Dilated Cardiomyopathy and Sudden Death Resulting From Constitutive Activation of Protein Kinase A

Christopher L. Antos, Norbert Frey, Steven O. Marx, Steven Reiken, Marta Gaburjakova, James A. Richardson, Andrew R. Marks, Eric N. Olson

Abstract—β-Adrenergic receptor (βAR) signaling, which elevates intracellular cAMP and enhances cardiac contractility, is severely impaired in the failing heart. Protein kinase A (PKA) is activated by cAMP, but the long-term physiological effect of PKA activation on cardiac function is unclear. To investigate the consequences of chronic cardiac PKA activation in the absence of upstream events associated with βAR signaling, we generated transgenic mice that expressed the catalytic subunit of PKA in the heart. These mice developed dilated cardiomyopathy with reduced cardiac contractility, arrhythmias, and susceptibility to sudden death. As seen in human heart failure, these abnormalities correlated with PKA-mediated hyperphosphorylation of the cardiac ryanodine receptor/Ca2+-release channel, which enhances Ca2+ release from the sarcoplasmic reticulum, and phospholamban, which regulates the sarcoplasmic reticulum Ca2+-ATPase. These findings demonstrate a specific role for PKA in the pathogenesis of heart failure, independent of more proximal events in βAR signaling, and support the notion that PKA activity is involved in the adverse effects of chronic βAR signaling. (Circ Res. 2001;89:997-1004.)

Key Words: protein kinase A ■ transgenic mice ■ dilated cardiomyopathy

Adrenergic signaling plays a fundamental role in regulating cardiac performance, and abnormalities in this pathway have been implicated as important determinants of diminished function of the failing heart.1-5 The β1- and β2-adrenergic receptors (βARs), which bind circulating catecholamines, activate adenylyl cyclase by coupling through the stimulatory G-protein, GoS. The resulting increase in intracellular cAMP activates cAMP-dependent protein kinase (PKA). In the inactive state, PKA exists as a tetramer, consisting of two regulatory and two catalytic subunits.6 Binding of cAMP by the regulatory subunits results in their dissociation from the catalytic subunits and activation of the enzyme. Thus, increasing the ratio of catalytic to regulatory subunits is sufficient to activate PKA.7

PKA has several substrates in cardiomyocytes that influence contractility in response to activated βAR signaling; these include the L-type Ca2+ channel in the sarcolemma, the ryanodine receptor (RyR2), and phospholamban in the sarcoplasmic reticulum (SR). Phosphorylation of phospholamban (PLB) leads to the increased activity of the sarcoplasmic reticulum Ca2+-ATPase (SERCA) and consequent accelerated Ca2+ accumulation in the SR. This increases the SR Ca2+ content available for subsequent cardiac cycles and thereby increases contractility. PKA also phosphorylates the RyR2, the Ca2+-release channel in the SR, resulting in dissociation of the Ca2+ channel regulator FKBP12.6 and consequent increase in Ca2+ sensitivity for channel activation.8 PKA phosphorylation of the RyR2 is markedly increased in failing human hearts,8 suggesting a link between PKA signaling and altered inotropy associated with long-term βAR signaling.

Chronic heart failure is associated with an increase in circulating catecholamines.9 Prolonged βAR stimulation results in the uncoupling of βARs from downstream effectors through 2 mechanisms: downregulation of receptor number (β1)10 and desensitization of the receptors (β2).11,10 Although the mechanisms regulating the loss of β1-AR number are not clear, β2-AR desensitization results from phosphorylation of the cytoplasmic domain of the receptor by βAR kinase (βARK).11 βARK is upregulated in the failing heart, which has been proposed as a mechanism to account for diminished βAR responsiveness.12-14 Consistent with this notion, βARK inhibitors increase cardiac contractility in vivo.15,16 β2-ARs are also phosphorylated by PKA,17,18 which results in diminished βAR activity and implicates PKA in impaired cardiac contractility.

Several transgenic mouse models have addressed the roles of βAR/adenylyl cyclase signaling in cardiac function. Increased transgenic expression of the β1-AR in the heart initially increases contractility,19 but its chronic activity leads to decreased function and eventual cardiac failure.19,20 Simi-
larly, transgenic cardiac overexpression of Goα in mice causes cardiomyopathy due to sustained activation of the βAR pathway. In contrast, chronic cardiac adenyl cyclase activity (which is activated by Goα) has been shown to result in long-term enhanced function,21,22 and in transgenic mouse models that suffer from cardiac failure, the overexpression of adenyl cyclase improves contractility.23 These data indicate that components of the β-adrenergic signaling pathway may have different consequences on cardiac performance.

Despite extensive investigations into the role of βAR/adenyl cyclase signaling in the heart, it is unknown whether PKA activation alone is sufficient to evoke pathological changes in cardiac function associated with chronic βAR stimulation or to ameliorate cardiomyopathic stimuli as attributed to overexpression of adenyl cyclase. To investigate the role of cardiac PKA signaling in the absence of other βAR-dependent signaling events, we generated transgenic mice that overexpressed the PKA catalytic subunit under control of the α-myosin heavy chain (α-MHC) promoter. These mice developed profound chamber dilation, with decreased cardiac function, edema, arrhythmias, and susceptibility to sudden death. Cardiac RyR2 and PLB were hyperphosphorylated in PKA transgenic mice, consistent with the notion that they mediate the effects of PKA on cardiac contractility. Our data demonstrate that upregulated PKA activity is detrimental to cardiac physiology and suggest a role for hyperphosphorylation of PKA targets in the pathogenesis of dilated cardiomyopathy.

Materials and Methods

Generation of Transgenic Mice
A cDNA encoding the PKA-Cα subunit was cloned 3’ to the α-MHC promoter and 5’ to the human growth hormone poly A’ signal. DNA isolation and oocyte injections were performed as described.24 Genomic DNA was isolated from mouse tail samples and was analyzed by Southern blot with a probe for the human growth hormone 3’ region. Mice were obtained from the National Cancer Institute, Frederick, Md.

PKA Enzyme Assay
PKA activity of transgenic and wild-type ventricular samples was determined by measuring γ32P incorporation into a biotinylated substrate (LRRASLG) using the SignaSECT cAMP-dependent protein kinase (PKA) assay system (Promega).

RNA Analysis
Total RNA was obtained from ventricular tissue using Trizol Reagent (Roche). Dot blot analysis was performed as described.25

Histology
Hearts were isolated, incubated in Krebs-Hanseleit solution lacking Ca2+ to relax the cardiac muscle, and fixed (10% formalin) overnight at 4°C. Samples were dehydrated, mounted in paraffin, and sectioned (10 μm thickness). The sections were stained with either eosin and hematoxylin dyes to determine cell and nuclear size or Masson-trichrome dye to visualize collagen deposits (fibrosis) in the ventricular and septal walls.

Transthoracic Echocardiography
Cardiac function of control and transgenic mice aged 8 to 10 weeks was evaluated with echocardiography. Echocardiography was performed on anesthetized mice (2.5% Avertin-15 μL/g body weight) using a Hewlett Packard Sonos 5500 Ultrasound system with a 12 MHz transducer. Heart rates were determined by ECG analysis. Three independent M-mode measurements per animal were obtained. End-systolic and end-diastolic chamber diameters, interventricular septum and posterior wall thicknesses, as well as left ventricular fractional shortening (FS% = ([LVEDD-LVESD]/LVEDD)×100) were determined in a short-axis view at the level of the papillary muscles. Animal handling was performed according to UT Southwestern Institutional guidelines.

Immunoprecipitation and Back-Phosphorylation Assays
Heart homogenates were prepared and immunoprecipitation assays were performed as previously described.8

Western Blots
Heart homogenates were immunoprecipitated with anti-RyR (5029) antibody and samples were size fractionated by SDS-PAGE. Separated proteins were transferred to nitrocellulose membranes and Western blots were performed using anti-RyR and anti-FKBP antibodies.26 To quantify phospholamban phosphorylation, 50 μg protein from heart homogenates were size fractionated by SDS-PAGE on a 15% gel and immunoblotted with a phospholamban phosphopeptide specific antibody (1:5000 dilution, PS-16, Cyclcel Ltd).

Single Channel Recordings
RyR2 single channel recordings under voltage-clamp conditions were performed and analyzed as described.8 RyR2-containing SR vesicles were added to the cis chamber and fused with planar lipid bilayers composed from 3:1 phosphatidyl ethanolamine/phosphatidyl serine (Avanti Polar Lipids). The bilayer cup was polystyrene with a 0.15 mm aperture. Fusion was activated by KCl added in cis chamber. After incorporation of a Ca2+-release channel, the KCi gradient was removed by perfusion of cis chamber with cis solution (10 mL). Solutions for channel analyses were as follows: trans solution, 250 mmol/L Hepes and 53 mmol/L Ca(OH)2, pH=7.35; and cis solution, 250 mmol/L HEPES, 125 mmol/L Tris, 1 mmol/L EGTA, 0.5 mmol/L CaCl2, pH=7.35. Free Ca2+ concentration (cis) 150 mmol/L was calculated with CHELATOR method.27 At the conclusion of each experiment, ryanodine and/or ruthenium red were applied to demonstrate the identity of RyR2 channels. Results are mean±standard deviation; the Student’s t test was used for statistical analyses.

Results

Generation of αMHC-PKA-Transgenic Mice
To study the effects of chronic PKA activation in the adult heart, we generated transgenic mice with a transgene encoding the catalytic subunit of PKA under control of the αMHC promoter. Two independent transgenic lines were generated with 1 and 3 copies of the transgene, as determined by Southern analysis of genomic DNA (data not shown).

Because the level of PKA activity is determined by the ratio of catalytic and regulatory subunits, overexpression of the catalytic subunit results in constitutive activation of the enzyme without a requirement for cAMP. Assays for PKA activity in mouse heart extracts confirmed that PKA activity was elevated 2.4-fold (line-33) and 8-fold (line-2) in the 2 transgenic lines (Figure 1). A third founder harboring the αMHC-PKA transgene died with a dilated heart at 17 weeks.

Cardiomyocyte Hypertrophy in αMHC-PKA-Transgenic Mice
Hearts from younger (4 to 8 weeks old) PKA-transgenic mice appeared normal. PKA-transgenics 10 weeks and older

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showed a slight increase in heart-to-body-weight ratios (Figure 1B). Histological analysis showed that the ventricular walls of the PKA transgenic mice were only moderately thicker at 10 weeks of age (Figure 2A). However, individual cardiomyocytes from PKA-transgenic mice were significantly enlarged (Figure 2A). The average cross-sectional area of cardiomyocytes from papillary and peripheral regions of the ventricles was $3.5 \pm 0.9 \times 10^{-2} \text{ m}^2$ in PKA-transgenics compared with $2.5 \pm 0.6 \times 10^{-2} \text{ m}^2$ ($P<0.01$) in wild-type mice. There was also an increase in cardiomyocyte nuclear dimensions in PKA-transgenic animals. The average cross-sectional area of cardiomyocyte nuclei from PKA-transgenic mice was $2.5 \pm 0.6 \times 10^{-2} \text{ m}^2$ compared with $1.5 \pm 0.7 \times 10^{-2} \text{ m}^2$ from wild-type siblings ($P<0.005$).

PKA overexpression resulted in elevated expression of the hypertrophic marker genes ANF and $\beta$-MHC. Conversely, transcripts for SERCA2, PLB, and $\alpha$-MHC were downregulated in hearts from PKA-transgenic mice, as seen in human heart failure28 (Figure 2B).

**αMHC-PKA-Transgenic Mice Develop Dilated Cardiomyopathy**

By 13 weeks of age, transgenic animals typically developed enlarged hearts (Figure 3A). Histological sections showed that the PKA-induced enlargement was a result of cardiac dilation (Figure 3B). The dilated hearts showed mild fibrosis, which contrasts with the extensive amounts of fibrotic scar tissue that result from continual $\beta$-adrenergic stimulation.29 PKA-transgenic mice also displayed symptoms indicative of congestive heart failure: the atria of transgenic mice frequently contained organized thrombi (Figure 3C, arrows) and, in particularly severe cases, transgenic animals also developed edema (Figure 3D). In accordance with the pathology, PKA-transgenic animals displayed higher mortality (Figure 4). The increased frequency of death correlated with the amount of PKA activity: the activity of transgenic line-2 was greater than that of transgenic line-33 (Figure 4).

**Impaired Contractility in αMHC-PKA-Transgenic Mice**

In order to examine the effect of PKA overexpression before the onset of dilated cardiomyopathy, in vivo cardiac morphology and performance were analyzed by echocardiography at 8 weeks of age. PKA-transgenic animals displayed increased systolic dimensions ($P<0.04$) and a tendency toward in-
creased diastolic diameters of the left-ventricular chamber (Table). Fractional shortening was significantly reduced in PKA-transgenic animals, indicating contractile dysfunction (27.0% versus 42.5%, P<0.005) (Table).

In contrast to regular sinus rhythm in wild-type siblings (Figure 5A), ECG-analysis during echocardiography revealed atrial fibrillation in 5 of 8 transgenic animals (Figure 5B). Transgenic animals frequently exhibited premature ventricular beats, suggesting a susceptibility to ventricular arrhythmias. In one severe case, spontaneous pauses in contraction lasting longer than 5 seconds were observed (Figure 5C), which likely were the consequence of intermittent higher degree sinoatrial block. PKA-transgenic animals also displayed a statistically significant decrease in heart rate (Table).

M-mode echocardiography showed that 8-week-old PKA transgenic animals did not suffer from gross hypertrophy of the cardiac wall. The thicknesses of the septal and posterior walls of the hearts, as determined by echocardiography, were comparable in wild-type and transgenic animals during diastole and systole (Table).

Hyperphosphorylation of the RyR2 and PLB in PKA-Transgenic Mice

Because PKA phosphorylation of RyR2 and PLB can alter cardiac physiology, we compared the phosphorylation status of these molecules in PKA-transgenic and wild-type mice. To determine the endogenous phosphorylation status of the RyR2, we performed back-phosphorylation assays. As shown in Figure 7, RyR2 from wild-type hearts was efficiently phosphorylated in vitro. In contrast, less 32P incorporation was detected in the RyR2 from hearts of PKA-transgenics. The level of 32P incorporation onto RyR2 in vitro is inversely proportional to the extent of phosphorylation of the proteins in vivo. Comparison of the 32P incorporation on RyR2 indicates a 3.5-fold increase in PKA phosphorylation of RyR2 from the PKA-transgenic heart lysates compared with wild-type controls in vivo (Figures 6A and 6B, P<0.01).

PLB is phosphorylated by PKA on serine-16, a site that is specific for PKA activity. Immunoblots using an antibody specific for PKA-phosphorylated PLB showed that phosphorylation of serine-16 was increased 4-fold in the hearts of PKA transgenic mice (line-2). Measurements From M-Mode Echocardiography

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<th>PKA Transgenic Mice</th>
<th>Wild-Type Mice</th>
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<tr>
<td>Fractional shortening</td>
<td>27.0±5.4%*</td>
<td>42.5±2.3%</td>
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<tr>
<td>Heart rate, bpm</td>
<td>412±38†</td>
<td>484±28</td>
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Assessment of ventricular function of wild-type and PKA-transgenic mice (line-2). Measurements are from interventricular septum (IVS), chamber (LVED), and posterior wall (LVPW) during distole and systole, respectively. *P<0.005, †P<0.002, ‡P<0.04.
PKA-transgenic mice compared with wild-type littermate controls (Figure 6C, n=3, P<0.01). As shown to occur in human heart failure, dissociation of FKBP12.6 from the channel results in increased P_o for the RyR2 channels. Similarly, the P_o of RyR2 in PKA-transgenic mouse hearts was 10-fold greater than that in wild-type mouse hearts (Figure 7B, P_o=0.05±0.003 to P_o=0.004±0.001, n=6, P<0.01).

These results show that the PKA sites in the RyR2 and PLB are hyperphosphorylated in PKA-transgenic mice and are substoichiometrically phosphorylated in the hearts of wild-type animals. Furthermore, these events are associated with increased RyR2 activity, which suggests that the dysfunctional physiology suffered by PKA-transgenic mice results from reduced cardiac performance due to altered Ca^{2+} fluxes.

**Discussion**

Heart failure is associated with diminished βAR responsiveness, loss of cardiac contractility, and abnormalities in Ca^{2+} handling. β-Adrenergic stimulation modulates Ca^{2+} fluxes and Ca^{2+} responsiveness of the sarcomere, which links β-adrenergic signaling to contractility. Whereas short-term activity of the βAR pathway increases inotropy/chronotropy, chronic βAR activation is maladaptive. Transgenic mice that overexpress βARs or Gs in the heart ultimately develop dilated cardiomyopathy. Unless active at very high levels, β2-AR signaling results in a prolonged increase in inotropy and chronotropy without cardiomyocyte toxicity. Active βAR or Gs increases basal adenyl cyclase activity, but extended transgenic overexpression of adenyl cyclases does not reproduce any pathology. Because of RyR2 and FKBP12.6, we performed coimmunoprecipitation experiments with RyR2. These experiments showed a 60% decrease in the amount of FKBP12.6 in the RyR2 macromolecular complex in PKA-transgenic hearts (Figure 7A, n=3, P<0.01). In order to determine whether hyperphosphorylation of the RyR2 stoichiometrically altered the interaction between PKA-transgenic mice compared with wild-type littermate controls (Figure 6C, n=3, P<0.01).

The open probability (P_o) of RyR2 is decreased by the interaction of the RyR2 with FKBP12.6, a cis-trans peptidylprolyl isomerase that is expressed in cardiac muscle. In order to determine whether hyperphosphorylation of the RyR2 stoichiometrically altered the interaction between RyR2 and FKBP12.6, we performed coimmunoprecipitation experiments with RyR2. These experiments showed a 60% decrease in the amount of FKBP12.6 in the RyR2 macromolecular complex in PKA-transgenic hearts (Figure 7A, n=3, P<0.01). As shown to occur in human heart failure, dissociation of FKBP12.6 from the channel results in increased P_o for the RyR2 channels. Similarly, the P_o of RyR2 in PKA-transgenic mouse hearts was 10-fold greater than that in wild-type mouse hearts (Figure 7B, P_o=0.05±0.003 to P_o=0.004±0.001, n=6, P<0.01).

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**Figure 5.** Detection of cardiac arrhythmias in PKA-transgenics. Panels depict M-mode views and simultaneous electrocardiograms of a wild-type (A) and 2 PKA-transgenic line-2 mice at 8 weeks of age (B and C). Compared with the regular sinus rhythm of wild-type animals (A), the electrocardiograms of PKA-transgenic mice frequently exhibited atrial fibrillation (B), and in one case prolonged pauses in cardiac contractions (C). Five wild-type and 8 transgenic mice were analyzed.

**Figure 6.** PKA hyperphosphorylation of RyR2 and PLB. A, RyR2 was immunoprecipitated from 4-week-old PKA-transgenic (line-2) and wild-type mouse hearts and back-phosphorylated with PKA (upper panel). The PKA inhibitor, PKI was used to demonstrate specificity of PKA phosphorylation. Immunoblot (lower panel) shows equal amounts of immunoprecipitated RyR2. B, PKA phosphorylation of RyR2 was quantified using densitometry and normalized for the amount of RyR2 immunoprecipitated in each sample, respectively, and expressed as the inverse of the back-phosphorylation. The number of mice analyzed of each genotype were as follows: WT, n=5; line-33, n=3; line-2, n=4. C, PLB phosphorylation was assessed using a phosphoepitope specific antibody that recognizes the phosphorylated serine-16 on PLB. A representative immunoblot (inset, upper panel) and the bar graph (lower panel) shows the quantification of results from 2 separate experiments from three transgenic (line-2) and 3 wild-type hearts.
these results and the fact that downregulation of the βAR is coupled with heart failure, it is unclear which of the effects of this signaling system are mediated by PKA activation and which reflect alterations in βAR-adenylyl cyclase coupling or other early events associated with βAR occupancy.8,43 Our results demonstrate that prolonged activation of PKA in the heart results in dilated cardiomyopathy, arrhythmias, and sudden death, which accompany hyperphosphorylation of RyR2 and PLB.

**Hyperphosphorylation of RyR2 and PLB in Response to PKA**

Heart failure is associated with alterations in excitation-contraction coupling, which are dependent on release and uptake of Ca²⁺ from and into the SR.32 The RyR2 mediates Ca²⁺ release from the SR. Entry of Ca²⁺ into cardiomyocytes via the voltage-gated L-type Ca²⁺ channel triggers activation of RyR2 during each cardiac action potential: an event known as Ca²⁺-induced Ca²⁺ release.8 RyR2 is localized to the SR and is the primary regulator of the Ca²⁺ signal that induces contraction. RyR2 is part of a large macromolecular complex that includes PKA, the protein phosphatases PP1 and PP2A, and the FK-506–binding protein FKBP12.6.8 PKA phosphorylates serine 2809 on the cytoplasmic surface of the RyR2, which results in dissociation of FKBP12.6 from RyR2. FKBP12.6, is required for normal gating of the Ca²⁺ channel,30 so the consequence of dissociation is an increase in Ca²⁺ sensitivity for channel activation.8

Recent studies have shown that the PKA site on the RyR2 is also hyperphosphorylated in failing human hearts and that treatment with a left ventricular assist device, which restores cardiac function, is accompanied by a reduction in RyR2 phosphorylation by PKA to normal levels.8 This association of RyR2 hyperphosphorylation with heart failure suggests that negative regulation of the RyR2 is an effector of heart failure. Chronic RyR2 activation and FKBP12.6 depletion can lead to a leftward shift of Ca²⁺-dependent activation and a reduction in coupled gating of the RyR2.8 This may lead to a diastolic leak of SR Ca²⁺, contributing to diastolic dysfunction as is frequently observed in heart failure patients. The demonstration of PKA-hyperphosphorylation of RyR2, FKBP12.6 depletion, and increased P₂ of the channels from the PKA-transgenic hearts suggests that altered regulation of SR Ca²⁺ release may play a role in heart failure and arrhythmias observed in these mice. The PKA-hyperphosphorylated channels would be predicted to be more active at low cytosolic [Ca²⁺] as previously reported for RyR2 channels from failing human and canine failing hearts.8 This increased activity is due to increased sensitivity to Ca²⁺-induced Ca²⁺ release.8 Furthermore, diastolic Ca²⁺ release could provide a stimulus to remove the excess Ca²⁺ via the Na⁺-Ca²⁺ exchanger in the sarcolemma. This would eventually lead to a depletion of SR Ca²⁺, making it unavailable for subsequent release during systole and ultimately resulting in a deterioration of systolic performance. Moreover, activation of reverse mode Na⁺-Ca²⁺ exchange can result in inward depolarizing currents leading to delayed after-depolarizations that could trigger arrhythmias.

Uptake of Ca²⁺ into the SR is mediated by SERCA2a, which is negatively regulated by PLB. Phosphorylation of PLB relaxes its inhibitory effects on SERCA2a, which results in an increased removal of Ca²⁺ from the sarcoplasm by SERCA2a after β-adrenergic stimulation.43–45 Loss of PLB has been reported to ameliorate the heart failure phenotype in mice lacking the muscle LIM-domain protein, MLP.46 This suggests that inactivation of PLB via PKA phosphorylation is beneficial. Yet, despite PLB hyperphosphorylation, PKA-transgenic animals developed dilated cardiomyopathy. We propose that the chronic activity of RyR2 (which would cause a leak of SR Ca²⁺) predominates over any benefit conveyed by inactivation of PLB. Sustained depletion of Ca²⁺ from the SR and its removal from the cardiomyocyte may gradually render any positive effect of increased SERCA2a activity inconsequential.

**Signals for Altered Physiology and Dilated Cardiomyopathy**

The dilated cardiomyopathy, depressed cardiac contractility and increased frequency of arrhythmias of PKA-transgenic mice is reminiscent of the pathophysiology of β₁-AR– and Gsα-overexpressing mice. Interestingly, chronic PKA overexpression in the heart results in reduced heart rate, whereas chronic Gsα-overexpression results in increased heart rate.55 This could suggest the activation of different effectors even though both Gsα and PKA are involved in βAR signal transduction.

Chronic βAR activation stimulates multiple signaling events and ultimately leads to dilated cardiomyopathy.33 However, experiments in which adenylyl cyclases are over-
expressed in the heart have indicated that activation of certain downstream components of β-adrenergic signaling does not result in failure. Adenylyl cyclase overexpression results in improved performance without the pathological consequences of continuous βAR stimulation.21,22 Yet, one study found increased PKA activity in the cardiomyopathic hamster UM-X7, which implicates the involvement of PKA activity in the generation of cardiomyopathy.47 Although increased expression of adenylyl cyclases may be beneficial, our data indicate that increased activity of PKA in the heart is not, so understanding the difference between adenylyl cyclase signaling and PKA signaling may be crucial for elucidating the difference between compensatory and decompensatory cardiac physiology. One possibility is that adenylyl cyclases may interact with other factors that buffer the deleterious effects of sustained, high levels of PKA enzymatic activity. In this respect, PKA phosphorylates adenylyl cyclase isoforms that are present in cardiomyocytes, which reduces adenylyl cyclase activity48,49 and, thereby, may attenuate any beneficial effects conveyed by adenylyl cyclase signaling.

There is growing evidence that β-adrenergic signaling is spatially regulated. βARs appear to be localized to distinct regions of the plasma membrane.50 Further intracellular compartmentalization of β-adrenergic signaling is achieved through A-kinase anchoring proteins (AKAPs) that confine PKA activity.51 PKA is targeted to the RyR2 via mAKAP.8 Thus, although global PKA activity appears to be unchanged in myocardial tissue of patients suffering from dilated cardiomyopathy,52,53 site- or substrate-specific signaling by PKA may contribute to the progression of heart failure.

Our results indicate that PKA is a key mediator of the deleterious effects of chronic βAR signaling and that exacerbated PKA activity selectively results in dilated cardiomyopathy. This suggests that chronic phosphorylation of PKA substrates including RyR2 is associated with dilation and failure. Furthermore, the diminished contractility, arrhythmias, histological changes, and susceptibility to sudden death in PKA-transgenic mice resemble the clinical picture of human dilated cardiomyopathy. It is likely that the sustained pleotropic effect of deregulated Ca2+ fluxes and deregulated Ca2+ utilization due to PKA activity causes the progression to heart failure. Taken together, PKA-transgenic mice represent a model for further exploring the downstream consequences of chronic β-adrenergic signaling and dilated cardiomyopathy.

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References


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