Transgenic Mice with Cardiac Overexpression of \( \alpha_{1B} \)-Adrenergic Receptors

IN VIVO \( \alpha_{1B} \)-ADRENERGIC RECEPTOR-MEDIATED REGULATION OR \( \beta \)-ADRENERGIC SIGNALING*

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Transgenic mice were generated with cardiac-specific overexpression of the wild-type (WT) \( \alpha_{1B} \)-adrenergic receptor (AR) using the murine \( \alpha \)-myosin heavy chain gene promoter. Previously, we described transgenic mice with \( \alpha \)-myosin heavy chain-directed expression of a constitutively active mutant \( \alpha_{1B} \)-AR that had a phenotype of myocardial hypertrophy (Milano, C. A., Dolber, P. C., Rockman, H. A., Bond, R. A., Venable M. E., Allen, L. F., and Lefkowitz, R. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10109–10113). In animals with >40-fold WT \( \alpha_{1B} \)-AR overexpression, basal myocardial diacylglycerol content was significantly increased, indicating enhanced \( \alpha_{1B} \)-adrenergic signaling and phospholipase C activity. In contrast to the mice overexpressing constitutively active mutant \( \alpha_{1B} \)-ARs, the hearts of these mice did not develop cardiac hypertrophy despite an 8-fold increase in ventricular mRNA for atrial natriuretic factor. In vivo physiology was studied in anesthetized intact animals and showed left ventricular contractility in response to the \( \beta \)-agonist isoproterenol to be significantly depressed in animals overexpressing WT \( \alpha_{1B} \)-ARs. Membranes purified from the hearts of WT \( \alpha_{1B} \)-AR-overexpressing mice demonstrated significantly attenuated adenyl cyclase activity basally and after stimulation with isoproterenol, norepinephrine, or phenylephrine. Interestingly, these in vitro changes in signaling were reversed after treating the mice with pertussis toxin, suggesting that the extraordinarily high signaling were reversed after treating the mice with pertussis toxin. Interestingly, these in vitro changes in signaling were reversed after treating the mice with pertussis toxin, suggesting that the extraordinarily high levels of WT \( \alpha_{1B} \)-ARs can lead to coupling to pertussis toxin-sensitive G proteins. Another potential contributor to the observed decreased myocardial signaling and function could be enhanced \( \beta \)-AR desensitization as \( \beta \)-adrenergic receptor kinase (\( \beta \)ARK1) activity was found to be significantly elevated (>3-fold) in myocardial extracts isolated from WT \( \alpha_{1B} \)-AR-overexpressing mice. This type of altered signal transduction may become critical in disease conditions such as heart failure where \( \beta \)ARK1 levels are elevated and \( \beta \)-ARs are downregulated, leading to a higher percentage of cardiac \( \alpha \)-ARs. Thus, these mice serve as a unique experimental model to study the in vivo interactions between \( \alpha \) and \( \beta \)-\( \beta \)-ARs in the heart.

There have been numerous in vitro studies characterizing the role of \( \alpha_{1B} \)-adrenergic receptor (AR)\(^1\) signaling in cardiac myocytes. Agents that stimulate \( \alpha_{1B} \)-ARs, leading to the activation of the guanine nucleotide-binding protein \( G_{\beta\gamma} \), have been shown to induce nuclear transcription factors such as c-myc, c-fos, and c-jun and to mediate morphological changes including increases in myocyte size and volume (1). Signaling through the \( \alpha_{1B} \)-AR/G\( G_{\beta\gamma} \) pathway leads to the activation of the effector enzyme phospholipase C and protein kinase C, both of which may act as biochemical initiators of myocardial hypertrophy (2). In addition to G\( G_{\beta\gamma} \)-mediated hypertrophy, recent studies have implicated a p21\(^{ras}\) (Ras)-dependent hypertrophic pathway initiated by \( \alpha_{1B} \)-AR activation (3). Other in vitro \( \alpha_{1B} \)-AR/G\( G_{\beta\gamma} \)-mediated signaling events reported to exist in myocytes include positive inotropy and chronotropy and induction of the c-\( gr \)-1 gene (4). In addition to coupling to G\( G_{\beta\gamma} \), \( \alpha_{1B} \)-ARs have also been reported to activate pertussis toxin (PTx)-sensitive G proteins, leading, in myocytes, to negative chronotropy, positive inotropy, Na\(^{+}\)-K\(^{-}\)-ATPase activation, and modulation of intracellular calcium transients and cell shortening (5).

In contrast to these studies, very few reports have investigated in vivo cardiac \( \alpha_{1B} \)-AR signaling particularly with respect to physiological sequelae and the potential in vivo significance of dual G protein coupling. \( \alpha_{1A} \) and \( \alpha_{1B} \)-ARs have been shown to exist in neonatal myocytes (6), whereas in adult human myocardium, the \( \alpha_{1A} \)-AR appears to predominate (7). Both the \( \alpha_{1A} \) and \( \alpha_{1B} \) subtypes have been implicated in myocyte growth and hypertrophy (6). One possible functional role of \( \alpha_{1B} \)-ARs is as a source of inotropic reserve in pathophysiological conditions where the \( \beta \)-AR system is down-regulated and uncoupled (8). Thus, an interrelationship may exist in the heart between \( \alpha_{1} \) and \( \beta \)-ARs. Previous work from our laboratory revealed that cardiac-specific expression of a constitutively active mutant (CAM) of the \( \alpha_{1B} \)-AR in transgenic mice leads to myocardial hypertrophy, demonstrating that cardiac \( \alpha_{1B} \)-adrenergic signaling in vivo can trigger responses similar to myocytes in culture (9).

In this study, we continue our characterization of in vivo heart function.
myocardial adrenergic signaling in transgenic mice (9–13) by describing animals with cardiac overexpression of the wild-type (WT) α1B-AR. As in our previous studies, cardiac expression was targeted by using the murine α-MHC promoter (9–13). To determine the consequences of WT α1B-AR overexpression, we studied both biochemical signaling and in vivo physiology. We have previously observed for the WT β2-AR that when overexpression of these WT receptors reaches extraordinarily high levels, agonist-independent signaling can occur due to a small percentage of spontaneously activated receptors that is significant at high levels of receptor density (10, 14). Both α1- and β-AR signal transduction was assessed, including measurements of myocardial diacylglycerol (DAG) content, ventricular atrial natriuretic factor (ANF) mRNA levels, adenyl cyclase activity, and G protein-coupled receptor kinase (GRK) activity. In addition, the presence of myocardial hypertrophy was assessed. Finally, in vivo basal and β-AR-mediated cardiac function was assessed by catheterization of anesthetized mice. The results from these studies reveal findings that point to potentially important interactions between α- and β-adrenergic signaling in the heart.

EXPERIMENTAL PROCEDURES

Transgene Constructs—A 5.5-kilobase SalI-SacI fragment containing the murine α-MHC promoter (15) was ligated into a previously described plasmid containing the SV40 intron poly(A) signal (9–13) to generate a new plasmid, pGEM-α-MHC-SV40. A 2.0-kilobase SalI-SalI fragment containing the coding sequence for the wild-type hamster α1B-AR was then ligated into pGEM-α-MHC-SV40 to generate pGEM-α-MHC-α1B-AR-SV40. The transgene was then linearized and purified before pronuclear injections done by the Duke Comprehensive Cancer Center Transgenic Facility (9–13). Two lines of mice were established, TG α43 and TG α47. Litter sizes and postnatal development were indistinguishable from nontransgenic littermate controls. Offspring were screened by Southern blot analysis with a probe to the SV40 minisatellite (9). The results from these studies reveal findings that point to potentially important interactions between α- and β-adrenergic signaling in the heart.

Ligand Binding Assays—Membrane fractions were prepared from hearts heated in the presence of 100 μl of binding buffer (50 mM Tris-HCl, pH 7.4, and 5 mM EDTA or 75 mM Tris-HCl, pH 7.4, 12.5 mM MgCl2, and 2 mM EDTA). Binding assays were performed on 25 μg of membrane protein using saturating amounts of 125I-HEAT (300 pM), an α1B-AR-specific ligand, or 125I-CYP (300 pM), a β-AR-specific ligand. Nonspecific binding was determined in the presence of 50 μM prazosin for α- and 20 μM alprenolol for β-binding. Reactions were conducted in either 25 or 500 μl of binding buffer at 37 °C for 1 h and then terminated by vacuum filtration through glass-fiber filters. All assays were performed in triplicate, and receptor density (fmol) was normalized to mg of membrane protein following the method of Bradford (29).

DAG Quantitation—Lipid fractions were extracted from 50 mg of homogenized myocardial tissue as described (6, 9). Aliquots of lipid and DAG standards were dried under nitrogen, resuspended in dimethylformamide, and then completely phosphorylated using Escherichia coli DAG kinase and [γ-32P]ATP. 32P-Labeled phosphatidic acid (phosphorylated DAG) was isolated by silica gel thin-layer chromatography and quantitated with a PhosphorImager (Molecular Dynamics, Inc.). DAG content was normalized to tissue phospholipid, and the final DAG concentration was expressed as pmol of DAG/nmol of lipid phosphatase as described previously (9).

Ventricular ANF mRNA—Ventricular tissue was separated from the atria under a dissecting microscope. Total RNA was extracted using RNAxol (Biotex Laboratories, Houston TX) in a single-step guanidinium-based isolation procedure (16). Total RNA was then fractionated on a 1% formaldehyde-agarose gel and transferred to nitrocellulose as described (9). Blots were then hybridized overnight with a random primer, radiolabeled ANF cDNA probe (9). Blots were washed three times in 0.2 × SSC at 65 °C for 30 min before exposure to x-ray film. All blots were then stripped in water at 95–100 °C for 15 min and reprobed with a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe. The ANF and GAPDH bands were quantitated with the PhosphorImager, and the ANF/GAPDH signal intensity ratio was deter-

Adenylyl Cyclase Activity—Crude myocardial membranes were prepared as described above from both transgenic and nontransgenic control hearts. Membranes (20–30 μg of protein) were incubated for 15 min at 37 °C with [α-32P]ATP under basal conditions or in the presence of one of the following: 100 μM norepinephrine, 100 μM phenylephrine, or 10 mM NaF. Cyclic AMP was quantitated by standard methods described previously (17).

GK Activity in Rhodopsin Phosphorylation Assays—Cytosolic extracts were prepared as described previously (11). These were concentrated using a Centricron microconcentrator (Amicon, Inc.). Cytosolic extracts (300 μg of protein) were incubated with rhodopsin-enriched rod outer segments in 75 μl of lysis buffer (25 mM Tris-HCl, pH 7.4, 5 mM EDTA, 5 mM EGTA, 10 μg/ml leupeptin, 20 μg/ml aprotonin, and 1 mM phenylmethylsulfonyl fluoride) with 10 mM MgCl2 and 0.1 mM ATP containing [γ-32P]ATP. The reactions were incubated in white light for 15 min and quenched with 300 μl of ice-cold lysis buffer and then centrifuged for 15 min at 13,000 × g. Sedimented proteins were resuspended in 25 μl of protein gel loading dye and electrophoresed through SDS-12% polyacrylamide gels. Phosphorylated rhodopsin was visualized by autoradiography of dried polyacrylamide gels and quantified using the PhosphorImager.

Physiological Evaluation—Mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (2.5 mg/kg) given intraperitoneally. After tracheal intubation, mice were connected to a rodent ventilator. Following bilateral vagotomy, the chest was opened, and a 1.8-French high-fidelity micromanometer catheter (Millar Instruments, Inc., Houston, TX) was inserted into the left atrium, advanced through the mitral valve, and secured in the left ventricle (LV). Hemodynamic measurements were recorded at base line and 45–60 s after injection of incremental doses of isoproterenol. Doses of isoproterenol were specifically chosen to maximize the contractile response but to limit the increase in heart rate. Continuous high-fidelity LV pressure and fluid-filled aortic pressure were recorded simultaneously on an eight-channel chart recorder and in digitized form at 2000 Hz for later analysis. Experiments were then terminated with an overdose of pentobarbital. Hearts were rapidly excised, and individual chambers were separated, weighed, and then frozen in liquid N2 for later analysis. Parameters measured were heart rate, LV systolic and end diastolic pressure, and the minimal and maximal first derivative of LV pressure (LV dP/dtmax and LV dP/dtmin). Ten sequential beats were averaged for each measurement.

Statistical Analysis—Data are expressed as mean values ± S.E. Student’s t test was used to analyze all biochemical data. Two-way repeated measures analysis of variance was used to evaluate the in vivo hemodynamic measurements under basal conditions and with isoproterenol stimulation. When appropriate, post hoc analysis was performed with a Newman-Keuls test. For all analyses, p < 0.05 was considered significant.

RESULTS AND DISCUSSION

Two transgenic lines were established expressing the WT α1B-AR and were named TG α43 and TG α47. Cardiac-specific transgene expression was documented by Northern analysis of RNAs from different tissues, including heart, lung, diaphragm, quadriceps muscle, kidney, and liver (data not shown). This cardiac-specific expression is consistent with the previously documented pattern of transgene expression achieved with the murine α-MHC promoter (9–13). At 10 weeks of age, transgene expression was quantitated by radioligand binding assays performed on purified myocardial membranes using 125I-HEAT, and the results are shown in Fig. 1. Total cardiac α1-AR density in TG α43 animals was 43-fold greater than in nontransgenic littermate controls (NLC) and 26-fold greater in the TG α47 line compared with NLC. Unless otherwise stated, all subsequent studies were performed on TG α43 animals.

Classical α1-AR/Gq coupling leads to stimulation of phospholipase C, generating two second messengers, inositol trisphosphate and DAG, which subsequently leads to the activation of protein kinase C (18, 19). To assess the functional coupling of overexpressed WT α1B-ARs, myocardial DAG content was quantitated, and as shown in Fig. 2, base-line DAG content in TG α43 hearts was significantly higher than in control hearts. This indicates that α1B-AR/Gq signaling is enhanced under
basal conditions.

Since signaling through $\alpha_1$-ARs has been shown to evoke a hypertrophic response in cultured myocytes including activation of fetal gene expression (2, 20), we investigated the levels of ANF mRNA present in the ventricles of these transgenic mice. ANF is a gene normally inactive in the ventricles after maturation and has been shown to be associated with cardiac hypertrophy (21). To examine ANF gene activation present in TG $\alpha_43$ mice, Northern blots of ventricular RNA were generated and probed with a mouse ANF cDNA (Fig. 3A). Control ventricles showed minimal or undetectable ANF signals, which is consistent with the inactivation of this gene in normal adult ventricular myocytes (21). In contrast, there was a strong ANF signal in RNA isolated from TG $\alpha_43$ ventricles, which, when normalized to the control GAPDH mRNA, was ~9-fold higher compared with controls (Fig. 3B). The increase in ventricular ANF mRNA in TG $\alpha_43$ mice was twice that seen in our previously described CAM $\alpha_{1b}$-AR-overexpressing mice (9). Surprisingly, despite the extraordinarily high ANF mRNA levels, TG $\alpha_43$ animals did not have significantly different LV/body weight ratios or increased LV myocyte cross-sectional areas compared with controls (data not shown). Nonsignificant changes in heart mass were also found in TG $\alpha_47$ animals (data not shown). This is unlike the phenotype in the CAM $\alpha_{1b}$-AR transgenic animals, which had increased heart mass and increased cross-sectional areas of ventricular myocytes that accompanied the increased ANF signal (9). The lack of a hypertrophic phenotype in these animals is not clearly understood since signaling through $\alpha_1$-ARs is clearly elevated, and ventricular ANF expression is high (Figs. 2 and 3). This suggests that signaling through CAM $\alpha_{1b}$-ARs is somehow different from that through WT receptors.

In addition to investigating a possible hypertrophic phenotype in TG $\alpha_43$ mice, the primary aim of this study was to determine the in vivo physiological effects of enhanced signaling through myocardial $\alpha_1$-ARs. In vivo measurements of $\alpha_1$-AR-mediated cardiac hemodynamics using a pharmacological approach is difficult since $\alpha$-agonists are potent vasoconstrictors that can change the loading conditions and secondarily affect cardiac function. In TG $\alpha_43$ mice, we chose to initially study basal cardiac physiological parameters to determine whether the enhanced $\alpha_1$-AR/Gq signaling indicated by increased DAG content can affect basal function. In addition, we examined cardiac responses to the $\beta$-agonist isoproterenol to determine if enhanced $\alpha_1$-AR signaling affects this response, which is the primary mechanism for increasing performance of

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**FIG. 1.** Myocardial $\alpha_1$-adrenergic receptor density. Sarcolemmal membranes were purified as described under “Experimental Procedures” from NLC ($n = 10$), TG $\alpha_47$ ($n = 5$), and TG $\alpha_43$ ($n = 15$) hearts. The estimated $B_{\text{max}}$ was determined using 300 pM $\text{[^{125}I]-HEAT}$ and 25 $\mu$g of membrane protein. Data are expressed as mean $\pm$ S.E. *, $p < 0.001$ compared with control (Student’s $t$ test).

**FIG. 2.** Basal myocardial diacylglycerol content. Lipid extraction was performed from NLC ($n = 5$) and TG $\alpha_43$ ($n = 5$) hearts as described under “Experimental Procedures.” Diacylglycerol content was quantified using 50 nmol of lipid phosphate as described. Data shown are mean $\pm$ S.E. *, $p < 0.005$ compared with control (Student’s $t$ test).

**FIG. 3.** Ventricular ANF mRNA Levels. A, representative Northern blot of total RNA (15 $\mu$g) isolated from the ventricles of NLC and TG $\alpha_43$ hearts ($n = 2$ each) and probed with a mouse ANF cDNA (top panel). The blots were stripped and reprobed with rat GAPDH cDNA (bottom panel). B, quantitation of the ANF signal. The signals from the ANF blots were counted on a Molecular Dynamics PhosphorImager and normalized to the GAPDH signal as described (9). Data shown are mean $\pm$ S.E. for $n = 8$ in each group. *, $p < 0.05$ versus control (Student’s $t$ test).
normal hearts. Following catheterization of anesthetized mice (10–13), we measured several hemodynamic parameters, including heart rate and \( \text{LV } \frac{dP}{dt_{\text{max}}} \) and \( \text{LV } \frac{dP}{dt_{\text{min}}} \), measures of cardiac contractility and relaxation, respectively. The results found in TG \( \alpha_43 \) animals and NLC mice under basal conditions and in response to isoproterenol are shown in Fig. 4. There was no difference in LV systolic pressure between the two groups (Fig. 4A). Base-line and \( \beta \)-agonist-stimulated heart rates were significantly depressed in TG \( \alpha_43 \) mice compared with NLC mice (Fig. 4B). There was no statistically significant difference in basal \( \Delta \frac{dP}{dt_{\text{max}}} \) or \( \Delta \frac{dP}{dt_{\text{min}}} \) in TG \( \alpha_43 \) mice versus NLC mice (Fig. 4, C and D), although the trend was for lower values in TG \( \alpha_43 \) animals. There was, however, a significant decrease in these parameters in TG \( \alpha_43 \) animals compared with NLC mice in response to progressive isoproterenol infusion. Thus, \( \beta \)-AR-mediated LV function is depressed in these animals, suggesting that there is significant cross-talk between the signaling of \( \alpha_1 \)-ARs and \( \beta \)-ARs in the hearts of these transgenic animals. The increased constitutive \( \alpha_1 \)-AR signaling present in TG \( \alpha_43 \) mice has a significant effect on cardiac physiological responses elicited by \( \beta \)-AR stimulation. Although previous studies have demonstrated that \( \alpha_1 \)-AR stimulation can lead to negative chronotropy (5), \( \alpha_1 \)-AR-mediated negative inotropy is a novel finding. To further study \( \alpha_1 \)-AR-mediated effects on cardiac contractility, it would be relevant to study these \( \text{in vivo} \) parameters in the presence of an \( \alpha \)-agonist such as phenylephrine. However, the use of phenylephrine presents major fundamental problems due to its predominant peripheral effects on systolic pressure, which would influence myocardial function independent of any myocardial \( \alpha_1 \)-AR signaling. The data presented above could have important clinical significance since, in pathophysiological conditions such as heart failure, there is significant loss of both \( \beta \)-AR density and functional coupling, which could potentially increase the role of \( \alpha_1 \)-AR signaling in response to endogenous catecholamines.

To investigate possible molecular mechanisms involved in the altered cardiac physiology seen in TG \( \alpha_43 \) animals, we carried out \( \text{in vitro} \) biochemical assays on heart extracts to examine the \( \beta \)-AR system. Signaling through \( \beta \)-ARs involves the activation of the G protein \( G_s \), which activates adenylyl cyclase, leading to increases in intracellular cAMP and activation of cAMP-dependent protein kinase A (22). Activated protein kinase A phosphorylates several myocardial proteins, leading to positive inotropy as well as chronotropy. The \( \beta \)-AR

![Fig. 4. In vivo assessment of cardiac function of TG \( \alpha_43 \) (\( n = 19 \)) (●) and NLC (\( n = 12 \)) (○) mice. Cardiac catheterization was performed on intact anesthetized animals. Four measured parameters are shown at base line and after progressive doses of isoproterenol. A, LV systolic pressure; B, heart rate; C, LV \( \frac{dP}{dt_{\text{max}}} \); D, LV \( \frac{dP}{dt_{\text{min}}} \). Data were analyzed with a two-way repeated measure analysis of variance. *, \( p < 0.0005 \); #, \( p < 0.05 \) (control versus transgenic). A significant between-group main effect in response to isoproterenol was found in B for heart rate (\( p < 0.001 \)). The pattern of change between groups (interaction) was statistically significant in B for heart rate (\( p < 0.05 \)), in C for LV \( \frac{dP}{dt_{\text{max}}} \) (\( p < 0.001 \)), and in D for LV \( \frac{dP}{dt_{\text{min}}} \) (\( p < 0.05 \)).]
system as well as several other G protein-coupled receptors undergo rapid desensitization, which is the loss of response in the continued presence of agonist. This is initiated by targeted receptor phosphorylation via a family of serine/threonine kinases known as the GRKs, of which the β-AR kinase (βARK1) is a prototypic member (23). We first examined β-AR density in myocardial membranes purified from TG α43 hearts and control nontransgenic myocardial membranes using 125I-CYP and found no significant difference in total β-AR density (46.5 ± 0.8 fmol/mg of membrane protein in TG α43 mice versus 44.3 ± 1.6 in controls). Therefore, changes in myocardial β-AR density cannot account for the differences in cardiac contractility seen in TG α43 mice.

We then studied myocardial membrane adenylyl cyclase activity. There was significantly lower basal adenylyl cyclase activity in membranes purified from TG α43 hearts compared with nontransgenic myocardial membranes (Table I). This could account for the decreased basal heart rate seen in these animals. As shown in Table I, agonist-stimulated adenylyl cyclase activity in TG α43 membranes compared with control membranes was also significantly depressed following addition of isoproterenol or norepinephrine. Interestingly, the depressed response to norepinephrine, a mixed α/β-agonist, was greater than with the strict β-agonist isoproterenol. These results indicate that the depressed in vivo cardiac function shown above (Fig. 4) is likely due, at least in part, to the lower adenylyl cyclase activity and an attenuated β-AR-mediated cAMP response. To examine whether decreased adenylyl cyclase activity in TG α43 membranes was secondary to enhanced α1-AR signaling, we studied adenylyl cyclase activity following addition of an α-agonist. Surprisingly, the addition of 100 μM phenylephrine resulted in significant lowering of basal activity in TG α43 membranes, whereas control membranes had no α1-AR-mediated cAMP response (Table I). This strongly suggests that further α1-AR stimulation leads to inhibition of membrane adenylyl cyclase activity. This also suggests that the significant decrease in basal adenylyl cyclase activity is due to enhanced basal α1-AR signaling present in TG α43 hearts. Strengthening these conclusions of a receptor-mediated phenomenon, NaF-stimulated adenylyl cyclase activities were similar in membranes from TG α43 animals and nontransgenic controls (Table I).

One hypothesis for the decrease in adenylyl cyclase activity in TG α43 myocardial membranes is coupling of WT α1B-ARs to the adenylyl cyclase inhibitory G protein, Gi. As mentioned above, dual coupling of α1-ARs to Gi and Gq has been demonstrated in vitro, but not yet investigated in vivo. To examine the potential involvement of Gi in the α1-AR-mediated cyclase responses, we intraperitoneally injected TG α43 and NLC mice with either 100 µg/kg PTx or 150 µl of saline and sacrificed the animals 24 h later. Myocardial membranes were purified from these animals, and adenylyl cyclase activities were measured. As shown in Table II, PTx treatment reversed the depressed basal cyclase activity in TG α43 membranes, and in fact, the percent increase in TG α43 basal activity was significantly higher compared with PTx-treated controls (Table II), indicating the enhanced Gi coupling of overexpressed WT α1B-ARs. Isoproterenol- and norepinephrine-stimulated adenylyl cyclase activities were also significantly increased in TG α43 membranes following PTx treatment. Additionally and in contrast to findings in saline-treated transgenic animals, phenylephrine did not decrease membrane adenylyl cyclase activity (Table II).

PTx treatment also enhanced NaF-stimulated activities compared with saline treatment, as expected, but TG α43 membranes did not differ from controls, indicating that there is no change in the levels or function of myocardial G proteins. To confirm this, we carried out protein immunoblotting of membranes for Gα16 and Gα11 and found no difference in protein levels in TG α43 versus control hearts (data not shown). Overall, PTx treatment converted TG α43 membranes to having the biochemical characteristics of control membranes. Thus, these results indicate that the involvement of PTx-sensitive G proteins is significantly higher in hearts overexpressing the WT α1B-AR, which could lead to the dampened myocardial performance seen in TG α43 animals (Fig. 4).

Potentially, there could be additional contributors to the decreased β-AR-mediated myocardial signaling and function seen in TG α43 animals. Since β-AR density is unaltered in TG α43 hearts, desensitization and functional uncoupling may be enhanced. In fact, the attenuated adenylyl cyclase activities and in vivo β-AR cardiac responses are similar to the phenotype we have previously described for transgenic mice with cardiac overexpression of either the βARK1 (11) or GRK5 (12). These two members of the GRK family are expressed in the heart and can produce desensitization and functional uncoupling of myocardial β-ARs (11, 12). Interestingly, it has recently been reported that βARK1 can be regulated by protein kinase C (24, 25). This regulation involves the enhancement of βARK activity following the phosphorylation of βARK1 by protein kinase C (24, 25). This is of significance in the present study since protein kinase C activity is apparently increased in the hearts of TG α43 animals as indicated by the measured increase in myocardial DAG content (Fig. 2). To investigate any in vivo regulation of βARK in the hearts of these transgenic mice, we carried out in vitro phosphorylation assays using myocardial extracts and the G protein-coupled receptor substrate rhodopsin (11, 12). Fig. 5 contains our findings using soluble myocardial fractions that represent GRK activity primarily attributable to βARK1. As shown, GRK activity was increased 3-fold in soluble extracts from the hearts of TG α43 animals compared with NLC heart extracts. Thus, this enhanced GRK activity seen in TG α43 hearts could contribute to the attenuated β-AR signaling observed in these animals, and interestingly, the increase in βARK activity is in the same range as that in transgenic mice overexpressing βARK1 (11) and following the development of pressure overload cardiac hypertrophy (26). The increased soluble GRK activity appears not to involve βARK1 up-regulation as protein immunoblots revealed no changes in the levels of βARK1 in the hearts of TG α43 animals compared with NLC (data not shown). Thus, these

### Table I

| Hearts Basal | ISO | NE | PE | NaF |
|--------------|-----|----|----|-----|
| Control     | 38.53 ± 2.96 | 68.25 ± 6.55 | 78.60 ± 4.36 | 269.60 ± 12.78 |
| TG α43      | 16.87 ± 2.13^a | 24.55 ± 4.47^b | 20.02 ± 5.57^c | 245.65 ± 11.83 |

^a ISO, isoproterenol; NE, norepinephrine; PE, phenylephrine.

^b p < 0.05 compared with control activity.

^c p < 0.05 compared with TG α43 basal activity (Student’s t test).
**Table II**

Adenylyl cyclase activity in nontransgenic (control) and TG α43 myocardial membranes after treatment with PTx

| Hearts | Basal | ISO* | NE | PE | NaF |
|--------|-------|------|----|----|-----|
| Control |       |      |    |    |     |
| Saline | 37.91 ± 3.41 | 70.08 ± 6.88 | 74.52 ± 7.06 | 32.40 ± 4.78 | 250.16 ± 15.12 |
| PTx    | 46.03 ± 6.36 | 83.63 ± 13.89 | 94.27 ± 16.02 | 43.59 ± 6.30 | 338.01 ± 25.91* |
| TG α43 |       |      |    |    |     |
| Saline | 20.07 ± 4.61 | 28.10 ± 5.43 | 23.85 ± 4.97 | 13.17 ± 2.22* | 241.91 ± 20.36 |
| PTx    | 50.67 ± 7.21* | 90.53 ± 10.86* | 83.74 ± 16.45* | 46.98 ± 9.78* | 365.92 ± 27.06* |

*ISO, isoproterenol; NE, norepinephrine; PE, phenylephrine.

a p < 0.05 compared with control activity (saline).
b p < 0.05 compared with control activity (saline) and p < 0.05 compared with TG α43 basal activity (saline).
c p < 0.05 compared with control activity (saline) and p = not significant compared with control (PTx) activities.
d p < 0.05 compared with TG α43 (saline) (Student’s t test).

data are the first to suggest possible enhancement of βARK activity as an in vivo consequence of increases in the α1-AR/Gi/protein kinase C cascade. Thus, previous in vitro findings of protein kinase C regulation of βARK1 (24, 25) may have important in vivo implications. In additional studies, we examined the protein content of GRK5, which is found exclusively in myocardial membranes after treatment with PTx.

In summary, we have demonstrated that α-MyHC-directed cardiac overexpression of the WT α1AR can have profound effects on adrenergic signaling and in vivo cardiac physiology. This is evident even under basal conditions, suggesting that WT α1-ARs can signal spontaneously at this level of overexpression (>40-fold), much like the findings in mice overexpressing the WT β2-AR (10, 14). In TG α43 animals, Gq signaling was enhanced as assessed by myocardial DAG content and ventricular ANF mRNA expression, but in contrast to our previously described transgenic mice overexpressing a CAM α1B-AR, myocardial hypertrophy was not associated with this enhanced Gq signaling. In vivo hemodynamic evaluation of TG α43 mice revealed significantly dampened left ventricular function in response to β-agonist stimulation compared with nontransgenic controls. Probing possible mechanisms for altered myocardial function revealed that dual coupling of the α1B-ARs exists as PTx-sensitive G protein-mediated attenuation of adenylyl cyclase was seen in myocardial membranes purified from TG α43 animals. The surprising lack of hypertrophy in TG α43 and TG α47 animals suggests that the agonist-independent signaling of the CAM α1B-AR differs from the enhanced signaling through WT α1B-ARs. One possible explanation for this phenomenon is the significant dual G protein coupling seen in animals overexpressing the WT α1B-AR, which greatly alters β-AR signaling in these mice. This α1-AR regulation of β-AR signal transduction is not evident in the CAM α1B-AR transgenic mice, which suggests a true difference in the biochemical phenotype of these tissues. Interestingly, we have recently observed significant coupling of overexpressed WT β2-ARs to Gq in the hearts of transgenic animals. We also observed enhanced GRK activity, presumably βARK1, in the hearts of TG α43 mice, which also could contribute to the observed phenotype. Enhanced βARK activity is probably due to elevated protein kinase C activity as a result of enhanced α1-AR/Gq signaling, in as much as protein kinase C activation of βARK1 has been observed in vitro (24, 25). In support of the notion that this is a Gq-mediated phenomenon, CAM α1B-AR animals have a similar increase in soluable GRK activity.

These results demonstrate that myocardial α1-AR signaling can significantly alter the signaling through myocardial β-ARs via two distinct receptor-mediated mechanisms. First, α1-AR coupling to PTx-sensitive G proteins can occur in vivo, and second, enhanced α1-AR signaling can lead to the enhanced activity of βARK1, which can cause functional uncoupling of β-ARs. Such cross-talk between different adrenergic signaling pathways could have important implications in organs like the heart that contain α- and β-ARs and might become critical in pathological conditions where additional signaling alterations take place. For example, βARK1 expression and activity have been shown to be increased in end-stage human congestive heart failure (28). Thus, the TG α43 animals represent a unique model to study specific in vivo interactions between α1- and β-AR signaling as well as other pathways that can be regulated by GRK activity.

S. A. Akhter, C. A. Milano, and W. J. Koch, unpublished observations.

R. P. Xiao, S. A. Akhter, and W. J. Koch, unpublished observations.
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