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Hydrogen gas improves left ventricular hypertrophy in Dahl rat of salt-sensitive hypertension

Hiroki Matsuoka[a], Seiko Miyata[b], Nozomi Okumura[a], Takuya Watanabe[a], Katsunori Hashimoto[c], Miki Nagahara[d], Kazuko Kato[a], Sayaka Sobue[a], Kozue Takeda[a], Masatoshi Ichihara[a], Takashi Iwamoto[a], and Akiko Noda[a]

[a]Department of Biomedical Sciences, Chubu University Graduate School of Life and Health Sciences, Kasugai, Japan; [b]Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan; [c]Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan; [d]Center for Education in Laboratory Animal Research, Chubu University, Kasugai, Japan

ABSTRACT

Purpose: Hypertension is an important risk factor for death resulting from stroke, myocardial infarction, and end-stage renal failure. Hydrogen (H_2) gas protects against many diseases, including ischemia-reperfusion injury and stroke. The effects of H_2 on hypertension and its related left ventricular (LV) function have not been fully elucidated. The purpose of this study was to investigate the effects of H_2 gas on hypertension and LV hypertrophy using echocardiography.

Methods: Dahl salt-sensitive (DS) rats were randomly divided into three groups: those fed an 8% NaCl diet until 12 weeks of age (8% NaCl group), those additionally treated with H_2 gas (8% NaCl + H_2 group), and control rats maintained on a diet containing 0.3% NaCl until 12 weeks of age (0.3% NaCl group). H_2 gas was supplied through a gas flowmeter and delivered by room air (2% hydrogenated room air, flow rate of 10 L/min) into a cage surrounded by an acrylic chamber. We evaluated interventricular septal wall thickness (IVST), LV posterior wall thickness (LVPWT), and LV mass using echocardiography.

Results: IVST, LVPWT, and LV mass were significantly higher in the 8% NaCl group than the 0.3% NaCl group at 12 weeks of age, whereas they were significantly lower in the 8% NaCl + H_2 group than the 8% NaCl group. There was no significant difference in systolic blood pressure between the two groups.

Conclusion: Our findings suggest that chronic H_2 gas inhalation may help prevent LV hypertrophy in hypertensive DS rats.

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Salt sensitive hypertension; hydrogen gas; cardiac hypertrophy; reactive oxygen species; antioxidant effect

Introduction

Hypertension is an important risk factor for death resulting from stroke, myocardial infarction, and end-stage renal failure (1,2), and various factors are associated with the elevation of blood pressure (BP). For instance, excess salt intake is closely associated with hypertension (3), and it also affects the cardiovascular system independent from BP elevation by inducing hypertrophy of vascular smooth muscles, increasing oxidative stress, and decreasing large artery compliance (4,5).

Mechanism of developing hypertension is complex, but oxidative stress is one of the important risk factors. Reactive oxygen species (ROS) produced in endothelial cells and vascular smooth muscle cells contributes to the progressive cardiovascular disease (6). Hydrogen (H_2) can be used as an effective antioxidant therapy; because it can rapidly diffuse across membranes, reach and react with cytotoxic ROS and protect against oxidative damage (7).

Several studied have indicated that H_2 gas exerts protective effects against many diseases. Inhalation of 0.5–2% H_2 gas during ischemia and reperfusion reduces infarct size without altering hemodynamic parameters, thereby preventing deleterious left ventricular (LV) remodeling (8). Hydrogen-containing saline improved interstitial fibrosis of LV induced by pressure overload in abdominal aortic-constricted rats (9). H_2 gas inhalation significantly suppressed superoxide production in the LV myocardium and LV remodeling induced by intermittent hypoxia (10). However, the effect of chronic H_2 inhalation on the development of hypertension and LV hypertrophy has not been fully elucidated. In the present study, we investigated the effects of H_2 gas on the development of hypertension and LV hypertrophy.

Methods

Animals

Male Dahl salt-sensitive (DS) rats (Japan SLC, Hamamatsu, Japan) were fed a low-salt (0.3% NaCl) diet from weaning until 6 weeks of age. These rats were then randomly divided into three groups: those maintained on a diet containing 0.3% NaCl until 12 weeks of age (0.3% NaCl group, n = 10), those fed a high-salt diet until 12 weeks of age (8% NaCl group, n = 10), and those additionally treated with 2% H_2 gas (8% NaCl + H_2 group, n = 10). Similar to standard chow, the experimental diet consisted of protein, minerals, and fat (MF rat diet; Oriental Yeast, Tokyo, Japan). As previously described (11), rats in standard plastic cages were placed in a 60-L air-tight acrylic chamber, which was continuously supplied with 10 L/min 2% H_2 gas air. The 2% H_2 gas air was generated by mixing compressed air from

CONTACT Akiko Noda anoda@isc.chubu.ac.jp Department of Biomedical Sciences, Chubu University Graduate School of Life and Health Sciences, 1200 Matsumoto-cho Kasugai-shi, Aichi 487-8501, Japan

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a scroll compressor SLP-15 EB (Anest Iwata, Yokohama, Japan) and 100% H_2 (Taiyo Nippon Sanso, Tokyo, Japan) using a multi-flowmeter Model-1203 (Kofloc, Kyoto, Japan), and thereafter this gas mixture (2% H_2 gas air) was delivered into the chamber. Air samples from the chamber were periodically collected and H_2 concentrations monitored with an Optical Gas Monitor Model FI-21 (Riken Keiki, Tokyo, Japan). All experimental procedures and protocols were approved by the Animal Care and Use Committee of Chubu University and conformed to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996).

Physiological measurements

Body weight and systolic blood pressure (SBP) using tail-cuff method (BP-98A; Softron Co., Ltd., Tokyo, Japan) of conscious rats were measured weekly. Images were acquired with a 12 MHz transducer connected to a Xario ultrasound system (Toshiba Medical Systems Co., Tochigi, Japan) at 12 weeks of age. M-mode and 2-dimensional echocardiography images were acquired at the papillary muscle level with a frame rate of 80 to 120/s under anesthesia by intraperitoneal injection of pentobarbital (25 mg/kg body weight). Interventricular septal thickness (IVST), LV posterior wall thickness (LVPWT), and LV end-diastolic and end-systolic diameters (LVDd and LVDs) were obtained from a short-axis view. Percent LV fractional shortening (%LVFS) was calculated as an index of LV systolic function, and LV mass was measured to assess LV hypertrophy. Peak flow velocities at the mitral level during rapid filling (E) and deceleration time (Dct) were measured using pulsed Doppler echocardiography.

Histology

LV tissue was fixed with ice-cold 10% paraformaldehyde for 24 h, embedded in paraffin, sectioned transversely (thickness, 3 μm), and stained with hematoxylin-eosin to evaluate cardiomyocyte hypertrophy. Cross-sectional areas of cardiomyocytes were calculated in 10 randomly chosen microscopic fields from three different sections of LV free wall endocardium in each animal, as previously described (12,13). Image analysis was performed with Image J (14).

8-hydroxy-2'- deoxyguanosine

Plasma 8-hydroxy-2′- deoxyguanosine (8-OHdG), a marker for oxidative stress that reflects 8-hydroxyguanosine in the DNA, was measured using enzyme-linked immunosorbent assay, as previously described. The assay was performed using the highly sensitive ELISA kit for 8-OHdG (JaICA, NIKKEN SEIL Co., Ltd., Shizuoka, Japan) (15).

Statistical analysis

Data are presented as mean ± SEM. Comparisons at 7 and 12 weeks of age were made using one-way analysis of variance (ANOVA) to evaluate interactions among the three groups. Post-hoc tests (Scheffe's test) were conducted to isolate groups with significant differences. $P < 0.05$ was considered statistically significant.

Results

Physiological findings

There were no significant differences in body weight among the three groups at 12 weeks of age. SBP in the 8% NaCl and 8% NaCl + H_2 groups were significantly higher than those in the 0.3% NaCl group at 12 weeks of age. However, there was no significant difference in SBP between the 8% NaCl and 8% NaCl + H_2 groups (Table 1, Figure 1).

Echocardiographic findings

No significant differences were observed in echocardiographic parameters among any of the groups at 7 weeks of age. IVST, LVPWT, and LV mass were significantly higher in the 8% NaCl group than the 0.3% NaCl group at 12 weeks of age (IVST; 2.32 ± 0.22 vs 1.57 ± 0.08 mm, $P < 0.05$, LVPWT; 2.27 ± 0.16 vs 1.60 ± 0.13 mm, $P < 0.05$, LV mass; 1.56 ± 0.04 vs 1.22 ± 0.02 g, $P < 0.05$). IVST, LVPWT, and LV mass were significantly lower in the 8% NaCl + H_2 group than the 8% NaCl group at 12 weeks of age (IVST; 1.96 ± 0.27 vs 2.32 ± 0.22 mm, $P < 0.05$, LVPWT; 2.02 ± 0.22 vs 2.27 ± 0.16 mm, $P < 0.05$, LV mass; 1.40 ± 0.06 vs 1.56 ± 0.04 g, $P < 0.05$), although these parameters were significantly higher in the 8% NaCl + H_2 group than the 0.3% NaCl group (IVST; 1.96 ± 0.27 vs 1.57 ± 0.08 mm, $P < 0.05$, LVPWT; 2.02 ± 0.22 vs 1.60 ± 0.13 mm, $P < 0.05$, LV mass; 1.40 ± 0.06 vs 1.22 ± 0.02 g, $P < 0.05$). LVEDV, LVESV, LVEF, %LVFS, E, and Dct did not differ among the three groups at 12 weeks of age (Table 2).

Table 1. Hemodynamic measurements at 12 weeks of age.

	0.3% NaCl group n = 10	8% NaCl group n = 10	8% NaCl+ 2% H_2 group n = 10
SBP (mmHg)	122.3 ± 2.5	199.4 ± 11.1*	183.8 ± 15.3*
HR (bpm)	391.1 ± 22.9	452.2 ± 38.6	429.5 ± 34.5
Weight (g)	371.1 ± 14.7	352.5 ± 22.2	338.4 ± 8.7

SBP, systolic blood pressure; HR, heart rate; H_2, hydrogen. Data are presented as mean±SEM. * $P < 0.05$ vs. Control group.

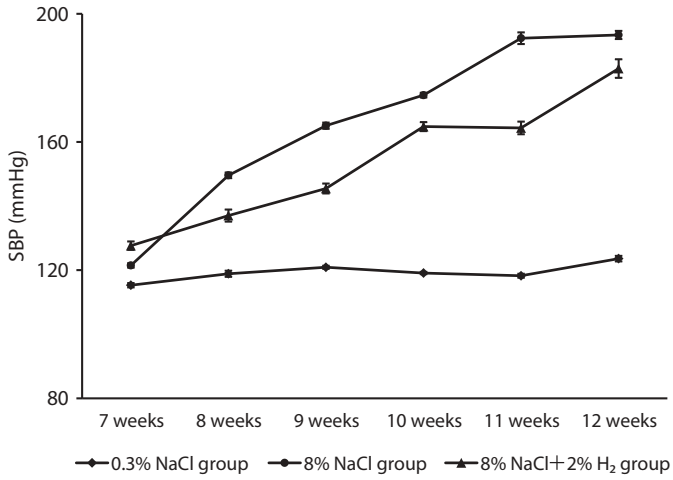
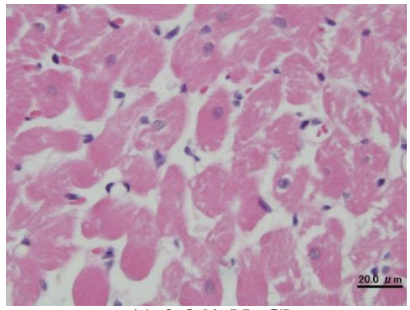


Figure 1. Changes in systolic blood pressure from 0.3%NaCl, 8% NaCl, and 8% NaCl+ + 2% H_2 groups.
Data are presented as means ± SEM.

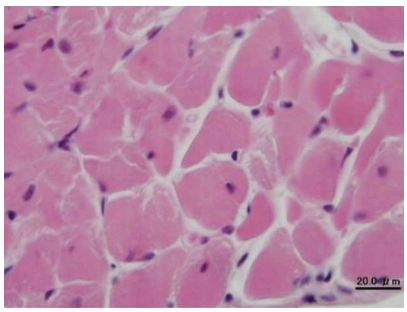
Table 2. Echocardiographic parameters at 12 weeks of age.

	0.3% NaCl group n = 10	8% NaCl group n = 10	8% NaCl+2% H_2 groupn = 10
IVST (mm)	1.57 ± 0.08	2.32 ± 0.22*	1.96 ± 0.27*†
LVPWT (mm)	1.60 ± 0.13	2.27 ± 0.16*	2.02 ± 0.22*†
LVDd (mm)	7.12 ± 0.11	6.76 ± 0.11	6.43 ± 0.23
LVDs (mm)	3.69 ± 0.14	3.10 ± 0.15	3.04 ± 0.28
LV mass (g)	1.22 ± 0.02	1.56 ± 0.04*	1.40 ± 0.06*†
LVEF (%)	84.2 ± 1.1	88.4 ± 1.2	87.0 ± 2.5
LVFS (%)	48.4 ± 1.3	54.4 ± 1.6	53.1 ± 3.1
E (cm/sec)	78.7 ± 3.3	85.7 ± 5.1	84.7 ± 3.6
DcT (sec)	0.032 ± 0.0020	0.030 ± 0.0013	0.031 ± 0.0027
HR (bpm)	356.8 ± 9.9	382.8 ± 11.7	374.9 ± 16.1

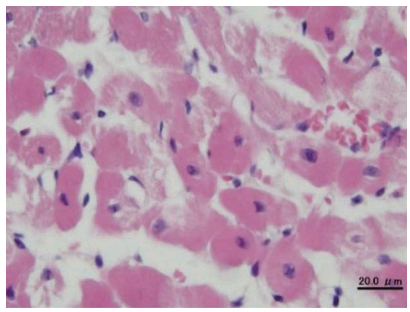
IVST, interventricular septal wall thickness; LVPWT, left ventricular posterior wall thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; E, peak E-wave velocity of mitral inflow; DcT, deceleration time of E; HR, heart rate; H_2, hydrogen. Data are presented as mean±SEM. * $P < 0.05$ vs. Control group and † $P < 0.05$ vs. 8% NaCl group



A) 0.3% NaCl



B) 8% NaCl



C) 8% NaCl+2% H_2

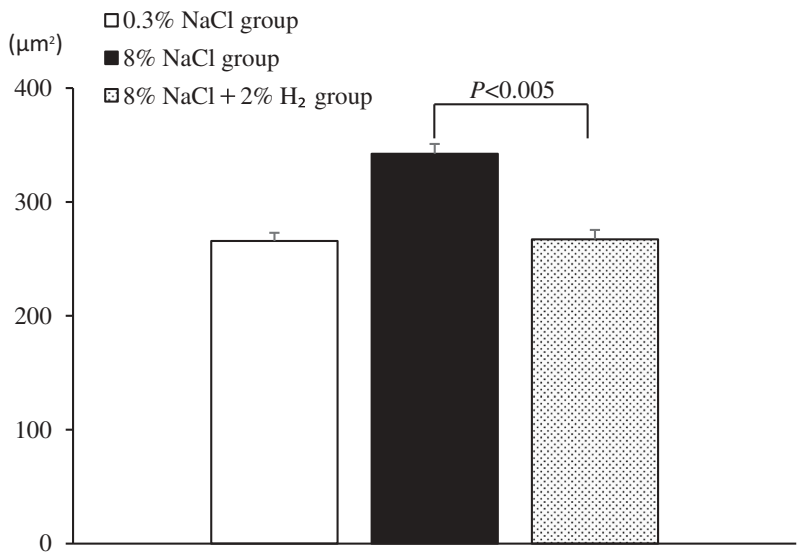


Figure 2. Effects of H_2 gas on hypertrophy of cardiac myocytes.

Micrographs of the left ventricle stained with hematoxylin-eosin in the (A) 0.3%NaCl, (B) 8% NaCl, and C) 8% NaCl□2% H_2 groups. Magnification, × 200. The extent of interstitial fibrosis. Data are presented as means ± SEM.

Histology

Microscopic analysis revealed that cardiac myocytes in the 8% NaCl group had a significantly larger cross-sectional area than that in the 0.3% NaCl group (358.7 ± 8.2 vs 263.9 ± 8.9 μm^2, $P < 0.001$), and hypertrophy of cardiac myocytes in the 8% NaCl + H_2 group was significantly suppressed than that in the 8% NaCl group at 12 weeks of age (267.1 ± 8.3 vs 358.7 ± 8.2 μm^2, $P < 0.001$) (Figure 2).

8-ohdg

8-OHdG was significantly lower in the 8%NaCl + H_2 group than the 8% NaCl group (0.092 ± 0.012 vs 0.155 ± 0.018 pg/ml, $P < 0.05$) though there was no significant difference between the 8% NaCl + H_2 and 0.3% NaCl groups (Figure 3).

Discussion

We demonstrated that chronic H_2 gas inhalation markedly prevented LV hypertrophy induced by the high-salt diet without a significant decrease in SBP. H_2 gas inhalation also suppressed oxidative stress, suggesting that H_2 gas may help prevent hypertensive LV hypertrophy in DS rats fed the high-salt diet.

The treatment of DS rats taking the high-salt diet with chronic H_2 gas inhalation improved indices of LV hypertrophy without an antihypertensive effect. 8-OHdG was significantly lower in the 8% NaCl + H_2 group than the 8% NaCl group. An imbalance in superoxide and nitric oxide production may account for reduced vasodilation, which in turn can favor the development of hypertension (16). Several studies have shown that patients with essential hypertension have a decreased antioxidant capacity (17) and increased ROS production (18). Increased ROS is also associated with LV hypertrophy, and this correlated with overexpression of NADPH oxidase (NOX) 2 in Angiotensin II-induced LV hypertrophy, and in pressure overload LV hypertrophy (19). Isoproterenol-induced LV hypertrophy was ameliorated by intraperitoneal injection of the H_2 medium concentration of 0.6–0.9 ppm, and this protective effect was mediated by direct interruption of NADPH oxidase expression and alleviating mitochondrial damage (20). Our results indicated that H_2 gas might inhibit LV hypertrophy lead by salt-induced hypertension with anti-oxidative stress.

Excess ROS production under pathophysiological conditions executes the detrimental effects on myocytes via mitochondrial dysfunction and bioenergetic decline (21). The chronic exposure of myocytes to ROS leads to the impairment of excitation–contraction coupling, contributing to cardiac remodeling by inducing cardiac hypertrophy, apoptosis, necrosis, and fibrosis (21,22). Treatment with hydrogen-rich saline attenuated LV hypertrophy without significant BP suppression in spontaneously hypertensive rats; moreover, it abated oxidative stress through upregulating activities of anti-oxidant enzymes and suppressing NADPH oxidase activity and mitochondrial ROS formation (23). H_2 gas inhalation suppressed LV hypertrophy and showed an anti-oxidative effect in salt-induced hypertensive rats of this study. Anti-oxidative stress effect of H_2 gas may be associated with direct cardioprotection rather than through the anti-hypertension effect.

H_2 is a specific scavenger of ·OH and ONOO-, which are very strong oxidants that react indiscriminately with nucleic acids, lipids, and proteins, resulting in DNA fragmentation, lipid peroxidation, and protein inactivation. H_2 administration decreases expression of various oxidative stress markers, such as myeloperoxidase, malondialdehyde, 8-OHdG, 8-iso-prostaglandin F2a, and thiobarbituric acid reactive substances in all human diseases and rodent models (24). H_2 can modulate signal transduction across multiple pathways, the effects can be mediated by modulating activities and expressions of various molecules such as Lyn, ERK, p38, JNK, ASK1, Akt, GTP-Rac1, iNOS, Nox1, NF-κB p65, IκBα, STAT3, NFATc1, c-Fos, and ghrelin (25). However, the exact molecular mechanisms of the effects of H_2 remain unclear.

The fixed concentration of 2% H_2 gas was administered during the experiment period. It is unclear that the dose-dependent effect of H_2 gas on oxidative stress is exert. Moreover, hydrogen sulfide, one of the anti-oxidants, postponed the transition from prehypertension to hypertension in the spontaneously hypertensive rat (26). Thus, additional experiments could be needed to confirm the effects of H_2 gas on SBP, LV hypertrophy, and oxidative stress.

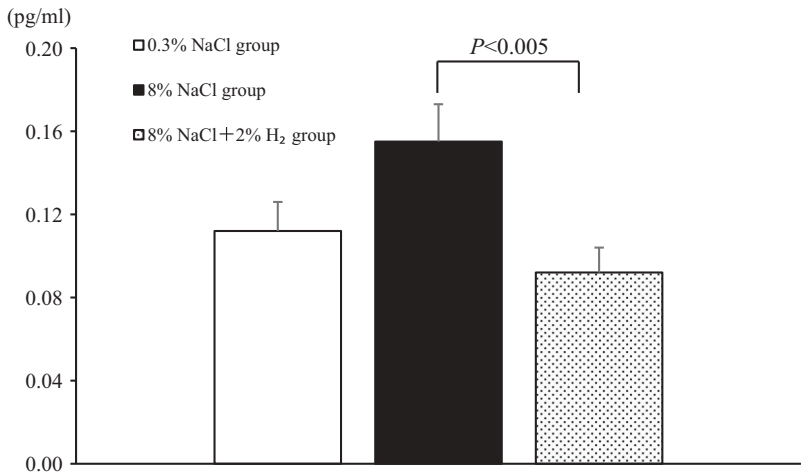


Figure 3. Comparing of 8-OHdG at 12 weeks of age.
Data are presented as means ± SEM.

In conclusion, chronic H_2 gas inhalation ameliorated the LV hypertrophy in DS rats fed the high-salt diet although it did not decrease BP. The beneficial effect of H_2 gas was associated with a decrease in oxidative stress.

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Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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