G-Protein Signaling Participates in the Development of Diabetic Cardiomyopathy

Ian S. Harris,1 Ilya Treskov,1 Michael W. Rowley,1 Scott Heximer,2 Kevin Kaltenbronn,2 Brian N. Finck,1 Richard W. Gross,1,3 Daniel P. Kelly,1 Kendall J. Blumer,2 and Anthony J. Muslin1,2

Diabetic patients develop a cardiomyopathy that consists of ventricular hypertrophy and diastolic dysfunction. Although the pathogenesis of this condition is poorly understood, previous studies implicated abnormal G-protein activation. In this work, mice with cardiac overexpression of the transcription factor peroxisome proliferator–activated receptor-α (PPAR-α) were examined as a model of diabetic cardiomyopathy. PPAR-α transgenic mice develop spontaneous cardiac hypertrophy, contractile dysfunction, and “fetal” gene induction. We examined the role of abnormal G-protein activation in the pathogenesis of cardiac dysfunction by crossing PPAR-α mice with transgenic mice with cardiac-specific overexpression of regulator of G-protein signaling subtype 4 (RGS4), a GTPase activating protein for Gq and Gi. Generation of compound transgenic mice demonstrated that cardiac RGS4 overexpression ameliorated the cardiomyopathic phenotype that occurred as a result of PPAR-α overexpression without affecting the metabolic abnormalities seen in these hearts. Next, transgenic mice with increased or decreased cardiac Gq signaling were made diabetic by injection with streptozotocin (STZ). RGS4 transgenic mice were resistant to STZ-induced cardiac fetal gene induction. Transgenic mice with cardiac-specific expression of mutant Gαq1αqG188S, that is resistant to RGS protein action were sensitized to the development of STZ-induced cardiac fetal gene induction and bradycardia. These results establish that Gq-mediated signaling plays a critical role in the pathogenesis of diabetic cardiomyopathy. Diabetes 53:3082–3090, 2004

Both insulin-dependent (type 1) and non–insulin-dependent (type 2) forms of diabetes in humans are accompanied by a greatly increased risk of cardiovascular death (1–3). Both types of diabetes are associated with cardiomyopathy that share many similar characteristics, including ventricular hypertrophy, decreased ventricular diastolic relaxation, and a reduced peak filling rate (4–7). Diabetic cardiomyopathy is distinct from ischemic cardiomyopathy because it is present in diabetic patients and animal models of diabetes in the absence of coronary artery disease.

Recently, rodent models of diabetes were used to uncover the pathogenesis of diabetic cardiomyopathy. One animal model of diabetic cardiomyopathy was developed that consists of cardiac-specific overexpression of the transcription factor myosin heavy chain (MHC)—peroxisome proliferator–activated receptor-α (PPAR-α) (8). PPAR-α is a ligand-activated transcription factor that regulates genes involved in cardiac fatty acid uptake and oxidation. PPAR-α is activated in the diabetic heart (8). MHC-PPAR transgenic mice develop a metabolic phenotype that is similar to the diabetic state in the heart but not in other tissues, with increased lipid uptake and oxidation and reduced glucose uptake and oxidation (8). Furthermore, MHC-PPAR mice develop a cardiomyopathy with ventricular hypertrophy, activation of gene markers of pathological hypertrophic growth, and transgene expression—dependent alteration in systolic ventricular dysfunction. It is unclear whether the cardiomyopathy seen in MHC-PPAR mice develops as a direct consequence of altered myocardial metabolism or rather because of abnormal intracellular signal transduction, which may occur secondarily to these metabolic changes.

A second rodent model of type 1 diabetes is based on the systemic administration of streptozotocin (STZ), a compound that contains a glucose molecule with a nitrosourea side chain that has cytotoxic action (9). The glucose portion of STZ directs the agent to pancreatic β-cells, which are then destroyed by the highly reactive nitrosourea side chain. STZ-induced diabetic rats develop a cardiomyopathy that is characterized by decreased left ventricular (LV) contractility, diminished ventricular compliance with markedly abnormal diastolic function, and decreased inotropic and chronotropic responses to certain ligands (9,10). The abnormalities in diastolic function in diabetic rats are manifest by prolonged isovolumic relaxation time, increased atrial contribution to diastolic filling, and elevated in vivo LV end-diastolic pressure (10). STZ-induced diabetic rats do not develop atherosclerosis or hypertension, so the cardiomyopathy is presumably caused by a direct effect of diabetes on the cardiac myocyte. The cardiomyopathy in STZ-induced diabetic rats may be caused, in part, by abnormalities in G-protein-mediated signal transduction. In particular, β-adrenergic...
Cardiac catheterization. Live anesthetized mice were evaluated by echocardiography 28 days after injection with STZ or vehicle to determine cardiac mass and function (21,22). We used a Sequoia cardiac echocardiography system (Acuson, Mountain View, CA) that was equipped with a 15-MHz linear transducer.

Cardiac catheterization. Live anesthetized mice were evaluated by cardiac catheterization 28 days after injection with STZ or vehicle to determine intraventricular pressures and cardiac function as previously described (21). A 1.4-F Millar catheter was used, and continuous aortic pressure and LV systolic and diastolic pressures were recorded.

Quantitative real-time PCR. RNA was purified from quick-frozen cardiac tissue by use of Trizol reagent (Sigma). The TaqMan Gold RT-PCR kit (PE Biosystems) was used according to the manufacturer's instructions. Quantitative PCR was performed by use of real-time detection technology and analyzed on a model 7700 Sequence Detector (Applied Biosystems) with specific primers and fluorescent probes for atrial natriuretic factor (ANF), β-MHC, mitochondrial carnitine palmitoyltransferase 1 (mCPT1), sarcoplasmic and endoplasmic reticulum calcium exchanger 2a (SERCA2a), and GLUT4. mRNA levels were compared at various time points after correction by use of concurrent glyceraldehyde-3-phosphate dehydrogenase message amplification.

Protein analysis. Murine ventricular cytosolic extracts were generated as previously described, and proteins were separated by SDS-PAGE (21). Proteins were electrophoretically transferred to nitrocellulose. Filters were blocked in Tris-buffered saline containing 1% Tween 20 and 2% nonfat dried milk. Filters were washed and incubated with primary antibody. Primary antibodies used included murine monoclonal (M5) anti-FLAG antibody (Sigma), rabbit polyclonal anti-p53 mitogen-activated protein kinase antibody, and rabbit polyclonal Gαq/G12 (C-19; Santa Cruz Biotechnology). Filters were extensively washed in Tris-buffered saline containing 1% Tween 20 and then incubated with horseradish peroxidase–conjugated anti-mouse or anti-rabbit secondary antibody (Amersham, Piscataway, NJ). Bands were visualized by use of the enhanced chemiluminescence system (Amersham) (21).

Histological analysis. Ventricular tissue was fixed in formalin and embedded in paraffin (21). Embedded mid-LV tissue was sectioned with a microtome, deparaffinized, stained with Masson's trichrome or hematoxylin and eosin, and examined by compound microscopy.

Statistical analysis. All data are reported as the means ± SD. Differences between values were evaluated for statistical significance by use of a nonpaired Student's t test or one-way ANOVA with the Fisher's post hoc procedure when appropriate. Significance was defined as P < 0.05.

RESULTS

MHC-PPAR × MHC-RGS4 compound transgenic mice. MHC-PPAR transgenic mice model diabetic cardiomyopathy and are characterized by enhanced cardiac fatty acid oxidation rates with reduced cardiac glucose uptake (8). These mice (line 404-3) develop cardiac hypertrophy, reduced systolic ventricular function, and "embryonic" cardiac gene expression (8). MHC-PPAR transgenic mice develop cardiac hypertrophy and dysfunction by an uncertain mechanism. We speculated that altered metabolism leads to the local release of ligands, such as endothelin-1 or angiotensin II, that activate G-proteins or other signaling intermediaries to promote cardiac hypertrophy and gene induction. To test this possibility, we bred MHC-PPAR mice with MHC-RGS4 transgenic mice to generate compound transgenic mice in the C57BL/6 genetic background.

The cardiac function and gene expression phenotype of MHC-PPAR × MHC-RGS4 compound transgenic mice were compared with MHC-PPAR and MHC-RGS4 single transgenic mice and with nontransgenic mice at 12 weeks of age. MHC-PPAR × MHC-RGS4 compound transgenic and MHC-PPAR single transgenic mice had identical cardiac PPAR-α protein levels, as measured by immunoblotting of ventricular cytosolic lysates (Fig. 1A).
Echocardiographic analysis demonstrated that MHC-
P PPAR single transgenic mice had markedly reduced sys-
tolic function at 12 weeks of age. The fractional shortening
was 44.0 ± 3.9% in MHC-PPAR mice, compared with
64.9 ± 3.7% in nontransgenic C57BL/6 mice. MHC-PPAR
MHC-RGS4 compound transgenic (PPAR+/RGS4+) mice were evaluated by conscious transthoracic echocardiography and morphometry at 12 weeks of age. A: Ventricular cytosolic lysates were generated from MHC-PPAR single transgenic and MHC-PPAR × MHC-RGS4 compound transgenic mice, as well as nontransgenic littermates. Proteins were electrophoretically transferred to nitrocellulose membranes, and immunoblots were performed by use of a murine monoclonal anti-FLAG antibody. The membranes were stripped and rebotted with rabbit polyclonal anti-p38 antibody to verify equal protein loading in each well. MAPK, mitogen-activated protein kinase. B: Echocardiographically determined fractional shortening in nontransgenic C57BL/6, MHC-RGS4 single transgenic, MHC-PPAR single transgenic, and MHC-PPAR × MHC-RGS4 compound transgenic mice. C: Echocardiographically determined LV mass in nontransgenic C57BL/6, MHC-RGS4 single transgenic, MHC-PPAR single transgenic, and MHC-PPAR × MHC-RGS4 compound transgenic mice. D: Morphometrically determined biventricular weight (BVW)–to–body weight (BW) ratio in nontransgenic C57BL/6, MHC-RGS4 single transgenic, MHC-PPAR single transgenic, and MHC-PPAR × MHC-RGS4 compound transgenic mice. E: Morphometrically determined biventricular weight (BVW)–to–body weight (BW) ratio in nontransgenic C57BL/6, MHC-RGS4 single transgenic, MHC-PPAR single transgenic, and MHC-PPAR × MHC-RGS4 compound transgenic mice.

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MHC-RGS4 compound transgenic mice exhibited an inter-
mediate phenotype with a fractional shortening of 51.5 ±
0.3%. The fractional shortening in MHC-PPAR × MHC-
RGS4 compound transgenic mice was significantly greater
than that observed in MHC-PPAR single transgenic mice
(P = 0.038) (Fig. 1B).

MHC-PPAR single transgenic mice had LV hypertrophy
at 12 weeks of age by echocardiography with a calculated
LV mass of 83.5 ± 13.8 mg compared with 57.4 ± 5.8 mg
for nontransgenic mice (P < 0.05). MHC-PPAR × MHC-
RGS4 compound transgenic mice exhibited a trend toward
less LV mass by echocardiographic analysis than MHC-PPAR single transgenic mice (67.6 ± 9.8 mg, P = 0.1) (Fig. 1C).

Morphometric analysis confirmed that MHC-PPAR single transgenic mice, but not MHC-PPAR × MHC-RGS4 compound transgenic mice, had cardiac hypertrophy at 12 weeks of age. The biventricular weight–to–body weight ratio in MHC-PPAR single transgenic mice was 6.5 ± 0.62 mg/g, but it was only 5.3 ± 0.31 mg/g in MHC-PPAR × MHC-RGS4 compound transgenic mice (P = 0.024) (Fig. 1D). Similarly, the LV weight–to–body weight ratio was 4.9 ± 0.35 mg/g in MHC-PPAR single transgenic mice, but it was only 4.2 ± 0.36 in MHC-PPAR × MHC-RGS4 compound transgenic mice (P = 0.027) (Fig. 1E).

Analysis of gene expression by quantitative real-time RT-PCR revealed that MHC-PPAR single transgenic mice that had elevated cardiac mCPT-1 gene expression, a PPAR-α target gene involved in fatty acid uptake and utilization known to be upregulated in the diabetic heart (8). MHC-PPAR × MHC-RGS4 compound transgenic mice had a similar elevation in mCPT-1 mRNA (Fig. 2). MHC-PPAR single transgenic mice had reduced cardiac glucose transporter GLUT4 gene expression, a gene inhibited by PPAR-α (8), and MHC-PPAR × MHC-RGS4 mice had a similar reduction in GLUT4 mRNA (Fig. 2). MHC-PPAR single transgenic mice had reduced SERCA2a gene expression, a marker of cardiac hypertrophy. In contrast, SERCA2a gene expression was normalized in MHC-PPAR × MHC-RGS4 compound transgenic mice (Fig. 2). Furthermore, MHC-PPAR single transgenic mice had increased ANF and β-MHC gene expression that was normalized in MHC-PPAR × MHC-RGS4 compound transgenic mice (Fig. 2).

**Effect of STZ injection on cardiac function and gene expression.** Although MHC-PPAR mice are an important model of the metabolic, structural, and functional abnormalities seen in diabetic cardiomyopathy, these mice do not develop systemic hyperglycemia. To induce diabetes in nontransgenic mice, we injected the pancreatic β-cell toxin STZ. We injected 12-week-old nontransgenic C57BL/6 mice with STZ (or vehicle) at a dose of 200 mg/kg, and random tail blood glucose measurements were obtained every 7 days after STZ injection. Mice with a random blood glucose level >250 mg/dl were defined as diabetic. Of the nontransgenic mice, ~90% developed diabetes within 14 days of the initial STZ injection by this protocol (Table 1). All but one STZ-injected animal survived for at least 28 days after injection, despite the finding that several animals had blood glucose >500 mg/dl. Most STZ-injected animals exhibited substantial whole-body weight loss after 28 days (Table 1).

Echocardiographic analysis of STZ- or vehicle-injected animals was performed 28 days after injection, and this demonstrated that cardiac function was largely unchanged in diabetic animals. Fractional shortening, a measure of cardiac systolic function, was 64 ± 4% in vehicle-injected C57BL/6 animals, and it was 61 ± 7% in STZ-injected animals (Table 2). The heart rate in anesthetized diabetic mice was mildly reduced at 639 ± 45 bpm compared with 655 ± 17 in vehicle-injected mice, but this difference was not statistically significant (P = NS). Analysis by invasive cardiac catheterization also revealed that cardiac function was unchanged in diabetic animals (Table 3). The calculated dP/dt_max (maximal change in pressure per unit time) was 3,558 ± 467 in vehicle-injected mice and 3,735 ± 877 in diabetic animals. Morphometric analysis of diabetic hearts revealed that the biventricular weight of diabetic mice was less than that of vehicle-injected mice, but that the biventricular weight–to–body weight ratio was unchanged because of a reduction in body weight (Table 1).

Analysis of gene expression by quantitative real-time PCR revealed that ANF and β-MHC expression, marker genes of cardiac hypertrophy and failure, were markedly induced 28 days after STZ injection. Indeed, ANF gene expression was induced by 14.1 ± 7.4-fold in STZ-injected animals, and β-MHC gene expression was induced by 13.5 ± 7.0-fold in STZ-injected animals when compared with vehicle-injected mice (Fig. 3).

**Induction of diabetes in MHC-RGS4 transgenic mice.** Although nontransgenic diabetic C57BL/6 mice did not develop overt cardiac dysfunction, they did exhibit abnormalities in gene expression. To determine whether G-protein–mediated signaling was involved in these phenotypic abnormalities, we injected MHC-RGS4 transgenic mice with STZ. MHC-RGS4 mice were injected with 200 mg/kg of STZ or vehicle. MHC-RGS4 mice developed diabetes at the same rate as nontransgenic animals (Table 1). Echocardiographic analysis 28 days after STZ or vehicle injection demonstrated that MHC-RGS4 diabetic animals had preserved systolic function, with a fractional shortening of 60 ± 2% when compared with vehicle-injected RGS4 mice (59 ± 2% fractional shortening). In addition, MHC-RGS4 diabetic animals did not have a reduced heart rate when compared with vehicle-injected animals. Indeed, the heart rate was slightly increased at 665 ± 20 bpm in STZ-injected MHC-RGS4 mice, and it was 653 ± 14 bpm in vehicle-injected MHC-RGS4 mice (Table 2). Morphometric analysis of diabetic hearts revealed that the biventricular weight of diabetic MHC-RGS4 mice was less than that in vehicle-injected mice, but that the biventricular weight–to–body weight ratio was unchanged because of a reduction in body weight (Table 1).

Analysis of gene expression revealed that MHC-RGS4 transgenic animals were resistant to STZ-stimulated ANF and β-MHC induction. ANF gene expression was induced by 14.1-fold in STZ-injected nontransgenic C57BL/6 animals, but it actually declined by 50 ± 40% in STZ-injected MHC-RGS4 animals when compared with vehicle-injected MHC-RGS4 mice (Fig. 3). β-MHC gene expression was induced by 13.5-fold in STZ-injected nontransgenic C57BL/6 animals, but it declined by 16% in STZ-injected MHC-RGS4 animals when compared with vehicle-injected MHC-RGS4 animals (Fig. 3).

**Generation and preliminary characterization of MHC-G188S transgenic mice.** RGS proteins deactivate heterotrimeric G-proteins by stabilizing the transition state between the GDP- and GDP-bound forms of the α-subunit (17,18). We generated a point mutant form of G_qα and G_qα−188S, that is unable to bind to RGS proteins (23). The G_qα−188S point mutant form of G_qα, in contrast to the Q209L mutant form, is not constitutively active when expressed in cultured cells, but it is resistant to deactivation by RGS proteins after ligand-mediated activation. We generated transgenic mice with cardiac-specific expression of G_qα−188S.
G188S. The α-MHC promoter was linked to the coding region of G\textsubscript{\alphaq}-G188S. Two transgenic lines were generated in the C57BL/6 strain: one with genomic integration of three copies of the transgene (MHC–G188S-3x) and a second with genomic integration of seven copies of the transgene (MHC–G188S-7x). Both lines have increased total G\textsubscript{\alphaq} protein in ventricular lysates (Fig. 4A). Mice from both lines appear normal and live normal life spans. The basal cardiac structure and function of these mice is normal at 12 weeks of age in the MHC–G188S-3x (data not shown).
shown) and MHC–G188S-7x lines, as determined by echocardiographic and histological examination (Figs. 4B–C).

The phenotype of the MHC–G188S-3x and MHC–G188S-7x transgenic mice is much milder than that observed in the Gq-40 mice previously generated by another group, and this is likely a consequence of the lower cardiac Gq protein levels achieved (25,26). In Gq-40 mice, significant LV systolic dysfunction and bradycardia are observed at 4 weeks of age (22).

**Response of MHC-G188S transgenic mice to STZ injection.** MHC–G188S-7x animals were evaluated by echocardiography 28 days after STZ or vehicle injection. MHC–G188S-7x diabetic mice had preserved systolic function when compared with vehicle-injected controls (Fig. 3). ANF gene expression was induced by 33-fold in STZ-injected MHC–G188S-7x mice, but it was induced by 14.1-fold in STZ-injected nontransgenic animals when compared with vehicle-injected controls (Fig. 3). β-MHC gene expression was induced by 218.7-fold in STZ-injected MHC–G188S-7x mice, but it was induced by 13.5-fold in STZ-injected nontransgenic animals when compared with vehicle-injected controls (Fig. 3).

**DISCUSSION**

Diabetic cardiomyopathy is a condition characterized by cardiac hypertrophy, decreased ventricular compliance, diastolic dysfunction, and, occasionally, systolic dysfunction (1–3). The underlying mechanism by which diabetes results in cardiomyopathy is unclear. It is unlikely that diabetic cardiomyopathy occurs as a result of increased insulin production or decreased target-organ insulin signaling because this condition occurs in type 1 as well as type 2 diabetes. Diabetes is often associated with increased production of angiotensin II in many tissues, and it is possible that hyperglycemia contributes to the production of this ligand. In diabetic nephropathy, considerable evidence suggests that increased angiotensin II production and signaling contributes to this disorder. Indeed, large clinical trials have demonstrated that ACE inhibitor therapy or angiotensin II receptor blocking therapy reduce the incidence of nephropathy in patients with either type 1 or type 2 diabetes (27–30).

In addition to angiotensin II, it is possible that additional secreted ligands, such as endothelin-1, are involved in the pathogenesis of diabetic cardiomyopathy (31). Diabetic

**TABLE 1**

Morphometric characterization of diabetic mice with altered Gq signaling

<table>
<thead>
<tr>
<th></th>
<th>Nontransgenic (n = 18)</th>
<th>MHC-G188S (n = 23)</th>
<th>MHC-RGS4 (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>19.82 ± 3.14</td>
<td>20.03 ± 3.6</td>
<td>23.5 ± 3.1</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>22.5 ± 3.0</td>
<td>18.8 ± 3.2</td>
<td>23.9 ± 3.1</td>
</tr>
<tr>
<td>% diabetic</td>
<td>92</td>
<td>92</td>
<td>80</td>
</tr>
<tr>
<td>Final biventricular weight (mg)</td>
<td>96.1 ± 9.6</td>
<td>75.9 ± 8.8</td>
<td>104.1 ± 11.2</td>
</tr>
<tr>
<td>Biventricular weight–to–body weight ratio (mg/g)</td>
<td>4.3 ± 0.3</td>
<td>4.1 ± 0.5</td>
<td>4.0 ± 0.1</td>
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</tbody>
</table>

Data are means ± SD unless otherwise indicated. Nontransgenic, MHC-G188S-7x transgenic, and MHC-RGS4 transgenic mice were weighed and injected with STZ or vehicle. Twenty-eight days later, the mice were weighed (body weight) and biventricular weights were determined. *P = 0.04 vs. vehicle-injected MHC-G188S mice.

**TABLE 2**

Physiological characterization of diabetic mice with altered Gq signaling

<table>
<thead>
<tr>
<th></th>
<th>Nontransgenic</th>
<th>MHC-RGS4</th>
<th>MHC-G188S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>655 ± 17</td>
<td>639 ± 45</td>
<td>653 ± 14</td>
</tr>
<tr>
<td>LV posterior wall thickness in systole (mm)</td>
<td>1.37 ± 0.18</td>
<td>1.36 ± 0.93</td>
<td>1.43 ± 0.13</td>
</tr>
<tr>
<td>Diastolic LV internal dimension (mm)</td>
<td>3.15 ± 0.12</td>
<td>3.23 ± 0.12</td>
<td>3.46 ± 0.26</td>
</tr>
<tr>
<td>Systolic LV internal dimension (mm)</td>
<td>1.12 ± 0.10</td>
<td>1.27 ± 0.25</td>
<td>1.65 ± 0.21</td>
</tr>
<tr>
<td>Interventricular septum thicknesses in diastole (mm)</td>
<td>0.62 ± 0.98</td>
<td>0.48 ± 0.06</td>
<td>0.70 ± 0.12</td>
</tr>
<tr>
<td>LV mass index (mg/g)</td>
<td>2.5 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>2.4 ± 0.07</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>64 ± 4</td>
<td>61 ± 7</td>
<td>59 ± 2</td>
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</table>

Data are means ± SD. Nontransgenic, MHC-G188S-7x transgenic, and MHC-RGS4 transgenic mice underwent echocardiography 28 days after STZ or vehicle injection. *P = 0.01 vs. vehicle-injected MHC-G188S mice.
patients have elevated serum endothelin-1 levels (31). In addition, treatment of STZ-injected rats with bosentan, an endothelin-1 receptor antagonist, resulted in improved cardiac diastolic and systolic function (32). These findings suggest that angiotensin II inhibition may not completely block the development of diabetic cardiomyopathy.

In this work, we investigated the role of Gq proteins in the pathogenesis of diabetic cardiomyopathy. Both angiotensin II and endothelin-1 activated seven transmembrane receptors that are coupled to Gq. We first tested a robust genetic model of diabetic cardiomyopathy, MHC-PPAR transgenic mice (8). These mice exhibit abnormal cardiac metabolism, although they are systemically euglycemic, and they spontaneously develop ventricular hypertrophy, systolic contractile dysfunction, and “fetal” gene induction. The observation that MHC-PPAR mice are not hyperglycemic is a limitation that makes them an imperfect model of human diabetic cardiomyopathy. We hypothesized that G-protein signaling might play a role in the pathogenesis of MHC-PPAR cardiomyopathy. MHC-PPAR mice were bred with MHC-RGS4 transgenic mice because RGS4 antagonizes Gq signaling in the heart (22). MHC-PPAR × MHC-RGS4 compound transgenic mice exhibited an intermediate phenotype with near-complete rescue of the ventricular hypertrophy and partial rescue of the contractile dysfunction. In addition, embryonic gene induction was markedly reduced in compound transgenic mice when compared with MHC-PPAR single transgenic animals. However, RGS4 did not reverse the direct transcriptional effects of PPAR-α on the regulation of fatty acid metabolism gene expression. These results suggest that the effects of PPAR-α overexpression on metabolic gene expression do not inexorably lead to cardiac hypertrophy and dysfunction.

We next used a murine model of type 1 diabetes that involved the intraperitoneal injection of the pancreatic β-cell toxin STZ (8). We induced diabetes in nontransgenic animals and in transgenic animals with decreased (MHC-RGS4) and increased (MHC–G188S-7x) Gq signaling. Nontransgenic animals developed diabetes after a single injection of STZ, and when analyzed at 28 days, they exhibited mild bradycardia, intact systolic contractile function, and marked induction of embryonic marker genes, including ANF and β-MHC. In the present study, we demonstrated that MHC-RGS4 mice were resistant to STZ-induced bradycardia and embryonic gene induction.

We used MHC–G188S-7x transgenic mice as a model system of mildly increased Gq-mediated cardiac signaling. We thought that these mice would be superior to Gq-40 mice for this purpose because they have a less dramatic cardiac phenotype in the absence of provocative stimulation (26,27). As predicted, MHC–G188S-7x mice were sensitized to STZ-induced bradycardia and embryonic gene induction. However, MHC–G188S-7x mice did not develop ventricular systolic dysfunction 28 days after STZ injection.

The cardiac phenotype observed in nontransgenic mice after STZ injection was much milder than that observed in rats, and this difference may be a result of the profound cachexia and dehydration that develops in diabetic mice. Indeed, STZ-injected mice did not develop the diastolic dysfunction that is characteristic of human diabetic cardiomyopathy and that is also observed in STZ-injected rats. Whether the profound cachexia and dehydration observed in STZ-injected mice prevented the development of diastolic dysfunction is not known, and further study is needed.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>STZ</th>
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<tbody>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>458 ± 27</td>
<td>455 ± 47</td>
</tr>
<tr>
<td>LV peak systolic pressure (mmHg)</td>
<td>67 ± 16</td>
<td>64 ± 10</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>3.1 ± 1.1</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>dP/dtₘₚₐₓ (mmHg/s)</td>
<td>3558 ± 467</td>
<td>3735 ± 877</td>
</tr>
<tr>
<td>dP/dtₘᵦₜᵦ (mmHg/s)</td>
<td>−3042 ± 441</td>
<td>−300 ± 979</td>
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</table>

Data are means ± SD. Nontransgenic mice underwent cardiac catheterization 28 days after STZ or vehicle injection. For all parameters, the differences in vehicle- and STZ-injected mice were not statistically significant. dP/dtₘₚₐₓ, maximal change in LV pressure per unit time; dP/dtₘᵦₜᵦ, minimal change in LV pressure per unit time.
FIG. 4. Physical characterization and physiological response to STZ-induced diabetes of MHC-G188S transgenic mice. A: Gaq-G188S protein levels in transgenic cardiac tissue. Cardiac tissue was obtained from nontransgenic (NTG), MHC–G188S-3x, and MHC–G188S-7x mice and was used to generate protein lysates. Either 25 or 50 μg of total protein was loaded per lane. Immunoblots were performed by use of a Gaq/G11 (C-19) polyclonal antibody. B: Echocardiographically determined LV mass index in MHC–G188S-7x transgenic and nontransgenic (NTG) mice. C: Representative photomicrographs of hematoxylin and eosin (H&E) and Masson's trichrome–stained ventricular tissue from MHC–G188S-7x transgenic and nontransgenic (NTG) mice. D: Morphometrically determined biventricular weight (BVW)–to–body weight (BW) ratio in STZ- and vehicle-injected MHC–G188S-7x transgenic mice. E: Echocardiographically determined heart rate (HR) in STZ- and vehicle-injected MHC–G188S-7x transgenic mice.
required to address this issue. It is possible that subtherapeutic insulin administration to STZ-injected mice—by reducing cachexia and volume depletion without completely restoring euglycemia—may better model human diabetic cardiomyopathy. Taken together, these experiments demonstrate that increased or decreased Gs- and Gq-mediated signaling affect the development of diabetic cardiomyopathy, and they suggest that medical therapy targeting this signaling pathway may benefit patients with diabetic cardiomyopathy and may prevent the development of this condition in patients with diabetes.

ACKNOWLEDGMENTS

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