Increased expression of protein kinase C isoforms in heart failure due to myocardial infarction

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Wang, Jingwei, Xuéliang Liu, Emmanuelle Sentex, Nobuakira Takeda, and Naranjan S. Dhalla. Increased expression of protein kinase C isoforms in heart failure due to myocardial infarction. Am J Physiol Heart Circ Physiol 284: H2277–H2287, 2003; 10.1152/ajpheart.00142.2002.—The activities of cardiac protein kinase C (PKC) were examined in hemodynamically assessed rats subsequent to myocardial infarction (MI). Both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent PKC activities increased significantly in left ventricular (LV) and right ventricular (RV) homogenates at 1, 2, 4, and 8 wk after MI was induced. PKC activities were also increased in both LV and RV cytosolic and particulate fractions from 8-wk infarcted rats. The relative protein contents of PKC-\(\alpha\), \(\beta\), \(\epsilon\), and \(\zeta\) isoforms were significantly increased in LV homogenate, cytosolic (except PKC-\(\alpha\)), and particulate fractions from the failing rats. On the other hand, the protein contents of PKC-\(\alpha\), \(-\beta\), and \(-\epsilon\) isoforms, unlike the PKC-\(\zeta\) isozyme, were increased in RV homogenate and cytosolic fractions, whereas the RV particulate fraction showed an increase in the PKC-\(\alpha\) isozyme only. These changes in the LV and RV PKC activities and protein contents in the 8-wk infarcted animals were partially corrected by treatment with the angiotensin-converting enzyme inhibitor imidapril. No changes in protein kinase A activity and its protein content were seen in the 8-wk infarcted hearts. The results suggest that the increased PKC activity in cardiac dysfunction due to MI may be associated with an increase in the expression of PKC-\(\alpha\), \(-\beta\), and \(-\epsilon\) isoforms, and the improvement of heart function in the infarcted animals by imidapril may be due to partial prevention of changes in PKC activity and isozyme contents.

protein kinase A; angiotensin-converting enzyme inhibitor; isozymes

AMONG A WIDE VARIETY OF protein kinases present in mammalian cells, two multifunctional protein kinases, calcium/phospholipid-dependent protein kinase [protein kinase C (PKC)] and cAMP-dependent protein kinase [protein kinase A (PKA)], are thought to mediate several phosphorylation reactions in the myocardium (7, 9, 37). Both of these protein kinases are known to regulate cation transport, contractile force development, metabolic processes, gene expression, and cellular growth in the heart (9, 37). Molecular cloning studies (9, 24) have indicated that PKC exists as a family of at least 12 distinct isoforms. The conventional PKC isoforms (\(\alpha\), \(\beta\), and \(\gamma\)) contain a Ca\(^{2+}\)-binding domain, which accounts for their activation by Ca\(^{2+}\). The novel PKC isoforms (\(\delta\), \(\epsilon\), \(\eta\), and \(\theta\)) lack the putative Ca\(^{2+}\)-binding domain and do not require Ca\(^{2+}\) for maximal enzymatic activation. Atypical PKC isoforms (\(\zeta\), \(\lambda\), and \(\iota\)) are distinguished from other members of the PKC gene family by the presence of only a single copy of a cysteine-rich motif. Activation of angiotensin II (ANG II) receptors, \(\alpha_1\)-adrenergic receptors, and endothelin-1 receptors has been shown to stimulate PKC via Gq-coupled phospholipase \(\mathrm{C}_\beta\) (PLC-\(\beta\)) (9, 37, 42). In contrast, PKA is activated by catecholamines through stimulatory \(\mathrm{G}\) protein-coupled \(\beta\)-adrenergic receptors (9, 14).

Previous work from our laboratory (44) has demonstrated increased activities of cardiac PKC and PKA due to congestive heart failure in cardiomyopathic hamsters. Increased cardiac PKC activity has also been shown in pressure-overloaded cardiac hypertrophy in the rat (13) and pressure-overloaded heart failure in guinea pigs (38), as well as in human failing hearts (5). Varying degrees of changes in PKC activities have been observed in cardiac dysfunction due to diabetes (17, 21, 22, 40, 45). Furthermore, transgenic mice with cardiac overexpression of PKC-\(\beta\) or PKC-\(\epsilon\) were found to exhibit gross cardiac hypertrophy and diminished ventricular function (39, 43). The PKA activity has also been observed to increase in cardiac hypertrophy due to volume overload in rats (19). Transgenic mice with overexpression of PKA in the heart have been reported to develop dilated cardiomyopathy and reduced cardiac contractility; however, no changes in PKA activity were seen in the failing human heart or in myocardial infarction (MI) (3, 28). Although cardiac hypertrophy, heart failure, and cardiac dysfunction are known to occur as a consequence of MI (1, 11, 30, 36, 41), there is no literature regarding changes in PKC activities in the infarcted heart. Ac-
cordingly, this study was undertaken to examine the status of PKC activities during the development of congestive heart failure in a rat model of MI. Some experiments were also carried out to examine whether the changes in PKC in the failing heart are due to corresponding changes in the contents of PKC isozymes.

It is now well known that PKC is activated by ANG II through the PLC-β-mediated mechanisms in cardiomyocytes (37). Furthermore, ANG II-induced activation of PKC has been demonstrated to result in the stimulation of cardiac gene expression, cell growth, and remodeling of the myocardium (4, 9, 37). Accordingly, the stimulation of the renin-angiotensin system (RAS) is considered to play a critical role in the activation of PKC that regulates the hypertrophic process and cardiac performance (38). Because the RAS is activated in congestive heart failure (12) and treatment of infarcted animals with ANG-converting enzyme (ACE) inhibitors has been shown to produce beneficial effects on heart function and attenuate the hypertrophic process (50 mM Tris·HCl, 0.25 M sucrose, 10 mM EGTA, 4 mM EDTA, 20 μg/ml leupeptin, and 200 μM aprotinin, pH 7.5) and homogenized (Polytron PT3000, Brinkmann Instruments; Mississauga, Ontario, Canada) at setting 8 for 2 × 30 s and sonicated for 2 × 15 s. In one set of experiments, the homogenate was incubated with 1% Triton X-100 (Sigma; St. Louis, MO) on ice for 60 min to solubilize PKC enzyme, which is bound with subcellular structures. This Triton X-100-treated homogenate was then centrifuged at 100,000 g for 60 min in an ultracentrifuge (model L70, Beckman Instruments), and the supernatant obtained was labeled as the homogenate fraction. In another set of experiments, the homogenate without Triton X-100 treatment was centrifuged at 100,000 g for 60 min to separate the soluble and particulate-bound enzyme. The resulting supernatant was labeled as the cytosolic fraction, whereas the pellet was resuspended in 1 ml of buffer A with 1% Triton X-100 and incubated on ice for 60 min. The resuspended pellet was centrifuged at 100,000 g for 60 min and this supernatant was labeled as the particulate fraction. For preparation of tissue extract for PKA determination, ~50 mg of frozen cardiac tissue were homogenized in 1 ml buffer B at pH 7.4 containing (in mM) 5 histidine-HCl, 0.1 phenylmethylsulfonyl fluoride, 50 K2HPO4, 25 NaF, 1 EDTA, 750 KCl, and 0.2 dithiothreitol. After gentle mixing, the homogenate was centrifuged at 100,000 g for 60 min at 4°C and the supernatant was used for analysis of PKA activity and protein level (3, 19).

Assays of PKC and PKA activities. PKC activities in small samples of nonpurified homogenate, cytosolic, and particulate fractions from the ventricular tissue were measured in the presence of okadaic acid, a highly specific inhibitor of type 1 and type 2A phosphatases (2, 15), by following methods described elsewhere (21, 44). The Ca2+-dependent PKC activity was determined with a PKC assay kit (Upstate Biotechnology; Lake Placid, NY) in the reaction buffer C containing (in mM) 20 MOPS, pH 7.2, 25 β-glycerophosphate, 1 sodium orthovanadate, 1 dithiothreitol, and 4 CaCl2. Substrate cocktail containing 500 μM PKC substrate peptide in buffer C, inhibitor cocktail containing 2 μM PKA inhibitor peptide in buffer C, and lipid activator containing 0.5 mg/ml phosphatidylserine and 0.05 mg/ml diglyceride in buffer C was used. The Ca2+-independent PKC activity was determined in a reaction buffer D containing (in mM) 20 MOPS (pH 7.2), 25 β-glycerophosphate, 1 sodium orthovanadate, 1 dithiothreitol, and 1.25 EGTA. Substrate cocktail (specific for

MATERIALS AND METHODS

Experimental model and hemodynamic assessment. Experiments were conducted in accordance with the “Guide to the Care and Use of Experimental Animals” issued by the Canadian Council of Animal Care, and the protocols were approved by the University of Manitoba Animal Care Committee. MI was induced in male Sprague-Dawley rats (175–200 g) by occlusion of the left coronary artery as described earlier (1, 11, 35, 36). The mortality of rats on coronary occlusion was ~30% within 48 h. The sham control rats were operated out at 4°C. Also, the scar tissue from the infarcted heart was separated and weighed. The removal of scar from the LV was necessary to obtain the noninfarcted myocardium for analysis, as used in other investigations (1, 11, 35, and to determine whether or not to use the viable LV tissue (including the septum) for biochemical studies. Because the scar weight-to-total LV weight ratio (including septum and scar tissue) was found to exhibit a linear relationship with infarct size, as measured morphometrically (1, 11, 35, 36), the scar weight-to-total LV weight ratio was used as a marker to determine the extent of scar size. It should be pointed out that ~10% of the untreated and treated animals showed that ~10% of the untreated and treated animals showed small infarct size (scar weight-to-total LV weight ratio <15% corresponding to scar size <30% of the free LV wall). Thus the hemodynamic data from the animals showing small infarct were not included and the cardiac tissue from these animals was discarded. The lung wet weight-to-dry weight ratio (an index of pulmonary congestion) and heart weight-to-body weight ratio (an index of cardiac hypertrophy) were also measured in experimental animals. The heart weight-to-body weight ratio included both ventricles, including infarct. For hemodynamic studies, the animals were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). The LV systolic pressure, LV end-diastolic pressure (LVEDP), heart rate, rate of pressure development (+dP/dt), rate of pressure decay (−dP/dt), and mean arterial blood pressure (MAP) were measured in these anesthetized animals according to the procedure described earlier (1, 11, 35, 36).

Preparation of tissue extract for enzyme determination. The preparations of tissue extract for PKC was carried out by the method described earlier (21, 44); all procedures were carried out at 4°C. The particulate fraction (50 mM Tris·HCl, 0.25 M sucrose, 10 mM EGTA, 4 mM EDTA, 20 μg/ml leupeptin, and 200 μM aprotinin, pH 7.4) by occlusion of the left coronary artery as described earlier (1, 11, 35, 36). The mortality of rats on coronary occlusion was ~30% within 48 h. The sham control rats were operated in the same way except that the coronary artery was not ligated. These animals were fed rat chow and water ad libitum and then maintained for 1, 2, 4, and 8 wk after the coronary artery ligation before the assessment of cardiac function and biochemical changes. In another series of experiments, some sham and MI rats were divided into untreated and treated groups at 4 wk after the operation. The untreated infarcted animals received distilled water, whereas treated animals were given imidapril hydrochloride dissolved in distilled water at a concentration of 1 mg/ml once a day by gavage at a volume of 1 ml/kg−1·day−1 for 4 wk. All animals were assessed hemodynamically before death and the heart was dissected out. The left ventricle (LV) and right ventricle (RV) were separated, weighed, frozen in liquid nitrogen, and stored at ~70°C. Also, the scar tissue from the infarcted heart was separated and weighed. The removal of scar from the LV was necessary to obtain the noninfarcted myocardium for analysis, as used in other investigations (1, 11, 35, and to determine whether or not to use the viable LV tissue (including the septum) for biochemical studies. Because the scar weight-to-total LV weight ratio (including septum and scar tissue) was found to exhibit a linear relationship with infarct size, as measured morphometrically (1, 11, 35, 36), the scar weight-to-total LV weight ratio was used as a marker to determine the extent of scar size. It should be pointed out that ~10% of the untreated and treated animals showed small infarct size (scar weight-to-total LV weight ratio <15% corresponding to scar size <30% of the free LV wall). Thus the hemodynamic data from the animals showing small infarct were not included and the cardiac tissue from these animals was discarded. The lung wet weight-to-dry weight ratio (an index of pulmonary congestion) and heart weight-to-body weight ratio (an index of cardiac hypertrophy) were also measured in experimental animals. The heart weight-to-body weight ratio included both ventricles, including infarct. For hemodynamic studies, the animals were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). The LV systolic pressure, LV end-diastolic pressure (LVEDP), heart rate, rate of pressure development (+dP/dt), rate of pressure decay (−dP/dt), and mean arterial blood pressure (MAP) were measured in these anesthetized animals according to the procedure described earlier (1, 11, 35, 36).
PKC isoforms in heart failure

PKC-α and -γ isoforms; Quality Controlled Biochemicals; Hopkinton, MA) containing 500 μM PKC substrate peptide in buffer D, inhibitor cocktail containing 2 μM PKC inhibitor peptide in buffer D, and lipid activator containing 0.5 mg/ml phosphatidyl serine and 0.05 mg/ml diglyceride in buffer C was used. The sequence of the peptide substrate (supplied by Upstate Biotechnology) used for PKC activity assay was QKRPSQRSKYL. The reactions for both Ca2+-dependent and Ca2+-independent PKC activities were initiated by the addition of [γ-32P]ATP (10 μl) and allowed to proceed at 30°C for 10 min. The incorporation of 32P from γ-32P into a synthesized substrate, which is a more specific substrate for PKC than Histone H-1 protein (40, 45), was measured as described elsewhere (21, 44). On the other hand, PKA activity was determined with the use of the PKA assay kit (Upstate Biotechnology). The reaction was initiated by adding [γ-32P]ATP (1 part of [γ-32P]ATP in 9 parts of kit ATP solution). The PKA activity was assayed as described earlier (44) by measuring the incorporation of 32P from [γ-32P]ATP into the substrate (3, 19).

Analysis of PKC isozyme and PKA protein content. The relative content for each PKC isozyme (PKC-α, -β, -ε, and -γ) was obtained by running 10% mini-SDS-PAGE, followed by Western blot analysis with homogenate, cytosolic, and particulate fractions (21, 44). The SDS-PAGE loading buffer contained 0.25 M Tris-HCl (pH 6.8), 8% (wt/vol) SDS, 45% glycerol, 20% β-mercaptoethanol, and 0.006% bromophenol blue. The proteins in both fractions separated by SDS-PAGE were electroblotted to Immobilon-P transfer membranes (Millipore; Bedford, MA), which were incubated with polyclonal anti-PKC-α, -β, -ε, and -γ isozyme antibodies (Life Technologies) for 1 h at a concentration of 1:1,000, respectively, and were subsequently incubated with biotinylated anti-rabbit IgG (1:5,000; Amersham) for 40 min and then finally with streptavidin conjugated horseradish peroxidase (1:5,000; Amersham) for 30 min. It should be pointed out that a recombinant standard (Bio-Rad Laboratories; Hercules, CA) was run on SDS-PAGE with each sample to confirm the molecular weight of PKC isoforms. Ponceau staining of the blots was performed to ensure no difference between control and experimental samples with respect to protein loading and protein transfer. The content of PKC isozyme was determined with an imaging densitometer (model GS-670, Bio-Rad) with the Image Analysis Software version 1.0. The relative protein content of PKC-α, -γ, and -ε isozymes was obtained by running 10% mini-SDS-PAGE, followed by Western blot analysis with homogenate, cytosolic, and particulate fractions (21, 44). The SDS-PAGE loading buffer contained 0.25 M Tris-HCl (pH 6.8), 8% (wt/vol) SDS, 45% glycerol, 20% β-mercaptoethanol, and 0.006% bromophenol blue. The proteins in both fractions separated by SDS-PAGE were electroblotted to Immobilon-P transfer membranes (Millipore; Bedford, MA), which were incubated with polyclonal anti-PKC-α, -β, -ε, and -γ isozyme antibodies (Life Technologies) for 1 h at a concentration of 1:1,000, respectively, and were subsequently incubated with biotinylated anti-rabbit IgG (1:5,000; Amersham) for 40 min and then finally with streptavidin conjugated horseradish peroxidase (1:5,000; Amersham) for 30 min. It should be pointed out that a recombinant standard (Bio-Rad Laboratories; Hercules, CA) was run on SDS-PAGE with each sample to confirm the molecular weight of PKC isoforms. Ponceau staining of the blots was performed to ensure no difference between control and experimental samples with respect to protein loading and protein transfer. The content of PKC isozyme was determined with an imaging densitometer (model GS-670, Bio-Rad) with the Image Analysis Software version 1.0. The relative protein content for each experimental sample was expressed as a percentage of the relative protein content of PKC isozyme was determined with an imaging densitometer (model GS-670, Bio-Rad) with the Image Analysis Software version 1.0. The relative protein content for each experimental sample was expressed as a percentage of the respective control value (band density of the sham control sample was considered as 100%). The information about the cross-reactivity of PKC isozyme antibodies (supplied by Life Technologies) indicated that antibodies directed against PKC-γ recognize PKC-α and to a lesser extent PKC-β. Nonetheless, the bands for PKC-γ, -α, and -β were distinguished on the basis of molecular weight. The antibody against PKC-β did not distinguish PKC-βI and PKC-βII, the band for PKC-β was considered to be due to both βI and βII isozymes.

To quantitate the levels of PKC isoforms in the heart, the samples were run on the SDS gels along with recombinant PKC-α and PKC-β (Calbiochem; La Jolla, CA). The membranes were incubated with polyclonal anti-PKC-α and anti-PKC-β (Calbiochem) for 1 h at a concentration of 1:1,000. Different amounts of PKC-α and -β (20, 40, and 60 mg) and different exposure times were used for obtaining standard curves for these isozymes; the optimal time period exposure for PKC-α was 30 s, whereas that for PKC-β was 3 min. The relative protein content of PKA was obtained by running 12% SDS-PAGE and Western blotting (44). The anti-PKA polyclonal antibody was from Transduction Laboratories and a concentration of 1:1,000 was utilized for the primary antibody. Cell lysates (5 μl) derived from a pituitary tumor of a female Wistar-Furth rat (supplied along with the anti-PKA antibody purchased) was used as a positive control in Western blotting experiments.

Data analysis. Data were expressed as means ± SE. The differences among different groups were evaluated statistically by one-way ANOVA, followed by the Newman-Keuls test. A P value <0.05 was taken to represent a significant difference.

RESULTS

Time course changes in general characteristics, hemodynamics, and cardiac PKC activities in experimental rats. Coronary occlusion in rats resulted in extensive LV infarction and the noninfarcted cardiac muscle underwent significant hypertrophy at 1, 2, 4, and 8 wk after the operation. These changes are reflected by the presence of a large scar (average infarct size varied from 38 to 42% of the LV free wall corresponding to the average values in the range of 19–21% for scar weight-to-total LV weight ratio in different groups) and increased ratio of heart weight-to-body weight ratio at all time points (Table 1). These values for infarct size in animals used in this study are comparable to those reported by others (20). The RV weight and viable LV weight were increased at 2, 4, and 8 wk after MI was induced compared with the respective sham control animals. There was a significant increase in the wet weight-to-dry weight ratio of the lungs in 4- and 8-wk infarcted animals, indicating the presence of pulmonary edema (Table 1). An increase in LVEDP and a decrease in both +dP/dt max and −dP/dt max were observed in 1-, 2-, 4-, and 8-wk MI animals (Table 1). These results are consistent with earlier observations in this experimental model (1, 11, 35), which have indicated that the experimental animals at 4 and 8 wk after the coronary occlusion are at early and moderate stages of congestive heart failure, respectively. The Ca2+-dependent PKC activity increased by 53, 79, 47, and 159% in the LV homogenate, whereas the Ca2+-independent PKC activity was elevated by 47, 88, 30, and 201% in the LV homogenate at 1, 2, 4, and 8 wk of MI compared with control values, respectively (Table 1). On the other hand, the Ca2+-dependent PKC activity in the RV homogenate was increased by 54, 24, 34, and 97% and the Ca2+-independent PKC activity was augmented by 47, 24, 29, and 238% of control values at 1, 2, 4, and 8 wk of MI, respectively.

Effect of imidapril treatment on general characteristics, hemodynamics, and cardiac PKC activities in experimental rats. The beneficial effects of imidapril treatment on heart function and cardiac PKC activities were tested by treating the 4-wk experimental animals with imidapril for 4 wk. The results in Table 2 indicate cardiac hypertrophy, lung congestion, elevated LVEDP, depressed +dP/dt and −dP/dt as well as increased Ca2+-dependent and Ca2+-independent PKC activities in both LV and RV homogenates in the 8-wk infarcted animals. All of these changes were partially normalized by treatment with imidapril. Treatment of sham control animals with imidapril did not show any sig-
Table 1. General and hemodynamic characteristics and cardiac homogenate PKC activity of infarcted rats 1, 2, 4, and 8 wk after left coronary artery ligation

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
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<th>MI</th>
<th>Sham</th>
<th>MI</th>
<th>Sham</th>
<th>MI</th>
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</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>279±8.5</td>
<td>273±15.6</td>
<td>297±8.5</td>
<td>298±13.7</td>
<td>378±13.9</td>
<td>393±12.2</td>
<td>491±7.5</td>
<td>482±12.7</td>
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<tr>
<td>RV, mg</td>
<td>157±13.7</td>
<td>181±10.1</td>
<td>163±5.2</td>
<td>216±17.2*</td>
<td>172±12.6</td>
<td>206±33.1*</td>
<td>278±17.1</td>
<td>600±19.8*</td>
</tr>
<tr>
<td>Viable LV, mg</td>
<td>672±21.1</td>
<td>669±25</td>
<td>660±25.3</td>
<td>705±11.6*</td>
<td>771±37.8</td>
<td>867±24.7*</td>
<td>861±27.3</td>
<td>942±39*</td>
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<tr>
<td>Scar wt, mg</td>
<td>ND</td>
<td>156±13.4</td>
<td>ND</td>
<td>184±14.2</td>
<td>ND</td>
<td>201±11.2</td>
<td>ND</td>
<td>237±21.4</td>
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<tr>
<td>Heart wt-to-body wt ratio, mg/g</td>
<td>2.92±0.06</td>
<td>3.67±0.13*</td>
<td>2.79±0.12</td>
<td>3.71±0.1*</td>
<td>2.49±0.07</td>
<td>3.5±0.17*</td>
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<td>Lung wet-to-dry wt ratio</td>
<td>5.23±0.07</td>
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<td>5.17±0.05</td>
<td>5.01±0.08</td>
<td>5.47±0.06*</td>
<td>4.58±0.25</td>
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<td>+dP/dt, mmHg/s</td>
<td>9,438±341</td>
<td>6,227±436*</td>
<td>9,922±576</td>
<td>6,882±447*</td>
<td>9,102±579</td>
<td>6,910±336*</td>
<td>8,763±412</td>
<td>5,468±389*</td>
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<tr>
<td>−dP/dt, mmHg/s</td>
<td>10,259±527</td>
<td>6,639±303*</td>
<td>9,908±575</td>
<td>7,361±638*</td>
<td>10,065±562</td>
<td>7,182±344*</td>
<td>8,906±501</td>
<td>6,574±409*</td>
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<td>LVEDP, mmHg</td>
<td>8.4±0.9</td>
<td>18±1.2*</td>
<td>9.8±2.1</td>
<td>19.8±2.6*</td>
<td>8.6±0.8</td>
<td>17±2.3*</td>
<td>8.1±0.9</td>
<td>19±2.2*</td>
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<tr>
<td>Ca²⁺-dependent PKC activity, pmol · min⁻¹ · mg⁻¹</td>
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<tr>
<td>LV</td>
<td>117±15.4</td>
<td>179±6*</td>
<td>104±9</td>
<td>187±5.6*</td>
<td>87.4±4</td>
<td>128±12.4*</td>
<td>110±7.5</td>
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<td>RV</td>
<td>99±6.3</td>
<td>152±9.3*</td>
<td>126±4.7</td>
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<td>98.7±6.8</td>
<td>132±13.4*</td>
<td>93.5±6.4</td>
<td>184±14*</td>
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<tr>
<td>Ca²⁺-independent PKC activity, pmol · min⁻¹ · mg⁻¹</td>
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<tr>
<td>LV</td>
<td>82.9±10.7</td>
<td>122±8.5*</td>
<td>84.4±9.5</td>
<td>159±15.1*</td>
<td>66.7±4.9</td>
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<tr>
<td>RV</td>
<td>93.1±4.1</td>
<td>137±4.4*</td>
<td>118±4.9</td>
<td>146±8.9*</td>
<td>106±2.6</td>
<td>137±4.7*</td>
<td>70.5±5.2</td>
<td>238±18.5*</td>
</tr>
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</table>

Values are means ± SE; n = 6 animals for each group. MI, myocardial infarction; RV, right ventricle; LV, left ventricle; LVEDP, LV end-diastolic pressure; +dP/dt, rate of pressure development; −dP/dt, rate of pressure decay; ND, not detectable; PKC, protein kinase C. *P < 0.05, significantly different from sham control group. LV pressure was not altered in the failing heart.

Table 2. General and hemodynamic characteristics and homogenate PKC activity of MI rats with or without Imp treatment for 4 wk starting at 4 wk after coronary occlusion

<table>
<thead>
<tr>
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<th>Sham</th>
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<tr>
<td>Body wt, g</td>
<td>495±8.6</td>
<td>486±8.6</td>
<td>487±18</td>
<td>480±15</td>
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<tr>
<td>RV, mg</td>
<td>274±16.6</td>
<td>270±20</td>
<td>605±29.3*</td>
<td>402±28.2†</td>
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<tr>
<td>Viable LV, mg</td>
<td>858±32.1</td>
<td>871±28.5</td>
<td>946±41.2*</td>
<td>834±38.4†</td>
</tr>
<tr>
<td>Scar wt, mg</td>
<td>ND</td>
<td>ND</td>
<td>241±25</td>
<td>247±18</td>
</tr>
<tr>
<td>Heart wt-to-body wt ratio, mg/g</td>
<td>2.31±0.15</td>
<td>2.43±0.09</td>
<td>3.68±0.07*</td>
<td>3.15±0.11†</td>
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<tr>
<td>Lung wet-to-dry ratio</td>
<td>4.69±0.24</td>
<td>4.47±0.19</td>
<td>5.37±0.21*</td>
<td>4.86±0.27†</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>8,019±554</td>
<td>7,125±461</td>
<td>5,321±470*</td>
<td>6,435±594*</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>8,319±622</td>
<td>8,054±467</td>
<td>6,296±302*</td>
<td>7,810±569*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>7.9±0.6</td>
<td>8.2±0.7</td>
<td>18.2±1.1*</td>
<td>10.3±0.9†</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>121±5.4</td>
<td>114±8.1</td>
<td>113±9.7</td>
<td>107±3.6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>104±7</td>
<td>95±8</td>
<td>101±11</td>
<td>97±8</td>
</tr>
<tr>
<td>Ca²⁺-dependent PKC activity, pmol · min⁻¹ · mg protein⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>102±8.1</td>
<td>101±4.4</td>
<td>295±20.3*</td>
<td>220±18.4†</td>
</tr>
<tr>
<td>RV</td>
<td>91.6±5.6</td>
<td>89.8±6.2</td>
<td>177±10.6*</td>
<td>129±9.8*</td>
</tr>
<tr>
<td>Ca²⁺-independent PKC activity, pmol · min⁻¹ · mg protein⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>139±9.4</td>
<td>148±12.3</td>
<td>442±29.6*</td>
<td>305±28.9†</td>
</tr>
<tr>
<td>RV</td>
<td>65.3±5.7</td>
<td>65.8±6.5</td>
<td>246±16.9*</td>
<td>117±5.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 animals for each group. Imp, imidapril; LVSP, LV systolic pressure; MAP, mean arterial pressure. *P < 0.05, significantly different from sham control group. †P < 0.05, significantly different from MI group.

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and 192% and 190% in particulate fraction from LV and RV of the infarcted animals compared with sham controls, respectively. Likewise, the Ca\(^{2+}\)-independent PKC activities were increased by 186% and 80% in cytosolic fraction and 170% and 241% in particulate fraction of LV and RV from the infarcted animals 8 wk after coronary ligation compared with the values of sham controls (Fig. 1, C and D). The increase in both LV and RV cytosolic and particulate fractions due to MI were significantly attenuated by imidapril treatment (Fig. 1). There was no significant difference between sham and imidapril-treated sham animals with respect to Ca\(^{2+}\)-dependent PKC and Ca\(^{2+}\)-independent PKC activities in LV and RV cytosolic and particulate fractions.

To examine whether the observed changes in PKC activities in the homogenate, particulate, and cytosolic fractions from the untreated and treated infarcted animals are due to alterations in the protein concentrations of these fractions, the protein yields in different fractions were determined. The results in Table 3 indicate no difference in the LV homogenate, particulate, or cytosolic fractions obtained from the untreated and imidapril-treated animals.

### Table 3. Protein concentration per unit of heart tissue in homogenate, cytosolic, and particulate fractions isolated from MI rats with or without Imp treatment for 4 wk starting at 4 wk after coronary occlusion

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Sham</th>
<th>Sham + Imp</th>
<th>MI</th>
<th>MI + Imp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate, µg/mg</td>
<td>52.21 ± 4.36</td>
<td>53.08 ± 2.18</td>
<td>52.72 ± 3.82</td>
<td>51.89 ± 4.17</td>
</tr>
<tr>
<td>Cytosolic, µg/mg</td>
<td>26.13 ± 1.32</td>
<td>25.56 ± 2.03</td>
<td>25.04 ± 2.71</td>
<td>24.87 ± 1.85</td>
</tr>
<tr>
<td>Particulate, µg/mg</td>
<td>19.76 ± 1.65</td>
<td>19.48 ± 1.40</td>
<td>19.07 ± 0.98</td>
<td>19.61 ± 2.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 animals for each group.
unaltered (Fig. 3). On the other hand, in PKC-α, -β, and -ε isoforms, unlike the PKC-ζ isozyme, the contents were increased in the RV cytosolic and particulate fractions from infarcted animals, except that no changes were seen in the RV particulate fraction (Fig. 4). The MI-induced increases in PKC isoforms in both LV and RV cytosolic and particulate fractions were attenuated by imidapril treatment, which showed no effect on the sham control animals (Figs. 2–4).

Although from the Western blots (Figs. 2–4) it appears that PKC-β expression is equal to or slightly greater than the expression of PKC-α in the LV and RV fractions, these data should be interpreted with a great deal of caution. In this regard, it is pointed out that the relative protein content for each of the PKC isoforms was determined in the untreated sham, sham + imidapril-treated, untreated infarcted, and infarcted + imidapril-treated animals under identical conditions where the densitometric intensity value for each isoform in the untreated sham control was taken as 100%. Thus the relative protein content for one isoform should not be compared with that of another in any of the fractions from different groups. To better evaluate the significance of changes in PKC isoforms in the experimental group, we have measured the absolute levels of PKC-α and -β isoforms in the control myocardium. The results shown in Fig. 5 indicate that PKC-α content is ~50 times of PKC-β in the LV and ~17 times in the RV. In view of the relatively low value for PKC-β content in the normal heart, the observed increase in the relative protein content for PKC-β isoform in the failing heart as well as its reduction by imidapril treatment are significant findings.

**PKA activity and relative protein content.** The activity and relative protein content of PKA were examined in homogenates from the LV and RV of sham, imidapril-treated sham, untreated infarcted, and infarcted + imidapril-treated infarcted animals. The results showed that there were no significant changes in LV and RV PKA activity and protein content in the infarcted animals (Fig. 6). Imidapril treatment did not affect PKA activ-

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**Fig. 2.** Typical immunoblots (top) and analysis of results (A–D) for the relative protein contents of cardiac PKC isoforms in homogenate fraction in LV and RV from sham, sham + Imp, MI, and MI + Imp rats at 8 wk after surgery. Values are means ± SE of 6 experiments in each group. *P < 0.05, significantly different from sham control; #P < 0.05, significantly different from MI.
ity and protein content in the sham or infarcted animals compared with values from untreated animals.

**DISCUSSION**

In this study, we have observed an increase in both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent PKC activities in the viable LV as well as LV dysfunction in 1-, 2-, 4-, and 8-wk infarcted animals. Because the activation of PKC by phorbol esters has been shown to exert a negative inotropic effect on the heart due to phosphorylation of troponin I and T and subsequent inhibition of myofibrillar ATPase activity (25), it is possible that the sustained increase in PKC activity may be involved in depressing the LV function on inducing MI. Stimulation of PKC by phorbol esters has also been shown to produce changes in cytosolic Ca\(^{2+}\) and negative inotropic effect in cardiomyocytes (6). Although the activation of PKC by phorbol esters has been reported to decrease cardiac sarcoplasmic reticulum Ca\(^{2+}\) transport (31) and can explain the depression in LV +dP/dt and −dP/dt in the infarcted hearts, the mechanism of decrease in sarcoplasmic reticular Ca\(^{2+}\) uptake by the activation of PKC are not clear. Nonetheless, increased PKC activities and cardiac dysfunction have also been observed in diabetic animals (17, 21, 22). Furthermore, attenuation of both increased PKC activities and LV dysfunction in the infarcted animals was found to occur on treatment with imidapril, which has been reported to produce beneficial effects as a consequence of ACE inhibition in this model of heart failure (41). Increased PKC activities have also been observed in failing human hearts (5) as well as in different experimental models of heart failure (32, 38, 44). Thus, in view of such observations, it appears that a sustained increase in PKC activities may be involved in the genesis of contractile dysfunction in heart failure. This suggestion is further supported by the fact that overexpression of PKC isoforms resulted in diminished heart function in transgenic mice (43). Whereas the attenuation of increased PKC activity and depressed cardiac

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**Fig. 3.** Typical immunoblots (top) and analysis of results (A–D) for the relative protein contents of cardiac PKC isoforms (α, β, ε, and ζ) in cytosolic and particulate fractions in LV from sham, sham + Imp, MI, and MI + Imp rats 8 wk after surgery. Values are means ± SE of 6 experiments in each group. *P < 0.05, significantly different from sham control; #P < 0.05, significantly different from MI.
function by treatment of MI animals with imidapril can be explained on the basis of suppression of the RAS, imidapril treatment was found to exert no effect in the control animals. Such results may indicate that both cardiac function and PKC isozymes in normal physiological conditions may not be under the influence of the RAS.

The increased PKC activities in the LV homogenate in infarcted animals does not appear to be due to translocation of cytosolic enzyme to the particulate compartment of the cell because the PKC activities in both LV cytosolic and particulate fractions were increased due to MI. Such an increase in PKC activities in LV is likely to be due to increase in the expression of PKC-α, β, ε, and ζ isozymes in the myocardium because the relative contents of these isozymes (except cytosolic PKC-α content) were increased in LV homogenate, cytosolic, and particulate fractions on inducing MI. Furthermore, treatment of infarcted animals with imidapril not only partially prevented the increase in LV PKC activities, it also had a similar effect on the

Fig. 4. Typical immunoblots (top) and analysis of results (A–D) for the relative protein contents of cardiac PKC isoforms (α, β, ε, and ζ) in cytosolic and particulate fractions in the RV from sham, sham + Imp, MI, and MI + Imp rats 8 wk after operation. Values are means ± SE of 6 experiments in each group. *P < 0.05, significantly different from control; #P < 0.05, significantly different from MI.

Fig. 5. Immunoblots of standard and different samples (lanes A–E) (top) and analysis of results (bottom) for the absolute levels of PKC isoforms (α and β) in the LV and RV of control rats.
PKC isoforms are known to serve in the signal transduction mechanism and thus play a crucial role in the development of cardiac hypertrophy (23, 29, 34, 39). Transfection of cardiomyocytes with constitutively active PKC was demonstrated to activate genes for atrial natriuretic factor and β-myosin heavy chain, which are associated with cardiac hypertrophy (8, 18, 26). Previous studies (13) have shown that the expression of both PKC-β and -ε isoforms is increased in cardiac hypertrophy induced in rats by aortic banding. Furthermore, mechanical stretch has been reported to increase PKC-ε, but not PKC-α, and induce cardiac hypertrophy (27). It is thus possible that cardiac hypertrophy observed in the LV due to MI may be caused by an increase in the content of PKC isoforms including content of PKC-ε. This view is consistent with the observations that treatment of infarcted animals with imidapril was found to not only reduce the extent of LV hypertrophy but also the level of LV PKC isoforms.

Whereas the Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent PKC activities were increased in both LV and RV homogenates on inducing MI, some differences between LV and RV were apparent with respect to changes in PKC isoyme contents. For example, the infarcted LV showed an increase in PKC-ζ content in homogenate, cytosolic, and particulate fractions, but no such changes were seen in the RV. Furthermore, unlike RV, no increase in cytosolic PKC-α content was detected in the LV. On the other hand, no changes in PKC-β and -ε isoyme contents were seen in the RV, whereas the contents of these isoforms were increased in the LV after MI. Such differential changes in the LV and RV in the infarcted heart indicate that PKC isoymes in different regions of the heart may be regulated differentially. Differences in the behavior of LV and RV with respect to changes in the sarcoplasmic reticular Ca\(^{2+}\) pump as well as adenylyl cyclase activities have also been reported during the development of congestive heart failure due to MI (1, 35). Nonetheless, the increase in PKC activities as well as PKC isoymes in the LV and RV in the infarcted animals may be of some specific nature because neither the PKA activities nor PKA protein contents were altered in both LV and RV on inducing MI. Although activation of PKA has been shown to represent a growth promoting signal (19, 33), our data are in agreement with other reports that PKA activity had no relation to the development of cardiac hypertrophy due to MI or pressure overload (28). Furthermore, unlike PKC, no change in PKA activity was observed in the failing human heart (3).

Taken together, the data in this study are consistent with the view that the increased PKC activities in the hypertrophied and failing heart subsequent to MI are due to increased expression of PKC isoymes and that the sustained increase in PKC activity may be involved in cardiac dysfunction on occluding the coronary artery. However, it should be recognized that the observed changes in cardiac function in the MI-induced heart failure may not be entirely due to cardiomyocyte-

isoyme contents in the LV homogenate, cytosolic, and particulate fractions. Whether the observed increase in LV PKC isoymes is due to translational or transcriptional changes in the myocardium remains to be investigated. However, it should be pointed out that PKC-ε isoyme, a predominant isoyme in cardiomyocytes (34), has been shown to be associated with sarcomeres on activation (10) and be responsible for the phosphorylation of troponin I (22, 25). On the other hand, PKC-β isoforms have been reported to stimulate the promoter of β-myosin heavy chain in the myocardium (18). Accordingly, it seems possible that the depressed LV function in the infarcted heart may be due to increases in both PKC-ε and -β isoyme contents. Because the role of PKC-α and -ζ isoforms in altering the function of any subcellular organelle or metabolic site in the myocardium has not been well established, it is difficult to speculate on the exact functional significance of increased PKC-α and -ζ isoymes in failing LV from the infarcted animals. However, the translocation of PKC-α and -ε has been reported to occur in failing hearts due to aortic banding in guinea pigs and this change was attenuated by treatment with ramipril, an ACE inhibitor (38).
specific adaptation in PKC signaling because the con-
tribution of alterations in PKC activity from other cell
types such as fibroblasts (16) cannot be excluded. Fur-
thermore, despite the association of increased PKC
activity and cardiac dysfunction in the failing heart,
the exact significance of the observed changes in PKC
isozymes in heart failure due to MI remains to be
established by the use of PKC inhibitors in this ex-
perimental model. The partial prevention of changes
in cardiac PKC isoforms and cardiac dysfunction in heart
failure due to MI by the ACE inhibitor imidapril indi-
cates that mechanisms other than those mediated by
increased formation of ANG II in heart failure may also
be implicated in the genesis of cardiac dysfunction.
Although ACE inhibition is generally considered to
confer beneficial effects on the failing heart by reducing
afterload, we did not observe any changes in the MAP
in the MI animals on treatment with imidapril for a
period of 4 wk. Also, this study does not provide any
information regarding the cause-and-effect relation-
ship between changes in heart function and PKC
isozyme expression. Accordingly, the exact mecha-
nisms responsible for the observed increase in PKC
isozymes during the development of heart failure due
to MI as well as for the partial prevention of PKC
activities in the failing hearts on treatment with imid-
april require further studies.

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