Characterization of a Novel, Water-Soluble Hydrogen Sulfide–Releasing Molecule (GYY4137)
New Insights Into the Biology of Hydrogen Sulfide

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Background—The potential biological significance of hydrogen sulfide (H$_2$S) has attracted growing interest in recent years. The aim of this study was to characterize a novel, water-soluble, slow-releasing H$_2$S compound [morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate (GYY4137)] and evaluate its use as a tool to investigate the cardiovascular biology of this gas.

Methods and Results—The acute vasorelaxant effect of drugs was assessed in rat aortic rings and perfused rat kidney in vitro and in the anesthetized rat in vivo. The chronic effect of GYY4137 on blood pressure in normotensive and spontaneously hypertensive rats was determined by tail-cuff plethysmography. GYY4137 released H$_2$S slowly both in aqueous solution in vitro and after intravenous or intraperitoneal administration in anesthetized rats in vivo. GYY4137 caused a slow relaxation of rat aortic rings and dilated the perfused rat renal vasculature by opening vascular smooth muscle K$_{ATP}$ channels. GYY4137 did not affect rat heart rate or force of contraction in vitro. GYY4137 exhibited antihypertensive activity as evidenced by ability to reduce N$^G$-nitro-L-arginine methyl ester–evoked hypertension in the anesthetized rat and after chronic (14-day) administration in spontaneously hypertensive rats.

Conclusions—These results identify GYY4137 as a slow-releasing H$_2$S compound with vasodilator and antihypertensive activity. GYY4137 is likely to prove useful in the study of the many and varied biological effects of H$_2$S. GYY4137 may also prove of therapeutic value in cardiovascular disease. (Circulation. 2008;117:2351-2360.)

Key Words: blood pressure ■ endothelium ■ hypertension ■ hydrogen sulfide

Hydrogen sulfide (H$_2$S) has long been recognized as a metabolic poison similar in potency to cyanide.\(^1\) Nitric oxide (NO) and carbon monoxide (CO), also once considered solely as metabolic poisons, are now recognized as important biological mediators. H$_2$S is formed from cysteine by 2 pyridoxal-5$^\alpha$-phosphate–dependent enzymes, cystathionine $\gamma$ lyase and cystathionine $\beta$ synthetase.\(^2\) H$_2$S dilates rat and human blood vessels both in vitro and in vivo by opening smooth muscle cell K$_{ATP}$ channels.\(^3\)-\(^5\) Other cardiovascular effects of H$_2$S, such as protection of the heart against ischemia/reperfusion injury,\(^6\) also involve activation of K$_{ATP}$ channels. H$_2$S also plays undefined roles in hypertension,\(^7\) myocardial infarction,\(^8\) and endotoxic\(^9\) and hemorrhagic\(^10\) shock.

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Much of our current knowledge of the biology of H$_2$S stems from the use of inhibitors of cystathionine $\gamma$ lyase and/or cystathionine $\beta$ synthetase such as DL-propargylglycine and aminoxyacetic acid, which target the pyridoxal phosphate binding site of these enzymes and, as such, may be of doubtful specificity. Over the years, NO and CO research has been facilitated by the development of organic compounds that release free NO/CO, the effects of which on cells, tissues, and intact animals (including humans) can then be studied. To date, H$_2$S-releasing “drugs” used in biological experiments have been largely restricted to simple sulfide salts, most commonly sodium hydrosulfide (NaHS), which releases H$_2$S instantaneously in aqueous solution. However, the release of endogenous H$_2$S from cells is likely to occur in lesser amounts and at a much slower rate than that from sulfide salts, and therefore NaHS may not mimic the biological effects of naturally produced H$_2$S. Recognizing the need for organic molecules capable of releasing H$_2$S over extended periods of time, we now report that morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate (GYY4137)
releases H$_2$S slowly both in vitro and in vivo, and we have used this compound to examine the effect of H$_2$S in the cardiovascular system. GYY4137 was one of a series of compounds synthesized in this laboratory on the basis of the structure of Lawesson’s compound, which releases H$_2$S in organic solvents.

Methods

Chemical Synthesis of GYY4137 and Release of H$_2$S In Vitro and In Vivo

Morpholine (20 mmol) in methyl chloride (CH$_2$Cl$_2$, 6 mL) was added dropwise (room temperature) to a CH$_2$Cl$_2$ solution (6 mL) of 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane (4.0 mmol). The reaction mixture was stirred at room temperature for 2 hours. The precipitate was filtered and washed several times with CH$_2$Cl$_2$. The product was a white solid (67% yield) and was pure as determined by $^1$H nuclear magnetic resonance. GYY4137 (melting point, 159.8°C to 160.0°C) is soluble in water up to 30 mg/mL (pH 7.4) (Figure 1). The nuclear magnetic resonance characteristics of GYY4137 are as follows: $^1$H nuclear magnetic resonance (300 MHz, acetone-D$_6$, 300K); $\delta = 8.03$ to 8.11 (m, 2H, aromatic CH), $\delta = 6.88$ to 6.90 (m, 2H, aromatic CH), $\delta = 3.94$ (m, 4H, CH$_3$), 3.50 to 3.53 (m, 4H, CH), 3.36 to 3.40 (m, 4H, CH), 2.87 to 2.92 (dd, J = 9.7, 5.4 Hz, CH), 2.04 to 2.09 (m, 4H, CH)$_3$$^1$C nuclear magnetic resonance (75 MHz, acetone-D$_6$, 300K); $\delta = 132.7$ (aromatic CH), 132.5 (aromatic CH), 112.2 (aromatic CH), 112.0 (aromatic CH), 66.8 (CH$_3$), 63.6 (CH$_3$), 54.6 (CH$_3$), 54.0 (CH$_3$), 44.9 (CH$_3$), 43.3 (CH$_3$). The infrared (film) values were 3019 cm$^{-1}$, 1215 cm$^{-1}$, and 756 cm$^{-1}$.

H$_2$S release from GYY4137 in vitro was assessed with the use of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and by amperometry. For DTNB experiments, phosphate buffer (100 mmol/L, pH 3.0, 7.4, or 8.5) was incubated (4°C to 37°C) with GYY4137 or NaHS (1 mmol/L, 100 µL), and, at appropriate times, aliquots (20 µL) were removed and added to 96-well microplates containing DTNB (1 mmol/L, 50 µL) and HEPES buffer (1 mmol/L, 50 µL, pH 8.0), and absorbance was measured at 1412 nm. The concentration of H$_2$S formed from GYY4137 was calculated from a standard curve of NaHS (1 to 500 µmol/L).

For amperometry experiments, GYY4137 (1 mmol/L) or NaHS (100 µmol/L) was added to an incubation chamber (World Precision Instruments; WPI) containing phosphate buffer (100 mmol/L, pH 7.4, 400 µL). H$_2$S formation was detected with the use of a 2-mm H$_2$S-selective microelectrode (ISO-H$_2$S-2; WPI) attached to an Apollo 1100 Free Radical Analyser (WPI) and shown as picoamps distribution, and p53 expression were determined as described previously. In separate experiments, cultured cells were exposed to sodium nitroprusside, forskolin, or GYY4137 (all 100 µmol/L) for 45 minutes in the presence of isobutyl methylxanthine (300 µmol/L), and cAMP and cGMP were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn).

Effect of GYY4137 on Smooth Muscle Cell Viability

Normal rat aortic vascular smooth muscle cells (A10) were obtained from the American Type Culture Collection (ATCC, Manassas, Va). Cells were cultured in Dulbecco’s modified Eagle’s medium containing fetal calf serum and antibiotics, and cell viability, cell cycle distribution, and p53 expression were determined as described previously. In separate experiments, cultured cells were exposed to sodium nitroprusside, forskolin, or GYY4137 (all 100 µmol/L) for 45 minutes in the presence of isobutyl methylxanthine (300 µmol/L), and cAMP and cGMP were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn).

Effect on Cultured Vascular Smooth Muscle Cells

Rat (male, Sprague-Dawley; weight, 250 to 300 g) aortic rings were prepared as described previously. Dose-response curves to NaHS were performed cumulatively while each aortic ring was exposed to a single concentration of GYY4137. In some experiments, responses to NaHS (300 µmol/L) or GYY4137 (200 µmol/L) were evaluated in aortic rings preincubated (30 minutes) with the K$_{atp}$ channel blockers glibenclamide (10 µmol/L) or PNU37883A (10 µmol/L), the NO synthase inhibitor N$^6$-nitro-L-arginine methyl ester (L-NAME) (50 µmol/L), the cyclooxygenase inhibitor indomethacin (2.8 µmol/L), the soluble guanylate cyclase inhibitor ODQ (3 µmol/L), the adenylyl cyclase inhibitor SQ22556 (50 µmol/L), or a mixture of apamin (100 nmol/L) and charybdotoxin (50 µmol/L) to block the effect of endothelium-derived hyperpolarizing factor. The effect of both NaHS and GYY4137 was also evaluated in endothelium-denuded rings, as was the time course of effect as determined by the ability to reduce the contraction to a standard concentration of phenylephrine (200 µmol/L).

Effect of GYY4137 on Perfused Kidney and Heart

For experiments using perfused kidney or heart, Sprague-Dawley rats (male; weight, 230 to 270 g) were anesthetized as described above. The renal artery was cannulated and the kidney perfused as described previously. The heart was also removed and perfused (Langendorff preparation) as described elsewhere. In kidneys, dose-response curves (volumes <10 µL) were obtained for bolus-injected noradrenaline, angiotensin II, or U46619. GYY4137 (100 to 500 µmol/L) was added to the perfusing Krebs’ solution, and the responses to each agonist was repeated. After 60 minutes, kidneys were reperfused with normal Krebs’ solution to assess the reversibility of the GYY4137 effect. Hearts were perfused with Krebs’ solution containing either GYY4137 (100 µmol/L) or NaHS (100 µmol/L), and left ventricular diastolic pressure was monitored. In separate experiments, the effect of GYY4137 (10 to 100 µmol/L) or NaHS (100 µmol/L) on heart rate (bpm) was determined.

Antihypertensive Effect of GYY4137

The methods used to assess the effect of GYY4137 on blood pressure in the anesthetized rat have been described previously. The effect of GYY4137 (26.6 to 135 µmol/kg IV) and NaHS (2.5 to 20 µmol/kg IV) was determined. The effect of pretreating animals (5 minutes before GYY4137) with PNU37883A (26.2 µmol/kg IP) or its vehicle (di-
methyl sulfoxide, 0.15 mL/kg IP) on the vasodepressor effect of GYY4137 was assessed, as was the effect of GYY4137 (133 μmol/kg) and NaHS (2.5 μmol/kg) administered intravenously 30 minutes before a hypertensive dose of L-NAME (185 μmol/kg IV). In other experiments, the effect of GYY4137 (133 μmol/kg IV) on the response to sodium nitroprusside (10 nmol/kg) was also evaluated.

The effect of GYY4137 on blood pressure of conscious, male spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats (age, 7 weeks; weight, 221 to 257 g) was studied. Systolic blood pressure was monitored with the use of a tail cuff connected to a PowerLab (AD Instruments Inc, Australia) attached to a computer running Chart (version 5.1). Blood pressure was determined (9:30 to 10:30 AM) before administration of GYY4137 (133 μmol/kg IP) or saline (1.0 mL/kg IP) and again for up to 28 days after drug injection was started. Drug administration was halted on day 14.

Statistical Analysis

Data are mean±SEM. Statistical analysis was by 1-way ANOVA or repeated-measures ANOVA, followed by the post hoc Tukey test or by the Student t test, as appropriate.

Figure 2. Release of H2S from NaHS (100 μmol/L) (A) and GYY4137 (1 mmol/L) (B) in phosphate buffer (pH 7.4, except C) in vitro as determined by amperometry (measured as picoamps) or spectrophotometrically (C, D) with the use of DTNB. Effect of pH and time (C) and temperature (D) on the release of H2S from GYY4137 is shown. In C, symbols for pH 7.4 and pH 8.5 overlap. Where no error bars are indicated, error lies within dimensions of symbol. Results show representative tracings (A, B) of at least 4 similar measurements and for C and D are mean±SEM; n=6.

Figure 3. Plasma concentrations of H2S (defined as H2S, HS−, and S2−) in animals administered either GYY4137 (133 μmol/kg IV or IP) (A) or (B) NaHS (20 μmol/kg IV) (B). Results show time course and are mean±SEM; n=6. *P<0.05 vs baseline values.
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Release of H₂S From GYY4137 In Vitro and In Vivo**

H₂S release from NaHS was instantaneous in the DTNB assay. Indeed, H₂S generation from NaHS was so rapid that a time course was not attempted. Real-time assessment of H₂S release from NaHS by amperometry showed peak signal generation (for H₂S) within 5 to 8 seconds (Figure 2A).

In contrast, release of H₂S from GYY4137 (pH 6.5) was slower, peaking at ~6 to 10 minutes with the use of the H₂S microelectrode (Figure 2B) or the DTNB (Figure 2C) assay. On incubation, GYY4137 releases low amounts of H₂S over a sustained period in aqueous solution (pH 7.4, 37°C). The rate of H₂S release from GYY4137 (1 mmol/L, ie, 100 nmol incubated) was 4.17±0.5 nmol/25 min (n=6, DTNB assay). When incubated in aqueous buffer (pH 7.4, 37°C), H₂S release climbed for 15 minutes and then plateaued at 75 minutes (Figure 2C). Release of H₂S from GYY4137 was pH dependent (Figure 2C) and temperature dependent (Figure 2D), with less release at 4°C and greater release at pH 3.0.

After administration (intravenous or intraperitoneal) of GYY4137 to anesthetized rats, plasma H₂S (defined as H₂S, HS⁻, and S₂⁻) concentration was increased at 30 minutes and remained elevated over the 180-minute time course of the experiment (Figure 3A). In contrast, NaHS administered to anesthetized rats did not elevate plasma H₂S levels at these time points (Figure 3B).

**Effect of GYY4137 on Vascular Smooth Muscle Cell Viability**

Treatment of rat vascular smooth muscle cells with GYY4137 (up to 100 μmol/L) for up to 72 hours did not cause detectable cytotoxicity (Figure 4A), change cell cycle distribution (Figure 4B), or induce p53 expression in these cells (Figure 4C).
Isolated Rat Aortic Ring

NaHS caused rapid, transient, and reversible (≈20 to 30 seconds) relaxation of aortic rings, whereas the effect of GYY4137 was slower in onset (≈10 minutes) and sustained (≈40 minutes). GYY4137 (EC50, 115.7 ± 6.7 μmol/L; Emax, 74.8 ± 4.7%; n = 8) was more potent than NaHS (EC50, 274.1 ± 22.2 μmol/L; Emax, 63.4 ± 3.3%; n = 11) (Figure 5A, 5B). The time course of effect of GYY4137 was also studied. The response to a standard concentration of phenylephrine was decreased 15 minutes after GYY4137 addition and remained reduced for at least an additional 45 minutes. In contrast, NaHS did not reduce the effect of added phenylephrine (Figure 5C).

The effect of antagonists on the vasorelaxant response to GYY4137 and NaHS was also examined. The effect of GYY4137 and NaHS was reduced by KATP channel blockers (glibenclamide or PNU37883A) but not by indomethacin, SQ23356, or a mixture of apamin and charybdotoxin. Removal of the endothelium or exposure of intact rings to L-NAME or ODQ reduced the vasorelaxant effect of both GYY4137 and NaHS (Figure 5D and 5E).

Incubation of cultured vascular smooth muscle cells with sodium nitroprusside or forskolin increased cGMP (24.5 ± 1.3 versus 9.7 ± 0.9 nmol/L; n = 6; P = 0.0001) and cAMP (79.6 ± 1.6 vs 11.3 ± 1.4 nmol/L; n = 6; P = 0.0001) concentrations, respectively. In contrast, GYY4137 (100 μmol/L) did not directly affect the concentration of either cGMP (10.7 ± 1.9 nmol/L; n = 6; P = 0.64) or cAMP (12.7 ± 1.4 nmol/L; n = 6; P = 0.50) under identical experimental conditions.

Perfused Rat Kidney and Heart

The effect of bolus injection of NaHS in the rat kidney was complex, with low doses causing transient falls in perfusion pressure (e.g., 10 nmol; 10.2 ± 2.8 mm Hg; n = 10), whereas higher doses (e.g., 5 μmol) caused a biphasic response made up of a fall (11.6 ± 4.5 mm Hg; n = 8) followed by a rise (22.7 ± 6.7 mm Hg; n = 8) in perfusion pressure. In contrast, bolus injection of GYY4137 (0.4 to 4.0 μmol) did not consistently affect renal perfusion pressure. The effect of GYY4137 was therefore assessed indirectly by reduction of the vasoconstrictor response to standard agonists. Bolus
injection of U46619, angiotensin II, or noradrenaline caused dose-related vasoconstriction. GYY4137 (100 to 500 μmol/L) in the perfusing Krebs’ solution caused concentration-related vasorelaxation, the effect of which was lost when the drug was removed from the perfusing solution (Figure 6A through 6C) or when angiotensin II was tested in the presence of PNU37883A (Figure 6D). Exposure of isolated hearts to NaHS (100 μmol/L) reduced cardiac contractility (left ventricular diastolic pressure) by 42.0±7.8% (n=7) and heart rate by 53.2±6.6% (n=9) at 30 minutes. GYY4137 by itself did not affect cardiac contractility or heart rate (Figure 7).

Effect of GYY4137 on Blood Pressure of Normotensive and SHR
NaHS (2.5 to 20 μmol/kg) caused immediate, transient (10 to 30 seconds), and dose-related falls in blood pressure in anesthetized rats (Figure 8A). GYY4137 (26.6 to 133 μmol/kg) caused a slowly developing fall in blood pressure that was apparent at 30 minutes and continued declining to 120 minutes after injection. GYY4137 modestly increased heart rate in these animals (eg, at 133 μmol/kg, 250.1±12.0 bpm, 60 minutes versus 213.5±3.2 bpm before drug injection; n=6; P=0.01). Pretreatment with PNU37883A did not significantly affect blood pressure (eg, at 15 minutes after PNU37883A, 117.2±3.7 mm Hg versus 105.6±3.5 mm Hg; n=5; P=0.053, before injection). However, PNU37883A injection blocked the vasodepressor effect of GYY4137 (eg, at 120 minutes, 120.6±4.1 mm Hg versus 117.2±3.7 mm Hg; n=5; P=0.56). In separate experiments, GYY4137 (133 μmol/kg) did not affect the vasodepressor response to sodium nitroprusside (33.1±7.02 mm Hg fall before versus 29.3±5.6 mm Hg fall 30 minutes after injection; n=5; P=0.68).

Two approaches were taken to examine the antihypertensive effect of GYY4137. First, acute injection of GYY4137 (133 μmol/kg IV) but not NaHS (2.5 μmol/kg IV) or saline 30 minutes before L-NAME administration reduced the L-NAME-mediated hypertension (Figure 8B). Second, chronic treatment of conscious animals with GYY4137 reduced systolic blood pressure (Figure 8C). The fall in blood pressure was apparent after 2 days and was still present after 14 days of treatment and was considerably greater in SHR than in normotensive animals. Treatment of rats with saline...
did not affect blood pressure. On cessation of drug therapy, blood pressure of WKY rats returned to preinjection values within 7 days, at which point blood pressure of SHR was still significantly reduced. Blood pressure of all animals returned to normal within 14 days of cessation of treatment. Daily treatment with GYY4137 did not affect weight gain (e.g., at 14 days, WKY, 64.9\(\pm\)14.5 g and SHR, 55.2\(\pm\)13.2 g versus saline-injected WKY, 60.1\(\pm\)10.7 g and SHR, 59.8\(\pm\)8.9 g; \(n=8\); \(P=0.79\) and \(P=0.78\), respectively) and, although not evaluated objectively, did not cause discernible signs of toxicity such as deterioration of fur condition, sedation, altered locomotor activity, or other gross behavioral changes.

**Discussion**

We describe here the chemical synthesis and characterization of the cardiovascular effects of GYY4137, a novel molecule that, unlike NaHS, decomposes slowly to generate small amounts of H\(_2\)S in vitro and in vivo. GYY4137 was originally described \(\approx 50\) years ago as an accelerant for the vulcanization of rubber, but there have been no previous reports of its biological activity.

In aqueous solution at physiological temperature and pH, H\(_2\)S release from GYY4137 is a slow process with \(\approx 4\%\) to 5\% H\(_2\)S generated from a starting concentration of 1 mmol/L within 25 minutes. In contrast, H\(_2\)S generation from NaHS is more or less instantaneous and certainly far too rapid to establish a time course of release even at room temperature. Release of H\(_2\)S from GYY4137 in vitro is both temperature and pH dependent, with limited generation on ice (4°C) and enhanced release under acidic conditions (pH 3.0). The finding that H\(^+\) promotes H\(_2\)S release from the parent molecule implies an electrophilic attack directed against the thione ring structure of GYY4137 structure. Additional experiments to pinpoint the precise chemical mechanisms involved are ongoing.

A sustained increase in plasma H\(_2\)S (defined as H\(_2\)S, HS\(^-\), and S\(_2\)\(^-\)) concentration was observed for up to 180 minutes after intravenous or intraperitoneal administration of GYY4137 in anesthetized rats. As shown previously in vitro, these data therefore suggest that GYY4137 (unlike NaHS) releases H\(_2\)S slowly when injected in the anesthetized rat.

GYY4137 did not cause significant cytotoxic effect or alter the cell cycle profile or p53 expression of cultured rat vascular smooth muscle cells. We\(^{12}\) and others\(^{22}\) have previously reported that NaHS (at similar concentrations and time course) promoted the apoptotic cell death of cultured fibroblasts and smooth muscle cells. That GYY4137 did not cause apoptosis in the present experiments may be explained by differences in the relative rates of H\(_2\)S release from the 2 drugs. Thus, large amounts of H\(_2\)S released over a short time frame (seconds) by NaHS may trigger signaling pathways leading to cell death, whereas this does not occur with the slower but sustained release of lower amounts of H\(_2\)S from GYY4137. The ability of H\(_2\)S to regulate cell viability in vivo may therefore be concentration and time dependent. At low concentrations, as may occur in physiological conditions
(mimicked by GYY4137), cells remain unscathed by H$_2$S, but, at high concentrations, as may occur in pathological states (and mimicked by NaHS), a cytotoxic/proapoptotic effect becomes evident. These experiments highlight the usefulness of a slow-releasing H$_2$S donor in advancing our understanding of the biological significance of this gas.

The effect of GYY4137 on cardiovascular function was also studied with a range of in vitro and in vivo pharmacological preparations. GYY4137 caused a slow relaxation of precontracted rat aortic rings, whereas the effect of NaHS was more rapid in onset and transient. GYY4137 was more potent presumably because aortic rings were in contact with the drug for longer times (compared with NaHS) and hence were exposed to accumulated H$_2$S over a longer time period. The effect of GYY4137 and NaHS was inhibited by glibenclamide and PNU37883A and reduced by endothelium removal and pretreatment with L-NAME and ODQ, which block the formation/vascular response to NO, respectively. In contrast, inhibition of cyclooxygenase enzyme activity (with indomethacin) or blocking the effect of vasodilator prostanooids such as prostaglandin I$_2$ and prostaglandin E$_2$ on adenylate cyclase (with SQ23356) did not affect the response to either GYY4137 or NaHS, suggesting that augmented endothelial prostanoid generation plays no part in the response to either H$_2$S donor. Similarly, direct measurement of cAMP/cGMP in cultured vascular smooth muscle revealed no significant effect of GYY4137, again indicating no direct action on guanylate or adenylate cyclase enzyme activity. Finally, a combination of apamin and charybdotoxin did not alter the response to either drug, again suggesting the lack of involvement of endothelium-derived hyperpolarizing factor. Taken together, these data support the hypothesis that both NaHS and GYY4137 open vascular smooth muscle cell K$_{ATP}$ channels and that at least part of the effect of both agents in this tissue involves the release of endogenous NO from endothelial cells. Essentially similar conclusions have been reached by other authors studying the effect of NaHS on rat aortic rings. In contrast, several H$_2$S-releasing organic per-
sulfides present in garlic have been shown to relax rat aortic rings by an endothelium-independent mechanism. Other researchers have reported an endothelium-dependent component of the vasodilator response to garlic. The precise role of NO in the response of blood vessels to H2S is therefore unclear. The O2 concentration at which experiments are conducted may determine the endothelium/NO dependence of the effect of H2S because a high (ie, 95%) level of oxygen reportedly promotes the involvement of NO in this response.

GY4137 was a vasodilator in the perfused rat kidney. Low doses of NaHS produced short-lived falls in renal perfusion pressure, as described previously in the rat mesenteric vasculature. However, higher doses caused a biphasic relaxation/constriction response, the mechanism of which warrants further study. H2S can contract isolated blood vessels either by quenching released NO, by inhibiting endothelial NO synthase, or by oxidation of H2S to a vasoconstrictor molecule in conditions of high oxygen tension. Bolus injection of GYY4137 did not affect renal perfusion pressure, presumably because the drug is washed out of the tissue before sufficient breakdown to H2S occurs. However, addition of GYY4137 to the perfusing Krebs’ solution dilated the renal vasculature, as evidenced by reduced response to vasoconstrictor drugs. This effect of GYY4137 was readily reversible and antagonized by PNU37883A, which indicates that, as in aortic rings, GYY4137 is a vasodilator by opening vascular smooth muscle KATP channels in the kidney. These observations demonstrate that, in vitro, GYY4137 relaxes not only large-capacitance vessels but also small-resistance arterioles, implying a potential effect of this compound on blood pressure and tissue perfusion in vivo. In contrast to H2S (released rapidly from NaHS), we propose that the vascular effect of H2S (released slowly from GYY4137) more closely parallels the biological effects of endogenous H2S. In the isolated heart, exposure to NaHS (but not GYY4137) caused a negative inotropic and chronotropic effect, as reported previously, presumably reflecting the explosive release of large amounts of H2S from NaHS. The slower release of H2S from GYY4137 leading to lower local concentrations of this gas may explain the lack of a direct effect of GYY4137 on cardiac contractility.

Bolus (intravenous) injection of GYY4137 had no immediate effect on blood pressure in the anesthetized rat but caused a slow fall in blood pressure for up to 2 hours accompanied by a progressive, presumably reflex rise in heart rate. In vivo, GYY4137 was 15 times less effective than NaHS as a vasodepressor, but the action was considerably more prolonged (ie, 120 minutes versus 15 to 30 seconds). In addition, GYY4137 did not affect the vasodilator response to sodium nitroprusside, implying no direct interference with the action of NO. PNU37883A reduced the vasodepressor effect of both GYY4137 and NaHS, suggesting that, in vivo as well as in vitro, the vasorelaxant effect of this agent occurs largely by opening vascular KATP channels.

To determine whether the vasodepressor activity of GYY4137 in anesthetized rats translates into an antihypertensive effect, we further observed that (1) acute GYY4137 administration reduced the hypertensive effect of L-NAME in the anesthetized rat and (2) chronic GYY4137 administration reversibly decreased systolic blood pressure of both conscious SHR and normotensive WKY rats. This effect occurred within 2 days of starting treatment, and blood pressure of treated animals remained lower for the full 14 days. Thereafter, blood pressure slowly returned to normal, with significant hypotension (in SHR) still present 7 days after the last injection. After 14 days without treatment, blood pressure of SHR and WKY rats had normalized, with no rebound rise detected. The persistent hypotension of SHR after cessation of drug treatment suggests that GYY4137 produces longer-term changes in blood pressure control.

Animals tolerated daily injection of GYY4137 well throughout the treatment period, with normal weight gain and no overt signs of toxicity. The present results show clearly that GYY4137 exerts a significant antihypertensive effect both acutely and after chronic administration.

In conclusion, we have utilized GYY4137 as a tool to further investigate the cardiovascular significance of H2S. In particular, we show that (1) exposing vascular smooth muscle cells to low concentrations of H2S over a prolonged period does not cause cell toxicity/apoptosis, which is in contrast to the effect of large quantities of H2S generated from NaHS; (2) low concentrations of H2S, unlike NaHS, do not have any direct effect on cardiac rate/force of contraction in the isolated rat heart; (3) isolated blood vessels respond to the presence of low concentrations of H2S with a slowly developing but sustained vasorelaxation as opposed to the rapid and transient effect of NaHS on these blood vessels; (4) low quantities of H2S reduce the hypertensive effect of L-NAME in anesthetized rats (an effect not shared by NaHS); and (5) chronic treatment of SHR with GYY4137 causes a sustained fall in blood pressure. We suggest that GYY4137 will be a useful tool to probe the biological significance of H2S in cardiovascular and other systems and may have therapeutic applications in cardiovascular disease.

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Disclosures
None.

References
The potential biological and clinical significance of hydrogen sulfide (H₂S) has been the subject of intense debate in recent years. Despite considerable progress in our understanding of this gas, much still needs to be learned. By analogy with other gases (eg, nitric oxide, carbon monoxide), a better understanding of the biological effects of H₂S would be expected by using drugs with the ability to release this gas over extended periods of time. Unfortunately, until now, no such drug has halted, and there was no rebound rise in blood pressure. We believe that the slow release of H₂S from GYY4137 better mimics the generation of this gas in vivo, and as such the spectrum of activity of this compound provides a more accurate reflection of the biological effects of this gas than has hitherto been available. Moreover, the clear antihypertensive effect of GYY4137 raises the possibility that this compound, or a like slow-releasing H₂S donor, may be of therapeutic benefit in clinical conditions associated with excessive vasoconstriction.
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