Abstract—To test the hypothesis that activation of the protein kinase C (PKC) ε isoform leads to cardiac hypertrophy without failure, we studied transgenic mice with cardiac-specific overexpression of a constitutively active mutant of the PKCε isoform driven by an α–myosin heavy chain promoter. In transgenic mice, the protein level of PKCε in heart tissue was increased 9-fold. There was a 6-fold increase of the membrane/cytosol ratio, and PKC activity in the membrane fraction was 4.2-fold compared with wild-type mice. The heart weight was increased by 28%, and upregulation of the mRNA for β-myosin heavy chain and α-skeletal actin was observed in transgenic mouse hearts. Echocardiography demonstrated increased anterior and posterior wall thickness with normal left ventricular function and dimensions, indicating concentric cardiac hypertrophy. Isolated cardiomyocyte mechanical function was slightly decreased, and Ca2+ signals were markedly depressed in transgenic mice, suggesting that myofilament sensitivity to Ca2+ was increased. No differences were observed in either the levels of cardiac Ca2+-handling proteins or the degree of cardiac regulatory protein phosphorylation between wild-type and transgenic mice. Unlike mice with PKCβ2 overexpression, transgenic mice with cardiac-specific overexpression of the active PKCε mutant demonstrated concentric hypertrophy with normal in vivo cardiac function. Thus, PKC isoforms may play differential functional roles in cardiac hypertrophy and failure. (Circ Res. 2000;86:1218-1223.)

Key Words: hypertrophy ■ signal transduction ■ transgenic mouse ■ heart failure ■ protein kinase C

Activation of the protein kinase C (PKC) signaling pathway has been implicated in the development of cardiomyocyte hypertrophy.1,2 Currently, at least 11 isoforms of this family of serine/threonine kinases have been identified, and their expression in the heart is developmentally regulated.3 Although PKC isoforms may play different functional roles in cell signaling, the exact significance of individual isoforms is not yet known. We have reported in the failing human myocardium with end-stage heart failure that the expression and activity of Ca2+-sensitive PKCa and -β isoforms are elevated.4 In isolated guinea pig hearts, oxidative stress using H2O2 induces left ventricular dysfunction associated with translocation of Ca2+-sensitive PKC isoforms.5 We have also demonstrated that postnatal cardiogenic overexpression of the PKCβ2 isoform in transgenic mice causes a cardiomyopathy that is characterized by left ventricular hypertrophy, myocardial fibrosis, and decreased in vivo left ventricular performance.6 In these mice, PKCβ2-induced phosphorylation of the myofilament regulatory protein troponin I decreases cardiomyocyte Ca2+ sensitivity and may cause the depressed cardiomyocyte function.7 These observations have suggested a critical role of the PKCβ isoform in the genesis of contractile dysfunction.

On the other hand, the Ca2+-independent PKCe isoform has been implicated in cardiac hypertrophy and ischemic preconditioning.8,9 An in vitro study using neonatal cardiomyocytes has shown that PKC, but not tyrosine kinase or Ras, is critical for angiotensin II–induced activation of extracellular signal–regulated kinase (ERK), which promotes cardiac hypertrophy by activating transcription factors.10 Among PKC isoforms, PKCe, but not PKCa, is a mediator for ERK activation induced by endothelin-1 and phenylephrine.11 Moreover, we have demonstrated in the isolated adult guinea pig heart that pathophysiologic elevation of left ventricular diastolic pressure activates phospholipase C and accumulates inositol phosphate with resultant translocation of the PKCe isoform.12 This PKCe translocation by mechanical stretch is attenuated by an AT1 antagonist. In addition, we have shown that the PKCe isoform is essential for ERK activation in in vitro rabbit cardiomyocytes13 and in vivo mouse hearts.14 Interestingly, activation of PKCe is not observed in explanted myocardial tissue from patients with end-stage heart failure.4 On the basis of these findings, we hypothesized that activation of the PKCe isoform may lead to compensated ventricular hypertrophy. To test this hypothesis, we generated transgenic mice
with cardiac-specific overexpression of a constitutively active mutant of the PKCε isoform using an α-myosin heavy chain (MHC) promoter. Cardiac-specific PKCε transgenesis made possible an in vivo evaluation of PKCε-mediated signaling pathways on cardiac hypertrophy and function without interference from phosphorylation events mediated either by other PKC isoforms or by upstream GqG

Materials and Methods
All procedures were performed in accordance with Case Western Reserve University animal care guidelines, which conform with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health.

Production of PKCε Transgenic Mice
PKCε transgenic mice were generated by P.P. and R.B. Briefly, a full-length PKCε cDNA was cloned from a rabbit heart cDNA library. Because the majority of the wild-type PKCε isoform resides in the cytosolic fraction and is usually self-inhibited, transgenesis with the wild-type PKCε may not lead to effective substrate phosphorylation in the membrane-particulate fraction. Thus, a constitutively active PKCε cDNA was generated through a mutation by converting A to E (amino acid 159) as previously described. This mutation prevents the pseudosubstrate domain from binding to the catalytic domain, and thus renders the molecule active. A linear 11.4-kb DNA fragment containing the entire α-MHC promoter (a gift from J. Robbins, Children’s Hospital Research Foundation, Cincinnati, Ohio), the complete PKCε cDNA with the mutation, and a polyadenylation signal was released by digestion with NotI and was used for microinjection into pronuclei of fertilized FVB mouse eggs as previously reported. The presence of the transgene was screened by Southern analysis of genomic DNA extracted from mouse tail using a 32P-labeled 1.9-kb EcoRI fragment as a probe.

Northern Blotting
The total RNA (10 µg/lane) was extracted from mouse hearts and hybridized under conditions previously described using a 32P-labeled BamHI-SalI fragment as a probe. Quantitative assessment of cardiac hypertrophic gene expression was performed using gene-specific oligonucleotides (gifts from G.J. Babu and M. Periasamy, University of Cincinnati) as previously described.

Quantitative Immunoblotting
Quantitative immunoblotting of cardiac homogenates was used to determine the levels of PKCε and Ca2+ handling proteins as previously described.

PKC Activity
The isoform-selective PKCε phosphorylation activity in the myocardium was measured as previously described. Briefly, proteins were immunoprecipitated with PKCε-specific antibody, and the activity was defined as phosphatidylserine- and phorbol 12-myristate 13-acetate–stimulated transfer of 32P from [γ-32P]ATP into the PKCε-specific substrate (ERMRFPRKQGSVRRRV).

Echocardiography
Mice were anesthetized with tribromoethanol, and cardiac ultrasound studies were performed with an Acuson Sequoia ultrasonograph equipped with a 15-MHz linear array imaging transducer as previously reported.

Isolated Cardiomyocyte Mechanical Properties and Ca2+ Signals
Left ventricular cardiomyocytes were isolated from mouse hearts, and cardiomyocyte mechanical properties were examined, as we previously described. Half of the isolated cells were used for measurements of cytosolic free Ca2+ by ratio imaging of fura-2

Figure 1. A, Representative immunoblots of the PKCε isoform in whole cardiac homogenates from wild-type (WT) and transgenic (TG) mice. B, PKCε immunoblots in purified membrane-particulate (M) and cytosolic (C) fractions. C, Same membrane as in Figure 1B was reprobed with anti-PKCα antibody. PKCε transgenesis did not change subcellular distribution of PKCα.

Table 1. A, WT TG

<table>
<thead>
<tr>
<th>PKCε</th>
<th>M</th>
<th>C</th>
<th>M</th>
<th>C</th>
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<tbody>
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<td></td>
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B, WT TG

<table>
<thead>
<tr>
<th>PKCε</th>
<th>M</th>
<th>C</th>
<th>M</th>
<th>C</th>
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<tbody>
<tr>
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</table>

C, WT TG

<table>
<thead>
<tr>
<th>PKCα</th>
<th>M</th>
<th>C</th>
<th>M</th>
<th>C</th>
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<tr>
<td></td>
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Flow cytometry, as reported previously. Eight to ten cardiomyocytes were analyzed for each mouse, and statistical analyses were performed on the basis of the number of hearts studied.

Phosphorylation of Membranous and Myofibrillar Proteins
Isolated cardiomyocytes were incubated with [32P]orthophosphate as previously described. PAGE of 32P-labeled proteins was performed using 4% to 20% gradient SDS gels, and 32P-labeled proteins were identified using a phosphor imager and autoradiography.

Statistics
Statistical analysis was done with unpaired t tests. If data were not normally distributed or failed equal variance tests after log10 transformations, they were analyzed by nonparametric statistics (Mann-Whitney rank sum test). A P value of <0.05 was considered significant.

Results
Transgenic mice 9 to 12 weeks of age and age-matched wild-type littermate control mice were used for the present study. A transgenic line expressing intermediate levels of the transgene (No. 388) was chosen for detailed characterization. Northern blot analysis revealed that the PKCε mRNA level in transgenic hearts was 15-fold in this line. Immunoblot analysis performed with specific antibodies for PKCε showed that the protein levels of the PKCε isoform in the heart were increased by 9-fold in the transgenic mice compared with the wild-type mice (Figure 1A). As shown in Figure 1B, the membrane-particulate/cytosol ratio of PKCε was 0.56±0.21 in wild-type mice and 3.04±0.44 in transgenic mice (n=4, P<0.01). PKCε activity in membrane-particulate and cytosolic fractions were 4.2±0.3-fold and 1.6±0.1-fold compared with wild-type controls (n=5, P<0.01), respectively. The PKCε transgenesis did not alter the expression and subcellular distribution of any of the other PKC isoforms expressed in the mouse heart (α, β, γ, δ, θ, and ζ). Immunoblots of PKCα are shown in Figure 1C as an example. The protein expressions of the PKCε isoform in lungs, liver, kidney, large intestine, and small intestine were similar between wild-type
and transgenic mice (data not shown). Systolic blood pressure of 3 transgenic and 3 wild-type littermate mice was measured with the mice in the conscious state using the standard tail cuff method in a blinded fashion. There were no differences in systolic blood pressure between transgenic and wild-type mice (136±9 versus 139±11 mm Hg, respectively).

**Heart Weight and Lung Weight**

The gravimetric data of wild-type littermate and PKCε transgenic mice are summarized in Table 1. The absolute heart weight and ratio of heart to body weight were increased in transgenic mice compared with wild-type mice by 28% and 21%, respectively. The lung weight and ratio of lung to body weight were the same between the wild-type and transgenic mice. There was no evidence of fibrosis on microscopic examinations of multiple histological sections from transgenic mouse hearts (data not shown).

**Expression of Hypertrophic Genes**

Quantitative assessment of cardiac hypertrophic gene expression, such as atrial natriuretic factor (ANF), c-fos, β-MHC, and α-skeletal actin, was performed by Northern blot analysis. Representative Northern blots and quantitative data are shown in Figure 2. Each value was normalized to the mRNA expression of GAPDH. Increased transcript levels of β-MHC (4-fold) and α-skeletal actin (7-fold) were observed in transgenic mouse hearts without significant changes in levels of ANF and c-fos.

**Echocardiography**

M-mode echocardiographic measurements include the left ventricular minor axis dimension at end-diastole (EDD) and end-systole (ESD) and wall thickness at end-diastole of the anterior (AWTh) and posterior (PWTh) walls. Representative M-mode echocardiograms from a wild-type mouse and a transgenic mouse are shown in Figure 3A, and group data of echocardiographic measurements are summarized in Table 2. There were no differences in EDD, ESD, and fractional shortening between wild-type and transgenic mice. In contrast, the AWTh, PWTh, and left ventricular mass were increased in transgenic mice compared with wild-type mice. The relative wall thickness was higher in transgenic mice than in wild-type mice, indicating the presence of concentric hypertrophy in PKCε transgenic hearts.

**Isolated Cardiomyocyte Mechanical Function and Ca2⁺ Transients**

Representative analog recordings of isolated left ventricular cardiomyocyte mechanics and Ca2⁺ transients for wild-type and PKCε transgenic mice are shown in Figure 3B. Group data for the cardiomyocyte mechanical properties and Ca2⁺ transients are summarized in Table 3. The percentage of cardiomyocyte shortening (P<0.05) was slightly decreased in PKCε transgenic mice compared with wild-type control mice. The baseline Ca2⁺ level was slightly lower, and the amplitude of the Ca2⁺ transient was markedly decreased in transgenic mice compared with wild-type mice (P<0.01). The times from start to 80% decay of the Ca2⁺ signal (T80) and 50% decay of the Ca2⁺ signal (T50) were prolonged in transgenic mice. The observed disparities between cardiomyocyte mechanics and Ca2⁺ transient data suggested that myofilament sensitivity to Ca2⁺ was relatively increased in transgenic mouse hearts.

**Protein Levels Involved in Ca2⁺ Homeostasis**

To determine whether the observed changes in the cardiomyocyte Ca2⁺ signal were associated with altered expression of Ca2⁺-handling proteins, the relative levels of these proteins in the heart were determined by quantitative immunoblotting (Table 4). No significant differences in phospholamban, sarcoplasmic reticulum Ca2⁺ ATPase (SERCA2a), the Na⁺-Ca2⁺ exchanger, or the Na⁺-H⁺ exchanger were found between PKCε transgenic and wild-type mice.

**Phosphorylation of Cardiac Proteins**

To clarify whether the suspected changes in myofilament Ca2⁺ sensitivity were associated with altered phosphorylation
status of cardiac regulatory proteins, we examined the degree of cardiac protein phosphorylation. The incorporation of "P"orthophosphate into a variety of cardiac proteins was studied in cardiomyocytes isolated from wild-type and transgenic mice hearts. The degree of protein phosphorylation at basal condition was expressed as a percentage of that after maximal stimulation with dibutylryl cAMP. As shown in Figure 3C and Table 5, no differences were found in the degree of phosphorylation of troponin I, troponin T, phospholamban, and 15-kDa protein between wild-type and transgenic mice.

Discussion

The major findings of the present study were as follows: transgenic mice with cardiac-specific overexpression of a constitutively active mutant of PKCe demonstrated (1) 4.2-fold increase of PKC activity in the membrane fraction; (2) mild concentric hypertrophy; (3) no evidence of fibrosis; (4) normal in vivo left ventricular performance; (5) slightly decreased isolated cardiomyocyte function and markedly depressed Ca2+ transients, suggesting an increase in myofilament Ca2+ sensitivity; (6) partial recapitulation of fetal gene expression; (7) unchanged abundance of Ca2+ cycling proteins; and (8) no differences in the degree of cardiac myofilament or sarcoplasmic reticulum regulatory protein phosphorylation.

**TABLE 2. Echocardiographic Data**

<table>
<thead>
<tr>
<th>Wild Type</th>
<th>Transgenic</th>
</tr>
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<tbody>
<tr>
<td>EDD, mm</td>
<td>3.65±0.18</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>2.15±0.16</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>41.7±1.5</td>
</tr>
<tr>
<td>AWTh, mm</td>
<td>0.49±0.03</td>
</tr>
<tr>
<td>PWTth, mm</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>50.9±5.6</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26.2±1.5</td>
</tr>
<tr>
<td>LV mass/body weight</td>
<td>1.93±0.15</td>
</tr>
<tr>
<td>Relative wall thickness, h/r</td>
<td>0.25±0.03</td>
</tr>
</tbody>
</table>

LV indicates left ventricular; h, wall thickness; and r, radius. Values are mean±SE. Data were obtained from 9 wild-type and 9 transgenic mice.

**Figure 3. A, Representative M-mode echocardiograms of a wild-type mouse and a transgenic mouse. EDD indicates end-diastolic dimension; ESD, end-systolic dimension. B, Representative analog recordings of cardiomyocyte mechanics and Ca2+ transients of cells isolated from a wild-type littermate and a PKCe transgenic mouse. C, Autoradiograms of SDS polyacrylamide gels (4 to 20% gradient) for transgenic (TG) and wild-type (WT) mice. Phosphorylated bands were identified on the basis of their migration patterns relative to pure protein standards as reported previously.19 Troponin I was identified by stimulation of phosphorylation with dibutyryl cAMP. Phospholamban was identified by its characteristic mobilities shift on boiling before electrophoresis.

**TABLE 3. Mechanical Properties and Calcium Transients of Isolated Left Ventricular Cardiomyocytes**

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Transgenic</th>
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<tbody>
<tr>
<td>Mechanical parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of shortening, %</td>
<td>11.8±1.2</td>
<td>9.6±1.6*</td>
</tr>
<tr>
<td>Rate of shortening: +dL/dt, μm/s</td>
<td>306±57</td>
<td>239±60</td>
</tr>
<tr>
<td>Rate of relengthening: −dL/dt, μm/s</td>
<td>273±65</td>
<td>204±55</td>
</tr>
<tr>
<td>Cell length, μm</td>
<td>128±7</td>
<td>127±6</td>
</tr>
<tr>
<td>Calcium kinetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, ratio unit</td>
<td>1.19±0.60</td>
<td>0.96±0.14†</td>
</tr>
<tr>
<td>Amplitude, ratio unit</td>
<td>0.91±0.14</td>
<td>0.48±0.10†</td>
</tr>
<tr>
<td>T90 ms/ratio unit</td>
<td>0.82±0.27</td>
<td>1.85±0.47†</td>
</tr>
<tr>
<td>T95 ms/ratio unit</td>
<td>0.43±0.09</td>
<td>1.12±0.29†</td>
</tr>
</tbody>
</table>

Values are mean±SD. Data were recorded at 15 bpm and were obtained from 6 wild-type and 6 transgenic mice. Baseline indicates 340/380 ratio at rest; amplitude, height of the calcium peak upon stimulation; and T90 and T95, time from start to 80% and 50% decay of Ca2+ signal, respectively.

*P<0.05.
†P<0.01.
Myofibrillar Proteins
PKC

Week-old transgenic mice was depressed compared with age-retired breeders demonstrated that fractional shortening in 48-hypertrophy) and reflex control of the circulation may affect compensatory alterations in chamber geometry (concentric

PKC activity and abundance of other Ca^{2+}-dependent phospholipid binding proteins such as annexins. For example, it has been demonstrated that transgenic overexpression of annexin VI in mice resulted in decreased basal and peak Ca^{2+} transients.20

TABLE 4. Protein Abundance of Cardiac Calcium Handling Proteins

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Transgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholamban</td>
<td>31.8±2.5</td>
<td>33.4±2.2</td>
</tr>
<tr>
<td>Sarcomplasmic reticulum Ca^{2+} ATPase</td>
<td>25.6±3.8</td>
<td>27.4±4.6</td>
</tr>
<tr>
<td>Na^{+}Ca^{2+} exchanger</td>
<td>5.4±1.6</td>
<td>4.9±1.2</td>
</tr>
<tr>
<td>Na^{+}-H^{+} exchanger</td>
<td>8.9±1.3</td>
<td>9.5±1.4</td>
</tr>
</tbody>
</table>

Values are mean±SD and are expressed as scan units per microgram protein. Relative protein levels were determined by quantitative immunoblotting as described in Materials and Methods.

diography, (2) decreased isolated cardiomyocyte function and normal Ca^{2+} kinetics, and (3) decreased myofilament responsiveness to Ca^{2+} resulting from increased phosphorylation of troponin I. Taken together, these data suggest differential functional roles for distinct PKC isoforms and support the notion that increased activity of PKC_{\beta}, but not PKC_{\epsilon}, could depress contractile function in heart failure. However, in a separate study, a transgenic line with an extraordinarily high level of constitutively active PKC_{\epsilon} demonstrated a heart failure phenotype.19 Whether this represents a “dose effect” from excessive PKC_{\epsilon} gene expression, a nonspecific effect of very high levels of cardiomyocyte protein loading, or an insertional effect of the transgene is unclear at this time.

Despite normal in vivo cardiac performance by echocardiography, isolated cardiomyocyte mechanical function was modestly reduced in transgenic mice compared with wild-type control (9.6% versus 11.8%). This discordance might be explained by differences in experimental conditions such as partial contracture after enzymatic myocyte extraction, which has been observed in this and other studies.7,20 In addition, compensatory alterations in chamber geometry (concentric hypertrophy) and reflex control of the circulation may affect in vivo systolic left ventricular performance.

A preliminary echocardiographic study in a small number of retired breeders demonstrated that fractional shortening in 48-week-old transgenic mice was depressed compared with age-matched wild-type littermate controls. These data suggest that PKC_{\epsilon} mice may develop an age-related impairment of systolic function, similar to that observed in other transgenic models.21

Although ANF is thought to be a marker of hypertrophy, the mRNA level of ANF was unexpectedly unchanged in PKC_{\epsilon} transgenic mouse hearts in the present study. However, it should be recognized that hypertrophy may not always be associated with increased ventricular expression of ANF.22

Possible Mechanisms for Decreased Cardiomyocyte Ca^{2+} Transients
Although the amplitude of Ca^{2+} signals of isolated cardiomyocytes was decreased in transgenic mice compared with wild-type mice, the levels of Ca^{2+} handling proteins such as SERCA2a, phospholamban, the Na^{+}-Ca^{2+} exchanger, and the Na^{+}-H^{+} exchanger were similar between wild-type and transgenic mice. We cannot exclude the possibility that changes in the intrinsic activities of these proteins may account for the depressed Ca^{2+} amplitude in transgenic mouse hearts. Other possible mechanisms for reduced Ca^{2+} signals include (1) altered biophysical environment of the sarcoplasmic reticulum;23 (2) altered spatial coupling between voltage-gated Ca^{2+} channels and the ryanodine receptor;24 and (3) altered activity and abundance of other Ca^{2+}-dependent phospholipid binding proteins such as annexins. For example, it has been demonstrated that transgenic overexpression of annexin VI in mice resulted in decreased basal and peak Ca^{2+} transients.20

ALTERATIONS IN MYOFILAMENT Ca^{2+} SENSITIVITY
It is well known that altered Ca^{2+} kinetics modify cardiac contractility.1 Although the amplitude of Ca^{2+} signals of isolated cardiomyocytes was depressed in transgenic mice (53% of wild type), isolated cardiomyocyte function was relatively preserved (81% of wild type), and in vivo cardiac function assessed by echocardiography was normal in these transgenic mice. These findings suggest that increased myofilament Ca^{2+} sensitivity may contribute to the preserved left ventricular chamber and cardiomyocyte function that we observed. This compensatory mechanism would offset the functional results of diminished cellular Ca^{2+} handling.

The mechanisms by which myofibrillar Ca^{2+} sensitivity may be altered include (1) phosphorylation of myofibrillar proteins,25 (2) changes in regulatory contractile protein isoforms,26 and (3) regulation of intracellular pH.27 It has been reported that phosphorylation of troponin I or T by PKC reduces Ca^{2+} sensitivity and maximal activity of actomyosin MgATPase and thus impairs actin-myosin interactions.28 However, in the present study, unlike in mice with PKC_{\beta} overexpression, the degree of phosphorylation of both troponin I and T was unchanged in PKC_{\epsilon} transgenic hearts. Phosphorylation specificities of PKC isoforms for cardiac regulatory proteins have been reported in vitro studies.29 PKC_{\alpha} phosphorylates Ser43/Ser45 of troponin I and reduces Ca^{2+} sensitivity and maximal activity of MgATPase. In contrast, PKC_{\zeta} phosphorylates 2 unknown sites of troponin T and results in a slight increase of Ca^{2+} sensitivity. PKC can potentially modify the regulation of intracellular pH through the activation of the Na^{+}–H^{+} exchanger and secondarily alter myofibrillar Ca^{2+} sensitivity.30 However, we did not find a change in the protein level of the Na^{+}–H^{+} exchanger in the present study. It is possible that changes in intracellular pH mediated by PKC_{\epsilon}-induced phosphorylation and resultant activation of the Na^{+}–H^{+} exchanger might contribute, at least in part,

TABLE 5. 32P Incorporation Into Cardiac Membranous and Myofibrillar Proteins

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Transgenic</th>
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<tbody>
<tr>
<td>Membranous proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholamban</td>
<td>86.3±6.3</td>
<td>81.8±2.3</td>
</tr>
<tr>
<td>15-kDa protein</td>
<td>80.4±9.7</td>
<td>85.3±1.6</td>
</tr>
<tr>
<td>Myofibrillar proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troponin I</td>
<td>74.1±6.0</td>
<td>77.9±8.9</td>
</tr>
<tr>
<td>Troponin T</td>
<td>85.0±3.0</td>
<td>78.4±9.1</td>
</tr>
</tbody>
</table>

Values are mean±SD. Four wild-type and four transgenic mice were used for the analysis. 32P-labeled proteins were subjected to SDS-PAGE and autoradiography, as described in Materials and Methods. 32P incorporation was expressed as percentage of the maximal phosphorylation with the dibutyryl cAMP.
to an increase in myofilament sensitivity to Ca\(^{2+}\) observed in the present study. We are currently examining this possibility.

**Conclusion**

Cardiac-specific overexpression of a constitutively active mutant of PKCε causes mild concentric hypertrophy with normal in vivo cardiac performance. These and other data from our laboratory support the notion that activation of Ca\(^{2+}\)-sensitive PKC isoforms, but not the PKCε isoform, predominates in mediating contractile dysfunction of failing myocardium. Furthermore, they suggest that distinct PKC isoforms may play differential functional roles in cell signaling pathways leading to cardiac hypertrophy and failure.

**Acknowledgments**

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**References**

Transgenic Overexpression of Constitutively Active Protein Kinase C ε Causes Concentric Cardiac Hypertrophy
Yasuchika Takeishi, Peipei Ping, Roberto Bolli, Darryl L. Kirkpatrick, Brian D. Hoit and Richard A. Walsh

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