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# Genetic and Pharmacologic Hydrogen Sulfide Therapy Attenuates Ischemia-Induced Heart Failure in Mice

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## Abstract

**Background—**Hydrogen sulfide ($H_2S$) is an endogenous signaling molecule with potent cytoprotective effects. The present study evaluated the therapeutic potential of $H_2S$ in murine models of heart failure.

**Methods and Results—**Heart failure was induced by subjecting mice either to permanent ligation of the left coronary artery for 4 weeks or to 60 minutes of left coronary artery occlusion followed by reperfusion for 4 weeks. Transgenic mice with cardiac-restricted overexpression of the $H_2S$-generating enzyme cystathione $\gamma$-lyase ($\alpha$MHC-CGL-Tg$^+$) displayed a clear protection against left ventricular structural and functional impairment as assessed by echocardiography in response to ischemia-induced heart failure, as well as improved survival in response to permanent myocardial ischemia. Exogenous $H_2S$ therapy ($Na_2S$; 100 $\mu$g/kg) administered at the time of reperfusion (intracardiac) and then daily (intravenous) for the first 7 days after myocardial ischemia also protected against the structural and functional deterioration of the left ventricle by attenuating oxidative stress and mitochondrial dysfunction. Additional experiments aimed at elucidating some of the protective mechanisms of $H_2S$ therapy found that 7 days of $H_2S$ therapy increased the phosphorylation of Akt and increased the nuclear localization of 2 transcription factors, nuclear respiratory factor 1 and nuclear factor-E2-related factor (Nrf2), that are involved in increasing the levels of endogenous antioxidants, attenuating apoptosis, and increasing mitochondrial biogenesis.

**Conclusions—**The results of the present study suggest that either the administration of exogenous $H_2S$ or the modulation of endogenous $H_2S$ production may be of therapeutic benefit in the treatment of ischemia-induced heart failure.

### Keywords

cystathionine $\gamma$-Lyase; heart failure; hydrogen sulfide; ischemia; myocardial infarction

Heart failure continues to be a major health problem in the United States, especially in the elderly population.[1,2] Unfortunately, current treatments for heart failure are insufficient, and

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the availability of hearts for transplantation is severely inadequate.[3] Therefore, adjunct pharmacotherapies designed to coincide with the standard means of care are needed to decrease the extent of injury leading to the development of heart failure. Small gaseous signaling molecules are labile biological mediators that are able to freely diffuse through cell membranes to invoke cellular signaling, thus alleviating the need for membrane receptors and second messengers. Hydrogen sulfide ($H_2S$), a recently classified small molecule effector,[4] is produced in the body by the enzymes cystathionine $\gamma$-lyase (CGL; cytstathione, CTH), cystathionine $\beta$-synthase, and 3-mercaptopyruvate sulfurtransferase. $H_2S$ has been reported to provide cardioprotection in various models of cardiac injury through its ability to preserve mitochondrial function and to reduce cardiomyocyte apoptosis.[5,6] Although the cytoprotective effects of $H_2S$ have been demonstrated in models of acute cardiac injury, the effects of $H_2S$ therapy on cardiac function in the setting of chronic heart failure are currently unknown. Therefore, the purpose of the present study was to investigate the potential cardioprotective effects of endogenous and exogenous $H_2S$ on survival and cardiac function in 2 murine models of ischemia-induced heart failure.

## Methods

### Animals

Male C57BL6/J mice, 8 to 10 weeks of age, were used (Jackson Laboratories, Bar Harbor, Me). The generation of cardiac-specific transgenic mice overexpressing CGL ($\alpha$MHC-CGL-$Tg^+$, FVB background) has been described previously.[6] $\alpha$MHC-CGL-$Tg^+$ and nontransgenic littermates were bred and used at 8 to 10 weeks of age. All experimental mouse procedures were approved by the Institute for Animal Care and Use Committee at Emory University and conformed to the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health (NIH Publication No. 86–23, revised 1996), and to federal and state regulations.

### Materials

Sodium sulfide ($Na_2S$) was produced by Ikaria Holdings, Inc (Seattle, Wash) by using $H_2S$ gas (Matheson, Newark, Calif) as a starting material as previously described.[7] $Na_2S$ (100 $\mu$g/kg) was administered with a 32-gauge needle in a final volume of 50 $\mu$L as an intracardiac injection once at the time of reperfusion ($Na_2S$) or once at the time of reperfusion followed by daily tail vein (intravenous) injections for the first 7 days of reperfusion ($Na_2S$ 7 days). This dose of $Na_2S$ was selected on the basis of our previous experience investigating $Na_2S$ in murine models of cardiac ischemia/reperfusion injury.[7] Saline was administered in the same manner for the respective vehicle groups.

### Heart Failure Protocols

Heart failure was induced either by permanent ligation of the left coronary artery (LCA) or by subjecting mice to 60 minutes of LCA occlusion followed by reperfusion for up to 4 weeks as described previously.[8] All mice were randomly allocated to treatment groups. Myocardial infarct size assessment, echocardiographic assessment of left ventricular (LV) structure and function, and histological analysis of infarct scar were all performed as previously described.[7,9]

**Lipid Hydroperoxide Assay—**Quantification of lipid peroxidation was performed to assess the extent of cardiac oxidative stress as described previously.[7]

**Quantitative Real-Time Polymerase Chain Reaction for Mitochondrial DNA—**Mitochondrial DNA content was quantified by real-time reverse-transcription polymerase chain reaction with cardiac DNA as described previously.[10]

**Cardiac Mitochondria Isolation, Mitochondrial Respiratory Rate, and ATP Synthesis—**Cardiac mitochondria were isolated from the following groups of mice: sham-operated, vehicle-treated, and $Na_2S$-treated mice. Oxygen consumption and ATP synthesis rates were determined as previously described.[8]

**Western Blot Analysis—**Western blot analysis was performed as described previously.[7]

### Statistical Analysis

All data in this study are expressed as mean ±SEM. Means were compared by use of Prism 4 (GraphPad Software Inc) with a Student unpaired 2-tailed *t* test (Western blot analysis), 1-way ANOVA (ratio of heart to body weight, lipid hydroperoxidation [LPO] data, mitochondrial DNA, and mitochondrial respiration data), or 2-way ANOVA (echocardiography data) when appropriate. For the ANOVA, if a significant result was found, the Tukey (1-way ANOVA) or Bonferroni (2-way ANOVA) test was used as the posthoc analysis. Survival curves were compared by use of a log-rank (Mantel-Cox) test. For all data, a value of $P<0.05$ was considered significant.

## Results

### Endogenous Overexpression of the $H_2S$-Generating Enzyme CGL Improves Survival After Permanent LCA Occlusion

The effects of $H_2S$ on heart failure were first evaluated in mice that overexpress the $H_2S$-generating enzyme CGL ($\alpha$MHC-CGL-Tg$^+$). These cardiac-specific transgenic mice have an ≈15-fold overexpression of CGL in their hearts, which results in a 2-fold increase in cardiac $H_2S$ production.[6] For these experiments, $\alpha$MHC-CGL-Tg$^+$ and nontransgenic mice were subjected to permanent occlusion of the LCA. At 4 weeks after myocardial ischemia, both groups of mice exhibited significant mortality (Figure 1). The $\alpha$MHC-CGL-Tg$^+$ mice exhibited an overall survival rate of 67% (22 of 33), whereas the nontransgenic mice exhibited an overall survival rate of 40% (14 of 35). Therefore, cardiac-specific overexpression of CGL resulted in a 68% improvement in survival after myocardial ischemia ($P=0.033$ between groups).

### Endogenous Overexpression of CGL Reduces LV Dilatation and Cardiac Hypertrophy but Does Not Improve Function After Permanent LCA Occlusion

At the end of the 4-week follow-up period, the surviving mice were subjected to 2-dimensional, high-resolution echocardiography to determine the degree of LV dilatation and LV dysfunction. Analysis revealed that the LV end-diastolic (LVEDD) and end-systolic (LVESD) diameter of both the $\alpha$MHC-CGL-Tg$^+$ and nontransgenic mice were significantly higher than their respective baseline readings ($P<0.001$), suggesting that LV dilation had occurred (Figure 2A and 2B). However, the hearts of $\alpha$MHC-CGL-Tg$^+$ mice had significantly smaller increases in both LVEDD ($P<0.05$ versus nontransgenic) and LVESD ($P<0.01$ versus nontransgenic). Cardiac hypertrophy was also analyzed by determining the ratios of heart to body weight (Figure 2C). Both $\alpha$MHC-CGL-Tg$^+$ and nontransgenic mice displayed cardiac hypertrophy 4 weeks after myocardial ischemia compared with sham-operated animals ($P<0.05$), but $\alpha$MHC-CGL-Tg$^+$ mice displayed significantly less hypertrophy ($P<0.01$ versus nontransgenic). Despite these significant reductions in LV dilatation and cardiac hypertrophy, no improvement in LV ejection fraction was evident in the $\alpha$MHC-CGL-Tg$^+$ mice compared with the nontransgenic mice (Figure 2D). Additionally, the heart rate of the 2 groups of mice was evaluated at baseline and 4 weeks after myocardial ischemia (Figure IA in the online-only Data Supplement). No differences at baseline were observed, and both groups of mice exhibited an elevated heart rate 4 weeks after myocardial ischemia.

We also measured the infarct area relative to the entire LV at 4 weeks after infarction (Figure IIA in the online-only Data Supplement). Analysis revealed that the nontransgenic mice displayed a 22±3% infarct area/LV and the $\alpha$MHC-CGL-Tg$^+$ mice displayed a 25±2% infarct area/LV. These findings suggest that overexpression of CGL improves survival after permanent LCA occlusion and that this survival benefit is independent of any effect on infarct size.

## Endogenous Overexpression of CGL Improves LV Structure and Function After Ischemia-Induced Heart Failure

To study the effects of $H_2S$ in a more clinically relevant model of heart failure that mimics the effects of coronary revascularization therapy, $\alpha$MHC-CGL-Tg$^+$ and nontransgenic mice were subjected to 60 minutes of LCA occlusion followed by 4 weeks of reperfusion. Myocardial infarction was evaluated in 2 different groups of mice at 24 hours and 4 weeks of reperfusion with the Evans blue/triphenyltetrazolium chloride method and histologically, respectively. Following 24 hours of reperfusion, the area at risk per LV was similar ($P$=NS) in both groups, and the $\alpha$MHC-CGL-Tg$^+$ mice (n=7) displayed a 19% reduction (52±2% versus 42±4%; $P$<0.05) in infarct area relative to the area at risk and a 30% reduction (33±3% versus 23±2%; $P$<0.05) in infarct area/LV compared with the nontransgenic mice (n=10). Following 4 weeks of reperfusion, analysis revealed that the nontransgenic mice displayed an 11.8±1.2% infarct area/LV and the $\alpha$MHC-CGL-Tg$^+$ mice displayed a 7.3±1% infarct area/LV at 4 weeks of reperfusion, which corresponded to a 38% reduction in infarct area ($P$<0.01 versus nontransgenic; Figure IIB in the online-only Data Supplement). Following 4 weeks of reperfusion, LV dilatation, cardiac hypertrophy, and LV dysfunction were all prevalent in both groups of mice (Figure 3). However, $\alpha$MHC-CGL-Tg$^+$ mice displayed significantly smaller increases in LVEDD ($P$<0.05), LVESD ($P$<0.05), and ratio of heart to body weight ($P$<0.01) and displayed better LV ejection fraction ($P$<0.001) compared with nontransgenic mice. In addition, both groups of mice exhibited an elevated heart rate 4 weeks after myocardial I/R, but only the nontransgenic mice had a significant increase from baseline ($P$<0.05; Figure IB in the online-only Data Supplement). These findings suggest that increased production of $H_2S$ during the reperfusion phase has a positive impact on LV structure and function after ischemia-induced heart failure.

## Single Injection of $Na_2S$ Reduces Infarct Size but Does Not Improve LV Structure and Function

In an effort to translate these findings to a more clinically relevant model we next utilized pharmacologic administration of $H_2S$ (Figure 4). In these experiments, C57BL/6J mice were subjected to 60 minutes of LCA occlusion followed by 4 weeks of reperfusion. $H_2S$ ($Na_2S$; 100 $\mu$g/kg) or vehicle (saline) was administered at the time of reperfusion (intracardiac). Again, myocardial infarction was evaluated in 2 different groups of mice at 24 hours and 4 weeks of reperfusion. Following 24 hours of reperfusion, $Na_2S$ (n=9) decreased infarct area/area at risk by 14% (69±2% versus 59±2%; $P$<0.05) and decreased infarct area/LV by 20% (41±2% versus 33±2%; $P$<0.05) compared with vehicle-treated mice (n=8). At 4 weeks of reperfusion, analysis revealed a similar 25% reduction in infarct area/LV (12±1% versus 9±1%; $P$<0.05) in the mice treated with $Na_2S$ compared with the vehicle-treated mice (Figure IIC in the online-only Data Supplement). However, following 4 weeks of reperfusion, the $Na_2S$-treated mice did not show any improvements in LVEDD, LVESD, ratio of heart to body weight, LV ejection fraction, or heart rate compared with the vehicle-treated group (Figure 4 and Figure IC in the online-only Data Supplement). This finding suggests that a single administration of $H_2S$ at reperfusion is not sufficient to improve LV function at 4 weeks, even though a single administration of $H_2S$ reduces infarct size.

### Daily Injections of $Na_2S$ During the First 7 Days of Reperfusion Improve LV Structure and Function

Subsequent experiments evaluated the effectiveness of daily administrations of $H_2S$ during the first 7 days of reperfusion (Figure 5). In these experiments, C57BL/6J mice were subjected to 60 minutes of LCA occlusion followed by 4 weeks of reperfusion. Analysis at 4 weeks of reperfusion revealed that treatment during the first 7 days of the reperfusion period led to a decrease in LV dilatation, a decrease in cardiac hypertrophy, and an improvement in cardiac function. No differences in heart rates were observed at baseline, and both groups of mice exhibited an elevated heart rate 4 weeks after myocardial ischemia (Figure ID in the online-only Data Supplement). To determine whether the 7-day treatment of $Na_2S$ had any additional effects on infarct size reduction, the area of infarction was evaluated at 4 weeks of reperfusion. Analysis revealed that the vehicle-treated mice displayed a 12±1% infarct area/LV and the $Na_2S$-treated mice displayed a 9±1% infarct area/LV at 4 weeks of reperfusion, which corresponded to a 25% reduction in infarct area ($P<0.01$ versus vehicle; Figure IID in the online-only Data Supplement). These results suggest that treatment with exogenous $H_2S$ during the first 7 days of reperfusion is critical for sustained improvements in LV structure and function.

### Daily Injections of $Na_2S$ Induce the Nuclear Localization of Nrf2 and NRF-1 and Increase the Phosphorylation of Akt

$H_2S$ has a diverse physiological profile, which may account for the cardioprotection observed in the current models of heart failure. Recently, nuclear factor-E2–related factor (Nrf2) has been identified as an important cellular target of $H_2S$.7 Nrf2 is a key transcription factor that regulates antioxidant genes as an adaptive response to oxidative stress11$^{-13}$ and regulates mitochondrial biogenesis through an upregulation of nuclear respiratory factor 1 (NRF-1).[14] Therefore, experiments were conducted to evaluate Nrf2 signaling after $H_2S$ treatment. For these experiments, $Na_2S$ was administered to mice for 7 days (intravenous), at which time hearts were excised and processed for Western blot analysis (Figure 6). Because Nrf2 is a transcription factor, its protein expression was evaluated in both cytosolic and nuclear fractions. Analysis revealed that Nrf2 protein levels were increased ($P<0.05$) in both the cytosolic and nuclear fractions in the hearts treated with $Na_2S$ compared with the sham hearts (Figure 6A). Subsequently, the nuclear expression, but not the cytosolic expression, of NRF-1 was elevated in the hearts of mice treated with $Na_2S$. Recently, NRF-1 transcriptional activity was reported to be regulated by Akt.[15] Therefore, the ability of $Na_2S$ to increase the phosphorylation of Akt at serine residue 473 (Akt-P$^{Ser473}$) was evaluated (Figure 6B). A significant ($P<0.05$ versus sham) increase in the phosphorylation of Akt at serine residue 473 (Akt$^{Ser473}$) was observed in the hearts of mice treated with $Na_2S$ for 7 days. No differences in total Akt levels were noted.

To determine whether $Na_2S$ could alter the expression levels of Nrf2, NRF-1 and Akt in nonvascular tissue, additional studies were performed using hepatic tissue taken from mice administered $Na_2S$ for 7 days. These studies revealed that $Na_2S$ therapy increased the nuclear expression of both Nrf2 and NRF-1 ($P<0.05$ versus vehicle) but did not increase the levels of Akt or alter its phosphorylation status (Figure III in the online-only Data Supplement), suggesting that the activation of Nrf2 and NRF-1 by $Na_2S$ was not restricted to the heart. Additionally, we have previously reported that hearts from $\alpha$MHC-CGL-Tg$^+$ mice have an increased nuclear expression of Nrf2.[7] Further analysis in the present study revealed that hearts from $\alpha$MHC-CGL-Tg$^+$ mice had an increased nuclear expression of NRF-1 ($P<0.01$ versus nontransgenic) and an increased expression of Akt ($P<0.05$ versus nontransgenic) but no changes in Akt-P$^{Ser473}$ (Figure IV in the online-only Data Supplement). No changes in Nrf2, NRF-1, and Akt were observed in the livers of $\alpha$MHC-CGL-Tg$^+$ mice (Figure V in the online-only Data Supplement), which confirms our previous findings that the increased generation of $H_2S$ is confined to the heart.[6]

### Daily Injections of $Na_2S$ Attenuate Oxidative Stress

Lipid hydroperoxidation (LPO) was used as a measure of cardiac oxidative stress during the development of heart failure. In these experiments (Figure 7A), 2 groups of C57BL/6J mice were subjected to 60 minutes of LCA occlusion followed by 1 and 4 weeks of reperfusion. At 1 week of reperfusion, both the vehicle-treated ($P<0.001$) and 7-day $Na_2S$-treated ($P<0.05$) mice exhibited significantly higher levels of LPO compared with sham-operated controls. However, the $Na_2S$-treated mice displayed significantly lower levels of LPO compared with the vehicle-treated mice ($P<0.05$). LPO levels remained elevated above sham levels in both groups of mice at 4 weeks of reperfusion ($P<0.01$), and although not statistically significant, there was a trend for lower LPO levels in the hearts of mice treated with $Na_2S$ compared with the vehicle-treated mice. These findings suggest that treatment with $H_2S$ during the first 7 days of reperfusion reduces oxidative stress associated with heart failure.

### Daily Injections of $Na_2S$ Did Not Increase Mitochondrial Biogenesis but Did Improve Mitochondrial Respiration and ATP Synthesis

NRF-1 regulates the expression of several genes responsible for mitochondrial biogenesis.[14,16] Because $H_2S$ increased the nuclear accumulation of NRF-1, the next series of experiments evaluated mitochondrial biogenesis. For these experiments, mice were subjected to 60 minutes of LCA occlusion followed by 4 weeks of reperfusion. $Na_2S$ or vehicle was administered at the time of reperfusion (intracardiac) and then daily for 7 days (intravenous). At 4 weeks of reperfusion, the hearts from all groups of mice were found to have similar ratios of cytochrome b DNA to $\beta$-actin DNA quantity, suggesting that $H_2S$ did not induce mitochondrial biogenesis (Figure 7B).

$H_2S$ can preserve mitochondrial function after acute myocardial ischemia/reperfusion injury.[6] Therefore, we investigated the effects of $H_2S$ on mitochondrial function during the development of heart failure (Figure 7C and 7D). Mitochondria isolated from the hearts of vehicle-treated mice were found to have a 61% reduction ($P<0.001$ versus sham) in maximal ADP-stimulated (state 3) oxygen consumption, a slightly increased oligomycin-inhibited respiration, and reduced respiratory control ratio ($P<0.001$ versus sham), suggestive of uncoupling. ATP synthesis rates and the ATP/oxygen consumption ratios in the mitochondria from vehicle-treated mice were significantly ($P<0.001$) reduced compared with sham-operated mice (Figure 7D). Mitochondria isolated from the hearts of $Na_2S$-treated mice were found to have slightly higher rates of ADP-stimulated oxygen consumption compared with vehicle-treated mice ($P=0.07$). Despite similar rates of oligomycin-inhibited respiration, the mitochondria isolated from $Na_2S$-treated mice displayed higher respiratory control ratios ($P<0.05$), greater ATP synthesis rates ($P<0.05$), and slightly higher ATP/oxygen consumption ratios compared with vehicle-treated mice. These data indicate that the respiration of cardiac mitochondria during heart failure was inefficient, likely a result of uncoupled respiration, and that $H_2S$ treatment is able to limit this dysfunction.

## Discussion

In recent years, the cardioprotective effects of $H_2S$ have been demonstrated in various models of myocardial injury.[17] These studies have provided important mechanistic insights into its cardioprotective actions and important information on dosage, timing, and route of administration. For instance, a single administration of $H_2S$ before, during, or after myocardial ischemia decreases myocardial infarct size and attenuates LV dysfunction in both rodents and pigs.[6,7,18] Although these studies provide strong evidence for the cardioprotective effects of short-term $H_2S$ therapy (ie, single treatment, short follow-up), these studies do not offer any insights into the long-term effects (ie, daily administration, long follow-up) of $H_2S$ therapy. Thus, the present study provides the first evidence that $H_2S$ therapy can provide long-term

protection against myocardial injury. Importantly, the present study demonstrates that although a single administration of $H_2S$ at the time of reperfusion is beneficial in attenuating infarct size, this alone is not sufficient to cause a significant improvement in cardiac function. On the other hand, daily $H_2S$ therapy initiated at the time of reperfusion and continued for the first 7 days of reperfusion provided significant improvements in cardiac function and LV dimensions despite not providing any additional infarct-sparing benefits over a single treatment of $H_2S$. This suggests that the first 7 days of reperfusion is a critical period for the development of heart failure in this murine model and that initiating therapeutic interventions during this time is paramount for improvements in outcome. This is further supported by the additional findings that genetic overexpression of a critical $H_2S$-producing enzyme, CGL, results in increased endogenous $H_2S$ production[6] and a profound protection against ischemia-induced heart failure and decreased mortality. Together, these results suggest that $H_2S$ treatment could potentially be initiated at the time of coronary artery reperfusion and then continued daily to achieve a long-term improvement in cardiac function and to decrease the morbidity and mortality resulting from heart failure.

$H_2S$ possesses a diverse physiological profile that contributes to its cardioprotective actions.[19] Of the reported physiological effects of $H_2S$, several could provide protection during the development of heart failure. First, it has become evident that $H_2S$ itself serves over the short term as a potent antioxidant[20,21] and under more long-term conditions upregulates antioxidant defenses[20,22] through the activation of the transcription factor Nrf2.[7] Nrf2, a member of the NF-E2 family of nuclear basic leucine zipper transcription factors, regulates the gene expression of a number of enzymes that serve to detoxify pro-oxidative stressors.[11] This regulation is mediated by Nrf2 binding to the antioxidant responsive element found in the promoter region of genes[12] such as heme oxygenase-1, thioredoxin, thioredoxin reductase, glutathione reductase, glutathione peroxidase, glutathione S-transferase, and catalase.[13,23,24] The reported antioxidant effects of $H_2S$ may be of critical importance in the setting of heart failure because oxidative stress plays a prominent role in the development of LV remodeling and dysfunction associated with heart failure,[25] suggesting that increasing the activity of cellular antioxidant enzymes should protect the failing myocardium.[26] The results of the present study support the previous finding that the cardioprotective effects of $H_2S$ are related to a reduction in oxidative stress because it was observed that $H_2S$ reduced LPO levels at both 1 and 4 weeks of reperfusion. The results of the present study also support a role for Nrf2 in mediating the antioxidant effects of $H_2S$ because $H_2S$ treatment was observed to induce the nuclear localization of Nrf2. Together, these findings indicate that $H_2S$ therapy creates an environment in the heart that is resistant to the oxidative stress associated with the development of heart failure.

Another physiological characteristic of $H_2S$ that could provide protection in the failing heart relates to the evidence that $H_2S$ can alter the metabolic state of organisms by modulating mitochondrial function.[27] In heart failure, there is a decrease in the activity of the complexes of the respiratory chain and Krebs cycle enzymes. The reduced expression of mitochondrial proteins results in decreased mitochondrial respiration efficiency and limited ATP synthesis capacity and myocardial energy production.[28] The decreased oxidative capacity of the failing myocardium therefore limits the ability of the heart to meet hemodynamic demands and leads to symptoms of heart failure. Mitochondria are essential for cell survival because of their roles as metabolic energy producers and regulators of programmed cell death.[29] Mitochondria rely on an intrinsic genome that is replicated and transcribed semiautonomously but whose maintenance requires nuclear factors such as NRF-1. Recently, the promoter region of NRF-1 was found to contain 4 antioxidant responsive elements that, when bound by Nrf2, led to an increase in NRF-1 protein levels and an increase in gene activation responsible for mitochondrial biogenesis.[14] Additionally, NRF-1 transcriptional activity was reported to be regulated by Akt.[15] Despite increasing the phosphorylation of Akt and the nuclear

accumulation of both Nrf2 and NRF-1, $H_2S$ therapy failed to increase mitochondrial biogenesis 4 weeks after myocardial infarction. $H_2S$ therapy also failed to provide significant improvements in mitochondrial function, although slight improvements in ATP synthesis were noted. These slight improvements suggest that the effects of $H_2S$ on the mitochondria were not direct. Rather, the slight improvements are more likely attributed to the ability of $H_2S$ to reduce oxidative stress, suggesting that, in this model of heart failure, the antioxidant effects of $H_2S$ may play a more prominent role in mediating its cardioprotective actions.

We live in the midst of the proclaimed epidemic of heart failure, as evidenced by a rise in the number of hospitalizations for heart failure, the number of deaths attributed to heart failure, and the costs associated with care.[30,31] Couple this with the ever-increasing prevalence of diabetes mellitus and obesity, 2 of the main risk factors for the development of coronary artery disease, and it is readily apparent that treatment strategies aimed at combating the development and progression of heart failure are important and severely needed. The findings of the present study provide the first evidence that either the modulation of endogenous $H_2S$ production or direct $H_2S$ administration significantly attenuates the severity of ischemia-induced heart failure in mice by reducing oxidative stress and attenuating mitochondrial dysfunction. Therefore, these findings further support the emerging concept that $H_2S$ therapy may be of clinical importance in the treatment of cardiovascular disease and may have a practical clinical use after myocardial infarction to reduce the morbidity and mortality associated with ischemia-induced heart failure.

### CLINICAL PERSPECTIVE

Heart failure continues to be a major health problem as evidenced by a rise in the number of hospitalizations for heart failure, the number of deaths attributed to heart failure, and the ever-increasing costs associated with care. Therapeutic strategies designed to coincide with the standard means of care are, therefore, needed to combat the development and progression of heart failure. Hydrogen sulfide ($H_2S$) is an endogenous gaseous signaling molecule with a diverse physiological profile that has recently been shown to be cardioprotective in various models of cardiac injury. In the present study, we found that either the modulation of endogenous $H_2S$ production or direct pharmacologic $H_2S$ administration significantly reduced mortality and attenuated the severity of ischemia-induced heart failure in mice. Importantly, the present study demonstrates that although a single administration of $H_2S$ at the time of reperfusion is beneficial in attenuating infarct size, this alone is not sufficient to improve cardiac function significantly. On the other hand, daily $H_2S$ therapy for the first 7 days of reperfusion or increased endogenous $H_2S$ production provided significant improvements in cardiac function, suggesting that multiple therapeutic interventions are paramount for improvements in outcome. Together, these findings further support the emerging concept that $H_2S$ therapy may be of clinical importance in the treatment of cardiovascular disease and may have a practical clinical use after myocardial infarction to reduce the morbidity and mortality associated with ischemia-induced heart failure.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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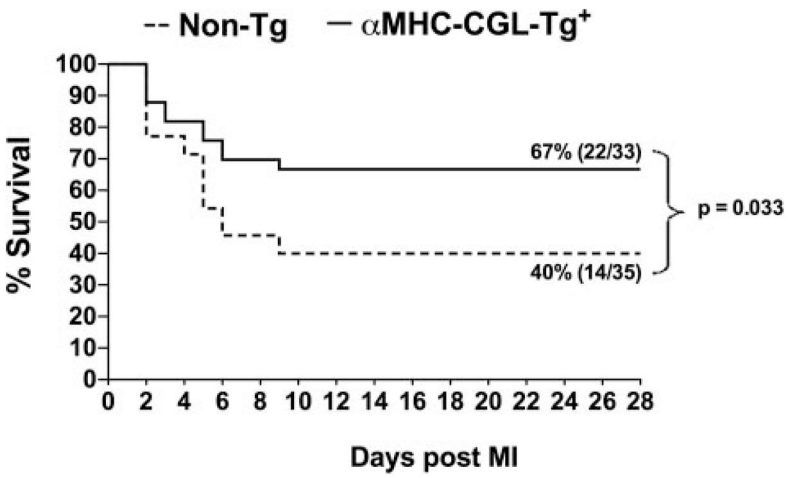


**Figure 1.**

Overexpression of CGL improved survival after permanent occlusion of the LCA. Survival curve for $\alpha$MHC-CGL-Tg$^+$ and nontransgenic (Non-Tg) mice during the 4-week period after permanent occlusion of the LCA. The $\alpha$MHC-CGL-Tg$^+$ mice exhibited an overall survival rate of 67% (22 of 33) during the 4-week follow-up; the nontransgenic mice exhibited an overall survival rate of 40% (14 of 35). Comparisons between survival curves were made with a log-rank (Mantel-Cox) test.

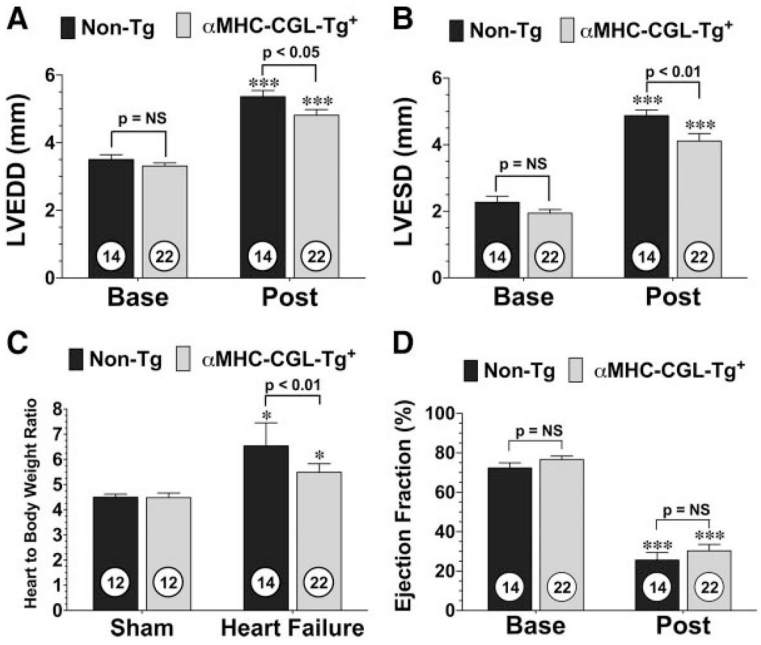


**Figure 2.**

Overexpression of CGL reduces LV dilatation and cardiac hypertrophy but does not improve LV function after permanent occlusion of LCA. LVEDD (A), LVESD (B), ratio of heart to body weight (C), and LV ejection fraction (D) for $\alpha$MHC-CGL-Tg$^{+}$ and nontransgenic (Non-Tg) mice 4 weeks after permanent LCA occlusion (Post). LVEDD, LVESD, and LV ejection fraction were calculated with 2-dimensional B-mode echocardiography images at baseline (Base) and after myocardial ischemia in all groups. Ratios of heart to body weight were used as a measure of cardiac hypertrophy. Values are mean±SEM. Numbers inside bars indicate the number of animals investigated in each group. Means for the echocardiography data were compared by use of a 2-way ANOVA with a Bonferroni test as the posthoc analysis. Means for the ratio of heart to body weight were compared through the use of a 1-way ANOVA with a Tukey test as the posthoc analysis. ***$P$<0.001 vs baseline; *$P$<0.05 vs sham.

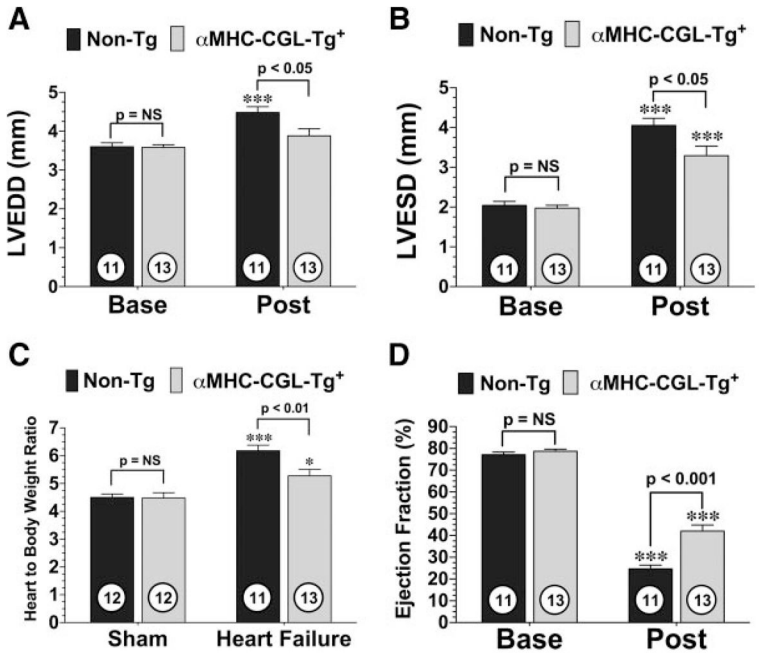


**Figure 3.**

Overexpression of CGL reduces LV dilatation, reduces cardiac hypertrophy, and improves LV function after myocardial ischemia and reperfusion. LVEDD (A), LVESD (B), ratio of heart to body weight (C), and LV ejection fraction (D) for $\alpha$MHC-CGL-Tg$^{+}$ and nontransgenic mice 4 weeks after 60 minutes of LCA occlusion and reperfusion (Post). Values are mean±SEM. Means for the echocardiography data were compared by use of a 2-way ANOVA with a Bonferroni test as the posthoc analysis. Means for the ratios of heart to body weight were compared by use of a 1-way ANOVA with a Tukey test as the posthoc analysis. ***$P$<0.001 vs baseline (Base) or sham; *$P$<0.05 vs sham.

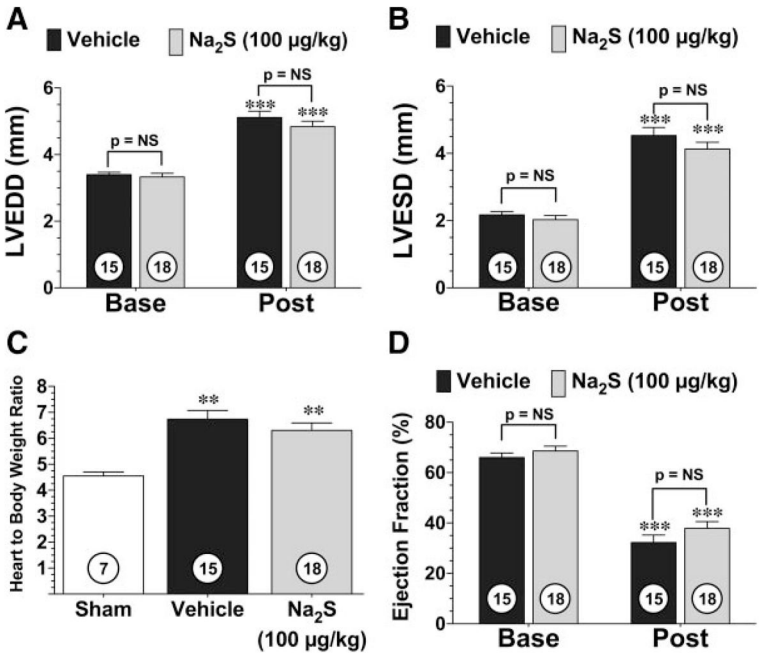


**Figure 4.**

Single administration of $Na_2S$ does not attenuate the development of ischemia-induced heart failure. LVEDD (A), LVESD (B), ratio of heart to body weight (C), and LV ejection fraction (D) for $Na_2S$- and vehicle-treated mice 4 weeks after 60 minutes of LCA occlusion and reperfusion. (Post) Mice were treated with 100 $\mu$g/kg $Na_2S$ or vehicle at the time of reperfusion. Values are mean±SEM. Means for the echocardiography data were compared by use of a 2-way ANOVA with a Bonferroni test as the posthoc analysis. Means for the ratios of heart to body weight were compared by use of a 1-way ANOVA with a Tukey test as the posthoc analysis. ***$P$<0.001 vs baseline (Base); **$P$<0.01 vs sham.

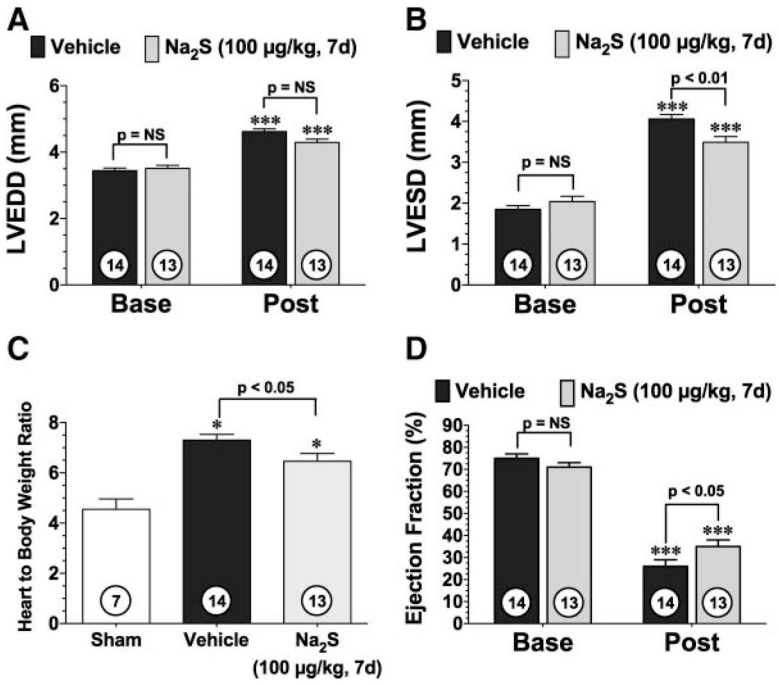


**Figure 5.**

Daily administrations of $Na_2S$ attenuate the development of ischemia-induced heart failure. LVEDD (A), LVESD (B), ratio of heart to body weight (C), and LV ejection fraction (D) for $NA_2S$-and vehicle-treated mice 4 weeks after 60 minutes of LCA occlusion and reperfusion (Post). Mice were treated with 100 $\mu$g/kg $Na_2S$ at the time of reperfusion and then daily for the first 7 days of reperfusion. Values are mean±SEM. Means for the echocardiography data were compared by use of a 2-way ANOVA with a Bonferroni test as the posthoc analysis. Means for the ratios of heart to body weight were compared by use of a 1-way ANOVA with a Tukey test as the posthoc analysis. ***$P$<0.001 vs baseline (Base); *$P$<0.05 vs sham.

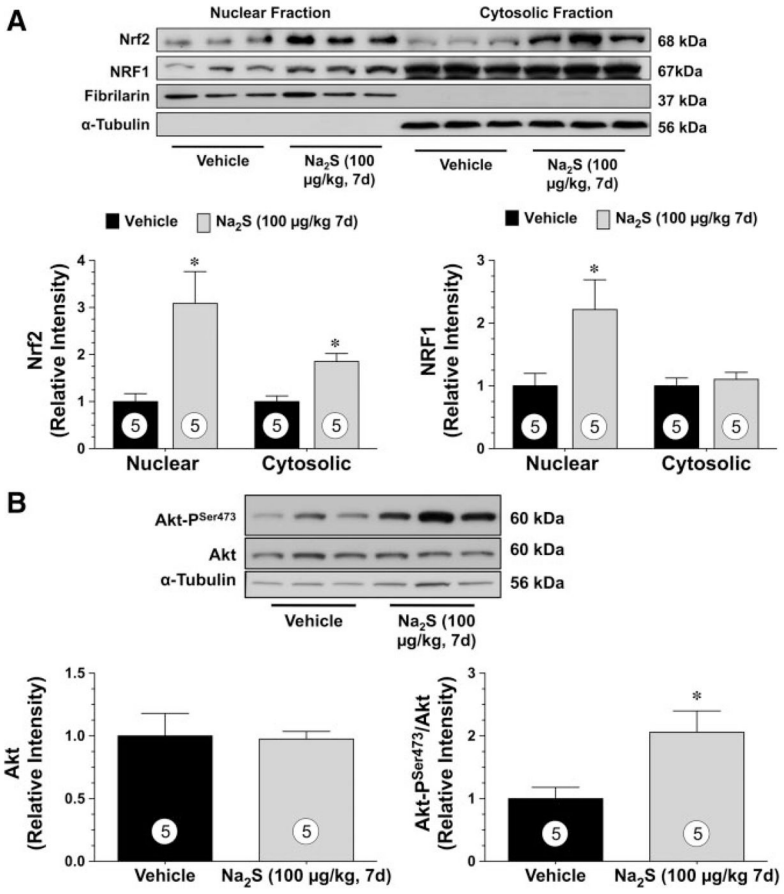


**Figure 6.**

Daily administrations of $Na_2S$ induce the nuclear localization of Nrf2 and NRF-1 and increase the phosphorylation of Akt. A, Representative immunoblots and densitometric analysis of cardiac Nrf2 and NRF-1 in the cytosolic and nuclear fractions after 1 week of $Na_2S$ treatment. B, Representative immunoblots and densitometric analysis of phosphorylated Akt-$^{Ser473}$ and total Akt after 1 week of $Na_2S$ treatment. Values are mean±SEM. Means for all data were compared by use of an unpaired *t* test. **P*<0.05 vs vehicle.

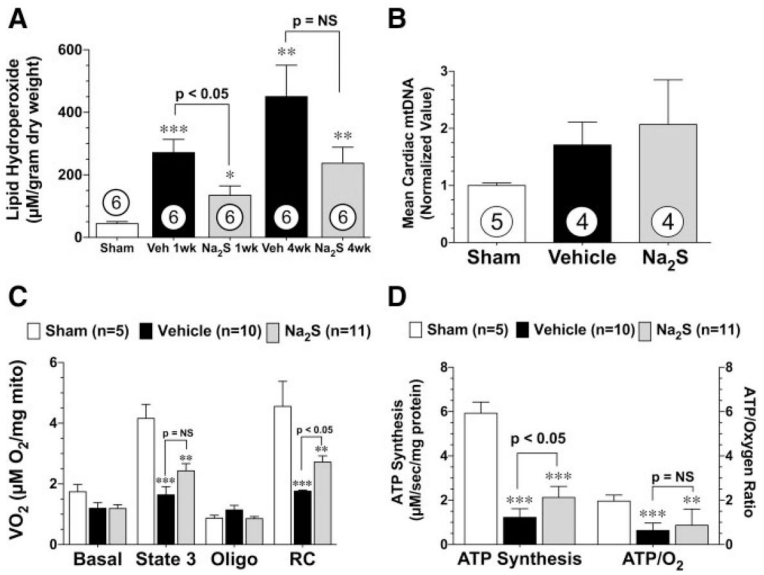


**Figure 7.**

Daily administrations of $Na_2S$ attenuate oxidative stress and mitochondrial dysfunction during the development of ischemia-induced heart failure. A, Lipid hydroperoxide levels ($\mu$mol/L) from sham controls and vehicle (Veh)- and $Na_2S$-treated mice at 1 and 4 weeks of reperfusion after myocardial ischemia. B, Mean cardiac mitochondrial DNA levels (mtDNA) determined by real-time polymerase chain reaction analysis shown as arbitrary units normalized to the sham value (1.0), C, Mitochondrial oxygen consumption rates, oligomycin (oligo)-inhibited respiration, and respiratory control (RC) ratios of mitochondria isolated from sham-operated, vehicle-treated, and $Na_2S$-treated mice at 4 weeks of reperfusion after myocardial ischemia. Mean±SEM values are shown for state 3 (ADP-stimulated) respiration in the presence of succinate and glycerol-3-phospate. D, ATP synthesis rates and the ratio of ATP synthesis to maximal oxygen consumption obtained from the same isolated mitochondria shown in C. Values are mean±SEM. Means for all data were compared by use of a 1-way ANOVA with a Tukey test as the posthoc analysis. *$P$<0.05, **$P$<0.01, and ***$P$<0.001 vs sham.