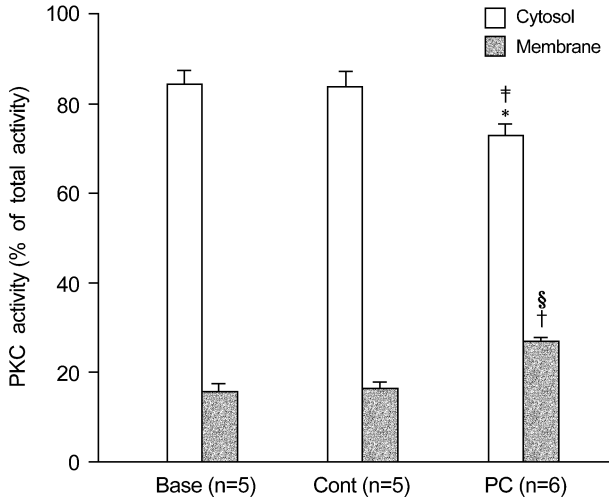

Fig. 5. PKC activity among groups in Experiment 2. Data are expressed as mean±SEM. Base, baseline; Cont, control; PC, preconditioned. *p<0.05, †p<0.01, base vs PC; ‡p<0.05, §p<0.01, cont vs PC.

also reported that IP has no effect on LVDP. It is possible to explain that the partial improvement in cardiac function is secondary to reduced infarct size and relatively lower stiffness of the left ventricle. Relatively smaller increase in LVEDP and lower calcium concentration in the cardiac tissue (data not shown) in the PC hearts support this speculation. It is still unclear why functional data in this study are different from our previous study; it could be controversial whether PC regimen in Experiment 1 of the present study can further increase myocardial stunning, despite partial improvement. However, the results are not sufficient to conclude that PC regimen in Experiment 1 is less reliable to expect functional improvement in isolated rabbit heart, since early functional differences can lead to increased long term recovery even if they are not sustained. Interestingly, on the other hand, PC by a single dose of brief ischemia and reperfusion in Experiment 2 improved the recovery of LVDP, contractility and coronary flow throughout 120 min of reperfusion. These parameters reached near 90% of the baseline and the range of increase in the LVEDP was less than 20 mm Hg at the end point of reperfusion. The infarct size was also smaller than in the preconditioned hearts of Experiment 1. Cave and Hearse (6) demonstrated that PC improves cardiac function even after a global ischemia for 160 min in isolated rat hearts: they employed a single dose of 5 min ischemia and 5 min reflow and functional parameters as end points to assess protection, and as a result, aortic flow was improved by PC. However, we still have question whether the effect of a single episode of brief ischemia could be continued for such a long period of sustained ischemia enough to delay infarction, since cardioprotective effect of PC lasts for about 1 hr in rabbits (21) and rats (2). And it is unclear why PC has an effect on functional recovery and whether PC can attenuate stunning. However, we think that improving post-ischemic functional recovery by PC is not a direct effect but secondary to reduced infarct size and relatively lower stiffness of the left ventricle. Recently, Jenkins et al. (4) described that PC does not attenuate stunning in a single dose of 5 min ischemia

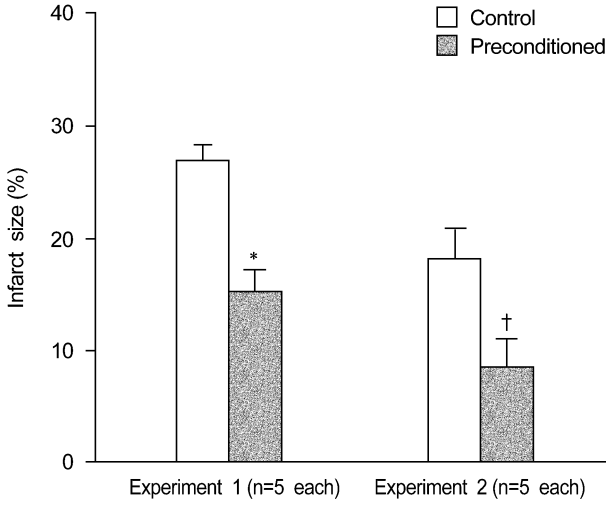

Fig. 6. Infarct size as % of the left ventricular volume. Data are expressed as mean±SEM. *p<0.01, control vs preconditioned (Experiment 1); †p<0.05, control vs preconditioned (Experiment 2).

and 10 min reperfusion for PC. However, it cannot simply be concluded that a single brief ischemia and reperfusion is more reliable than multiple doses to improve functional recovery because in addition to the number of ischemic treatments, Experiment 1 and Experiment 2 differ in the time of sustained ischemia, which is very likely to alter effectiveness of PC.

Ischemic preconditioning and PKC

Almost all previous studies assessing the role of PKC in PC used an indirect pharmacological approach. They attempted to block the effect of PC by administration of PKC inhibitors. PKC inhibitors, staurosporine and polymixin B, were shown to block the reduction in infarct size achieved by PC in both in situ and isolated rabbit heart subjected to regional ischemia, when administered before the onset of sustained ischemia (10, 11, 22). Similarly, calphostin-C was reported to inhibit the effects of PC in rabbit (23).

In the present study, we have provided direct evidence that PC probably has a role in translocation of PKC from the cytosol to myocyte membranes. In Experiment 1, the cytosolic PKC activity decreased from 84 to 74% of the total PKC activity after 60 min of ischemia in the preconditioned hearts; the membrane PKC activity increased from 16 to 24-26% of the total PKC activity (i.e., membrane PKC activity increased 1.7-fold after 60 of min ischemia and 1.5-fold after 60 min of ischemia and 120 min of reperfusion in the preconditioned hearts). In Experiment 2, the preconditioned hearts showed decrease in the cytosolic PKC activity from 84 to 73% of the total PKC activity; the membrane PKC activity increased from 16 to 28% (1.8-fold) of the total PKC activity. Activation and subsequent translocation of PKC to membrane-associated sites, presumably in response to brief transient ischemia and manifest during the early minutes of the subsequent sustained occlusion, has been proposed to play a pivotal role in PC (10, 11). In contrast, there has been no changes both in the cytosolic and membrane PKC activity just after the PC regimen (in Experiment 1); these results are consistent with previous studies (24, 25). Przyklenk et al. (24) were unable to show any difference in the total amount and subcellular distribution of PKC obtained from canine myocardium during and/or at the end of PC regimen. They determined PKC activity directly by measuring ^{32}P-γ-ATP incorporation into a PKC-specific peptide in subcellular fractions and used a fluorescein probe conjugated to bisindolylmaleimide, which selectively binds to active PKC. Overall increase in PKC activity in both fractions can be achieved by various physiological and pharmacological stimuli. These include α_1-adrenergic receptors, stimulation of muscarinic receptors, administration of diacylglycerol analogues, and exposure to phorbol esters (24). It has been proposed that activation of PKC is also manifested by the subsequent redistribution of isoenzymes from the cytoplasm to binding sites associated with the sarcolemma, cytoskeleton, perinuclear region and, in smooth muscle cells and cardiac myocytes, to sites associated with contractile proteins (26, 27). There is also evidence that PC causes translocation of PKC-δ isoenzyme to myocyte membrane (28), however, we have found by immunoblotting with monoclonal antibodies that expression of PKC-δ and -ε (Ca^{2+}-independent) both in the cytosol and myocyte membrane are not significantly different among study groups in Experiment 1 (data not shown). These results suggest at least that activation and translocation of PKC occur during subsequent sustained ischemia after PC; and that the Ca^{2+}-independent PKC isozymes are less responsible for the effect of PC in rabbit hearts, since the PKC assay used in the present study is responsible for Ca^{2+}- dependent isozymes (α, β, γ). In contrast, however, the opposite results were made from swine heart model. In swine heart, staurosporine or bisindolylmaleimide did not block the protective effects of PC (15, 17), rather they further reduced infarct size (15). Similar conclusion that PC does not cause PKC activation also has been drawn from studies in rats (29). We do not know the reason why previous studies (15, 17) failed to demonstrate activation and translocation of PKC, however, it could be explained by: (i) differences in species might blocking effectiveness of different isoforms of PKC by staurosporine, although such an explanation is not satisfying; (ii) there is a possibility that small amount of activity change could not reflect its translocation, since PKC pool in the cytosol is much larger than in the membrane. Although the purpose of the present study was beyond to rigorously elucidate the time course of PKC translocation in response to brief ischemia, we could state that translocation of PKC is an important mechanism for reduction in infarct size seen with PC in the isolated rabbit heart model.

Limitations of the present study

In the present study, we did not examine the effect of PC in in vivo hearts. Therefore, we could not assess biological phenomena among blood components, coronary endothelial cells, and cardiac myocytes during PC, sustained ischemia, and reperfusion. However, since previous studies (30, 31) have demonstrated that PC is equally effective in protecting the heart against infarction in in vivo and in vitro, using rabbit and rat heart models, the mechanism of PC probably is independent of blood components. Furthermore, Sandhu et al. (9) have demon-

strated that PC was equally effective in both the isolated buffer-perfused and blood-perfused rabbit hearts. However, it cannot totally be excluded that the lack of blood components in isolated buffer-perfused heart model in some way influence the results (32). Also, we could not assess the change of PKC activity in administration of PKC inhibitors, since we did not use PKC inhibitors. Therefore, time course of PKC activation and translocation by PC remains to be elucidated in both in vivo and isolated heart models.

In conclusion, it could be confirmed that translocation of PKC from the cytosol to myocyte membranes is an important factor responsible for PC, at least in the rabbit heart model of global ischemia.

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