Protein Kinase Cα Negatively Regulates Systolic and Diastolic Function in Pathological Hypertrophy

Harvey S. Hahn, Yehia Marreez, Amy Odley, Amber Sterbling, Martin G. Yussman, K. Chad Hilty, Ilona Bodi, Stephen B. Liggett, Arnold Schwartz, Gerald W. Dorn II

Abstract—The protein kinase C (PKC) family is implicated in cardiac hypertrophy, contractile failure, and β-adrenergic receptor (βAR) dysfunction. Herein, we describe the effects of gain- and loss-of-PKCα function using transgenic expression of conventional PKC isoform translocation modifiers. In contrast to previously studied PKC isoforms, activation of PKCα failed to induce cardiac hypertrophy, but instead caused βAR insensitivity and ventricular dysfunction. PKCα inhibition had opposite effects. Because PKCα is upregulated in human and experimental cardiac hypertrophy and failure, its effects were also assessed in the context of the Gαq overexpression model (in which PKCα is transcriptionally upregulated). Normalization (inhibition) of PKCα activity in Gαq hearts improved systolic and diastolic function, whereas further activation of PKCα caused a lethal restrictive cardiomyopathy with marked interstitial fibrosis. These results define pathological roles for PKCα as a negative regulator of ventricular systolic and diastolic function. **(*Circ Res*. 2003;93:1111-1119.)**

Key Words: systolic and diastolic heart failure ■ protein kinase C ■ interstitial fibrosis ■ restrictive cardiomyopathy

Cardiomyocytes challenged with increased hemodynamic load or neurohormonal stress respond by hypertrophying. A better understanding of the mechanisms that transduce hypertrophy has implications for developing strategies that can prevent functional decompensation. One important signal transducer for development and decompensation of pressure overload cardiac hypertrophy has been determined to be Gαq, the α-subunit of the Gq heterotrimeric G protein.[1,2] Coupled to phospholipase C, Gαq is the common signal transducer for multiple heptahelical cardiomyocyte receptors that stimulate cardiomyocyte hypertrophy, such as angiotensin II, endothelin, α-adrenergic agonists, and prostaglandin F2α.[3–6] In vivo genetically manipulated mouse models demonstrate that Gαq signaling is both necessary and sufficient for pressure overload hypertrophy,[2,7,8] and some critical downstream mediators have been defined.[9]

Activated by Gαq through phospholipase C, the protein kinase C (PKC) family of ubiquitous serine-threonine kinases has long been implicated in cardiac hypertrophy and, more recently, human heart failure.[10] The superfamily of twelve PKC isoforms is divided into three subfamilies based on functional and structural characteristics[11,12]: "conventional" PKCs (cPKC), which include PKCα, βI, βII, and γ, require both calcium and phospholipid for activation, whereas "novel" PKCs (nPKC), which include PKCδ and ϵ, require phospholipid, but not calcium (atypical PKCs are activated by phosphatidyl serine, and may represent a separate class altogether). Global knockouts of individual PKC isoforms have generally failed to define critical cardiac functions (likely due to opportunistic compensation by related isoforms[13–16]), whereas overexpression or activation of PKC isoforms has demonstrated that PKCβ, ϵ, and δ can each stimulate cardiac hypertrophic phenotypes.[1,17–21] PKCα has yet to be examined as a mediator of cardiac hypertrophy or failure by either in vivo gain- or loss-of-cardiac-specific function approaches, despite being the most abundant isoform in myocardium, in vitro evidence that it can regulate cardiac myocyte growth,[22] and its regulation in human and experimental heart failure.[7,10,23]

We analyzed the effects of myocardial PKCα activity utilizing novel transgenic mice expressing positive and negative regulators of cPKC translocation. Although these peptides are not specific for PKCα, its abundance relative to other cPKCs in adult mouse heart (>80% of myocardial cPKC) resulted in a biochemical phenotype of PKCα gain and loss of function. We demonstrate that activation of PKCα had no effect on hypertrophy. Instead, PKCα activation diminished myocardial responsiveness to β-adrenergic receptor agonists, whereas PKCα inhibition enhanced β-adrenergic responsiveness. In the context of Gαq-mediated hypertrophy and upregulated PKCα,[7,23] further PKCα translocation/activation caused a restrictive cardiomyopathy and premature lethality from fulminant heart failure. Gαq mice carrying a

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From the Departments of Internal Medicine/Cardiology (H.S.H., Y.M., A.O., A.S., M.G.Y., K.C.H., G.W.D.), Institute of Molecular Pharmacology and Biophysics (I.B., A.S.), and Internal Medicine/Pulmonary (S.B.L.), University of Cincinnati Medical Center, Cincinnati, Ohio.

Correspondence to G.W. Dorn II, Division of Cardiology, University of Cincinnati Medical Center, 231 Albert B. Sabin Way, Cincinnati, OH 45267-0542. E-mail dorngw@ucmail.uc.edu

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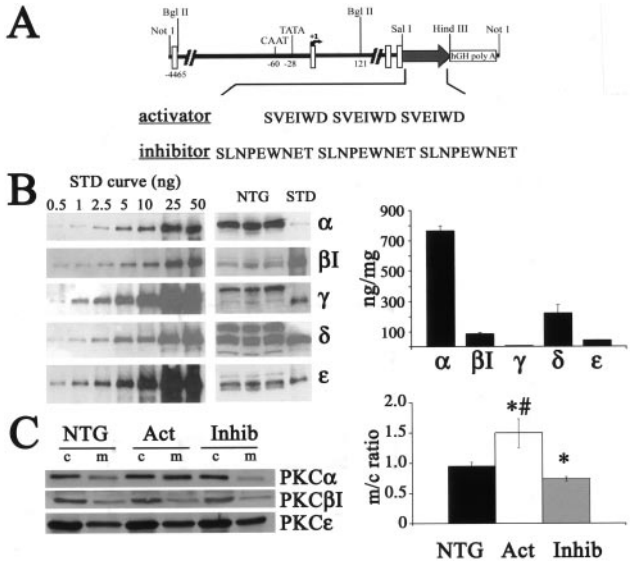

Figure 1. PKCα abundance, location, and activation. A, Schematic of the concatamerized PKCα activating and inhibiting constructs. B, Quantitative Western blots for conventional and novel PKC isoforms in the mouse heart. Bar graph depicting absolute expression levels is shown on the right. Note, PKCα was diluted 1:2 to stay within the standard curve. C, Representative Western blots of PKCα translocation. PKCϵ and PKCβI are shown as controls. *$P<0.05$ vs NTG; #$P<0.05$ vs inhibitor.

cPKC inhibitory peptide had modestly improved cardiac function and normal longevity. These observations establish pathological effects of PKCα in the adult heart.

Materials and Methods

Generation of Transgenic Mice With Altered PKCα Translocation

Transgenic mice were generated using the cardiomyocyte-specific α-myosin heavy chain (MHC) promoter to express single and concatamerized (three copies) peptides corresponding to either the PKCβC2-C4 region (cPKC inhibitor, amino acids 218 through 226 of rat PKCβII; inhibits translocation of all cPKC isoforms) or homologous regions of PKCβ and RACK1 (cPKC activator, enhances translocation of all cPKC isoforms)[24] (see Figure 1A, bottom). Founders were identified by genomic Southern analysis of tail clip DNA. Animals were treated in accordance with approved University of Cincinnati IACUC protocols.

Immunoblot Analysis

Quantitative immunoblot analysis of PKC isoform content used human recombinant PKC isoforms (Sigma) for generation of standard curves.[23] PKC isoform partitioning in subcellular fractions was assayed in cytosolic (100 000*g* supernatant) and Triton X-100–extracted membrane (100 000*g* pellet) ventricular fractions size-separated on 10% SDS-PAGE gels. Proteins were visualized using enhanced chemifluorescence and quantitated using a Storm PhosphorImager. Antibodies for PKCα, βI, βII, γ, δ, and ϵ were obtained from Transduction Laboratories.

Functional Assessments

Two-dimensional guided M-mode echocardiography of conscious unsedated mice was used to measure left ventricular (LV) diastolic and systolic dimensions (LVEDD and LVESD) and septal and posterior wall thickness (SWT and PWT), from which fractional shortening (FS) and LV mass were derived. Pulsed wave Doppler was used to measure aortic ejection time (ET) and calculate velocity of circumferential shortening, Vcf (FS/ET). Diastolic transmitral inflow Doppler indices included the following: peak E wave velocity, peak A wave velocity, E wave deceleration time, and isovolumic relaxation time (IVRT). Invasive hemodynamic studies were performed on anesthetized, spontaneously breathing 6- to 10-week-old transgenic mice, and their nontransgenic littermate controls as described.[7]

βAR Density and Adenylyl Cyclase Activity

βAR density (fmol/mg) was assayed by 125-I-cyanopindolol (CYP) binding, with nonspecific binding determined with 1 μmol/L alprenolol. Adenylyl cyclase activities were determined from membrane preparations as described.[7] Reactions were performed for 10 minutes at 37°C with various concentrations of isoproterenol, 10 nmol/L NaF, or 100 μmol/L forskolin. Adenylyl cyclase activities are reported as pmol/min per milligram and as a unitless value after normalization to NaF and forskolin stimulation.

Isolation of Ventricular Myocytes and Electrophysiology

Cardiomyocytes were enzymatically dissociated from ventricles of 4-month-old nontransgenic (NTG) and transgenic mice. Current recordings were obtained in the whole-cell, voltage-clamp configuration of the patch-clamp technique by using 1.60 OD borosilicate glass electrodes (Garner Glass Company), essentially as described.[20] Cell capacitance was calculated by integrating the area under an uncompensated capacity transient elicited by a 25-mV hyperpolarizing test pulse (25 ms) from a holding potential of 0 mV.

Implantable Telemetry

Mice were anesthetized and underwent sterile implantation of radio telemetry devices (Data Science Incorporated). Continuous nontethered telemetry was recorded as streaming data to a dedicated computer hard drive for off-line analysis.

Histopathology, Immunohistochemistry, and Apoptosis Studies

Histological examination was performed on formalin-fixed Masson's trichrome-stained sections. Collagen content of the extracellular matrix was assessed using picrosirius red staining and Image-Pro Plus software (Media Cybernetics). Apoptosis was measured using a TUNEL assay (Promega) imaged on a dual laser Nikon PCM2000 confocal system and a Nikon Eclipse E800 microscope using an emission wavelength of 515±30 nm (fluorescein).

Statistical Analysis

Results are presented as mean±SEM. Experimental groups were compared using Student's *t* test or one-way ANOVA. A Bonferroni or Newman-Keuls test was applied to all significant ANOVA results using SigmaStat software. A value of $P<0.05$ was considered significant.

Results

Effects of Cardiac-Specific cPKC Activator and Inhibitor Peptide Expression on In Vivo PKC Isoform Expression and Activation

Based on our prior experience with in vivo myocardial expression of translocation modifiers for PKCδ and PKCϵ,[20,21,25–27] we initially generated multiple lines of mice expressing FLAG-epitope tagged cPKC activator and inhibitor peptides. In none of these mice was there detectable modulation of any PKC isoform translocation, nor was there any demonstrable molecular or physiological phenotype (data not shown). Confocal analysis of myocardial samples failed to demonstrate expression of the epitope-tagged peptides, despite abundant mRNA expression (data not shown), suggesting that the small peptides might be unstable. Therefore,

TABLE 1. cPKC Inhibitor and Activator Morphometric and Physiological Parameters

	NTG	cPKC Inhibitor	cPKC Activator
Body weight, g	26.94±0.72	25.91±1.19	25.66±1.93
Heart/body weight ratio, mg/g	4.8±0.05	4.85±0.07	4.71±0.07
Lung/body weight ratio, mg/g	6.22±0.15	6.43±0.23	6.28±0.20
Liver/body weight ratio, mg/g	44.61±3.31	51.83±0.91	47.17±1.23
Cell capacitance, pF	210.58±14.91	215.69±9.90	231.05±8.51
Heart rate, bpm	732.7±8.6	724.8±6.9	756.9±5.9
LVM, g	48.5±3.2	55.4±3.6	63.1±13.1
FS, %	47.9±2.1	54.9±3.6	48.2±2.8
Vcf, circ/s	10.9±0.6	13.3±0.9	12.3±0.7
FS, % at 1 year	56.2±2.6	48.6±9.9	43.0±4.0*
Vcf, at 1 year	14.4±0.7	13.0±4.3	8.0±0.7*

LVM indicates left ventricular mass; FS, fractional shortening; and Vcf, velocity of circumferential fiber shortening.

*$P<0.05$ vs both NTG and inhibitor.

the transgenic constructs were reengineered to express thrice-concatamerized versions of the peptides (Figure 1A) and reinjected. Multiple founders were identified for cPKC activator and cPKC inhibitor constructs, and three viable lines were established and phenotyped for each. As transgene expression, PKC translocation, and basal phenotype were similar between lines, baseline data (Table 1) were combined. All other data are from one each cPKC activator and inhibitor line.

Because the PKCβ-derived peptides had the potential to target any conventional PKC isoform,[24] we performed quantitative analysis of relative PKC isoform expression in adult FVB/N mouse hearts to determine the most abundant target. As shown in Figure 1B, and consistent with prior reports,[23,28] PKCα was the most abundant ventricular isoform, followed by PKCδ. Of the cPKCs, besides PKCα, only PKCβI was measurable.

The concatamerized cPKC translocation-modifying peptides had the anticipated effects on PKCα subcellular partitioning, without measurable effects on PKCβI or other isoforms (likely due to low expression of the other cPKCs in ventricular tissue; Figure 1C). Compared with nontransgenic controls, basal PKCα translocation in cPKC activator hearts, measured by the ratio of immunoreactive partitioning to a particulate subcellular fraction, was increased 44±1.5% (n=8, $P=0.013$ versus NTG), whereas in cPKC inhibitor hearts, it was decreased by 25±2.6% (n=8, $P=0.043$ versus NTG). This degree of basal translocation modification is somewhat greater than the 15% to 20% values for our previously described PKCδ and ϵ translocation modifier mice.[21,27] PKCα, PKCβ1, and PKCϵ content were not altered in the transgenic mouse hearts (data not shown).

PKCα Translocation Enhancement Is Not Sufficient to Cause Cardiac Hypertrophy In Vivo

Multiple lines of transgenic mice expressing either the cPKC activator or inhibitor peptides were outwardly normal and healthy in all respects. Gross chamber geometry and dimensions (Figure 2A and Table 1), and histological appearance (not shown) were normal. Despite the previously noted association between PKCα upregulation and cardiac hypertrophy in the Gαq mouse and other pathological cardiac states,[7,10,23] and a prior report that PKCα expression causes hypertrophy in cardiac myocytes,[22] chronically enhanced PKCα translocation did not increase gravimetric cardiac mass (Figure 2B), echocardiographically estimated left ventricular mass (Table 1), or isolated myocyte capacitance (Table 1) in mice followed up to 1 year. Although there was no cardiac or cardiomyocyte hypertrophy, enhanced translocation of PKCα caused a subtle increase in expression of some fetal cardiac genes that are upregulated in myocardial hypertrophy: Compared with normal controls, βMHC mRNA was increased ≈25 fold in cPKC activator hearts, and ANF mRNA was increased ≈4 fold (Figures 2C and 2D).

As with PKCα activation, PKCα inhibition failed to cause measurable cardiac or cardiomyocyte hypertrophy, but was associated with a ≈6 fold increase in βMHC mRNA (Figures 2C and 2D). ANF gene expression, thought to be a marker of cardiac failure rather and possibly of hypertrophy,[29] was not increased by chronic PKCα inhibition.

Functional Consequences of Myocardial PKCα Translocation Modulation

Consistent with the absence of any overt signs of heart failure in cPKC activator and inhibitor mice, there was also no increase in lung/body weight ratios (Table 1). There were no differences in basal left ventricular systolic function (measured as fractional shorting and Vcf) between either of the cPKC translocation modifier mice and their NTG littermates at 10 weeks (see Table 1) or 6 months. cPKC activator mice did, however, develop mild echocardiographic left ventricular dysfunction at 1 year of age (Table 1; $P<0.001$). Together with increased ANF gene expression in these mice, the time-dependent decrease in left ventricular ejection performance suggested that a subthreshold contractile abnormality could be caused by chronic cPKC activation. Notably, cPKC inhibitor mice retained normal ventricular function through 1 year of age (Table 1).

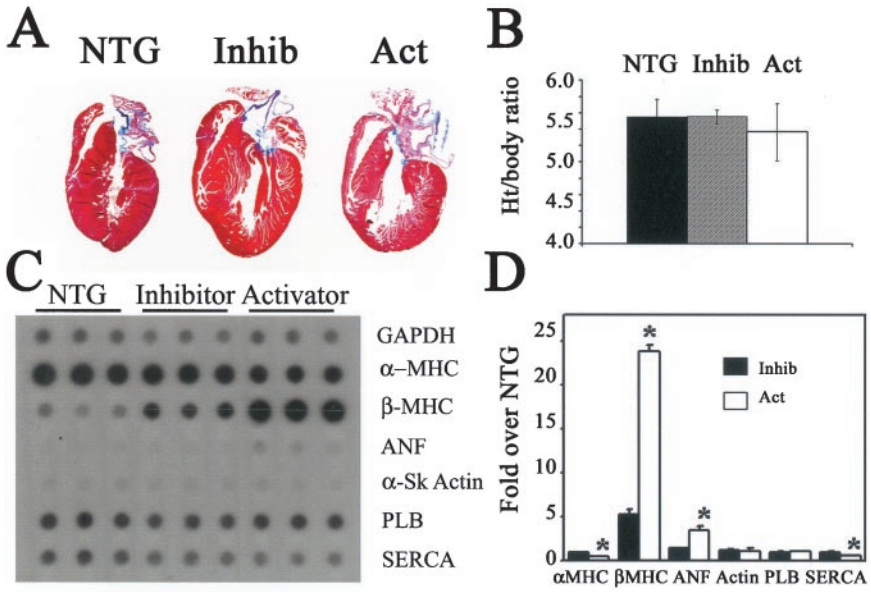

Figure 2. PKCα is not sufficient to cause cardiac hypertrophy. A, Representative hearts from 12-week-old animals. B, Quantitative heart/body weight ratios. C, mRNA expression with quantitative bar graphs (D). *$P<0.05$ vs NTG.

PKCα Activation Impairs βAR Responsiveness

The functional integrity of the cardiac β-adrenergic receptor (βAR) system was assessed in closed chest homodynamic studies of 10- to 12-week-old mice at baseline and during dobutamine infusion. (We typically use dobutamine as the β1AR provides the majority of inotropic drive in the mouse heart.[30]) Consistent with the echocardiographic findings, basal left ventricular contractile function of PKCα activator or inhibitor mice did not differ from nontransgenic controls (Figure 3A). However, responsiveness to β1AR stimulation was diminished in cPKC activator mice, with a rightward shift in the dobutamine-response curve (Figure 3A). cPKC inhibitor mice had an antithetic phenotype of enhanced chronotropic and inotropic responses to β1AR agonist infusion, with a leftward shift in the concentration-response relationship (ED50 [ng/g per minute] for NTG=7.7±2.0, inhibitor=1.9±0.1, activator=17.3±2.3; $P<0.05$ for activator and inhibitor versus NTG). βAR density, assessed by 125I-CYP binding, did not differ between cPKC activator, inhibitor, and nontransgenic mice (Figure 3B), ruling out a change in receptor expression. To assess receptor-effector coupling, adenylyl cyclase activity was measured in cardiac membranes at baseline and in response to isoproterenol, NaF, and forskolin. Basal and isoproterenol stimulated adenelyl cyclase activities (pmol/min per milligram) were as follows: NTG, 10.6±1.6 and 28±4.0; cPKC activator, 15.2±1.6 and 34.2±4.5; and cPKC inhibitor, 12.2±2.0 and 25.9±4.1. Both NaF- and forskolin-stimulated activities trended toward being higher in the activator and lower in the inhibitor samples, suggesting regulation at the level of Gs or adenylyl cyclase. Therefore, βAR function was isolated by normalizing isoproterenol-stimulated activates to NaF and forskolin. This revealed (Figure 3C) a moderate decrease in βAR function in activator membranes and enhanced function in inhibitor (39±7.6 versus 68±6.7; $P<0.03$). NTG responses were intermediate (54±9.0). The EC_{50} values for isoproterenol stimulation of adenylyl cyclase did not differ between the three groups. These results are consistent with previously described effects of PKCs to phosphorylate and uncouple βAR from adenylyl cyclase.[31,32] Indeed, it was not possible to overwhelm the βAR dysfunction by simply increasing the number of membrane receptors through crossbreeding the PKCα translocation modifying mice with transgenic β2AR overexpressors having nearly maximal βAR signaling at baseline[33] (Figure 3D). Even in the context of a ≈30-fold increase in βAR receptor number, βAR coupling was similarly and reciprocally modulated by the cPKC translocation modifying peptides (Figure 3D), consistent with a potent direct effect of PKCα on βAR coupling.

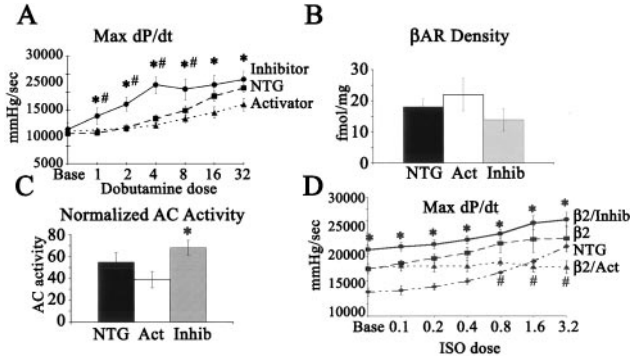

Figure 3. PKCα activation uncouples β-adrenergic receptors. A, In vivo contractile response to dobutamine. *$P<0.05$ vs activator; #$P<0.05$ vs NTG. B, βAR receptor density. C, Normalized adenylyl cyclase activity. *$P<0.05$ vs activator. D, Effect of PKCα modulation on the β_2-overexpressing mouse hearts response to isoproterenol. *$P<0.05$ vs activator; #$P<0.05$ vs β_2.

Lethal Effects of PKCα Activation in Gαq-Mediated Hypertrophy

Although the phenotype was relatively subtle by comparison, there were intriguing similarities between the cPKC activator mice and the Gαq mice, in which PKCα is upregulated and activated.[7,23] Both mice exhibit a time-dependent decrease in ventricular function associated with lack of responsiveness to βAR agonists and an increase in ANF and βMHC gene

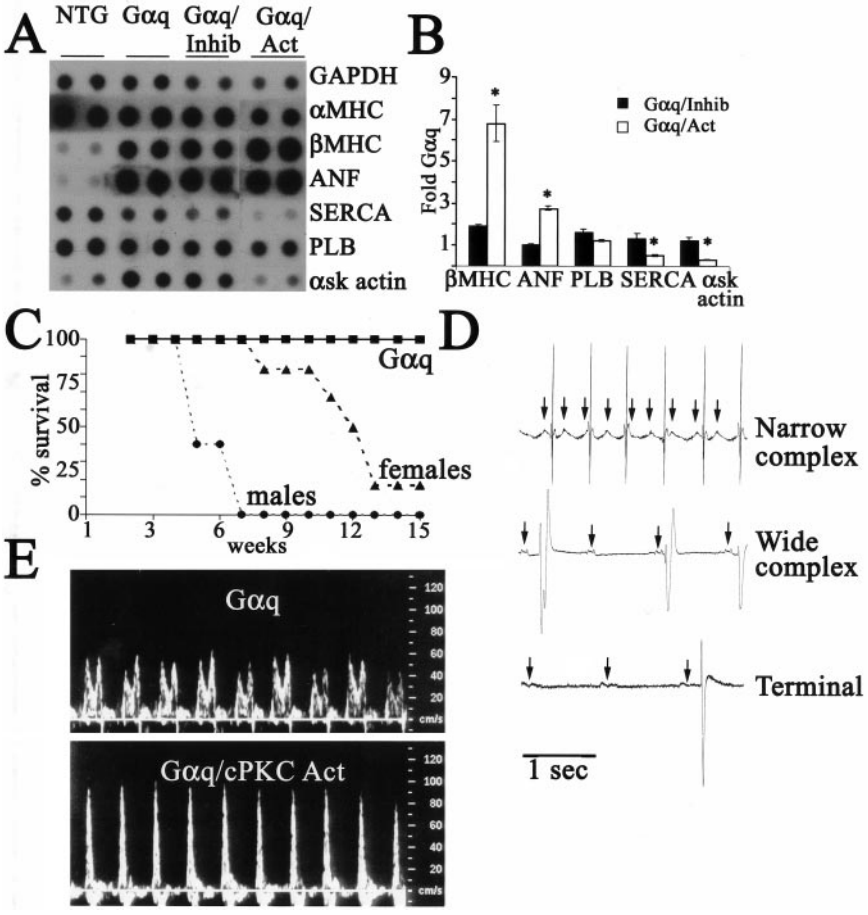

Figure 4. PKCα activation exacerbates Gαq-mediated hypertrophy and leads to rapidly progressive heart failure and death. A, mRNA expression. B, Quantitative bar graphs. C, Survival curve for Gαq and Gαq/cPKC mice. D, Radio telemetry received from Gαq/cPKC activator mouse. Arrows denote P waves. E, Representative transmitral inflow patterns from Gαq and Gαq/cPKC activator mouse. *$P<0.05$ vs Gαq.

expression. However, the cPKC activator mouse has no evidence of hypertrophy, which is the hallmark characteristic of Gαq activation in the heart. This suggested that the major function of PKCα activation could be to regulate cardiac function, rather than myotrophy. To test whether concurrent Gαq-mediated events would unmask a more significant effect of PKCα modulation, we expressed both cPKC translocation modifier peptides on the Gαq overexpressor background, analogous to our prior studies of Gαq in combination with PKCε translocation modifiers.[25] Our expectation was that the relatively subtle deleterious effects observed with enhanced PKCα translocation might be augmented in the context of Gαq-mediated myocardial hypertrophy, whereas PKCα inhibition might prevent some Gαq-mediated pathological effects.

The combination of Gαq and the cPKC inhibitor peptide in compound transgenic mice resulted in animals with normal viability and molecular characteristics essentially identical to the parent Gαq mouse (Figures 4A and 4B). Compared with Gαq, Gαq/cPKC inhibitor mice had modest, but significantly smaller hearts, measured as heart weight indexed to body weight (Table 2). A contrasting phenotype was observed in the compound Gαq/cPKC activator mice, which displayed stunted growth and premature lethality compared with Gαq mice (Table 2, Figure 4C), accompanied by increased heart to body weight ratios (Table 2). Additionally, expression of embryonic cardiac genes was exaggerated compared with Gαq alone (Figures 4A and 4B). It was not possible to obtain an unbiased assessment of ventricular function in the Gαq/cPKC activator compound transgenics because all male compound transgenic mice died by the age of 5 weeks (Figure 4C). Ventricular tachyarrhythmia was ruled out as the proximate cause of death in these mice, because chronic telemetered EKG monitoring showed only progressive bradycardia and high grade AV block that coincided with tachypnea and lethargy, suggesting that the mice succumbed to heart failure (Figure 4D). Indeed, at 4 weeks of age, male Gαq/cPKC activator mice exhibited a substantial decline in LV shortening velocity and depressed heart rate, compared with Gαq alone (Table 2), which along with stunted growth (Table 2), are common features of heart failure in juvenile mice.[34]

Although aggressive heart failure in male compound Gαq/cPKC activator mice precluded a careful assessment of physiological cause and mechanism, we were able to investigate events preceding lethality by studying the females, who exhibited a slower progression of heart failure, dying by 13 weeks of age (Figure 4C). (Similar gender separation has been described in other transgenic mouse heart failure models.[35]) Serial echocardiographic evaluation of female Gαq/cPKC activator mice revealed a progressive decline in LV function that became significant by 7 weeks of age (FS% Gαq, 26.17±1.03 versus Gαq/cPKC activator, 15.07±2.43; $P=0.006$; Vcf Gαq, 4.0±0.2 circ/s versus Gαq/cPKC activator, 1.9±0.31; $P=0.001$) and increasing lung to body

TABLE 2. Gαq/cPKC Inhibitor and Activator Morphometric and Physiological Parameters

	NTG	Gαq	Gαq/cPKC Inhibitor	Gαq/cPKC Activator
Body weight, g	19.08±0.42	23.06±1.66	26.02±1.06	14.4±0.79*†
Heart weight, mg	119.6±3.2	154.4±3.6	138.9±6.5	166.3±11.5
Heart/body weight ratio, mg/g	6.28±0.19	6.98±0.53	5.35±0.19*	10.75±0.41*†
Lung/body weight ratio, mg/g	8.71±0.55	7.83±0.39	7.09±0.29	9.25±0.58*†
Liver/body weight ratio, mg/g	53.38±2.12	52.87±1.1	52.16±2.0	59.83±7.3
LVEDD, mm	2.9±0.1	4.0±0.2	4.3±0.3	4.1±0.1
LVESD	1.6±0.1	2.9±0.2	3.1±0.2	3.1±0.1
Heart rate, bpm	732.7±8.6	411.3±8.2	400.4±36.3	320.9±22.47*†
LVM, g	47.9±3.2	72.8±9.8	87.4±8.4	82.0±7.2
FS, %	47.9±2.1	27.8±2.6	29.7±0.9	25.0±1.9
Vcf, circ/s	10.9±0.6	4.0±0.3	4.7±0.3	3.0±0.4*†
Heart rate, bpm	494.3±15.4	245.7±8.9	312.4±10.4	277.6±14.6
LVP, mm Hg	96.2±2.7	79.5±0.6	84.6±1.8	66.7±1.7*†
+dP/dt max	11 583.1±679.8	6419.3±153.5	7730.1±97.1*	4709.1±351.4*†
−dP/dt min	−8471.9±592.3	−3887.4±142.6	−5735.3±126.4*	−2904.3±194.3†
LVEDP, mm Hg	2.45±1.3	6.7±0.7	3.2±0.1	11.8±1.5*†

LVEDD indicates left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LVM, left ventricular mass; FS, fractional shortening; Vcf, velocity of circumferential fiber shortening; LVP, left ventricular pressure; and LVEDP, left ventricular end-diastolic pressure. NTG shown for reference only.

*$P<0.05$ vs Gαq; †$P<0.05$ vs Gαq/cPKC inhibitor.

weight ratios in the compound transgenic Gαq/cPKC activator mice (Gαq 7.83±0.39 versus Gαq/cPKC activator 10.41±1.38; $P=0.027$).

The disparity between progressive heart failure and relatively preserved ventricular dimension and systolic function, ie, the absence of characteristic ventricular dilation measured either by echocardiography or by gross morphometric analysis (see Table 2 and Figure 5), suggested that diastolic functional abnormalities could contribute to lethal heart failure. Fortunately, the inherent bradycardia of Gαq mice permitted interrogation of transmitral inflow Doppler patterns for characteristic abnormalities associated with diastolic dysfunction. Compared with Gαq, Gαq/cPKC activator mice had higher peak E wave velocities with significantly shorter E wave deceleration times (Figure 4E; Gαq, 35.02±3.01 ms versus Gαq/cPKC activator, 18.02±2.0; $P=0.022$), reflecting increased left atrial pressures and impaired ventricular filling. These animals completely lacked an A wave, indicating atrial mechanical failure that correlated with extensive atrial thrombosis seen in almost every heart (Figures 5C and 5D). Consistent with these noninvasive studies, invasive hemodynamics revealed that Gαq/cPKC activator mice had diminished positive and negative dP/dt, and increased left ventricular end-diastolic pressures (LVEDP), compared with Gαq (Table 2). (In contrast, Gαq/cPKC inhibitor mice had a modest, but significant improvement in both systolic and diastolic function, measured by positive and negative dP/dt, respectively [Table 2].) Because the above diastolic parameters are relatively load-dependent, we performed pressure-dimension analysis to determine the compliance curves of each ventricle. Gαq/cPKC activator mice had a significant left and upward shift in compliance curves, compared with Gαq (slope constant Gαq, 13.04±1.4 versus Gαq/cPKC, 30.1±1.9; $P=0.017$), representing increased ventricular stiffness.

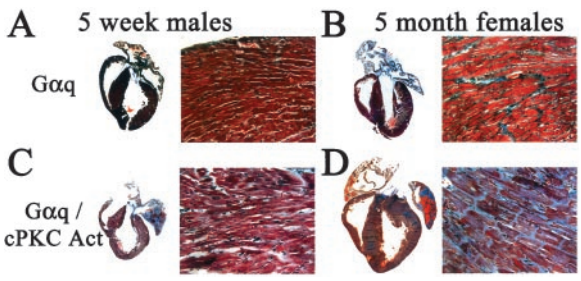

Figure 5. Interstitial fibrosis resulting from PKCα activation. Gross heart specimens and Masson's trichrome sections from 5-week-old Gαq (A), 5-month-old Gαq (B), 5-week-old Gαq/cPKC activator (C), and 5-month-old Gαq/cPKC activator (D). Note the left atrial thrombi in the Gαq/cPKC activator hearts. Histological sections are ×400.

Although there were no differences in TUNEL positivity (Gαq 0.36±0.12% versus Gαq/cPKC activator 0.55±0.08%), a cellular mechanism for combined systolic and diastolic failure in compound transgenic Gαq/cPKC activator mice was identified: Masson's Trichrome staining revealed marked interstitial fibrosis (Figures 5A through 5D) and staining with picrosirius red showed ≈4-fold increased interstitial collagen content in the Gαq/cPKC activator mice, compared with normal, and ≈2-fold increased compared with Gαq (Figure 6). Gαq/cPKC inhibitor mice had the reciprocal finding of decreased collagen deposition fraction (Figure 6).

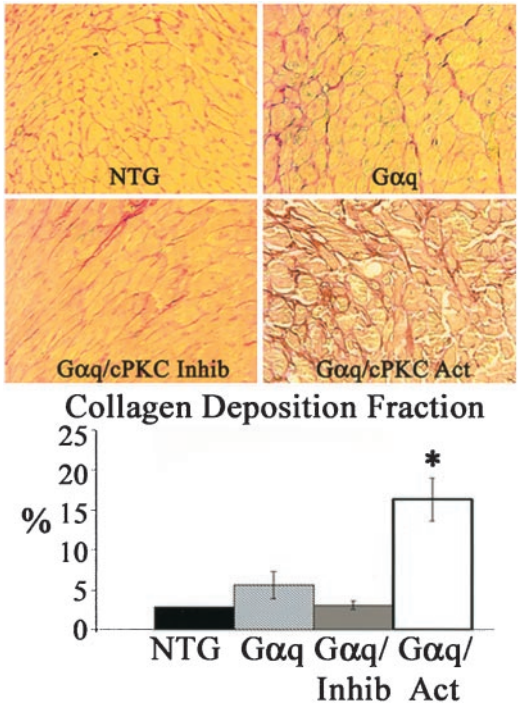

Figure 6. Extracellular matrix content in Gαq/PKCα mouse hearts. Picrosirius red–stained myocardium from NTG (A), Gαq (B), Gαq/cPKC inhibitor (C), and Gαq/cPKC activator (D) hearts. *$P<0.05$ vs both Gαq and Gαq/cPKC inhibitor.

Discussion

To our knowledge, these are the first studies to examine forced gain or loss of PKCα function in the in vivo heart. As with our previous studies of PKCδ and ε,[20,21,26,27] the approach was to modify PKC isoform activity by positively or negatively modulating translocation. In contrast to these prior studies, the PKCβ-derived peptides used herein lack absolute specificity for the targeted enzyme, but had the potential to modulate translocation of PKCα, β, and γ.[19] However, that adult FVB/N mouse hearts contained vastly more PKCα than PKCβI, PKCβII, or PKCγ, and the biochemical phenotype was therefore that of PKCα regulation, with no measurable effects on other PKC isoforms. Nevertheless, the conclusions regarding in vivo PKCα effects will need to be confirmed by standard transgenic and knockout studies. In this context, our results suggest that isolated alterations of myocardial PKCα activity can modulate βAR coupling, and hence contractile function, in the heart. Additionally, PKCα appears to mediate interstitial fibrosis and restrictive ventricular physiology. Indeed, in our studies, inhibiting PKCα translocation in Gαq-mediated hypertrophy might be viewed as conferring a modest therapeutic benefit.

In vitro studies have previously implicated PKC-mediated phosphorylation in regulating βAR receptors.[31,32] The reciprocal effects observed herein on βAR response with PKCα activating and inhibiting peptides, and the absence of similar βAR regulation in analogous models targeting PKCδ and ε[20,21,26,27] suggest that the α isoform of PKC can be an important modulator of βAR responsiveness in the heart. Whereas inhibition of PKCα enhanced βAR-mediated inotropic responsiveness to infused dobutamine, activation of PKCα resulted in βAR uncoupling in young mice that was associated with overt basal contractile dysfunction at 1 year of age. Importantly, these effects were independent of cardiac hypertrophy and failure, and thus do not represent actions of endogenous catecholamines. Although the association is correlative only, it suggests the possibility that chronic βAR uncoupling can be deleterious in the normal heart.

In the present study, myocardial fibrosis and restrictive physiology were related to PKCα activation only in the context of Gαq-mediated hypertrophy and contractile dysfunction. Myocardial fibrosis is a hallmark of heart failure in human and experimental models, and contributes to functional decompensation because accumulation of interstitial collagen stiffens the ventricle, impairing both systolic and diastolic function.[36,37] A physiological connection between PKC and fibrosis is found in the angiotensin system. Yazaki has suggested that angiotensin stimulates cardiomyocytes via Gαq/PKC/ERK signaling[38] and lack of AT-2 receptors prevents both hypertrophy and myocardial fibrosis in angiotensin-induced hypertension.[39] We found increased levels of phosphorylated ERK in cPKC activator hearts (data not shown), confirming the integrity of this signaling pathway in the in vivo heart, and suggesting for the first time a causal relationship between myocyte PKCα activity and development of myocardial fibrosis. Furthermore, the nature of the fibrosis observed in Gαq/activator mouse hearts was "reactive" or interstitial, which is not associated with cell death, as opposed to "reparative" or replacement fibrosis, which is the scarring that typically follows cell death. An unresolved issue that arises from these observations is how a cardiomyocyte-specific signaling perturbation can result in increase interstitial collagen, which is presumably synthesized not by cardiomyocytes, but by fibroblasts. Again the angiotensin system provides a compelling example. Ichikawa generated chimeric mice with both intact and AT-1 receptor null cells,[40] in which infusion of angiotensin caused fibroblast proliferation clustered around the AT-1–expressing cardiac myocytes, providing evidence for local myocyte-to-fibroblast communication. Thus, altered myocyte signaling can contribute to ventricular remodeling through communication with fibroblasts and resulting myocardial fibrosis.

It is useful to compare the results of the current investigations with prior attempts to delineate the roles of PKC isoforms using the approach of translocation modification. PKCε seemed to primarily have trophic functions, because its activation produced hyperplastic cardiac enlargement,[20,27] and its inhibition cause a hypoplastic dilated cardiomyopathy.[20] Increasing PKCε activity in the Gαq mouse reversed some of the pathological features of that model.[25] Thus, PKCε seems to be a beneficial isoform, which is supported by conventional transgenic overexpression studies.[19] In contrast, modulated translocation of PKCδ had little measurable effect on myocardial growth or function, but was critical to maintaining proper cytoskeletal structure in cardiac myocytes.[26] Activation of PKCδ, like PKCε, resulted in modest, normally functioning cardiomegaly, whereas its inhibition produced a

cytoskeletal cardiomyopathy. These results contrast with those for PKCα, which seems to affect cardiac function more than growth, notwithstanding in vitro studies that suggested a trophic function.[22]

Whereas PKCα is the most abundant conventional PKC isoform in the adult FVB/N mouse heart (vide supra), PKCβ is the major cPKC isoform in human myocardium. Indeed, substantial evidence exists that PKCβ mediates certain pathological aspects of human cardiac hypertrophy and heart failure. PKCβ expression and activity is increased in human heart failure.[10] Expression of PKCβ in the mouse heart (more an ectopic expression experiment than overexpression) results in hypertrophy, failure, and death, depending on the age of expression and the activity of the expressed enzyme.[17,18] It is interesting to speculate that PKCα in the mouse heart has many of the same effects as PKCβ in the human, which suggests that cPKC isoforms may be generally pathological in the heart.

In summary, these studies demonstrate that PKCα can have profound modulatory effects on cardiac contractility by regulating β-adrenergic receptors. In the context of Gαq-mediated cardiac hypertrophy, PKCα appears to stimulate reactive fibrosis, thereby impairing both systolic and diastolic function, and leading to early heart failure and premature lethality. These results suggest that conventional PKC isoforms may be useful therapeutic targets in heart failure syndromes.

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