Increased Protein Kinase C and Isozyme Redistribution in Pressure-Overload Cardiac Hypertrophy in the Rat

Xin Gu, Sanford P. Bishop

Abstract Protein kinase C (PKC) activity and isozyme distribution were evaluated during development of pressure-overload-induced cardiac hypertrophy. Three-week-old rats were loosely banded on the ascending aorta (left ventricular hypertrophy [LVH] group). Two weeks later, when left ventricular mass was 50% greater than in the sham-operated control group and cardiac mass was still rapidly increasing beyond that of normal growth, PKC activity and [3H]phorbol 12,13-dibutyrate (PDBu) binding capacity were determined. In LVH, PKC activity was 119±14%, 158±17%, and 152±9% of the control value in cytosol, membrane, and nuclear-cytoskeletal fractions, respectively (n=9 or 10). [3H]PDBu binding assay revealed increased PKC concentration in LVH cytosolic (control, 0.51±0.06 pmol/L per milligram; LVH, 0.78±0.09 pmol/L per milligram; n=5; P<.05) and membrane fractions (control, 1.33±0.15; LVH, 2.32±0.39; n=5; P<.05). Scatchard analysis indicated no difference in Kd values between control and LVH groups. Immunoblot analysis using PKC isozyme-specific antibodies showed that both Ca2+-dependent (α and β) and Ca2+-independent (δ, ε, and ζ) isozymes were present in the left ventricle. Compared with the control value, there was increased concentration in the membrane and nuclear-cytoskeletal fractions for β1, and ε and in the cytosol for β2. PKC-δ could be detected only in the nuclear-cytoskeletal fraction and was not changed in LVH. PKC-α and -ζ were present in all three fractions but were not altered in LVH. These data indicate that PKC activity and concentration increase during development of LVH induced by pressure overload. The increased PKC isozymes were mainly limited to PKC-β1 and PKC-ε, and the increase was present mainly in the membrane and nuclear-cytoskeletal fractions. (Circ Res. 1994;75:926-931.)

Key Words • protein kinase C • protein kinase C isoforms • cardiac hypertrophy • immunoblotting

Cardiac hypertrophy is a common complication of hypertension and is recognized as a risk factor for the development of congestive heart failure.1 In response to hormonal and mechanical stimuli, the myocardium adapts to increased work loads through the hypertrophy of individual muscle cells. Characteristics of the myocardial hypertrophic response include an increase in contractile protein content, the induction of more efficient contractile protein isoforms, and the expression of several embryonic markers, which appear to depend largely on the activation of transcription of the corresponding cardiac genes that encode these proteins.

Since the early studies of Nishizuka,2,3 protein kinase C (PKC) has been implicated as the intracellular mediator of several neurotransmitters, growth hormones, and tumor promoters, such as α-adrenergic agonists, angiotensin II, and phorbol esters. Activation of PKC leads to phosphorylation of transcription factors and subsequent gene expression in a variety of tissues.4,5 PKC is now recognized to be a group of enzymes encoded by a family of genes, which can be further divided into two groups, conventional PKC (cPKC) and novel PKC (nPKC).5,6 cPKC contains four isozymes, PKC-α, -β1, -β2, and -γ, which require Ca2+, phospholipids, and diacylglycerol for activation; nPKC isozymes (nPKC-δ, -ε, -ζ, -η, and -θ) seem to be independent of Ca2+ for activation.

Accumulated evidence suggests that PKC is involved in myocardial cell hypertrophy. Stimulation with α-adrenergic agonists and angiotensin II promotes the endogenous PKC activator diacylglycerol,5 leading to activation of cardiac gene transcription,6 the accumulation of contractile protein, and the induction of a program of immediate-early genes (c-fos and Egr-1).7 These studies suggest that PKC activation may represent a common signaling event in the activation of cardiac gene expression and subsequent protein synthesis during the development of cardiac hypertrophy.

When cell culture systems were used, activated PKC was found to translocate from the cytosol to the membranes in response to a variety of stimuli,8,9 Moreover, this translocation was found to be isozyme specific in response to α1 agonist stimulation.9 These results raise the possibility that, after activation, each isozyme may be in a specific location and phosphorylate different substrates to regulate diverse biological functions.

Although PKC has been implicated in the regulation of gene expression and several hypertrophic stimuli elicit changes in the activity and distribution of PKC in cultured myocytes, the characteristics of PKC in response to chronic hypertrophic stimuli in vivo remain to be defined. The goal of the present study was to characterize the distribution of PKC activity and iso-
forms in subcellular fractions in a model of chronic pressure-overload–induced development of cardiac hypertrophy.

Materials and Methods

[γ-32P]ATP and [3H]phorbol 12,13-dibutyrate (PDBu) were purchased from Amersham. Phosphatidylserine was obtained from Avanti Polar-Lipids. Monoclonal antibody to PKC-α was purchased from Upstate Biotechnology Inc. Polyclonal antisera to PKC-Bβ2, PKC-δ, PKC-ε, and PKC-ζ were purchased from Gibco BRL. All other reagents were from Sigma Chemical Co.

Animal Model of Cardiac Hypertrophy

Cardiac hypertrophy was produced, as previously described,10 by banding the ascending aorta of 23-day-old Sprague-Dawley rats (45 to 55 g body weight) using a Week hemoclip set to a 0.61-mm inside diameter. A surgical plane of anesthesia was maintained with brevital (0.1 mg/kg). Sham-operated rats underwent the same operation without placement of the clip. The experiments were performed 2 weeks after surgery.

Preparation of PKC

Partially purified PKC was prepared from subcellular fractions according to the methods described previously,11,12 with slight modification. Briefly, the hearts were removed and immediately cooled in ice-cold saline. Subsequent extraction was performed at 4°C. The left ventricles were dissected from the right ventricle and atria and minced quickly. The minced tissue was homogenized with a Tissumizer polystyrene (two times for 30 seconds each, at highest speed) in 15 vol of buffer containing (mmol/L) HEPES 20, sucrose 250, EDTA 2, EGTA 2, phenylmethylsulfonyl fluoride (PMSF) 2, and diithiothreitol 2, along with 25 µg/mL leupeptin and 10 µg/mL aprotinin (pH 7.5). The homogenates were passed through a 300-µm mesh nylon filter and then subjected to differential centrifugation. Crude particles and nuclei sedimenting at 800g were sonicated (two bursts of 20 seconds each) to complete cellular disruption and then washed and centrifuged at 800g for three times with the homogenization buffer containing 30% glycerol and 0.05% Triton X-100. The supernatant contained nuclei and cytoskeletal filaments, which did not dissolve in mild detergent. The 800g supernatant was centrifuged at 100 000g for 40 minutes. The pellet was the membrane fraction, and the particulate-fraction–free supernatant was the cytosolic fraction. In our experiments, the activity of lactate dehydrogenase (LDH) in the membrane and nuclear-cytoskeletal fraction was <0.2% of total homogenate LDH. Microscopic examination of the membrane fraction stained with 0.1% 4’,6-diamidino-2-phenylindole (DAPI) and examined with a 365-nm UV filter revealed no intact nuclei, which were numerous in the nuclear-cytoskeletal fraction. Triton X-100 was added to the cytosol to give a final concentration of 0.5%. The separate membrane and nuclear-cytoskeletal fractions from two rats were combined, and each fraction was suspended in homogenization buffer containing 5% Triton X-100, held on ice for 60 minutes with stirring, and centrifuged at 100 000g for 40 minutes. The resulting detergent-treated supernatants from the membrane, nuclear-cytoskeletal, and cytosolic fractions were each chromatographed on an ion-exchange DEAE Sepharose column (1-mL bed volume) equilibrated with Tris-HCl buffer containing (mmol/L) Tris-HCl 20, pH 7.5, EDTA 1, EGTA 2, β-mercaptoethanol 10, and PMSF 2, along with 25 µg/mL leupeptin and 10 µg/mL aprotinin. Columns were washed with 4 mL of the same buffer, and PKC was then eluted by 2 mL Tris-HCl buffer containing 0.2 mol/L NaCl. Protein concentration was determined with a Bio-Rad protein assay based on the procedure by Bradford using bovine serum albumin as a standard. The aliquots of partially purified PKC samples from each fraction containing an appropriate amount of protein were used for activity, [3H]PDBu binding, and immunoblot analysis.

Determination of PKC Activity

PKC activity was measured in a reaction mixture (100 µL) containing 50 µg histone III-S, 25 mmol/L Tris-HCl, pH 7.5, 5 mmol/L MgCl2, 1.2 mmol/L CaCl2, 5 µg phosphatidylserine, 0.5 µg/mL diolein, 10 µmol/L [γ-32P]ATP (2·1011 cpm), and PKC (10 to 50 µg protein). The Ca2+-independent kinase activity was measured under the same conditions without the addition of Ca2+. After a 3-minute preincubation at 30°C, the reaction was initiated by the addition of [3H]PDBu and stopped after 3 minutes by the transfer of the reaction mixture onto a 3×3-cm Whatman P81 phosphocellulose paper, which was immediately dropped into 75 mmol/L H3PO4. The filters were washed three times for 5 minutes each with 75 mmol/L H3PO4, dried, and counted for radioactivity. PKC Ca2+-dependent activity was determined by subtracting the Ca2+-independent 32P incorporation into histone III-S from 32P incorporation measured in the presence of Ca2+.

[3H]PDBu Binding Assay

Binding of [3H]PDBu was measured as previously described.13 The reaction mixture (0.25 mL) containing 25 mmol/L Tris-HCl buffer, pH 7.5, 5 mmol/L MgCl2, 1.2 mmol/L CaCl2, 250 µg/mL phosphatidylserine, 4 mg/mL bovine serum albumin, 0 to 25 mmol/L [3H]PDBu, and an appropriate amount of partially purified PKC sample was incubated at 34°C for 3 hours. Under our conditions, the binding was linear for protein concentrations ranging between 0 and 60 µg, and the association of the ligand to PKC reached an equilibrium at 2 hours. Bound [3H]PDBu samples were separated from free ligand by filtering through Whatman GF/C glass-fiber filters and washed five times each with 5 mL of ice-cold 20 mmol/L phosphate-buffered saline, pH 7.4. Nonspecific binding was determined under the same conditions with the addition of 50 µmol/L nonradioactive PDBu.

Immunoblot Analysis of PKC Isoforms

Each partially purified PKC sample (100 µg) was precipitated with 10% trichloroacetic acid and dissolved in sodium dodecyl sulfate (SDS)–polyacrylamide gel sample buffer (0.5 mol/L Tris-HCl, pH 6.8, 10% glycerol, and 2% SDS) and boiled for 5 minutes. The samples were separated by using SDS–10% polyacrylamide gels. They were then electrophoblotted onto polyvinylidene difluoride membranes (Immobilon-P, Millipore). The membranes were incubated in 20 mmol/L Tris-HCl (pH 7.5) containing 137 mmol/L NaCl and 0.2% Tween 20 (TBS-T) supplemented with 5% (wt/vol) nonfat dry milk for 60 minutes at room temperature. After five washes with TBS-T (5 minutes each), isotype-specific antibodies diluted in TBS-T (1:2500 for anti-α and 1:1000 for anti-β2, δ, and ζ) were added and incubated for 2 hours at room temperature, followed by five washes with TBS-T. Primary binding of the PKC isoform-specific antibodies was detected by using anti-mouse Ig (Amersham, 1:5000 dilute for PKC-α) or anti-rabbit IgG (Amersham, 1:2500 for PKC-β2, δ, ε, and ζ) conjugated with horseradish peroxidase. Blots were made visible by the ECL system (Amersham) according to the manufacturer’s instructions. Immunoblot analyses were repeated three to five times. In each experiment, a PKC sample (10 µg), which was extracted from rat brain by the same method as described previously, was carried out simultaneously as a positive control. To ensure reproducibility of the immunoblotting procedure, two samples from the same fraction prepared in each experiment were separately immunoblotted. Results from the two blots were similar in all cases. In some experiments, the PKC membranes were incubated with 100 mmol/L Tris-HCl buffer (pH 6.7) containing 100 mmol/L 2-mercaptoethanol and 2% SDS at 50°C for 30 minutes and then reblotted with another antibody.
TABLE 1. Heart Weight in Pressure-Overload Hypertrophy

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>LV Weight, mg</th>
<th>LV Weight/Body Weight, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated control (n=12)</td>
<td>161±4</td>
<td>480±13</td>
<td>3.02±0.12</td>
</tr>
<tr>
<td>Hypertrophy (n=12)</td>
<td>148±5</td>
<td>735±28</td>
<td>4.97±0.19</td>
</tr>
<tr>
<td>Increase, %</td>
<td></td>
<td>53</td>
<td>64</td>
</tr>
</tbody>
</table>

LV indicates left ventricular. Values are mean±SEM.

Statistical Analysis

The data were expressed as mean±SEM. Statistical analysis was performed by unpaired Student's t test. Differences were considered significant at P<.05. Curve fitting was carried out with SIGMAFIT software (Jandel Scientific). Since quantification of immunoblot analysis is only valid for any single PKC isoform examined in a single immunoblot, results for experiments such as these are therefore expressed as percentages of suitable control performed and analyzed simultaneously.

Results

Animal Model

As previously described,10 along with growth, constriction of the ascending aorta in 23-day-old rats resulted in left ventricular hypertrophy (LVH) (Table 1). Two weeks after surgery, the left ventricular weight was 53% more than in sham-operated control rats. Hypertrophy of both atria and the right ventricle also occurred during this period. In our preliminary studies, we found different patterns of PKC activity and isoforms in atria and right and left ventricles during normal development from birth to 11 weeks of age. Therefore, only left ventricular tissue was included in the present study.

Expression of PKC Activity in Subcellular Fractions

In the left ventricle of normal heart, Ca²⁺-dependent PKC activity distribution in cytosol, membrane, and nuclear-cytoskeletal fractions was 66%, 17%, and 17%, respectively, of the total combined Ca²⁺-dependent activity of all three fractions (Fig 1A). When the activity was expressed as specific activity as picomolar per milligram protein per minute, the activity in the cytosol was the lowest, and the activity in the membrane and nuclear-cytoskeletal fraction was higher (Fig 1B).

In hypertrophied left ventricle, the distribution of total Ca²⁺-dependent activity in subcellular fractions was the same as in control animals (Fig 1A). The specific activity in cytosol from hypertrophied left ventricles remained unchanged compared with that from normal tissue. However, the specific activity in the membrane and nuclear-cytoskeletal fractions was significantly increased by >50% (Fig 1B). In all activity experiments, the Ca²⁺-independent activity measured in the presence of histone as a substrate was not different between control and hypertrophied hearts in any fraction; Ca²⁺-independent PKC activity was as follows [(pmol/L) · mg protein⁻¹· min⁻¹]: control/cytosol, 2.8±0.4; LVH/cytosol, 3.2±0.5; control/membrane, 17.9±1.9; LVH/membrane, 19.0±2.4; control/nuclear-cytoskeletal, 11.9±1.6; and LVH/nuclear-cytoskeletal, 14.3±1.9 (P=NS). It should be noted that histone as a substrate does not measure the activity of PKC-ε.

[³H]PDBu Binding Capacity

To further test the possibility of a quantitative increase of PKC concentration during development of cardiac hypertrophy, we analyzed the capacity of subcellular fractions to bind to [³H]PDBu. In both control and LVH groups, Scatchard analysis revealed one class of binding site in cytosol, membrane, and nuclear-cytoskeletal fractions (Fig 2). The [³H]PDBu binding capacity in the cytosol and membrane fractions was significantly increased in hypertrophied left ventricles (Fig 2A and 2B). However, we did not find a significant difference between control and hypertrophied left ventricles in the nuclear-cytoskeletal fraction. The Kᵥ value was lowest in the nuclear-cytoskeletal fraction and highest in the membrane fraction in both control and hypertrophied groups (Fig 2). There was no difference in Kᵥ value between control and hypertrophied left ventricles in any membrane fraction.

Immunoblot Analysis of PKC Isoform Expression in Subcellular Fractions

The expression of Ca²⁺-dependent (α and β₁₂) and Ca²⁺-independent (δ, ε, and ζ) isoforms in subcellular fractions was examined in control and hypertrophied

![Fig 1](attachment:image.png) Bar graphs showing protein kinase C (PKC) Ca²⁺-dependent activity in subcellular fractions in control and hypertrophied left ventricle (LVH). A. PKC Ca²⁺-dependent activity distribution in subcellular fractions is shown as percentage of total combined activity for all three fractions. B. Specific Ca²⁺-dependent activity is expressed as picomolar per milligram protein per minute in subcellular fractions. Cyt indicates cytosol; Mem, membrane; and N-cke, nuclear-cytoskeleton. Values are mean±SEM of 9 to 11 separate experiments. *P<.05 vs control.
left ventricles. The results are summarized in Table 2. As shown in Fig 3, the distribution of these isozymes in subcellular fractions was different. The immunoreactivity of PKC-α in the nuclear-cytoskeletal fraction was weaker compared with that in cytosol and membrane fractions, PKC-δ appeared only in the nuclear-cytoskeletal fraction, and other isozymes (β, ε, and ζ) were found in all three fractions with no difference in distribution. Compared with the control group, only PKC-β₁,₂ and PKC-ε isozymes increased in hypertrophied left ventricles.

### Table 2. Changes in Protein Kinase C Isoform Expression From Control Value in Developing Cardiac Hypertrophy

<table>
<thead>
<tr>
<th>PKC Isoform</th>
<th>α</th>
<th>β</th>
<th>δ</th>
<th>ε</th>
<th>ζ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>-</td>
<td>↑</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Membrane</td>
<td>-</td>
<td>↑</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Nuclear cytoskeleton</td>
<td>-</td>
<td>↑</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
</tbody>
</table>

PKC indicates protein kinase C; ↑, increase \((P<.05, n=3 \text{ to } 5)\); -, no change. Data are from Fig 3.

Fig 2. Graphs showing specific binding of \(^{3}H\)phorbol 12,13-dibutyrate (PDBu) to protein kinase C (PKC) in cytosol (A), membrane (B), and nuclear-cytoskeletal fractions (C) in control and hypertrophied left ventricle (LVH). Partially purified PKC sample was incubated with varied concentrations of \(^{3}H\)PDBu for 3 hours at 4°C. Nonspecific binding, determined in parallel incubations containing a 500-fold excess of unlabeled PDBu, was subtracted from the total binding to yield values for specific binding. The specific binding was also analyzed by the method of Scatchard. Values are mean±SEM from 5 or 6 separate experiments. Bmax indicates maximal binding capacity; Kd, dissociation constant.

Fig 3. Immunoblot analysis of protein kinase C (PKC) isoform distribution in cytosol (Cyt), membrane (Mem), and nuclear-cytoskeletal (N-cyte) fractions in control (C) and hypertrophied left ventricle (H) as described in "Materials and Methods." Immunoblot analysis was performed (100 μg protein loaded per lane) by using monoclonal antibody to PKC-α or polyclonal antiserum to PKC-β₁,₂, -δ, -ε, and -ζ. Std indicates PKC standard from rat brain.
ventricles. Densitometry showed that the amount of PKC-β2 at 78 kD, which was consistent with a PKC from brain extract, was more abundant in hypertrophied tissue than in control tissue in all three subcellular fractions (cytosol, 123±9.7% of control [P<.05]; membrane, 178±19% of control [P<.01]; and nuclear-cytoskeleton, 138±16% of control [P<.05]). PKC-ε was increased in both membrane and nuclear-cytoskeletal fractions (membrane, 167±11% of control [P<.01]; nuclear-cytoskeleton, 136±12% of control [P<.05]) but was not significantly changed in the cytosolic fraction. PKC-β2 was expressed as doublets, with a prominent band at ≈78 kD and a fainter band at 70 kD in both membrane and nuclear-cytoskeletal fractions. It is possible that these doublets represent different phosphorylation states of the isoforms.14

**Discussion**

The present study characterized the activation and isoyme localization of PKC during the active phase of development of LVH in a progressive model of pressure overload in the rat. In normal rat heart, both Ca2+-dependent (α and β) and Ca2+-independent (δ, ε, and ζ) isoforms of PKC were found. During the developmental phase of cardiac hypertrophy, the specific activity of PKC was increased in the membrane and nuclear-cytoskeletal fractions but not in the cytosolic fraction. Immunoblot analysis revealed that increased PKC was limited to PKC-β2 and PKC-ε and mainly distributed in membrane and nuclear-cytoskeletal fractions.

**PKC Activity and [3H]PDBu Binding Capacity in Subcellular Fractions in Hypertrophied Left Ventricle**

Activation of PKC in cultured myocytes is associated with redistribution to the particulate fractions, including membranes, cytoskeleton, and nuclei.5,6 Recently, translocation of activated PKC from cytosol to nuclei6 and the cytoskeleton5 has been demonstrated in cultured neonatal myocytes by using biochemical and immunohistochemical techniques. In the present study, the fraction containing nuclei and cytoskeleton elements was separated, and chelate- and detergent-stable PKC was extracted. Further separation of these components resulted in a total loss of activity. A low LDH activity in this fraction indicated a good separation from cytosol. However, we cannot rule out the possible presence of other intracellular components in this preparation.

Previous studies have demonstrated that activation of PKC caused a translocation of the enzyme, appearing as an increased activity in the particulate fraction, with concomitant decreased activity in the cytosol.12,15 However, in the present study, we did not find such an inverse relation of PKC activity between cytosolic and particulate fractions. The additional PKC activity was restricted to the particular fractions in this model of progressive pressure-overload hypertrophy, without a decrease in the activity in the cytosolic fraction.

**Distribution of PKC Isozymes in Subcellular Fractions in Hypertrophied Left Ventricle**

The existence of PKC-α and PKC-β isoymes has been identified in rat and bovine hearts by chromatography.13,16 A recent report, using immunoblotting methods, has identified the existence of PKC-ε and PKC-ζ in rat heart tissue and has suggested that PKC-ε is the major isoform in normal rat cardiac myocytes and that PKC-ζ is mainly distributed in nonmyocyte cells.17 As in previous reports from other laboratories,13,16 we were unable to find PKC-γ in heart tissue (result not shown); PKC-γ is believed to exist only in the brain. However, two other Ca2+-dependent isoymes, PKC-α and PKC-β2, were present. Among Ca2+-independent PKC isoymes (δ, ε, ζ, and θ), the existence of PKC-δ, -η, and -θ in heart tissue has not previously been demonstrated. In the present study, we identified abundant PKC-ε and PKC-ζ in cardiac tissue, as reported previously,17 and these isoymes were present in all three subcellular fractions. PKC-δ was identified in the present study. Consistent with previous findings in other tissues,18 we found PKC-δ in the particulate fraction of the left ventricle, with little or no immunoreactivity in the cytosol. Specific distribution and tight binding in the nuclear-cytoskeletal fraction could explain the failure to find this isoyme in previous studies.17

As shown in “Results,” there was no increased PKC Ca2+-dependent activity in the cytosol fraction to correspond with the increase of [3H]PDBu binding capacity in this fraction; additionally, there was no increased Ca2+-independent activity in the membrane and nuclear-cytoskeletal fractions, as measured with the histone substrate, to correspond with the increased protein of Ca2+-independent PKC-ε in the immunoblot analysis. It is well known that PDBu binds to both Ca2+-dependent and Ca2+-independent isoymes.19 It is also known that PKC isoymes differ intrinsically in their substrate specificity20 and that PKC-ε only phosphorylates myelin basic protein and therefore is not measured in the analysis for activity of PKC Ca2+-independent forms using histone as a substrate.21 Coexistence of multiple isoymes with different respect to Ca2+, phospholipid, and substrate dependence might be an explanation for the discrepancy between the results in our study of activity, [3H]PDBu binding, and immunoblot analysis. In the present study, we did not evaluate the existence of other Ca2+-independent isoymes that could also be present in cardiac tissue. A limitation of the present study is that we did not separate myocytes from other cells. However, since ≈90% of the mass of cardiac tissue is from myocytes, it appears unlikely that nonmyocytes were a major source of the PKC in the present study.

Activation of PKC initiates a cascade of regulatory events, including the control of gene expression. In cultured neonatal cardiomyocytes, transfection with DNA encoding constitutively active forms of PKC-β induced a change of gene expression for β-myosin isoymes typical of the hypertrophic response.22 In our previous studies with this model, a shift of myosin isoyme to the β isoform was demonstrated.19 In light of these studies, the increase and redistribution of PKC-β2 during the development of hypertrophy in vivo appears to be associated with gene regulation and subsequent increased protein synthesis in response to pressure overload. However, a direct relation has not been demonstrated. Increased PKC-ε isoyme in membrane and nuclear-cytoskeletal fractions implicated the activation of this Ca2+-independent isoyme. In a recent report,17 translocation of PKC-ε occurred in response to the stimulation of phorbol esters and epinephrine, both
of which are known to stimulate myocyte protein synthesis in vitro. Increase of PKC-ε in the particulate fractions in response to pressure overload suggests that PKC-ε was also involved in the development of hypertrophy in vivo. However, the physiological significance of this Ca$^{2+}$-independent isozyme in the development of cardiac hypertrophy requires further study.

In summary, using biochemical subcellular fractionation methods, we found a quantitative increase of PKC in the cytosolic and membrane fractions as well as in the nuclear-cytoskeletal fraction of hypertrophied left ventricular myocardium of the rat. PKC-α, -β2, -ε, and -ζ isozymes were found in both soluble and particulate fractions, whereas PKC-δ isozyme was found only in the nuclear-cytoskeleton of the particulate fraction. PKC-β2 and PKC-ε concentrations were increased in response to pressure overload. These results are consistent with the hypothesis that PKC plays an important role in the regulation of cardiac myocyte growth and function in development of hypertrophy and that individual isozymes may perform different functions in response to pathological stimulation.

Acknowledgments

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