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Oxidative Post-Translational Modifications Mediate Decreased SERCA Activity and Myocyte Dysfunction in Gαq-Overexpressing Mice

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Abstract

Background—Myocyte contractile dysfunction occurs in pathological remodeling in association with abnormalities in calcium regulation. Mice with cardiac myocyte-specific overexpression of Gαq develop progressive left ventricular (LV) failure associated with myocyte contractile dysfunction and calcium dysregulation. We tested the hypothesis that myocyte contractile dysfunction in the Gαq mouse heart is mediated by reactive oxygen species (ROS), and in particular, oxidative post-translational modifications (OPTM) that impair the function of sarcoplasmic reticulum Ca^{++}-ATPase (SERCA).

Methods and Results—Freshly isolated ventricular myocytes from Gαq mice had marked abnormalities of myocyte contractile function and calcium transients. In Gαq myocardium, SERCA protein was not altered in quantity, but displayed evidence of oxidative cysteine modifications reflected by decreased biotinylated iodoacetamide labeling, and evidence of specific irreversible oxidative modifications consisting of sulfonylation at cysteine 674 and nitration at tyrosines 294/295. Maximal calcium-stimulated SERCA activity was decreased 47% in Gαq myocardium. Cross-breeding Gαq mice with transgenic mice that have cardiac myocyte-specific overexpression of catalase a) decreased SERCA oxidative cysteine modifications, b) decreased SERCA cysteine 674 sulfonylation and tyrosine 294/295 nitration, c) restored SERCA activity, and d) improved myocyte calcium transients and contractile function.

Conclusions—In Gαq-induced cardiomyopathy, myocyte contractile dysfunction is mediated, at least in part, by one or more OPTM of SERCA. Protein OPTM contribute to the pathophysiology of myocardial dysfunction, and thus may provide a target for therapeutic intervention.

Keywords

Cardiac myocytes; sarcoplasmic reticulum ATPase; SERCA; oxidative modification

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Disclosures

None.

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Introduction

Myocyte contractile dysfunction occurs in several models of pathological remodeling including pressure overload [1–4] and after myocardial infarction [5–8]. While myocyte dysfunction appears to be caused, at least in part, by abnormalities in calcium regulation [9], the underlying mechanism remains unclear. There is evidence that reactive oxygen species (ROS) mediate some aspects of pathological myocardial remodeling including myocyte hypertrophy and apoptosis [10–12]. Mice with cardiac myocyte-specific overexpression of Gαq develop progressive left ventricular (LV) dilation and failure [13] that is associated with myocyte contractile dysfunction and calcium dysregulation [14]. These mice have increased oxidative stress in the myocardium [15], and recently, we demonstrated that concomitant myocyte-specific expression of catalase ameliorated pathological LV remodeling, inhibited myocyte hypertrophy and apoptosis, and preserved LV contractile function [16]. Accordingly, we tested the hypothesis that myocyte contractile dysfunction in the Gαq mouse heart is also mediated by ROS, and in particular, involves oxidative post-translational modifications (OPTM) that impair the function of sarcoplasmic reticulum Ca^{++}-ATPase (SERCA) [17].

Methods

Detailed methods are provided in the online supplement. Briefly, transgenic mice with cardiac myocyte-specific overexpression of Gαq (Gαq-40 mice, FVB/N) [13] and WT (FVB/N) mice were cross-bred with transgenic mice having myocyte-specific overexpression of catalase [18], as we previously described [16]. Myocytes were isolated, and contraction and intracellular calcium transients were measured as we have described previously [19]. SERCA2 activity was measured using calcium-stimulated, thapsigargin-inhibitable calcium[45] uptake in an SR membrane preparation by a modification of published methods [14;20], as we have described [21]. BIAM-labeling, immunoblotting, and immunohistochemical detection of SERCA OPTM are described in the online supplement. All data are presented as mean ± SEM.

Results

Concurrent myocyte-specific catalase overexpression ameliorates contractile dysfunction and calcium dysregulation in myocytes from Gαq mice

Ventricular myocytes were isolated from mice with myocyte-specific overexpression of Gαq [13–16] and myocyte contractile function and intracellular calcium transients were assessed, as we have described [19;21]. In myocytes from Gαq mice (vs. WT), the amplitude of cell shortening was decreased by 53%, and the rates of myocyte shortening and relaxation were reduced by 62% and 63%, respectively (Figure 1, A–C). Likewise, in Gαq myocytes the calcium transient amplitude was decreased by 37%, and the rates of rise and decline were decreased by 34% and 33%, respectively (Figure 1, D–F). Gαq mice were cross-bred with mice that have myocyte-specific overexpression of catalase [18], as we have described [16]. In myocytes from Gαq/catalase mice, the amplitude of cell shortening, and the rates of cell shortening and relaxation were improved (Figure 1, A–C); and the abnormalities in calcium transient amplitude and kinetics were ameliorated (Figure 1, D–F).

Expression of calcium regulating proteins in Gαq myocardium

The protein levels of SERCA, the ryanodine receptor (RyR), phospholamban (PLB) and the sodium/calcium exchanger (NCX) were determined by immunoblotting. RyR protein expression was decreased by 47% in Gαq mice, whereas the expression of SERCA, PLB and NCX was unchanged (Table 1). The decrease in RyR protein was associated with a 42% decrease in mRNA (Online Figure I), suggesting that the decrease in protein was mediated at

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the transcript level. Concurrent expression of catalase in Gαq/catalase mice had no effect on RyR protein or transcript levels (Table 1; Online Figure I).

OPTM of SERCA in Gαq myocardium

To test whether OPTM may contribute to contractile dysfunction in Gαq myocytes, oxidative thiol modifications of SERCA and RyR were assessed using biotinylated iodoacetamide (BIAM), as we have described [17;22]. Compared to WT, the fraction of BIAM-labeled SERCA in Gaq was decreased by 36% (Figure 2, A and B), whereas BIAM-labeling of RyR was unchanged (data not shown). We have developed antibodies directed at SERCA that is sulfonylated at cysteine 674 [23] or nitrated at tyrosine 294/295 [24]. Using these antibodies, immunohistochemistry revealed increased staining for both OPTM diffusely over myocytes in Gαq hearts (Figure 2, C and D). In myocardium from Gαq/catalase mice (compared to Gαq mice), there was a) increased BIAM labeling of SERCA (Figure 2, A and B), indicating a decrease in cysteine oxidation, b) decreased sulfonylation of SERCA cysteine 674 (Figure 2C), and c) decreased nitration of SERCA tyrosine 294/295 (Figure 2D).

Decreased SERCA activity in Gαq myocardium is restored by catalase

To assess the functional consequence of the observed OPTM, SERCA activity was measured using maximal calcium-stimulated calcium uptake in sarcolemmal membranes, as we have described [21]. SERCA-mediated calcium uptake was reduced by 47% in Gαq membranes (Figure 3). In myocytes from Gαq/catalase mice, maximal calcium-stimulated SERCA activity was restored to WT levels (Figure 3).

Discussion

We used the Gαq mouse model of dilated cardiomyopathy to examine the role of protein OPTM in mediating myocyte contractile dysfunction. Prior work from Satoh [15] and our group [16] demonstrated increased oxidative stress in the myocardium of these mice. Likewise, our finding of contractile dysfunction and abnormal calcium regulation in Gαq myocytes confirms and extends the prior report by Yatani et al [14]. Recently, we demonstrated that concurrent cardiac myocyte-specific overexpression of catalase improved cardiac function in these mice [16]. Accordingly, we hypothesized that catalase would improve myocyte function and thereby allow the identification of catalase-sensitive OPTM of myocyte proteins involved in the pathophysiology of contractile dysfunction.

Our initial finding was that cardiac myocyte-specific overexpression of catalase ameliorated the abnormalities in cardiac myocyte contractile function and calcium regulation. This indicates that a catalase-sensitive pathway is involved in mediating myocyte dysfunction, and directed our attention to proteins involved in calcium handling. Of the proteins primarily involved in myocyte calcium regulation, only the expression of RyR was decreased. However, the decreases in RyR protein and its mRNA were not affected by catalase, and thus, are not responsible for the effect of catalase on myocyte function that we observed

SERCA activity was decreased in Gαq myocardium, consistent with prior observations in this [14] and other models of heart failure [9;25]. However, the expression of SERCA was not decreased, which is also consistent with prior observations in this mouse [13]. Of note, SERCA activity was corrected by concurrent catalase expression, suggesting that OPTM of SERCA might be responsible for decreased SERCA activity. This thesis was further supported by 3 observations. First, in Gαq myocardium there was a decrease in the quantity of BIAM binding to SERCA, which indicates oxidative modification of the most reactive SERCA cysteine, cysteine 674, and potentially other cysteines [17]. Importantly, the quantity

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of BIAM binding to SERCA was restored towards normal in Gαq/catalase mice, confirming that the modification was oxidative in nature. Second, there was immunohistochemical evidence of sulfonylation of SERCA at cysteine-674 [23]. This OPTM is noteworthy because we have shown that sulfonylation of SERCA cysteine 674 in atherosclerotic aortic smooth muscle is associated with decreased activity [23]. Third, there was immunohistochemical evidence of nitration of SERCA tyrosine 294/295 [24]. Sulfonylation of SERCA at cysteine 674 and nitration of tyrosine at 294/295 provide evidence of irreversible oxidation by elevated oxidants [23]. As with BIAM binding, both SERCA sulfonylation and nitration were markedly decreased in Gαq/catalase mice. These findings thus directly identify two specific OPTM of SERCA in the Gαq mouse, and demonstrate that both can be prevented by catalase. This observation implicates H_2O_2 in the oxidation of SERCA cysteine-674 [26] and the nitration of SERCA tyrosines [27]. H_2O_2 is most likely derived via the dismutation of superoxide that is produced by mitochondria and/or oxidases.

While we have identified two specific irreversible OPTM that are associated with decreased SERCA activity, we can not exclude phosphorylation of calcium-regulating proteins due to oxidative regulation of a phosphatase. However, we think it is unlikely that this mechanism could explain our primary observation – correction of maximal calcium-stimulated SERCA activity - since a) SERCA is not known to have regulatory phosphorylation sites, and b) SERCA activity was measured using maximal calcium stimulation, which is not sensitive to phospholamban. On the other hand, oxidative regulation of phosphorylation might contribute to other aspects of contractile dysfunction in this model.

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Prior studies in mice have implicated abnormal calcium handling, and in particular, decreased SERCA function in the pathophysiology of myocyte contractile dysfunction [3;4;28;29]. Likewise, numerous studies have identified decreased SERCA activity in failing human myocardium [30–32] which, in some cases, has been associated with normal SERCA protein levels [31;32]. In preliminary studies we have found immunohistochemical evidence that SERCA is sulfonylated at cysteine 674 in myocardium from patients with heart failure due to dilated cardiomyopathy, but not in myocardium from patients without heart failure (unpublished data), thus suggesting that our findings in the Gαq mouse are relevant to human disease.

Novel findings of this study include the demonstration of a) multiple specific oxidative modifications of SERCA, b) the relationship between SERCA OPTM and reduced SERCA activity and altered cellular calcium handling, and c) the rescue of OPTM, SERCA activity and cellular calcium handling by myocyte-specific overexpression of catalase. Taken together, our data suggest that cardiac myocyte contractile dysfunction in the Gαq mouse is mediated, in part, by catalase-sensitive OPTM of SERCA. These observations suggest that OPTM caused by H_2O_2 contribute to myocardial dysfunction in pathologic states, such as heart failure, that are associated with increased oxidant levels in the heart.

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Novelty and Significance

What Is Known?

- Myocyte dysfunction and calcium dysregulation occur in human heart failure in association with increased reactive oxygen species (ROS) in the myocardium.
- Mice with myocyte-specific overexpression of Gαq develop a dilated cardiomyopathy that progresses to heart failure associated with myocyte contractile dysfunction and calcium dysregulation.
- ROS are increased in the Gαq overexpressing mouse heart.

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What New Information Does This Article Contribute?

- Gαq overexpressing mice exhibit specific irreversible oxidative post-translational modifications (OPTM) of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) consisting of sulfonylation at cysteine 674 and nitration at tyrosine 294/295.
- OPTM of SERCA in Gαq overexpressing mice are associated with reduced SERCA activity in myocardium, myocyte calcium dysregulation and myocyte contractile dysfunction.
- Myocyte-specific overexpression of catalase prevents OPTM of SERCA, restores SERCA activity and improves myocyte calcium dysregulation and contractile dysfunction.

We tested whether myocyte dysfunction in Gαq mice is mediated by OPTM of SERCA. In Gαq myocardium there were specific OPTM of SERCA associated with reduced SERCA activity and impaired calcium-related myocyte function. Myocyte-specific overexpression of catalase prevented SERCA OPTM and rescued SERCA activity and isolated myocyte function. Thus, myocyte contractile dysfunction in Gαq-induced cardiomyopathy is mediated, at least in part, by OPTM of SERCA. More broadly, these observations suggest that protein OPTM may contribute to the pathophysiology of myocardial dysfunction in heart failure and other conditions associated with increased myocardial ROS, and may provide a novel therapeutic target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Non-standard Abbreviations and Acronyms

BIAM	biotinylated iodoacetamide
H_2O_2	hydrogen peroxide
LV	left ventricular
OPTM	oxidative post-translational modifications
PLB	phospholamban
RyR	ryanodine receptor
ROS	reactive oxygen species
SERCA	sarcoplasmic reticulum Ca^{2+}-ATPase
NCX	sodium/calcium exchanger

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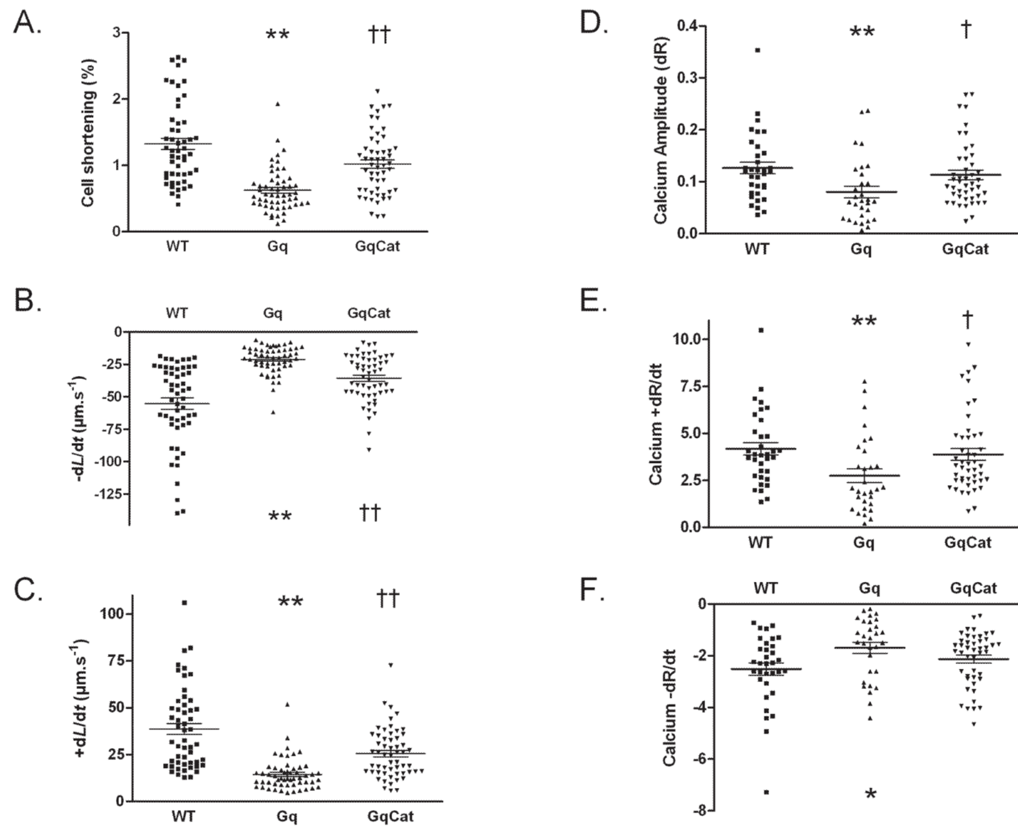

Figure 1.

Abnormal contractile function and intracellular calcium transients in cardiac myocytes from Gαq overexpressing mice are ameliorated by cross-breeding with mice that overexpress catalase in the myocardium. Ventricular myocytes were isolated from wild-type (WT), Gαq (Gq) or Gαq/catalase (GqCat) mice. **Panel A.** Cell shortening (% of baseline). **Panel B.** Velocity of contraction (−d*L*/d*t*). **Panel C.** Velocity of relaxation (+d*L*/d*t*). **Panel D.** Calcium transient amplitude (delta of the ratio (R) of fluorescence 360/380nm). **Panel E.** Rate of calcium transient rise (+d*R*/d*t*). **Panel F.** Rate of calcium transient decline (−d*R*/d*t*). *$p<0.05$ vs. WT; **$p<0.01$ vs. WT; †$p<0.05$ vs. Gαq; ††$p<0.01$ vs. Gαq; 5–10 cells per heart, 4–5 hearts per group.

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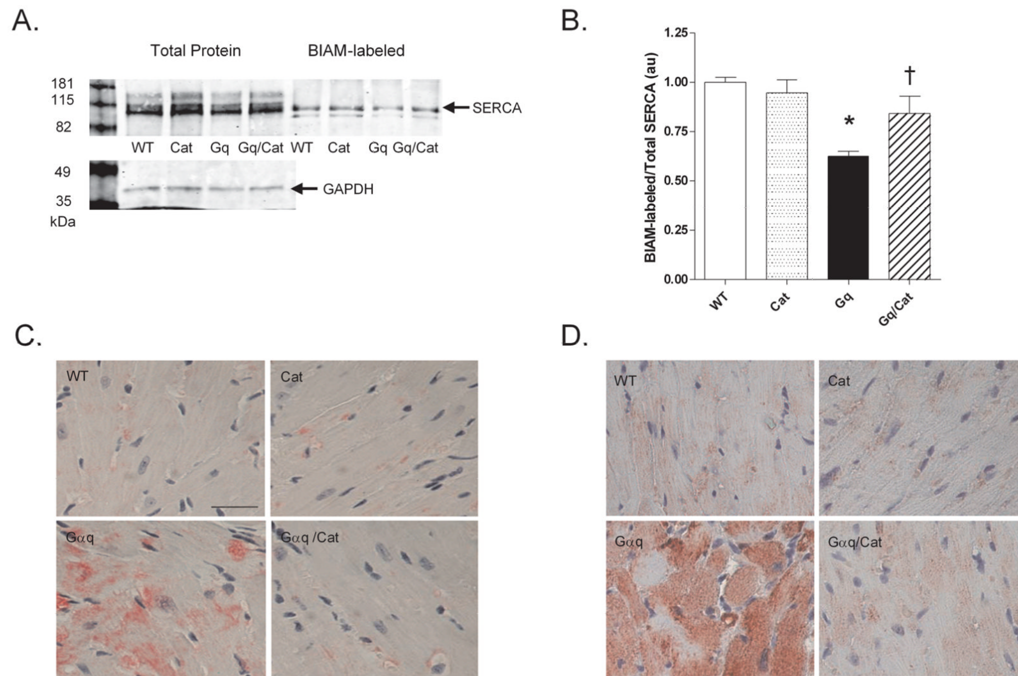

Figure 2.

OPTM of SERCA in myocardium from Gαq mice. **Panel A.** Representative immunoblot for total and BIAM-labeled SERCA in WT, Cat, Gαq and Gαq/Cat mice. **Panel B.** Ratio of BIAM-labeled to total SERCA. Shown are mean data from 4 hearts in each group (*$p<0.001$ vs. WT; †$p<0.05$ vs. Gαq). **Panel C and D.** Representative micrographs showing increased levels of SERCA sulfonylated at cysteine 674 **(Panel C)** and nitrated at tyrosine 294/295 **(Panel D)** distributed diffusely in myocytes from Gαq mice, and the prevention of both OPTM by concurrent expression of catalase in Gαq/catalase mice (bar = 25 μM).

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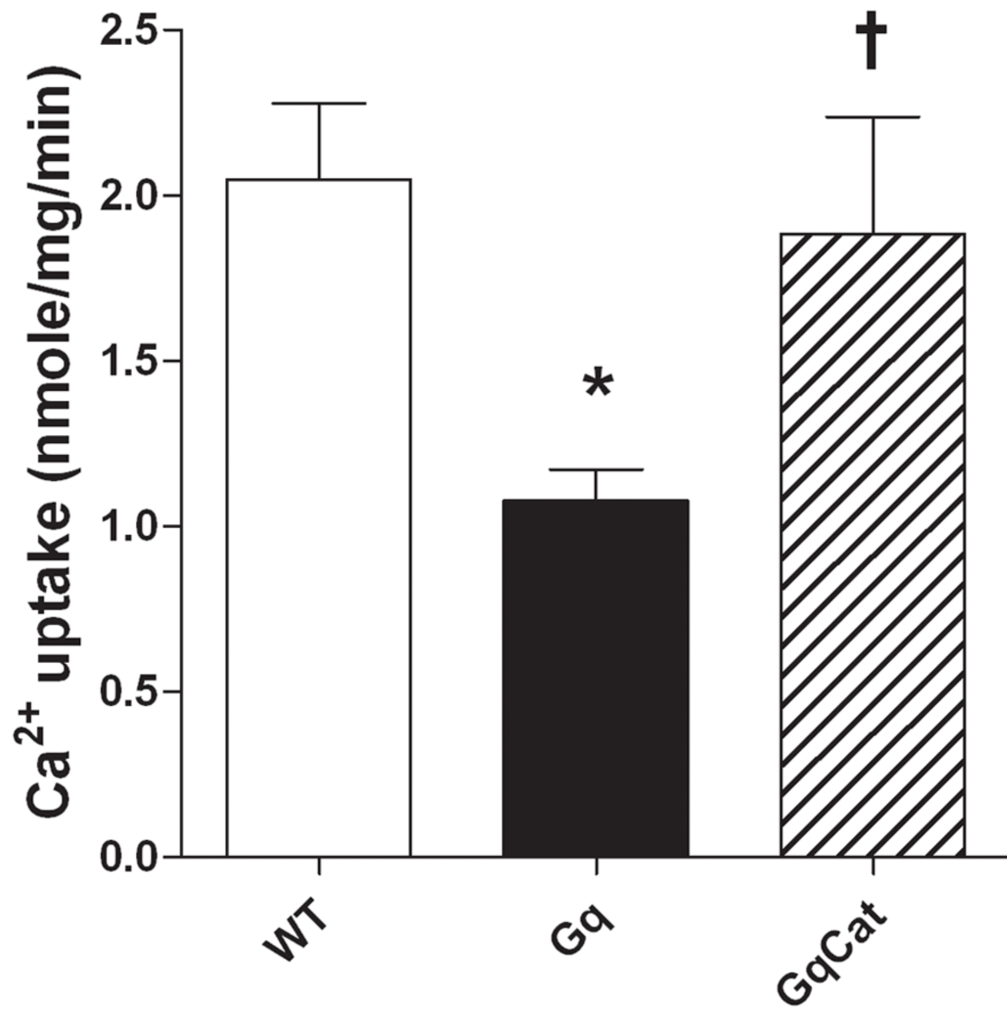

Figure 3.

Decreased SERCA activity in Gαq myocardium is corrected by concurrent expression of catalase in Gαq/catalase mice. Maximum SERCA activity was assessed using maximum calcium-stimulated, thapsigargin-inhibited calcium uptake [21]. Shown are mean data for 7 – 8 hearts in each group (*$p<0.05$ vs. WT; †$p<0.05$ vs. Gαq).

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Table 1

Total protein expression of calcium handling proteins in WT and Gαq mice.

	Total protein		
	WT	**Gαq**	**Gαq/Cat**
SERCA	1 ± 0.07	1.05 ± 0.07	0.99 ± 0.11
RyR	1 ± 0.09	0.53 ± 0.01*	0.54 ± 0.10*
PLB	1 ± 0.05	1.23 ± 0.31	ND
NCX	1 ± 0.08	0.95 ± 0.15	ND

Total protein is expressed as the ratio of the protein of interest/GAPDH, normalized to the average value in WT group. Data are the means of 4 hearts in each group (

* $p<0.05$ vs. WT mice).

ND = not done.