TRANSLATIONAL PHYSIOLOGY

Chronic sodium hydrosulfide treatment decreases medial thickening of intramyocardial coronary arterioles, interstitial fibrosis, and ROS production in spontaneously hypertensive rats

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1Department of Physiology and Pathophysiology, 2School of Pharmacy, and 3The Children’s Hospital, Fudan University Shanghai Medical College, Shanghai, China; Cardiovascular Biology Research Group, Departments of 4Pharmacology and 5Biochemistry, National University of Singapore, Singapore

Submitted 21 January 2007; accepted in final form 2 July 2007

Shi Y-X, Chen Y, Zhu Y-Z, Huang G-Y, Moore PK, Huang S-H, Yao T, Zhu Y-C. Chronic sodium hydrosulfide treatment decreases medial thickening of intramyocardial coronary arterioles, interstitial fibrosis, and ROS production in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 293: H2093–H2100, 2007. First published July 13, 2007; doi:10.1152/ajpheart.00088.2007.— Hydrogen sulfide (H2S) is a gasotransmitter that regulates cardiovascular functions. The present study aimed to examine the hypothesis that chronic treatment with sodium hydrosulfide (NaHS, an H2S donor) is able to prevent left-ventricular remodeling in spontaneously hypertensive rats (SHR). Four-week-old SHR were treated with NaHS (10, 30, and 90 μmol·kg−1·day−1), a combination of NaHS (30 μmol·kg−1·day−1) and glibenclamide (5 mg·kg−1·day−1), glibenclamide alone (5 mg·kg−1·day−1), hydralazine alone (10 mg·kg−1·day−1), and placebo for 3 mo. At the end of the treatment period, variables such as cardiac geometry and function, intramyocardial arterioles ranging in diameter from 25 to 100 μm, perivascular and interstitial collagen content, reactive oxygen species (ROS), thiol groups, conjugated dienes, and DNA base modification were examined. The novel finding of the present study is that chronic NaHS treatment prevented the hypertrophy of intramyocardial arterioles and ventricular fibrosis, as well as decreased myocardial ROS and conjugated diene levels. The cardioprotective effects were blunted by coadministration of glibenclamide, suggesting a role of ATP-sensitive potassium channels in mediating the action of NaHS. Hydralazine caused a comparable reduction of blood pressure compared with NaHS treatment; however, it exerted no effect on the remodeling process or on ROS and conjugated diene levels. Moreover, NaHS treatment caused an increase in myocardial thiol group levels, whereas DNA base modification was not altered by NaHS treatment. In conclusion, the superior cardioprotective effects of NaHS treatment are worthy to be further explored to develop novel therapeutic approaches for the treatment of cardiac remodeling in hypertension.

left-ventricular remodeling; gasotransmitter; hypertension; hydrogen sulfide

HYPERTENSION-INDUCED left-ventricular hypertrophy is recognized as an independent risk factor for myocardial ischemia, dysfunction, infarction, and heart failure (26). Structural remodeling of coronary arterioles and extracellular matrix becomes obvious in hypertrophied left ventricle (LV) in 4-mo-old spontaneously hypertensive rats (SHR) (15). Hypertension-induced medial thickening of intramyocardial coronary arterioles results in stenosis of the coronary arterioles and consequent myocardial ischemia and dysfunction (19), whereas perivascular fibrosis may induce a compression on the coronary arterioles and therefore account for a reduction in coronary vasodilator reserve. Moreover, an accumulation of fibrillar collagen in the interstitial space of hypertrophied myocardium has been held responsible for abnormal ventricular wall stiffness and for impaired cardiac pumping capacity (12, 20). In this context, the aim of antihypertensive therapy is not only to decrease blood pressure (BP) but also to reduce hypertrophy of intramyocardial coronary arterioles and left-ventricular interstitial fibrosis.

Hydrogen sulfide (H2S) is endogenously generated from cysteine by the pyridoxal-5’-phosphate-dependent enzymes, including cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (9). CBS is highly expressed in the brain (1), whereas CSE is mostly concentrated in the vasculature (13). In recent years, accumulating evidence has suggested that H2S plays a pivotal role in cardiovascular regulation (27, 16). H2S has been further shown to dilate rat aortic tissues by opening ATP-sensitive potassium channels (KATP channels) in vascular smooth muscle cells (VSMC) (31). Administration of NaHS has been shown to reduce BP and heart-to-body weight (BW) ratio in various hypertensive models such as spontaneous (29) and nitric oxide synthase inhibition-induced hypertension (25) in rats. In SHR, NaHS treatment decreased aortic thickening (29). However, although of interest, thickening of the aorta does not play a pivotal role in increasing peripheral resistance and decreasing perfusion to the important organs such as heart, kidney, and brain in hypertension. Whether chronic NaHS treatment is able to inhibit remodeling processes such as arteriole thickening or perivascular fibrosis remains unknown.

To date, there is no information about the potential role of H2S in the remodeling process of intramyocardial coronary...
arterioles and interstitial collagen as well as in the production of reactive oxygen species (ROS) in hypertension. Therefore, the present study aimed to investigate the effect of chronic NaHS (as a H₂S donor) treatment on medial thickening of intramyocardial coronary arterioles and interstitial fibrosis in the LV of SHR. The effects of NaHS administration on myocardial ROS levels as well as the parameters for oxidative damage such as conjugated diene level and DNA base modification were also examined.

MATERIALS AND METHODS

Experimental protocol. Four-week-old male Wistar-Kyoto (WKY) rats and SHR (obtained from the Department of Experimental Animals, Chinese Academy of Sciences) were randomly assigned to eight groups: WKY control (WKY rats treated with placebo, i.e., saline, 1 ml·kg⁻¹·day⁻¹ ip and soybean oil, 1 ml·kg⁻¹·day⁻¹ po), SHR control (SHR treated with placebo, i.e., saline, 1 ml·kg⁻¹·day⁻¹ ip and soybean oil, 1 ml·kg⁻¹·day⁻¹ po), SHR-NaHS10 (SHR treated with NaHS, 10 μmol·kg⁻¹·day⁻¹ ip and soybean oil, 1 ml·kg⁻¹·day⁻¹ po), SHR-NaHS30 (SHR treated with NaHS, 30 μmol·kg⁻¹·day⁻¹ ip and soybean oil, 1 ml·kg⁻¹·day⁻¹ po), SHR-NaHS90 (SHR treated with NaHS, 90 μmol·kg⁻¹·day⁻¹ ip and soybean oil, 1 ml·kg⁻¹·day⁻¹ po), SHR-NaHS30 + GLIB (SHR treated with NaHS, 30 μmol·kg⁻¹·day⁻¹ ip and glibenclamide, 5 mg·kg⁻¹·day⁻¹ po), SHR-GLIB (SHR treated with saline, 1 ml·kg⁻¹·day⁻¹ ip and glibenclamide, 5 mg·kg⁻¹·day⁻¹ po), and SHR-HYD (SHR treated with hydralazine, 10 mg·kg⁻¹·day⁻¹ ip and soybean oil, 1 ml·kg⁻¹·day⁻¹ po). H₂S was administered in the form of the myocardial tissue was rapidly frozen in liquid nitrogen and stored at −80°C. The left-ventricular hypertrophy was assessed as previously described (15). Briefly, paraffin-embedded segments of heart were cut into 3-μm-thick cross-sections and were stained with hematoxylin and eosin for examination of overall morphology and arteriolar thickness or with a collagen-specific stain (Sirius red F3BA in aqueous saturated picric acid) for examination of perivascular and interstitial fibrosis. Media-to-lumen area ratio, an index of arteriolar thickening, was defined as the wall area-to-lumen area ratio. Each field was photographed with the imaging system (Leica, Wetzlar, Germany) and was analyzed by using ImageMeasure software (Department of Physiology and Pathophysiology, Fudan University Shanghai Medical College) by an observer blinded to the animal groups. Collagen volume fraction (CVF) was calculated as the sum of the connective tissue areas in the coronal section divided by the sum of all connective tissue and muscle areas. Perivascular collagen was excluded from this measurement. To normalize the area of perivascular collagen around vessels with different sizes, perivascular collagen content was represented as perivascular collagen area-to-luminal area ratio (PVALA). Intramyocardial arteriolar thickening was assessed as previously described (32). Briefly, left-ventricular frozen sections (20 μm) were incubated with 12.5 μM DHE at 37°C for 30 min in a light-tight humidified chamber. The sections were observed by using a Leica microscope with a 585-nm filter. Generation of superoxide in the tissue was shown as red fluorescent labeling. Nonstained left-ventricular sections were used as background control. Identical photomultiplier settings were used for image acquisition for all samples. The fluorescent signals were quantified by using ImageMeasure. The average of four sections stained with DHE was taken as the value for each animal.

ROS detection in the LV. Dihydroethidium staining (DHE, Sigma) was used to evaluate the in situ levels of superoxide in the myocardium as described (32). Briefly, left-ventricular frozen sections (20 μm) were incubated with 12.5 μM DHE at 37°C for 30 min in a light-tight humidified chamber. The sections were observed by using a Leica microscope with a 585-nm filter. Generation of superoxide in the tissue was shown as red fluorescent labeling. Nonstained left-ventricular sections were used as background control. Identical photomultiplier settings were used for image acquisition for all samples. The fluorescent signals were quantified by using ImageMeasure. The average of four sections stained with DHE was taken as the value for each animal.

Assessment of thiol groups in the myocardium and plasma. Total thiol groups were measured by using 2, 2′-dinitro-5, 5′-dithiobenzoiic acid (DTNB), which reacts with thiol groups to produce a yellow-colored complex (peak absorbance at 412 nm). Briefly, 1 ml Tris-EDTA buffer (pH 8.6) was added to 50 μl of homogenate/plasma in 2 ml cuvettes, and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then 20 μl DTNB reagent (10 mM in methanol) was added to the mixture, and after 15 min at room temperature, the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (in mM) was calculated from the following equation: total thiol concentration = (A2 – A1 – B) × 1.07/(0.05 × 13.6) = (A2 – A1 – B) × 1.57.
Assessment of conjugated dienes in the myocardium. Myocardial conjugated diene levels were measured as described (21, 18) with modifications. Briefly, 75 mg of tissue was homogenized on ice in 1.5 ml PBS with 0.001 M Na2EDTA. Then 1 ml homogenate was mixed with 2.25 ml of chloroform-methanol (1:2 vol/vol), shaken for 60 min at room temperature. Shaking was continued for another 30 min after adding 0.75 ml of chloroform again. Hydrochloric acid (0.75 ml of 0.003 M) was added and mixed slightly to wash the organic layer. The mixture was centrifuged at 1,500 g for 10 min at 10°C. Thereafter the lower chloroform layer was dried and the residue was reconstituted with 0.5 ml of cyclohexane. Absorbance was determined at a wavelength of 234 nm with a Tecan spectrophotometer (Infinite M200; Tecan, Grödig, Austria) against a cyclohexane blank. The content of conjugated dienes was represented as value of absorbance at 234 nm per gram of tissue.

Assessment of base modification of the DNA in the myocardium. Methods for the assessment of DNA base modification are provided in the online data supplement.

Statistical analysis. Data are presented as means ± SE. Comparison between the groups was performed by one-way ANOVA, followed by Student-Newman-Keuls test. A P value of < 0.05 was taken as statistically significant.

RESULTS

Effect of NaHS on hemodynamic parameters and indexes of left-ventricular hypertrophy. As shown in Table 1, chronic NaHS treatment for 3 mo at doses of 10, 30, and 90 μmol·kg⁻¹·day⁻¹ significantly reduced DBP and MAP in SHR (P < 0.05). In contrast, SBP was only reduced in SHR treated with NaHS at doses of 30 and 90 μmol·kg⁻¹·day⁻¹ (P < 0.05). The range of BP reduction in the three NaHS-treated SHR groups was comparable. BP reduction in the SHR-NaHS30 group was prevented by coadministration of glibenclamide (5 mg·kg⁻¹·day⁻¹). Hydralazine (10 mg·kg⁻¹·day⁻¹) treatment caused a reduction in BP comparable with that induced by NaHS treatment. However, glibenclamide (5 mg·kg⁻¹·day⁻¹) alone had no effect on BP. HR was increased in the SHR control compared with the WKY control (P < 0.05). Neither NaHS nor hydralazine exerted any significant effects on HR compared with the SHR control.

As shown in Table 2, BW was reduced in the SHR-NaHS30 + GLIB and SHR-GLIB groups compared with the WKY control group, whereas there was no statistical difference in BW among all SHR groups. Compared with the WKY control group, LVW and LVW/BW were significantly increased in the SHR control. However, NaHS treatment did not induce a reduction in LVW and LVW/BW compared with SHR control. In contrast, there was no significant difference in right-ventricular weight (RVW) between placebo-treated SHR and WKY rats, suggesting that the right ventricle was not involved in hypertension-induced cardiac hypertrophy in 4-mo-old SHR. Neither NaHS nor hydralazine exerted any significant effects on RVW compared with the SHR control.

Effect of NaHS on hypertrophy of intramyocardial and ventricular fibrosis. As shown in Fig. 1, A and B, coronary arteriolar thickening, was significantly increased in 4-mo-old placebo-treated SHR compared with the WKY control (1.626 ± 0.129 vs. 0.966 ± 0.043; P < 0.05). Chronic treatment with NaHS for 3 mo at a dose of 90 μmol·kg⁻¹·day⁻¹ significantly reduced this ratio to 1.245 ± 0.080 in SHR (P < 0.05). NaHS treatment at lower doses (10 and 30 μmol·kg⁻¹·day⁻¹) was not significantly effective in reducing intramyocardial thickening, nor was hydralazine treatment at a dose of 10 mg·kg⁻¹·day⁻¹ compared with that of SHR control.

PVCA/LA (Fig. 1, C and D) and CVF (Fig. 2, A and B), indexes of perivascular and interstitial fibrosis, were significantly increased in 4-mo-old placebo-treated SHR compared with the WKY control group (1.446 ± 0.096 vs. 0.882 ± 0.094 for PVCA/LA and 5.181 ± 0.172% vs. 4.055 ± 0.117% for CVF, respectively; P < 0.05). Chronic treatments with NaHS at doses of 10, 30, and 90 μmol·kg⁻¹·day⁻¹ for 3 mo were all effective in reducing PVCA/LA and CVF in SHR (P < 0.05; Fig. 1, C and D, and Fig. 2, A and B), whereas hydralazine treatment at a dose of 10 mg·kg⁻¹·day⁻¹ was not effective on those parameters. Coadministration of glibenclamide (5 mg·kg⁻¹·day⁻¹) blunted the effect of NaHS (30 μmol·kg⁻¹·day⁻¹) in reducing CVF (P < 0.05; Fig. 2, A and B).

Effect of NaHS on myocardial ROS production. Myocardial ROS production was assessed by DHE staining of the left-ventricular sections. As shown in Fig. 2, C and D, There was an increase in ROS production in the LV of 4-mo-old placebo-treated SHR compared with the WKY control group (0.080 ± 0.022 vs. 0.002 ± 0.001, P < 0.05). Chronic treatment with NaHS at doses of 10, 30, and 90 μmol·kg⁻¹·day⁻¹ significantly reduced ROS production in SHR to 0.024 ± 0.007, 0.018 ± 0.007, and 0.012 ± 0.004, respectively (P < 0.05). Coadministration of glibenclamide (5 mg·kg⁻¹·day⁻¹) blunted the effect of NaHS (30 μmol·kg⁻¹·day⁻¹) in reducing myocardial ROS production, whereas hydralazine treatment (10 mg·kg⁻¹·day⁻¹) had no effect on ROS production.

Effect of NaHS on geometry and function of the LV. Doppler echocardiogram showed that LVPWd and LVPWs, geometric...
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Table 2. Effect of H₂S treatment for 3 mo on indexes of LVH in SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY Control</th>
<th>SHR Control</th>
<th>SHR-NaHS10</th>
<th>SHR-NaHS30</th>
<th>SHR-NaHS90</th>
<th>SHR-NaHS30 + GLIB</th>
<th>SHR-GLIB</th>
<th>SHR-HYD</th>
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<td>n</td>
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<td>7</td>
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<td>BW, g</td>
<td>318 ± 12</td>
<td>289 ± 14</td>
<td>297 ± 8</td>
<td>308 ± 8</td>
<td>305 ± 8</td>
<td>279 ± 13*</td>
<td>284 ± 9*</td>
<td>290 ± 12</td>
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<tr>
<td>LVW, mg</td>
<td>189 ± 28</td>
<td>148 ± 5</td>
<td>157 ± 11</td>
<td>160 ± 10</td>
<td>191 ± 32</td>
<td>160 ± 8</td>
<td>162 ± 8</td>
<td>160 ± 9</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>2.192 ± 0.670</td>
<td>2.726 ± 0.102*</td>
<td>2.627 ± 0.049*</td>
<td>2.476 ± 0.054</td>
<td>2.456 ± 0.118</td>
<td>2.877 ± 0.240*</td>
<td>2.810 ± 0.088*</td>
<td>2.469 ± 0.076</td>
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</table>

Values are means ± SE; n = number of rats. LVH, left-ventricular hypertrophy; LVW, left-ventricular weight; RVW, right-ventricular weight; BW, body weight; LVW/BW, LVW-to-BW ratio. *P < 0.05 vs. WKY control. #P < 0.05 vs. SHR control.

indexes of LVH, were significantly increased in placebo-treated SHR compared with WKY control group (1.75 ± 0.08 vs. 1.22 ± 0.09 for LVPWd and 2.31 ± 0.07 vs. 1.85 ± 0.12 for LVPWs, respectively; P < 0.05). Chronic treatment with NaHS at doses of 30 and 90 μmol·kg⁻¹·day⁻¹ significantly reduced LVPWd and LVPWs in SHR, whereas hydralazine treatment (10 mg·kg⁻¹·day⁻¹) did not reduce LVPWd and LVPWs (P < 0.05; Table 3). Neither NaHS nor hydralazine treatment showed any significant effect on LV chamber size as indicated by LVDD and LVSD. In placebo-treated SHR, indexes of LV systolic function such as FS, EF, CO, and SV, as well as indexes of diastolic function such as IRT and MEV/MAV, were not significantly different from that of the WKY control group. Treatment with NaHS (10, 30, and 90 μmol·kg⁻¹·day⁻¹) had no effect on those functional parameters in SHR. However, hydralazine treatment (10 mg·kg⁻¹·day⁻¹) reduced FS and EF compared with placebo-treated SHR, suggesting a negative inotropic effect of hydralazine (P < 0.05; Table 3).

Effect of NaHS on thiol group levels in the myocardium and plasma. Myocardial thiol concentration was significantly decreased in SHR control compared with the WKY control. Whereas treatment of SHR with NaHS at doses of 30 and 90 μmol·kg⁻¹·day⁻¹ was effective in increasing myocardial thiol levels compared with SHR control (P < 0.05; Fig. 3A), NaHS treatment (10, 30, and 90 μmol·kg⁻¹·day⁻¹) did not change plasma thiol levels in SHR. There was no significant difference in plasma thiol levels between SHR control and WKY control (Fig. 3B).

Effect of NaHS on myocardial conjugated diene levels. In contrast, myocardial conjugated diene levels were significantly increased in SHR control compared with WKY controls. Treatment of SHR with NaHS at a dose of 30 μmol·kg⁻¹·day⁻¹ was effective in decreasing myocardial conjugated diene levels compared with SHR control (P < 0.05; Fig. 3C).

Effect of NaHS on myocardial DNA base modification. There was no significant difference in the levels of myocardial DNA base modification products, i.e., 5-Cl uracil, 5-OH Me

Fig. 1. Effect of NaHS on medial thickening of intramyocardial coronary arterioles and perivascular fibrosis. A and B: representative micrographs (A) and values (B) of media-to-lumen area ratio of intramyocardial coronary arterioles. C and D: representative micrographs (C) and values (D) of perivascular collagen area-to-lumen area ratio (PVCA/LA). WKY control (n = 8), Wistar-Kyoto rats treated with placebo; SHR control (n = 9), spontaneously hypertensive rats (SHR) treated with placebo; SHR-NaHS10 (n = 9), SHR-NaHS30 (n = 7), and SHR-NaHS90 (n = 8), SHR treated with NaHS at doses of 10, 30, and 90 μmol·kg⁻¹·day⁻¹, respectively; SHR-NaHS30 + GLIB (n = 9), SHR treated with NaHS (30 μmol·kg⁻¹·day⁻¹) and glibenclamide (5 mg·kg⁻¹·day⁻¹); SHR-GLIB (n = 9), SHR treated with glibenclamide (5 mg·kg⁻¹·day⁻¹); SHR-HYD (n = 10), SHR treated with hydralazine (10 mg·kg⁻¹·day⁻¹). Values are means ± SE. *P < 0.05. Bar, 50 μm.

a
product.

Effect of NaHS on blood chemistry profile. Terminal blood samples were collected for biochemical analysis for glucose, triglycerides, cholesterol, creatinine, and alanine aminotransferase. Glucose was abnormally increased in placebo-treated SHR compared with the WKY control group. However, NaHS treatment of SHR showed no significant effect on glucose levels compared with SHR control. No significant difference in other blood chemistry parameters was found among all WKY and SHR groups (see Table 5 in the online data supplement).

**DISCUSSION**

Structural remodeling of small arterioles is one of the most prevalent forms of target organ damage in hypertension (19). Medial hypertrophy of intramyocardial coronary arterioles is a

**Table 3. Effect of exogenous H2S treatment for 3 mo on cardiac parameters measured by Doppler echocardiograph**

<table>
<thead>
<tr>
<th></th>
<th>WKY Control</th>
<th>SHR Control</th>
<th>SHR-NaHS30</th>
<th>SHR-NaHS90</th>
<th>SHR-NaHS30 + GLIB</th>
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<td>7</td>
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<td>LVPWd, mm</td>
<td>1.22±0.09</td>
<td>1.75±0.08*</td>
<td>1.76±0.12*</td>
<td>1.43±0.09#</td>
<td>+</td>
<td>1.44±0.11#</td>
<td>1.82±0.12*</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>1.85±0.12</td>
<td>2.31±0.07*</td>
<td>2.22±0.14</td>
<td>2.06±0.10#</td>
<td>+</td>
<td>1.93±0.13#</td>
<td>2.33±0.134*</td>
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<td>LVDD, mm</td>
<td>5.43±0.32</td>
<td>4.69±0.37</td>
<td>4.61±0.41</td>
<td>4.81±0.42</td>
<td>+</td>
<td>4.81±0.24</td>
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<td>LVSD, mm</td>
<td>3.16±0.23</td>
<td>2.55±0.19</td>
<td>2.58±0.23</td>
<td>2.81±0.28</td>
<td>+</td>
<td>2.71±0.09</td>
<td>2.45±0.14</td>
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<td>FS, %</td>
<td>42.01±1.29</td>
<td>45.28±1.25</td>
<td>44.16±1.56</td>
<td>41.83±1.93</td>
<td>+</td>
<td>43.30±1.78</td>
<td>44.02±1.38</td>
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<td>EF, %</td>
<td>80.30±1.34</td>
<td>83.41±1.13</td>
<td>82.29±1.47</td>
<td>79.87±1.81</td>
<td>+</td>
<td>81.38±1.86</td>
<td>82.21±1.39</td>
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<tr>
<td>CO, l/min</td>
<td>0.09±0.02</td>
<td>0.08±0.02</td>
<td>0.08±0.022</td>
<td>0.075±0.011</td>
<td>+</td>
<td>0.067±0.011</td>
<td>0.082±0.017</td>
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<tr>
<td>SV, ml</td>
<td>0.337±0.054</td>
<td>0.245±0.055</td>
<td>0.239±0.062</td>
<td>0.240±0.052</td>
<td>+</td>
<td>0.238±0.036</td>
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<td>IRT, ms</td>
<td>28.49±1.02</td>
<td>26.19±1.38</td>
<td>26.84±2.07</td>
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<td>+</td>
<td>26.95±1.23</td>
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<tr>
<td>MEV/MAV</td>
<td>2.018±0.146</td>
<td>1.870±0.137</td>
<td>2.084±0.157</td>
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<td>+</td>
<td>2.039±0.101</td>
<td>1.642±0.256</td>
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</table>

Values are means ± SE; n = number of rats. LVPWd and LVPWs, left-ventricular end-diastolic and systolic posterior wall thickness; LVDD and LVSD, left-ventricular end-diastolic and systolic dimensions; FS, fractional shortening; EF, ejection fraction; CO, cardiac output; SV, stroke volume; IRT, isovolumic relaxation time of the left ventricle; MEV/MAV, ratio of peak E velocity and peak A velocity of mitral diastolic flow. *P < 0.05 vs. WKY control. #P < 0.05 vs. SHR control. +P < 0.05 vs. SHR-NaHS30 + GLIB.
prominent contributor to cardiac remodeling. It is responsible for impaired coronary reserve and increases the incidence of myocardial infarction and dysfunction. Yan et al. (29) reported that chronic treatment of SHR with NaHS for 5 wk (56 μmol·kg⁻¹·day⁻¹) prevented the development of hypertension and the remodeling of the aorta. However, whether NaHS treatment is able to inhibit the remodeling of intramyocardial arterioles and extracellular matrix remains unknown. In the present study, we found that chronic NaHS treatment for 3 mo at doses of 10, 30, and 90 μmol·kg⁻¹·day⁻¹ was effective in reducing BP, whereas Yan and colleagues (29) found a BP-reducing effect of NaHS treatment at a dose of 56 μmol·kg⁻¹·day⁻¹. Thus lower doses of NaHS treatment (e.g., 10 and 30 μmol·kg⁻¹·day⁻¹) were also effective in reducing BP if a longer treatment period (e.g., 3 mo) was applied. However, there was no significant dose-related difference in BP reduction in SHR treated with different NaHS doses (10, 30, and 90 μmol·kg⁻¹·day⁻¹).

NaHS treatment (90 μmol·kg⁻¹·day⁻¹) alleviated medial thickening of intramyocardial coronary arterioles in the LV of SHR. NaHS treatment was effective at doses of 10, 30, and 90 μmol·kg⁻¹·day⁻¹ for the inhibition of perivascular and interstitial fibrosis. In the present study, parameters for cardiac remodeling were examined in SHR at an age of 4 mo in which an increase in left-ventricular mass, arteriolar hypertrophy, and perivascular and interstitial fibrosis had already become obvious (15), whereas the 9-wk-old SHR examined in the study by Yan and colleagues (29) may be too young to show any significant changes in such remodeling parameters. Moreover, the longer treatment duration (i.e., 3 mo) and various doses applied in the present study may also account for the significant inhibitory effect of NaHS on the remodeling of intramyocardial arterioles and on collagen.

On the other hand, the inhibitory effects of NaHS on intramyocardial arteriole and collagen cannot be solely ascribed to the BP-decreasing effect of NaHS treatment, because a comparable decrease in BP by hydralazine treatment was not effective in preventing intramyocardial arteriolar hypertrophy as well as perivascular and interstitial fibrosis. However, hydralazine treatment may not be regarded as the best control for the BP-reduction effect based on the antioxidative effects of this vasodilator (3). In the present study, NaHS treatment caused a rather significant ROS reduction compared with hydralazine, suggesting that the added reduction in inhibition of remodeling provided by NaHS may be explained by an additional reduction in ROS levels. Moreover, in vitro studies suggest a direct inhibitory effect of H₂S on proliferation of cultured VSMC (6). However, it still remains to be clarified whether NaHS treatment causes a BP-independent inhibitory effect on hypertension-induced intramyocardial arteriolar hypertrophy in vivo.

Another novel finding of the present study is an inhibitory effect of NaHS on myocardial ROS production in SHR, which has not previously been observed by other investigators (29). Interestingly, the antioxidative effect of NaHS was abolished by coadministration of glibenclamide, suggesting a role of Kₐ₅p channels in the antioxidative effect of NaHS. Although direct evidence for a Kₐ₅p channel-opening effect of H₂S in the heart is lacking, the Kₐ₅p channel-opening effect of H₂S in VSMC (31) supports the hypothesis that this gasotransmitter may also open the Kₐ₅p channels in the cardiac cells. On the
other hand, ROS production has been shown to be increased in hypertrophied cardiomyocytes, and treatment with antioxidants inhibits cardiomyocyte hypertrophy (17). In rat models of hypertension, ROS levels are increased in the hypertrophied myocardium (24) and vasculature (5). Moreover, ROS has been shown to play a role in stimulating VSMC proliferation (2) and myocardial fibrogenesis (30). Therefore, the inhibitory effects of NaHS on medial thickening of intramyocardial coronary arterioles as well as perivascular and interstitial fibrosis are associated with a reduction in ROS levels.

In contrast, ROS have been reported to induce oxidative damage, as evidenced by an increase in conjugated diene levels (22) as well as oxidatively modified DNA bases (23). The present study revealed that myocardial conjugated diene levels were significantly increased in SHR control compared with WKY controls, whereas this increase was inhibited by NaHS treatment. However, the later parameters of oxidative damage such as DNA base modification in the myocardium of SHR were not significantly greater than that in WKY rats in spite of increased ROS levels in SHR. It remains to be further investigated whether it is too early to identify ROS-induced DNA base modification in 4-mo-old SHR.

It is worth noting that H$_2$S is transformed to thiol groups (28), which have been shown to be involved in the maintenance of enzymatic activation of antioxidant enzymes (10) and are recognized as a natural reservoir of the reductive capacity of the cell (11, 28). In the present study, NaHS treatment (30 and 90 μmol·kg$^{-1}$·day$^{-1}$) significantly increased the concentration of thiol groups in the myocardium, suggesting that H$_2$S-derived thiols may also contribute to the antioxidant properties of H$_2$S.

We observed that LVW/BW as well as LVPWd and LVPWs, geometric indexes of left-ventricular hypertrophy, were increased in placebo-treated SHR compared with WKY control. There was a reduction in LVPWd and LVPWs in SHR treated with NaHS compared with SHR control. In line with the present study, NaHS treatment reduced heart-to-BW ratio in nitric oxide synthase inhibition-induced hypertension in rats (25). On the other hand, there is no significant difference in the parameters for left-ventricular function, i.e., FS, EF, CO, SV, IRT, and MEV/MAV, between the placebo-treated SHR and WKY rats. At the time point of assessment in the present study (4 mo), the heart of SHR is under a compensatory status of remodeling (15). This may explain why NaHS treatment did not improve the parameters for left-ventricular function. In this context, it will be interesting to investigate whether NaHS treatment for a longer duration (e.g., 12 mo) might be able to improve left-ventricular function.

In addition, hypertension has been demonstrated to be an independent risk factor for diabetes (8). We observed here a significant increase in plasma glucose levels in SHR. However, whether NaHS treatment has an effect on glucose homeostasis remains to be investigated. The chronic treatment of NaHS was performed by intraperitoneal injection once a day. Plasma H$_2$S has been shown to be significantly increased in NaHS-treated SHR (29). The present study further showed an increase in thiol groups in the myocardium of SHR treated with NaHS. Although the protocol for chronic NaHS administration remains to be optimized, single daily administration of NaHS is able to increase both the levels of H$_2$S and thiol groups and to prevent left-ventricular remodeling and ROS increase.

In conclusion, chronic NaHS treatment for 3 mo in SHR decreases medial thickening of intramyocardial coronary arterioles, interstitial fibrosis, ROS production, conjugated diene levels, and BP. The beneficial effect of NaHS treatment is associated with an opening of K$_{ATP}$ channels and a decrease in myocardial ROS production. The cardioprotective effect of NaHS as a H$_2$S donor is worthy to be further explored to develop novel therapeutic approaches for the treatment of cardiac remodeling in hypertension.

ACKNOWLEDGMENTS

We thank Xiao-Wei Huang and Shun-Na Ge for expert technical assistance.

GRANTS

This study was supported by grants from the National Natural Science Foundation of China (30470628) and the Ministry of Science and Technology (2006CB503804) of China.

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AJP-Heart Circ Physiol • VOL 293 • OCTOBER 2007 • www.ajpheart.org


