Cytosolic \( \text{H}_2\text{O}_2 \) mediates hypertrophy, apoptosis, and decreased SERCA activity in mice with chronic hemodynamic overload


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**Qin F, Siwik DA, Pimentel DR, Morgan RJ, Biolo A, Tu VH, Kang YJ, Cohen RA, Colucci WS.** Cytosolic \( \text{H}_2\text{O}_2 \) mediates hypertrophy, apoptosis, and decreased SERCA activity in mice with chronic hemodynamic overload. *Am J Physiol Heart Circ Physiol* 306: H1453–H1463, 2014. First published March 14, 2014; doi:10.1152/ajpheart.00084.2014.—Oxidative stress in the myocardium plays an important role in the pathophysiology of hemodynamic overload. The mechanism by which reactive oxygen species (ROS) in the cardiac myocyte mediate myocardial failure in hemodynamic overload is not known. Accordingly, our goals were to test whether myocardocyte-specific overexpression of peroxisomal catalase (pCAT) that localizes in the sarcoplasm protects mice from hemodynamic overload-induced failure and prevents oxidation and inhibition of sarc(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA), an important sarcoplasmic protein. Chronic hemodynamic overload was caused by ascending aortic constriction (AAC) for 12 wk in mice with myocardocyte-specific transgenic expression of pCAT. AAC caused left ventricular hypertrophy and failure associated with a generalized increase in myocardial oxidative stress and specific oxidative modifications of SERCA at cysteine 674 and tyrosine 294/5. pCAT overexpression ameliorated myocardial hypertrophy and apoptosis, decreased pathological remodeling, and prevented the progression to heart failure. Likewise, pCAT prevented oxidative modifications of SERCA and increased SERCA activity without changing SERCA expression. Thus cardiac myocyte-restricted expression of pCAT effectively ameliorated the structural and functional consequences of chronic hemodynamic overload and increased SERCA activity via a post-translational mechanism, most likely by decreasing inhibitory oxidative modifications. In pressure overload-induced heart failure cardiac myocyte cytosolic ROS play a pivotal role in mediating key pathophysiolgic events including hypertrophy, apoptosis, and decreased SERCA activity.

**THE ROLE OF REACTIVE OXYGEN species (ROS) in mediating myocardial failure in response to chronic hemodynamic overload is well appreciated (29, 32, 34).** Systemic administration of small molecule antioxidants ameliorated pathologic remodeling and failure in mice with hemodynamic overload due to chronic aortic constriction (8, 35). Transgenic total body overexpression of mitochondrial catalase (mCAT) likewise decreased left ventricular (LV) hypertrophy and failure in mice with aortic constriction (6), further suggesting that mitochondria are a source of ROS in hemodynamic overload. However, several nonmitochondrial sources of ROS in the myocardium have been implicated in hemodynamic overload including uncoupled endothelial nitric oxide synthase (23, 33), NADPH oxidases (4), and xanthine oxidase (21). Because these nonmitochondrial sources of ROS are located in the sarcoplasm, we reasoned that their effects should be susceptible to catalase that is targeted to the sarcoplasm of the cardiac myocyte. Accordingly, our first goal was to test whether LV hypertrophy and failure following ascending aortic constriction (AAC) is inhibited in transgenic mice in which catalase is targeted to cardiac myocyte peroxisomes (pCAT), and thus located in the cytosolic compartment.

An important consequence of increased myocyte ROS may be decreased sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) activity. In animal models of pressure overload-induced heart failure the activity of SERCA is decreased and contributes to depletion of sarcoplasmic reticulum calcium stores, decreased intracellular calcium transients, and impaired contractile function (12, 14, 15, 25, 26). The importance of impaired SERCA function in pressure overload is supported by the observation that increasing myocardial SERCA levels corrects calcium handling and myocardial function (9, 14, 22, 25), whereas SERCA\(^{+/−}\) mice have accelerated progression to failure (31).

Decreased SERCA activity in pressure overload is often associated with decreased expression of SERCA (26). However, there is increasing recognition that SERCA activity can also be inhibited by irreversible oxidative post-translational modifications including sulfonylation at cysteine 674 and nitration at tyrosine 294/5 (1, 18, 28, 38), which might act alone or in concert with a decrease in protein expression, to limit SERCA activity. Therefore, our second goal was to test whether pCAT can prevent SERCA oxidation and rescue SERCA function in mice with pressure overload due to aortic constriction.

**METHODS**

**Experimental animals.** Transgenic FVB/N mice with myocardocyte-specific overexpression of catalase in the cytosolic compartment (pCAT) (16, 20, 39) and wild-type (WT) FVB/N mice were used in this study. The protocol was approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.

**AAC.** The surgical procedure was performed as we have described (17). Briefly, 10-wk-old male WT and pCAT mice were anesthetized...
by an intraperitoneal injection with pentobarbital (50 mg/kg), and the chest was shaved. Animals were intubated with a 20-gauge intravenous catheter, ventilated on a rodent respirator (Harvard Apparatus, Holliston, MA) with a tidal volume of 0.2 ml at a respiratory rate of 130 breaths/min. The thorax was opened by an anterolateral thoracotomy, and AAC was performed by tying a 7-0 silk suture around the ascending aorta and a 27-gauge needle, which was then promptly removed after ligation. The chest was closed and mice were allowed to recover on a warming pad until they were fully awake. Sham-operated mice underwent a similar procedure without ligation of the ascending aorta.

**Echocardiographic measurements.** LV dimensions and function were measured in nonanesthetized mice shortly before surgery and 1, 2, 4, 8, and 12 wk after surgery using an Acuson Sequoia C-256 echocardiograph machine equipped with a 15-MHz linear transducer (model 15L8) as we have described (27). Briefly, the heart was imaged in the two-dimensional parasternal short-axis view, and an M-mode echocardiogram of the midventricle was recorded at the level of papillary muscles. Anterior wall thickness (AWT), posterior wall thickness (PWT), LV end-diastolic dimension (EDD) and end-systolic dimension (ESD) were measured from the M-mode image. LV fractional shortening (FS) was calculated as \( \frac{\text{EDD} - \text{ESD}}{\text{EDD}} \times 100 \).

**Exercise capacity.** Maximal exercise capacity was tested 12 wk after surgery by using a rodent treadmill with air puff motivation, as we have described (3). Exhaustion is defined as the point at which the animal cannot keep pace with the treadmill (within 15 s) despite air puff motivation. Maximal exercise capacity was calculated as the total distance run by the animal.

**Organ weight and histology.** Mice were euthanized 12 wk after surgery. Heart, LV with septum, lung, and liver were weighed. LV samples were fixed in 10% buffered formalin, embedded with paraffin, and sectioned. To assess myocyte size, sections were stained with hematoxyline and eosin and examined under a light microscope (BX 40; Olympus). Five random fields from each of four sections per animal were analyzed so that 60 myocytes per animal were measured. Myocyte cross-sectional area was measured using National Institutes of Health (NIH) ImageJ software as we have described (27). To assess fibrosis, sections were stained with Masson’s trichrome kit (Sigma) and examined under a light microscope (BX 40; Olympus).

**Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling staining.** Apoptosis was assessed using an In Situ Cell Death Detection Fluorescein Kit (Roche Applied Science, Indianapolis, IN) according to the manufacturer’s instructions, as we have described (27). Briefly, LV sections were incubated with the reaction mixture containing terminal deoxynucleotidyl transferase and fluorescein-labeled dUTP. To identify cardiomyocytes, sections were incubated with monoclonal antibody to mouse anti-sarcomeric actin (Sigma-Aldrich, St. Louis, MO) and incubated with goat anti-mouse IgG conjugated TRITC (Sigma). Finally, to identify all nuclei (nonapop-
Table 1. Body and organ weights

<table>
<thead>
<tr>
<th></th>
<th>WT-Sham</th>
<th>Catase-Sham</th>
<th>WT-AAC</th>
<th>Catase-AAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>29.8 ± 0.5</td>
<td>30.1 ± 1.1</td>
<td>27.4 ± 1.4</td>
<td>27.2 ± 1.5</td>
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<tr>
<td>Heart weight, mg</td>
<td>139.8 ± 2.9</td>
<td>139.5 ± 3.7</td>
<td>237.4 ± 10.5*</td>
<td>191.9 ± 12.0†</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>4.43 ± 0.10</td>
<td>4.51 ± 0.11</td>
<td>8.93 ± 0.74*</td>
<td>6.98 ± 0.48†</td>
</tr>
<tr>
<td>Lung weight, mg</td>
<td>174.5 ± 9.3</td>
<td>168.1 ± 4.3</td>
<td>261.5 ± 35.6*</td>
<td>186.3 ± 18.4</td>
</tr>
<tr>
<td>Lung weight/body weight, mg/g</td>
<td>5.51 ± 0.27</td>
<td>5.44 ± 0.14</td>
<td>10.20 ± 1.87*</td>
<td>5.99 ± 0.24†</td>
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<tr>
<td>Lung weight/wet/dry</td>
<td>4.67 ± 0.09</td>
<td>4.84 ± 0.10</td>
<td>5.14 ± 0.21*</td>
<td>4.68 ± 0.07†</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.38 ± 0.04</td>
<td>1.35 ± 0.53</td>
<td>1.27 ± 0.61</td>
<td>1.27 ± 0.28</td>
</tr>
<tr>
<td>Liver weight/body weight, g/g</td>
<td>0.046 ± 0.002</td>
<td>0.044 ± 0.001</td>
<td>0.046 ± 0.001</td>
<td>0.047 ± 0.001</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>0/10</td>
<td>0/11</td>
<td>79*</td>
<td>1/15†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10–15. *P < 0.05 vs. wild-type (WT)-sham; †P < 0.05 vs. WT-ascending aortic constriction (AAC).
Ten color images of NY or HNE staining were randomly selected from four sections of the heart and photographed at a magnification of ×40. The area and intensity of staining were scored in a blinded manner for quantification as follows: 0, no visible staining; 1, faint staining; 2, moderate staining; and 3, strong staining.

Oxidative post-translational modifications of SERCA. Oxidative modifications of SERCA were assessed by immunohistochemical staining using site-specific antibodies that detect SERCA sulfonic acid at cysteine 674 or nitrotyrosine at tyrosine 294/295, as we have described in mouse myocardium (18, 36, 38).

SERCA mRNA and protein. Frozen hearts were ground under liquid nitrogen, and total RNA was extracted with a mirVana miRNA Isolation Kit (Applied Biosystems). Total RNA was treated with DNase before cDNA synthesis with the High Capacity RNA-to-cDNA Kit (Applied Biosystems). Quantitative PCR was performed with TaqMan Universal PCR Master Mix and TaqMan primers (Applied Biosystems) specific for mouse SERCA2a (Mm01201431_m1) and GAPDH (4352339E) using the Applied Biosystems Step One Plus Real Time PCR System. Data is normalized to GAPDH using the equation 2exp-(CT target gene-CTGAPDH) and expressed as arbitrary units.

Immunoblotting for SERCA protein, hearts were homogenized in lysis buffer (1% Triton X-100, 0.5% Nonidet P-40, 10 mmol/l Tris, 1 mmol/l EDTA, 1 mmol/l EGTA, 150 mmol/l NaCl, 0.4 mmol/l PMSF, 0.2 mmol/l sodium orthovanadate, and 1 g/l leupeptin). Protein concentration was determined using Bradford assay (Bio-Rad). Equal amounts of total protein were separated by SDS-PAGE on 10% gels and transferred to nitrocellulose membrane. The following primary antibodies were used: mouse monoclonal anti-SERCA2 (Affinity Bioreagents) and rabbit polyclonal anti-GAPDH antibody (Abcam). Protein-primary antibody complex was detected by using infrared-dye conjugated goat polyclonal antibody IRDye 680 or IRDye 800.
**SERCA activity.** Maximal calcium-stimulated SERCA activity was measured in myocardium as we have described (18). Briefly, LV myocardium was homogenized on ice by sonication in Tris-sucrose homogenization buffer [8% (wt/vol) sucrose in (in mM) 3 Tris-HCl, pH 7.0, 1 PMSF]. The homogenate was centrifuged for 5 min at 4,000 rpm, and the protein concentration of the supernatant was determined by Bradford assay. Samples were pretreated with and without 10 µM of the SERCA inhibitor, thapsigargin. Calcium uptake was initiated by the addition of sample to assay buffer of (in mM) 100 KCl, 5 NaH2PO4, 6 MgCl2, 0.15 EGTA, 0.12 CaCl2, 30 Tris-HCl (pH 7.0), 10 oxalate, and 2.5 ATP containing 1 µCi 45CaCl2 (New England Nuclear, Boston, MA) in a 37°C water bath. Aliquots of each sample taken at 30, 60, and 90 s were vacuum filtered on glass filters (Whatman GF/C; Fisher Scientific, Pittsburgh, PA), washed three times with wash buffer of (in mM) 30 imidazole, 250 sucrose, and 0.5 EGTA and counted with a scintillation counter. SERCA activity is expressed as the rate of thapsigargin-sensitive 45Ca2+ uptake.

**Statistical analysis.** Results are presented as means ± SE. The statistical significance of differences among groups or between two means was determined using analysis of variance and the Bonferroni correction for multiple comparisons. Survival was calculated by Kaplan-Meier analysis. *P < 0.05 was considered statistically significant.

**RESULTS**

**Myocyte pCAT alleviates myocardial oxidative stress.** Myocardial oxidative stress was assessed by measuring oxidant-mediated HNE and NY protein modifications. In WT mice, AAC caused marked increases in HNE and NY staining, both of which were labeled diffusely over myocytes (Fig. 1). In pCAT mice, the levels of HNE and NY were decreased to

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**Fig. 4.** Representative photomicrographs of LV myocardium with Masson trichrome staining for fibrosis 12 wk after surgery. Myocardium stains red, and collagen stains blue. Bar = 100 µm. **Bottom:** quantification of cardiac fibrosis measured using NIH ImageJ. Values are means ± SE; n = 6–8. *P < 0.001 vs. WT-sham or CAT-sham; #P < 0.05 vs. WT-sham or CAT-sham; †P < 0.001 vs. WT-AAC.
levels similar those in sham-operated mice, indicating that increased oxidative stress in mice with AAC is attributable at least in part to H₂O₂ and is effectively mitigated by pCAT.

*Myocyte pCAT attenuates myocardial hypertrophy.* In WT mice, LV wall thickness measured by echocardiography increased 1 wk after AAC, increased further at 3 wk, and remained constant to slightly decreased over weeks 4 through 12 (Fig. 2). In pCAT mice, increased LV wall thickness with AAC was decreased by ~32%. Likewise, heart weight (ratio of heart to body weight) in WT mice was increased 12 wk after AAC (vs. sham-operated WT mice), and the increase was attenuated by ~28% in pCAT mice (Table 1). Myocyte cross-

![Fig. 5. A: representative photomicrographs of LV showing terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) staining for apoptotic myocytes. Apoptotic nuclei (arrows) are shown by green fluorescence in a, e, i, and m. Nuclei (arrowheads) stained by Hoechst 33258 are shown blue in b, f, j, and n. Cardiomyocytes identified by α-sarcomeric actin staining are shown red in c, g, k, and o. The overlays in d, h, l, and p allow identification of apoptotic nuclei present in myocytes. Bar (A) = 25 μm. B: mean changes in the number of apoptotic myocytes in LV myocardium 12 wk after surgery. Values are means ± SE; n = 5–7. *P < 0.001 vs. WT-sham or CAT-sham; #P < 0.05 vs. WT-sham or CAT-sham; †P < 0.002 vs. WT-AAC.](image-url)
sectional area measured histologically was increased in WT mice with AAC, and the increase was partially (60%) attenuated in pCAT mice (Fig. 3). Myocardial fibrosis, assessed by Masson’s trichrome staining, was increased in WT mice with AAC and was markedly decreased in pCAT mice (Fig. 4).

**Myocyte pCAT prevents progression to LV failure.** In WT mice, ACC caused progressive LV dilation and systolic failure as assessed by echocardiography. LV EDD and LV ESD increased progressively beginning 1 to 2 wk after AAC, in association with a decrease in LV FS (Fig. 2). Myocyte-specific overexpression of pCAT attenuated both LV dilation and the decrease in FS. Myocyte apoptosis, a major mechanism responsible for LV failure with AAC (10), was assessed by measurement of TUNEL staining. The frequency of TUNEL-positive myocytes increased approximately eightfold in WT mice after AAC, and the increase was markedly decreased by pCAT (Fig. 5).

In WT mice, AAC caused functional heart failure as reflected by a ~60% decrease in maximal exercise capacity, which was partially corrected by pCAT (Fig. 6). Lung weight (wet/dry) was increased by AAC in WT mice, indicative of lung congestion, and the increase was attenuated in pCAT mice (Table 1). Likewise, pleural effusions were present in 90% of WT mice surviving 12 wk after AAC, but were present in only 6% of pCAT mice, and absent in sham-operated WT or pCAT mice. Survival rate at 12 wk was 100% in sham-operated mice, 6% of pCAT mice, and absent in sham-operated WT or pCAT mice surviving 12 wk after AAC, but were present in only 6% of pCAT mice, and absent in sham-operated WT or pCAT mice.

**Myocyte pCAT prevents oxidative modification of SERCA.** Oxidative modifications of SERCA were assessed immunohistochemically using site-specific antibodies for oxidatively modified SERCA with sulfonic acid at cysteine 674 (SERCA-C674-SO3H) (38) or nitrotyrosine 294/295 (SERCA-N294/295-Y) (36). In sham-operated mice there were low levels of both SERCA-C674-SO3H and SERCA-N294/295-Y, and both levels were markedly increased in WT mice with AAC (Fig. 7). In pCAT mice with AAC, both oxidative modifications of SERCA were decreased to the low levels present in sham-operated mice.

**Myocyte pCAT preserves SERCA activity.** We have shown that oxidative modifications of SERCA are associated with decreases in Ca2+ uptake activity (18, 28, 38). Therefore, SERCA activity was assessed by measuring maximal calcium-stimulated 45Ca2+ uptake. In WT mice, AAC caused a ~46% decrease in SERCA activity (vs. sham-operated mice), which was restored, at least partially, in pCAT mice (Fig. 8A).

SERCA mRNA levels were not affected by AAC or pCAT (Fig. 8B). SERCA protein levels were decreased by AAC but were not affected further by pCAT (Fig. 8C), suggesting that the increase in SERCA activity with pCAT is mediated at the post-translational level.

**DISCUSSION**

There are two major new findings in this study. First, we show for the first time that cardiac myocyte-restricted expression of pCAT effectively ameliorates the structural and functional consequences of chronic hemodynamic overload. This finding suggests that sarcoplasmic ROS in the cardiac myocyte plays a central role in mediating the effects of hemodynamic overload. Second, we show for the first time that chronic hemodynamic overload causes oxidative modifications of SERCA that are associated with decreased activity and that both SERCA oxidation and function are improved by pCAT in the absence of changes in transcript or protein expression. This finding provides a new mechanistic basis for impaired calcium regulation and myocyte function in chronic hemodynamic overload, and further identifies SERCA as an important target of ROS that can be mitigated by sarcoplasmic pCAT.

**pCAT effectively inhibits hypertrophy and progression to failure.** In WT mice AAC initially led to LV hypertrophy followed by progressive chamber dilation and contractile failure, associated at the cellular level with myocyte hypertrophy and apoptosis. LV hypertrophy and failure were inhibited in pCAT-expressing mice, as were myocyte hypertrophy and apoptosis. The functional importance of these effects of pCAT is supported by the concomitant findings of improved exercise function, decreased lung congestion, and improved survival. Thus pCAT effectively ameliorates the effects of hemodynamic overload on myocardial structure and function.

The effects of pCAT that we observed are qualitatively and quantitatively similar to those observed in this model in transgenic mice with total body overexpression of mCAT (6). The localization of catalase in the pCAT mouse used in our study was determined by electron microscopy, which showed that the pCAT is expressed primarily in peroxisomes, sarcoplasm, and nucleus but not expressed in mitochondria (39). Conversely, the mCAT mouse was shown to express catalase only in mitochondria (7). The comparable effectiveness of mCAT and pCAT in pressure overload suggests that both catalase locations have access to ROS that ultimately reaches critical target.
molecules located in the sarcoplasm. These experiments do not identify the source of the ROS, which might originate in mitochondria (7) and/or from one or more sarcoplasmic sources including uncoupled endothelial nitric oxide synthase (33), NOX2 (13), or xanthine oxidase (37). However, because pCAT does not enter mitochondria our data indicate that scavenging of ROS in the sarcoplasm is sufficient to ameliorate important aspects of the phenotype including hypertrophy, apoptosis, and impaired SERCA activity.

This is the first demonstration of the effects of pCAT in AAC or any other model of hemodynamic overload. The transgenic pCAT mouse used in these experiments was one of several lines (line 742) developed by Kang et al. (16) and shown to oppose the cardiac effects of doxorubicin (16) and

![Figure 7](image-url)

**Fig. 7.** Representative photomicrographs of LV myocardium subjected to immunohistochemical staining with site-specific antibodies that recognize sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) that is nitrated at tyrosine 294/295 (SERCA-NY 294,295; A) or sulfonated on C674 (SERCA C674-SO3; B). Bar (WT-sham) = 25 μm.
corrected the GSH-to-GSSG ratio to normal (27). Ourobservations in the Gaq mouse pCAT mice (5, 27). Although we did not assess the antioxidant background in our study. We also found that pCAT mice have less LV hypertrophy and diastolic dysfunction with age (28).

**pCAT protects SERCA function.** A major finding of this study is that decreased SERCA activity after AAC was partially prevented by pCAT. Myocardial SERCA activity is decreased in pressure overload leading to sarcoplasmic reticulum calcium depletion, abnormal calcium transients, and impaired systolic and diastolic function at the myocyte level (15, 26). These abnormalities are ameliorated by transgenic over-expression of WT SERCA (14, 25) and worsened in SERCA<sup>+/−</sup> mice (31). Likewise, viral expression of SERCA improves LV function in rats with hemodynamic overload (22), aging (30), and other models of heart failure (24), and has led to the investigational use of virally mediated SERCA expression in patients with heart failure (40). Decreased SERCA activity in pressure overload has generally been attributed to decreased protein expression (26), and consistent with prior observations, the expression of SERCA protein was decreased with AAC in our study. However, because neither SERCA protein nor mRNA expression was affected by pCAT, the pCAT-induced increase in activity cannot be attributed to increased SERCA levels. In contrast with most other studies in mice with aortic constriction (15), in our study the decrease in SERCA protein was not associated with a decrease in mRNA, possibly reflecting increased protein degradation.

SERCA activity can also be regulated by OPTM. Low concentrations of ROS activate SERCA via reversible glutathiolation of cysteine 674 (2, 19), and this activation is inhibited by higher concentrations of ROS that irreversibly oxidize this cysteine (18, 28, 38). Using site-directed antibodies to identify sulfonic acid at SERCA cysteine 674 and nitrotyrosine at tyrosine-294/5, we found that AAC increases both oxidative modifications, and that both are prevented by pCAT. The observation that pCAT corrects SERCA OPTM and restores SERCA activity in mice with AAC, without increasing SERCA expression (i.e., WT-AAC vs. CAT-AAC), strongly supports the thesis that pCAT increases SERCA activity by decreasing inhibitory oxidative modifications. The ability of pCAT to decrease SERCA oxidation is consistent with the ability of H<sub>2</sub>O<sub>2</sub> to diffuse across membranes, and in this case, into peroxisomes, which may act as an antioxidant ‘sink’ in the sarcoplasm.

In summary, the ability of pCAT to protect the myocardium from the cardinal features of hemodynamic overload supports the utility of protecting sarcoplasmic targets from ROS. In this regard, we show that SERCA is an important target of ROS that contributes to the pathophysiology of hemodynamic overload and is protected by pCAT. Improved SERCA function has potentially important consequences for cardiac excitation/contraction (15). In addition, by affecting endoplasmic reticulum calcium stores, SERCA may play a role in a variety of other biologic processes including endoplasmic reticulum stress, the unfolded protein response, growth signaling, and apoptosis (11). These findings expand our understanding of the mechanisms by which ROS mediate heart failure, suggest that prevention of target protein oxidation may be a useful therapeutic approach, and have implications for the optimal repletion of SERCA in failing myocardium.

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**Fig. 8. Maximal calcium-stimulated SERCA activity (A), SERCA mRNA (B), and SERCA protein expression (C) measured in LV myocardium 12 wk after surgery. Values are means ± SE; n = 3 for SERCA activity and n = 4 for SERCA mRNA or protein expression. *P < 0.01 vs. WT-sham or CAT-sham; †P < 0.01 vs. WT-AAC.**

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ischemia-reperfusion (20). We found that the pCAT mouse ameliorates the pathologic phenotype in Gaq-overexpressing mice (5, 27). Although we did not assess the antioxidant effectiveness of pCAT in this study, in the Gaq mouse pCAT corrected the GSH-to-GSSG ratio to normal (27). Our observations in the Gaq mouse differ from a report by Dai et al. (7) who found no benefit with a different pCAT mouse. These discrepant results may reflect the use in their experiments of a different line of pCAT mouse (line 776) and/or the mixed background of their mice as opposed to the pure FVB background in our study. We also found that pCAT mice have less LV hypertrophy and diastolic dysfunction with age (28).
DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


