Precursors and inhibitors of hydrogen sulfide synthesis affect acute hypoxic pulmonary vasoconstriction in the intact lung

Jane A. Madden,1 Susan B. Ahlf,2 Mark W. Dantuma,1 Kenneth R. Olson,3 and David L. Roerig2

Departments of 1Neurology and 2Anesthesiology, The Medical College of Wisconsin and Research Service, Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin; and 3Department of Physiology, Indiana University School of Medicine-South Bend, South Bend, Indiana

Submitted 18 August 2011; accepted in final form 3 November 2011

Madden JA, Ahlf SB, Dantuma MW, Olson KR, Roerig DL. Precursors and inhibitors of hydrogen sulfide synthesis affect acute hypoxic pulmonary vasoconstriction in the intact lung. J Appl Physiol 112: 411–418, 2012. First published November 10, 2011; doi:10.1152/japplphysiol.01049.2011.—The effects of hydrogen sulfide (H2S) and acute hypoxia are similar in isolated pulmonary arteries from various species. However, the involvement of H2S in hypoxic pulmonary vasoconstriction (HPV) has not been studied in the intact lung. The present study used an intact, isolated, perfused rat lung preparation to examine whether adding compounds essential to H2S synthesis or to its inhibition would result in a corresponding increase or decrease in the magnitude of HPV. Western blots performed in lung tissue identified the presence of the H2S-synthesizing enzymes, cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfur transferase (3-MST), but not cystathionine β-synthase (CBS). Adding three H2S synthesis precursors, cysteine and oxidized or reduced glutathione, to the perfusate significantly increased peak arterial pressure during hypoxia compared with control (P < 0.05). Adding α-ketoglutarate to enhance the 3-MST enzyme pathway also resulted in an increase (P < 0.05). Both aspartate, which inhibits the 3-MST synthesis pathway, and propargylglycine (PGP), which inhibits the CSE pathway, significantly reduced the increases in arterial pressure during hypoxia. Diethylmaleate (DEM), which conjugates sulfhydryls, also reduced the peak hypoxic arterial pressure at concentrations >2 mM. Finally, H2S concentrations as measured with a specially designed polarographic electrode decreased markedly in lung tissue homogenate and in small pulmonary arteries when air was added to the hypoxic environment of the measurement chamber. The results of this study provide evidence that the rate of H2S synthesis plays a role in the magnitude of acute HPV in the isolated perfused rat lung.

Cystathionine γ-lyase; 3-mercaptopyruvate sulfur transferase; cysteine aminotransferase; gasotransmitter

Hydrogen sulfide (H2S) has long been known as a noxious and toxic gas. However, recent studies showing that vertebrates produce endogenous H2S and that it fulfills all the criteria of a signaling molecule, have led to H2S being called the third “gasotransmitter,” thereby joining NO and CO (24) in this role. H2S is both a neuromodulator and neuroprotectant (14), and H2S-mediated effects as well as endogenous H2S synthesis have been described throughout the entire body, from brain to gut (12). Within the vasculature, H2S is synthesized by the enzyme, cystathionine γ-lyase (CSE), and by the tandem enzymes, cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfur transferase (3-MST), which catalyze the sequential transfer of sulfur from cysteine to α-ketoglutarate to form 3-mercaptopyruvate and then H2S (Fig. 1). CSE is localized in the endothelium and smooth muscle while CAT and 3-MST are found in mitochondria and cytoplasm (11). Cystathionine β-synthase (CBS), an enzyme involved in H2S synthesis in other organs, has not yet been found in vascular smooth muscle but has been identified in the endothelium (13, 20).

To date, most studies on the vascular effects of H2S have focused primarily on systemic vessels, where it has been shown to dilate preconstricted arteries and veins (4, 10, 31–33). This effect appears to be mediated in part through activation of ATP-sensitive K+ (KATP) channels, and to a lesser extent to activation of Ca2+-dependent K+ (KCa) channels, and possibly H2S-mediated endothelial NO release (25, 32–34). In whole animals, intraperitoneal injection of a CSE inhibitor increased mean arterial blood pressure in rats (31) and CSE-deficient mice developed age-dependent hypertension (30).

Within the pulmonary vascular bed, H2S is known primarily for its toxic effects, which are often associated with pulmonary edema (22), suggesting that either capillary permeability or pulmonary blood pressure is increased. However, recent studies in isolated pulmonary arteries from various species (9, 17) showed that the effects of H2S on the pulmonary vasculature are strikingly similar to those produced by acute hypoxia. Both hypoxia and H2S produce almost identical contractile patterns that are accompanied by membrane depolarization and myosin light chain phosphorylation (9, 17). Inhibitors of H2S biosynthesis inhibit the hypoxic response while sulfur donors including cysteine, cysteine plus α-ketoglutarate, and glutathione augment it. H2S production by a variety of tissues is inversely coupled to O2 availability at physiologically relevant PO2 (19). A similar activation of O2-sensing glomus cells in the carotid body and neural epithelial cells in the fish gill has led to the hypothesis that H2S is the critical couple between PO2 and tissue responses to hypoxia (18, 19). The involvement of H2S in HPV has not, however, been studied in the intact lung. In the present study, we used an intact, isolated, perfused rat lung preparation to examine whether adding compounds essential to H2S synthesis or compounds that inhibited its synthesis would result in a corresponding increase or decrease in the magnitude of HPV.

Materials and Methods

The Institutional Animal Care and Use Committee of the Zablocki Veterans Administration Medical Center approved the animal handling protocol for this study. Male Sprague-Dawley rats (366 ± 12 g) were anesthetized with pentobarbital sodium (40–50 mg/kg ip). The neck was opened and the trachea clamped to keep the lung inflated. The chest was immediately opened and the rat was heparinized (700 units/kg) by direct cardiac puncture into the right ventricle. The pulmonary artery was cannulated and the heart completely cut away to...
The perfusate also contained 4.5% Ficoll (sucrose, 0.57 MgSO4, 2.5 KH2PO4, 118 NaCl, 25 NaHCO3, and 5.5 glucose). The lung in italics: 3-MST, 3-mercaptopyruvate sulfur transferase; 3-MP, 3-mercaptopyruvate; thionine.

The lung was then suspended in the perfusion apparatus via the arterial and tracheal cannulas and perfused at 10 ml/min with a physiological salt solution containing (in mM) 4.7 KCl, 2.5 CaCl2, 0.57 MgSO4, 2.5 KH2PO4, 118 NaCl, 25 NaHCO3, and 5.5 glucose (pH 7.4 at 37°C). The perfusate also contained 4.5% Ficoll (sucrose, 0.57 MgSO4, 2.5 KH2PO4, 118 NaCl, 25 NaHCO3, and 5.5 glucose). The lung in italics: 3-MST, 3-mercaptopyruvate sulfur transferase; 3-MP, 3-mercaptopyruvate; CAT, cysteine amino transferase; RSH, thio compounds. Inhibitors are shown in italics: PPG, propargylglycine; AOAA, aminooxyacetic acid; AA, aspartate.

The presence of O2, H2S is ultimately metabolized to sulfate (SO4). CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; αKG, α-ketoglutarate; 3-MST, 3-mercaptopyruvate sulfuryl transferase; 3-MP, 3-mercaptopyruvate; CAT, cysteine amino transferase; RSH, thio compounds. Inhibitors are shown in italics: PPG, propargylglycine; AOAA, aminooxyacetic acid; AA, aspartate.

**Fig. 1. Pathways and enzymes involved in H2S synthesis.**

Experimental protocols. The protocol used and the increase in pulmonary arterial pressure in the isolated perfused rat lung induced by acute hypoxia were similar to that described by Weigand et al. (27). Briefly, under normoxic conditions a bolus (0.2 ml) dose of 0.05 μg of ANG II was injected into the isolated lung via the pulmonary arterial cannula and the spike in arterial pressure allowed to return to baseline (~3 min). The ventilation gas mixture was then rapidly switched to the hypoxic mixture (6% CO2, balance N2) and the transfusion in pulmonary arterial pressure recorded with time. Just after the maximum hypoxic arterial pressure was achieved the ventilation gas was switched back to normoxia. Once the arterial pressure returned to a stable baseline (~3 min) the sequence of ANG II followed by hypoxia was repeated for a total of five hypoxic episodes designated H1 through H5. The magnitude of the pulmonary arterial pressure (cmH2O) after the stabilization of the ANG II contraction and before hypoxia, subtracted from the hypoxia-induced peak arterial pressure was used as the measure of HPV for each episode.

Cysteine and reduced (GSH) or oxidized (GSSG) glutathione, diethylmaleate (DEM) and α-ketoglutarate (αKG), aspartate, aminooxyaceticacid (AOAA), and propargylglycine (PPG), were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were of reagent grade. They were dissolved in saline except for DEM, which is a liquid. All were added directly to the lung perfusate either during the 15 min equilibration period or between the fourth and fifth hypoxic episodes. The final lung perfusate concentrations of cysteine, GSSG, GSH, and αKG were 1 mM; aspartate, AOAA and PPG were 10 mM; and DEM from 0.25 to 5 mM.

**RESULTS**

The presence in the lung of enzymes known to produce H2S was determined from the Western blot shown in Fig. 2. Both CSE and 3-MST were clearly identified in the rat lung homogenate. The absence of the CBS enzyme is consistent with the work of others showing that this enzyme is found primarily in brain tissue (12).

Figure 3A shows a typical recording of pulmonary arterial pressure during five episodes of exposure to ANG II and hypoxia in a control rat lung. The magnitude of the transient rise in pulmonary arterial pressure during hypoxia increased with subsequent hypoxic episodes. The gradual increase in baseline arterial pressure during an experiment is due to accumulation of circulating ANG II in the perfusate, the long duration of perfusion (75 min), and the development of some edema caused by the transient high pulmonary artery pressures in the latter episodes of acute HPV. The histogram in Fig. 3B shows the increase in the mean hypoxia-induced arterial pressure for hypoxic episodes H1 to H5 in control lungs (n = 19).

**Fig. 3B** shows the increase in the mean hypoxia-induced arterial pressure for hypoxic episodes H1 to H5 in control lungs (n = 19).
The large $n$ results from the fact that the effect of hypoxia in control lungs was determined throughout the course of the study. The arterial pressure increase in the control lungs was significantly greater ($P < 0.05$) for each hypoxic episode, reaching a maximum of $14.4 \pm 1.2$ cmH$_2$O at H5.

Initially, we focused on how altering H$_2$S production by the CSE and 3-MST enzyme pathways in the isolated perfused lung affected the arterial pressure in response to hypoxia. For the first of these experiments, the sulfur donors, cysteine, GSSG, or GSH, were added to the perfusate and allowed to recirculate through the lung during the 15 min equilibration period. This was followed by the five ANG II/hypoxia episodes and the increases in arterial pressure measured for each one. Cysteine is the predominate precursor of H$_2$S production by CSE and 3-MST while glutathione serves as an in situ source of cysteine. The arterial pressure increase for each hypoxic episode (H1 through H5) in control lungs was compared to its corresponding hypoxic episode in lungs perfused with cysteine, GSSG, or GSH, and the results are shown in Fig. 4A. The presence of the three H$_2$S precursors significantly ($P < 0.05$) increased arterial pressure by 75–100% compared with control for all hypoxic episodes, with one exception. The arterial pressure during H5 with added cysteine while ~75% higher was not statistically different from the H5 control. All other data points demonstrated a significant correlation between these added precursors of H$_2$S synthesis and the magnitude of the hypoxic response. The ANG II responses in the presence of cysteine, GSSG, and GSH were not significantly different from those measured in control lungs (Fig. 4B; $P > 0.05$ for all).

Fig. 3. A: representative tracing showing changes in pulmonary artery pressure (Ppa) by isolated perfused rat lungs in response to ANG II (A), hypoxia (Hn), and normoxia (Nn). B: histogram of average increase in Ppa (cmH$_2$O) during 5 hypoxic challenges for all control lungs ($n = 19$).

Fig. 4. A: histogram of changes in pulmonary artery pressure during hypoxic episodes 1 through 5 of control lungs (Con; $n = 19$) and lungs treated with cysteine (Cys; $n = 7$), oxidized (GSSG; $n = 6$) and reduced glutathione (GSH; $n = 4$); all at 1 mM; *Significantly different from control. B: histogram of changes in pulmonary artery pressure during ANG II episodes 1 through 5.
A more specific approach for altering pulmonary H$_2$S production was to add $\alpha$-ketoglutarate to the lung perfusate to enhance the 3-MST enzyme pathway. Cysteine aminotransferase (CAT) converts $\alpha$-ketoglutarate and cysteine to 3-mercaptoppyruvate, which is converted to H$_2$S by 3-MST (Fig. 1). Figure 5A shows a recording of the pulmonary artery pressure for episodes H1 through H5. Adding $\alpha$-ketoglutarate after H4 resulted in a large increase in arterial pressure during H5. If $\alpha$-ketoglutarate was added at the start of the perfusion the resulting high arterial pressures for all hypoxic episodes resulted in more rapid lung edema, thereby limiting the number of hypoxic episodes. Therefore, the $\alpha$-ketoglutarate-induced pressure change is expressed as the percent increase of episode H5 over H4. Figure 5B shows that adding $\alpha$-ketoglutarate resulted in a 130% increase in arterial pressure, further supporting a role of H$_2$S in HPV.

The effect of using aspartic acid to inhibit the 3-MST pathway for H$_2$S synthesis was also investigated. Aspartic acid competitively inhibits CAT, thereby blocking conversion of cysteine to 3-mercaptoppyruvate and thus H$_2$S production by 3-MST. Aspartic acid was added to the lung perfusate and recirculated through the lung during the 15 min equilibration period. Immediately after episode H4, $\alpha$-ketoglutarate was added to the perfusate as before and the change in arterial pressure again expressed as the percent change of H5 over H4. As shown in Fig. 5B in the presence of both $\alpha$-ketoglutarate and aspartate the percent change in arterial pressure from H4 to H5 was greatly reduced and was similar to control, further implicating 3-MST-generated H$_2$S in HPV. Neither aspartate nor $\alpha$-ketoglutarate had any effect on the magnitude of the ANG II responses (Fig. 5C; $P > 0.05$ for both).

The effect of inhibition of the CSE pathway on arterial pressure during hypoxia was also examined by adding the CSE inhibitor, PPG, to the lung perfusate between episodes H3 and H4. The change in arterial pressure for episodes H4 and H5 is expressed as the percent change in pressure at H3 for both control and PPG-treated lungs (Fig. 6A). In control lungs the arterial pressures for episodes H4 and H5 were, respectively, 64 ± 11 and 121 ± 27% greater than episode H3 ($P < 0.05$ for both). In contrast, adding PPG to the lung perfusate resulted in a 50 ± 14% and a 60 ± 10% decrease ($P < 0.05$ for both) in arterial pressures between H4 and H5 compared to its respective H3. The ANG II responses were similar before and after adding PPG to the perfusate (Fig. 6B; $P > 0.05$). Finally, lungs treated with the CBS inhibitor, AOAA, did not show any diminution of the hypoxic response (data not shown), consistent with the absence of the CBS enzyme in the Western blot (Fig. 2).

Another approach to decrease in situ H$_2$S production in the isolated lung involved recirculation of various concentrations of DEM (0.25 to 5.0 mM) through the lung during the 15 min equilibration. DEM is one of many $\alpha$-unsaturated compounds that form conjugates with sulfhydryls such as cysteine and glutathione catalyzed by glutathione $\gamma$-transferases, and it has been widely used to deplete glutathione (1, 3, 7). Figure 7A shows a plot of perfusate DEM concentration versus mean peak pulmonary artery pressure for episode H5. The three horizontal lines denote the mean ± SE arterial pressure at episode H5 for the control lungs. At the highest DEM concentration (5 mM) the hypoxia-induced arterial pressure increase was only 1.3 ± 0.03 cmH$_2$O compared with 14.2 ± 1.2 cmH$_2$O in control lungs.
cmH₂O in the control lungs \((P < 0.05)\). Although the ANG II responses at 4 and 5 mM DEM were 36 ± 0.05 and 37 ± 0.08% lower than control (Fig. 7B; \(P < 0.05\)), these decreases were significantly less than those noted for the hypoxic responses, which were 79.0 ± 0.04 and 91.0 ± 0.02%, respectively (\(P < 0.05\)).

At decreasing DEM concentrations the peak arterial pressure during hypoxia increased and was similar to control levels at 1.0 mM DEM, further supporting a relationship between endogenous H₂S precursors and hypoxic pulmonary vasoconstriction. However, at DEM concentrations <1.0 mM, the pulmonary arterial pressure at H5 began to increase further and was significantly greater than control at both 0.5 and 0.25 mM (\(P < 0.05\)). Concentrations of DEM less than 4 mM had no effect on ANG II responses (Fig. 7B; \(P > 0.05\)). Several reports have shown that at low concentrations (0.05 to 0.5 mM) DEM increases the cellular concentrations of glutathione, cysteine, and glutamate (6, 21). The increase in glutathione at low DEM has been attributed to increased cystine and glutamate transport activity into cells (2). Increased glutathione concentrations at low DEM concentrations have been reported in bovine lung artery endothelial cells (6, 21), bovine lung artery smooth muscle cells, rat lung fibroblasts, and rat lung epithelial-like cells (21).

Figure 8A shows lung homogenate concentrations of H₂S and O₂ with time starting at a low O₂ concentration. After \(\alpha\)-ketoglutaric acid was added the H₂S concentration increased markedly. Injection of 8 \(\mu\)l of air into the system increased the

---

**Fig. 6.** A: histogram of percent increases in Ppa in isolated lungs between episode H3 (baseline) and episodes H4 and H5 for control lungs \((n = 19)\) and lungs exposed to the CSE inhibitor propargylglycine [PPG (10 mM); \(n = 4\)]; *Significantly different from H3 at \(P < 0.05\). B: histogram of mean ANG II-induced increases in Ppa before and after PPG.

---

**Fig. 7.** A: effect of increasing concentrations of DEM on Ppa during hypoxic challenge H5 in isolated perfused rat lungs; \(n = 5\) for each concentration of DEM. Horizontal line is mean ± SE of the response in control lungs \((n = 19)\). *Significantly different from control \(P < 0.05\). B: mean ANG II-induced increases in Ppa in control and DEM-treated lungs.
strates the release of H$_2$S from isolated pulmonary arterial tissue during hypoxia is involved in HPV. The intact organ model was useful for this study since the lung exhibits a transient increase in pulmonary arterial pressure in response to hypoxia and the increasing peak arterial pressures with successive hypoxic episodes allows a given lung to be used both as its own control, and/or be compared with a separate control group. Furthermore, compounds known to inhibit or stimulate H$_2$S could be added directly into the recirculating perfusate at any time point during the experiment.

The results of this approach provided consistent support for a role for H$_2$S in HPV. First, two major enzymes involved in H$_2$S synthesis, CSE and 3-MST, were found in the lung tissue, but another enzyme, CBS, was not (Fig. 2). The absence of the CBS enzyme was further confirmed when addition of the CBS inhibitor AOAA to the perfusate had no effect on any of the hypoxia-induced increases in arterial pressure. These results agree with those of others who did not detect CBS in rat pulmonary arteries (33) and that AOAA did not block H$_2$S production in rat vessel homogenates (10). However, we should note that our group has detected the CBS enzyme in bovine pulmonary artery endothelial cells (20) and that AOAA partially inhibited H$_2$S production in bovine pulmonary arteries and veins (17). Also, Wang et al. (23) showed that mouse pulmonary vascular smooth muscle and endothelial cells expressed CBS. Thus the enzymes involved in pulmonary vascular H$_2$S synthesis may be species specific.

In the present study, compounds that stimulated or inhibited the CSE or 3-MST-catalyzed synthesis of H$_2$S significantly affected pulmonary arterial pressure during acute hypoxia (Figs. 3–7). Cysteine, GSH, or GSSH added to the perfusate to increase the cysteine concentration of the vascular smooth muscle cells resulted in a near doubling of arterial pressure during acute hypoxia. These three compounds ultimately result in an increased synthesis of GSH in lung tissue, thereby leading to a more reduced redox state of the lung, which would favor both H$_2$S production and increasing its stability.

It has been postulated that HPV is mediated by an increased production of reactive oxygen species (ROS) and that “an antioxidant should suppress HPV without mimicking hypoxia” (26). However, Weir et al. (26, 28) have proposed “that HPV is signaled by a decrease in ROS or by more reduced redox couples.” This latter theory is consistent with our finding of a large increase in HPV in the rat lung when cysteine or glutathione was added to the perfusate. Further support for a reduced redox status contributing to HPV comes from the experiments in which we depleted sulphydryls (cysteine or GSH) by adding DEM to the lung perfusate. In these studies the hypoxia-induced increase in arterial pressure was significantly reduced and approached zero increase at the highest DEM concentration (Fig. 7). At the highest doses of DEM (4 and 5 mM) the ANG II response was also reduced, which might suggest that DEM was not totally specific for inhibition of just the hypoxic response; however, this result is not surprising since the general mechanism of action of DEM is via reaction with free sulphydryls that could be present in factors involved in the ANG II response and be manifested at high concentrations of DEM. More importantly, however, the unusual finding that at very low DEM perfusate concentrations HPV was greater than in control lungs (with no effect on ANG II responses) is also consistent with the view that a reduced redox status contributes to HPV since very low concentrations...
of DEM result in elevated cellular GSH due to the increased uptake of glutamate and cysteine (2, 6). This would increase lung cysteine concentrations, favoring higher H2S production and/or a more reduced redox status in lung tissue.

As noted in the introduction, CAT catalyzes the formation of 3-mercaptopyruvate from α-ketoglutaric acid and cysteine, which then becomes the substrate for 3-MST catalyzed production of H2S. The greater than fourfold increase in HPV in the presence of α-ketoglutarate in the perfusate (Fig. 5) provides more specific evidence for a role of H2S in HPV. This is further supported by the inhibition of HPV (Fig. 5) when aspartic acid, a competitive inhibitor of CAT, was added to the perfusate after α-ketoglutarate. The fact that the magnitude of HPV in the presence of both α-ketoglutarate and aspartate was similar to that of control might suggest that in the isolated lung preparation the contribution of the CAT-3-MST enzyme pathway in HPV is limited by the endogenous α-ketoglutarate concentration. Whether this is due to washout of α-ketoglutarate during the 15 min washout perfusion at the start of the experiment or that α-ketoglutarate levels are merely low in the lung tissue cannot be determined from these experiments. Finally, adding PPG to the perfusate to inhibit the CSE-mediated synthesis of H2S produced the expected decrease in HPV (Fig. 6) and is consistent with our previous finding that another CSE inhibitor, β-cyanoalanine, inhibited the hypoxic response in rat isolated pulmonary arteries (17). It is difficult to envision that the observed differential effects of these three similar low molecular weight organic acids (aspartate, α-ketoglutarate, and PPG) on HPV could occur via mechanisms other than their known interactions with the enzymes involved with H2S production.

The results of the experiments described above do not individually constitute proof of the involvement of H2S in HPV. However, taken together the constancy of each result does provide reasonable circumstantial evidence that the rate of H2S synthesis plays a role in the magnitude of acute HPV in the isolated perfused rat lung. Our findings are also substantiated by a recent study by Derwall et al. (8) who found that administration of H2S via ventilation of an extracorporeal membrane lung in sheep caused significant dose-dependent pulmonary vasoconstriction. In contrast, it should be noted that a study done in chronically hypoxic rats found that one of the enzymes responsible for H2S synthesis was reduced in systemic vessels (5). Plasma [H2S] was also lower in these animals, which led the authors to suggest that overall H2S production was reduced and that a hypoxia-induced loss in tonic H2S dilation contributed to chronic hypoxic pulmonary vasoconstriction. While at first glance these results would appear to argue against a role for H2S in HPV, all information was collected from systemic vessels; no pulmonary vessels were studied. Furthermore, recent studies have questioned the validity of plasma H2S measurements (29).

Further proof of the dependence of HPV on H2S synthesis ideally would involve correlation of the magnitude of the HPV response during hypoxia with the amount of H2S released from the isolated lung. This is not a simple task since the amount of H2S produced during hypoxia is very small and it is rapidly released from the lung tissue. It then becomes so highly diluted in both the perfusate fluid and the expired gas under the existing perfusate flow and respiration rates used in the intact lung (10 ml/min and 40 breaths/min) that it is not feasible to measure it with any currently available techniques. It has, however, been shown that isolated vessels and smooth muscle cells placed in a small volume closed system and perfused at a flow rate 1 ml/min or lower produced H2S in response to hypoxia as measured with a specially designed polarographic electrode (29). This technique would not be feasible in the isolated perfused rat lung preparation used in our study.

Other studies have used sodium hydrosulfide (NaHS) in order to produce H2S and study its effects. This approach is problematic since there can be significant loss of H2S to the atmosphere and thus result in an inability to determine the exact concentration of H2S present in the experimental preparation. Indeed, when we tried this approach in the isolated perfused lung there was out-gassing from the 37°C perfusate and by respiration, which resulted in a constantly decreasing H2S concentration. A number of H2S-releasing compounds are now available (16), and these might prove useful in future studies, although perhaps not in an acute hypoxic situation as described in this study. While definitive evidence of the role of H2S and elucidation of the mechanisms involved in its action in an intact organ such as the lung await the development of new, more sensitive and directed techniques, the results of this study coupled with those from Derwall et al. (8) strongly suggest that pharmacological manipulations aimed at altering H2S biosynthesis could serve as an effective mean to modulate HPV in the intact lung.

ACKNOWLEDGMENTS

This work was supported in part by Advancing a Healthier Wisconsin (J. A. Madden), NSF-IOS0641436 (K. R. Olson and J. A. Madden), and Department of Veterans Affairs (D. L. Roerig).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES