In Vivo Inhibition of Elevated Myocardial β-Adrenergic Receptor Kinase Activity in Hybrid Transgenic Mice Restores Normal β-Adrenergic Signaling and Function

Shahab A. Akhter, Andrea D. Eckhart, Howard A. Rockman, Kyle Shotwell, Robert J. Lefkowitz and Walter J. Koch

Circulation. 1999;100:648-653
doi: 10.1161/01.CIR.100.6.648
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539

In Vivo Inhibition of Elevated Myocardial β-Adrenergic Receptor Kinase Activity in Hybrid Transgenic Mice Restores Normal β-Adrenergic Signaling and Function

Shahab A. Akhter, MD; Andrea D. Eckhart, PhD; Howard A. Rockman, MD; Kyle Shotwell, BA; Robert J. Lefkowitz, MD; Walter J. Koch, PhD

Background—The clinical syndrome of heart failure (HF) is characterized by an impaired cardiac β-adrenergic receptor (βAR) system, which is critical in the regulation of myocardial function. Expression of the βAR kinase (βARK1), which phosphorylates and uncouples βARs, is elevated in human HF; this likely contributes to the abnormal βAR responsiveness that occurs with β-agonist administration. We previously showed that transgenic mice with increased myocardial βARK1 expression had impaired cardiac function in vivo and that inhibiting endogenous βARK1 activity in the heart led to enhanced myocardial function.

Methods and Results—We created hybrid transgenic mice with cardiac-specific concomitant overexpression of both βARK1 and an inhibitor of βARK1 activity to study the feasibility and functional consequences of the inhibition of elevated βARK1 activity similar to that present in human HF. Transgenic mice with myocardial overexpression of βARK1 (3 to 5-fold) have a blunted in vivo contractile response to isoproterenol when compared with non-transgenic control mice. In the hybrid transgenic mice, although myocardial βARK1 levels remained elevated due to transgene expression, in vitro βARK1 activity returned to control levels and the percentage of βARs in the high-affinity state increased to normal wild-type levels. Furthermore, the in vivo left ventricular contractile response to βAR stimulation was restored to normal in the hybrid double-transgenic mice.

Conclusions—Novel hybrid transgenic mice can be created with concomitant cardiac-specific overexpression of 2 independent transgenes with opposing actions. Elevated myocardial βARK1 in transgenic mouse hearts (to levels seen in human HF) can be inhibited in vivo by a peptide that can prevent agonist-stimulated desensitization of cardiac βARs. This may represent a novel strategy to improve myocardial function in the setting of compromised heart function. **(*Circulation*. 1999;100:648-653.)**

Key Words: receptors, adrenergic, beta ■ G proteins ■ protein kinases ■ desensitization ■ heart failure ■ mice, transgenic ■ myocardium

The myocardial β-adrenergic receptor (βAR) signaling pathway plays a critical role in the regulation of cardiac contractility. βARs (β_1 and β_2 subtypes) are the primary myocardial targets of the sympathetic neurotransmitter norepinephrine and the adrenal hormone epinephrine. Activation of βARs in the heart by these 2 catecholamines leads to positive chronotropic and inotropic action via stimulation of adenylyl cyclase and subsequent increases in cAMP and intracellular Ca^{2+} release.[1] Continued exposure of βARs to agonists results in a rapid decrease in responsiveness, which is known as desensitization.[2] Agonist-dependent desensitization can be initiated by the phosphorylation of activated receptors by members of the family of G protein–coupled receptor kinases (GRK).[2] The βAR kinase-1 (βARK1; GRK2) is a GRK that specifically phosphorylates activated β_1- and β_2-ARs, leading to desensitization in vitro and in vivo.[2–4]

See p 579

Heart failure (HF) in humans has been characterized by specific alterations in the βAR signaling system. These include selective down-regulation of β_1ARs by ≈50% and desensitization of the remaining βARs, which leads to the blunting of further agonist-mediated stimulation.[1,5,6] The enhanced desensitization of myocardial βARs is likely due, in part, to the elevated expression of βARK1 (≈3-fold) present in human HF.[7,8] It is generally thought that these changes in the βAR system in HF are triggered by increased sympathetic

Received January 29, 1999; revision received April 7, 1999; accepted April 9, 1999.

From the Departments of Surgery (S.A.A., A.D.E., K.S., W.J.K.) and Medicine and Biochemistry and the Howard Hughes Medical Institute (R.J.L.), Duke University Medical Center, Durham, NC, and the Department of Medicine/Cardiology, The University of North Carolina at Chapel Hill, Chapel Hill, NC (H.A.R.).

Drs Akhter and Eckhart contributed equally to this work.

Correspondence to Walter J. Koch, PhD, Laboratory of Molecular Cardiovascular Biology, Box 2606, MSRB Room 471, Duke University Medical Center, Durham, NC 27710. E-mail koch0002@mc.duke.edu

Circulation **is available at http://www.circulationaha.org**

stimulation of the heart in this disease state.[9] The dysfunctional βAR signaling, including increased βARK1 expression and activity, is a contributing factor to the impaired myocardial contractility seen in HF.

Our laboratory previously reported that transgenic mice with cardiac-specific overexpression of βARK1 to the levels seen in human HF (3-fold) have significantly depressed agonist-stimulated left ventricular (LV) function in vivo.[4] This study demonstrated the in vivo action of βARK1 on β_1ARs and the importance of this GRK in the regulation of myocardial function. In the same study, transgenic mice with cardiac-specific expression of the carboxyl-terminus region of βARK1 (βARKct), which acts as a functional inhibitor of βARK1 activity, showed enhanced in vivo basal and agonist-stimulated LV function.[4] βARKct contains the binding domain responsible for the specific binding of βARK1 to the dissociated βγ subunits of heterotrimeric G proteins ($G_{\beta\gamma}$), a process required for the activation of this GRK.[4,10,11] Therefore, inhibiting normal endogenous βARK1 activity can lead to increased cardiac function in transgenic mice, presumably through the attenuation of myocardial βAR desensitization, although other receptor targets for βARK1 cannot be ruled out.

In this study, we sought to determine if concomitant myocardial expression of βARKct could inhibit increased levels of βARK1 activity (in the elevated range present in human HF) in transgenic mouse hearts. To do this, novel hybrid transgenic mice were created with cardiac-specific overexpression of both βARK1 and the βARKct peptide; this effectively turned the heart into a novel "in vivo reaction vessel" that we used to study the interaction between the 2 transgenes. We first examined the functional activity of the βARK inhibitor peptide at a biochemical level and ultimately studied LV contractility in the intact animal. Our results indicated that the inhibition of elevated myocardial βARK1 activity by the βARKct peptide occurs in vivo and that this leads to the reversal of abnormal βAR responsiveness that accompanies enhanced βARK1 expression. Thus, βARK1 is a potential therapeutic target for enhancing myocardial contractility in conditions in which cardiac function is compromised, such as HF.

Methods

Experimental Animals

Transgenic mice with cardiac-targeted overexpression of βARK1 were mated with transgenic mice with cardiac-targeted overexpression of the βARKct peptide to generate mice that overexpressed both βARK1 and βARKct in their hearts.[4] In these 2 individual lines of transgenic mice, the βARK1 and βARKct transgenes were targeted to the myocardium by using the murine α-myosin heavy chain gene promoter.[4] In the βARK1 mice, myocardial βARK1 protein and activity was ≈3-fold over endogenous βARK1 levels; the molar ratio of the βARKct peptide to endogenous βARK1 in the myocardium of βARKct animals, as determined by protein immunoblotting, was approximately 5:1.[4] Using 1 parent from each of these 2 individual lines, offspring were generated; the genotype of these hybrid mice was determined by polymerase chain reaction on genomic DNA isolated from tail biopsies.[4] Hybrid transgenic mice from these matings containing both transgenes were used in this study, as were littermates that were positive for individual transgenes. Importantly, as shown in Figure 1, expression of both the βARK1 and βARKct transgenes in the hybrid mice did not differ from the levels expressed in the individual lines. The animals in this study were handled according to the approved protocols and animal welfare regulations at Duke University Medical Center and the University of North Carolina at Chapel Hill.

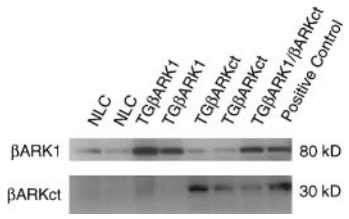

Figure 1. Myocardial expression of βARK1 and βARKct. Protein immunoblots were performed on soluble cytosolic myocardial extracts to detect βARK1 and βARKct peptide expression. Representative autoradiograms are shown for NLC, βARK1 transgene (TGβARK1), βARKct peptide (TGβARKct), and hybrid βARK1/βARKct peptide (TGβARK1/TGβARKct) gene-targeted mice. Molecular sizes are indicated in kilodaltons (kD).

Hemodynamic Evaluation in Intact Anesthetized Mice

Cardiac catheterization was performed as described previously.[4,12] Mice were anesthetized with a mixture of ketamine (100 mg/kg IP) and xylazine (2.5 mg/kg IP) and, after endotracheal intubation, were connected to a rodent ventilator. After bilateral vagotomy, the chest was opened, and a 1.4 French (0.46 mm) high-fidelity micromanometer catheter (Millar Instruments) was inserted into the left atrium, advanced across the mitral valve, and secured in the left ventricle. The external jugular vein was cannulated to administer isoproterenol (ISO). Hemodynamic measurements were recorded at baseline and 45 to 60 s after the injection of an incremental dose of ISO.[4,12]

Protein Immunoblotting

Transgenic mouse hearts were homogenized in ice-cold buffer (25 mmol/L Tris-HCl [pH 7.5], 5 mmol/L EDTA, 5 mmol/L EGTA, 10 μg/mL leupeptin, 20 μg/mL aprotinin, and 1 mmol/L phenylmethylsulfonyl fluoride). Nuclei and tissue were separated by centrifugation at 800*g* for 15 minutes. The crude supernatant was then centrifuged at 20 000*g* for 15 minutes. Protein concentrations were determined on the supernatant (cytosolic fraction). Sedimented proteins (membrane fraction) were resuspended in 50 mmol/L HEPES (pH 7.3) and 5 mmol/L $MgCl_2$.[4] The immunodetection of myocardial levels of βARK1 was performed on an equal amount of protein from cytosolic extracts from non-transgenic littermate controls (NLC) and from transgenic mice after immunoprecipitation by using a monoclonal βARK1/2 antibody, as described previously.[12] The βARK1 protein (≈80kDa) was visualized with the monoclonal antibody raised against an epitope within the carboxyl terminus of βARK1 and chemiluminescent detection of anti-mouse IgG conjugated with horseradish peroxidase (Renaissance, Amersham). βARKct was identified with rabbit polyclonal antiserum to the carboxyl terminus of βARK1[4,10] and by chemiluminescent detection of anti-rabbit IgG.

GRK Activity by Rhodopsin Phosphorylation

The supernatants of the myocardial extracts that contained the soluble kinases were used to determine GRK activity. Extracts (100 to 150 μg of protein) were incubated with rhodopsin-enriched rod outer-segment membranes in reaction buffer containing the following (in mmol/L): $MgCl_2$ 10, Tris-Cl 20, EDTA 2, EGTA 5, and ATP 0.1 (containing [γ-^{32}P]ATP), as previously described.[4] Reactions were carried out in the absence and presence of purified $G_{\beta\gamma}$ (20 pmol) to maximally activate βARK1.[10,11] After incubating in white light for 15 minutes at room temperature, reactions were quenched with ice-cold lysis buffer and centrifuged for 15 minutes at 13 000*g*. Sedimented proteins were resuspended in 25 μL of protein–gel-loading dye and treated with 12% SDS-PAGE. Phosphorylated rhodopsin was visualized by autoradiography of dried polyacryl-

amide gels and quantified using a Molecular Dynamics PhosphorImager.

Radioligand Binding

Total βAR density was determined by incubating 25 μg of cardiac sarcolemmal membranes with a saturating concentration of [^{125}I]cyanopindolol and 20 μmol/L alprenolol to define nonspecific binding.[4] Competition binding-isotherms in sarcolemmal membranes were done in triplicate with 80 pmol/L [^{125}I]cyanopindolol and 22 varying concentrations of ISO (10^{-14} to 10^{-4} mol/L) in 250 μL of binding buffer (50 mmol/L HEPES [pH 7.3], 5 mmol/L $MgCl_2$, and 0.1 mmol/L ascorbic acid].[4] Assays were done at 37°C for 1 hour and then filtered over GF/C glass fiber filters (Whatman) that were washed twice and counted in a γ counter. Data were analyzed by nonlinear least-square curve fit (GraphPad Prism).

Adenylyl Cyclase Activity

Cardiac sarcolemmal membranes (20 μg of protein) were incubated for 15 minutes at 37°C with [α-^{32}P]ATP under basal conditions, 10^{-4} mol/L ISO to stimulate βAR, or 10 mmol/L NaF to maximally activate adenylyl cyclase. cAMP production was quantified by standard methods described previously.[4]

Statistical Analysis

Data are expressed as mean±SEM. Unpaired Student's *t* tests and 1-way ANOVA were performed for statistical comparisons except as described otherwise. For all tests, $P<0.05$ was considered significant.

Results

Myocardial Expression of βARK1 and βARKct

As previously shown, transgenic mice with cardiac-specific expression of βARK1 had a ≈3-fold increase in myocardial βARK1 expression, as assessed by protein immunoblotting, compared with NLC mice.[4] In addition, cardiac-specific expression of the ≈30kDa βARKct peptide was documented by protein immunoblot in this line of transgenic mice.[4] In the hybrid transgenic mice, myocardial expression of both βARK1 and the βARKct peptide was identical to that seen in the individual breeder transgenic lines with cardiac-specific expression of either protein alone (Figure 1). Thus, expression of either of these transgenes, both driven by the same α-myosin heavy chain promoter, does not alter expression of the other transgene, and the hybrid transgenic mice generated are an intact concomitant model of the 2 independent transgenic lines.

Myocardial βARK1 Activity

To assess the in vitro functional effect of the βARKct peptide on elevated myocardial βARK1 activity, we assayed heart extracts for phosphorylation of the G protein-coupled receptor rhodopsin in the absence and presence of exogenous purified $G_{\beta\gamma}$. We previously showed that $G_{\beta\gamma}$ maximally activates βARK1 by a membrane-targeting event and that βARKct peptide action should result from inhibiting $G_{\beta\gamma}$ activation of βARK1.[4,10,11] In addition, our previous studies demonstrated that myocardial cytosolic GRK activity is primarily due to βARK1.[12] In this study, adding $G_{\beta\gamma}$ maximally stimulated βARK1 activity, especially in transgenic extracts overexpressing βARK1 (Figure 2). As shown in Figure 2, in transgenic mice expressing βARKct alone or with βARK1 overexpression (βARK1/βARKct), activation of βARK1 activity by exogenous $G_{\beta\gamma}$ was significantly inhibited. These data indicate that even in the presence of elevated βARK1 protein levels, βARK1 activity can be attenuated in vitro by the presence of βARKct.

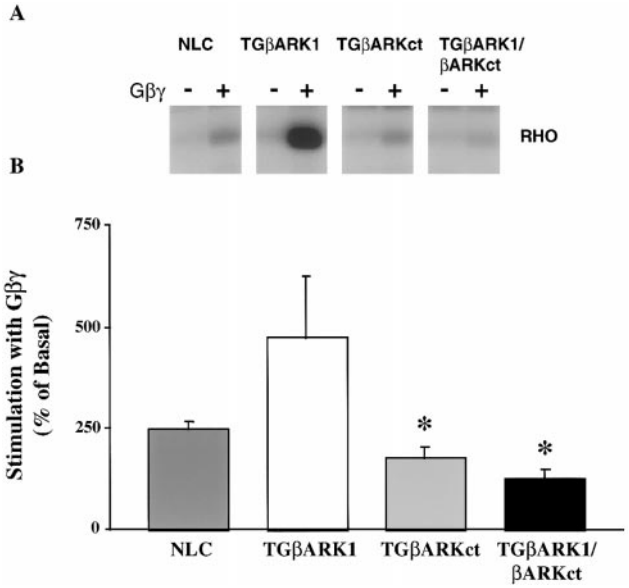

Figure 2. Assessment of in vitro myocardial βARK activity. Soluble cytosolic myocardial extracts were prepared and βARK1 activity was assessed using rhodopsin (RHO) phosphorylation assay in absence (−) and presence (+) of purified $G_{\beta\gamma}$ to maximally activate βARK1 (see Methods). A, Representative autoradiogram. B, Histogram for mean±SEM for NLC (n=3), transgenic (TG) βARK1 (n=3), TGβARKct (n=4), and TGβARK1/βARKct (n=3) mice. *$P<0.05$ versus TGβARK1.

Myocardial βAR Functional Coupling

To examine the biochemical effects of the 2 transgenes on the myocardial βAR system, we assessed receptor-effector coupling in sarcolemmal membranes from the hearts of NLC and transgenic mice. As shown in the Table, no difference existed in total βAR density between NLC cardiac membranes and those from the 3 transgenic lines, including the hybrid mice. Previously, we showed that β_1AR in the hearts of βARK1 mice are less able to form the high-affinity state of the receptor, which is coupled to G proteins.[4] We confirmed this finding in the present study and, importantly, showed that concomitant overexpression of βARK1 and the βARKct peptide in βARK1/βARKct mice restores this high-affinity population back to control (NLC) values (Table).

We assessed functional βAR coupling by studying the activity of adenylyl cyclase in myocardial sarcolemmal mem-

Myocardial βAR Binding Site Characteristics

Mouse Line	Total βAR Density, fmol/mg of Membrane Protein	% High Affinity ^{125}I-CYP Binding Sites
NLC	51.1±6.8	47.5±2.1
βARK1	56.3±5.2	27.1±2.3*†
βARKct	45.8±7.1	40.0±6.0
βARK1/βARKct	52.8±4.6	43.5±4.5

Data are given as mean±SEM of n=3 to 8 individual cardiac membrane preparations in triplicate. ^{125}I-CYP indicates [^{125}I]cyanopindolol. *$P<0.01$ vs NLC; †$P<0.05$ vs βARK1/βARKct.

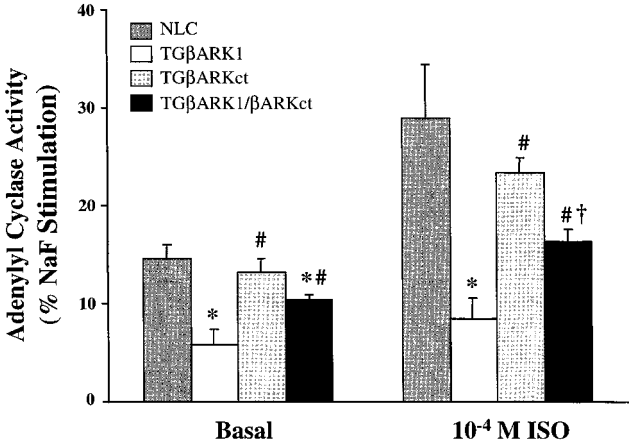

Figure 3. Myocardial βAR functional coupling. The mean±SEM is shown for levels (basal and those achieved by 10^{-4} mol/L of ISO) of adenylyl cyclase activity in myocardial sarcolemmal membranes normalized to the percentage of activation achieved with 10 mmol/L NaF. NaF activation did not differ between different transgenic groups and NLC membranes (122±9 pmol/mg of cAMP/min in NLC; 141±12 pmol/mg of cAMP/min in transgenic [TG] βARK1; 163±11 pmol/mg of cAMP/min in TGβARKct, and 148±13 pmol/mg of cAMP/min in the hybrid TGβARK1/βARKct; *P*=not significant, ANOVA). n=5 for each group. **P*<0.05 versus NLC; #*P*<0.05 versus TGβARK1; †*P*<0.05 versus TGβARKct.

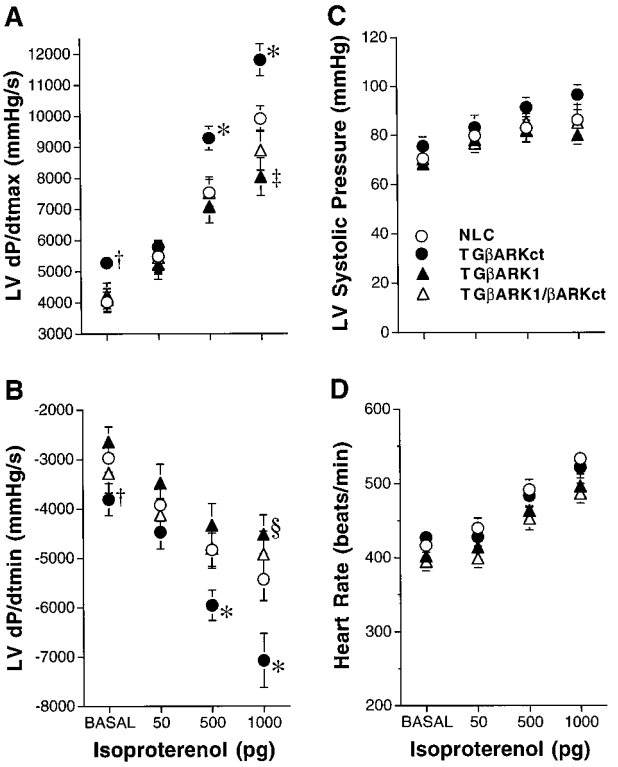

Figure 4. In vivo assessment of LV contractile function in response to β-agonist stimulation. Cardiac catheterization was performed in intact, anesthetized, open-chest mice using a 1.4 French high-fidelity micromanometer. Parameters measured were LV systolic and end-diastolic pressures, the maximal and minimal first derivative of LV pressure (LV dP/dt_{max}; dP/dt_{min}), and heart rate. Four parameters are shown at baseline and after progressive doses of ISO in NLC (○; n=17), transgenic (TG) βARK1 (▲; n=13), TGβARKct (●; n=12), and TGβARK1/βARKct (△; n=9) mice. A, LV dP/dt_{max}; B, LV dP/dt_{min}; C, LV systolic pressure; and D, heart rate. Data were analyzed with 4×4 repeated measures ANOVA. If appropriate, post hoc analysis was performed by Newman-Keuls test. **P*<0.005, †*P*<0.04 NLC versus TGβARKct; ‡*P*<0.0001, §*P*<0.03 NLC versus TGβARK1. A significant between-group main effect in response to ISO was found for LV dP/dt_{max} (*P*<0.01) and LV dP/dt_{min} (*P*<0.02). The pattern of change between groups was statistically different for LV dP/dt_{max} (*P*<0.0001) and LV dP/dt_{min} (*P*<0.01).

branes. Basal and ISO-stimulated cyclase values normalized to the percentage of activation achieved with NaF, which was not different between groups (Figure 3). In cardiac membranes from βARK1 animals, basal cyclase activity was significantly depressed (Figure 3). As in our prior study,[4] no difference existed in basal activity in the βARKct hearts compared with NLC hearts (Figure 3) because βARKct is a cytosolic peptide and is not present in the membrane fraction. The hybrid transgenic mice (βARK1/βARKct) had basal cyclase activity, which although still depressed compared with NLC mice, was significantly higher than that found in βARK1 cardiac membranes (Figure 3). ISO-stimulated cyclase activity was also significantly depressed in βARK1 versus NLC membranes, whereas the βARKct membranes had similar ISO-stimulated adenylyl cyclase activity as NLC membranes (Figure 3). In the hybrid βARK1/βARKct mouse hearts, ISO-stimulated cyclase activity in myocardial sarcolemmal membranes was significantly increased over the βARK1 group but lower than NLC mice (Figure 3). These data indicate that, in vitro, increased myocardial βARK1 activity impairs functional coupling of βARs, both basally and in response to ISO, and this impairment can be attenuated by inhibiting membrane targeting of βARK1.

In Vivo Cardiac Physiology

To investigate the potential effects of the inhibition of elevated βARK1 activity in the hybrid transgenic mice on in vivo myocardial function, we used cardiac catheterization in anesthetized intact mice. As shown in Figure 4, βARK1 overexpression led to a significantly blunted inotropic response to the highest dose of ISO as compared with responses in NLC mice. In contrast, βARKct mice had enhanced βAR responsiveness, consistent with the peptide's effect on reducing βAR desensitization. Importantly, in the hybrid βARK1/βARKct mice, the response of the maximum first derivative of LV pressure (LV dP/dt_{max}) to ISO was restored to the control values found in NLC mice (Figure 4). Neither heart rate nor LV systolic pressure were different between groups. Thus, these data suggest that overexpression of the βARKct peptide results in inhibition of the augmented βAR desensitization that is induced by elevated βARK1 activity, which leads to the normalization of in vivo cardiac contractility.

Discussion

The results of this study demonstrate the effectiveness of the βARKct peptide as an inhibitor of increased βARK1 expression and activity, both in vitro and in vivo. Using novel hybrid transgenic mice with myocardial-targeted concomitant overexpression of βARK1 and βARKct, we showed that the presence of a βARK inhibitor could reverse depressed βARK1-mediated βAR coupling, as determined by myocar-

dial βAR affinity states, adenylyl cyclase activity, and βAR responsiveness in vivo.

In this study, the hearts of these hybrid transgenic mice were, effectively, novel in vivo reaction vessels, which allowed us to study the physiological consequences of the direct action of 1 transgene on another. This is the first demonstration of 2 competing transgenes being expressed in the hearts of gene-targeted animals via this methodology. Importantly, expression levels of the transgene products (driven by the same α-myosin heavy chain promoter) in the hybrid mice were equal to their individual parental lines, demonstrating that there was no apparent promoter competition, which could be a problem in hybrid transgenic mice using an endogenously occurring promoter. The generation of such hybrid mice by this relatively simple cross-breeding strategy provided a powerful model for studying in vivo myocardial interactions between proteins and for dissecting individual phenotypes.

It is becoming increasingly more evident that βARK1 plays a critical role in myocardial function. As described above, alteration of myocardial βARK1 activity can have profound effects on in vivo cardiac performance. The importance of βARK1 in heart function is further supported by the recent findings that increased expression of βARK1 accompanies attenuated cardiac function in several cardiovascular diseases, including hypertension,[13] myocardial ischemia,[14] ventricular hypertrophy,[15] and HF.[7,8,16] It is not clear what triggers increased βARK1 in these conditions; however, an increased catecholamine level caused by enhanced sympathetic outflow is a likely candidate. In fact, we recently demonstrated that chronic activation of myocardial βARs led to increased βARK1 expression in the heart and enhanced myocardial GRK activity.[17] Elevated βARK1 in the failing heart contributes to physiological dysfunction as βARs become uncoupled from downstream effectors. Moreover, a typical feature of HF is diminished responsiveness to βAR stimulation.

As a further demonstration of the importance of βARK1 in the cardiovascular system, we previously showed that the complete disruption of the βARK1 gene in mice leads to a lethal phenotype characterized by cardiac malformations.[18] Heterozygous (±) βARK1-deleted mice have no developmental abnormalities and age normally and, interestingly, these mice were recently found to have a cardiac phenotype of enhanced contractility similar to transgenic mice with βARKct overexpression.[12] Mating the heterozygous βARK1 (±) knockout mice with the βARKct mice showed that mice with 50% less βARK1 expressed in their hearts and expression of βARKct had further significant enhancement of in vivo cardiac contractility.[12] Because the previous study could not delineate a definitive mechanism of βARKct action on βARK1 activity, we used the novel hybrid strategy reported here.

In the present study, we directly demonstrated that the βARKct peptide can act as an in vivo inhibitor of βARK1. Moreover, using transgenic mice showed that βARKct expression can inhibit enhanced myocardial βARK activity that is at the level seen in human disease. In vitro studies demonstrated that βARKct could inhibit enhanced βARK1 activity in these transgenic hearts by competing for, and inhibiting, $G_{\beta\gamma}$-mediated membrane translocation. $G_{\beta\gamma}$ binding to βARK1 and subsequent membrane targeting are required steps for βARK1 activity directed toward agonist-occupied receptors.[10,11] The myocardial deficits caused by βARK1 overexpression, including attenuated ISO-stimulated LV contractility in vivo, decreased adenylyl cyclase activity, and reduced functional coupling of βAR,[4] were overcome simply by concomitant overexpression of the βARKct peptide. This suggests that inhibiting βARK1 activity is sufficient to restore the integrity of myocardial function in vivo.

The critical finding in the present study, that βARKct can inhibit enhanced βARK1 activity in the heart, points to βARK1 being a potential target for inhibition in diseases such as HF where βARK1 is elevated. Interestingly, the level of βARK1 enhancement seen in our transgenic mice (≈3 to 5-fold) was similar to the increased expression observed in human HF.[7,8] Thus, the expression of βARKct could be useful in the targeted inhibition of myocardial βARK1. Recently, a genetic mouse model of HF was described[19]; this model made it possible to test βARKct action using hybrid transgenic mice. This murine model of dilated cardiomyopathy resulted from the ablation of the gene that encodes the muscle-specific LIM-domain containing protein (MLP) (−/−).[19] We mated cardiac overexpression of either the βARKct peptide or β_2AR into the MLP (−/−) background.[16] Like βARKct, transgenic mice overexpressing β_2ARs at >100-fold over endogenous levels had enhanced in vivo cardiac contractility.[20] Interestingly, overexpression of βARKct prevented the development of cardiomyopathy, whereas β_2AR overexpression further exacerbated murine HF. Importantly, MLP (−/−) mice exhibit a 2-fold increase in cytosolic βARK1 levels.[12] The extraordinary finding that βARKct prevents HF in this model, coupled with the present findings directly demonstrating that βARKct inhibits enhanced βARK activity in vivo, strongly supports the idea that βARK1 is an attractive, novel, therapeutic target. Thus, by using hybrid-generating technology, it will be possible to further exploit the usefulness of transgenic mouse models to study complex human diseases, such as hypertension and HF.

Further support for the determination that βARK1 inhibition results in improved outcomes in HF is the finding that, in mice, long-term treatment with carvedilol, a novel β-blocking drug that has been used successfully in the treatment of human HF, results in more efficient βAR coupling associated with a significant decrease in the expression of myocardial βARK1.[17] Thus, several lines of evidence in different models have demonstrated that regardless of how myocardial βARK1 activity is diminished, βAR signaling in the heart (and hence cardiac function) is enhanced. In addition to βARKct expression, heterozygous βARK1 knockout animals have enhanced cardiac contractility.[12] As noted above, carvedilol and other β-blockers can have a positive effect on the failing heart, which may, in part, be due to the lowering of βARK1 expression in the heart. It is important to note that inhibition of βARK1 activity in the heart and enhancement of endogenous βAR signaling does not seem to produce negative effects on the heart,[4,12,16] which is in contrast to the cardiomyopathy seen in transgenic mice with cardiac-specific

overexpression of β_1AR.[21] This difference in phenotype needs to be further investigated, but it suggests that these 2 mechanisms of increased receptor-effector coupling are intrinsically different. Thus, gene therapy approaches using the βARKct transgene[22] or the development of small-molecule inhibitors of βARK1 activity could, therefore, be novel therapeutic strategies for the treatment of HF or other cardiovascular diseases that are characterized by desensitized βARs and enhanced βARK1 expression and/or activity.

Acknowledgments

The authors thank Sandy Duncan and Lan Mao for their excellent technical assistance. R.J.L. is an Investigator of the Howard Hughes Medical Institute. This study was supported, in part, by National Service Research Award HL-09436 (S.A.A.) and grant HL-56687 (H.A.R.) from the National Institutes of Health and a Grant-in Aid from the North Carolina Affiliate of the American Heart Association (W.J.K.).

References

1. Brodde O-E. β-Adrenergic receptors in failing human myocardium. *Basic Res Cardiol.* 1993;91:35–40.
2. Inglese J, Freedman NJ, Koch WJ, Lefkowitz RJ. Structure and mechanism of the G protein-coupled receptor kinases. *J Biol Chem.* 1993;268:23735–23738.
3. Freedman NJ, Liggett SB, Drachman DE, Pei G, Caron MG, Lefkowitz RJ. Phosphorylation and desensitization of the human β-adrenergic receptor: involvement of G protein-coupled receptor kinases and cAMP-dependent protein kinase. *J Biol Chem.* 1995;270:17953–17961.
4. Koch WJ, Rockman HA, Samama P, Hamilton RA, Bond RA, Milano CA, Lefkowitz RJ. Cardiac function in mice overexpressing the β-adrenergic receptor kinase or an βARK inhibitor. *Science.* 1995;268: 1350–1353.
5. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Luni K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. *N Engl J Med.* 1982;307:205–211.
6. Feldman AM. Modulation of adrenergic receptors and G-transduction proteins in failing human ventricular myocardium. *Circulation.* 1993;87: IV27-IV34.
7. Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ. Expression of β-arrestins and β-adrenergic receptor kinases in the failing human heart. *Circulation.* 1993;87:454–463.
8. Ungerer M, Parruti G, Bohm M, Puzicha M, DeBlasi A, Erdmann E, Lohse MJ. Expression of β-arrestins and β-adrenergic receptor kinases in the failing human heart. *Circ Res.* 1994;74:206–213.
9. Leimbach WN Jr, Wallin BG, Victor RG, Aylward PE, Sundlof G, Mark AL. Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. *Circulation.* 1986;73: 913–919.
10. Koch WJ, Inglese J, Stone WC, Lefkowitz RJ. The binding site for the beta gamma subunits of heterotrimeric G proteins on the β-adrenergic receptor kinase. *J Biol Chem.* 1993;268:8256–8260.
11. Pitcher JA, Inglese J, Higgins JB, Arriza JL, Casey PJ, Kim C, Benovic JL, Kwatra MM, Caron MG, Lefkowitz RJ. Role of beta gamma subunits of G proteins in targeting the β-adrenergic receptor kinase to membrane-bound receptors. *Science.* 1992;257:1264–1267.
12. Rockman HA, Choi D-J, Akhter SA, Jaber M, Giros B, Lefkowitz RJ, Caron MG, Koch WJ. Control of myocardial contractile function by the level of β-adrenergic receptor kinase 1 in gene-targeted mice. *J Biol Chem.* 1998;293:18180–18184.
13. Gros R, Benovic JL, Tan CM, Feldman RD. G-protein-coupled receptor kinase activity is increased in hypertension. *J Clin Invest.* 1997;99: 2087–2093.
14. Ungerer M, Kessebohm K, Kronsbein K, Lohse MJ, Richardt G. Activation of β-adrenergic receptor kinase during myocardial ischemia. *Circ Res.* 1996;79:455–460.
15. Choi D-J, Koch WJ, Hunter JJ, Rockman HA. Mechanism of β-adrenergic receptor desensitization in cardiac hypertrophy is increased β-adrenergic receptor kinase. *J Biol Chem.* 1997;272:17223–17229.
16. Rockman HA, Chien KR, Choi D-J, Iaccarino G, Hunter JJ, Ross J Jr, Lefkowitz RJ, Koch WJ. Expression of a β-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. *Proc Natl Acad Sci U S A.* 1998;95:7000–7005.
17. Iaccarino G, Tomhave ED, Lefkowitz RJ, Koch WJ. Reciprocal in vivo regulation of myocardial G protein-coupled receptor kinase expression by β-adrenergic receptor stimulation and blockade. *Circulation.* 1998;98: 1783–1789.
18. Jaber M, Koch WJ, Rockman HA, Smith B, Bond RA, Sulik K, Ross J Jr, Lefkowitz RJ, Caron MG, Giros B. Essential role of β-adrenergic receptor kinase 1 in cardiac development and function. *Proc Natl Acad Sci U S A.* 1996;93:12974–12979.
19. Arber S, Hunter JJ, Ross J Jr, Hongo M, Sansig G, Borg J, Perriard J-C, Chien KR, Caroni P. MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure. *Cell.* 1997;88:393–403.
20. Milano CA, Allen LF, Rockman HA, Dolber PC, McMinn TR, Chien KR, Johnson TD, Bond RA, Lefkowitz RJ. Enhanced myocardial function in transgenic mice overexpressing the β_2-adrenergic receptor. *Science.* 1994; 264:582–586.
21. Port JD, Weinberger HD, Bisognano JD, Knudson OA, Bohlmeyer TJ, Pende A, Bristow MR. Echocardiographic and histopathologic characterization of young and old transgenic mice over-expressing the human β1-adrenergic receptor. *J Am Coll Cardiol.* 1998;31(suppl A):177A. Abstract.
22. Akhter SA, Skaer CS, Kypson AP, McDonald PH, Peppel KC, Glower DD, Lefkowitz RJ, Koch WJ. Restoration of β-adrenergic signaling in failing cardiac ventricular myocytes via adenoviral-mediated gene transfer. *Proc Natl Acad Sci U S A.* 1997;94:12100–12105.