MPST but not CSE is the primary regulator of hydrogen sulfide production and function in the coronary artery

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Key Points

- MPST rather than CSE generates H2S in coronary artery, mediating its effects through direct modulation of NO. This has important implications for H2S-based therapy in healthy and diseased coronary arteries.
- Because oxygen extraction in the myocardium is maximal, increased metabolic demand can only be matched by increasing coronary blood flow. The impaired vasodilatory response of the coronary vasculature results in insufficient supply of blood and oxygen, leading to myocardial ischemia, as manifested in patients with coronary artery disease (30). Therefore, identifying and characterizing mechanisms underlying coronary tone regulation are of clinical relevance.
- Coronary tone is regulated locally by the endothelium predominantly through vasodilatory mediators nitric oxide (NO) and endothelial-derived hyperpolarizing factors (EDHFs). NO is produced by endothelial nitric oxide synthase (eNOS) and has been demonstrated to regulate resting coronary tone (11, 23) and metabolic demand-mediated vasodilation (21). Of the EDHFs, hydrogen peroxide (H2O2) has been the most widely researched. H2O2 has similarly been shown to be involved in coronary autoregulation (27), metabolic demand-mediated vasodilation (28) and flow-mediated vasodilation (3).
- Another EDHF, hydrogen sulfide (H2S), has recently emerged as another important gasotransmitter in the vasculature. H2S is produced from L-cysteine by cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MPST) (22). CSE converts L-cysteine into H2S with pyridoxal-5'-phosphate (PLP) as a cofactor. L-cysteine-dependent production of H2S by MPST is a two-step reaction. First, cysteine aminotransferase converts L-cysteine along with α-ketoglutarate into 3-mercaptopyruvate (3-MP). MPST then converts 3-MP into H2S (20). CSE appears to have an important role in vascular tone regulation as mice lacking CSE are hypertensive and have attenuated endothelium-dependent vasodilation (29).
- Vasoactive effects of H2S vary by vascular bed. In the mesenteric bed, H2S is a vasodilator (7), while in the pulmonary bed, it is a vasoconstrictor (17). In the aorta, the vasoactive effects of H2S depends on dose (14): H2S is a vasoconstrictor at low doses and a vasodilator at high doses. In the coronary vasculature, H2S has been reported to be a vasodilator (5, 6). Exogenous H2S induces coronary vasodilation independent of the endothelium through 4-aminopyridine-sensitive Kcs, BKCa, and KATP channels (4, 5, 6, 8). The role of CSE as a producer of endogenous H2S has also been investigated, although conclusions regarding its contribution are inconsistent. Some studies support little contribution of CSE in coronary vasoregulation (4, 6) while others support involvement (5, 8).
- The role of MPST in coronary vasoregulation has not been examined. Moreover, the current literature on H2S involvement in coronary vasoregulation lacks direct measurement of H2S production in the coronary artery. In this study, we hypothesized that MPST is a source of endogenous H2S in the coronary...
vasculature. We measured the protein abundance of the two H₂S-producing enzymes and measured in vitro H₂S production through both pathways. We next investigated the role of CSE-derived H₂S in vivo by measuring in vivo coronary vasorelaxation function in CSE inhibited and CSE knockout models. We then investigated the role of MPST-derived H₂S by characterizing the vasoactive effects of MPST substrate 3-MP ex vivo.

**MATERIALS AND METHODS**

**Animals.** All animal use was approved by the Animal Care and Use Committee at Johns Hopkins University. In vivo coronary vasoreactivity experiments were performed in male, 12-to 18- wk-old CSE-deficient (CSE−/−) and B6/129S (Jackson) wild-type mice. Male 250- to 300-g Sprague-Dawley rats (Harlan) were used for ex vivo experiments.

**Cell culture.** Human coronary artery (HCAECs; Lonza), pulmonary artery (HPAECs; Invitrogen), and aortic endothelial cells (HAECs; Lonza) were cultured in endothelial basal medium supplemented with 5% fetal bovine serum, endothelial cell growth supplements, and penicillin/streptomycin (Lonza).

**Protein expression.** Protein expression was determined in human endothelial cells and rat vascular tissue by Western blot. Rat aorta and pulmonary artery (HPAECs; Invitrogen), and aortic endothelial cells (HAECs; Lonza) were cultured in endothelial basal medium supplemented with 5% fetal bovine serum, endothelial cell growth supplements, and penicillin/streptomycin (Lonza).

**In vitro amperometric H₂S measurement.** Tissue H₂S production was measured by amperometry using the Apollo 4000 Free Radical Analyzer (WPI) and a 3-mm H₂S-selective electrode (WPI). Reactions were performed as previously described (22). For MPST-mediated H₂S production, rat liver and coronary tissue samples were homogenized in cold 10 mM PBS buffer pH 7.4 containing 1 mM dithiothreitol (DTT; New England BioLabs) and protease inhibitor (Roche). Total reaction volume was 200 μl and total protein amount used was 300 μg for liver and 30 μg for coronary. Homogenate was incubated with MPST substrate 3-MP (Sigma) for 50 min at 37°C in 2-ml gas-tight vials (Fisher). Liver homogenate was incubated with the listed 3-MP concentrations. Coronary homogenate was incubated with 1 mM 3-MP. After 50 min of incubation, 200 μl of 10× PBS adjusted to pH 6.0 were added to the reaction to promote release of H₂S and to stop the reaction. The reaction was incubated at 37°C for another 10 min. Two milliliters of headspace gas for liver samples and 3 ml of headspace gas for coronary samples were withdrawn from the gas-tight vial using a 10-ml syringe (BD) with a 22-G needle (BD) attached and injected into a scintillation vial containing 15 ml of 10× PBS (ThermoScientific) in which the amperometric probe was equilibrated. The amount of H₂S produced by the reaction was measured by the probe in units of pA. Data were recorded 2 min after the headspace gas was injected.

For CSE-mediated H₂S production, the tissue was homogenized in 10× PBS buffer containing protease inhibitor and incubated with 2 mM pyridoxal-5’-phosphate (PLP; Sigma) and L-cysteine (Sigma) (16). Liver homogenate was incubated with listed 1-cysteine concentrations. Coronary homogenate was incubated with 50 mM L-cysteine. Reaction incubation and measurement procedures were described as described.

**In vivo coronary vasoreactivity.** Coronary tone was evaluated in vivo by measuring coronary flow velocity using transthoracic echocardiography as previously described (26). Mice were anesthetized with 1.5% isoflurane and coronary flow velocity was measured using the Vevo 2100 (Visual Sonics) and a 40 MHz transducer (Visual Sonics). The transducer was mounted on a stand (Visual Sonics) to measure flow velocity from the same location for the entire experiment. Chest hair was removed by depilatory cream. The animal was secured onto a heated platform to monitor ECG, heart rate, and body temperature.

The left main coronary artery was visualized by color Doppler flow from a modified long axis view of the left ventricle. Flow velocity profile of the blood flow through the coronary artery was captured by pulse wave velocity. The difference in angle between blood flow and ultrasound beam was accounted for with angle correction done on the Vevo mainframe.

**Coronary vasorelaxation was induced by intravenous infusion of adenosine triphosphate (ATP; Sigma) or the β-adrenergic agonist dobutamine (Dob; Hospira).** ATP was administered to evaluate agonist-induced coronary vasorelaxation and dobutamine to evaluate metabolic demand-mediated vasorelaxation. Increase in diameter corresponded with an increase in flow velocity. Drugs were infused through a catheter inserted into the lateral tail vein. Catheters were custom-made by attaching a 30-G needle (BD) to size PE-10 polyethylene tubing (BD). The dose of drug administered was controlled with a syringe pump (Harvard Apparatus) by adjusting infusion rate.

Working concentration of ATP was 0.5 mg/ml. ATP was infused at 20, 40, 80, and 160 μg·kg⁻¹·min⁻¹ for 1 min/dose. Coronary flow velocity was allowed to return to baseline between each dose. Working concentration of dobutamine was 0.2 mg/ml. Dobutamine was infused at 5, 10, 20, and 30 μg·kg⁻¹·min⁻¹ at 2 min per dose. The CSE inhibitor propargylglycine (PPG; Sigma) dissolved in saline was administered intravenously at 50 mg/kg 30 min before flow velocity measurements. All drugs were prepared in a heparinized 0.9% saline solution. Peak diastolic flow velocities of three cardiac cycles were measured for data analysis.
Wire myography. Rats were euthanized by isoflurane overdose, and the left main and descending coronary artery was dissected and sectioned into ~1-mm rings. Precise vessel section length was measured and vessel sections were mounted onto a wire myograph (DMT). Vessels were bathed in 95%-5% O2/CO2 oxygenated Krebs physiological solution containing the following (in mM): 118 NaCl, 4.7 KCl, 25.0 NaHCO3, 1.2 MgSO4, 1.1 KH2PO4, 11 glucose, and 2.5 CaCl2·2H2O maintained at 37°C. Vessels were equilibrated for 30 min and then stretched in 0.3-mM increments to the tension equivalent to 50 mmHg (6.7 kPa), as determined by the DMT normalization module for LabChart. After being stretched, vessels were equilibrated for 15 min, and then subjected to two rounds of 60 mM KCl bolus administration into the bath. Vessels were preconstricted at 10⁻⁷ M or 10⁻⁶ M U46619 (Cayman) for 15 min. Vessels were then subjected to cumulative dose response of NaHS (10⁻⁵ M, 10⁻⁴ M, and 10⁻³ M; Sigma) or 3-MP (10⁻⁶ M, 10⁻⁵ M, 10⁻⁴ M; Sigma).

To test the involvement of arachidonic acid, rings were next incubated with 10 μM phospholipase A2 inhibitor 4-4′-(octadecylacetyl)-4′-oxobutenoic acid (OBAA; Tocris) for 30 min prior to U46619 preconstriction, and NaHS and 3-MP dose responses were carried out. Rings were then incubated with 100 μM N⁶-nitro-l-arginine methyl ester (l-NAME; Caymen) for 15 min before U46619 preconstriction to test the involvement of NOS-dependent NO. In a separate experiment, coronary artery rings were mechanically denuded to examine the role of the endothelium in the H2S- and 3-MP-mediated response.

Acetylcholine (ACh; Sigma) 10⁻⁵ M was added after NaHS 10⁻³ M and 3-MP 10⁻⁴ M to determine if endothelial-mediated vasorelaxation function remained intact. In separate challenges, vessels were preconstricted with U46619, and after steady constriction was reached, 100 μM l-NAME were added. After a steady l-NAME-induced constriction was reached, ACh 10⁻⁵ M was added to evaluate endothelial-mediated vasorelaxation after NOS inhibition.

Data analysis and statistics. Statistical significance was determined by two-way analysis of variance with Bonferroni posttest or Student’s t-test (GraphPad). Data are reported as means ± SE.

RESULTS

CSE and MPST expression in cells and tissue. CSE expression varied between human endothelial cell lines: expression was robust in HAECs and HPAECs and minimal in HCAECs in comparison (Fig. 1A). MPST and eNOS abundances were in similar in all the cell types. Rat liver was used as a positive control.

In rats (Fig. 1B) MPST was expressed in similar abundance in coronary artery and aorta. In mice, MPST expression was more robust in the coronary artery compared with the aorta (Fig. 1C). CSE expression was more robust in aortic tissue in both rats and mice compared with the coronary artery (Fig. 1, B and C). GAPDH was used as the loading control.

MPST- and CSE-mediated H2S production in tissue and cells. Enzymatic kinetics of MPST and CSE were characterized in rat liver homogenate, where both enzymes are robustly expressed (Fig. 1A) (10, 19). The Kₘ value of MPST for 3-MP in the reaction conditions was 0.46 ± 0.08 mM 3-MP and Vₘₐₓ was 254.1 ± 9.6 nM H₂S/h (Fig. 2A). For the CSE reaction with L-cysteine as substrate, Kₘ was 21.38 ± 4.31 mM L-cysteine, and Vₘₐₓ was 1,777 nM H₂S/h (Fig. 2B).

To measure enzyme activity, the coronary artery homogenate was incubated with 1 mM 3-MP or 50 mM L-cysteine. H₂S produced in these conditions was 32.4 ± 6.1 nM for 3-MP incubation and 3.6 ± 6.5 nM for L-cysteine incubation. MPST-mediated production was significantly higher than CSE-mediated production (Fig. 2C; P = 0.0056).

Similarly, in HCAECs, 3-MP-dependent H₂S production (1.38 ± 0.12 nM) was significantly higher than CSE-mediated production (0.25 ± 0.1 nM; P = 0.0017; Fig. 2D).

Role of CSE-derived H₂S in coronary vasorelaxation in vivo. ATP-mediated vasorelaxation (Fig. 3A) was not different among WT, WT treated with PPG, and CSE⁻/⁻ mice (P = 0.33 for WT vs. PPG treated and P = 0.31 for WT vs. CSE⁻/⁻). Metabolic demand-mediated vasorelaxation induced by dobutamine (Fig. 3B) was also not different between cohorts (P = 0.66 for WT vs. PPG-treated and P = 0.23 for WT vs. CSE⁻/⁻).

Vasoactive effects of exogenous H₂S and 3-MP. 3-MP caused dose-dependent vasoconstriction in coronary rings (Fig. 4A): 10⁻⁶ M decreased preconstriction tone by 3.6 ± 2.2% while 10⁻⁵ M significantly increased preconstriction tone by 19.7 ± 9.5% (P = 0.022 compared with 3-MP 10⁻⁶ M), and 10⁻⁴ M increased preconstriction tone by 68.6 ± 26.6% (P = 0.059 compared with 3-MP 10⁻⁵ M). Pyruvate did not have any effects on vasoconstriction (Fig. 5E). The vasoconstrictive response to 3-MP was not significantly altered following incubation with the PLA2 inhibitor OBAA (P = 0.29 OBAA vs. untreated) but was dramatically reduced following NOS inhibition with l-NAME. A dose of 10⁻⁴ M 3-MP produced an 8.2 ± 4.3% increase in preconstriction tone with l-NAME incubation compared with 68.6 ± 26.2% increase in tone in untreated vessels (P = 0.026). Removal of the endothelium similarly abolished vasoconstrictive effect of 3-MP and unmasked its vasodilatory effect: 10⁻⁴ M produced a 7.9 ± 6.5% decrease in tone in denuded vessels compared with the 68.6 ± 26% increase in tone in endothelial-intact vessels (P = 0.034).
NaHS induced a dose-dependent vasoconstriction in the coronary arteries (Fig. 4B): a 4.9 ± 3.7% increase in preconstriction tone occurred with 10⁻⁵ M, 18.3 ± 4.7% increase occurred with 10⁻⁴ M (P = 0.029 vs. 10⁻⁵ M), and 218.5 ± 52.0% occurred with 10⁻³ M (P = 0.0025 vs. NaHS 10⁻⁴ M). Following PLA2 inhibition with OBAA, NaHS also produced dose-dependent vasoconstriction but to a lesser degree: 10⁻⁵ M produced a 95.9 ± 30.9% increase in preconstriction tone with OBAA incubation compared with 218.0 ± 52.0% increase in preconstriction tone in untreated vessels (P = 0.039). Following L-NAME incubation, NaHS induced dose-dependent vasodilation: 10⁻⁵ M NaHS increased preconstriction tone by 2.8 ± 0.9% while 10⁻⁴ M NaHS decreased preconstriction tone by 10.0 ± 7.5% (P = ns compared with NaHS 10⁻⁵ M). 10⁻³ M NaHS further decreased tone to 76.9 ± 12.9% of preconstriction tone (P = 0.001 compared with NaHS 10⁻⁴ M). In endothelial-denuded coronary arteries, NaHS also had little vasoconstrictive effect at 10⁻⁵ M and 10⁻⁴ M and induced vasodilation at 10⁻³ M: 10⁻³ M NaHS reduced preconstriction tone by 76.5 ± 52.0% in denuded vessels compared with 218.5 ± 52.0% increase in tone in endothelial-intact vessels (P = 0.00081).

Representative traces of 3-MP dose responses are shown in Fig. 5 for untreated (Fig. 5A) and with OBAA incubation (Fig. 5B), L-NAME incubation (Fig. 5C), and endothelial denuding (Fig. 5D). Representative trace of pyruvate dose response is shown in Fig. 5E. Representative traces of NaHS dose responses are shown in Fig. 6 for untreated (Fig. 6A), with OBAA incubation (Fig. 6B), L-NAME incubation (Fig. 6C), and endothelial denuding (Fig. 6D).

**NO-H2S interaction.** Since enhanced preconstriction tone could be explained by decreased NO bioavailability, we tested whether H₂S had a direct effect on NOS activity and NO production. Maximum endothelial-mediated vasorelaxation could be obtained by intravenous administration of 50 mg/kg propargylglycine (PPG) 30 min before flow velocity measurement. ATP-induced vasorelaxation response was not different among wild-type (WT), PPG-treated wild-type, and CSE knockout mice (A). Vasorelaxation response to dobutamine-induced increase in cardiac metabolic demand was also not different between cohorts (B).
Vasoactive effects of 3-MP and NaHS. Physiological role of MPST in coronary vasoregulation was determined by wire myography in the rat left coronary artery. Data are displayed as percent change from preconstriction tone. 3-MP induced vasoconstriction in preconstricted arteries in a dose-dependent manner (A). Incubation with 10 μM phospholipase 2A inhibitor 4-[4-(octadecylphenyl)-4-oxobutenoic acid (OBAA) did not alter 3-MP vasoconstrictive effects. 100 μM N\(^o\)-nitro-L-arginine methylester (l-NNAME) incubation significantly reduced 3-MP-mediated vasoconstriction. Endothelial removal resulted in 3-MP-mediated vasodilation. Exogenous H\(_2\)S donor NaHS also induced vasoconstriction in preconstricted arteries in a dose-dependent manner (B). OBAA incubation attenuated NaHS-induced vasoconstriction. l-NNAME and endothelial denuding removed NaHS-induced vasoconstriction and only presented NaHS-induced vasodilation. 

Fig. 4. Vasoactive effects of 3-MP and NaHS. Physiological role of MPST in coronary vasoregulation was determined by wire myography in the rat left coronary artery. Data are displayed as percent change from preconstriction tone. 3-MP induced vasoconstriction in preconstricted arteries in a dose-dependent manner (A). Incubation with 10 μM phospholipase 2A inhibitor 4-[4-(octadecylphenyl)-4-oxobutenoic acid (OBAA) did not alter 3-MP vasoconstrictive effects. 100 μM N\(^o\)-nitro-L-arginine methylester (l-NNAME) incubation significantly reduced 3-MP-mediated vasoconstriction. Endothelial removal resulted in 3-MP-mediated vasodilation. Exogenous H\(_2\)S donor NaHS also induced vasoconstriction in preconstricted arteries in a dose-dependent manner (B). OBAA incubation attenuated NaHS-induced vasoconstriction. l-NNAME and endothelial denuding removed NaHS-induced vasoconstriction and only presented NaHS-induced vasodilation. 

Fig. 5. 3-MP dose response traces. Representative vasotension traces of 3-MP dose response in untreated coronary artery (A), after incubation with 10 μM OBAA (B), 100 μM l-NNAME (C), and after endothelial denudation (D). Pyruvate (E) was used as a control for 3-MP.
DISCUSSION

We investigated the role of H2S and the contribution of vascular H2S producers CSE and MPST in coronary tone vasoregulation. The new findings of this study are as follows: 1) CSE is not a significant contributor to coronary vasoregulation; the data shown here provide evidence that supports the involvement of MPST in coronary vasoregulation; 2) in HCAECs and in rat and mouse coronary arteries, MPST is the predominant source of H2S; and 3) this study supports the role of H2S as a coronary vasoconstrictor when NO bioavailability is physiological and vasodilator in the absence of NO.

Protein abundance measurement by Western blot showed that CSE and MPST are expressed in human endothelial cells and rat and mouse vascular tissue, although the relative expression between vascular beds varies between the species. CSE expression was robust in the aorta compared with the coronary artery in rats and mice. In contrast, MPST expression was similar in the aorta and coronary in rats and MPST...
expression was much higher in the mouse coronary artery compared with the aorta. The differences in the relative abundance of the proteins in the conduit arteries and arterioles could be due to the distinct properties and functions of these vessels. Conduit vessels are highly compliant and offer little resistance to flow. The arterioles on the other hand are resistance vessels and regulate organ perfusion and blood pressure by vasoconstriction and relaxation in response to various stimuli. The presence of H$_2$S-producing enzymes can provide an alternative pathway to NO-dependent vasoregulation and thus organ perfusion in conditions where NO bioavailability is compromised.

Alternatively, these differences could be due to differences in vascular health. CSE and MPST expression have been shown to be influenced by pathological conditions. In a mouse heart failure model, mice with heart failure induced by thoracic aortic constriction had elevated CSE expression and reduced MPST expression in the cardiac tissue (13). While the rats from which the tissue was harvested can be considered healthy, we did not ascertain the vascular health status of the donors of the human cell lines. Nevertheless, the expression data demonstrate that MPST is expressed in the coronary artery and support the possibility of endogenous H$_2$S production in the coronary vasculature.

The Michaelis-Menten kinetics of CSE and MPST were examined in vitro using rat liver homogenate. For 3-MP, the $K_m$ value was 0.46 ± 0.14 mM 3-MP. For CSE, the $K_m$ value was 21.38 ± 4.31 mM L-cysteine, a concentration far exceeding physiologically relevant L-cysteine values. The previously reported $K_m$ value is 1.2 ± 0.1 mM 3-MP for wild-type rat liver-derived MPST (19), which is comparable to our findings. On the other hand, for CSE the previously reported $K_m$ value is 1.9 mM L-cysteine for wild-type human CSE (9). Differences in reported values can be attributed to the use of a liver homogenate in our study compared with purified enzyme preparations in previous studies. In the homogenate, other proteins may compete for both PLP cofactor and L-cysteine substrate, markedly shifting the observed $K_m$ for CSE. Moreover, in the previous studies, both reactions were performed at basic pH, pH 9.55 for the MPST reaction and pH 8.2 for the CSE reaction. The reactions in this study were performed at pH 7.4, which is also the pH at which the vasoactivity studies were performed. The difference in reaction pH may also account for the discrepancy in the observed $K_m$ value. The $K_m$ value of human CSE for L-cysteine has also been shown to vary significantly with polymorphic variants (31).

In vitro amperometric H$_2$S measurement in rat coronary artery homogenate and HCAEC lysates showed that H$_2$S produced through the MPST pathway was significantly greater than through the CSE pathway. These reactions were performed at 2-mM 3-MP or 50-mM L-cysteine, concentrations greater than twofold the observed $K_m$ value in this study, to ensure maximal (specific) activity of the enzymes. The higher expression of the 3-MPST in the coronary artery coincides with higher rates of 3-MP-dependent H$_2$S production in coronary artery homogenates, further supporting the idea that 3-MPST is the primary source of H$_2$S in the coronary vasculature.

In vivo coronary vasorelaxation responses in CSE-deficient and CSE-inhibited mice were not different from untreated wild-type mice. In conjunction with the lack of CSE-mediated H$_2$S production in vitro, these in vivo data further support the notion that CSE-derived H$_2$S does not significantly contribute to coronary vasoregulation. Our findings are confirmed by previous studies that reported lack of coronary vasodilation upon addition of L-cysteine, suggesting minimal involvement of CSE in coronary tone modulation (4, 6). The insignificant role of CSE-produced H$_2$S in the coronary vasculature seems counterintuitive given the significant role of CSE-produced H$_2$S in regulating tone of the resistance arteries (29). However, endothelial protein expression and associated phenotypic traits have been shown to vary by vascular bed. For instance, in the heart, endocardial endothelial cells express connexin (CX) 43, CX40, and CX37 while myocardial capillary endothelial cells do not, consistent with the observation that endocardial endothelial cells possess a larger number of gap junctions than myocardial endothelial cells (1). It is therefore not unreasonable that the coronary arteries, which are controlled locally, have different main vasoactive mediators than the resistance arteries, which are controlled neurohumorally.

We demonstrated in vitro that the coronary artery can produce H$_2$S by the MPST pathway. Because direct MPST inhibitors are currently unavailable and transgenic deletion model was not readily accessible, we determined if MPST was important physiologically using ex vivo wire myography. The vasoactive response to MPST substrate 3-MP was similar to the vasoactive response to H$_2$S donor NaHS. There was no response to pyruvate, suggesting that the effects of 3-MP are due to H$_2$S synthesis rather than pyruvate. The vasoconstrictive effects were not significantly altered with PLA2 inhibition but disappeared with NOS inhibition and endothelial denudation. These data support that the effects of 3-MP are mediated through MPST production of H$_2$S as opposed to direct vascular effects of 3-MP. Although 3-MP-induced vasoconstriction was removed by L-NAME incubation and endothelial removal, 3-MP was not observed to induce vasodilatation to the same degree as NaHS. Lack of vasodilatory effects of 3-MP could be due to insufficient substrate amount to produce the amount of H$_2$S necessary for vasodilation. Coronary vasodilation was observed in this study and has been previously reported to occur at 1-mM NaHS. This concentration of H$_2$S may not have been possible to produce in the tissue with the 3-MP concentration used.

Interestingly, we observed the vasoconstrictive effects of NaHS in untreated coronary arteries, which contradicts previous studies that reported vasodilatory effects of NaHS (4, 5, 6, 8). We demonstrated, however, that the vasoconstrictive effect of NaHS is eNOS and therefore NO dependent. NOS inhibition with L-NAME yielded no vasoconstriction and only vasodilatation. A similar response observed with endothelial denuding identified the involvement of eNOS and eNOS-derived NO. Such NO dependence has also been observed in CO-mediated vasoactivity, where NO plays a “permissive” role in CO-induced dilatation in arteries (15).

Reducing NO bioavailability through eNOS inhibition has been shown to further increase preconstriction tension (18). The enhanced vasoconstriction with addition of NaHS observed in this study could therefore occur through eNOS inhibition or NO scavenging. Both H$_2$S interference with eNOS activity as well as H$_2$S reaction with NO have been previously described. H$_2$S was shown to cause a dose-dependent decrease in activity of recombinant bovine eNOS. The resulting reduction in NO bioavailability was associated with...
the observed vasoconstrictive effects of NaHS in rat and mouse aorta (14). Aortic vasoconstriction observed at low NaHS doses was attributed to the vasoconstrictive effects of reduced NO bioavailability overriding the vasodilatory effects of H2S. Aortic vasodilation observed at high NaHS doses was attributed to the vasodilatory effects of H2S overriding the vasoconstrictive effects of decreased NO bioavailability. The reported H2S inhibition of eNOS appears to contradict other studies that demonstrated that exogenous H2S promoted eNOS function and eNOS production of NO (12), (2). However, the duration of the exogenous H2S administration was different between the studies. H2S donor treatment was chronic in the studies showing cooperative effects of H2S on eNOS function and acute in the study that showed dose-dependent decrease in eNOS activity as well as this study. H2S has also been shown to react with NO to form HNO or a nitrosothiol, demonstrating the scavenging effect of H2S. Mixing NaHS with SNP reduced the vasodilatory effects of SNP in preconstricted rat aortic rings, further supporting that H2S reacted with NO and reduced NO bioavailability through scavenging (2, 25).

Our ex vivo vasoreactivity data showed that endothelium-mediated relaxation was maximal after NaHS-induced vasoconstriction. NOS inhibition with l-NAME significantly attenuated endothelium-mediated relaxation. In addition, 10 $^{-5}$ M ACh consistently induced complete relaxation between ring sections and animals for both NaHS and 3-MP, supporting that eNOS activity was truly unimpaired. These ex vivo vasoreactivity data therefore support that H2S-induced vasoconstriction is not through inhibition of eNOS activity.

Our fluorometric data demonstrated the scavenging interaction of H2S and NO. Whether the reaction product of H2S and NO is also a vasoconstrictor was not tested in this study. However, as noted earlier, this reaction product was shown previously to not have vasoactive effects (2, 25). Vasoconstriction observed in this study can therefore be attributed directly to decreased NO bioavailability. Because only the vasodilatory effects of NaHS were observed in the absence of NO, either by l-NAME inhibition or by endothelial denuding, we agree that H2S can act as a vasodilator in the coronary vasculature. We further conclude that its vasoconstrictive effects are due to its scavenging interaction with NO. The NO dependency of the vasoactive effects of H2S suggests a change in role from regulatory to vasodilatory with development of coronary artery disease. Under healthy conditions with physiological NO bioavailability, H2S appears to primarily regulate NO. In disease conditions with diminished NO bioavailability, H2S could serve as a compensatory vasodilator. By extension, these findings support that exogenous H2S induces vasoconstriction in healthy coronary arteries and vasodilation in diseased coronary arteries. Therapies involving administration of exogenous H2S would therefore benefit coronary artery disease patients but would be counterproductive as a supplement for patients with healthy coronary arteries.

Limitations of this study. The absence of H2S measurement in the bath during the wire myograph experiments to confirm that the effects of 3-MP are due to MPST-mediated H2S production is a limitation of this study. This detection was limited by the sensitivity of the amperometric probe in a noisy environment. Nevertheless, similar effects and mechanism of action between 3-MP and NaHS support the conclusion that 3-MP is acting on the coronary artery through H2S production by MPST. In vivo H2S concentrations are estimated to range from nanomolar to low micromolar levels. Thus the concentrations of exogenous H2S from NaHS used in the vasoreactivity experiments in this study are supraphysiological. While micromolar to millimolar concentrations of 3-MP were used in the bath, it is unclear how much intracellular H2S this translates to. Thus, the responses described are more likely to be relevant during sulfide-based therapies.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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