The Cardiac-specific Nuclear δB Isoform of Ca\(^{2+}\)/Calmodulin-dependent Protein Kinase II Induces Hypertrophy and Dilated Cardiomyopathy Associated with Increased Protein Phosphatase 2A Activity*

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The δ isoform of Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) predominates in the heart. To investigate the role of CaMKII in cardiac function, we made transgenic (TG) mice that express the nuclear δB isoform of CaMKII. The expressed CaMKII\(\delta_B\) transgene was restricted to the myocardium and highly concentrated in the nucleus. Cardiac hypertrophy was evidenced by an increased left ventricle to body weight ratio and up-regulation of embryonic and contractile protein genes including atrial natriuretic factor, β-myosin heavy chain, and α-skeletal actin. Echocardiography revealed ventricular dilation and decreased cardiac function, which was also observed in hemodynamic measurements from CaMKII\(\delta_B\) TG mice. Surprisingly, phosphorylation of phospholamban at both Thr\(^{17}\) and Ser\(^{16}\) was significantly decreased in the basal state as well as upon adrenergic stimulation. This was associated with diminished sarcoplasmic reticulum Ca\(^{2+}\) uptake in vitro and altered relaxation properties in vivo. The activity and expression of protein phosphatase 2A were both found to be increased in CaMKII TG mice, and immunoprecipitation studies indicated that protein phosphatase 2A directly associates with CaMKII. Our findings are the first to demonstrate that CaMKII can induce hypertrophy and dilation in vivo and indicate that compensatory increases in phosphatase activity contribute to the resultant phenotype.

Although cardiac hypertrophy is a beneficial adaptive response of the heart to a variety of intrinsic and extrinsic stimuli, chronic hypertrophy often leads to dilated cardiomyopathy and eventually to heart failure if the stimulus is not relieved (1). A hallmark of the pathological transition from hypertrophy to heart failure is decreased cardiac contractility. Cardiac hypertrophy has also been reported to be an independent risk factor for ischemic heart disease, arrhythmia, and sudden death (2). The development of these pathophysiological end points highlights the need to understand the cellular signaling events that regulate cardiac growth and function.

Ca\(^{2+}\) signals have long been known to play a central role in the regulation of cardiac contractility and more recently have been considered as likely regulators of growth and gene expression. A role for Ca\(^{2+}\) signaling in the pathogenesis of cardiac hypertrophy is supported by a growing body of evidence. For example, transgenic (TG) mice with a 3–5-fold overexpression of the Ca\(^{2+}\)-binding protein, calmodulin, develop severe cardiac hypertrophy (3). This chronic elevation of calmodulin in the ventricles of TG mice was more recently shown to increase CaMKII phosphorylation, an index of the Ca\(^{2+}\)-independent activity of CaMKII, and the expression of atrial natriuretic factor (ANF) (4), an established indicator of ventricular hypertrophy. In cultured ventricular myocytes, electrical pacing can elevate intracellular Ca\(^{2+}\), and this is essential for cardiac myocytes to respond with increased expression of ANF and myosin light chain-2, another marker of hypertrophy (5). In addition, the calmodulin antagonist W-7 can block hypertrophy of primary cultured cardiomyocytes in response to electrical pacing and α-adrenergic stimulation (5), further implicating Ca\(^{2+}\) as a mediator of cardiac gene expression in the hypertrophic response. An alternate effector of the Ca\(^{2+}\)/calmodulin complex is calcineurin, a protein phosphatase that has recently attracted attention as a mediator of hypertrophic stimuli in vitro and in vivo (6, 7).

The Ca\(^{2+}\)/calmodulin-dependent protein kinases (CaMKs) are critical transducers of Ca\(^{2+}\) signals (8). The CaMK family, consisting of CaMKI, CaMKII, and CaMKIV, has an extremely wide tissue distribution and is represented to varying degrees in all eukaryotic systems examined. These multifunctional kinases can phosphorylate a range of substrates in vitro and in situ, including ryanodine receptors (9), sarcoplasmic reticular Ca\(^{2+}\) ATPase (SERCA) (10), phospholamban (PLB) (11, 12), L-type Ca\(^{2+}\) channels (13), activating transcription factor-1 (14), and cAMP response element-binding protein (14, 15). Whereas CaMKI and CaMKIV are monomeric, CaMKII exists in various isoforms that vary in their Ca\(^{2+}\)/calmodulin affinities, calmodulin-binding properties, subcellular localizations, and kinase activities. The CaMKII family includes a wide variety of Ca\(^{2+}\)-independent isoforms (CaMKI and CaMKIV) with broad tissue distribution and unique substrate specificities. The CaMKII family includes a wide variety of Ca\(^{2+}\)-independent isoforms (CaMKI and CaMKIV) with broad tissue distribution and unique substrate specificities.
as a multimer of 8–12 subunits, encoded by four separate genes: α, β, γ, and δ (8,16). Several laboratories have identified CaMK II δ as the predominant isoform in the heart (17–21) and several distinct splice variants (δα, δc, et al.), characterized by the presence of a second variable domain are expressed (17,21). The δα isoform contains an 11-amino acid nuclear localization signal that is absent from CaMKIIδc. Thus the δα isoform localizes to the nucleus in both fibroblast cells and cardiomyocytes, whereas the δc isoform localizes to the cytoplasm (17,22, 23).

Our lab previously showed that transient expression of CaMKIIδb in neonatal rat ventricular myocytes induced gene expression and resulted in an enhanced response to phenylephrine, as assessed by transcriptional activation of an ANF-luciferase reporter gene (23). The nuclear localization signal of CaMKIIδb was required for this response because expression of CaMKIIδc did not result in enhanced ANF expression (23). Activated CaMKI and IV can also induce hypertrophic responses in cultured cardiomyocytes (24) and the CaMKII inhibitor KN-62 can block cardiomyocyte hypertrophy in response to endothelin-1 (25). Of particular significance is the demonstration that hypertrophy develops in TG mice that express increased levels of CaMKIV in the myocardium (24). CaMKIV is expressed at very low levels in the heart relative to CaMKII (8,18), but it has, in common with the predominant cardiac CaMKIIδb isoform, the ability to enter the nucleus.

To investigate the role of CaMKII in the intact heart, we used the well characterized cardiac-specific α-mysin heavy chain (MHC) promoter to generate TG mice that express CaMKIIδb isoform. We demonstrate here that overexpression of wild type CaMKIIδb in the mouse heart induces cardiac hypertrophy and dilation with decreased ventricular function and that this is associated with changes in protein phosphorylation, phosphatase activity, and sarcoplasmic reticulum (SR) Ca2+ uptake.

EXPERIMENTAL PROCEDURES

Generation of CaMKIIδb Transgenic Mice—Hemagglutinin (HA)-tagged rat wild type CaMKIIδb cDNA was subcloned into a Bluescript-based TG vector (a gift from J. Robbins, University of Cincinnati, Cincinnati, OH) between the 5.5-kb murine MHC promoter and a polyadenylation signal. Purified linear transgene fragments were injected into pronuclei of fertilized mouse oocytes. The resulting pups were screened for the presence of the transgene by PCR as described previously (26), using a CaMKII-specific primer (5’TATGGGTCATCTTGACA-3’) and a TG vector-specific primer (5’CCGCTCTA- GAACACTGAGGACT-3’). Founder mice were bred with C57BL/6 wild type mice. Heterozygous animals from at least the third generation were used for all studies, with their wild type (WT) littermates serving as controls. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (45) and approved by the Institutional Animal Care and Use Committee.

Kinase Assay—Frozen powdered ventricular tissue was resuspended in ice-cold kinase sample buffer (50 mM PIPES, 10 mM EGTA, 20 mM benzamidene, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride, and 10% glycerol) prior to sonication for 10 s. Protein concentration was measured by Bradford assay, and the homogenates were diluted to 10 mg/ml protein. Kinase activity was measured using a previously published method (27).

Phosphatase Assays—Frozen powdered ventricular tissue was homogenized in ice-cold lysis buffer (20 mM HEPES, pH 7.5, 100 mM NaCl, and protease inhibitors as a mixture from Sigma-Aldrich). The homogenized hearts were centrifuged in an air centrifuge for 10 min in a cold room. The supernatant was considered to be cytosol, and the pellet was treated with lysis buffer containing 1% Triton X-100 to solubilize the proteins. This last extract was centrifuged as before, and the supernatant was considered as particulate. Protein phosphatase 1 (PP1) activity was measured using 32P-labeled phosphorylase as a substrate, and protein phosphatase 2A (PP2A) activity was measured using a 32P-labeled synthetic peptide (RRATpVA) that is selective for PP2A as described recently (28).

Immunoprecipitation and Western Blotting—Cardiac homogenates were prepared as described previously (29). Cytosolic and particulate fractions were prepared as stated above. In some experiments, a nuclear fraction was prepared from mouse hearts using a Wheaton Dounce homogenizer as described previously (30). The antibodies used for immunoprecipitation and immunoblotting were as follows: mouse anti-HA antibody (Roche Molecular Biochemicals), CaMKIIα antibody (rabbit antiseraum against a 15-amino acid peptide in the carboxyl-terminal region of CaMKIIα), monoclonal anti-PLB (Upstate Biotechnology, Inc.), phospho-tyrosine PLB (Thr20 and Ser19) antibodies (Phosphor, Leeks, UK), CaMKII antibody (Santa Cruz), phosphorylated CaMKII antibody (Upstate Biotechnology, Inc.), and PP2A (BD Transduction Laboratories, CA) and PP2A/A antibody (Oxford Biomedical Research). The secondary antibody is a hors eradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse IgG (Sigma-Aldrich). Enhanced chemiluminescence was performed using the SuperSignal Chemiluminescent Detection System (Pierce).

RNA Dot Blot Analysis—RNA was prepared from ventricular tissue using Trizol reagent (Invitrogen), and dot blot analysis was performed as described previously (26,31).

Homodynamic Measurements—Mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg) intraperitoneally. After endotracheal intubation, the mice were connected to a volume-cycled rodent ventilator. A PE-50 catheter was placed in the left jugular vein for intravenous access. Through the right carotid artery, 1.5-French high fidelity catheter tip micromanometer was inserted via a small incision, and the tip was manipulated across the aortic valve into the left ventricle (LV). A bilateral vagotomy was performed. When LV pressure and heart rate became stable, dobutamine was given intravenously by an infusion pump, at a rate of 0.75, 2, 4, 8, and 12 μg/kg/min. Atrial pacing was achieved by using a guide wire placed in the right atrium via the right jugular vein. Prior to pacing, the heart rate was controlled at a slow, stable rate using UL-FS 49, a selective sinus node inhibitor, at a dosage of 0.05 mg given intravenously to achieve a rate of 150–250 beats/min. Heart rate was then increased by atrial pacing in steps of 50 beats/min. There was 30 s between two steps. Then dobutamine was given intravenously at 2 μg/kg/min for 3 min, and the pacing was repeated. Data analysis was done by computer, and 12 beats were averaged.

Transthoracic Echocardiography—Mice were anesthetized by intraperitoneal injection of 5% Avertin (15 μl/g body weight). The current echocardiographic system is an Agilent Technologies, Sonos 5500 with a 15 MHz free probe that utilizes ultraband technology. M-mode and Doppler tracing were recorded at a sweep speed of 150 mm/s. At least three independent M-mode and Doppler measurements in each animal were obtained by an examiner blinded to the genotype of the animals.

Measurements of SR Ca2+ Uptake—Ventricular tissue from TG and littermate control mouse hearts was homogenized at 4°C in 1.5 ml of homogenizing solution (25 mM imidazole, pH 7.0) with a Teflon glass Thomas tissue grinder. SR Ca2+ uptake assays were performed in ventricular homogenates at room temperature based on a protocol modified from That of Paganini and Solagro (32). Aliquots (350 μl) of homogenates were transferred into tubes containing 2.8 ml of uptake buffer (100 mM KCl, 10 mM potassium oxalate, 40 mM imidazole, 10 mM Tris, 0.1 mM MgCl2, 0.1 mM EGTA, 200 μM PMSF, 10 μM leupeptin, 10 μM aprotinin, 0.1 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100). The tubes were incubated at 37°C for 5 min (preincubation) and then 1 μCi/ml 45Ca2+ (20 and 200 μCi/ml, which was calculated on the basis of the amount of added EGTA). After 5 min of preincubation, the uptake reaction was initiated by the addition of 2.5 mM sodium ATP. Ca2+ uptake was terminated at various times (1, 3, and 5 min for 200 μCi/ml free Ca2+; 1, 10, and 20 min for 20 μCi/ml free Ca2+) by filtering 500-μl aliquots on 0.45-μm nitrocellulose membranes (Millipore-type HA), followed by two washes (5 ml) with uptake buffer without Ca2+ or ATP. The radioactivity remaining on the nitrocellulose filters was determined by liquid scintillation spectrometry. Protein concentration in the ventricular homogenate was assayed with a Bradford reagent. Ca2+ uptake was calculated from the slope of the linear regression analysis relating 45Ca2+ uptake/milligram of protein to reaction time.

Immunocytochemical Staining—Ventricular myocytes were isolated from WT and CaMKIIδb TG mice following recently published methods (33). Cells cultured on laminin-coated (3.5 mg/ml) plates were incubated in 5% CO2, 95% air; 1% glutaeraldehyde for 10 min, and then incubated in 2% osmium tetroxide for 20 min. Indirect immunofluorescence staining was carried out by incubation with 1:100 dilution of rabbit IgG antibody (Cappel) overnight. The cells were observed by a Zeiss Axiovert 135 fluorescence microscope and photographed using a CCD camera.
**RESULTS**

**Generation and Identification of CaMKII\(\delta_9\) Transgenic Mice**—TG mice expressing HA-tagged rat wild type CaMKII\(\delta_9\) under the control of the cardiac-specific \(\alpha\)-MHC promoter were generated as described under "Experimental Procedures." Three TG founders showed germline transmission of the transgene. Significant expression of the transgene was seen only in the heart based on examination with the anti-HA antibody (Fig. 1A). The CaMKII\(\delta_9\) TG line studied in detail here showed at least a 10-fold overexpression of CaMKII\(\delta_9\) in the heart, based on Western blots using the anti-CaMKII antibody (Fig. 1B). Enzymatic activity of CaMKII measured in ventricular homogenates was ∼4-fold higher in the TG animals than in the littermate controls (Fig. 1C). The discrepancy between CaMK activity and expression may be due at least in part to the fact that the activity assay includes other isoforms of CaMKII. The phosphorylation state of CaMKII reflects its Ca\(^{2+}\)-independent activity. Experiments using a phospho-CaMKII antibody revealed a ∼1.5-fold increase in the CaMKII\(\delta_9\) TG mice (Fig. 1D). Immunocytochemical staining of cardiomyocytes isolated from the TG animals using a HA antibody confirmed that the CaMKII\(\delta_9\) transgene was, as predicted based on its nuclear localization signal, present and highly concentrated in the nucleus (Fig. 2).

**Cardiac Overexpression of CaMKII\(\delta_9\) Induces Cardiac Hypertrophy**—Most CaMKII\(\delta_9\) TG mice showed significantly enlarged hearts at 3–4 months of age (that shown in Fig. 3A is a typical one). On average, CaMKII\(\delta_9\) TG mice exhibited a 22% increase in heart weight to body weight ratio and a 27% increase in left ventricle to body weight ratio at 12 weeks of age (Fig. 3B). Body weights in the TG and WT mice were equivalent; thus the heart to body weight ratio increase in the TG mice is due to increased ventricular mass.

To determine whether specific alterations in cardiac gene expression were associated with CaMKII\(\delta_9\) overexpression, RNA was isolated from mouse ventricles, and a selected panel of hypertrophic genes was examined by dot blot analysis. As shown in Fig. 3C, ANF, \(\beta\)-MHC, and \(\alpha\)-skeletal actin mRNA levels were significantly increased in TG ventricles, and there was a modest but significant decrease in \(\alpha\)-MHC, SERCA, and PLB mRNA levels.

**Cardiac Overexpression of CaMKII\(\delta_9\) Causes Ventricular Dilatation and Decreased Contractile Function**—To assess chamber size and cardiac function in CaMKII\(\delta_9\) TG mice, we performed echocardiography on 3–4-month-old mice. As shown in Fig. 4, left ventricular end diastolic diameter and left ventricular end systolic diameter in TG mice were increased by 14 and 31%, respectively. The calculated left ventricular mass was increased by 20%, consistent with changes described above. Fractional shortening and velocity of circumferential shortening, which reflect left ventricular contractile function, also decreased significantly in TG mice compared with the control mice.

Hemodynamic measurements of cardiac contractility and relaxation demonstrated that both +dP/dt and −dP/dt responses to pacing were blunted in hearts of TG mice (Fig. 5, A and B). Stimulation with dobutamine, a \(\beta\)-adrenergic receptor agonist, should increase Ca\(^{2+}\), either directly or indirectly and might therefore activate CaMKII\(\delta_9\) in the TG mice. However, dose response curves to this \(\beta\)-adrenergic agonist were not significantly altered in TG versus WT mouse hearts (Fig. 5, C and D).

**Cardiac Overexpression of CaMKII\(\delta_9\) Results in Reduced Ca\(^{2+}\) Uptake and Decreased Phosphorylation of Phospholamban**—The decreased relaxation properties observed in the hemodynamic measurements led us to assess changes in SR Ca\(^{2+}\) uptake. Experiments examining Ca\(^{2+}\) uptake in SR containing cardiac homogenates revealed that SR Ca\(^{2+}\) uptake was significantly decreased in ventricles from 3–4-month-old TG mice (Fig. 6A). Because SR Ca\(^{2+}\) uptake is regulated by PLB phos-
versus /H11006 (1000) ratios were measured at 12 weeks of age. The data are pre-
maximal phosphorylation of neither the PKA nor the CaMKII
TG PLB phosphorylation is generally low, we also examined PLB
SERCA protein (data not shown). Because the basal state of
hearts (Fig. 6
creased in extracts prepared from 3
4-month-old TG mouse
–
TG mice. We therefore assayed PP1 and PP2A phos-
thase activity in WT and TG mouse hearts. As shown in Fig.
7A, PP2A activity was selectively increased in the particulate
fraction from TG mouse hearts. There was no change in PP2A
activity in the cytosol (Fig. 7A), nor was PP1 activity altered in
either fraction (data not shown). Western blots examining
phosphatase expression levels also showed no change in PP1 or
PP2A in the cytosol from TG hearts (data not shown). However,
in the particulate fraction from TG hearts, increases in expres-
ion of the catalytic as well as the A subunit and the B56α
targeting subunit of PP2A were observed (Fig. 7B). This did not
occur at the transcriptional level, because Northern blot anal-
ysis using a 32P-labeled PP2A catalytic subunit cDNA fragment
as a probe revealed no difference in PP2A mRNA levels in TG
and WT hearts (data not shown).

There is growing evidence for association of phosphatases
and kinases in signaling complexes. In particular, CaMKIV has
been shown to interact with PP2A catalytic subunit (35). To
determine whether there was a direct interaction between
PP2A and CaMKII in cardiomyocytes, we first examined asso-
ciation of these molecules in neonatal rat ventricular myocytes.
Immunoprecipitation with either CaMKII or PP2A catalytic
subunit antibody demonstrated that CaMKII associates with PP2A
in cardiomyocytes, we first examined asso-
ciation of these molecules in neonatal rat ventricular myocytes.
Immunoprecipitation with either CaMKII or PP2A catalytic
subunit antibody followed by Western blots with the opposite
antibody demonstrated that CaMKII associates with PP2A in
neonatal rat ventricular myocytes (data not shown). In addi-
tion, we demonstrated that there was an increase in PP2A
catalytic subunit that coimmunoprecipitated with CaMKII in
the particulate fraction (Fig. 7C) as well as in the nuclear
fraction (Fig. 7D) prepared from TG mouse ventricles.

**DISCUSSION**

A variety of studies performed throughout the last decade
suggest that Ca2+ signaling is a central mechanism triggering
hypertrophic growth (3–6). A likely sensor for the effects of
elevated calcium is CaMK, an enzyme suggested to mediate
changes in cell growth and gene expression in a number of
neuronal systems (36, 37). Most studies linking CaMK to con-
tral of gene expression have focused on the effects of CaMKIV
because it is a monomeric enzyme that, in contrast to the
multimeric CaMKII, is readily able to enter the nucleus. Re-
cently published work demonstrated that CaMKIV could
induce hypertrophic responses in cardiomyocytes in vitro and
that CaMKIV expression can cause cardiac hypertrophy in TG
mice (24). In contrast, there is no information concerning the
effects of CaMKIIδ, the CaMK subtype that predominates in

| Parameter | Wild-type (n=13) | CaMKIIδ (n=13) |
|-----------|-----------------|----------------|
| LVEDD (mm) | 3.65±0.08       | 4.16±0.09      |
| LVESD (mm) | 2.20±0.11       | 2.89±0.12      |
| FS (%) | 40.2±1.9        | 30.8±1.7       |
| VCF (circles) | 7.4±0.45      | 8.63±0.40      |
| HR (bpm) | 352±11.3        | 346±11.3       |
| LVM (g) | 0.980±0.033     | 0.996±0.031    |

**FIG. 4.** Transthoracic echocardiography in WT and CaMKIIδ
TG mice at 3-4 months of age. A, representative M-mode images
(bottom) and echocardiographs (top) of mice at 15 weeks of age. B,
echocardiographic parameters for WT and CaMKIIδ TG mice. IVS,
ventricular septum; PW, posterior wall; ESD, end-systolic diame-
ter; EDD, end-diastolic diameter; VCF, percentage of fractional shorten-
ing calculated as 100 × (LVEDD-LVESD)/LVEDD; VCF, heart rate-
corrected mean velocity of circumferential shortening; HR, heart rate;
LVM, left ventricular mass. The data are presented as the means ± S.E.
*, p < 0.01 versus WT; †, p < 0.001 versus WT.
FIG. 5. Hemodynamic measurements in 12-week-old WT and CaMKIIδB TG mice. A and B (n = 7 for WT, n = 10 for TG) show that +dP/dt and −dP/dt are significantly decreased in maximal responses in paced TG mouse hearts. *, p < 0.05 TG versus WT. C and D (n = 8 for each group) show that β-adrenergic responsiveness is not altered in TG mouse hearts. WT and TG mice were challenged by administration of a range of concentrations of dobutamine.

FIG. 6. SR Ca$^{2+}$ uptake and Western blots of phosphorylated PLB in WT and CaMKIIδB TG mice. A, Ca$^{2+}$ uptake in ventricular homogenates from WT and CaMKIIδB TG mice. SR Ca$^{2+}$ uptake assays were performed in ventricular homogenates at room temperature. Ca$^{2+}$ uptake was calculated from the slope of the linear regression analysis relating $^{45}$Ca$^{2+}$ uptake per milligram of total protein to reaction time (n = 5 for each group). *, p < 0.05 versus WT. B, Thr$^{17}$- and Ser$^{16}$-phosphorylated PLB were significantly decreased in TG hearts (n was between 5 and 12 for all groups). *, p < 0.05 versus WT; **, p < 0.01 versus WT. Total PLB was unchanged (data not shown). C, ventricles were removed and rapidly frozen for 3 min following infusion of dobutamine (12 µg/kg/min) (n = 6 for each group). PLB phosphorylation at Thr$^{17}$ and Ser$^{16}$ remained significantly diminished in TG versus WT following dobutamine treatment. *, p < 0.05 versus WT; **, p < 0.01 versus WT.

FIG. 7. Increased PP2A in CaMKIIδB TG mouse hearts. A, PP2A activity was assessed in ventricles from WT and TG mice as described under “Experimental Procedures.” Activity was significantly increased in particulate fraction from TG mouse hearts (n = 6 for cytosol in each group, n = 3 for particulate in each group). *, p < 0.05 versus WT. B, Western blots of particulate mouse hearts from CaMKIIδB TG mice (n = 3 for each group) show ~60% increase in expression of all PP2A subunits (PP2A/C, PP2A/A, and PP2A/B56α) compared with WT mice. PP2A/C, catalytic subunit; PP2A/A, A subunit; PP2A/B56α, B56α targeting subunit. C and D, immunoprecipitation studies show that there is an increase in the PP2A catalytic subunit, which coimmunoprecipitates with CaMKII in the particulate fraction (C) as well as in the nuclear fraction (D) of ventricular homogenates from TG mice. IP, immunoprecipitation; IB, immunoblot.
the heart, on myocardial cell growth in vivo.

The findings reported here utilized the α-MHC promoter to drive the expression of the wild type isofrom of CaMKIIδ2. This approach has been applied to a number of genes and has been shown to lead to considerable increases in mRNA and generally also in the protein of interest in the TG mouse heart. In the present report we assessed not only increases in protein expression but also examined concomitant changes in enzyme activity and in the extent to which there is active enzyme in the myocardium in vivo. Interestingly, these further measures allow one to “rationalize” the use of 10-fold overexpression by demonstrating that this leads to only a 4-fold increase in the total cellular CaMKII pool and only a 1.5-fold increase in the amount of active CaMKII in vivo. The modest increase in the amount of active kinase associated with overexpression of the wild type form of CaMKII probably accounts for the relatively mild phenotype. On the other hand, the increase is clearly within a range that would reflect physiological increases in CaMKII activity.

A variety of end points confirm that CaMKIIδ2 TG mice develop cardiac hypertrophy by 3–4 months of age. These include statistically significant increases in heart and left ventricle to body weight ratios, up-regulation of embryonic and contractile protein genes including ANF, β-MHC, and α-skeletal actin, and down-regulation of α-MHC, SERCA, and PLB genes. These hypertrophic changes in ventricular mass and gene expression were associated with development of a dilated cardiomyopathy in the CaMKIIδ2 TG mice. Specifically, these mice showed ventricular dilation and decreased contractile function, as documented by echocardiography, and impairment of the force-frequency response, as assessed by hemodynamic measurements. The observation that overexpression of CaMKIIδ2 can induce cardiac hypertrophy and dilated cardiomyopathy indicates that the phenotype previously reported for CaMKIV TG mice (24) mirrors that elicited by expression (and presumably activation) of the endogenous cardiac CaMK.

PLB is a substrate for CaMKII. Although we did not anticipate enhanced phosphorylation of this cytosolic substrate by the nuclear CaMKIIδ2, we were surprised to observe that PLB phosphorylation was actually decreased. Notably phosphorylation of both the CaMKII and the PKA site were decreased by 33–50% under conditions of both basal activity and adrenergic stimulation. Furthermore, functional effects of the diminished phosphorylation were evident as a decrease in contractility and relaxation in vivo and a decrease in SR Ca2+ uptake in vitro. The decreased PLB phosphorylation associated with heart failure has been suggested to result from β-adrenergic receptor desensitization (38, 39); however, hemodynamic measurements did not reveal significantly diminished β-adrenergic responsiveness in the CaMKII TG mice. The basis for the global decrease in PLB phosphorylation seen in the CaMKIIδ2 TG mice would therefore appear to be the increase in phosphatase activity. Our preliminary observation (data not shown) that PLB phosphorylation is not decreased, and the fact that phosphatase activity is not significantly elevated in 6-week-old TG mice further suggests that PLB phosphorylation is diminished secondary to increases in phosphatase activity.

Decreased basal levels of Ser16 and Thr17 phosphorylated PLB and prolonged relaxation were recently reported in postinfarction remodeled myocytes and partially explained by increased PP1 activity (40). Although there is evidence that PP1 is mainly responsible for the dephosphorylation of PLB (41, 42), PP2A can also dephosphorylate this substrate (41, 43). We observed no change in PP1 activity in CaMKII TG mice. However, hearts from CaMKII TG mice had increased PP2A activity and increased expression of the catalytic, and regulatory A, and B56α subunits in the particulate fraction. The particulate fraction is comprised of membranes from various cellular organelles. Increased PP2A is evident in nuclei in association with CaMKII (see below); however, the changes in PLB phosphorylation suggested that PP2A might also be increased in the SR membrane. This was supported by the observation that PP2A protein level increased ~60% in the cytoplasmic membrane fraction, which would include SR (data not shown).

The observation that the increase in PP2A was confined to the membrane-associated particulate fraction suggested the possibility that PP2A was localized in a complex of signaling molecules. Accordingly we asked whether there was a direct association between PP2A and CaMKII. Studies using neonatal rat ventricular myocytes demonstrated that endogenous CaMKII coimmunoprecipitated with endogenous PP2A and vice versa. Furthermore, there was an increase in PP2A associated with CaMKII in the particulate fraction as well as in the nuclear fraction from TG mice. Although the mechanism for the increase in PP2A activity and its association with CaMKII is not known, we suggest that this alteration contributes to the development of the cardiomyopathy, particularly to the decreased contractile function observed in CaMKIIδ2 TG mice. In this regard, it is of interest that a recent study reported that TG mice expressing a mutant A subunit of PP2A exhibit a dilated cardiomyopathy (44).

The generation and analysis of TG mice overexpressing CaMKIIδ2 support the conclusion that physiologically relevant levels of activation of the predominant cardiac isoform of CaMK II can function within the nucleus to induce cardiac gene expression and hypertrophy. In addition, kinase increases in both the amount and the activity may be associated with compensatory increases in phosphatase activity, and these may contribute to changes in SR function and Ca2+ handling that underlie the impairment of ventricular function.

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