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Reciprocal In Vivo Regulation of Myocardial G Protein–Coupled Receptor Kinase Expression by β-Adrenergic Receptor Stimulation and Blockade

Guido Iaccarino, MD; Eric D. Tomhave, BS; Robert J. Lefkowitz, MD; Walter J. Koch, PhD

Background—Impaired myocardial β-adrenergic receptor (βAR) signaling, including desensitization and functional uncoupling, is a characteristic of congestive heart failure. A contributing mechanism for this impairment may involve enhanced myocardial β-adrenergic receptor kinase (βARK1) activity because levels of this βAR-desensitizing G protein–coupled receptor kinase (GRK) are increased in heart failure. An hypothesis has emerged that increased sympathetic nervous system activity associated with heart failure might be the initial stimulus for βAR signaling alterations, including desensitization. We have chronically treated mice with drugs that either activate or antagonize βARs to study the dynamic relationship between βAR activation and myocardial levels of βARK1.

Methods and Results—Long-term in vivo stimulation of βARs results in the impairment of cardiac βAR signaling and increases the level of expression (mRNA and protein) and activity of βARK1 but not that of GRK5, a second GRK abundantly expressed in the myocardium. Long-term β-blocker treatment, including the use of carvedilol, improves myocardial βAR signaling and reduces βARK1 levels in a specific and dose-dependent manner. Identical results were obtained in vitro in cultured cells, demonstrating that the regulation of GRK expression is directly linked to βAR signaling.

Conclusions—This report demonstrates, for the first time, that βAR stimulation can significantly increase the expression of βARK1, whereas β-blockade decreases expression. This reciprocal regulation of βARK1 documents a novel mechanism of ligand-induced βAR regulation and provides important insights into the potential mechanisms responsible for the effectiveness of β-blockers, such as carvedilol, in the treatment of heart failure. **(*Circulation*. 1998;98:1783-1789.)**

Key Words: heart failure ■ receptors, adrenergic, beta ■ myocardium ■ catecholamines

β-Adrenergic receptors (βARs), which couple to the heterotrimeric guanine nucleotide binding (G) protein G_s, are major determinants of cardiac contractility. In the heart, βARs are targets for catecholamines such as the sympathetic neurotransmitter norepinephrine and the adrenal hormone epinephrine.[1,2] Catecholamine stimulation of myocardial βARs triggers a series of transmembrane signaling events through G_s that lead to the increased production of cAMP. In the myocyte, this results in positive inotropy, dromotropy, and chronotropy.[1,2] Acute agonist (ie, catecholamine) exposure also triggers a series of counterregulatory mechanisms that lead to the functional uncoupling of βARs, a process known as desensitization.[3,4] Homologous desensitization of G protein–coupled receptors, such as βARs, is initiated by the actions of a family of serine/threonine kinases known as the G protein–coupled receptor kinases (GRKs).[3,4] GRKs normally expressed in the heart, such as the βAR kinase (βARK1, or GRK2) and GRK5, are enzymes that are rapidly activated after agonist occupancy of receptors and GRK-mediated receptor phosphorylation and subsequent β-arrestin binding leads to the loss of G protein coupling.[3,4]

A growing body of evidence supports the hypothesis that the actions of GRKs are extremely important in modulating myocardial adrenergic signaling and cardiac function both under normal conditions and in disease states. Several recent studies have shown that GRK levels (eg, βARK1) and activity are elevated in a variety of cardiovascular disorders. These pathological conditions include human congestive heart failure,[5] experimental myocardial ischemia,[6] mild human hypertension,[7] and pressure overload ventricular hypertrophy.[8] In the latter study, we have shown that the cardiac hemodynamic dysfunction that accompanies pressure overload ventricular hypertrophy in mice is primarily due to an increase in the expression of βARK1.[8] Furthermore, in studies with transgenic mice, we have shown that increased βARK1 or GRK5 expression and activity in the heart can lead to functional uncoupling and desensitization of myocardial βARs and subsequent in vivo cardiac dysfunction.[9,10]

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From the Departments of Medicine (G.I., R.J.L.), Surgery (E.D.T, W.J.K.), and Biochemistry (R.J.L.) and Howard Hughes Medical Institute, Duke University Medical Center (R.J.L.), Durham, NC.

Correspondence to Walter J. Koch, PhD, Department of Surgery, Duke University Medical Center, Room 472, MSRB, Research Drive, Durham, NC 27710. E-mail koch0002@mc.duke.edu

The mechanisms of GRK upregulation associated with these cardiovascular disorders are unknown, but we hypothesize that they may involve enhanced sympathetic nervous activity and high catecholamine levels, triggering enhanced activation and signaling through myocardial βARs. To explore the possibility that the myocardial expression of GRKs is coupled to the functional state of βARs, we investigated specific GRK regulation due to long-term activation or antagonism of βARs. The β-agonist isoproterenol or the β-antagonist atenolol was infused into mice through the use of implanted miniosmotic pumps. After long-term treatment with these drugs, we assessed the levels of βARK1 and GRK5 in the heart through immunoblotting. We also measured myocardial GRK activity. GRK regulation in response to βAR ligands also was studied in cultured mammalian cells to circumvent the hemodynamic changes associated with the in vivo administration of these drugs. We studied the specific effects on myocardial GRK expression of carvedilol, a novel β-blocking agent that enhances cardiac performance and survival in human heart failure.[11] The mechanisms that account for the effectiveness of β-blockers in heart failure are not completely understood. In this study, we test the hypothesis that these drugs might be exerting beneficial effects in heart failure through attenuation of βAR desensitization due to decreased myocardial βARK1 expression.

Methods

Study Design and Miniosmotic Pump Implantation

C57/Bl6 mice (weight, 25 to 30 g) were used in the study. All animal procedures were approved by the Institutional Animal Usage Committee at Duke University. Mice were anesthetized with a mixture of ketamine (10 mg/kg) and xylazine (0.5 mg/kg), and a small incision was made in the skin between the scapulae. A small pocket was created by spreading apart the subcutaneous connective tissue. After insertion of the miniosmotic pump (model 2002; Alzet), the skin incision was closed with a 4.0 catgut suture. Atenolol and isoproterenol were dissolved in 0.002% ascorbic acid, and carvedilol (a generous gift from SmithKline Beecham) was dissolved in 60% DMSO. Pumps were filled to deliver atenolol at the rate of 0.1, 1.0, and 10.0 $mg \cdot kg^{-1} \cdot d^{-1}$, isoproterenol at the rate of 0.3, 3.0, and 30.0 $mg \cdot kg^{-1} \cdot d^{-1}$, or carvedilol at the rate of 10.0 $mg \cdot kg^{-1} \cdot d^{-1}$ over a period of 14 days. As controls, pumps that delivered vehicle (0.002% ascorbic acid or 60% DMSO) were implanted in mice. Heart rates in anesthetized animals were measured by ECG leads after 1 week to ensure drug delivery. At the end of the treatment, the animals were anesthetized and weighed, and their hearts were explanted, rinsed three times in cold PBS, and blotted dry. After weighing, isolated hearts were frozen in liquid nitrogen and stored at −70°C until needed for biochemical studies. The heart weight–to–body weight ratio was then calculated (mg/g).

βAR Radioligand Binding

Receptor binding on myocardial membranes was performed as previously described using the nonselective βAR ligand [^{125}I]cyanopindolol.[8,9] Nonspecific binding was determined in the presence of 10 μmol/L alprenolol. Reactions were conducted in 500 μL of binding buffer at 37°C for 1 hour and then terminated by vacuum filtration through glass-fiber filters. All assays were performed in triplicate, and receptor density (in fmol) was normalized to milligrams of membrane protein.

Adenylyl Cyclase Activity

Crude myocardial membranes (20 to 30 μg of protein) were incubated for 15 minutes at 37°C with [α-^{32}P]ATP under basal conditions or in the presence of either 100 μmol/L isoproterenol or 10 mmol/L NaF, and cAMP was quantified by standard methods as we have described previously.[8,9]

Protein Immunoblotting

Immunodetection of myocardial levels of βARK1 was performed on detergent-solubilized extracts after immunoprecipitation, as previously described.[8] Excised hearts were solubilized in ice-cold RIPA buffer (50 mmol/L Tris-HCl, pH 8.0, 5 mmol/L EDTA, 150 mmol/L NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mmol/L NaF, 5 mmol/L EGTA, 10 mmol/L sodium pyrophosphate, 1 mmol/L phenylmethylsulfonyl fluoride), and βARK1 was immunoprecipitated from 1 mL of clarified extract (equal protein amounts) using 1:2000 of an anti-βARK1/2 (GRK2/3) monoclonal antibody[8,12] and 35 μL of a 50% slurry of Protein A–agarose conjugate agitated for 1 hour at 4°C. After extensive washing,[8] immune complexes were electrophoresed through 12% polyacrylamide Tris/glycine gels and transferred to nitrocellulose. The 80-kDa βARK1 protein was visualized using standard enhanced chemiluminescence (ECL kit; Amersham). Immunodetection of GRK5 was performed by Western blotting of myocardial membranes using a polyclonal anti-GRK5 antibody.[10,13] Quantification of immunoreactive βARK1 and GRK5 was done by scanning the final autoradiography films and using ImageQuant software (Molecular Dynamics).

Rhodopsin Phosphorylation Assays

Myocardial extracts were prepared through homogenization of excised hearts in 2 mL of ice-cold lysis 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) as described previously.[8–10] Soluble cytosolic fractions and membrane fractions were separated, and GRK activity was assessed in cytosolic fractions (100 to 150 μg of protein) by light-dependent phosphorylation of rhodopsin-enriched rod outer segment membranes in lysis buffer with 10 mmol/L $MgCl_2$ and 0.1 mmol/L ATP (containing [γ-^{32}P]ATP) as described previously.[8–10] Phosphorylated rhodopsin was visualized by autoradiography of dried gels and quantified using a Molecular Dynamics PhosphorImager.

RNA Preparation and Semiquantitative Reverse Transcription–Polymerase Chain Reaction

Total RNA was isolated using RNAzol (Biotech), a one-step guanidinium-based extraction solution.[14] After the treatment of final RNA aliquots with DNase I, 1 μg of total RNA was used for reverse transcription (RT) into cDNA according to standard methods.[15] Equal aliquots of cDNA then was used as templates for the specific amplification of fragments of βARK1 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using *Taq* DNA polymerase in the presence of [^{32}P]dCTP. Primer pairs specific for rat βARK1 and GAPDH sequences have been previously described and were used for amplification of mouse transcripts.[15] These primer pairs amplify the appropriate mRNA in the mouse as revealed by sequencing of amplified products (data not shown). Optimal annealing temperatures for βARK1 and GAPDH were previously found to be 63°C and 55°C, respectively.[15] The final cycle number used for quantification of the amplified cDNA products was 36 for βARK1 and 27 for GAPDH, which were previously determined to be in the linear portion of the amplification curve that went to 42 and 35, respectively.[15] Samples were electrophoresed through 1% agarose gel containing ethidium bromide, the polymerase chain reaction (PCR) products were removed from the gel, and ^{32}P incorporation was measured using liquid scintillation. Relative quantities of βARK1 were normalized to levels of GAPDH in individual samples as described previously.[15] βARK1/GAPDH values (in arbitrary units [AU]) from drug-treated hearts are expressed as fold of control (vehicle) mRNA values.

In Vitro Cell Studies

Chinese hamster fibroblast (CHW) cells stably overexpressing β_1ARs (201±48 fmol/mg) were used.[16] On the day before the

TABLE 1. Trophic Heart Responses After Drug Treatment

Drug	Value
Atenolol	
Vehicle	4.8±0.06
0.1 mg · kg^{-1} · d^{-1}	4.78±0.14
1.0 mg · kg^{-1} · d^{-1}	4.9±0.04
10 mg · kg^{-1} · d^{-1}	4.91±0.12
Isoproterenol	
Vehicle	4.83±0.06
0.3 mg · kg^{-1} · d^{-1}	5.27±0.13*
3.0 mg · kg^{-1} · d^{-1}	5.34±0.17*
30.0 mg · kg^{-1} · d^{-1}	5.58±0.15*
Carvedilol	
Vehicle	4.70±0.05
10 mg · kg^{-1} · d^{-1}	4.39±0.08*

Data are given in heart weight–to–body weight ratios (mg/g) as mean±SEM of 6 to 10 experiments.
*$P<0.05$ vs vehicle.

experiment, cells were serum starved overnight to induce a state of quiescence, and on the next day, the medium was replaced with fresh medium that contained propranolol (10^{-4} M), isoproterenol (10^{-4} M), or vehicle (0.002% ascorbic acid). Cells were incubated for 48 hours at 37°C. Cells were washed twice with PBS and then solubilized with ice-cold RIPA buffer. Immunodetection of the specific GRKs were assessed as described.

Statistical Analysis

Data are expressed as mean±SEM. Data for isoproterenol and atenolol were analyzed using ANOVA with posthoc testing performed with Bonferroni's analysis. An unpaired Student's *t* test was performed to analyze the carvedilol data as well as the RT-PCR data.

Results

Heart Weight–to–Body Weight Ratios

Isoproterenol induced a dose-dependent increase in heart size without affecting the body weight (Table 1). This isoproterenol-dependent increase in the heart weight–to–body weight ratio demonstrates the presence of myocardial hypertrophy. Atenolol treatment did not modify body or heart weight. Conversely, carvedilol treatment significantly reduced the cardiac mass, as indicated by the decreased heart weight–to–body weight ratio (Table 1).

Myocardial βAR Signaling Properties

Classically, long-term exposure to agonists causes downregulation of βARs, whereas long-term β-blockade produces upregulation.[2] Therefore, βAR density was measured in the hearts of treated animals. As expected, isoproterenol decreased βAR density and atenolol treatment induced an increase in the number of βARs in a dose-dependent manner (Table 2). Carvedilol is an atypical β-antagonist that has been shown to decrease βAR density,[17] which was seen after 14 days of treatment (Table 2).

We assessed adenylyl cyclase activity in cardiac membranes to examine the signaling properties of myocardial βARs after long-term stimulation or blockade. Long-term infusion of isoproterenol resulted in a dampening of adenylyl cyclase activity under basal conditions and after βAR stimulation, which is consistent with both receptor downregulation and enhanced desensitization (Table 2). In atenolol- and carvedilol-treated animals, there was a dose-dependent increase in adenylyl cyclase activity both under basal conditions and in response to isoproterenol (Table 2). This increase in membrane adenylyl cyclase activity in carvedilol-treated animals occurred despite a significant loss in βAR density (Table 1).

Myocardial GRK Protein Levels

Long-term stimulation of βARs with isoproterenol resulted in a significant increase in βARK1 expression that was related to the dose of the drug (Figure 1A). The analysis of total myocardial βARK1 levels in atenolol-treated animals dem-

TABLE 2. βAR Density and Membrane Adenylyl Cyclase Activity in Treated Mice

	βAR Density, fmol/mg of Membrane Protein	Adenylyl Cyclase Activity, pmol of cAMP · mg^{-1} · min^{-1}		
		Basal	Isoproterenol (10^{-4} mol/L)	NaF (10^{-2} mol/L)
Isoproterenol				
Control	38±1	41±2	53±1	450±70
0.3 mg · kg^{-1} · d^{-1}	26±3*	37±5	42±7	424±22
3.0 mg · kg^{-1} · d^{-1}	23±1*	25±6*	42±5*	414±72
30 mg · kg^{-1} · d^{-1}	23±2*	27±3*	36±6*	390±82
Atenolol				
Control	33±5	40±2	52±6	362±27
0.1 mg · kg^{-1} · d^{-1}	46±4*	42±1	59±7	316±52
1.0 mg · kg^{-1} · d^{-1}	55±4*	49±9	67±7	363±21
10 mg · kg^{-1} · d^{-1}	74±2*	62±8*	88±11*	406±65
Carvedilol				
Control	36±3	47±3	69±5	397±30
10 mg · kg^{-1} · d^{-1}	22±4*	60±5*	79±3*	430±21

Data are given as mean±SEM of 6 to 10 individual cardiac membrane preparations prepared in triplicate.
*$P<0.05$ vs basal.

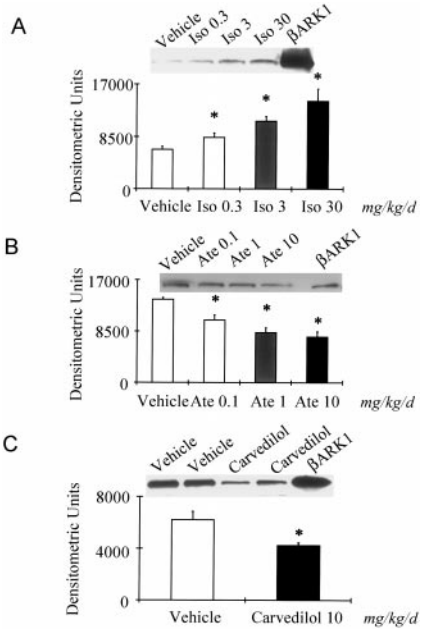

Figure 1. Myocardial βARK1 protein levels. Histograms represent mean±SEM in densitometry units of scanned chemiluminescent immunoblots from 4 to 6 hearts at each given dose of (A) isoproterenol (Iso), (B) atenolol (Ate), or (C) carvedilol. Insets show representative immunoblots for each set of mouse hearts. Purified βARK1 is included as control for protein migration. $*P<0.05$ versus vehicle.

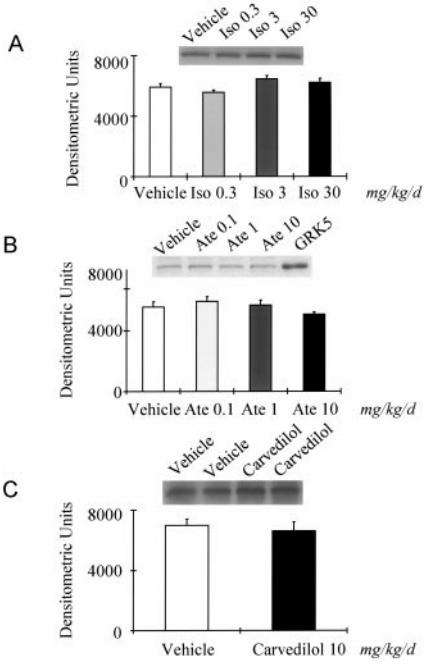

Figure 2. Myocardial GRK5 protein levels. Histograms represent mean±SEM in densitometry units of scanned chemiluminescent immunoblots from 4 to 6 hearts at each given dose of (A) isoproterenol (Iso), (B) atenolol (Ate), or (C) carvedilol. Insets show representative immunoblots for GRK5 for each drug treatment. P=NS at all doses.

onstrated that βARK1 regulation is dependent on the degree of βAR blockade in that the amount of βARK1 was reduced in a dose-dependent manner (Figure 1B). Interestingly, carvedilol treatment also induced a significant reduction in myocardial βARK1 expression (Figure 1C). Because other GRKs are expressed in the heart, we investigated whether changes in βARK1 expression were specific by examining the myocardial levels of GRK5. This GRK is a membrane-bound kinase expressed in the heart that has been shown to desensitize myocardial βARs in vivo.[10] In contrast to βARK1, none of the drug treatments affected the expression of GRK5 in cardiac membranes, suggesting that regulation of this enzyme is not dependent on the functional state of βARs (Figure 2).

To prove that the regulation of βARK1 is an intrinsic feature of βAR stimulation and inhibition and is independent of cellular type and to rule out direct or indirect interference of the drugs with mechanisms other than the functional state of βARs, such as peripheral hemodynamic changes, we studied cultured mammalian cells (CHW) expressing exclusively the human β_1AR, thus simulating the predominant βAR signaling pathway in cardiomyocytes. The analysis of total βARK1 expression in this model showed that βAR inhibition using the β-blocker propranolol induced a significant reduction (≈25%), whereas βAR stimulation with isoproterenol induced a similar significant increase in βARK1 levels (Figure 3). These two opposite-acting drugs did not alter GRK5 expression (data not shown). These findings in a cultured cell system clearly parallel our in vivo findings in the mouse heart.

Myocardial GRK Activity

To assess whether the changes in the protein levels of βARK1 correspond to an increase in myocardial GRK activity, we examined the soluble GRK activity of cardiac extracts in an in vitro phosphorylation assay using the G protein–coupled receptor rhodopsin as a substrate. We have found that GRK activity in cytosolic fractions is almost entirely due to βARK1.[8–10] In isoproterenol-treated animals, there was a dose-dependent increase in myocardial GRK activity that was proportional to the increase in βARK1 protein (Figure 4A). Reciprocally, in atenolol- and carvedilol-treated animals, there was a reduction in GRK activity (Figure 4B and 4C).

Semiquantitative RT-PCR

To examine the molecular regulation of myocardial βARK1 expression in response to the modulation of βAR signaling, we used semiquantitative RT-PCR[15] to analyze mRNA levels in the hearts of mice treated with the highest doses of isoproterenol and atenolol because these hearts have the

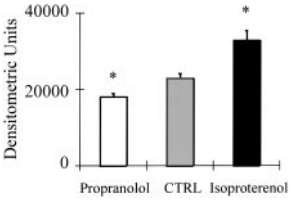

Figure 3. βARK1 levels in treated CHW-β_1AR cells. Histograms show mean±SEM in densitometry units of scanned immunoblots from 4 experiments performed in duplicate. CTRL indicates control. $*P<0.05$ versus vehicle.

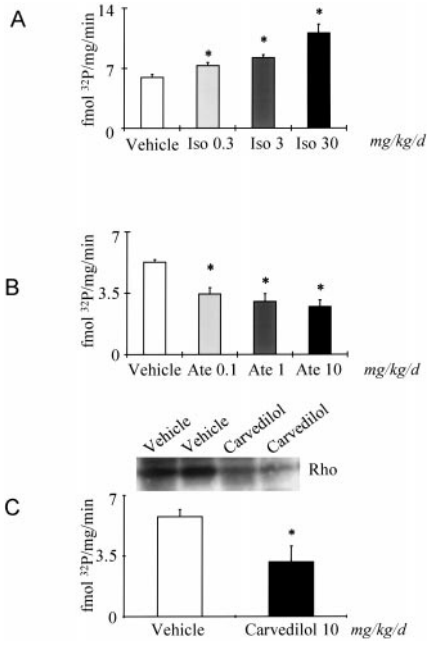

Figure 4. Myocardial GRK activity. Results shown are mean±SEM from 4 to 6 cytosolic extracts taken from mouse hearts after treatment with doses of (A) isoproterenol (Iso), (B) atenolol (Ate), or (C) carvedilol. Inset in C is representative autoradiograph from dried gel showing reduced rhodopsin (rho) phosphorylation activity in cytosolic extracts from carvedilol-treated hearts. $*P<0.05$ versus vehicle.

largest changes in levels of βARK1 protein. The amplified βARK1 product was normalized to amplified GAPDH (which was similar in all samples), and values from drug-treated hearts were compared with control (vehicle-treated) mRNA levels. The final cycle lengths used for the quantification of βARK1 and GAPDH (36 and 27, respectively) were previously found to be in the linear portion of the amplification curve (see Methods). In isoproterenol-treated hearts, βARK1 mRNA levels were 2-fold higher than those in vehicle-treated control hearts (in fold of control values: 2.10±0.04 for isoproterenol treatment versus 1.00±0.02 for vehicle treatment, n=3 each; $P<0.05$). Atenolol treatment induced significant lowering of βARK1 mRNA levels (in fold of control values: 0.63±0.28 for atenolol versus 1.00±0.13 for vehicle, n=5 each; $P<0.05$). This reciprocal regulation of βARK1 mRNA expression after isoproterenol and atenolol treatment explains the changes in protein levels already described.

Discussion

The results of the present study demonstrate that βARK1 expression in the myocardium is tightly linked to the functional state of βARs. Using βAR ligands with opposing actions, we found that βARK1 expression in the heart was reciprocally regulated after long-term infusion of isoproterenol or the β-blocker atenolol through the use of implanted miniosmotic pumps. This dynamic relationship between βAR signaling and the expression of βARK1 is selective because βAR inhibition or activation did not affect the expression of GRK5.

A growing body of evidence supports a critical role of GRK activity in the determination of cardiac contractility. Studies conducted in transgenic mice have shown that the manipulation of βARK1 activity in the heart can have profound effects on in vivo cardiac performance. Adding to the importance of βARK1 in heart function are the recent findings that increased levels of βARK1 accompany depressed cardiac contractility in several diseases or conditions, such as myocardial ischemia,[6] ventricular hypertrophy,[8] hypertension,[7] and heart failure.[5] The mechanisms that induce upregulation of βARK1 in these states are not known. However, because enhanced sympathetic outflow is associated with these conditions, especially heart failure,[18] increased catecholamines may be a triggering mechanism through long-term stimulation of myocardial βARs.

Long-term isoproterenol administration results in sustained cardiac adrenergic activation, which may mimic the heightened sympathetic nervous system activity observed in cardiovascular disease. Fourteen days of isoproterenol infusion produced cardiac hypertrophy and impairment of βAR signaling. βAR density was reduced, and the remaining receptors were desensitized. The increase in βARK1 expression and activity appears to be responsible for the desensitization because long-term infusion of isoproterenol did not affect GRK5 expression. The mRNA levels for βARK1 were also increased, supporting the hypothesis of a direct and selective relationship between βAR signaling and molecular GRK regulation.

Because our results with isoproterenol suggest a biofeedback mechanism linking the functional state of βARs and myocardial βARK1 expression, we hypothesized that β-blockers would reduce βARK1 levels, leading to improved βAR signaling. Indeed, long-term treatment with atenolol reduced βARK1 protein and activity levels in a dose-dependent manner, reaching a maximum reduction of ≈50%. Lower βARK1 activity in atenolol-treated animals was associated with enhanced βAR signaling as measured by adenylyl cyclase activity. The decrease in βARK1 expression and activity can be attributed to decreased mRNA. Like isoproterenol, atenolol did not affect the expression of GRK5, demonstrating specificity for the regulation of βARK1. These results demonstrating the specific effects of a β-blocker on one form of a GRK but not another are in contrast to an earlier study in pigs in which myocardial GRK activity was examined after long-term β-blockade.[19] Although the authors of this study found an apparent decrease in GRK activity, no specific GRK isoform was examined. Taken together, the present results obtained with atenolol- and isoproterenol-treated mice provide in vivo evidence for the reciprocal regulation of myocardial βARK1 by the functional state of βARs. Our data do not rule out minor contributions of other GRKs that are expressed at lower levels in the heart, such as GRK3 and GRK6.

To rule out any possibility that these two opposite-acting drugs regulate βARK1 through mechanisms independent of myocardial βAR signaling alterations, such as changes in cardiac hemodynamics, we studied the effects of βAR antagonism and activation in an in vitro cellular model. We chose CHW cells stably overexpressing the β_1AR because of the

prevalence of this βAR subtype in the heart. Using isoproterenol or propranolol treatment, we found similar reciprocal regulation of βARK1. These results demonstrate that regulation of βARK1 expression is an intrinsic feature of βAR signaling, apparently independent of cell type. Furthermore, they indicate that regulation of myocardial βARK1 expression in vivo is due to the direct action of these drugs on myocardial βARs and not to peripheral effects such as changes in systolic pressure.

The results of the present study demonstrate that long-term βAR activation triggers mechanisms that lead to the selective increase in βARK1 mRNA, protein, and activity. Relating this to pathophysiological settings such as in heart failure, the elevated catecholamine levels[18] presumably trigger a series of events, including the upregulation of βARK1, aimed at compensating for long-term βAR activation. Importantly, increased βARK1 leads to both βAR desensitization and diminished cardiac contractility.[8–10] This explanation supports the "adrenergic hypothesis" of heart failure,[20] which proposes that increased cardiac sympathetic drive results in abnormalities of βAR signaling. We demonstrate here that this includes GRK regulation. Although GRK5 can also desensitize myocardial βARs in vivo,[10] our findings demonstrate that GRK5 expression is not regulated by βAR signaling and that this mode of feedback regulation is specific for βARK1.

If the enhanced βARK expression and activity in response to βAR activation in heart failure are maladaptive, then one might predict that treatments that show benefit in the treatment of heart failure through decreased βAR stimulation would lower levels of βARK1. We hypothesized that this may play a role in the beneficial effects of certain β-blockers in the treatment of heart failure. Thus, in addition to atenolol, we examined myocardial GRK expression in mice after long-term treatment with carvedilol, a novel β-blocker (also possessing α-adrenergic receptor antagonism)[17] that has been shown to dramatically increase survival in patients with heart failure.[11] Interestingly, 14 days of a carvedilol infusion in the mouse significantly decreased βARK1 levels in a selective manner. Importantly, the effects of carvedilol on βARK1 expression are not due to its effects on βAR density because unlike atenolol, carvedilol decreased βAR number, suggesting that βAR density is not a determinant for the improvement in βAR signaling observed with these drugs. Furthermore, carvedilol and isoproterenol treatments produced similar decreases in βAR density, yet cAMP production was reciprocally altered, as was βARK1 expression. The increased adenylyl cyclase activity seen with lower βARK1 expression by the two β-blocking agents (Table 2) is consistent with our previous findings in isolated cardiomyocytes in which infection with an adenovirus containing a peptide inhibitor of βARK1 was found to increase intracellular cAMP accumulation without an alteration in βAR density.[21]

Our present findings strongly suggest that the increase in βARK1 levels in heart failure can contribute to attenuated βAR signaling and cardiac dysfunction. They also specifically demonstrate that antagonism of βAR signaling leads to a selective reduction in βARK1, thus raising the hypothesis that reduction in βARK1 activity participates in the ameliorating effects on heart failure associated with carvedilol treatment. Future studies, perhaps in patients treated with carvedilol, will be required to demonstrate a correlation between lower βARK1 activity in the heart with salutary effects in heart failure, but our findings support this intriguing possibility. Therefore, inhibition of cardiac βARK1 activity represents a novel therapeutic target in heart failure. βARK1 inhibition can be achieved with classic drugs such as β-blockers or experimentally with more specific and novel therapeutic tools such as gene delivery of DNA encoding a peptide inhibitor of βARK1, which we have been studying in different model systems.[21,22] In addition, small molecule pharmaceutical inhibitors of βARK1 activity can be developed that have the potential to serve as novel therapeutic agents for the treatment of heart failure or other cardiovascular disorders that have a component of βAR desensitization.

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References

1. Brodde O-E. β-Adrenergic receptors in failing human myocardium. *Basic Res Cardiol*. 1993;91:35–40.
2. Stiles GL, Lefkowitz RJ. Cardiac adrenergic receptors. *Annu Rev Med*. 1984;35:149–164.
3. Lefkowitz RJ. G protein-coupled receptor kinases. *Cell*. 1993;74:409–412.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. Rockman HA, Choi DJ, Rahman NU, Akhter SA, Lefkowitz RJ, Koch WJ. Receptor-specific in vivo desensitization by the G protein-coupled receptor kinase-5 in transgenic mice. *Proc Natl Acad Sci U S A*. 1996;93:9954–9959.
11. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH, for the US Carvedilol Heart Failure Study Group. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. *N Engl J Med*. 1996;334:1349–1355.
12. Oppermann M, Diverse-Pierluissi M, Drazner MH, Dyer SL, Freedman NJ, Peppel KC, Lefkowitz RJ. Monoclonal antibodies reveal receptor specificity among G-protein-coupled receptor kinases. *Proc Natl Acad Sci U S A*. 1996;93:7649–7654.
13. Premont RT, Koch WJ, Inglese J, Lefkowitz RJ. Identification, purification, and characterization of GRK5, a member of the family of G protein-coupled receptor kinases. *J Biol Chem*. 1994;269:6832–6841.

14. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*. 1987;161:156–159.
15. Xiao R-P, Tomhave ED, Ji X, Boluyt M, Cheng H, Lakatta EG, Koch WJ. Age-associated reductions in cardiac β_1- and β_2-adrenergic responses without changes in inhibitory G proteins or receptor kinases. *J Clin Invest*. 1998;101:1273–1282.
16. Freedman NJ, Liggett SB, Drachman DE, Pei G, Caron MG, Lefkowitz RJ. Phosphorylation and desensitization of the human β_1-adrenergic receptor: involvement of G protein-coupled receptor kinases and cAMP-dependent protein kinase. *J Biol Chem*. 1995;270:17953–17961.
17. Yoshikawa T, Port JD, Asano K, Chidiak P, Bouvier M, Dutcher D, Roden RL, Minobe W, Tremmel KD, Bristow MR. Cardiac adrenergic receptor effects of carvedilol. *Eur Heart J*. 1996;17:8–16.
18. 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.
19. Ping P, Gelzer-Bell R, Roth DA, Kiel D, Insel PA, Hammond HK. Reduced β-adrenergic receptor activation decreases G-protein expression and β-adrenergic receptor kinase activity in porcine heart. *J Clin Invest*. 1995;95:1271–1280.
20. Bristow M. Mechanism of action of β-blocking agents in heart failure. *Am J Cardiol*. 1997;80:26L–40L.
21. Drazner MH, Peppel KC, Dyer S, Grant AO, Koch WJ, Lefkowitz RJ. Potentiation of β-adrenergic signaling by adenoviral-mediated gene transfer in adult rabbit ventricular myocytes. *J Clin Invest*. 1997;99:288–296.
22. Akhter SA, Skaer CA, 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.