Reversible Heart Failure in Gαq Transgenic Mice*

Ya-Ping Jiang‡, Lisa M. Ballou‡§, Zhongju Lu‡, Li Wan‡, Damon J. Kelly¶, Ira S. Cohen*, and Richard Z. Lin*†*

From the‡Department of Medicine, the§Department of Physiology and Biophysics and the Institute of Molecular Cardiology, and the¶Department of Biomedical Engineering, Stony Brook University, Stony Brook, New York 11794 and the*Department of Veterans Affairs Medical Center, Northport, New York 11768

For many patients with cardiac insufficiency, the disease progresses inexorably to organ dilatation, pump failure, and death. Although there are examples of reversible heart failure in man, our understanding of how the myocardium repairs itself is limited. A well defined animal model of reversible heart failure would allow us to better investigate these restorative processes.

Receptors that activate Gαq, a signal transduction molecule in the heterotrimeric G protein superfamily, are thought to play a key role in the development of heart failure. We demonstrated previously that mice expressing a recombinant Gαq-protein, the activity of which can be turned on or off at will in cardiac myocytes, develop a dilated cardiomyopathy with generalized edema and heart failure following activation of the protein (Fan, G., Jiang, Y.-P., Lu, Z., Martin, D. W., Kelly, D. J., Zuckerman, J. M., Ballou, L. M., Cohen, I. S., and Lin, R. Z. (2005) J. Biol. Chem. 280, 40337–40346). Here we report that the contractile dysfunction and pathological structural changes in the myocardium improved significantly after termination of the Gαq signal, even in animals with overt heart failure. Abnormalities in two proteins that regulate Ca2+ handling in myocytes, phospholamban and the voltage-dependent L-type Ca2+ channel, were also reversed, as was the increased expression of genes that are associated with heart failure. These results indicate that the heart has a substantial reparative capacity if the molecular signals responsible for the myocardial dysfunction can be identified and blocked.

Following cardiac injury, activation of Gαq-coupled receptors in the heart exerts deleterious effects that promote the development of heart failure. To better investigate the role of Gαq activation in the development of cardiac failure in adult animals, we developed transgenic mice that express a recombinant Gαq protein, the activity of which can be turned on or off at will. Fusing a mutant hormone-binding domain of the estrogen receptor to the carboxyl terminus of constitutively active GαqQ209L renders this Gαq protein inactive (1). However, in the presence of 4-hydroxytamoxifen, the GαQ209L-hbER (QL) protein becomes active. This inducible model allowed us to investigate the consequences of Gαq activation in myocytes after completion of normal development and growth. We found that tamoxifen activation of the QL protein in adult transgenic mice causes a dilated cardiomyopathy that leads to heart failure (1).

Heart failure is often associated with depressed cardiac contractility, which is also exhibited by the QL mice (1). Contractility of cardiac myocytes is regulated by changes in the intracellular Ca2+ concentration. Ca2+ enters the cell through the L-type voltage-dependent Ca2+ channel (LTCC) and induces a large release of Ca2+ from the sarcoplasmic reticulum (SR) through the ryanodine receptor, resulting in myocyte contraction. Relaxation is caused by removal of Ca2+ either by the SR Ca2+-ATPase (SERCA) or the Na+-Ca2+ exchanger located at the plasma membrane. SERCA is regulated by phospholamban (PLB), which inhibits SERCA when dephosphorylated and thus decreases Ca2+ uptake into the SR. In the absence of compensatory responses, decreased LTCC function would reduce Ca2+ entry, leading to decreased myocyte contractility. Dephosphorylation of PLB leading to inhibition of SERCA would deplete SR Ca2+ stores and also lead to a decrease in myocyte contractility. Both LTCC function and PLB phosphorylation are decreased in the QL hearts, which may explain why these animals develop heart failure (1).

Another advantage of this inducible model is that it allowed us to terminate the Gαq signal and determine whether any of the cardiac abnormalities are reversible. In this study, we found that even after the QL mice developed signs of overt heart failure, many of the cardiac defects returned to normal once the Gαq signal was turned off.

**EXPERIMENTAL PROCEDURES**

*Materials—Antibodies for phospho-Ser16 PLB and PLB were from Upstate Biotechnology, Inc. (Lake Placid, NY). SERCA-2 antibody was from ABR (Golden, CO). Tamoxifen was from Sigma.

GαQ209L-hbER Transgenic Mice—C57BL/6 mice expressing QL under the control of an α myosin heavy chain (MHC) promoter were described previously (1). Measurement of cardiac hemodynamics, PLB phosphorylation, percent extracellular space, gene expression, and whole-cell patch clamping of isolated cardiac myocytes were performed as described previously.
RESULTS AND DISCUSSION

Reversible Heart Failure in QL Mice—In our previous study of QL transgenic mice, we found that many of the male mice developed generalized edema indicative of heart failure following tamoxifen injection to activate the Goq protein (1). Because this phenotype can be easily monitored by weighing the animals, we used a 20% increase in body weight as the criterion to identify mice with overt heart failure. Starting at 8 weeks of age, male QL mice and wild type (WT) littermates were weighed and injected daily with 1 mg of tamoxifen for up to 28 days. Fifty percent (36/71) of the QL mice met the weight gain criterion within this time span. These mice were designated as QL (Tam), and their paired WT controls were designated as WT (Tam). Thirty-six percent (26/71) of the QL mice died without attaining a 20% weight gain. The paired QL (Tam) and WT (Tam) animals were randomly assigned to two groups. In the first group, the animals were analyzed immediately. In the second group, we stopped the tamoxifen injections and continued to monitor their body weight for the next 14 days to determine whether heart function improved upon termination of the Goq signal. None of the WT mice died during the experimental period, and none of the QL (Tam) mice died after the tamoxifen injections were stopped. Mice at the end of this 14-day recovery period were designated as QL (Rev) and WT (Rev).

Fig. 1A shows the change in body weight of a representative pair of QL (Rev) and WT (Rev) animals throughout the experimental period. As expected, the WT mouse gained weight gradually over the first 23 days. In contrast, the QL mouse began to gain weight rapidly after 14 days of tamoxifen injection. This was associated with a decrease in physical activity and an edematous appearance as described previously (1). At day 23, the QL mouse had gained more than 20% of its initial body weight, and the injections were stopped in both mice (Fig. 1A, arrow). Over the next 14 days, the body weight of the WT mouse changed relatively little. In contrast, the QL mouse lost weight rapidly (Fig. 1A), and the edematous appearance resolved. Fig. 1B summarizes the changes in body weight for multiple QL (Rev) and WT (Rev) mouse pairs. The elevated average body weight gain of the QL mice at the time when the tamoxifen injections were stopped significantly reversed 14 days later (Fig. 1B). The final average body weight gain in QL mice after the 14-day recovery period was statistically indistinguishable from that of the WT mice (~7%) (Fig. 1B).

We previously used invasive hemodynamic measurements to demonstrate the presence of heart failure in QL mice injected with tamoxifen (1). The same technique was used to confirm that cardiac function improved in QL (Rev) mice. Left ventricular end-diastolic pressure was elevated, and dp/dt values were altered in the edematous QL (Tam) animals as compared with the WT (Tam) controls (Table 1), indicating compromised contractile function. All of the hemodynamic parameters were significantly improved in the QL (Rev) mice as compared with the QL (Tam) group (Table 1).

TABLE 1

Hemodynamic measurements in QL and WT mice

| Parameter               | WT (Tam)        | WT (Rev)       | QL (Tam)        | QL (Rev)        |
|-------------------------|-----------------|----------------|-----------------|-----------------|
| HR (beats/min)          | 395 ± 21        | 425 ± 24       | 320 ± 33        | 442 ± 28*       |
| LVEP (mm Hg)            | 98 ± 4          | 99 ± 3         | 81 ± 5          | 106 ± 7*        |
| LVESP (mm Hg)           | 0.6 ± 1.2       | 0.1 ± 0.7      | 10.8 ± 3.7      | −1.4 ± 0.5*     |
| Max dp/dt (mm Hg/s)     | 9230 ± 740      | 10432 ± 910    | 4167 ± 610      | −11588 ± 1060*  |
| Min dp/dt (mm Hg/s)     | −6450 ± 427     | −6990 ± 210    | −3426 ± 440     | −8333 ± 647*    |

* Significantly different from QL (Tam).
Reversible Heart Failure

As we reported previously (1), the cardiomyopathy in QL (Tam) mice is associated with cardiac dilatation and chamber enlargement (Fig. 2A), an increased heart weight/tibia length ratio (Fig. 2B), and increased extracellular space between myocytes (Fig. 2, C and D). These morphological and histological changes were much improved in the QL (Rev) mice (Fig. 2). However, the percent extracellular space in the QL (Rev) animals was still elevated as compared with WT (Rev) mice (Fig. 2D). It is not known whether a recovery period longer than 14 days would allow a complete reversal of this phenotype. Relatively rapid reversal of these morphological changes supports our previous observation that heart failure in the QL mice is probably not associated with myocyte loss (1).

Unlike QL mice, in which the cardiac pathologies were reversed when the Go-q signal was turned off, overexpression of Go-q(Q209L) in myocytes of conventional transgenic mice induces cardiac dilatation that progresses to heart failure even after the initiating signal is lost (2). The level of exogenous Go-q(Q209L) protein in the ventricles drops dramatically between 2 and 4 weeks of age, and by 10 weeks it is undetectable. These mice die of heart failure between 8 and 30 weeks of age (2). This result is somewhat surprising because the structural changes in our QL mice reverted to near base line when the Go-q signal was turned off (Fig. 2). One possible explanation for this difference in phenotype is that activation of Go-q during development but not during adulthood irreversibly alters the myocardium in a deleterious manner.

Reversible Molecular Changes in QL Hearts—Heart failure is associated with increased expression of genes such as natriuretic peptide precursor type B (BNP) and βMHC. In humans, an increased level of circulating BNP is used as a diagnostic indicator of heart failure. As reported earlier (1), heart failure in the QL mouse is also associated with an increased expression of BNP and βMHC mRNAs and decreased expression of αMHC mRNA (Fig. 3A). More importantly, in the QL (Rev) hearts the amount of BNP and βMHC mRNA decreased, and the amount of αMHC mRNA increased nearly to WT (Rev) levels (Fig. 3A). All of the changes between the QL (Tam) and QL (Rev) groups are statistically significant.

Heart failure in the QL mice is associated with functional changes in two major regulators of Ca^{2+} homeostasis in myocytes: SERCA-2 and the LTCC (1). To assess the reversibility of these Ca^{2+} handling defects, we first measured the phosphorylation state of PLB in heart membranes prepared from QL and WT mice. Phosphorylation of PLB on Ser^{16} prevents it from binding to and inhibiting SERCA-2. PLB underwent almost complete dephosphorylation in QL (Tam) animals (Fig. 3B). However, phosphorylation was essentially completely restored in the QL (Rev) hearts to the level seen in WT (Rev) hearts. The total amount of PLB and SERCA-2 was not noticeably different in the four groups (Fig. 3B). Finally, we investigated whether the decreased LTCC function exhibited by QL (Tam) mice is also reversible. Whole-cell patch clamping was used to measure inward Ca^{2+} current (I_{Ca,L}) density as an assessment of LTCC function as described previously (3). First, the peak I_{Ca,L} density at +10 mV was calculated. The depressed I_{Ca,L} density seen in myocytes from QL (Tam) mice over the entire range of activation (greater than −30 mV) (Fig. 3D).
Although some forms of heart failure in humans are reversible, including myocardial dysfunction because of sepsis (4), myocarditis, transient ischemia (5), and takotsubo cardiomyopathy (6), the presence of structural changes with ventricular dilatation is considered to be a poor prognostic factor. However, recent 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 (7, 8). This therapy also normalizes Ca\(^{2+}\)/H\(^{+}\) transients and increases SERCA-mediated Ca\(^{2+}\)/H\(^{+}\) uptake. Results from the present study are consistent with these findings in humans, and they further indicate that the structural and electrical remodeling of even a severely damaged heart is potentially reversible. However, one must be cautious when extrapolating results using rodent models of heart failure to the human condition because there are important species differences in cardiac physiology. Our model differs from the type of heart failure that progresses through a hypertrophic stage caused by hypertension. However, experiments in sheep suggest that recovery is also possible in a failing hypertrophic heart.

Moorjani et al. (9) showed that after relief of pressure stress in an aortic banding model, both cardiac remodeling and contractile function improve.

The relatively rapid onset and recovery from heart failure in this unique animal model will allow additional studies to delineate the sequence of molecular events that are necessary and sufficient to cause these cardiac changes. For example, our previous study indicated that changes in Ca\(^{2+}\)/H\(^{+}\) homeostasis occur prior to structural remodeling of the heart (1), but it is not known whether normalization of electrical and other molecular changes precedes the structural recovery of the heart. These mice may also be used to ask whether stable epigenetic or post-translational changes in the myocytes occur at some point during disease progression to lead to an irreparable failure. Lastly, this animal model will also allow us to investigate how age, exercise, and diet affect the onset and recovery from heart failure. It is our hope that these investigations will lead to an improved understanding of the molecular mechanisms that lead to heart failure and to the discovery of strategies that will stop or reverse this pathological process.

**FIGURE 3.** Reversible molecular changes in the QL mouse heart. A, total RNA was extracted from hearts, and quantitative real-time PCR was performed as described previously (1) to determine relative changes in mRNA expression as compared with WT. n = 6 per group. B, Western blotting to detect PLB phosphorylated on Ser\(^{16}\) (top panel), total PLB (middle panel), and SERCA-2 (bottom panel) in heart membranes. Each lane corresponds to one animal. The experiment was repeated with similar results. C, myocytes were isolated from QL and WT mice. The peak \(I_{\text{Ca},\text{L}}\) values were measured by whole-cell patch clamping and normalized to cell capacitance. The membrane current was evoked by a 300-ms depolarizing pulse to +10 mV from a holding potential of −50 mV. WT (Tam), n = 21 cells; WT (Rev), n = 9; QL (Tam), n = 45; and QL (Rev), n = 15. D, current-voltage relationships were generated using 300-ms depolarizing voltage steps from −50 to +50 mV in 10 mV increments from a holding potential of −50 mV. WT (Tam), n = 17; WT (Rev), n = 7; QL (Tam), n = 12; and QL (Rev), n = 15.
REFERENCES

1. Fan, G., Jiang, Y.-P., Lu, Z., Martin, D. W., Kelly, D. J., Zuckerman, J. M., Ballou, L. M., Cohen, I. S., and Lin, R. Z. (2005) J. Biol. Chem. 280, 40337–40346
2. Mende, U., Kagen, A., Cohen, A., Aramburu, J., Schoen, F. J., and Neer, E. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13893–13898
3. Lu, Z., Jiang, Y. P., Ballou, L. M., Cohen, I. S., and Lin, R. Z. (2005) J. Biol. Chem. 280, 40347–40354
4. Parker, M. M., Shelhamer, J. H., Bacharach, S. L., Green, M. V., Natanson, C., Frederick, T. M., Damske, B. A., and Parrillo, J. E. (1984) Ann. Intern. Med. 100, 483–490
5. Hollenberg, S. M., and Parrillo, J. E. (1997) J. Heart Lung Transplant. 16, S7–S12
6. Akashi, Y. J., Nakazawa, K., Sakakibara, M., Miyake, F., Koike, H., and Sasaki, K. (2003) QJM 96, 563–573
7. Dipla, K., Mattiello, J. A., Jeevanandam, V., Houser, S. R., and Margulies, K. B. (1998) Circulation 97, 2316–2322
8. Zafeiridis, A., Jeevanandam, V., Houser, S. R., and Margulies, K. B. (1998) Circulation 98, 656–662
9. Moorjani, N., Catarino, P., El-Sayed, R., Al-Ahmed, S., Meyer, B., Al-Mohanna, F., and Westaby, S. (2003) Eur. J. Cardiothorac. Surg. 24, 920–925