Receptors coupled to Goq play a key role in the development of heart failure. Studies using genetically modified mice suggest that Goq mediates a hypertrophic response in cardiac myocytes. Goq signaling in these models is modified during early growth and development, whereas most heart failure in humans occurs after cardiac damage sustained during adulthood. To determine the phenotype of animals that express increased Goq signaling only as adults, we generated transgenic mice that express a silent Goq protein (GoqQ209L-hbER) in cardiac myocytes that can be activated by tamoxifen. Following drug treatment to activate GoqQ209L-hbER, these mice rapidly develop a dilated cardiomyopathy and heart failure. This phenotype does not appear to involve myocyte hypertrophy but is associated with dephosphorylation of phospholamban (PLB), decreased sarcoplasmic reticulum Ca2+ current density, and a decrease in L-type Ca2+ current density. Changes in Ca2+ handling and decreased cardiac contractility are apparent 1 week after GoqQ209L-hbER activation. In contrast, transgenic mice that express an inducible Goq mutant that cannot activate phospholipase Cβ (PLCβ) do not develop heart failure or changes in PLB phosphorylation, but do show decreased L-type Ca2+ current density. These results demonstrate that activation of Goq in cardiac myocytes of adult mice causes a dilated cardiomyopathy that requires the activation of PLCβ. However, increased PLCβ signaling is not required for all of the Goq-induced cardiac abnormalities.

Goq is a member of the heterotrimeric G protein superfamily. Heterotrimeric G proteins are composed of three subunits (α, β, and γ). Receptor activation leads to the exchange of GTP for GDP on the α subunit, which causes Gα to dissociate from the tightly bound βγ complex. Both Gα and βγ can then interact with specific effector proteins, resulting in changes in cellular function. Phospholipase Cβ (PLCβ) is the best known effector of the Goq subfamily of G proteins (1). Activated Goq binds to PLCβ and increases its enzymatic activity to hydrolyze phosphatidylinositol (4,5)-bisphosphate to form inositol (1,4,5)-trisphosphate and diacylglycerol. Although less well characterized, other signaling effectors of Goq include the p110α/p85α phosphatidylinositol 3-kinase (PI3K) complex, which is inhibited by Goq-GTP binding (2), and Bruton’s tyrosine kinase, which is activated by Goq (3).

Following cardiac injury, up-regulation of angiotensin II and catecholamines leads to activation of Goq and progression to heart failure. The signaling pathway that mediates Goq-induced cardiomyopathy is unclear. The presumption has been that activation of PLCβ by Goq is responsible for the cardiac pathology, but this hypothesis has not been directly tested. Activation of PLCβ leads to the release of Ca2+ from inositol (1,4,5)-trisphosphate-sensitive stores, but the role of this signaling event in cardiac myocytes is unknown. Activation of PLCβ also leads to the diacylglycerol-dependent activation of some protein kinase C (PKC) isoforms. Although there are a number of mouse models with genetically altered PKC function in cardiac myocytes, it remains unclear whether activation of PKC is responsible for Goq-induced cardiomyopathy (4). The presence of several PKC isoforms in the myocardium complicates the interpretation of animal studies that examine the cardiac effect of a particular PKC isoform.

Results from genetically modified mouse models suggest that Goq signaling mediates a hypertrophic response in cardiac myocytes (5–8). Targeted overexpression of wild-type Goq at supraphysiologic levels in cardiac myocytes using the α myosin heavy chain (MHC) promoter resulted in cardiac hypertrophy and subsequent decompensation into heart failure (5). Because wild-type Goq can sequester βγ subunits, its expression might interfere with signals initiated by βγ. Mutation of Gln209 to Leu of Goq produces a mutant (GoqQ209L) that no longer hydrolyzes GTP, resulting in constitutive signaling to downstream effectors. GoqQ209L does not bind to βγ, so expression of this mutant does not interfere with βγ signaling. Conventional αMHC-driven expression of GoqQ209L in cardiac myocytes also led to cardiac hypertrophy. Surprisingly, these mice developed heart failure with age even though the mutant protein became undetectable (6).

Standard transgenic and gene knock-out models impose a genetic alteration during embryonic and postnatal periods that can drastically affect the phenotype of the animals. This is a weakness for studying heart failure, which mostly occurs in humans subsequent to a cardiac injury suffered during adulthood. Indeed, when wild-type Goq was overexpressed in myocytes of adult mice using an inducible Cre-lox-based system, the animals did not develop cardiac hypertrophy or other phenotypes seen in the conventional transgenic animals (9). This dramatic difference in phenotype raises doubt regarding the role of Goq in causing cardiac dysfunction in adult animals.

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1 These authors contributed equally to this work.

2 To whom correspondence should be addressed. Tel: 631-444-2059; Fax: 631-444-7530; E-mail: richard.lin@sunyosb.edu.

3 The abbreviations used are: PLCβ, phospholipase Cβ; PK3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; MHC, myosin heavy chain; PLB, phospholamban; SERCA-2, sarcoplasmic reticulum Ca2+/-ATPase-2; HA, hemagglutinin; 4-HT, 4-hydroxytamoxifen; hbER, hormone-binding domain of the estrogen receptor; BNP, brain natriuretic peptide precursor type B; LVEDP and LVESP, left ventricular end-diastolic and end-systolic pressure, respectively; H&E, hematoxylin and eosin; WT, wild-type; SV, sarcomplasmic reticulum; TA, total activity; OA, oxidase activity; TAA, total ATPase activity; CIA, Ca2+/-independent ATPase activity; F, farad; ANOVA, analysis of variance.
To better investigate the role of $\alpha_q$ activation in the development of heart failure in adult animals, we generated transgenic mice that express recombinant $\alpha_q$Q209L proteins whose activity can be turned on by injection of the drug tamoxifen. This inducible model allows us to investigate the consequences of $\alpha_q$ activation in myocytes after completion of normal development and growth. In contrast to the standard transgenic models, we found that activation of $\alpha_q$ in 8-week-old animals causes a dilated cardiomyopathy and heart failure that does not appear to progress through a hypertrophic stage. Use of an additional transgenic mouse line that expresses an inducible $\alpha_q$Q209L protein that cannot activate PLCB demonstrates that PLCB activation is required for the development of heart failure but not for all of the $\alpha_q$-induced cardiac abnormalities. Another advantage this inducible model has over standard transgenic models is that it allows us to investigate early biochemical events initiated by $\alpha_q$ activation. We found that two aspects of Ca$^{2+}$ handling in myocytes are markedly altered 1 week after activation of the exogenous $\alpha_q$ protein. We speculate that these changes in Ca$^{2+}$ handling may be responsible for the contractile defect seen at this time.

**EXPERIMENTAL PROCEDURES**

**Materials**—Antibodies against the carboxyl terminus of the estrogen receptor and $\alpha_q$ were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to phospho-Ser$^{16}$ phospholamban (PLB), PLB and phospho-Ser$^{657}$ PKCa were from Upstate Biotechnology, Inc. (Lake Placid, NY). Sarcoplasmic reticulum Ca$^{2+}$-ATPase-2 (SERCA-2) antibody was from Affinity BioReagents (Golden, CO). General antibody against PKCa was from BD Biosciences. Anti-hemagglutinin (HA) antibody was from Covance (Richmond, CA). myo-[3H]inositol (10–25 Ci/mmol) was from PerkinElmer Life Sciences. Tamoxifen and 4-hydroxytamoxifen (4-HT) were from Sigma.

**DNA Constructs**—The 4-HT-activateable $\alpha_q$ constructs were made using $\alpha_q$Q209L and $\alpha_q$Q209L-AAA (10) and a mutant hormone-binding domain of the murine estrogen receptor (hbER) obtained from Dr. J. Robbins, University of Cincinnati at the Sall site for construction of transgenic mice with cardiac-specific expression of the transgenes. After digestion with NotI to remove the bacterial vector sequence, $\alpha_q$-hbER constructs were then subcloned into pcDNA3.1 (Invitrogen) for expression in mammalian cells. Akt-HA was described earlier (2).

**Go$_q$ Signaling and Heart Failure**

$\alpha_q$Q209L-hbER Transgenic Mice—$\alpha_q$Q209L-hbER and $\alpha_q$Q209L-AAA-hbER were subcloned into the αMHC promoter cassette (obtained from Dr. J. Robbins, University of Cincinnati) at the Sall site for construction of transgenic mice with cardiac-specific expression of the transgenes. After digestion with NotI to remove the bacterial vector sequence, $\alpha_q$-hbER constructs were then subcloned into pcDNA3.1 (Invitrogen) for expression in mammalian cells. Akt-HA was described earlier (2).

**Preparation of Tissue and Cell Lysates**—Frozen mouse organs were homogenized with a PRO250 in phosphate-buffered saline containing protease inhibitors (Sigma). The homogenate was centrifuged at 15000 × g for 15 min at 4 °C. The supernatant was centrifuged at 72,000 × g for 15 min at 4 °C, and the resulting pellet was suspended in 50 mM Tris-HCl, pH 7.4. Protein concentration was determined by a modified Lowry method (11).

**Western Blotting**—Membrane suspensions were mixed with a urea-containing loading buffer (110 mM Tris-HCl, pH 6.8, 4% sodium dodecyl sulfate, 200 mM dithiothreitol, 0.2% bromphenol blue, 20% glycerol, and 8% (v/v) urea) without boiling, and proteins were separated by polyacrylamide gel electrophoresis. The loading buffer used for non-membrane Western samples contained no urea. Equal amounts of protein were loaded in each lane. After immunoblotting, signals were detected using either an enhanced chemiluminescence kit (PerkinElmer Life Sciences) or the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE).

**SERCA Activity**—Crude ventricular membranes were prepared by homogenizing the tissue as described above followed by centrifugation at 14,000 × g for 20 min at 4 °C. The supernatant was centrifuged at 72,000 × g for 20 min at 4 °C. The resulting pellet was suspended in 50 mM Tris-HCl, pH 7.4 plus 0.6 M KCl and kept on ice for 45 min. The suspension was centrifuged again at 72,000 × g for 30 min at 4 °C. The pellet was assayed for Ca$^{2+}$-ATPase activity using a modification of an NADH-coupled assay (12). The standard assay medium contained 10 mM Tris, pH 7.5, 100 mM KCl, 5 mM MgCl$_2$, 5 mM Na$_2$ATP, 0.1 mM CaCl$_2$, ~0.2 mM NADH, 1.5 mM trisodium phosphoenolpyruvate, 15 units/ml pyruvate kinase, and 36 units/ml lactate dehydrogenase. Additionally, the assay mix contained 10 mM Na$_3$B$_4$O$_7$ and 0.1 mM ouabain to inhibit contaminating NADH oxidase and Na$^+$,K$^+$-ATPase, respectively. Total activity (TA) was assayed by monitoring the rate of loss of A$_{340}$ after addition of the membrane preparation to a thermostatically controlled (37 °C) cuvette in a Hewlett-Packard 8452A diode array spectrophotometer. Background oxidase activity (OA) was assayed in the absence of ATP. Total ATPase activity (TAA) was calculated as TA-OA. Ca$^{2+}$-independent ATPase activity (CIA) (corrected for OA) was assayed in the presence of 10 mM EGTA instead of Ca$^{2+}$. Ca$^{2+}$-dependent ATPase activity was calculated as TAA-CIA and is expressed as nmol ATP hydrolyzed per mg of protein per min.
Hemodynamic Measurements of Cardiac Function—Measurements of heart rate, left ventricular end-diastolic and end-systolic pressure (LVEDP and LVESP, respectively), dp/dt, and ejection fraction were performed using the Millar ARIA™ pressure-volume conductance system (Houston, TX). A closed-chest approach similar to the one described by Lorenz and Robbins was used (13). After adequate anesthesia was attained with ketamine and fentanyl, the right carotid artery was isolated and cannulated with a 1.4 French Millar P-V catheter that was passed down the aorta into the left ventricle.

Ventricular Myocyte Isolation—Mice were euthanized by intraperitoneal injection of 100 mg/kg sodium pentobarbital. The heart was removed and rinsed in three changes of PS solution (112 mM NaCl, 5.4 mM KCl, 1.7 mM NaH2PO4, 1.63 mM MgCl2, 42 mM NaHCO3, 20 mM HEPES, 5.4 mM glucose, 4.1 mM L-glutamine, 10 mM taurine, minimal essential medium vitamins, and minimal essential medium amino acids, pH 7.4) containing 20 units/ml sodium heparin. The heart was cannulated through the aorta and perfused on a Langendorf apparatus with PS containing 1 mg/ml 2,3-butanedione monoxime for 10 min and with PS solution containing 12.6 mM tetraethylammonium hydroxide). The heart was minced in KB solution and the cells were washed once with KB solution after settling.

Electrophysiology—Only clearly rod-shaped myocytes were studied. Whole-cell patch clamp recordings used 2–3 m2 borosilicate glass pipettes measured prior to sealing (Sutter Instrument), pCLAMP 8 software, the DigiData 1350 interface, and the Axopatch 1D amplifier (Axon Instruments). For the recording of L-type Ca2+ current (I_Ca,L) (14), pipettes contained 111 mM CsCl, 20 mM tetraethylammonium chloride, 10 mM glucose, 14 mM EGTA, 10 mM HEPES, and 5 mM MgATP, pH 7.2 (adjusted by CsOH). I_Ca,L was recorded in Na+-free bath solution (137 mM tetraethylammonium chloride, 1 mM MgCl2, 2 mM CaCl2, 10 mM HEPES, and 10 mM glucose, pH 7.4, adjusted with tetraethylammonium hydroxide). The I_Ca,L was recorded as a single voltage step every 4 s. In current/voltage (I-V) experiments, current stability was usually checked for 2 min before beginning the protocol. I-V curves were generated using 300 ms depolarizing voltage steps from -50 to +50 mV in 10 mV increments (holding potential -50 mV). The membrane capacity was measured by applying a voltage step from a holding potential of -50 mV, and the current amplitudes are normalized to the cell capacitances. Perfusion solutions contained 1 μM 4-HT throughout the experiment. All the experiments were performed at 22 °C.

Calculation of Percent Extracellular Space—Non-overlapping images on hematoxylin and eosin (H&E)-stained sections were digitally captured to obtain RGB color images with 1280 × 1024 resolution. The following algorithm was developed in ImageJ Version 1.34i (National Institutes of Health, Bethesda, MD) to quantify extracellular space. The green channel, having the greatest contrast between stained tissue and non-stained extracellular space, was isolated in each image. A high pass filter in the Fourier domain of the image was then used to smooth the shading caused by non-uniform illumination of the sample. The histogram of each image was then normalized such that the low intensity pixels were set to 0, and high intensity pixels were set to 255. Pixels having intensities above a standard threshold value in each image were counted as extracellular space. The ratio of extracellular space was then computed as EC = Pec/Ptotal, where Pec is the number of pixels considered extracellular space, and Ptotal is the number of pixels in the image.

Statistical Analysis—Values represent means ± S.E., and significance is defined as p < 0.05. Unless otherwise indicated, experimental groups were compared using Student’s t test.

RESULTS

Conditionally Active GαqQ209L-hbER Constructs—The Gαq proteins used in this study are Gαq_Q209L, which signals constitutively to all of its downstream effectors, and a Gαq_Q209L mutant in which Arg256 and Thr257 are changed to Ala. The latter protein (Gαq_Q209L-AA) cannot activate PLCβ (15) but still inhibits PI3K signaling (10). To obtain inducible forms of Gαq, we fused a mutant hbER to the carboxyl terminus of Gαq_Q209L and Gαq_Q209L-AA (Fig. 1A). This mutant hbER does not bind 17β-estradiol but is responsive to 4-HT (16).

The function of Gαq_Q209L-hbER was checked by measuring its ability to activate PLCβ and increase the level of intracellular inositol phosphates in HEK 293 cells. The basal level of inositol phosphates in cells transfected with Gαq_Q209L-hbER was similar to that in the control cells, but treatment with 4-HT caused inositol phosphates to increase more than 6-fold at 4 h (Fig. 1B). In contrast, the level of inositol phosphates in cells transfected with either vector or Gαq_Q209L-AA-hbER did not change in response to 4-HT treatment (Fig. 1B). To confirm that the inducible form of Gαq_Q209L-AA can still inhibit PI3K signaling, we
measured the activity of Akt, which acts downstream of PI3K. HEK 293 cells were cotransfected with epitope-tagged Akt-HA and either vector or GaqQ209L-AA-hbER. Akt activity was inhibited 40% when the fusion protein was activated by addition of 4-HT to the cells (Fig. 1C). A similar level of inhibition was obtained using GaqQ209L-hbER (Fig. 1C).

GaqQ209L-hbER and GaqQ209L-AA-hbER Transgenic Mice—Gaq transgenic mice were generated using an αMHC promoter construct to selectively express these proteins in cardiac myocytes (Fig. 1A). Three GaqQ209L-hbER founders (lines 5, 7, and 11) were identified by PCR analysis of tail DNA (Fig. 2A). Mice from line 7 express the GaqQ209L-hbER protein at a level higher than mice from lines 5 or 11, as demonstrated by Western blotting of heart lysates (Fig. 2B). Although all three lines of transgenic mice develop heart failure when injected with tamoxifen (see below), transgenic mice from line 7 developed this phenotype most rapidly. Therefore, this line was selected for further study. Cardiac-specific expression of the recombinant protein was confirmed in line 7 animals (Fig. 2C). The transgenic mice are born at the expected Mendelian ratio and breed normally.

Five GaqQ209L-AA-hbER founders (lines 27, 41, 56, 59, and 66) were identified by PCR analysis of tail DNA (data not shown). Expression of the recombinant protein in four of these lines was very low (Fig. 2D). However, line 66 GaqQ209L-AA-hbER mice express the protein at a high level comparable with that seen in line 7 GaqQ209L-hbER mice (Fig. 2D), so they were selected for all subsequent experiments. This allows us to directly compare the phenotypes of mice expressing either Gaq protein. It is important to note that expression of endogenous Gaq is not affected in any of the transgenic mouse lines (Fig. 2D).

To confirm that tamoxifen activates Gaq/PLCβ signaling in the GaqQ209L-hbER mice but not in WT or GaqQ209L-AA-hbER animals, we examined the membrane localization of PKCa. Phosphorylated PKCa translocates from the cytosol to membranes and becomes active in response to the PLCβ-catalyzed production of diacylglycerol and elevated intracellular Ca2+ (17). Western blotting showed that phospho-PKCa was undetectable in heart membranes isolated from transgenic and WT mice before tamoxifen injection, but after 7 days of drug treatment the protein was enriched in the membrane fraction from GaqQ209L-hbER animals only (Fig. 3A, top panel). Similar results were obtained using an antibody that recognizes total PKCa (Fig. 3A, middle panel). The total amount of PKCa in unfractionated lysates did not change in any of the three groups after drug treatment (Fig. 3A, bottom panel). Thus, Gaq/PLCβ signaling is elevated as early as 1 week after tamoxifen injection in the GaqQ209L-hbER hearts. This signaling pathway remains active for up to 28 days of drug treatment, as PKCa was still enriched in membranes from GaqQ209L-hbER hearts at this time point (Fig. 3B). The amount of PKCβ, but not PKCe, was also increased in the membrane fraction prepared from GaqQ209L-hbER as compared with WT hearts (data not shown).

Peripheral Edema, Heart Enlargement, and Increased Extracellular Space in GaqQ209L-hbER Mice—Starting at 8 weeks of age, transgenic and WT mice were injected intraperitoneally daily with 1 mg of tamoxifen. Western blots of heart membranes prepared at the indicated times were probed with antibodies to detect phosphorylated PKCa or total PKCa. The bottom panel in A shows the total amount of PKCa in unfractionated heart lysates. The experiments were repeated on another set of animals with similar results. QL, GaqQ209L-hbER; QL-AA, GaqQ209L-AA-hbER.
phenotypic abnormalities up to 2 years of age, indicating that the G\textsubscript{aq}Q209L-hbER protein is essentially inactive in the absence of drug. This result is consistent with a recent report by Syed et al. (9) showing that overexpression of wild-type G\textsubscript{aq} in adult mice does not have a pathological consequence. One would expect that wild-type G\textsubscript{aq} in the absence of receptor activation is in the inactive state.

All G\textsubscript{aq}Q209L-AA-hbER and WT mice were alive after 28 days of tamoxifen injection and appeared healthy and free of edema (Fig. 4A). These animals exhibited body weight increases of 9% (WT) and 12% (G\textsubscript{aq}Q209L-AA-hbER) over the course of the experiment (Fig. 4B). Transgenic or WT mice injected with vehicle suffered no ill effects.

When sacrificed after 28 days of drug treatment, the G\textsubscript{aq}Q209L-hbER hearts were found to be enlarged, with ventricular dilatation (Fig. 4C). The heart weight/tibial length ratio was significantly increased (1.65-fold) in G\textsubscript{aq}Q209L-hbER mice with edema as compared with WT mice injected with tamoxifen for 28 days (Fig. 4D). G\textsubscript{aq}Q209L-hbER mice as a group, including non-edematous animals that were injected for 28 days, exhibited a 1.89-fold increase in heart weight/tibial length ratio as compared with WT mice (12.97 \pm 0.74 mg/mm, n = 10). Although most G\textsubscript{aq}Q209L-AA-hbER hearts did not exhibit architectural changes after 28 days of tamoxifen injection (Fig. 4C), modest ventricular dilatation was seen in ~10% of these animals (data not shown). The heart weight/tibial length ratio was 1.3-fold higher in G\textsubscript{aq}Q209L-AA-hbER mice than in WT animals (Fig. 4D), an increase that is not statistically significant.

Transgenic mice expressing exogenous proteins in cardiac myocytes often exhibit increased expression of fetal genes such as βMHC in the heart, with no increase in the expression level of αMHC. Up-regulation of fetal genes is associated with cardiac hypertrophy and/or heart failure in many, but not all, of these animal models. In humans, increased circulating BNP levels are indicative of heart failure. Quantitative PCR analysis of RNA extracted from hearts after tamoxifen injection showed a large increase in expression of BNP and βMHC mRNA in both G\textsubscript{aq}Q209L-hbER and G\textsubscript{aq}Q209L-AA-hbER hearts as compared with WT (Fig. 4E). This result was surprising, since G\textsubscript{aq}Q209L-AA-hbER mice do not develop enlarged hearts or heart failure after tamoxifen injection. In addition, αMHC levels decreased in both transgenic animals (Fig. 4E). Thus, these genes do not represent useful markers for distinguishing between these phenotypes in the two transgenic G\textsubscript{aq} lines studied here.

The myocytes in heart sections from G\textsubscript{aq}Q209L-hbER mice injected for 28 days appeared normal, with no obvious evidence of hypertrophy, apoptosis, or necrosis (Fig. 5A, upper panel). Cleaved forms of caspase 3 or poly(ADP-ribose) polymerase were not detected on Western blots of heart extracts prepared after 7 days of tamoxifen treatment, indicating that apoptosis was also not occurring at this early time point (data not shown). Trichrome staining of heart sections showed that the interstitial space between myocytes was increased in G\textsubscript{aq}Q209L-hbER hearts as compared with G\textsubscript{aq}Q209L-AA-hbER and WT hearts, although only a minimal increase in interstitial fibrosis was evident (Fig. 5A, lower panel). The percent extracellular space was quantified using a computer algorithm that analyzes H&E-stained heart sections (see “Experimental Procedures”). The extracellular space in G\textsubscript{aq}Q209L-hbER mice exhibiting edema was significantly increased (2.45-fold) as compared with
WT mice injected for 28 days (Fig. 5B). GoqQ209L-hbER mice as a group, including non-edematous animals that were injected for 28 days, exhibited a 3.13-fold increase in extracellular space as compared with WT mice (25.7 ± 0.74%, n = 22). The difference in calculated extracellular space between WT and GoqQ209L-AA-hbER heart sections (1.61-fold) was not statistically significant.

The enlarged hearts seen in GoqQ209L-hbER animals could be due to cellular hypertrophy. It was surprising that cell hypertrophy was not apparent upon histological examination of heart sections from these mice, as this phenotype was reported in other Goq transgenic mice. As this phenotype was reported in other Goq knockout mice, this phenotype was observed in other mouse models of heart failure. Percent extracellular space was calculated as described under “Experimental Procedures.” n = 11 for WT and GoqQ209L-hbER; n = 9 for GoqQ209L-AA-hbER. * denotes a statistically significant difference between WT and GoqQ209L-hbER. Data were analyzed by one-way ANOVA, and pairwise comparisons were obtained using Fisher’s post-hoc tests. QL, GoqQ209L-hbER; QL-AA, GoqQ209L-AA-hbER.

Changes in PLB and SERCA-2 in GoqQ209L-hbER Mice—Heart failure is often associated with decreased myocyte contractility. Since myocyte contractility is regulated by changes in intracellular Ca2+ concentration generated with each heart beat, we examined two major pathways for Ca2+ handling in myocytes. One major regulator of Ca2+ concentration in cardiac myocytes is SERCA-2. Entry of extracellular Ca2+ through the L-type Ca2+ channel induces a large release of Ca2+ from the sarcoplasmic reticulum (SR) that is sequestered back into the SR by the SERCA-2 pump. Over time, inhibition of SERCA-2 activity decreases the amount of Ca2+ stored in the SR and leads to decreased contractile force. SERCA-2 is regulated by PLB, which occurs as monomeric and pentameric forms. Monomeric PLB binds to SERCA-2 to inhibit its activity. The pentameric form of PLB is phosphorylated on Ser16, which blocks PLB from binding to SERCA-2. Using a phospho-specific antibody, we found that pentameric PLB in membranes prepared from hearts of GoqQ209L-hbER mice becomes dephosphorylated in response to tamoxifen treatment (Fig. 7B, upper panel). The decrease in Ser16 phosphorylation occurred gradually over 7 days and remained low for the duration of the experiment. In contrast, tamoxifen injection did not affect PLB phosphorylation in either WT or GoqQ209L-AA-hbER hearts (Fig. 7, A and C, upper panels).

Using a general PLB antibody, we detected an increase in the monomeric form of PLB in GoqQ209L-hbER hearts after tamoxifen injection...
for 7–28 days (Fig. 7B, lower panel). Furthermore, after 7–28 days, the PLB pentamers from Gαq,Q209L-hbER myocytes migrated faster during polyacrylamide gel electrophoresis, indicating that the protein contains less phosphate than PLB from earlier time points (Fig. 7B, lower panel). Tamoxifen injection did not affect the PLB migration pattern or increase the amount of monomeric PLB in either WT or Gαq,Q209L-AA-hbER mice (Fig. 7, A and C, lower panels).

Finally, SERCA activity was measured by assaying Ca2+-dependent ATPase activity in heart membranes prepared from mice that were injected with tamoxifen for 14 days. SERCA specific activity was significantly decreased by 65% in Gαq,Q209L-hbER hearts as compared with WT hearts (Fig. 8A). In contrast, SERCA activity in the Gαq,Q209L-AA-hbER hearts was not reduced as compared with WT (Fig. 8A). The drop in activity in Gαq,Q209L-hbER hearts was not due to a lower level of SERCA-2 protein, as tamoxifen injection for up to 28 days did not affect the amount of SERCA-2 protein in any of the three groups of animals (Fig. 8B). These results suggest that activation of Gαq,Q209L-hbER by tamoxifen induces dephosphorylation of PLB and the release of PLB monomers that bind tightly to SERCA-2 to inhibit its activity. This response is probably PLCβ-dependent, as it is not seen in the Gαq,Q209L-AA-hbER hearts.

**Decreased I_{Ca,L} in Transgenic Myocytes**—Next we examined the major pathway for Ca2+-entry into myocytes, I_{Ca,L}, using the whole-cell patch clamp technique. Isolated myocytes from transgenic animals were always paired with cells from WT littermates. In the first experiment, myocytes were isolated from animals at different times after tamoxifen injection. The membrane was held at −50 mV and depolarized for 300 ms to +10 mV to measure I_{Ca,L}. I_{Ca,L} density in Gαq,Q209L-hbER myocytes was significantly reduced by 32% as compared with WT after only 7 days of drug treatment (TABLE ONE). The decrease was even larger at 14 days (72%) and 21 days (61%). After 28 days of tamoxifen treatment, I_{Ca,L} density in Gαq,Q209L-hbER myocytes was still 58%
lower than in WT cells (TABLE ONE). To our surprise, $I_{Ca,L}$ density was also significantly reduced by 51% in myocytes from $G_{\alpha_q}Q209L$-hbER animals injected with tamoxifen for 28 days ($5.8 \pm 0.8 \text{ pA/pF} (n = 14)$ as compared with $11.8 \pm 0.6 \text{ pA/pF}$ for WT ($n = 15$)).

We also constructed the peak inward $I-V$ relations for myocytes from WT and transgenic animals injected with tamoxifen for 28 days. Fig. 9, A and B, illustrate the patch clamp records, while Fig. 9, C and D, plot the peak inward current density for each data set at each potential. The $I_{Ca,L}$ density is decreased throughout the entire voltage range in myocytes from both transgenic mouse lines. This reduction in $I_{Ca,L}$ density was not due to a negative shift in the voltage dependence of inactivation nor to a difference in autonomic regulation (data not shown). These results demonstrate that $G_{\alpha_q}$ affects the L-type Ca$^{2+}$ current at least in part through a signaling pathway that is independent of PLC$\beta$ activation. We did not detect consistent differences in protein or mRNA levels of Ca$^{2+}$ channels in WT, $G_{\alpha_q}Q209L$-hbER, or $G_{\alpha_q}Q209L$-AA-hbER hearts (data not shown). Thus, activation of $G_{\alpha_q}$ leads to severe defects in two major Ca$^{2+}$-handling proteins that could explain the contractile defect in $G_{\alpha_q}Q209L$-hbER mice.

**DISCUSSION**

Previous studies suggested that activation of $G_{\alpha_q}$ induces cardiac hypertrophy and a contractile defect that is a consequence of this hypertrophic response. However, $G_{\alpha_q}$ signaling in the mice used in earlier studies was altered prior to adulthood, and it is becoming clear that the timing of genetic manipulations can have a profound effect on the phenotypic outcome (9). Here we used $G_{\alpha_q}Q209L$-hbER fusion proteins to activate $G_{\alpha_q}$ signaling selectively in cardiac myocytes of adult mice. hbER has been fused to a wide variety of partners to make tamoxifen-controlled fusion proteins. Inducible forms of the transcription factor myc (18) and the recombinase Cre (19) have been successfully used in transgenic mice to study heart phenotypes. Our transgenic mouse model is the first application of this system to regulate the activity of $G_{\alpha_q}$ proteins. We have found that this approach works for a variety of $G_{\alpha_q}$ proteins and small GTPases (data not shown). Our results indicate that it might be possible to utilize hbER fusion proteins to investigate the function of other signaling molecules in a temporally controllable manner in a variety of tissues.

Using the inducible $G_{\alpha_q}Q209L$-hbER model, we found that activation of $G_{\alpha_q}$ in adult mice causes a dilated cardiomyopathy that rapidly results in heart failure, apparently without progressing through a hypertrophic stage. It is possible that the rapid onset of cardiac failure precluded the animals from developing compensatory hypertrophy. This study also demonstrates that activation of $G_{\alpha_q}$ in adult mouse cardiac myocytes has early effects on Ca$^{2+}$ homeostasis and cardiac contractility. At least two aspects of Ca$^{2+}$ handling are markedly affected: the entry of Ca$^{2+}$ into myocytes through the L-type Ca$^{2+}$ channel is attenuated, and SERCA-2 activity is inhibited as a consequence of PLB dephosphorylation. Use of the $G_{\alpha_q}Q209L$-AA-hbER construct shows that $G_{\alpha_q}$ regulates the two Ca$^{2+}$-handling components via separate effector pathways: $G_{\alpha_q}$-induced PLB dephosphorylation is mediated by the canonical PLC$\beta$ pathway, whereas the effect on $I_{Ca,L}$ does not require PLC$\beta$ activation. Cumulatively, these two effects on Ca$^{2+}$ homeostasis would be expected to severely depress myocyte contractility and in combination are probably responsible for $G_{\alpha_q}$-induced heart failure.

Similar to our observations in $G_{\alpha_q}Q209L$-hbER mice, SERCA activity
was found to be decreased in human myocardium obtained from patients with end-stage heart failure (20). Schwinger et al. (21) also found that PLB Ser16 phosphorylation was reduced in failing as compared with control hearts. These investigators did not find significant changes in the amount of SERCA-2 or PLB protein (20), but controversy still exists as to whether levels of these proteins are altered in human heart failure. In transgenic mice overexpressing wild-type Goαq, SERCA-2 protein levels were decreased, and PLB protein was markedly increased and dephosphorylated (22, 23). In our heart failure model, we did not detect changes in the expression of SERCA-2 or PLB. Instead, PLB became dephosphorylated, monomeric PLB appeared, and SERCA activity was reduced. These Goαq effects are probably mediated by the PLCβ pathway that leads to the activation of PKC isozymes including PKCα. Interestingly, mice overexpressing PKCα in cardiac myocytes also showed decreased PLB phosphorylation on Ser16 (24).

Although our results suggest that inhibition of SERCA-2 plays an important role in the development of Goαq-induced cardiomyopathy, this effect alone is probably not sufficient to induce heart failure. The PKCα transgenic mice mentioned above develop contractile defects and cardiac hypertrophy but not heart failure, even though dephosphorylation of PLB should lead to inhibition of SERCA-2 (24). SERCA-2−/− heterozygous mice have a 35% reduction in SERCA activity but do not develop heart failure or cardiac hypertrophy (25). SERCA-2 haploinsufficiency does, however, cause a decrease in cardiac contractility (25) and myocyte contractility (26). Transgenic mice that overexpress PLB 2-fold in cardiac myocytes do not develop heart failure despite a decrease in left ventricular fractional shortening (27). Similarly, expression of PLB mutants L37A and I40A that persist in the inhibitory monomeric form reduces dP/dt and fractional shortening but does not cause heart failure (28).

The lack of a severe cardiac phenotype in the animals with decreased SERCA-2 activity discussed above as compared with our GoαqQ209L-hbER mice may be due to the fact that ICa,L is not concurrently inhibited. In fact, overexpression of a superinhibitory PLB V49G mutant in transgenic mice caused a significant compensatory increase in the ICa,L density in myocytes (29). Goαq may have additional effects on other components of the Ca2+/cycling machinery such as the ryanodine receptor and the Na+/Ca2+ exchanger that contribute to heart failure. However, we did not detect changes in expression of the exchanger or the ryanodine receptor or its phosphorylation in GoαqQ209L-hbER hearts (data not shown). It is interesting to note that PLB ablation in mice overexpressing wild-type Goαq did not improve global cardiac function, suggesting that Goαq-induced heart failure might involve more than just SERCA-2 inhibition (22).

Since Ca2+ influx through the L-type Ca2+ channel is essential for triggering SR Ca2+ release, a reduction in ICa,L density would be expected to lead to depressed cardiac contractility. However, the contribution of altered L-type Ca2+ channel expression or function to the development of heart failure is controversial. Measurements in myocytes isolated from patients with end-stage heart failure have generally not detected a reduction in basal

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**FIGURE 9.** ICa,L activation in transgenic and WT myocytes. Representative traces of ICa,L activation in myocytes from WT and GoαqQ209L-hbER (QL) mice (A) and WT and GoαqQ209L-AA-hbER (QL-AA) mice (B). The currents were elicited by voltage steps from −50 mV to +50 mV (300-ms duration) in 10-mV increments from a holding potential of −50 mV. The peak I-V relationships of ICa,L for myocytes from GoαqQ209L-hbER mice (C; ●) and matched WT littermates (C; ○) (n = 17 for both) and GoαqQ209L-AA-hbER (D; ●) and matched WT (D; ○) mice (n = 14 for both).
$I_{\text{Ca,L}}$ density (30–32). However, a study by Ouaddad et al. (33) did report a reduction in $I_{\text{Ca,L}}$ density in atrial and ventricular myocytes from patients undergoing cardiac transplantation. Most studies in animal models of heart failure have shown either unchanged or reduced L-type current density (34). Both the $\alpha_q$Q209L-hbER and $\alpha_q$Q209L-AA-hbER mice studied here exhibit a significant reduction in $I_{\text{Ca,L}}$ density after tamoxifen injection, but only the $\alpha_q$Q209L-hbER animals developed a contractile defect and heart failure. While these results suggest that inhibition of $I_{\text{Ca,L}}$ alone is not sufficient to induce heart failure, they do not rule out the possibility that a reduction in $I_{\text{Ca,L}}$ density contributes to $\alpha_q$-induced cardiomyopathy.

The suppression of $I_{\text{Ca,L}}$ density in $\alpha_q$Q209L-AA-hbER mice indicates that $\alpha_q$ inhibits the L-type $\text{Ca}^{2+}$ channel independently of PLCβ. Studies are ongoing in our laboratory to identify the signaling pathway that mediates this effect. We recently demonstrated that $\alpha_q$ inhibits the PI3K p110α isoform (2) and binds directly to the enzyme in vitro.4 $\alpha_q$ inhibition of PI3K may play a significant role in causing the inhibition of $I_{\text{Ca,L}}$. Indeed, we have found that injection of the second messenger phosphatidylinositol (3,4,5)-trisphosphate into $\alpha_q$Q209L-AA-hbER myocytes can reverse the inhibition of $I_{\text{Ca,L}}$ (37). This hypothesis can be further tested in vivo by producing transgenic mice that express a $\alpha_q$ mutant that cannot inhibit PI3K but that retains the ability to activate PLCβ.

Studies in end-stage heart failure patients treated with left ventricular assist devices have shown that mechanical unloading improves myocyte function and reverses structural remodeling of the failing heart (35, 36). This therapy also normalizes $\text{Ca}^{2+}$ transients and increases SERCA-mediated $\text{Ca}^{2+}$ uptake. These findings support the concept that some aspects of myocyte dysfunction in the failing heart are reversible. Preliminary studies using the $\alpha_q$Q209L-hbER mice support this view. We have found that terminating the tamoxifen injections in edematous mice allows the gradual reversal of the overt signs of heart failure, enlargement, ventricular dilation, and interstitial space expansion.5 Additional studies are needed to determine whether the biochemical and hemodynamic alterations in these animals are also reversible. Future studies that utilize these transgenic mice to examine genetic changes associated with the onset and reversal of heart failure may yield fruitful information.

Results from our study suggest that activation of $\alpha_q$-coupled receptors by elevated levels of neuroendocrine hormones found in heart failure will lead to dampening of the $\text{Ca}^{2+}$ transient and depression of cardiac contractility. Development of pharmacological interventions that block $\alpha_q$ or PLCβ signaling in myocytes may prove useful as a strategy to improve contractile function in heart failure.

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