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Low- and high-level transgenic expression of β_2-adrenergic receptors differentially affect cardiac hypertrophy and function in Gαq-overexpressing mice

GERALD W. DORN II*†, NICOLE M. TEPE†, JOHN N. LORENZ‡, WALTER J. KOCH§, AND STEPHEN B. LIGGETT*†¶

Departments of *Medicine, †Pharmacology, and ‡Physiology, University of Cincinnati, College of Medicine, Cincinnati, OH 45267; and §Department of Surgery, Duke University Medical Center, Durham, NC 27710

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ABSTRACT **Transgenic overexpression of Gαq in the heart triggers events leading to a phenotype of eccentric hypertrophy, depressed ventricular function, marked expression of hypertrophy-associated genes, and depressed β-adrenergic receptor (βAR) function. The role of βAR dysfunction in the development of this failure phenotype was delineated by transgenic coexpression of the carboxyl terminus of the βAR kinase (βARK), which acts to inhibit the kinase, or concomitant overexpression of the β_2AR at low (≈30-fold, Gαq/$\beta_2\mathrm{AR_L}$), moderate (≈140-fold, Gαq/$\beta_2\mathrm{AR_M}$), and high (≈1,000-fold, Gαq/$\beta_2\mathrm{AR_H}$) levels above background βAR density. Expression of the βARK inhibitor had no effect on the phenotype, consistent with the lack of increased βARK levels in Gαq mice. In marked contrast, Gαq/$\beta_2\mathrm{AR_L}$ mice displayed rescue of hypertrophy and resting ventricular function and decreased cardiac expression of atrial natriuretic factor and α-skeletal actin mRNA. These effects occurred in the absence of any improvement in basal or agonist-stimulated adenylyl cyclase (AC) activities in crude cardiac membranes, although restoration of a compartmentalized β_2AR/AC signal cannot be excluded. Higher expression of receptors in Gαq/$\beta_2\mathrm{AR_M}$ mice resulted in salvage of AC activity, but hypertrophy, ventricular function, and expression of fetal genes were unaffected or worsened. With ≈1,000-fold overexpression, the majority of Gαq/$\beta_2\mathrm{AR_H}$ mice died with cardiomegaly at 5 weeks. Thus, although it appears that excessive, uncontrolled, or generalized augmentation of βAR signaling is deleterious in heart failure, selective enhancement by overexpressing the β_2AR subtype to limited levels restores not only ventricular function but also reverses cardiac hypertrophy.**

β-adrenergic receptor (βAR)-mediated cardiac inotropic responsiveness is critical to meeting the acute hemodynamic demands of homeostasis. This reserve is lost in cardiac hypertrophy or failure because of alterations in βAR expression and/or coupling to downstream effectors (1–3). The mechanisms of such desensitization are not well understood, although in some models, βAR uncoupling appears to be because of enhanced activity of the βAR kinase (βARK1) (4, 5). Thus, despite increased activity of the sympathoadrenal system, failing hearts exhibit depressed responsiveness to endogenous catecholamines as well as to exogenously administered β-agonist inotropic agents. These observations have prompted various pharmacologic and genetic interventions aimed at restoring βAR function in failing hearts. While the efficacy of pharmacologic stimulation of βAR may be limited by receptor desensitization and proarrhythmic effects, transgenic overexpression of β_2AR or of a dominant-negative inhibitor of βARK (βARK minigene) have favorably modified cardiac function in normal mice (6, 7). Recently, expression of the βARK minigene was also reported to improve myocardial contractility in a mouse genetic model of dilated cardiomyopathy (5). These benefits of enhanced/restored βAR function in normal and dilated cardiomyopathic mouse hearts suggested that a similar approach might be beneficial in a model of primary cardiac hypertrophy and contractile depression such as that exhibited by transgenic mice overexpressing the α-subunit of Gq at ≈5-fold over background (8). Such expression triggers a series of signaling events that recapitulates many aspects of experimental hypertrophy/failure and the human syndrome. The development of load-independent hypertrophy, ventricular dysfunction, and expression of fetal genes via physiologically relevant means makes the Gαq-overexpressing mouse a useful model for assessing the relevance of individual pathways via further transgenesis. The current studies determined the functional and developmental cardiac effects of overexpressing β_2AR or the dominant-negative βARK minigene combined with transgenic expression of Gαq. βARK inhibition had no effects on the Gαq phenotype. The response to overexpression of β_2AR was expression-dependent: lower levels of β_2AR improved cardiac contractility and attenuated hypertrophy development, whereas high levels of expression exaggerated hypertrophy and contractile depression with lethal consequences in Gαq overexpressors.

METHODS

Transgenic Models. Heterozygous transgenic FVB/N mice overexpressing Gαq ≈5-fold over endogenous levels (Gαq40) have been described (8, 9), as have heterozygous β_2AR (6, 10) and homozygous βARK minigene (7) overexpressing C57BL/6J mice. To achieve higher levels of β_2AR expression, two additional lines of β_2AR-overexpressing FVB/N mice, herein designated $\beta_2\mathrm{AR_M}$ and $\beta_2\mathrm{AR_H}$, were made and screened exactly as described (10). Transgenic expression for all mice was driven by the full-length α myosin heavy chain promoter (11). As indicated, Gαq mice and βARK minigene, or Gαq mice and one of the β_2AR-overexpressing mice, were mated to generate dual transgenic animals (heterozygous for each gene). When indicated, control mice (nontransgenic and Gαq) consisted of FVB/N + C57BL/6J hybrid crosses in all cases. All studies were carried out with age-matched mice at the ages denoted.

Physiological Measurements. M mode echocardiography was performed on lightly anesthetized, spontaneously breath-

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Abbreviations: βAR, β-adrenergic receptor; $\beta_2\mathrm{AR_L}$, $\beta_2\mathrm{AR_M}$, $\beta_2\mathrm{AR_H}$; low, moderate, and high levels of β_2AR overexpression; βARK1, βAR kinase; GRK, G protein-coupled receptor kinase; ANF, atrial natriuretic factor; MLP, muscle LIM protein; AC, adenylyl cyclase; MAP, mitogen-activated protein.

[¶] To whom reprint requests should be addressed at: University of Cincinnati College of Medicine, 231 Bethesda Avenue, Room 7511, P.O. Box 670564, Cincinnati, OH 45267-0564. e-mail: stephen.liggett@uc.edu.

ing mice as described (8, 12). In some cases, mice were studied before and 5 min after intraperitoneal isoproterenol (100 ng/g body weight). Left ventricular mass (LVM) was calculated from M mode measurements as: LVM = $[(\mathrm{LVEDD} + \mathrm{SWT} + \mathrm{PWT})^3 - \mathrm{LVEDD}^3] \times 1.832$, where LVEDD is left ventricular end diastolic dimension, SWT is septal wall thickness, and PWT is posterior wall thickness. Closed chest invasive hemodynamic measurements were performed on 12-week-old sedated, lightly anesthetized mice as described (8, 10) by using 1.4 French Millar catheters placed into the left ventricle via a retrograde transaortic approach. Dobutamine was infused at concentrations from 1 to 32 mg·g^{-1}·min^{-1}. Right atrial pacing was carried out by methods described (8) using a pacing wire placed via the right internal jugular vein.

Molecular and Biochemical Measurements. Expression of atrial natriuretic factor (ANF), β-myosin heavy chain, and α-skeletal actin mRNA was compared by RNA dot-blot analysis (8, 12). For determination of adenylyl cyclase activities, ventricles were minced in 5 mM Tris (ph 7.4)/2 mM EGTA buffer with 5 μg/ml each leupeptin, soybean trypsin inhibitor, aprotinin, and benzamidine and then homogenized with a polytron for 5 seconds. The homogenate was diluted and centrifuged at 500 × *g* for 10 min. The supernatant was centrifuged at 40,000 × *g* for 10 min, and the membranes were resuspended in a buffer providing for 2 mM Tris (pH 7.4)/12 mM MgCl$_2$/0.9 mM EGTA in the final reaction. Reaction conditions and detection of cAMP were exactly as described except that incubations were for 10 minutes (13). Receptor density was determined by [^{125}I]cyanopindolol ([^{125}I]CYP) and expressed as fmol/mg membrane protein (10). G protein-coupled receptor kinase (GRK) activity of whole heart homogenates was determined by using rod outer segments (rhodopsin) as substrate in an *in vitro* assay as described (14, 15). Mitogen-activated protein (MAP) kinase assays were performed as described (8). In addition, phosphotyrosine-containing proteins from heart homogenates were immunoprecipitated with anti-phosphotyrosine conjugated agarose (Upstate Biotechnology, Lake Placid, NY) before immunoblotting with an ERK 1/2 antibody (Santa Cruz Biotechnology).

Morphometry and Histology. Wet heart weight indexed to body weight and histological examination of Masson's trichrome-stained ventricular coronal sections used standard techniques as described (8, 9, 12).

Statistical Analysis. Data are reported as mean ± SEM. Statistical comparisons used two-tailed Student's *t* test for two-group comparison or one-way ANOVA followed by the Bonferroni procedure for multiple group comparison. Statistical significance was accepted at $P < 0.05$.

RESULTS

A feature of Gαq-overexpressing mouse hearts that may contribute to their characteristic contractile depression is absence of inotropic and chronotropic responsiveness to βAR agonists (8). Agonist-promoted stimulation of AC is markedly depressed in myocardial membranes from these mice despite normal levels of cardiac βAR and a normal ratio of the β_1/β_2 AR subtypes. Recent studies have indicated that Gαq mice have decreased receptor–Gs coupling, increased expression of Gi, and decreased expression of adenylyl cyclase (G.W.D. and S.B.L., unpublished data). The receptor coupling defect can theoretically be reversed by overexpression of the receptors or by inhibition of those kinases considered likely mediators of the desensitization. Recent studies have implicated βARK as a mechanism for βAR uncoupling in the hypertrophied ventricles of pressure-overloaded mice (4) and the muscle Lim protein (MLP) knockout mouse (5). In the current study, heterozygous transgenic Gαq overexpressors were mated with homozygous βARK minigene expressors or with three different heterozygous β_2AR overexpressors. βAR expression (*n* = 4 mice from each group) in ventricular membranes measured using radioligand binding was 25 ± 5 fmol/mg in Gαq overexpressors, which was not significantly different from nontransgenic levels of 28 ± 3 fmol/mg. Expression of the βARK minigene did not change βAR expression. The level of βAR in the initial Gq/β_2AR crosses was 809 ± 76 fmol/mg (*n* = 4), representing an ≈30-fold increase over the Gαq mice and the nontransgenics. Because these double transgenic mice had a relatively lower level of β_2AR expression compared with the subsequently generated transgenic lines, these were designated Gαq/β_2AR$_L$.

Mice from the Gαq/βARK$_{\mathrm{mini}}$ and Gαq/β_2AR$_L$ crosses underwent screening for heart rate and left ventricular contractility with echocardiography. βARK minigene expression did not affect heart rate, left ventricular fractional shortening, or left ventricular mass of Gαq overexpressors (Table 1). These results suggest that βARK-mediated events likely play a minor role in the impairment of βAR function in the Gαq transgenic mouse. Although an increased level of βARK or GRK activity is not a requisite for this class of kinases to be implicated as an uncoupling mechanism, it is interesting to note that in the pressure-overload (4) and MLP-knockout (5) mouse models GRK activity is increased. Studies were thus undertaken to determine cardiac GRK activity in Gαq mice and nontransgenic littermates, so as to correlate the lack of an effect seen in the βARK$_{\mathrm{mini}}$/Gαq crosses (Fig. 1). By using rod outer segments (rhodopsin) as an *in vitro* substrate, GRK activity from Gαq hearts was not found to be elevated compared with those of nontransgenic littermates, and indeed trended toward being lower. Taken together, the above results indicate that βARK-mediated phosphorylation of β_1-or β_2AR in the hearts of the Gαq mice is not a major mechanism of uncoupling of these receptors.

In contrast to this lack of demonstrable physiologic effects with the βARK inhibitor, Gαq/β_2AR$_L$ mice displayed an improvement over Gαq mice in fractional shortening (44 ± 2 vs. 32 ± 2) and left ventricular mass (74 ± 1 vs. 90 ± 3 mg) (Table 1 and Fig. 2*A*). The observed inotropic effects were not simply a consequence of the increase in heart rate, because a similar increase in Gαq overexpressor heart rate stimulated by atropine administration did not increase left ventricular fractional shortening (data not shown). Thus, echocardiographic analysis indicated that increased β_2AR expression, but not inhibition of βARK, improved cardiac function in Gαq over-

Table 1. Echocardiographic left ventricular functional and morphologic parameters in transgenic mice

Strain	Heart rate, beats per minute	Fractional shortening, %	End systolic dimension, mm	End diastolic dimension, mm	Septal wall thickness, mm	Posterior wall thickness, mm	Left ventricular mass, mg	*n*
NTG	470 ± 30	50 ± 1	1.7 ± 1	3.4 ± 2	0.41 ± 0.01	0.40 ± 0.01	65 ± 4	7
Gαq	288 ± 17*	32 ± 2	2.5 ± 1*	4.0 ± 0.1	0.42 ± 0.01	0.42 ± 0.01	90 ± 3*	5
Gαq/βARK$_{\mathrm{mini}}$	282 ± 22*	27 ± 1*	2.8 ± 0.1*	4.1 ± 0.1	0.41 ± 0.01	0.40 ± 0.01	91 ± 4*	6
Gαq/βAR$_L$	348 ± 18†	44 ± 2†	2.1 ± 0.1	3.7 ± 0.1	0.40 ± 0.01	0.41 ± 0.01	74 ± 1†	5

HR, heart rate; FS, fractional shortening; ESD, end systolic dimension; EDD, end diastolic dimension; SWT, septal wall thickness; PWT, posterior wall thickness; LV mass, left ventricular mass. * = $P < 0.05$ vs NTG. † = $P < 0.05$ vs Gαq (*n* = 5–11 per group).

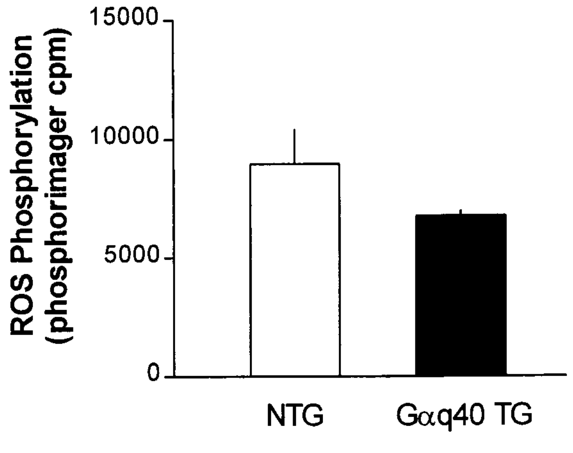

FIG. 1. GRK activity in transgenic mice overexpressing Gαq in the heart. Activities of cytosolic preparations were determined in an *in vitro* assay with rod outer segments as substrate (see *Materials and Methods*). No differences were found between Gαq mice and nontransgenic littermates. Shown are the results from three independent experiments.

expressors, and a more detailed analysis of combined β_2AR and Gαq overexpression was undertaken.

The functional effects of β_2AR overexpression in Gαq mice were further characterized by invasive hemodynamic assessment of basal and isoproterenol-stimulated ventricular contractility, assessed as the peak rate of left ventricular pressure development (+dP/dt$_{\max}$, Fig. 2*B*). Baseline left ventricular +dP/dt$_{\max}$ was significantly improved in Gαq/β_2AR$_L$ overexpressors compared with Gαq overexpressors at intrinsic heart rates (5,992 ± 653 vs. 4,557 ± 468 mmHg per sec, $n = 4$) (1 mmHg = 133 Pa) or at matched (atrial paced) heart rates of 450 beats per min (5,438 ± 137 vs. 4,595 ± 534 mmHg per sec, $n = 4$). To determine whether increased responsiveness to βAR agonists was present in the Gαq/β_2AR$_L$ mice, contractility was measured in paced hearts in response to intravenous administration of the nonselective agonist isoproterenol. However, only a small increment in +dP/dt$_{\max}$ was observed for the Gq/β_2AR$_L$ mice. At the highest concentration studied (32 ng·g^{-1}·min^{-1}), the +dP/dt$_{\max}$ of Gαq/β_2AR$_L$ mice was ≈37% higher than that of Gαq mice, whereas that of nontransgenic mice was ≈266% greater. This minimal improvement in isoproterenol responsiveness in Gαq/β_2AR$_L$ mice prompted a biochemical analysis of βAR receptor-stimulated AC activity. As shown in Fig. 3, basal and maximal isoproterenol-stimulated AC activities were depressed in the Gαq mice, and coexpression of low levels of β_2AR did not increase activities over those found with Gαq mice.

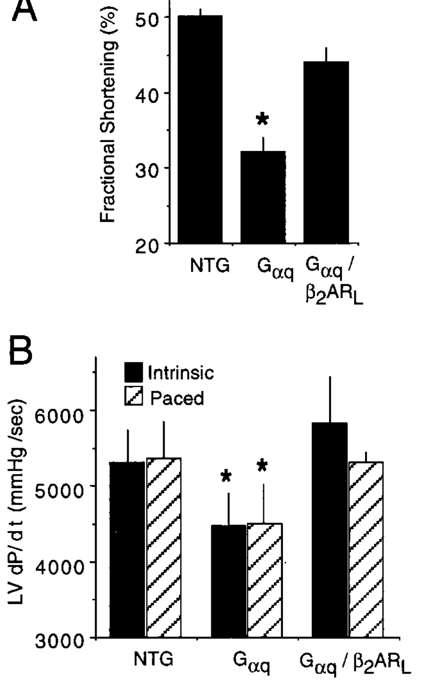

FIG. 2. Effects of 30-fold β_2AR overexpression on left ventricular function in Gαq overexpressing transgenic mice. (*A*) Echocardiographic left ventricular shortening is depressed in Gαq overexpressors compared with nontransgenic controls (NTG). In Gαq/β_2AR$_L$ mice, fractional shortening is normalized ($n = 6$–11). (*B*) Left ventricular +dP/dt$_{\max}$ at intrinsic heart rates (solid bars) and matched atrial paced heart rates (450 beats per min) (hatched bars) is depressed in Gαq overexpressors and normalized in the Gαq/β_2AR$_L$ mice under both conditions ($n = 3$–5). *, $P < 0.02$ vs. NTG.

The results with Gαq/β_2AR$_L$ overexpressors demonstrated that a ≈30-fold increase in β_2AR expression could improve resting ventricular contractility without significantly enhancing responsiveness to a βAR agonist or measurably increasing myocardial adenylyl cyclase activity. Therefore, to further increase β_2AR receptor expression levels and activate myocardial AC with the possibility of enhancing function (particularly that stimulated by agonist) in Gαq overexpressors, two lines of β_2AR transgenic mice with higher expression levels were mated with Gαq overexpressors. The mice so generated exhibited moderate (Gαq/β_2AR$_M$ = 3,564 ± 919 fmol/mg, $n = 4$) and high (Gαq/β_2AR$_H$ = 23,294 ± 2,438 fmol/mg, $n = 4$) levels of β_2AR expression, representing ≈140- and ≈1,000-fold overexpression, respectively, compared with Gαq mice.

Gαq/β_2AR$_H$ mice did not survive past the age of 5 weeks, and most of these animals died suddenly by 3 weeks with massively enlarged hearts. Gαq/β_2AR$_M$ mice did not exhibit this very early mortality, and their cardiac functional and biochemical characteristics were studied at 8 weeks of age. Echocardiographic analysis (Fig. 4) of left ventricular fractional shortening demonstrated no improvement in resting contractility in Gαq/β_2AR$_M$ mice. Furthermore, as with Gαq mice, isoproterenol failed to increase echocardiographic left ventricular fractional shortening (Fig. 4). As shown, in NTG mice, left ventricular fractional shortening increased ≈50% with intraperitoneal isoproterenol, whereas no statistically significant increase was observed with the Gαq mice or the Gαq/β_2AR$_M$ mice. AC activities were, however, increased both at baseline and in response to isoproterenol in Gαq/β_2AR$_M$ hearts, indicating that the 140-fold increase in β_2AR

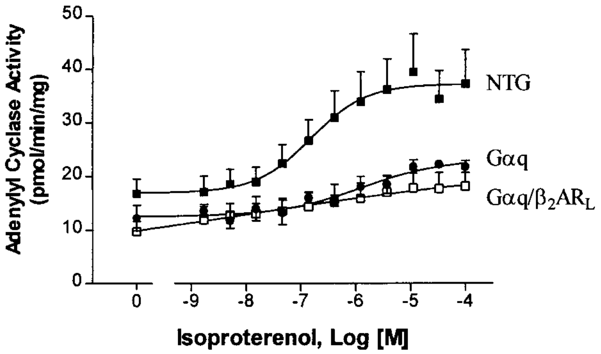

FIG. 3. βAR signaling to AC in cardiac membranes from nontransgenic, Gαq-transgenic, and dual-transgenic Gαq/β_2AR$_L$ mice. Basal (nonagonist) and maximal isoproterenol-stimulated activities were decreased in the Gαq mice ($P < 0.02$). ≈3-fold overexpression of β_2AR in the Gαq background (Gαq/β_2AR$_L$) had no effect on this signaling. Shown are results (mean ± SEM) from experiments performed with four mice from each group.

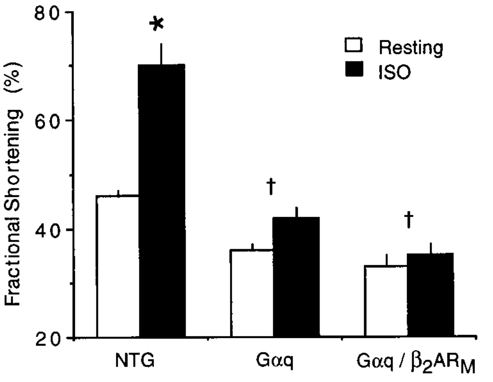

FIG. 4. Effects of 140-fold β_2AR overexpression on ventricular function in Gαq-overexpressing transgenic mice. As shown, isoproterenol-stimulated increases in left ventricular fractional shortening were not observed in Gαq/β_2AR$_M$ and Gαq mice. ($n = 6$ each); *, $P < 0.05$ vs. untreated; †, $P < 0.05$ vs. nontransgenic.

expression was sufficient to intrinsically activate AC and to restore biochemical agonist responsiveness to nearly nontransgenic levels (Fig. 5).

Gαq transgenic mice display not only contractile depression but also hypertrophy (8, 12). Gαq/β_2AR$_L$ mice exhibited a normalization of basal ventricular contractility without enhanced biochemical responsiveness to β-agonist stimulation, whereas Gαq/β_2AR$_M$ failed to show improvement in ventricular contractile function despite increased AC signaling. We examined whether β_2AR expression modified the development of cardiac hypertrophy in Gαq overexpressors by assessing morphometric (heart/body weight ratios), echocardiographic (calculated LV mass), and molecular (expression of the hypertrophy-associated genes ANF, β-myosin heavy chain, and α-skeletal actin) markers. These studies revealed normalization of Gαq-stimulated hypertrophy as assessed by heart/body weight ratios and calculated LV mass with 30-fold overexpression of β_2AR (Fig. 6*A*) and a corresponding inhibition of hypertrophy-associated ANF and α-skeletal actin gene expression (Fig. 6*B*). In contrast, Gαq/β_2AR$_M$ mice had massive enlargement of the heart, and hypertrophy gene expression remained at high levels.

Histologic analysis of the heart from the Gαq, Gαq/β_2AR$_L$, and Gαq/β_2AR$_M$ mice was consistent with the morphometric, functional, and molecular findings in that Gαq/β_2AR$_M$ mouse hearts exhibited widespread interstitial fibrosis with focal areas of replacement fibrosis suggesting chronic cardiomyocyte dropout (16). Fibrosis was not consistently observed in the Gαq or Gαq/β_2AR$_L$ groups (Fig. 7).

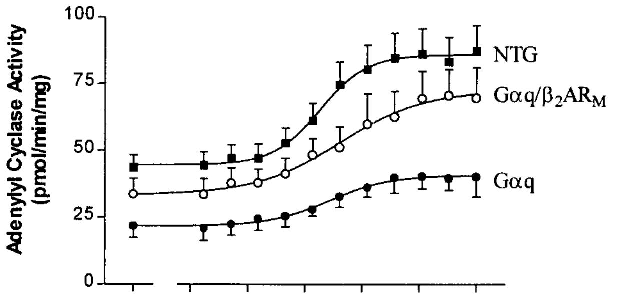

FIG. 5. βAR signaling to AC in cardiac membrane from nontransgenic, Gαq-transgenic, and dual-transgenic Gαq/β_2AR$_M$ mice. Overexpression of β_2AR to ≈140-fold in the Gαq mice resulted in enhanced basal and isoproterenol stimulated activities. Maximal activities were not different than NTG ($P = 0.61$), while the basal activities trended toward being lower, but not statistically different ($P = 0.08$), than NTG. Shown are mean results from experiments performed with four mice from each group.

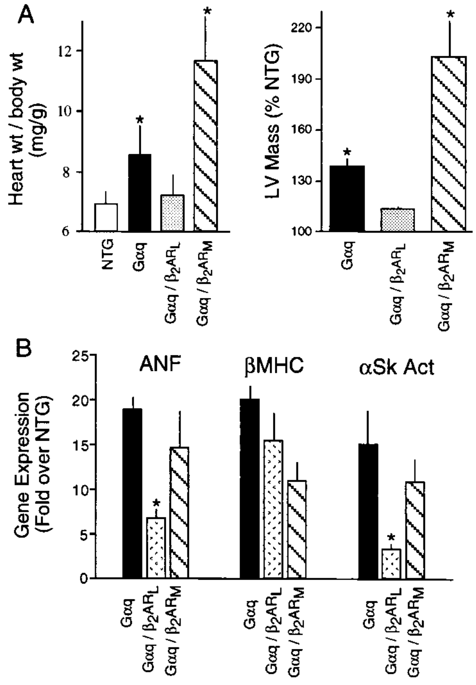

FIG. 6. Effects of 30- and 140-fold β_2AR overexpression on cardiac hypertrophy in Gαq-overexpressing transgenic mice. All mice were studied at 12–14 weeks of age. (*A*) Normalization of cardiac mass in Gαq overexpressors expressing β_2AR at lower levels (Gαq/β_2AR$_L$), but enhanced hypertrophy in Gαq with higher level β_2AR expression (Gαq/β_2AR$_M$) ($n = 6$–12). *, $P < 0.05$ vs. NTG. (*B*) Attenuation of hypertrophy-associated gene expression in hearts of Gαq/β_2AR$_L$, but not Gαq/β_2AR$_M$, mice. Sk act, α-skeletal actin. $n = 6$ per group. *, $P < 0.01$ vs. Gαq.

Recent studies have shown that β_2AR couple to activation of MAP kinase under conditions of receptor phosphorylation by protein kinase A and subsequent Gi coupling (17). We considered that transgenic β_2AR overexpression might evoke cardiac MAP kinase activation, which might affect myocyte growth and contribute to the exaggerated phenotype of the Gαq/β_2AR mice. We have previously shown that MAP kinase is not activated in the Gαq mice (8). Experiments to address this issue were carried out with Western blots by using cardiac extracts probed with antiserum reactive to activated ERK 1/2 and immunoprecipitated tyrosine phosphoproteins from extracts probed with nonselective ERK 1/2 antisera. These studies were performed with Gαq, β_2AR$_M$, or Gαq/β_2AR$_M$ mice. No evidence of activation was detected (data not shown).

DISCUSSION

Overexpression of Gαq in the heart and the resulting autonomous activation of downstream Gq signaling pathways causes eccentric hypertrophy with modest contractile depression but not overt heart failure. This cardiac phenotype may therefore represent the purely biochemical consequences of signaling by Gq-coupled receptor agonists such as angiotensin II, epinephrine, or endothelin in the absence of mechanical or hemodynamic cardiac stress. The resulting hypertrophy, in terms of increased cardiac chamber mass, cardiomyocyte cross sectional area, cardiomyocyte and ventricular mechanical function, and qualitative fetal gene expression (8, 12) resembles pressure-overload hypertrophy which is transitioning toward decompensated heart failure, a condition we have termed "compromised" (12). An obligatory role for Gαq signaling in

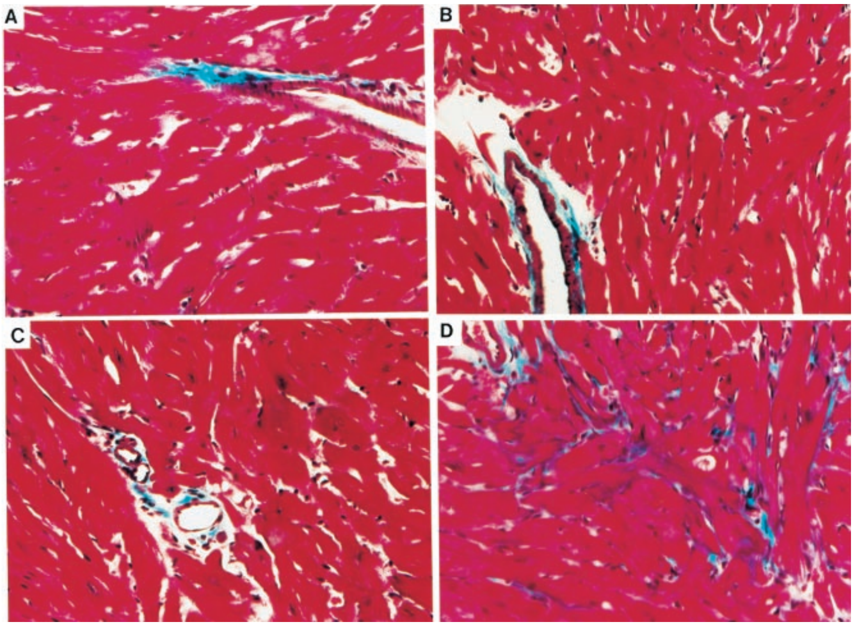

FIG. 7. Myocardial fibrosis in Gαq/β_2AR$_M$ transgenic mice. Masson's trichrome stain of myocardial section from mid-left ventricular free wall of 8-week-old NTG (*A*), Gαq (*B*), Gαq/β_2AR$_L$ (*C*), and Gαq/β_2AR$_M$ (*D*). The perivascular blue staining serves as a control for the stain in that it identifies vascular collagen. Gαq/β_2AR$_M$ exhibits significant fibrosis not observed in other groups (representative of 4–6 individual hearts examined). (Magnification, ×200.)

pressure-overload hypertrophy has recently been established by using a transgenic dominant-negative approach (18). Thus, the transgenic Gαq overexpression is a suitable approach for delineating the consequences of genetic or pharmacologic interventions within the context of physiologically relevant stimuli. As with human heart failure, Gαq-overexpressing hearts are hyporesponsive to βAR stimulation and thus provide a background for examining the effects of enhanced βAR signaling.

Expression of the βARK inhibitor did not alter contractility or hypertrophy development in Gαq mouse hearts, which contrasts with the beneficial effects of βARK inhibition in the MLP knockout mouse model of dilated cardiomyopathy (5). This is likely due to the differences in the underlying mechanisms that cause βAR impairment in the two models. As shown in the current study, GRK activity is not increased in the hearts of Gαq mice, whereas such activity is increased ≈2-fold in the MLP knockout mouse (5). In the Gαq mouse, the kinase responsible for βAR uncoupling appears to be protein kinase C (15). Furthermore, the mechanism for inhibition of βARK1 activity by the βARK inhibitor peptide is its binding of free $\beta\gamma$ proteins that are necessary for βARK1 translocation to the receptor (19). Overexpression of a Gα protein subunit might also bind free $\beta\gamma$ and thus minimize the effectiveness of the βARK inhibitor in the context of Gαq overexpression.

In contrast to coexpression of the βARK inhibitor, β_2AR overexpression corresponding to a ≈30-fold increase nearly normalized cardiac contractility assessed either by echocardiographic or by invasive hemodynamic techniques. An important feature of the functional salvage achieved by lower levels of β_2AR expression was inhibition of the hallmark Gαq-mediated cardiac hypertrophy, assessed by gravimetric heart weights and calculated left ventricular mass, and by attenuated expression of two molecular markers of cardiac hypertrophy, ANF and α-skeletal actin. This overexpression, then, effectively increases the number of functional receptors (despite ongoing uncoupling) to a level such that partial restoration of function is obtained. However, the expected corresponding increase in either basal or isoproterenol-stimulated AC activity in cardiac membranes was not detected at these levels of β_2AR expression. With ≈140-fold overexpression of β_2AR, basal and maximal isoproterenol-stimulated AC activities were significantly increased to levels very similar to nontransgenic littermates. Yet, these mice exhibited depressed contractile function, worsening cardiomegaly, and continued elevated expression of hypertrophy-associated genes. These observations with the Gαq/β_2AR$_M$ transgenic mice are particularly noteworthy because overexpressing the same number of receptors in the absence of Gαq overexpression results in mice that exhibit only subtle changes in hypertrophy gene expression with no measurable cardiac hypertrophy and no increase in mortality when followed for up to 25 weeks (unpublished results). All mice expressing Gαq in combination with β_2AR at the highest levels (≈1,000-fold overexpression) died before the age of 5 weeks with massively dilated hearts. Thus, the chronic increase in basal and agonist-stimulated AC activity achieved by β_2AR expression at these levels appeared to evoke an aggressive form of myocardial degeneration in the context of the compromised hypertrophy of Gαq overexpressors. In this respect, our results are similar to those of Rockman *et al.* (5), who have reported a lethal effect of β_2AR overexpression at very high levels in the MLP knockout mouse model.

Our current results are not confounded by strain differences because studies were always carried out with transgenic or nontransgenic mice of the same genetic background (FVB/N + C57BL/6J or FVB/N). Of potential concern might be that the rescue cross (Gαq/β_2AR$_L$) is of the hybrid background whereas the other two β_2AR crosses, which do not show rescue, are in the FVB/N background. However, the Gαq/βARK$_{mini}$ mouse is also a hybrid but displays no improvement in ventricular function. And finally, we have bred the Gαq mouse onto the C57BL/6J background and have observed no change in the expression of Gαq or the physiologic/molecular phenotype.

It is important to distinguish signaling because of chronic agonist infusion acting at β_1AR, transgenic overexpression of β_2AR, and transgenic overexpression of G$_{s\alpha a}$, in that the resulting phenotypes are quite different, potentially because of different signaling pathways being enhanced. βAR subtypes differ in agonist-binding affinity for norepinephrine, in coupling pathways, and in regulation by agonists (20–22). Recent studies have shown that β_2AR couple more efficiently to the stimulation of AC compared with the β_1AR expressed in otherwise identical recombinant cells (20, 21) but that the β_1AR appears to couple more efficiently to the opening of the L type calcium channel (22, 23). Coupling to inhibitory G proteins by β_2AR has been shown to activate MAP kinase as well as inhibit AC (17), and direct coupling of the carboxyl terminus of the receptor to the Na^+/H^+ exchanger regulatory factor affects proton exchange (24). In contrast, coupling of β_1AR to either of these latter two pathways in cells has not been reported. Regarding coupling to AC/cAMP, recent studies have suggested that in cardiac myocytes cAMP production may be compartmentalized in a subtype-specific manner (25). Thus, coupling to other potentially beneficial pathways and subsarcolemma-restricted activation of Gαs may be a potential explanation for the observed physiologic effects of lower level β_2AR expression on cardiac function in the absence of measurable increases in crude membrane AC activity. At the higher levels of β_2AR expression achieved in the Gq/β_2AR$_M$ mice, however, the striking increases in AC activity represents substantially enhanced coupling to Gαs. The worsening hypertrophy observed in these mice is likely because of such enhanced coupling, but promiscuous activation of as yet unknown effectors must be considered. Overexpression of Gαs might be expected to evoke a generalized increase in signaling to AC that is not necessarily β_1AR- or β_2AR-like. Indeed, such transgenic mice appear to develop a subtle cardiomyopathy that is apparent only in senescence (26). Finally, the observed effects could potentially have been caused by enhancement of signaling to known pathways that directly affect cell growth. We considered that MAP kinase activity might be elevated by overexpression of β_2AR in the heart (with or without Gαq co-overexpression). However, we have not observed such, despite several different detection methods (see ref. 8 and above). Recent studies have shown that β-arrestin acts as an adapter protein, binding the GRK phosphorylated β_2AR to c-Src for initiation of MAP kinase signaling (27). Thus, the lack of increased GRK activity in the Gαq model may be the basis for no apparent increase in MAP kinase activation in these hearts.

In conclusion, a substantial body of evidence exists supporting the potentially detrimental effects of chronic, unregulated sympathetic stimulation of the heart, particularly within the context of compromised ventricular function. This includes human studies showing detrimental effects of infusion of β-agonists (28) or other inotropes (29) in heart failure, transgenic mouse studies with Gαs overexpression (26), high levels of β_2AR overexpression within the context of hypertrophy/failure in MLP knockout (5), or Gαq-overexpressing mice (this study). Conventional wisdom based on these types of studies holds that chronic activation of βAR signaling is uniformly deleterious for the compromised or failing heart. The current studies show that favorable effects on cardiac function *and* hypertrophy may be achieved by β_2AR expression at levels that presumably preserve the specificity and fidelity of β_2AR signaling and support a reevaluation of overly broad generalizations regarding the deleterious effects of βAR signaling in the compromised heart.

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