Protein kinase C translocation and PKC-dependent protein phosphorylation during myocardial ischemia

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Albert, Carolyn J., and David A. Ford. Protein kinase C translocation and PKC-dependent protein phosphorylation during myocardial ischemia. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H642–H650, 1999.—The present study demonstrates that the α, ε, and δ isozymes of protein kinase C (PKC) are translocated to particulate fractions from the cytosol during brief intervals of global ischemia as well as reperfusion of ischemic rat myocardium. In contrast, phorbol ester treatment of perfused hearts resulted in the translocation of the α, δ, and ε isozymes of PKC to particulate fractions. Additionally, the α, δ, and ε isozymes of PKC are translocated to particulate fractions in phorbol ester-stimulated, isolated adult rat cardiac myocytes. Concomitant with the translocation of PKC isozymes to particulate fractions during myocardial ischemia, increased protein phosphorylation was observed, which was blocked by pretreatment of hearts with the selective PKC inhibitor bisindolylmaleimide I (50 nM). In particular, ischemia resulted in the phosphorylation of 26-, 20-, and 17-kDa particulate-associated proteins. Taken together, the present findings are the first to demonstrate that specific PKC isozymes are translocated to particulate fractions in the ischemic and the reperfused ischemic rat heart, resulting in the phosphorylation of specific particulate-associated proteins.

heart; reperfusion; myocytes; phorbol esters

Protein kinase C (PKC) is an ubiquitous enzyme that is involved in signal transduction pathways in many organs (1, 16, 18, 23). In the heart, PKC is believed to play a role in ischemic preconditioning by a mechanism involving modulation of K_{ATP} channel activity (11, 12, 15, 25). However, the role of PKC translocation and activity in ischemic preconditioning is not universally accepted (24). In addition to this putative role in myocardial ischemic preconditioning, PKC may play an important role in the pathophysiological sequelae of myocardial ischemia. For example, studies by Lucchesi and co-workers (2) have demonstrated that phorbol esters elicit ventricular arrhythmias in Langendorf-perfused rabbit hearts that are blocked by the PKC inhibitor staurosporine as well as the K_{ATP} channel blocker glibenclamide. These studies have implicated PKC-mediated phosphorylation of ion channels as a potential mechanism contributing to arrhythmogenesis during myocardial ischemia. PKC activation during myocardial ischemia may also mediate long-term effects on myocardial function following recovery from ischemia because the PKC pathway is coupled to the mitogen-activated protein kinase cascade and the activation of this pathway likely has long-term effects on myocardial function through the activation of proto-oncogenes (4, 14, 30).

Several reports have demonstrated activated PKC activity in membrane fractions isolated from ischemic myocardium. Utilizing in vitro histone phosphorylation assays as a measure of PKC activity, Prasad and Jones (20) demonstrated that membrane-associated PKC is activated during global ischemia. Similar techniques were also employed by Strasser and co-workers (26) in their studies, which showed PKC activation in ischemic hearts. Collectively, these studies demonstrated that PKC may play a role in the pathophysiological consequences of myocardial ischemia and suggested that individual PKC isozymes in the heart may have unique roles during the heart’s response to ischemic episodes.

The identification of the PKC isozymes that are translocated and activated during myocardial ischemia in the adult rat heart has not been conclusively determined. The α, δ, and ε isozymes of PKC have been shown to translocate differentially during myocardial ischemia in the adult rat heart (17, 29). While the α and ε isozymes of PKC translocate from the cytosolic to membrane compartments in response to ischemia, the δ isozyme of PKC has been shown to translocate from crude membranes to the cytosol in one study (29) and selectively to the sarcolemma in another study (17). Additionally, it is not universally believed that the α isozyme of PKC is translocated in ischemic adult rat heart, because several studies have not detected the α isozyme of PKC in adult rat heart (3, 22). However, it should be noted that others have detected the α isozyme of PKC in the adult rat heart (6, 13, 21).

The present studies were designed to determine the PKC isozymes that are translocated during myocardial ischemia utilizing the Langendorf-perfused, isolated adult rat heart model. The results herein demonstrate that the α, ε, and δ isozymes of PKC translocate to particulate fractions during global myocardial ischemia in the Langendorf-perfused adult rat heart model. Additionally, the translocation of these PKC isozymes to particulate fractions during myocardial ischemia results in concomitant PKC-dependent phosphorylation of particulate-associated proteins. The α, δ, and ε isozymes of PKC are also translocated in phorbol ester-stimulated perfused adult rat hearts as well as adult rat cardiac myocytes.
MATeRIALS and METHODS

Preparation of Langendorff-perfused rat hearts, induction of myocardial ischemia and reperfusion, and tissue extraction.

Male Sprague-Dawley rats (200–250 g body wt) were injected with heparin (200 U ip) 30 min before being anesthetized with pentobarbital sodium (25 mg ip), and their hearts were subsequently removed and placed in ice-cold saline before being perfused. Rat hearts were retrograde perfused via the aorta (Langendorff-perfused) with modified Krebs-Henseleit buffer consisting of (in mM) 137 NaCl, 4.7 KCl, 3 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 0.5 NaEDTA, 15 NaHCO₃, and 11 glucose equilibrated with 95% O₂-5% CO₂ (pH 7.4) at 37°C for 15 min at a constant aortic perfusion pressure of 60 mmHg. In selected experiments, hearts were pulse-chase radiolabeled with [³²P] before experimental conditions. In brief, hearts were perfused for 45 min in a recirculating buffer mode with modified Krebs-Henseleit buffer that contained only 120 µM K₂HPO₄ as well as 625 µCi (9 Ci/µmol) of [³²P]. Following [³²P] labeling, hearts were perfused with modified Krebs-Henseleit buffer containing 1.2 mM K₂HPO₄ for 15 min in a non-recirculating mode. In selected experiments, 50 nM bisindolylmaleimide I was included in the perfusion buffer during the last 5 min of the 15-min chase interval. Following these perfusion protocols, Langendorff-perfused hearts were either control-perfused at 60 mmHg (control) or subjected to ischemia with aortic clamping and myocardial tissue was pulverized to a fine powder at the temperature of liquid nitrogen. Myocardial tissue (≈0.8 g wet wt) was then homogenized at 4°C in 20 ml of homogenization buffer (20 mM Tris·HCl, 0.33 M sucrose, 5 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.005% leupeptin; pH 7.4) utilizing a Polytron (50% setting for 20 s) followed by Potter-Elvehjem homogenization with 5 strokes at a setting of 50%. Homogenates were then centrifuged at 24,000 × g for 20 min to collect cytosolic (supernatant) and particulate fractions.

Preparation of isolated adult rat cardiac myocytes. Rat hearts were prepared for Langendorff perfusion as described above and were subsequently used for the preparation of isolated adult rat cardiac myocytes as previously described (8). In brief, four hearts were used for each preparation and were perfused via the aorta at an aortic perfusion pressure of 40 mmHg with an initial perfusion buffer composed of Ca²⁺-free Joklik’s minimum essential medium (pH 7.4) equilibrated with 100% O₂ and supplemented with 60 mM taurocholic, 20 mM creatine, 5 mM adenosine, and 20 mM HEPES for 5 min. After the initial perfusion protocol, hearts were perfused in a buffer-recirculating mode with perfusion buffer supplemented with 1% BSA, 0.1% (wt/vol) collagenase (Worthington, CLS-2), and 50 µM CaCl₂ until the hearts became flaccid. The hearts were then removed from the aortic cannulas and were minced into ~2-mm³ chunks that were further digested in 0.1% (wt/vol) collagenase while incubating in a gyratory water bath at 37°C. Myocytes were isolated through sedimentation in perfusion buffer supplemented with 1% BSA and were made calcium tolerant by incrementally increasing CaCl₂ to 1 mM. Myocyte preparations routinely contained >80% rod-shaped cells that excluded trypan blue.

Phorbol ester stimulation of isolated adult rat cardiac myocytes. Adult rat cardiac myocytes were maintained in perfusion buffer containing 1% BSA and 1 mM CaCl₂ at a dilution of ~5 mg myocyte protein/ml. In selected experiments, 5-mI aliquots of myocyte suspensions were transferred to 15-ml conical tubes and incubated in the presence or absence of 100 nM phorbol myristate acetate (PMA) for 15 min at 37°C. At the end of each experimental interval, the myocytes were pelleted and subsequently resuspended in 2 ml of homogenization buffer (20 mM Tris·HCl, 0.33 M sucrose, 5 mM EDTA, 0.5 mM EGTA, 1 mM PMSF, and 0.005% leupeptin; pH 7.4) followed by immediate freezing in liquid nitrogen. Frozen myocytes were then thawed, homogenized by three cycles of freeze thawing. Homogenates were then centrifuged at 20,000 g for 20 min to collect cytosolic (supernatant) and particulate fractions.

Western blot analysis of PKC isozymes from isolated perfused rat hearts and isolated adult rat cardiac myocytes. Cytosolic- and particulate-associated proteins prepared from isolated perfused rat hearts and isolated adult rat cardiac myocytes were quantitated by a Bio-Rad protein assay, and subsequently the samples were adjusted to equal protein concentrations before being subjected to SDS-PAGE under reducing conditions using 10% polyacrylamide gels (10 μg protein loaded per lane). Proteins were then quantitatively transferred to PVDF plus filters (Micron Separations, Westborough, MA). The membranes were sequentially blocked for 1 h with 5% dry milk in Tris-buffered saline (pH 7.6) at room temperature and then incubated for 1 h with primary antibodies at indicated concentrations in 5% dry milk in Tris-buffered saline (pH 7.6) containing 0.05% Tween 20. Next, the membranes were washed with Tris-buffered saline containing 0.1% Tween 20 and then incubated 1 h with the appropriate horseradish peroxidase-conjugated secondary antibodies (Sigma goat anti-rabbit HRP, 1:7,000 dilution or Bio-Rad goat anti-mouse HRP, 1:7,000 dilution) in Tris-buffered saline containing 0.1% Tween 20 at room temperature at indicated concentrations. Immunoreactive bands were then visualized by chemiluminescence detected on X-ray film (Kodak X-OMAT AR) utilizing the enhanced chemiluminescence system (Amersham). Multiple exposures of film to the blots were developed. X-ray film exposures that had linear levels of silver grain development were used for quantitation of band intensity utilizing National Institutes of Health (NIH) Image software following scanning and conversion of autoradiographic data to TIFF files using a Macintosh 5500/225 scanner and a Linocolor-Hell Jade scanner. Quantitative analysis of the autoradiographic data was performed using the public-domain NIH Image program (developed at the NIH and available on the Internet at http://rsb.info.nih.gov/nih-image).

Silver staining and autoradiography of SDS-PAGE gels. To ensure that both equal amounts of protein were loaded onto gels utilized for autoradiography and Western blot analysis and that subcellular fractions prepared from control and ischemic myocardium were similar in their individual protein profiles, parallel SDS-PAGE gels were prepared and subsequently silver stained utilizing the Bio-Rad Silver Stain Plus kit. In experiments utilizing particulate and cytosolic fractions that were [³²P] labeled, proteins were subjected to gel electrophoresis followed by autoradiography utilizing X-ray film (Kodak X-OMAT AR). Multiple exposures of film to the dried gels containing [³²P]-labeled proteins were developed, and exposures that had linear levels of silver grain development were used for quantitation of protein phosphorylation utilizing NIH Image software as described above.

Quantification of [³²P]ATP radiospecific activity. ATP was separated from other nucleotides and creatine phosphate by HPLC utilizing an SAX column as the stationary phase and gradient elution with phosphate buffer as previously described by Harmsen et al. (10). In brief, perchlorate extracts were prepared from pulverized ventricular tissue, extracts

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were neutralized with KOH, and the neutralized extracts were subjected to HPLC. Ultraviolet absorbance was monitored at 210 nm, and eluate corresponding to the ATP peak (retention time = 28 min) was collected and subjected to Cerenkov counting to determine the amount of 32P incorporated into ATP. The mass of ATP in each analysis was determined by comparing the integrated area corresponding to the ATP peak to that from an external standard curve generated with known amounts of ATP subjected to HPLC.

Materials. Anti-α, -βI, -βII, -γ, -δ, and -ε PKC were from Sigma. Anti-η and -ζ PKC were purchased from Santa Cruz. Anti-δ and -ε PKC were purchased from Transduction Laboratories. Anti-Na+-K+-ATPase β-1 and anti-sarcoplasmic reticulum Ca2+-ATPase (SERCA) were obtained from UBI and Affinity Bioreagents, respectively. Secondary antibodies, including goat anti-rabbit horseradish peroxidase and goat anti-mouse horseradish peroxidase, were purchased from Sigma or Bio-Rad. Electrophoresis-grade reagents for gel electrophoresis were purchased from ICN, Pharmacia, and Bio-Rad. All other chemicals were purchased from either Sigma or Fisher. Lactate dehydrogenase activity was determined spectrophotometrically by the method of Wroblewski and LaDue (28) utilizing a kit from Sigma.

RESULTS

Characterization of commercially available, isozyme-specific PKC antibodies. To determine PKC isozyme translocation in perfused hearts and cardiac myocytes, Western blot analysis of particulate and cytosolic fractions was employed, which is dependent on the use of isozyme-specific PKC antibodies. Accordingly, numerous commercially available, isozyme-specific PKC antibodies were tested for reactivity against a panel of recombinant human PKC isozymes including α, βI, βII, γ, δ, ε, η, and ζ to assure the proper assignment of PKC isozymes in rat heart preparations. Figure 1 illustrates that the antibodies employed in this study were specific for their indicated PKC isozyme and did not cross-react with other PKC isoforms. It should be noted that rat brain lysate was added as a δ PKC-positive standard (Fig. 1). The anti-δ PKC (Sigma) employed in these studies did not react with recombinant human δ PKC. Subsequent tests with another anti-δ PKC (Transduction Laboratories) demonstrated that this antibody was isozyme-specific, reactive with human recombinant δ PKC, and provided similar results to those utilizing the anti-δ PKC (Sigma) as a reagent for Western blot analysis of heart samples. Rat brain lysate was also used as a positive control when testing the anti-α PKC (Transduction Laboratories) since human recombinant δ PKC is not commercially available (Fig. 1). Other antibodies, including anti-α PKC (Santa Cruz), anti-βII PKC (Santa Cruz), and anti-ζ PKC (Boehringer Mannheim), were identified in this screening process to cross-react with more than one PKC and, accordingly, were not used in the present studies.

Identification of the translocation of specific PKC isozymes during myocardial ischemia and reperfusion in the isolated perfused adult rat heart. Experiments were performed to identify which PKC isoforms are prevalent in the rat heart and are translocated during myocardial ischemia and ischemia-reperfusion protocols. Typical immunoblots from these experiments are shown in Fig. 2, and quantitative data from immunoblot analysis of multiple hearts subjected to each experimental protocol are shown in Fig. 3. With the isolated perfused rat heart model, 5 min of global ischemia resulted in slight increases in the ε PKC isozyme in particulate fractions, which increased further with prolonged global ischemia and was maintained at an increased level following 30 min of global ischemia followed by 15 min of reperfusion (Figs. 2 and 3). In conjunction with the increases in particulate-
associated PKC during ischemia and ischemia-reperfusion episodes, a decrease in cytosolic ε PKC isozymes was observed following 20 min of global ischemia (Figs. 2 and 3). Additionally, the α and ε PKC isozymes were translocated to particulate fractions within 10 min of global ischemia as well as following prolonged global ischemia (Figs. 2 and 3). The α, ε, and i isozymes of PKC were not translocated to particulate fractions following 5 min of global ischemia followed by 15 min of reperfusion (Fig. 2), but they were translocated to particulate fractions when these hearts were subsequently subjected to 30 min of global ischemia (Fig. 2). The α, ε, and i isozymes of PKC were also translocated to particulate fractions following 30 min of global ischemia followed by 15 min of reperfusion (Fig. 2; summarized in Fig. 3). In contrast to the spatial translocation of the α, ε, and i isozymes of PKC, the δ and η PKC isozymes were present in control and ischemic hearts but did not translocate during experimental intervals (data not shown). In all cases, the use of control peptides to these isozyme-specific PKC antibodies resulted in the loss of the PKC isozyme signal in Western blots. The βI, βII, γ, and i PKC isozymes were not detected in the adult rat heart. Additionally, enzyme marker analysis ensured that particulate fractions were not contaminated with cytosolic proteins, because the cytosolic enzyme lactate dehydrogenase was only observed in cytosolic fractions (Fig. 4B). Furthermore, enzyme marker analysis demonstrated that cytosolic fractions were not contaminated with particulate proteins, because the sarcolemmal and sarcoplasmic reticulum enzymes, Na⁺-K⁺-ATPase and SERCA, respectively, were only detected in particulate fractions and not in cytosolic fractions (Fig. 4A).

Translocation of specific PKC isozymes during phorbol ester stimulation of isolated perfused adult rat heart. Further experiments were performed to determine the translocation of PKC isozymes in isolated perfused rat hearts following perfusions with 1 µM PMA for 10 min. Both PMA treatment as well as ischemia resulted in the translocation of the α and ε PKC isozymes to particulate fractions (Fig. 5). However, in contrast to hearts subjected to 30 min of global ischemia, PMA treatment did not result in the translocation of the i PKC isozymes to particulate fractions while it did result in the translocation of the δ PKC isozyme to particulate fractions (Fig. 5). Additionally, under all conditions studied (control-perfused hearts, PMA treatment, and global ischemia) the η PKC isozyme was present in the particulate fraction (Fig. 5).

PKC-mediated protein phosphorylation during myocardial ischemia. To test whether the translocation of PKC isozymes during myocardial ischemia resulted
in the phosphorylation of particulate-associated proteins, isolated perfused hearts were prelabeled with \(^{32}\)P and then subjected to control perfusions and 20 min of global ischemia. The autorad of phosphorylated particulate-associated proteins shown in Fig. 6A demonstrates that protein phosphorylation is increased following 20 min of global ischemia. Although multiple proteins are phosphorylated, striking increases in the phosphorylation state of 26-, 20-, and 17-kDa proteins were observed in response to 20 min of global ischemia, as indicated in Fig. 6A and quantitated in Fig. 6C. Furthermore, the demonstration that particulate-associated protein phosphorylation during 20 min of global ischemia was inhibited by treating hearts with the selective PKC inhibitor bisindolylmaleimide I (50 nM) before ischemia or control perfusions suggests that the protein phosphorylation observed during ischemia is mediated by PKC (Fig. 6A). To ensure that particulate preparations used for SDS-PAGE and subsequent autoradiography shown in Fig. 6A contained equal amounts of protein as well as similar protein composi-
tion, parallel SDS-PAGE and subsequent silver staining was performed. This analysis revealed minimal differences between particulate fractions prepared from control-perfused and ischemic hearts (Fig. 6B). It should also be noted that the radiospecific activity of the ATP pool was in isotopic equilibrium under each condition (e.g., 3,967 ± 6153, 4,154 ± 153, 4,135 ± 339, and 4,174 ± 410 dpm/nmol ATP for control-perfused hearts, 20-min ischemic hearts, control-perfused hearts pretreated with bisindolylmaleimide I, and 20-min ischemic hearts pretreated with bisindolylmaleimide I, respectively). Taken together, these data support the hypothesis that PKC isozymes that are translocated during myocardial ischemia are active and mediate the phosphorylation of particulate-associated proteins.

Identification of PKC isozymes translocated to membrane domains during phorbol ester stimulation of isolated adult rat cardiac myocytes (Fig. 7). Additionally, nearly complete translocation of the α, δ, and ε isozymes of PKC to particulate fractions was observed in cardiac myocytes that were treated with 100 nM PMA for 15 min (Fig. 7). Similar to the results utilizing isolated perfused hearts, the η isozyme of PKC did not translocate to particulate fractions in isolated adult rat cardiac myocytes treated with PMA (Fig. 7). Additionally, the η isozyme of PKC was detected in adult rat cardiac myocytes and was associated with the particulate fractions under both control and experimental conditions (Fig. 7). Taken together, these studies utilizing isolated adult rat cardiac myocytes confirm that a large proportion of the PKC translocation that is observed in the isolated perfused rat heart during ischemia is occurring in the cardiac myocyte cell population. Furthermore, these studies utilizing the anti-α PKC from Sigma agree with those of Rybin and Steinberg (21) utilizing the anti-α PKC from Upstate Biotechnology to demonstrate the presence of the α isozyme of PKC in adult rat cardiac myocytes.
DISCUSSION

PKC has been suggested to mediate, at least in part, multiple processes in the pathophysiological sequelae of myocardial ischemia, including preconditioning, arrhythmogenesis, and long-term dysfunction (2, 4, 11, 14, 15, 25, 30). Accordingly, the processes regulating the translocation and activation of individual PKC isozymes during myocardial ischemia may represent key pharmacological targets for the treatment of ischemia-induced myocardial dysfunction. The delineation of the role of PKC activation as an important biochemical mechanism that mediates myocardial dysfunction has been complicated by the diversity of PKC isozymes that exist in different animal models. For example, the ε and η isozymes of PKC have been shown to be translocated to membrane domains during ischemia in the isolated perfused rabbit heart (19). Furthermore, considerable differences have been observed concerning the presence of the α isozyme of PKC in the adult rat heart (3, 6, 13, 22). The present studies demonstrate that in the isolated perfused rat heart the α, ε, and η isozymes of PKC are translocated to particulate fractions during global ischemia, while the δ and η isozymes of PKC are detected but do not change their intracellular spatial distribution during global ischemia. In respect to the η isozyme of PKC, the difference

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**Fig. 6. PKC-mediated protein phosphorylation during myocardial ischemia.** Isolated perfused hearts were pulse-chase radiolabeled with 32P, and subsequently subjected to control perfusions (C) or 20 min of global ischemia (20I) in the presence (+BIM 1) or absence (−BIM 1) of the PKC inhibitor bisindolylmaleimide I (50 nM) as described in MATERIALS AND METHODS. Following each experimental interval, crude particulate fractions were prepared from ventricular tissue and were sequentially subjected to SDS-PAGE (12% gel) followed by either fluorography (A) or silver staining (B) as described in detail in MATERIALS AND METHODS. Throughout the preparation of samples used for this experiment, buffers were supplemented with 50 mM NaF and 0.2 mM Na3VO4. Each lane in the gels of A and B represents samples from independently treated hearts (i.e., each condition was repeated 3 times). Phosphorylated proteins indicated by arrows 1–3 in A were quantitated as described in MATERIALS AND METHODS (C). Values in C represent means ± SE for at least 3 independent determinations from 3 hearts subjected to the indicated experimental conditions.
METHODS.

clearly demonstrate that the present studies, as well as those of others (21, 29), to be present in the adult rat heart (3, 22). However, the results of the present studies and those of others (19) may reflect differences between rat and rabbit myocardium.

The translocation of specific PKC isozymes can vary depending on the perturbation to the heart. For example, in the present studies the δ isozyme of PKC is translocated to particulate fractions in the adult rat heart during phorbol ester stimulation, but not during myocardial ischemia. Additionally, while the ε isozyme of PKC is translocated to particulate fractions in the ischemic adult rat heart, its spatial translocation is not changed in either isolated perfused hearts or isolated adult cardiac myocytes in response to phorbol esters. It is not surprising that the δ isozyme of PKC is not translocated to particulate fractions in response to phorbol ester stimulation because this isozyme does not have a phorbol ester binding domain and has been shown to have a unique zinc finger domain that interacts with a specific lambda-interacting protein that may be involved in the translocation of this isozyme (5). Taken together, these results underscore the diversity of the PKC isozymes in the heart, which are poised to be activated and translocated to subcellular membrane pools in response to specific stimuli.

The gross changes in the particulate-associated protein phosphorylation state during myocardial ischemia were striking, and, on the basis of the observed inhibition by the selective PKC inhibitor bisindolylmaleimide I, it appears that this protein phosphorylation is mediated by PKC. It should be noted that it is unlikely that the effects are due to the inhibition of protein kinase A (PKA) because the concentration of bisindolylmaleimide I employed in this study is 40-fold less than the Kᵢ for PKA (27). Importantly, although the translocation of PKC has been shown in multiple species and models of cardiac injury, the results herein are the first to demonstrate that this translocation event results in PKC-dependent phosphorylation. The identification of the proteins that are phosphorylated by PKC during ischemia will be critical to thoroughly understand the role of PKC in the pathophysiological sequelae of myocardial ischemia.

Taken together, specific PKC isozymes are translocated to membrane domains during global myocardial ischemia in the isolated perfused rat heart model, which likely occurs in the cardiac myocytes. Additionally, the PKC isozymes translocated during global ischemia are active, resulting in the phosphorylation of specific myocardial proteins. The precise role of each of these PKC isozymes that are translocated during ischemia, as well as the identity of their protein substrates, remains to be resolved. It is likely that one or more of these translocated isozymes and protein substrates are involved in the preconditioning response to ischemia as well as the pathophysiological sequelae of myocardial ischemia.

Between the results of the present studies and those previously reported (19) may reflect differences between rat and rabbit myocardium.

Until recently, the α isozyme of PKC was not believed to be present in the adult rat heart (3, 22). However, the present studies, as well as those of others (21, 29), clearly demonstrate that the α isozyme of PKC is present in the adult rat heart as well as in adult rat cardiac myocytes. Steinberg and co-workers (9) have clearly demonstrated that the α isozyme of PKC translocates to particulate fractions in neonatal cardiac myocytes prepared from Wistar rat ventricles that are subjected to hypoxia. Rybin and Steinberg (21) have also recently demonstrated that some of the conflicting results regarding the presence of the α isozyme of PKC in adult rat cardiac myocytes can be attributed to the use of different commercially available antibodies that do not universally recognize the α isozyme of PKC in rat cardiac myocytes (21). In the present study, another antibody, Sigma anti-α PKC, was utilized to confirm the presence of the α isozyme of PKC in the adult rat cardiac myocyte. Thus the present studies support the findings of Rybin and Steinberg (21) that the α isozyme of PKC is present in the adult rat cardiac myocyte.

The translocation of PKC isozymes in isolated adult rat cardiac myocytes subjected to phorbol ester stimulation. Adult rat cardiac myocytes were incubated in either modified Joklik’s minimum essential medium (MEM) equilibrated with 100% O₂ (Control) for 15 min or modified Joklik’s MEM equilibrated with 100% O₂ that contained 100 nM PMA for 15 min (PMA). Following experimental intervals, isolated cardiac myocytes were rapidly frozen at the temperature of liquid nitrogen. Cytosolic (C) and particulate (P) fractions were prepared from isolated cardiac myocytes and were sequentially subjected to SDS-PAGE and Western blot analysis utilizing the indicated primary antibodies, including either Sigma anti-α PKC (rabbit, 1 µl/10 ml), Sigma anti-δ PKC (rabbit, 1.5 µl/10 ml), Sigma anti-ε PKC (rabbit, 4 µl/10 ml), or Transduction Laboratories anti-ε PKC (mouse, 20 µl/10 ml) as described in detail in MATERIALS AND METHODS.
REFERENCES