Hydrogen sulfide mediates vasoactivity in an O2-dependent manner

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Koenitzer JR, Isbell TS, Patel HD, Benavides GA, Dickinson DA, Patel RP, Darley-Usmar VM, Lancaster JR Jr, Doeller JE, Kraus DW. Hydrogen sulfide mediates vasoactivity in an O2-dependent manner. Am J Physiol Heart Circ Physiol 292: H1953–H1960, 2007. First published January 19, 2007; doi:10.1152/ajpheart.01193.2006.—Hydrogen sulfide (H2S) has recently been shown to have a signaling role in mammalian cell signaling. It is produced in many tissues and may interact with NO and heme proteins such as cyclooxygenase. It is well known that the reactions of NO in the vasculature are O2 dependent, but this has not been addressed in most studies designed to elucidate the role of H2S in vascular function. This is important, since H2S reactions can be dramatically altered by the high concentrations of O2 used in cell culture and organ bath experiments. To test the hypothesis that the effects of H2S on the vasculature are O2 dependent, we have measured real-time levels of H2S and O2 in respirometry and vessel tension experiments, as well as the associated vascular responses. A novel polarographic H2S sensor developed in our laboratory was used to measure H2S levels. Here we report that, in rat aorta, H2S concentrations that mediate rapid contraction at high O2 levels cause rapid relaxation at lower physiological O2 levels. At high O2, the vasoconstrictive effect of H2S suggests that it may not be H2S per se but, rather, a putative vasoactive oxidant product that mediates constriction. These data are interpreted in terms of the potential for H2S to modulate vascular tone in vivo.

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HYDROGEN SULFIDE (H2S) has recently been shown to have a role in mammalian cell signaling. It is produced in many tissues and has been detected in micromolar amounts in blood and brain (2, 12, 19). In physiological solution, the equilibrium H2S ↔ H+ + HS− (reaction 1) ↔ H+ + S2− (reaction 2) is rapidly established with pKs 6.9 and 14 for reactions 1 and 2, respectively (31), and the concentrations of H2S and HS− represent ~30% and 70%, respectively, of the total sulfide, whereas S2− concentration is negligible. Because at physiological pH the major sulfide species are H2S and HS− and because the exact molecular targets for sulfide are not fully known, any mention of H2S here refers to H2S and HS−. H2S is capable of inducing posttranslational protein modifications, including dithiol reduction (20) and ligand displacement from heme iron (29), similar in many respects to nitric oxide (NO). Also similar to NO, H2S is highly soluble in aqueous and lipid environments, can readily pass between cells, is rapidly oxidized, and is a poten-...
that are O2 dependent include H2S interaction with heme proteins such as cyclooxygenase (COX) and the formation of sulfide oxidation products such as sulfite, thiosulfate, and sulfate. However, the O2 dependence of these reactions and their impact on the biological function of H2S have not been previously considered.

To better understand the kinetic and functional interactions between vessel tension, H2S, and O2, we have measured both dissolved gases in respirometry and vessel tension experiments. Because H2S, similar to NO, is rapidly oxidized, most standard analytic methods for measuring H2S are critically limited in terms of real-time physiological measurements. A novel polarographic H2S sensor (PHSS) has been developed in our laboratory to overcome these limitations (15, 28) and was used to measure H2S levels throughout the course of these experiments. Here we report that, in the rat aorta, low physiological H2S concentrations mediate rapid vasoactive responses, either contraction at air saturation O2 levels or relaxation at physiological O2 levels, underscoring the importance of O2 in mediating the biological effects of H2S.

MATERIALS AND METHODS

Chemicals

All chemical reagents were purchased from Sigma unless otherwise noted.

Animals and Tissue Preparation

Male and female Sprague-Dawley rats (250–350 g body wt) were housed in University of Alabama at Birmingham animal care facilities under 12:12-h light-dark cycles, with food and water available ad libitum. Anesthesia was induced by intraperitoneal injection of ketamine and xylazine (100 and 16 mg/kg, respectively), and thoracic aortas were immediately excised and placed in Krebs-Henseleit (K-H) mine and xylazine (100 and 16 mg/kg, respectively), and thoracic aortas were immediately excised and placed in Krebs-Henseleit (K-H) buffer (pH 7.3 at 37°C) containing 50 μM diethylenetriaminepentaacetic acid (DTPA) to chelate trace metals that catalyze H2S oxidation. Aorta segments were cleaned of adventitious tissue and blood and then cut into seven to eight ~3-mm-long segments. Excised tail artery was also sectioned into 3-mm-long segments and used in respirometric experiments to assess O2 dependence of a model peripheral artery. All animal procedures were performed according to University of Alabama at Birmingham Institutional Animal Care and Use Committee-approved protocols.

H2S Measurement

Solution H2S concentration in the respirometer chamber or in the vessel organ bath (see below) was recorded with a PHSS (15) connected to a multichannel analyzer (Apollo 4000, WPI, Sarasota, FL). The PHSS is selective for H2S, responds rapidly to changing H2S concentrations, and has a lower detection limit near 10 nM (15). PHSS calibrations were made in each experimental chamber using anoxic stock solutions prepared daily as 10 mM H2S in 50 mM potassium phosphate buffer with 50 μM DTPA (pH 7.3 at 37°C), as previously described (15). Stock solutions of H2S can be conveniently made with sodium hydrosulfide hydrate NaSH·xH2O (Sigma-Aldrich certificate of analysis for product S2006, lot 085K0667, is 101.1% by titration). This avoids the need for stepwise manner to give typically 5, 5, 10, 10, and 20 μM H2S, etc. Concentration, a 3-ml gas space above the liquid, adjusted with stopper height, was perfused with N2. When the desired O2 concentration was reached, the N2 stream was removed and the stopper was fully inserted.

To measure H2S consumption rates at specific H2S concentrations, aliquots of the H2S stock were injected into both chambers in a stepwise manner to give typically 5, 5, 10, 10, and 20 μM H2S, etc. The slope of the decreasing PHSS signal between injections provided a measure of the H2S consumption rate over the wide range of H2S concentrations. To carry out these experiments within the 40–60 μM O2 range, the respirometer stopper was lifted at 40 μM O2 and was lowered at 60 μM O2.

On completion of the experiment, the aorta segments were removed, blotted dry, and weighed. Rates of O2 and H2S consumption were corrected for the respective background consumption rates recorded in the absence of aorta and then normalized to the aorta fresh weight. The contribution of background rate to total rate was dependent on tissue weight, which was chosen to keep experimental O2 levels fairly constant and above the critical O2 concentration (see below). Average aorta fresh weight per experiment was 26.5 ± 2.5 mg (n = 9). Background consumption rates were ~30% of the total rates. Consumption rates were plotted as functions of H2S concentration.

Vessel Tension: Effects of O2 and H2S

Isometric tension of intact rat thoracic aortic ring segments in response to H2S at specific O2 concentrations was determined accord-
ing to established methodology using a vessel bioassay system (Radnoti, Monrovia, CA) (11, 24) modified to house the polarographic oxygen sensor and the PHSS for simultaneous readouts of O2 and H2S concentrations and vessel tension recordings. Data were acquired on a personal computer using AcqKnowledge software (Biopac Systems, Goleta, CA).

Aorta segments were mounted in baths containing 15 ml of K-H buffer with 50 µM DTPA bubbled with a mass flow-controlled (series 100, Sierra Instruments, Monterey, CA) mixture of N2 and air, each containing 5% CO2, to maintain O2 concentration at a specific level from 40 to 200 µM. To obtain an accurate measure of H2S concentration at the vessel, the PHSS was held within 5 mm of the aorta. After the aorta segments were mounted and allowed 30 min of equilibration, vessel response was tested for maximal contraction with 60 mM KCl. After relaxation was achieved by wash out of the KCl, the vessels were contracted with 100 nM phenylephrine, giving 50–70% maximum contraction. The aorta prepared by this method exhibited relaxation on addition of 10 µM acetylcholine, demonstrating the presence of a functional endothelium. Anoxic H2S stock was injected into vessel-containing baths to give concentrations of 5–40 µM, and the effect on vessel tension was recorded simultaneously.

To ensure that ATP levels were not compromised relaxation if there was concurrent increased KATP channel activity. To test H2S interaction with sGC under physiological conditions, COX-1 and COX-2, activity that H2S binds to and/or reduces the ferric heme of sGC also reduce ferric heme iron in myoglobin to the ferrous state, the oxygen sensor and the PHSS for simultaneous readouts of O2 and H2S levels of O2 and H2S that are physiological, yet nonlimiting to aerobic metabolism of the preparation. A decrease in conduction. To ensure that ATP levels were not compromised as a result of hypoxia, it was important to determine vessel O2 consumption rate as a function of solution O2 concentration to determine the threshold of O2 limitation (Fig. 1). The O2 consumption rate of noncontracted rat aorta segments (typically 2 mm OD) was maintained at 1.3 pmol O2·s⁻¹·mg fresh wt⁻¹ at higher O2 levels to a threshold of ~25 µM O2, below which the rate of O2 consumption decreased in an O2-dependent fashion, consistent with inhibition of mitochondrial respiration (Fig. 1). This threshold level is called the critical O2 concentration (Table 1) (24). These data were analyzed to calculate an apparent Km, defined as the O2 concentration at half-maximal O2 consumption rate, near 10 µM (Table 1). Next we determined that contraction of the aorta segments with phenylephrine caused an approximately twofold increase in the rate of O2 consumption, to 2.8 pmol O2·s⁻¹·mg fresh wt⁻¹, with an unchanged critical O2 concentration and Km for O2 (Table 1). To compare the O2-mediated effects on respiration of aorta with those of a model peripheral artery with relatively little connective tissue, compared with aorta and typically 1 mm OD, we measured the O2 consumption rate of contracted tail artery segments (Fig. 1). O2 consumption rate (3.5 pmol O2·s⁻¹·mg fresh wt⁻¹) was higher and critical O2 concentration and apparent Km values (near 18 and 3 µM, respectively) were lower in contracted tail artery than in contracted aorta segments (Table 1). On the basis of these results, subsequent experiments on contracted aorta segments were performed at O2 concentrations of ≥40 µM, which are reported as intravascular for arteries to arterioles (40, 46). This ensured that any measured aortic vasorelaxation was not the result of respiratory inhibition by O2 diffusion limitation.

Aorta H2S and O2 Consumption

To determine the effects of H2S on aorta respiration over the concentration range that mediates vasorelaxation, rates of O2 and H2S consumption were measured as a function of H2S concentration in contracted and noncontracted aorta at 40–60 µM O2 (Fig. 2). Noncontracted aorta, an increase in H2S

Data Analysis

The majority of data manipulations were performed with Oroboros DatLab software (Innsbruck, Austria). Statistical significance, determined by one-way ANOVA, was assigned at P < 0.05.

RESULTS

Effects of O2 on Mitochondrial Respiration in Isolated Vessel Preparations

To determine the O2 dependency of H2S effects on the tension of isolated vessels, it was first necessary to establish levels of O2 and H2S that are physiological, yet nonlimiting to the aerobic metabolism of the preparation. A decrease in vascular smooth muscle cell ATP levels could lead to vessel relaxation if there was concurrent increased KATP channel conduction. To ensure that ATP levels were not compromised.
concentrations caused a commensurate decrease in O2 consumption rates, reaching near-complete inhibition in 90 μM H2S (Fig. 2A), most likely via blockade of cytochrome oxidase. In contrast, O2 consumption rates of contracted aorta with increased ATP demand were stimulated to a maximum at ~40 μM H2S, above which the O2 consumption rates began to decline. H2S consumption rates also showed differential response to H2S concentrations as contracted aorta exhibited enhanced H2S consumption rates, reaching a maximum near 40 μM H2S, compared with noncontracted aorta (Fig. 2B). On the basis of these results, tension experiments on contracted aorta at 40–60 μM O2 were performed at 5–40 μM H2S.

H2S-Dependent Vasoreactivity

Effects of O2. Similar to reported results from experiments in air-equilibrated buffer (~200 μM O2) (55), vessels contracted with phenylephrine to 50–75% of maximum tension exhibited a biphasic response to H2S, with vasoconstriction at 5–100 μM H2S followed by vasorelaxation at ≥200–400 μM H2S (Fig. 3). However, at lower (40 μM) O2, H2S elicited a transient vasorelaxation at concentrations as low as 5 μM (Fig. 3). The short duration of the H2S-dependent relaxation is most likely the result of chemical or biological H2S oxidation, as well as the loss of H2S to the headspace above the vessel bath (15). The cumulative dose-response curves at high and low O2 (Fig. 4) indicate a 17-fold decrease in the EC50 for H2S-dependent vessel relaxation at 40 μM O2 compared with the EC50 at 200 μM O2 (Table 1).

Reversibility of H2S-dependent vasorelaxation. To more directly define the relation between H2S and aorta tension at different O2 levels, the H2S sensor was placed directly in the vessel bath to give simultaneous measurements of vessel tension and H2S concentration. As contracted aortas at 40 μM O2 were exposed to bolus additions of 20 or 40 μM H2S, the vessels initially relaxed and then recontracted as the H2S was purged from the solution (Fig. 5). This could be repeated multiple times on the same vessel, indicating that H2S-mediated vessel relaxation is a reversible process. With data from these experiments and others performed at 70 μM O2, the relation between aorta tension and H2S concentration was determined (Fig. 6). The H2S EC50 for aorta tension as a function of O2 concentration, shown in the inset in Fig. 6, further illustrates the O2 dependency of H2S-mediated vasorelaxation.

Mechanisms of H2S-Mediated Vasorelaxation

H2S interactions with COX in intact aorta. Vessels described above were routinely treated with 5 μM indomethacin to inhibit production of vasoactive prostaglandins via the constitutive heme-containing COX-1 pathway. Because heme groups are potential biological targets for H2S reactivity, studies were also performed without indomethacin to determine whether COX-1 plays a role in the effects of H2S on the vasculature. In contrast to vessels treated with indomethacin, non-indomethacin-treated vessels exhibited no vasoconstrictive response to H2S concentrations (Fig. 2C). A summary of these data (Fig. 4) shows the significantly different response to H2S between aorta with and without indomethacin at high O2. At lower (40 μM) O2, the effect of indomethacin was not pronounced (Fig. 4), with no significant difference observed between vessels with and without indomethacin. These data suggest that products of the COX pathway may limit the vasoconstrictive effects of H2S at high O2.

H2S interaction with sGC. To determine whether H2S vasodilatory effects at 40 μM O2 are mediated via the NO-dependent sGC pathway, cGMP levels were measured in the presence of H2S, the NO donor SNP, and the sGC inhibitor ODQ (Fig. 7). Although cGMP levels were increased by SNP and inhibited by ODQ as expected, 20 μM H2S had no effect on cGMP activity, indicating that, under these conditions, H2S does not mediate vasodilation via sGC activity directly or via an indirect effect on NO biosynthesis.
DISCUSSION

H₂S is newly recognized as a cell signal in the mammalian cardiovascular, neurological, hepatic, pancreatic, pulmonary, and renal systems (27, 30, 32). Although there have been no definitive studies showing endogenously produced H₂S-mediated vasorelaxation in situ, it is generally accepted that H₂S has vasoactive effects in the cardiovascular system. Here we demonstrate that H₂S-mediated vasorelaxation is an O₂-dependent process and that low (5–40 μM) H₂S concentrations are sufficient to relax vessels under physiological O₂ conditions.

Effects of O₂ on Vessel Respiration

The aorta has been extensively studied as a model vessel for the evaluation of mechanisms by which vasoactive compounds mediate their effects. The O₂ consumption rate and apparent $K_m$ reported here for noncontracted aorta are similar to values reported previously (5, 35). Aorta O₂ consumption rate increased on addition of phenylephrine, whereas apparent $K_m$ and critical O₂ remained nearly constant, indicating that the change in tissue geometry was not sufficient to change O₂ availability under these conditions. In the tail artery, a peripheral vessel with a smaller diameter, smaller wall thickness, and greater proportion of smooth muscle cells than collagen compared with aorta, O₂ consumption rate was higher, yet apparent $K_m$ and critical O₂ were lower, as expected, supporting the concept that peripheral arteries and arterioles normally operate at lower O₂ concentrations than the aorta with no hypoxia until O₂ drops below 20 μM (40, 46).

Effects of H₂S on Vessel Respiration

The range of reported endogenous H₂S levels in tissues is quite large (from undetectable to 300 μmol/kg) (47), and the range of exogenous H₂S levels used experimentally is equally large (up to 500 μM) (30). Because H₂S is a potential inhibitor of mitochondrial respiration by ligation to the ferric heme iron of cytochrome $a_3$ in complex IV (4, 34), it was important to show that the exogenous H₂S concentrations necessary to cause vasorelaxation in this study were not inhibitory to respiration. Respiratory inhibition could result in ATP depletion, thus mediating relaxation via KATP channel opening (7). However, H₂S has been shown to increase KATP channel conductance, even when ATP is elevated (55), thereby mediating vasorelaxation independent of any decrease in cellular ATP.

![Fig. 3. Representative traces of aorta tension as a function of time. Aorta was exposed to bolus additions of H₂S at 200 and 40 μM O₂ in the presence and absence of 5 μM indomethacin. Vessel tension is expressed as fraction of maximum phenylephrine (PE)-induced contraction, which is defined as 1. At 40 μM O₂, no differences were observed between vessels in the presence and absence of indomethacin (data not shown).](image)

![Fig. 4. Aorta tension as a function of H₂S concentration. Aorta was exposed to 200 or 40 μM O₂ in the presence and absence of 5 μM indomethacin. Data are from traces such as those in Fig. 3. Values are means (SD) of 6 experiments. *Significant differences (P < 0.5) between treatments and controls.](image)

![Fig. 5. Representative simultaneous traces of aorta tension and H₂S concentration as a function of time. Aorta tension, expressed as fraction of maximum phenylephrine-induced contraction (black line, left y-axis), was recorded with bolus additions of 20 and 40 μM H₂S (gray line, right y-axis) at 40 μM O₂. Overlay of data shows direct, reversible, and limited response of vessel tension to H₂S concentration. Data are summarized in Fig. 6.](image)
We demonstrate here that when contracted aorta with increased ATP demand is provided sufficient H2S to cause relaxation, respiration is stimulated, not inhibited. This increased O2 consumption is accompanied by a nearly identical increase in H2S consumption, indicating that H2S oxidation is enhanced in contracted aorta. H2S, which is a source of reducing equivalents, has been shown to be a rapidly metabolized respiratory substrate in several tissue and mitochondrial preparations from a variety of animals, both vertebrates (3, 52) and invertebrates (13, 21, 39). In fact, H2S-oxidizing enzymes, which deliver electrons directly to the electron transport chain, have been identified in prokaryotes and eukaryotes, with gene sequence homologies extending to mammals (45, 48). When these tissue and mitochondrial preparations are provided with noninhibiting H2S levels and are stimulated with ADP or made to do work, O2 and H2S consumption rates increase in a parallel fashion, and ATP is produced. We argue that, on the basis of the similarity in reported and present results, phenylephrine-contracted aorta may also metabolize H2S to produce ATP.

The possibility has also been raised that H2S could uncouple mitochondria by diffusion past cytochrome oxidase into the mitochondria matrix, where it deprotonates to HS\(^-\) and H\(^+\) (36). However, we have shown with isolated gill mitochondria that H2S stimulates coupled O2 consumption and ATP production (39). Although H2S is a weak acid, with pK near 7, it is not a classic uncoupler, because HS\(^-\) is membrane impermeant. Therefore, although protons would be carried into the matrix and the small ΔpH would decrease, the anions would accumulate in the matrix and would enhance the potential between metal and solution, thereby maintaining the proton-motive force (33). We suggest that, instead of uncoupling, H2S would more likely inhibit cytochrome oxidase, as it does in noncontracted aorta. Because H2S does not inhibit O2 consumption in contracted aorta, it is highly unlikely that the vessels are ATP deprived. The mechanism of H2S-induced vasorelaxation is most likely direct interaction of H2S with the K\(_{\text{ATP}}\) channel, as proposed by several groups (7, 55) and supported by data showing glibenclamide inhibition of H2S-mediated aorta vasorelaxation (unpublished observations).

Effects of O2 on H2S-Mediated Vasorelaxation

Although we have replicated the findings of Zhao et al. (55) and Olson et al. (37), demonstrating that 200–300 μM H2S was required to cause rat aorta vasorelaxation at 200 and 900 μM O2, respectively, we have also observed that lower H2S concentrations cause vasoconstriction at high O2 concentrations, under which H2S is rapidly oxidized. H2S-mediated vasoconstriction at high O2 has been observed in a variety of nonmammalian vertebrates, often as part of a multiphasic response (37), and may be facilitated by one or more H2S oxidation products, rather than by H2S alone. Although the vasoreactivity of known biological H2S oxidation products, such as sulfite, sulfate, or thiosulfate, has not been examined thoroughly, Wills et al. (49) showed that sodium meta(bis)sulfite is a vasoconstrictor of rat vessels. By repeating these experiments at the physiological O2 concentrations found in the vasculature (40, 46), we demonstrate that much less H2S is required to cause vasorelaxation. This O2-mediated shift in vasorelaxation EC50 for H2S may reflect the fact that, at high O2 concentrations, more of the added H2S is oxidized, leading to less H2S available for vasorelaxation. Additionally, the generated H2S oxidation products at high O2 may drive a competing vasoconstrictive reaction.

An interesting prediction is that O2-dependent H2S levels and/or effects will be manifest differently in the pulmonary vasculature, where O2 levels are much higher and where hypoxia induces vasoconstriction, and several groups have shown that H2S has a role in pulmonary vascular tone (37) and that changes in H2S levels have been linked to hypoxic pulmonary hypertension (25, 53). Other vasoactive H2S interactions, such as those between H2S and NO (1), and the effects of H2S on the production of vasoactive prostaglandins by heme-containing COX may also be O2 dependent.

We propose that 15 μM H2S, the EC50 at 40 μM O2, is more representative of physiological H2S concentrations than 200–300 μM H2S, which is effective in rat aorta vasorelaxation at high O2 (37, 55). If the peripheral arteries operate at <40 μM O2, as indicated by the respirometric experiments, then their...
H₂S EC₅₀ would most likely be comparably lower. Vascular free H₂S may be even lower. Preliminary determinations of free H₂S concentrations in whole rat blood, measured within minutes of extraction using a flow-through PHSS system, indicate that normal levels are ±5 µM (unpublished data). Indeed, as with NO, the level of H₂S required within the vasculature for vasorelaxation will be difficult to measure in vivo because of the extreme dilution once H₂S has left the cell, as well as cellular H₂S consumption. We previously reported that intact aorta produces H₂S, measured under low (5 µM) O₂ conditions with the PHSS, at a sustained steady-state level near 1 µM for >30 min when provided with substrate (15), perhaps via a feedback-regulated dynamic equilibrium between cellular H₂S production and consumption mechanisms. Because the H₂S inhibition constant for isolated cytochrome oxidase is submicromolar (34), these processes, along with diffusion and equilibria between bound and free H₂S, most likely serve to regulate intracellular and extracellular H₂S concentrations at noninhibitory levels.

**Mechanisms of H₂S-Mediated Vasorelaxation**

It has been proposed that H₂S-mediated vasorelaxation is primarily the result of hyperpolarization due to H₂S directly opening Kₐtₚ channels (7, 55), with a small proportion of the relaxation attributed to other mechanisms, including those dependent on NO. NO and H₂S have been shown to operate synergistically (1, 22), perhaps at the level of the H₂S-producing enzymes cystathionine-β-synthase and cystathionine-γ-lyase (CGL). Cystathionine-β-synthase contains a redox-sensitive hexacoordinate heme remote from the active site (43), and one of its axial ligands is the sulfhydryl of a cysteine residue that can be displaced by NO or carbon monoxide (43). CGL contains 12 cysteine residues that, if bearing free -SH groups, are potential targets for S-nitrosation-type regulation (55). The presence of S-nitrosothiols in vascular tissue may also contribute to vessel tone, if the bound NO group is effectively reduced and liberated from the S-nitrosothiol (18). We have determined that H₂S catalyzes the reactivation of NO-inhibited GAPDH in cultured cells, as well as the stoichiometric release of NO from S-nitrosothiolactone in a highly O₂-dependent manner (unpublished observations), further substantiating the interaction between H₂S and NO. Vascular smooth muscle cells cultured in the presence of an NO donor expressed increased production of CGL and H₂S (55). Interestingly, smooth muscle cell proliferation is limited by exogenous H₂S (16), and the overexpression of CGL commensurate with increased endogenous H₂S production limits cell proliferation via activation of the ERK-p21 signaling cascade (51). H₂S, when given to mice in their breathable air, has recently been shown to cause a reversible suspended-animation-like state (6), perhaps as a result of mitochondrial inhibition, in addition to cell protection against oxidative damage.

We report no significant increase in tissue cGMP levels after the administration of H₂S, confirming that H₂S operates via cGMP-independent mechanisms (54, 55). However, we did see COX-dependent effects of H₂S in vivo. The results of our vessel tension studies with and without indomethacin have implicated H₂S interaction with the COX pathway at 200 µM O₂, but not at lower O₂ levels. Preliminary results with intact aorta and with human umbilical vein endothelial cells indicate that H₂S directly affects COX activity only at high O₂. The relation between H₂S and the COX pathway may involve interactions between H₂S and the COX-produced vasoactive prostaglandins, as well as between H₂S and the heme-containing COX enzymes. However, regardless of potential O₂-dependent mechanistic interactions between H₂S and COX, the COX pathway does not appear to play a major role in H₂S-mediated vasorelaxation under normal physiological conditions.

In conclusion, H₂S is a potent vascular signal with O₂-dependent vasoactivity. In rat aorta, H₂S concentrations that mediate rapid contraction at high O₂ levels cause rapid relaxation at lower O₂ levels. At high O₂, the effect of H₂S at physiological concentrations is vasoconstrictive, suggesting that not H₂S but, rather, a putative oxidation product is also vasoactive and mediates constriction. These results indicate that the role of H₂S as a vascular signal and the predominant vasoactive mechanism is highly O₂ dependent, with vasorelaxation being the response at physiological H₂S and O₂. We suggest that all studies assessing H₂S biology must account for O₂ effects as well.

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