Inhaled Hydrogen Sulfide

A Rapidly Reversible Inhibitor of Cardiac and Metabolic Function in the Mouse

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Background: Breathing hydrogen sulfide (H₂S) has been reported to induce a suspended animation–like state with hypothermia and a concomitant metabolic reduction in rodents. However, the impact of H₂S breathing on cardiovascular function remains incompletely understood. In this study, the authors investigated the cardiovascular and metabolic effects of inhaled H₂S in a murine model.

Methods: The impact of breathing H₂S on cardiovascular function was examined using telemetry and echocardiography in awake mice. The effects of breathing H₂S on carbon dioxide production and oxygen consumption were measured at room temperature and in a warmed environment.

Results: Breathing H₂S at 80 parts per million by volume at 27°C ambient temperature for 6 h markedly reduced heart rate, core body temperature, respiratory rate, and physical activity, whereas blood pressure remained unchanged. Echocardiography demonstrated that H₂S exposure decreased both heart rate and cardiac output but preserved stroke volume. Breathing H₂S for 6 h at 35°C ambient temperature (to prevent hypothermia) decreased heart rate, physical activity, respiratory rate, and cardiac output without altering stroke volume or body temperature. H₂S breathing seems to induce bradycardia by depressing sinus node activity. Breathing H₂S for 30 min decreased whole body oxygen consumption and carbon dioxide production at either 27° or 35°C ambient temperature. Both parameters returned to baseline levels within 10 min after the cessation of H₂S breathing.

Conclusions: Inhalation of H₂S at either 27° or 35°C reversibly depresses cardiovascular function without changing blood pressure in mice. Breathing H₂S also induces a rapidly reversible reduction of metabolic rate at either body temperature.

HYDROGEN sulfide (H₂S) is a colorless gas with a characteristic rotten-egg odor and is found in volcanic gas emissions, sulfur springs, bacterial decomposition of proteins, and various sulfur-containing products.¹–³

The cellular toxicity of H₂S is attributed to its capacity to inhibit cytochrome c oxidase, the terminal enzyme of oxidative phosphorylation, resulting in cellular hypoxia.¹,² The cellular toxicity of H₂S is attributed to its capacity to inhibit cytochrome c oxidase, the terminal enzyme of oxidative phosphorylation, resulting in cellular hypoxia.¹,² In the past, the effects of H₂S inhalation have been extensively studied as an environmental pollutant.¹,⁴

Blackstone et al.⁶ reported that breathing H₂S at 80 parts per million by volume (ppm) decreases both body temperature and metabolic rate, inducing a “suspended animation–like state” in mice. Furthermore, the same group recently reported that preinhalation of H₂S improved the survival rate of mice exposed to acute hypoxia.⁷ Although these observations suggest an exciting possibility that the metabolic depressant effects of H₂S may be exploitable for organ protection,⁶ the effects of H₂S inhalation on cardiovascular function remain largely unknown. Further, whether H₂S breathing reduces metabolism independently of changes in core body temperature (Tb) is incompletely understood.

In the current study, we examined the impact of H₂S inhalation on cardiovascular function using telemetry and echocardiography in conscious mice. We also evaluated the impact of differing ambient temperatures on the metabolic inhibition caused by H₂S breathing in mice. We report that H₂S breathing decreases metabolism and cardiovascular function in mice independent of Tb.

Materials and Methods

Animals

This study was approved by the Subcommittee on Research Animal Care at the Massachusetts General Hospital, Boston, Massachusetts. We used 12 male C57BL/6 WT mice (Jackson Laboratories, Bar Harbor, ME) for radiotelemetric experiments and 7 male SV129 mice (Taconic Inc., Cambridge, MA) for the echocardiographic studies. The animals were maintained individually in polycarbonate mouse cages on a 12/12-h light–dark schedule at 27° ± 1°C ambient temperature (Tb). Mice had free access to food and water, except during exposures to study gas.

Telemetric Measurements

Core Body Temperature. To record Tb and activity, radiotelemetry devices (model TA10TA-F20; DSI, St. Paul, MN) were surgically implanted in the peritoneal cavity of four mice. During surgery, the animals were anesthetized with ketamine (0.1 mg/g) and xylazine (0.01 mg/g), and they were allowed to recover for a minimum of 2 weeks before commencing experiments.

Heart Rate and Mean Arterial Pressure. Radiotelemetry devices (model TA111PA-C10; DSI) equipped with a miniature intraarterial catheter were surgically implanted in the ventral subcutaneous space with a catheter (0.4 mm OD) inserted in the left carotid artery.

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of four mice to record heart rate (HR), mean arterial pressure (MAP), and physical activity.

**Electrocardiogram.** To record the electrocardiogram during H₂S breathing, radiotelemetry devices (model TA10ETA-F20; DSI) were surgically implanted in the peritoneal cavity of three mice. These devices are equipped with two electrocardiographic leads that were implanted within muscle in a lead II configuration (positive lead 1 cm to the left of the xiphoid process, and negative lead in the right pectoral muscle).

**Physical Activity.** Activity was measured in mice with the telemeter based on the signal strength recorded by the receiver (using Dataquest ART 4.0 software; DSI). The signal strength depends on the orientation and the distance of the animal from the receiving antenna. As the mouse moves in its cage or chamber, the relative change in signal strength is converted into activity units. The number of counts or units generated depends on both the distance and the speed of movement.

**Respiratory Rate.** Respiratory rate (RR) was counted manually and recorded at 30-min intervals during the experiments.

**Experimental Protocol**

**Telemetric Hemodynamic Measurements in Awake Mice.** Each mouse was placed in its cage to breathe air for 1 h to record the baseline values of HR, MAP, and RR. To minimize the effects of agitation due to handling and obtain stable hemodynamics, the baseline values were measured while the mice remained in their cages with the telemetric device turned on. After 1 h of recording baseline values in individual cages, each mouse was placed in a 500-ml metabolic chamber (model PLY 3211 V2.1; Buxco Electronics, Inc., Wilmington, NC) in which the mouse breathed air supplied at a rate of 1 l/min at either 27° or 35°C Ta. After 1 h of breathing air in the metabolic chamber, each mouse was exposed to an atmosphere containing 1,000 ppm H₂S in nitrogen (Airgas Inc., Radnor, PA), which was mixed with compressed air using a three-tube volumetrically calibrated flowmeter (Cole-Parmer, Vernon Hills, IL), to deliver a final concentration of 80 ppm H₂S and 17.5% oxygen (total gas flow delivered at 1 l/min). H₂S and oxygen concentrations were continuously measured using a portable gas monitor (ITX Multi-Gas Monitor; Industrial Scientific Corporation, Oakdale, PA). Mice were exposed to this atmosphere continuously for 6 h and then were replaced in their cages to breathe air at 27°C Ta for 3 h. Ta, MAP, HR, and physical activity were measured for 10-s intervals during each minute of the experiment and recorded using Dataquest ART 4.0 software on a computer.

**Echocardiography.** To study the effects of H₂S breathing on cardiac function, echocardiography was performed on seven awake SV129 mice. Echocardiography was first performed in mice breathing air at Ta 27°C. Body temperature was measured with a rectal probe (FHC, Bowdoin, ME). One week later, echocardiography was repeated after the mice breathed H₂S (80 ppm) for 3 h in a chamber at Ta 23°C (n = 6). Mice recovered for another week, and echocardiography was performed again after H₂S exposure at Ta 35°C (n = 5). The mice were kept inside the chamber, breathing H₂S throughout the procedure. Echocardiography was performed using a 14-MHz probe (Vivid 7; GE Medical Systems, Milwaukee, WI) in conscious mice. Three to four M-mode acquisitions were obtained at the midpapillary level of a parasternal short axis view as previously described.⁵ Left ventricular internal diameter at end-diastole and left ventricular internal diameter at end-systole were measured (All measures were averaged on a minimum of 5 consecutive beats). HR, shortening fraction, and left ventricular volumes were calculated⁶; stroke volume (SV) and cardiac output (CO) were derived.

**Telemetric Recording of Electrocardiogram.** Mice were placed in individual chambers (25 cm long and 5.0 cm in diameter). Animals breathed air (1 l/min total gas flow) for 1 h and then H₂S (80 ppm) for 3 h at Ta 27°C, followed by a recovery period of 2 h breathing air. A gas sample was continuously withdrawn from the expiratory limb of the circuit to measure H₂S concentration, and the electrocardiographic tracing was recorded continuously.

**Blood Sampling.** To explore the possible influence of blood gas tensions or electrolyte abnormalities on cardiovascular function, C57BL/6 mice (n = 7) were exposed to room air or H₂S (80 ppm) for 2 h, as described above. Each chamber was fitted with a rubber stopper at one end, through which the mouse’s tail protruded. After 2 h of breathing either air or H₂S, an arterial blood sample was obtained from the tail artery for analysis of arterial partial pressure of carbon dioxide, arterial partial pressure of oxygen, arterial pH, arterial oxygen saturation, Na⁺, K⁺, Ca²⁺, Mg²⁺, glucose, blood urea nitrogen, and lactic acid levels using a Stat Profile Critical Care Xpress machine (Nova Biomedical, Waltham, MA).

**Measurements of Carbon Dioxide Production.** To examine the impact of H₂S breathing on carbon dioxide production, C57BL/6 mice (n = 3) implanted with temperature telemeters were exposed to breathing air at either 27° or 35°C Ta for 1 h, as described above. Mice were then exposed for 30 min to H₂S (80 ppm at 27°C Ta and 40, 80, or 120 ppm at 35°C Ta), after which they breathed air without H₂S for a 30-min recovery period at the same Ta. Carbon dioxide production was measured in the effluent chamber gas airflow once each minute using an infrared carbon dioxide analyzer (LI-820 CO₂ Gas Analyzer; LI-COR Biosciences, Lincoln, NE).

**Simultaneous Measurements of Carbon Dioxide Production Rate and Heart Rate.** To study the relation between carbon dioxide production and HR during H₂S inhalation, C57BL/6 mice (n = 3) implanted with radiotelemetry devices (model TA11PA-C10; DSI) were first...
exposed to air at 27°C T\(_a\) for 1 h as described above. The animals were then exposed for 30 min to H\(_2\)S (80 ppm), followed by a 30-min recovery period breathing air at the same T\(_a\). Carbon dioxide production and HR were recorded every minute using the same methods described above.

**Measurements of Oxygen Consumption Rate.** C57BL/6 mice (n = 3) implanted with temperature tele­meters were studied at either 27° or 35°C T\(_a\) while breathing air for 1 h as described above, then breathing air supplemented with H\(_2\)S (80 ppm) for 30 min, and finally during a 30-min recovery period breathing air without added H\(_2\)S. The oxygen concentration in the chamber was assayed every 5 min, using a PA-10a oxygen analyzer (Sable Systems, Las Vegas, NV). Oxygen consumption (μL O\(_2\) · g\(^{-1}\) · min\(^{-1}\)) was calculated by open circuit respirometry (flow rate 0.5 l/min). The respiratory quotient was calculated as the ratio of carbon dioxide produced/oxygen consumed during H\(_2\)S breathing in mice at 35°C T\(_a\).

**Analgesic Effects of Inhaled H\(_2\)S.** To determine whether inhaled H\(_2\)S had analgesic effects, we examined the effects of H\(_2\)S breathing on the response of mice to noxious stimuli as described previously.\(^{10}\) Mice were placed in individual chambers (25 cm long and 5.0 cm in diameter). Each chamber was fitted with a rubber stopper at one end through which the mouse’s tail and a rectal temperature probe protruded. Groups of six mice breathed H\(_2\)S in oxygen (1 l/min total gas flow). A gas sample was continuously drawn from the expira­tory limb of the circuit to measure H\(_2\)S concentration. The rectal temperature of the mice was maintained between 36.5° and 38.5°C using a heat lamp. Rectal temperature probe protruded. Each chamber was fitted with a rubber stopper at one end through which the mouse’s tail and a rectal temperature probe protruded. Groups of six mice breathed H\(_2\)S in oxygen (1 l/min total gas flow). A gas sample was continuously drawn from the expira­tory limb of the circuit to measure H\(_2\)S concentration. The rectal temperature of the mice was maintained between 36.5° and 38.5°C using a heat lamp. Each chamber was fitted with a rubber stop­per at one end through which the mouse’s tail and a rectal tempera­ture probe protruded. Groups of six mice breathed H\(_2\)S in oxygen (1 l/min total gas flow). A gas sample was continuously drawn from the expira­tory limb of the circuit to measure H\(_2\)S concentration. The rectal temperature of the mice was maintained between 36.5° and 38.5°C using a heat lamp.

**Measurements of Plasma H\(_2\)S in Mice.** Plasma H\(_2\)S concentration was measured using a sulfide-sensitive Ag\(_2\)S electrode (model 9616 BNPW; Orion Research, Beverly, MA) as described previously.\(^{11–13}\) In plasma, approximately one third of H\(_2\)S molecules persist as the undisso­ciated form (H\(_2\)S) and the remaining two thirds as HS\(^-\) at equilibrium with H\(_2\)S.\(^3\) The Ag\(_2\)S electrode measures the combined concentrations of both H\(_2\)S and HS\(^-\).\(^{14}\) The electrode was calibrated against a standard solution produced by dissolving Na\(_2\)S in distilled, deox­ygenated water before each measurement. Fifteen C57BL/6 male mice were anesthetized using ketamine (0.1 mg/g) and xylazine (0.01 mg/g). Mice were intubated via the trachea, and volume-controlled ventilation (ventilator model 687; Harvard Apparatus, Holliston, MA) was initiated at an RR of 120 breaths/min at an inspired oxygen fraction of 1.0 for the control group (n = 5). A second group of ventilated mice breathed 80 ppm H\(_2\)S in oxygen (n = 4), and a third group of ventilated mice was exposed to 200 ppm H\(_2\)S in oxygen (n = 6). After breathing H\(_2\)S for 30 min, blood samples were collected with a heparinized syringe via an apical cardiac puncture. Samples were centrifuged for 3 min at 8,000 rpm. The plasma samples were collected and diluted at a 1:1 ratio with a previously prepared solution of sulfide antioxidant buffer (SAOB II; Fisher Scientific, Pittsburgh, PA), according to the manufacturer’s instructions.

**Statistical Analysis**

Statistical evaluations of multiple group comparisons (echocardiography data analysis) were performed with one­way analysis of variance using a Tukey post hoc test. A two­way analysis of variance was used to compare data from mice breathing air or H\(_2\)S at 27° or 35°C T\(_a\). Inter­actions among variables were studied, and a Holm-Sidak analysis was used as a post hoc test. If, during the statistical analysis of our data, the parametric assumption was violated (i.e., normality test failed), a two­way analysis of variance on ranks with a Holm-Sidak post hoc test was used. Data are presented as mean ± SE. Differences were considered significant if P was less than 0.05.

**Results**

**H\(_2\)S Breathing Reduced Core Body Temperature**

Before exposure to H\(_2\)S at 27°C T\(_a\), the average T\(_b\) was 37.4°± 0.4°C. Initiation of 80 ppm H\(_2\)S inhalation led to a progressive decrease in T\(_b\) reaching 33.8° ± 1.9°C after 2 h at T\(_a\) 27°C (fig. 1). After 6 h at H\(_2\)S breathing, T\(_b\) reached a steady state of 29.3° ± 0.6°C (P < 0.05 compared with baseline). When H\(_2\)S administration was discontinued, T\(_b\) recovered to 35.1° ± 0.2°C after 3 h. In contrast, breathing H\(_2\)S at T\(_a\) 35°C did not alter T\(_b\) after 6 h of H\(_2\)S breathing.

**Impact of H\(_2\)S Breathing on Heart Rate and Mean Arterial Pressure**

In mice breathing 80 ppm H\(_2\)S at T\(_a\) 27°C, HR decreased by nearly 50% within 2 h (511 ± 13 beats/min to 265 ± 13 beats/min; P < 0.05; fig. 2A) and remained low as long as mice breathed H\(_2\)S (measured up to 6 h). In addition, the cardiac rhythm became irregular, an observation that was confirmed by electrocardiogram. After H\(_2\)S was discontinued, HR increased to 433 ± 34 beats/ min after 2 h. Despite the marked reduction of HR, MAP did not change significantly from baseline during H\(_2\)S inhalation (fig. 2B).
At a $T_a$ of 35°C, breathing $H_2S$ reduced the HR approximately 50% after 2 h of exposure (498 ± 24 beats/min to 242 ± 13 beats/min; $P < 0.05$; fig. 2A), and the bradycardia persisted as long as mice breathed $H_2S$. The effect of $H_2S$ breathing on HR did not differ between mice maintained at 27° or 35°C. MAP increased modestly during $H_2S$ breathing at 35°C after 6 h and was consistently greater in mice breathing $H_2S$ at 35°C than in mice breathing $H_2S$ at 27°C ($P < 0.05$; fig. 2B).

**Electrocardiogram**

We recorded the electrocardiogram to better characterize the slow and irregular HR observed during $H_2S$ inhalation. Breathing $H_2S$ induced a sinus bradycardia with sinus arrest, typically starting after 1 h of $H_2S$ inhalation and lasting until $H_2S$ administration was terminated (fig. 3). After 5 min of breathing air, a rapid recovery to a regular and slow sinus rhythm (HR approximately 390 beats/min) had occurred. A complete recovery of the electrocardiographic tracing was seen after 2 h of breathing air (fig. 3).

**Echocardiography**

An echocardiographic analysis of mice breathing $H_2S$ at 23°C $T_a$ confirmed that 80 ppm $H_2S$ inhalation decreased HR from 690 ± 19 beats/min to 233 ± 23 beats/min ($P < 0.05$; fig. 4A). A slow and irregular HR was noted during $H_2S$ breathing, consistent with the observations we obtained with telemetry. $H_2S$ breathing decreased CO by 60% ($P < 0.05$; fig. 4B), although SV remained unaffected (fig. 4C). After breathing $H_2S$ for 3 h, $T_b$ was decreased markedly (35.9° ± 0.2°C to 26.5° ± 0.2°C; $P < 0.05$). When mice breathed $H_2S$ at a $T_a$ of 35°C, HR also decreased (690 ± 19 beats/min to 340 ± 16 beats/min; $P < 0.05$; fig. 4A). $H_2S$ breathing at 35°C $T_a$ also decreased CO as compared with baseline CO values ($P < 0.05$; fig. 4B), whereas SV was not affected (fig. 4C). $T_b$ remained stable during the 3-h period while mice breathed $H_2S$ at 35°C $T_a$.
creased, and after 3 h, mice became inactive and appeared asleep (fig. 5). Interestingly, these mice were able to move when stimulated by tapping on the chamber. When these animals subsequently breathed air without H₂S, they regained baseline activity levels within 1 h. Similar reversible effects of H₂S breathing on the activity level were observed when we repeated this experiment at 35°C Ta (fig. 5).

**H₂S Breathing Decreased Respiratory Rate**

Breathing 80 ppm H₂S at 27°C Ta decreased RR, reaching a maximal effect after 3 h of inhalation (RR 31 ± 4 breaths/min; P < 0.05). RR remained low as long as mice breathed H₂S, rapidly returning to baseline after H₂S gas breathing was discontinued. Mice regained a baseline level of RR after 2 h breathing air (115 ± 3 breaths/min). RR was also decreased in a group of mice that breathed H₂S at the higher Ta (35°C). After 120 min of H₂S breathing at 35°C Ta, the RR was significantly greater than that measured at 27°C Ta (P < 0.05; fig. 6).

**Blood Gas Tension and Electrolyte Analysis**

We explored the possibility that changes in blood gas tensions or electrolyte concentrations could be responsible for the cardiovascular abnormalities observed in mice breathing H₂S. We found that breathing H₂S at 80 ppm for 2 h did not change either arterial blood gas tensions or electrolyte concentrations in mice (table 1).

**Metabolic Response to H₂S Breathing**

In mice breathing 80 ppm to H₂S at Ta 27°C, the carbon dioxide production rate was reduced by 46% after 10 min of H₂S inhalation (P < 0.05; fig. 7A) and remained at that level during the 30-min exposure to H₂S. This reduction in carbon dioxide production was followed by a decrease in Tb of 3.4°C (39°C ± 1°C to 35°C ± 1°C; P < 0.05; fig. 7A). After breathing air for 15 min, the carbon dioxide production rate returned to baseline levels (49.8 ± 7 μL/min), and Tb returned to normal levels.

To separate the metabolic effects of H₂S inhalation from the reduction of Tb, experiments were conducted at Ta 35°C to maintain Tb unchanged during H₂S breathing. Breathing 80 ppm H₂S at Ta 35°C decreased the whole body carbon dioxide production rate by 18% after 10 min (from 60 ± 1 μL/min to 49 ± 1 μL/min; P < 0.05) and by 23% after 30 min of exposure (fig. 7B), whereas Tb did not change during the experimental period.

**H₂S Reduces Carbon Dioxide Production Rate before Affecting Heart Rate**

When mice breathed H₂S (80 ppm) at Ta 27°C, carbon dioxide production was reduced by approximately 35% after 2 min of inhalation (P < 0.05). The effect of H₂S breathing on carbon dioxide production persisted during the 30-min exposure to H₂S (fig. 8). Interestingly, during the initial 30 min of H₂S breathing, HR did not change. When H₂S was stopped after 30 min of inhala-
tion and mice were allowed to breathe air, HR was greater than the baseline for the first 20 min and returned to the baseline level thereafter \((P < 0.05; \text{fig. 8})\). HR and carbon dioxide production rate returned to their baseline levels after 30 min of air breathing recovery.

**Dose-Response Relation between H\(_2\)S Concentration and Carbon Dioxide Production**

To more completely characterize the effects of H\(_2\)S inhalation on metabolism, carbon dioxide production was measured in mice that breathed 40, 80, or 120 ppm H\(_2\)S for 30 min at a \(T_a\) of 23°C (fig. 9). Breathing 40 ppm H\(_2\)S did not decrease carbon dioxide production after 30 min, but breathing 80 and 120 ppm H\(_2\)S markedly reduced carbon dioxide production. When H\(_2\)S breathing ceased, carbon dioxide production returned to baseline levels within 5 min at all three H\(_2\)S concentrations.

**H\(_2\)S Reduces Oxygen Consumption Rate**

Breathing H\(_2\)S (80 ppm) at \(T_a\) 27°C reduced the oxygen consumption rate by 30% after 10 min \((25.8 \pm 3 \text{ vs. } 18.1 \pm 1 \mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}; P < 0.05; \text{fig. 10})\), and this reduction persisted during the 30-min exposure to H\(_2\)S. This reduction in oxygen consumption was followed by a decrease in \(T_b\) of 1.9°C \((37° \pm 0.4°C \text{ to } 35.1° \pm 0.5°C; P = \text{not significant})\). Within 10 min of discontinuing H\(_2\)S, the oxygen consumption rate had increased, to a level higher than the baseline. Oxygen consumption returned to baseline levels after 30 min of breathing air (fig. 10).

When oxygen consumption was measured at \(T_a\) 35°C, H\(_2\)S breathing reduced the oxygen consumption rate within 20 min, reaching a 25% reduction after 30 min of exposure to the gas \((26.4 \pm 1 \text{ vs. } 20.6 \pm 1 \mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}; P < 0.05)\). \(T_b\) did not change during the exposure period \((37.4° \pm 0.1°C \text{ at baseline to } 37.1° \pm 0.2°C \text{ before treatment}; P = \text{not significant})\). When H\(_2\)S breath-
ing was discontinued, the oxygen consumption rate recovered to baseline levels within 10 min (fig. 10).

The calculated respiratory quotient of mice breathing air at Ta 35°C was compared with their respiratory quotient after 30 min of exposure to 80 ppm H2S. We found that breathing H2S at 80 ppm did not significantly alter respiratory quotient (from 1.00 ± 0.01 at baseline to 1.04 ± 0.04 at the end of the 30-min exposure to H2S at 80 ppm).

**Inhaled H2S (60–160 ppm) Does Not Produce an Analgesic Effect in Mice**

Although mice breathing H2S at concentrations from 60 to 160 ppm seemed less active, they moved when their tail was noxiously stimulated by tail clamping, suggesting a lack of significant analgesic effects of H2S breathing at these inhaled concentrations. We did not examine the analgesic effects of higher concentrations of H2S because all of the mice we studied died with marked respiratory depression when exposed to higher concentrations of inhaled H2S (data not shown).

**Impact of H2S Breathing on Plasma Sulfide Concentration in Mice**

The baseline plasma sulfide concentration in C57BL/6 mice was measured at 23.8 ± 0.2 μM. Breathing 80 ppm H2S for 30 min did not alter plasma sulfide levels (23.5 ±

### Table 1. Whole Blood Analyses in Mice Breathing Air or H2S (80 ppm)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Air</th>
<th>H2S, 80 ppm</th>
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<td>PaCO2, mmHg</td>
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<td>27 ± 1</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>112 ± 25</td>
<td>96 ± 3</td>
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<td>SaO2, %</td>
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<td>96 ± 3</td>
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<td>Na+, mm</td>
<td>143 ± 4</td>
<td>144 ± 3</td>
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<tr>
<td>K+, mm</td>
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<tr>
<td>Ca2+, mm</td>
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<td>1.2 ± 0.08</td>
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<tr>
<td>Mg2+, mm</td>
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<td>0.3 ± 0.1</td>
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</tr>
<tr>
<td>Glucose, mg/dl</td>
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<td>202 ± 20</td>
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</table>

Whole blood gas and chemical analysis values of mice breathing air or hydrogen sulfide (H2S; 80 ppm) for 2 h. Data are presented as mean ± SD. n = 3 in the air group and n = 4 in the H2S group.

BUN = blood urea nitrogen; PaCO2 = arterial partial pressure of carbon dioxide; PaO2 = arterial partial pressure of oxygen; SaO2 = arterial oxygen saturation.

**Fig. 7.** Effects of hydrogen sulfide (H2S) inhalation on carbon dioxide (CO2) production (inverted triangles) and core body temperature (diamonds) in mice breathing H2S (80 ppm) for 30 min at ambient temperature (Ta) 27°C (A, closed symbols, n = 3) or Ta 35°C (B, open symbols, n = 3), followed by a 30-min recovery period breathing air at Ta 27°C or 35°C, respectively. *P < 0.05 versus time 0.

**Fig. 8.** Effects of hydrogen sulfide (H2S) inhalation on carbon dioxide (CO2) production (closed inverted triangles) and heart rate (open squares) in mice breathing H2S (80 ppm) for 30 min at ambient temperature (Ta) 27°C, followed by a 30-min recovery period breathing air at Ta 27°C. n = 3, *P < 0.05 versus time 0.

**Fig. 9.** Carbon dioxide (CO2) production response, in mice breathing hydrogen sulfide (H2S) at 40 ppm (open squares), 80 ppm (open circles), or 120 ppm (open triangles) for 30 min at ambient temperature (Ta) 35°C, followed by a 30-min recovery period breathing air at Ta 35°C. n = 3 in each group. *P < 0.05 versus time 0.

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Discussion

The primary aim of the current study was to examine the cardiovascular effects of H$_2$S breathing at a concentration that has been reported to depress metabolism in mice.$^{6}$ In addition, we sought to examine the thermal dependence of the cardiac and metabolic effects of H$_2$S breathing because hypothermia per se affects both hemodynamics and metabolism. We report that HR and CO, but not MAP and SV, were markedly reduced by H$_2$S breathing, primarily due to the occurrence of a sinus bradycardia independent of body temperature. We also found that when the core body temperature is held constant, breathing H$_2$S markedly and reversibly inhibits metabolism in mice. These observations provide evidence that H$_2$S breathing modifies cardiac and metabolic function independent of its effects on core body temperature.

The most striking hemodynamic change associated with H$_2$S breathing was the profound bradycardia in normothermic as well as hypothermic mice. The thermal independence of the bradycardia produced by H$_2$S was also suggested by the observation that the reduction of HR preceded the reduction of $T_b$ of mice breathing H$_2$S at $T_a$ 27°C. This bradycardic effect of H$_2$S breathing was accompanied by a reduction in CO (measured by echo-cardiography) because H$_2$S breathing did not change the SV. On the other hand, H$_2$S-induced bradycardia was preceded by a marked reduction of the carbon dioxide production rate (fig. 8). The latter observation suggests that the inhibitory effects of H$_2$S on metabolism may also cause the reduction of HR and CO.

Because H$_2$S breathing induces a marked and irregular bradycardia without changing the SV, it is tempting to speculate that H$_2$S breathing adversely affects sinus node function but not myocardial contractility per se. To examine this hypothesis, we obtained a more detailed analysis of the irregular slow HR using telemetric electrocardiographic recordings. They revealed a profound sinus bradycardia with periods of sinus arrest. Although the precise mechanism responsible for the H$_2$S-induced depression of sinus activity is unknown, it was not caused by abnormalities in blood pH or blood gas tensions or electrolyte concentrations after H$_2$S exposure (table 1). Further studies are needed to elucidate the precise impact of H$_2$S breathing on myocardial function and the cardiac conduction system.

Despite a marked reduction of HR and CO, MAP did not change in mice breathing H$_2$S at $T_a$ 27°C, suggesting that a compensatory systemic vasoconstriction occurred. H$_2$S-induced vasoconstriction is unlikely to be due to hypothermia alone, because MAP was also increased in mice breathing H$_2$S with an unchanged body temperature. Previous studies have shown that H$_2$S can produce a potent vasodilation$^{11,15,16}$ by relaxing smooth muscle cells, presumably by activation of adenosine triphosphate-sensitive K$^+$ channels$^{17,18}$ In contrast, Dombkowski et al.$^{19}$ demonstrated that H$_2$S has both vasodilatory and vasoconstrictory properties. A recent study by Ali et al.$^{20}$ also reported the biphasic effects of NaHS on vascular tone: vasoconstriction at lower concentration due to scavenging of endothelial nitric oxide by H$_2$S, and vasodilation at higher concentrations via activation of adenosine triphosphate-sensitive K$^+$ channels. It is conceivable that H$_2$S breathing at 80 ppm caused vasoconstriction in our current study because the concentration of H$_2$S in peripheral vascular smooth muscle cells was likely to be low. Alternatively, it is also possible that systemic vasoconstriction (and bradycardia) was triggered by cardiovascular reflexes (likely via the aortic baroreceptor) activated in response to reduced CO after H$_2$S-induced metabolic depression (i.e., decreased oxygen consumption).

Mice breathing 80 ppm H$_2$S for 6 h at $T_a$ 27°C decreased their $T_b$ more than 8°C. This reduction of $T_b$ and activity is in accord with the results previously reported by Struve et al.$^{21}$ and Blackstone et al.$^{6}$ in rats and mice, respectively, exposed to H$_2$S. It has been reported that H$_2$S (produced from the donor molecule Na$_2$S) inhibits cytochrome c oxidase activity. $^{22}$ It is possible that mitochondrial thermogenesis may be reduced as a consequence of the inhibition of cytochrome c oxidase in heat-producing tissues (e.g., brown fat), decreasing the ability of mice to generate sufficient heat to maintain their body temperature. Our observation that H$_2$S-induced decrements of both carbon dioxide production and oxygen consumption preceded the reduction in $T_b$
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when mice breathed H₂S at Tₐ 27°C suggests that inhibition of metabolism is primary and reduction in Tₐ is secondary. This hypothesis is further supported by the observation that breathing H₂S at Tₐ 35°C markedly reduced carbon dioxide production and oxygen consumption without decreasing Tₚ.

Inhalation of 80 ppm H₂S also decreased the RR in mice exposed to the gas at 27°C or 35°C, whereas the magnitude of the reduction was more marked at a lower Tₚ. It has been suggested that H₂S may selectively affect the brain stem and inhibit respiratory drive. The inhibitory effects of H₂S on brain stem function may be related to its inhibitory effects on cytochrome c oxidase and mitochondrial adenosine triphosphate production in the brain. Interestingly many organisms respond to adverse environmental conditions by reducing their RR. Although the mechanisms responsible for the reduction in RR in adverse conditions are incompletely understood, this response is usually correlated with a decreased metabolic rate and changes of the intrinsic properties of the respiratory rhythm generator in the brain. On the other hand, a previous study showed that inhalation of H₂S up to 400 ppm for 3 h did not change sulfide concentration or cytochrome c oxidase activity in the hindbrain of rats, whereas sulfide concentrations increased and cytochrome c oxidase activity decreased in lung and nasal respiratory epithelium. These results suggest that respiratory inhibition by H₂S inhalation may be partially mediated via effects on chemoreceptors in the lung and upper airways. Alternatively, H₂S-induced decrements in RR may also reflect the reduced need for minute ventilation due to the marked reduction of carbon dioxide production induced by H₂S breathing. The observation that a reduction in carbon dioxide production precedes the decrements of RR supports this hypothesis.

Our observation that H₂S breathing at 80 ppm markedly depressed spontaneous activity in mice is consistent with a previous report by Struve et al. in which rats exposed to higher concentrations of H₂S (e.g., 80–400 ppm) exhibited reduced spontaneous ambulation. Our results do not support a major role for hypothermia in the H₂S-induced reduction in spontaneous activity, because mice breathing H₂S with stable Tₚ also exhibited a marked reduction in spontaneous activity. Furthermore, the reduction in activity of mice breathing H₂S at Tₚ 27°C preceded their reduction in Tₚ. These results suggest that hypothermia is not solely responsible for the H₂S-induced depression of spontaneous activity. Although H₂S can produce a profound dormant state in mice, it does not seem to have profound anesthetic effects in the range of concentrations that we tested (60–160 ppm). Although higher concentrations of inhaled H₂S may have produced analgesia, the higher H₂S concentrations were not tested in our model due to H₂S-induced respiratory depression.

To estimate the amount of systemically absorbed H₂S by H₂S inhalation, we measured plasma sulfide concentration ([H(S)S] + [HS⁻]) in mice. The baseline plasma sulfide levels observed in the current study are comparable with values previously reported in mice (approximately 23 μM) and Sprague-Dawley rats (approximately 46 μM) as well as the levels reported in human plasma (approximately 34 μM). Compared with controls, breathing H₂S at 200 ppm for 30 min increased plasma sulfide levels in mice, demonstrating that inhaled H₂S is systemically absorbed. The absence of an increase in the plasma sulfide concentration after H₂S breathing at 80 ppm may suggest that absorbed H₂S is converted to other sulfur-containing molecules that are not readily measured by the Ag₂S electrode. The major metabolic pathway for H₂S is the rapid multistep hepatic oxidation of sulfide to sulfate (SO₄²⁻) and the subsequent elimination of sulfate in the urine. H₂S may also be methylated (e.g., CH₃SCH₃), or it can react with cytochrome c and other metallo- or disulfide-containing proteins. It is likely that the H₂S that reacts with cytochrome c and other proteins has a larger impact on metabolism than H₂S in plasma. Alternatively, the failure to detect any increase of plasma sulfide concentration after inhaling H₂S at 80 ppm for 30 min may be related to the limitation of our experimental procedures. Although care was taken to avoid the escape of H₂S from blood during our sulfide measurements, it is possible that some H₂S was lost during sample collection and processing (approximately 10 min elapsed after mice were killed for sampling and measurements).

In summary, inhalation of H₂S reversibly and rapidly decreases HR and CO without changing SV and MAP. This was independent of the effects of H₂S on core body temperature. H₂S breathing markedly and reversibly depresses metabolism in mice, an effect that is also independent of hypothermia. Inhalation of H₂S decreases HR and CO without changing SV and MAP, and this effect is also independent of any effects on core body temperature. The rapid inhibition of metabolism produced by H₂S breathing may be exploitable, either by gas inhalation or the injection of H₂S donors to protect cellular or organ function when the supply of nutrients and oxygen are jeopardized, such as after major trauma or a cardiac arrest. Further studies of the safety of H₂S inhalation and the ability of H₂S inhalation to be effective in larger species will be required.

References

3. Phillips SD: Hydrogen sulfide, Critical Care Toxicology: Diagnosis and