Reappraisal of H\textsubscript{2}S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling

Nathan L. Whitfield, Edward L. Kreimier, Francys C. Verdial, Nini Skovgaard, and Kenneth R. Olson

1Indiana University School of Medicine-South Bend, South Bend; and 2Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana

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Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, Olson KR. Reappraisal of H\textsubscript{2}S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling. Am J Physiol Regul Integr Comp Physiol 294: R1930–R1937, 2008. First published April 16, 2008; doi:10.1152/ajpregu.00025.2008.—Hydrogen sulfide (H\textsubscript{2}S) is rapidly emerging as a biologically significant signaling molecule. Studies published before 2000 report low or undetectable H\textsubscript{2}S (usually as total sulfide) levels in blood or plasma, whereas recent work has reported sulfide concentrations between 10 and 300 \textmu M, suggesting it acts as a circulating signal. In the first series of experiments, we used a recently developed polarographic sensor to measure the baseline level of endogenous H\textsubscript{2}S gas and turnover of exogenous H\textsubscript{2}S gas in real time in blood from numerous animals, including lamprey, trout, mouse, rat, pig, and cow. We found that, contrary to recent reports, H\textsubscript{2}S gas was essentially undetectable (<100 nM total sulfide) in all animals. Furthermore, exogenous sulfide was rapidly removed from blood, plasma, or 5% bovine serum albumin in vitro and from intact trout in vivo. To determine if blood H\textsubscript{2}S could transiently increase, we measured oxygen-dependent H\textsubscript{2}S production by trout hearts in vitro and in vivo. H\textsubscript{2}S has been shown to mediate ischemic preconditioning (IPC) in mammals. IPC is present in trout and, unlike mammals, the trout myocardium obtains its oxygen from relatively hypoxic systemic venous blood. In vitro, myocardial H\textsubscript{2}S production was inversely related to \textit{PO}\textsubscript{2}, whereas we failed to detect H\textsubscript{2}S in ventral aortic blood from either normoxic or hypoxic fish in vivo. These results provide an autocrine or paracrine mechanism for myocardial gasotransmitter; hydrogen sulfide metabolism; vascular signaling
was then conducted to determine if trout hearts produced sulfide, if myocardial sulfide production was inversely related to PO2, and if ambient hypoxia was sufficient to increase circulating [sulfide] in trout in vivo.

**MATERIALS AND METHODS**

*Animals and blood sampling.* All animal protocols were approved by Institutional Animal Care and Use Committee review. All blood was collected in heparinized (~50 USP/ml) syringes or plastic containers.

Sea lamprey (*Petromyzon marinus*, 0.13–0.45 kg) were trapped in streams feeding into the Great Lakes and maintained at Indiana University School of Medicine-South Bend in 500-liter tanks with aerated, through-flowing well water (14°C) and a 12:12 light-dark cycle. They were anesthetized in benzocaine (1:5,000 wt/vol) and opened with a midventral incision, and blood was drawn from the posterior cardinal veins.

Rainbow trout (*Oncorhynchus mykiss*, 0.4–0.7 kg) obtained from a commercial hatchery and maintained in circulating 2,000-liter tanks with aerated, through-flowing well water (14°C) and a 12:12 light-dark cycle and fed a maintenance diet with commercial trout pellets until 72 h before experimentation. Blood was drawn from the hemal arch of lightly restrained fish.

Lobund-Wistar (LW) rats (0.35–0.5 kg), Harlan-Sprague-Dawley (HSD) rats (0.4–0.5 kg), and C57 Black/6 mice (~25 g) were housed on-site and kept on a 12:12-h light-dark cycle with access to food and water ad libitum. LW rats and mice were killed with CO2, and blood was drawn via cardiac puncture while the heart was still beating. HSD rats were heparinized by intraperitoneal injection of 0.1 ml of 10 mg/ml heparin and then anesthetized with 1 ml of 50 mg/ml pento-barbital. Blood was drawn from the hepatic vein (flow cell experiments) or thoracic cavity (metabolism experiments).

**Pig** (*Sus scrofa*) and cow (*Bos taurus*) mixed venous and arterial blood was collected in heparinized containers within 5–10 min after the animals were killed at a local slaughterhouse. Flow cell and chamber experiments (below) were conducted on-site.

Blood was used within 1 min after collection for flow cell experiments (below) and kept on ice and used the same day. Heparin did not interfere with H2S measurements or sulfide consumption.

**Isolation of red blood cell ghosts.** Red blood cell ghosts were isolated from cow blood as described previously (23). Following isolation, an aliquot of ghosts was washed in Krebs' buffer and isolated from cow blood as described previously (23). Following isolation, an aliquot of ghosts was washed in Krebs' buffer and isolated from cow blood as described previously (23).

**Polarographic H2S sensor.** The polarographic H2S sensor as described above. Sulfide (as Na2S) was injected into the return cannula distal to the pump. The stability of sulfide in the extracorporeal loop was examined by substituting a vial containing a volume of HEPES buffer equivalent to the trout’s estimated blood volume and serial additions of sulfide (as Na2S).

**Plasma sulfide and sulfide metabolism in trout in vivo.** Trout were instrumented with an extracorporeal loop that drew blood from the dorsal aorta and returned it to the caudal vein. The dorsal aorta was cannulated (described below) and connected to a blood pressure transducer to ensure that this level of hypoxia produced the classical hypoxic bradycardia. In several experiments, the water PO2 was further decreased down to 20 mmHg before returning to normoxia. The H2S sensor was calibrated in position by substituting a vial containing a volume of HEPES buffer equivalent to the trout’s estimated blood volume and serial additions of sulfide (as Na2S).

**FREE H2S LEVELS IN BLOOD**
The addition of Na$_2$S to the blood and addition of TCA to the plasma was usually between 6 and 8 min and not more than 10 min. Chambers were incubated for 60 min on a shaker at 37°C (before incubation, chambers containing cow and pig plasma were at room temperature for ~1 h during transport from the slaughterhouse to the laboratory). Following incubation, the center well with the filter paper and zinc acetate was removed and placed in a 5-ml glass vial containing 3.5 ml water. Next, 0.4 ml of 20 mM N,N-dimethyl-p-phenylenediamine hydrochloride in 7.2 M HCl was added immediately followed by 0.5 ml of 30 mM FeCl$_3$ in 1.2 M HCl, and the vial was capped and gently inverted. Contents of the vials were transferred to 96-well plates, and the absorbance was read at 669 nm after 10 min. Absorbances were compared with those obtained from a Na$_2$S standard curve run in Cortland buffer (when using fish plasma) or Krebs buffer (when using mammalian plasma) in parallel with the experimental samples. The lower detection limit for this assay was 1 µM.

**Ion selective electrode assay of sulfide metabolism.** Sulfide antioxidant buffer (AOB) was made by dissolving 25 g sodium salicylate, 6.5 g ascorbic acid, and 8.5 g NaOH in water and diluting to 100 ml. The Accumet silver/sulfide combination ion selective electrode (Fisher Scientific, Pittsburgh, PA) was immersed in a blank solution of 5 ml of AOB and 10 ml Krebs buffer, and the potential was allowed to stabilize. The electrode was then transferred to a solution of 5 ml AOB and 10 ml Krebs buffer with 5% BSA that had been mixed immediately before transfer. At ~1-h intervals, the electrode was switched between the same blank and BSA solutions. The electrode was rinsed in a separate blank between transfers. The result was compared with a standard curve made by serial additions of 100 mM Na$_2$S to a 1:2 mixture of AOB and Krebs buffer following the manufacturer’s instructions. Continuous recording of the electrode potential was done with Biopac MP30 hardware and BSL Pro 3.7 software (Biopac Systems, Goleta, CA).

**Buffers.** The following buffers were used: Krebs-Henseleit (mammalian; in mM): 115 NaCl, 2.5 KCl, 2.46 MgSO$_4$, 2 CaCl$_2$, 2H$_2$O, 5.6 glucose, 1.38 NaH$_2$PO$_4$, and 25 NaHCO$_3$, pH 7.4; cortland bicarbonate (fish; in mM): 124 NaCl, 74.6 KCl, 0.57 MgSO$_4$, 2 CaCl$_2$, 5.5 glucose, 12 NaHCO$_3$, 0.09 NaH$_2$PO$_4$, and 1.8 Na$_2$HPO$_4$, pH 7.8; HEPES (trout in vivo; in mM): 145 NaCl, 3 KCl, 0.57 MgSO$_4$, 2 CaCl$_2$, 5 glucose, 3 HEPES, and 7 HEPES sodium salt (HEPES-Na), pH 7.8; and potassium phosphate (100 mM KH$_2$PO$_4$/K$_2$HPO$_4$).

**Chemicals.** Sodium sulfide nonahydrate crystals were placed in a glass syringe that was then sealed with a rubber stopper and sparged with N$_2$. N$_2$-bubbled distilled water containing 50 µM diethylenetriaminepentacetic acid (to chelate iron) was then injected in the syringe through the stopper to dissolve the Na$_2$S. Stock solutions were made fresh daily. This method was verified by iodometric titration (3). Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

**Data analysis.** Half-times for sulfide consumption were determined from exponential decay equations fit by Table Curve (Jandel, Chicago, IL). Statistical significance was determined by Students’ $t$-test or paired $t$-test, and correlation among groups was determined by a Spearman rank order correlation test using SigmaStat or SigmaPlot (Systat Software, San Jose, CA). Results are given as means ± SE; significance was assumed when $P ≤ 0.05$.

**RESULTS**

**Sulfide in blood, polarographic measurements.** Sulfide was not detected in blood or plasma from any animal (Table 1). Total sulfide in blood spiked to 10 µM Na$_2$S did not exceed 2 µM, indicating that while the polarographic H$_2$S sensor could detect sulfide in blood, most sulfide was rapidly removed (Table 1 and Fig. 1, A and B).
Table 1. Concentration of sulfide in whole blood before and after a 10 μM Na₂S spike, measured in flow-through cell with the polarographic H₂S sensor

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Baseline</th>
<th>Spiked</th>
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<tbody>
<tr>
<td>Trout</td>
<td>5</td>
<td>ND</td>
<td>0.26±0.15</td>
</tr>
<tr>
<td>Mouse</td>
<td>5</td>
<td>ND</td>
<td>0.54±0.15</td>
</tr>
<tr>
<td>LW rat</td>
<td>5</td>
<td>ND</td>
<td>0.42±0.07</td>
</tr>
<tr>
<td>HSD rat</td>
<td>3</td>
<td>ND</td>
<td>NM</td>
</tr>
<tr>
<td>Pig</td>
<td>5</td>
<td>ND</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>Cow</td>
<td>5</td>
<td>ND</td>
<td>0.38±0.11</td>
</tr>
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Values are means ± SE; n, no. of animals. Units are μM. ND, not detected; NM, not measured; LW, Lobund-Wistar; HSD, Harlan-Sprague-Dawley. Less than 10 s elapsed between spiking the sample and measurement. *Sensor response was atypical, slowly rising to 0.22 ± 0.02. This response is not characteristic of free sulfide.

Polarographic measurement of sulfide metabolism in whole blood, bovine plasma, albumin, and red blood cell ghosts. Blood from all animals tested consumed sulfide, as did bovine plasma and BSA. When Na₂S was added to whole blood, plasma, or BSA, the concentration of sulfide measured with the polarographic H₂S sensor decayed exponentially to baseline (Table 2 and Fig. 1, C–E). Sulfide consumption increased with BSA concentration, but consumption was independent of ambient oxygen (Fig. 1F) and was essentially nil in protein-free solutions (Fig. 1E, inset). The half-time for decay of a 10 μM Na₂S spike in buffer following addition of an excess of zinc acetate, which very rapidly removes sulfide from solution, was 23.7 ± 0.6 s at 15°C and 13.0 ± 0.2 s at 37°C (all n = 4). Red blood cell ghosts had no effect on sulfide consumption or sensor response (data not shown).

Colorimetric measurement of acid-labile sulfide in plasma and BSA. Acid-labile sulfide values in plasma are shown in Table 3. Control plasma was ~2 μM in lamprey, trout, cow, and pig, not detected in LW rat, and 4.3 μM in HSD rat. Acid-labile sulfide in plasma isolated from spiked whole blood had returned to control levels by the time of assay. Recoveries for the spiked plasma were >60%. Krebs buffer with 5% BSA that had been spiked to 10 μM in the metabolism chamber and assayed after the polarographic H₂S sensor reading had returned to baseline contained 2.8 ± 0.3 μM acid-labile sulfide, not significantly different from an unspiked control 3.3 ± 0.6 μM (n = 4).

Sulfide production in BSA and trout plasma measured with ion selective electrode. Sulfide was rapidly liberated from 5% BSA upon mixing with alkaline AOB and exceeded 1 mM by 12 h (Fig. 2). Sulfide was produced at a similar rate from trout plasma (data not shown). Acidification of the 12-h samples produced the characteristic odor of H₂S that was not evident when freshly mixed samples of BSA or trout plasma in AOB were acidified.

Liberation of sulfide from blood by DTT. Addition of the disulfide reductant DTT (final concentration 10 mM) did not generate sulfide from either unspiked trout blood or from blood spiked with 50 μM sulfide (data not shown). No further studies with DTT were conducted.

Sulfide production by steelhead and trout hearts in vitro. Sulfide generation from minced trout heart increased when cysteine was added to the reaction mixture (Fig. 3). Injection of oxygen transiently produced net sulfide consumption (Fig. 3A) but did not affect the overall rate of sulfide production once the oxygen was consumed (Fig. 3B). These experiments show that tissue [sulfide] is inversely related to oxygen availability.

Effects of hypoxia on plasma sulfide production in trout in vivo. Sulfide was not detected in blood drawn directly from the ventral aorta of unanesthetized trout via an extracorporeal pump (Fig. 4A). Reducing ambient PO₂ from normoxia (~145 mmHg) to 40 mmHg was sufficient to produce a profound reflex hypoxic bradycardia but did not increase the [sulfide] in ventral aortic blood. In two fish, ambient PO₂ was further reduced from 40 to 20 mmHg, also without an effect on blood [sulfide].

Effects of exogenous sulfide on blood sulfide in trout in vivo. Sulfide was undetectable in blood drawn via an extracorporeal pump from the dorsal aorta of an unanesthetized trout, and...
injection of an amount of Na2S sufficient to theoretically raise plasma sulfide level to 30 μM produced only a transient 1.6 μM increase in sulfide (Fig. 5). The same amount of Na2S added to a volume of buffer equivalent to the trout’s blood volume produced a sustained 30 μM sulfide reading (Fig. 5, inset).

**DISCUSSION**

In the present study, we used a polarographic H2S sensor to directly measure H2S gas in blood and plasma without any chemical modification other than adding an anticoagulant. We found that sulfide was undetectable in plasma and blood and that exogenously applied sulfide was rapidly removed. We also found that trout heart tissue in vitro produced sulfide in the presence of cysteine under hypoxic conditions, whereas sulfide was consumed when the tissue was normoxic In vivo, however, blood exiting the heart does not contain measurable levels of sulfide, even when trout were rendered hypoxic by reducing ambient oxygen. Our studies support the predictions of Szabó (35) and Li and Moore (20) and confirm earlier and generally ignored reports that sulfide does not circulate in the plasma at measurable concentrations. They also provide evidence that the balance between sulfide production and its metabolism by available oxygen may be a key component in oxygen-dependent (“oxygen sensing”) responses in a variety of tissues, including ischemic preconditioning in the myocardium. We could find no evidence, however, that sulfide exists in the circulation even as a transient response to environmental hypoxia and therefore it is doubtful that sulfide serves as a blood-borne signaling molecule.

Many of the studies that have implicated sulfide signaling in a variety of physiological processes, including neural, cardiovascular, gastrointestinal, genitourinary, and immune systems, have utilized “physiological” concentrations of sulfide in the range of 30–300 μM. These values are based on colorimetric assays or ion selective electrode assays performed within the past eight years (see list in supporting materials), each of which depends on harsh chemical treatment (strong acid or base, respectively) before analysis. However, using the polarographic H2S sensor constructed in our laboratory, which responded in real time under physiological conditions with a resolution of 100 nM total sulfide, we were unable to find any evidence that sulfide exists in vertebrate blood or plasma (Table 1 and Fig. 1), or that even when added to blood in vitro or in vivo it would remain as free sulfide for any appreciable time (Table 2 and Fig. 5). In fact, when sulfide was added to

![Fig. 2. Continuous recording of sulfide production in alkaline antioxidant buffer (AOB) using a sulfide ion-selective electrode (ISE). Sulfide was continuously generated from 5% (BSA; solid line) in Krebs buffer but not from a 30 μM sulfide standard in BSA-free Krebs buffer (dotted line). Removing the BSA from the ISE (blank) and returning it (second BSA) did not affect the rate of sulfide formation.](http://ajpregu.physiology.org/)

![Fig. 3. A: representative trace showing total sulfide production by minced trout heart in vitro as a function of time. Total sulfide was calculated from hydrogen sulfide (H2S) production measured with the polarographic H2S sensor. Arrows indicate addition of cysteine (Cys) or injection of 100% oxygen (O2; estimated final concentration 100 μM). B: effect of cysteine on total sulfide production by minced steelhead heart over the range of 0.1–10 mM. Shaded bar shows sulfide production in 10 mM cysteine after recovery from oxygen injection. Mean ± SE, n = 4.](http://ajpregu.physiology.org/)
whole blood, the peak concentration measured typically did not exceed 20% of that which was added (Fig. 1, A–D), and sulfide was also removed, albeit at a slower rate, by plasma and BSA (Table 2 and Fig. 1, A–E). Because we showed that red blood cell ghosts did not affect sulfide disappearance, the rapid fall in sulfide likely reflects entry in the red blood cell where it may be bound to hemoglobin or metabolized.

**Sulfide as a paracrine signal in ischemic preconditioning.**

To determine if blood [sulfide] could transiently increase, we selected the tissue with the greatest probability of increasing sulfide production, the hypoxic trout myocardium. Our choice was based on several observations. First, there is recent evidence that ischemic preconditioning, the ability of short bouts of ischemia to protect the heart from a subsequent prolonged ischemic period, is mediated in the rat heart by sulfide (27, 31); second, ischemic preconditioning has been demonstrated in trout hearts (9); third, trout hearts are continuously exposed to some degree of hypoxia (8); and fourth, tissue [sulfide] appears to be inversely related to PO2 (25).

Previously, we proposed that sulfide is involved in oxygen sensing in vascular smooth muscle (25) and in the urinary bladder (6). The ability of blood vessels to “sense” oxygen is important in mediating hypoxic vasodilation of systemic vessels and hypoxic vasoconstriction in pulmonary vessels. This helps couple perfusion to metabolism in the former and per- fusion to ventilation in the latter. However, the oxygen sensor mediating this process has been the subject of considerable debate (cf. Refs. 1 and 14). In our model, the concentration of vasoactive sulfide is regulated by the simple balance between constitutive sulfide production in the cytosol and the PO2−dependent oxidation of sulfide to inactive metabolites, i.e., sulfite and/or sulfate, in the mitochondria. This model has been supported by demonstration of sulfide production by vascular tissue, the identical effects of hypoxia and sulfide on a variety of vessels, and the ability of inhibitors of sulfide biosynthesis to inhibit the hypoxic responses (25). This is further supported by recent evidence for sulfur cycling between the cell cytoplasm (where it is reduced to H2S) and oxidation in the mitochondria of eukaryotic cells (30). It is also likely that the presence of mitochondria in lamprey and trout red blood cells accounts for the 5- to 10-fold faster rate of sulfide removal by fish blood compared with mammalian blood (Table 2). (These differences are not due to temperature effects on the polarographic H2S sensor, since the sensor recovery after rapid sequestration of sulfide by addition of zinc acetate was faster at 37°C than at 15°C.) Our observation of sulfide production by the hypoxic trout myocardium and the rapid reversal to net sulfide consumption in the presence of oxygen (Fig. 3), in conjunction with related findings in other tissues (6, 25), suggests that oxygen-dependent sulfide metabolism may be a general property of tissues in which PO2 is coupled to a physiological response.

The trout heart has two distinct layers of myocardium, a relatively thin outer compact layer supplied by coronary arteries and an inner spongy myocardium that derives its oxygen directly from systemic venous blood. Under most circumstances, cardiac function is unaffected by coronary artery ligation, implying that much, if not all, routine cardiac work is performed by the spongy layer (32). Even at rest in a normoxic environment, spongy myocardial cells are exposed to moderate hypoxia, since systemic venous PO2 is <50 mmHg; during exercise, venous PO2 may drop to 15 mmHg (8). Because ambient hypoxia only exacerbates this situation (32), it was predicted that ambient hypoxia sufficient to produce a reflex hypoxic bradycardia would also be a sufficient physiological stimulus to increase sulfide in blood. Because this did not occur (Fig. 4), it seemed unlikely that sulfide could serve, even transiently, as a circulating signal.

Our studies do, however, suggest that sulfide is involved in oxygen “sensing”/signal transduction in ischemic preconditioning. Recent studies have shown that application of sulfide mimics ischemic preconditioning in rat hearts by mitigating the pathological consequences of the final long-term myocardial ischemia (27, 31). It is not known how ischemia initiates the sulfide response. Our study suggests that the decrease in oxygen itself is sufficient to produce a net increase in intracellular sulfide and that H2S gas or HS− then acts locally. Ischemic preconditioning has also been demonstrated in trout hearts (9), indirectly suggesting that sulfide may be involved here as well.

**Fig. 4.** A: effect of normoxia (open bars) and hypoxia (PO2 ≤40 mmHg; filled bars) on sulfide concentration in ventral aortic blood measured with polarographic H2S sensor (left; n = 4) and heart rate (right; n = 2) in unanesthetized trout. An extracorporeal pump circulated blood from the ventral aorta across the sensor and returned it to the caudal vein. Sulfide was not detected in plasma of normoxic or hypoxic trout, whereas this level of hypoxia was sufficient to produce the classic chemoreceptor-mediated reflex bradycardia. B: calibration curve of sulfide in recirculated HEPES buffer.

**Fig. 5.** Effect of exogenous sulfide (as Na2S) on sulfide concentration of dorsal aortic blood in an unanesthetized trout. An extracorporeal pump circulated blood from the dorsal aorta across the sensor and returned it to the caudal vein. The arrow indicates bolus injection of Na2S in the caudal vein cannula. The amount of Na2S injected was theoretically sufficient to raise plasma sulfide to 30 μM when fully mixed in the plasma. Inset shows injection of the same amount of sulfide in a recirculated volume of HEPES buffer equivalent to the trout’s plasma volume and pH. Sulfide is rapidly removed from the plasma in vivo but not from buffer.
Historical perspective of sulfide in the circulation. There is a very clear demarcation in blood sulfide levels reported in studies published before or after 2000 (summarized in supplemental information) (Supplemental information for this article can be found on the American Journal of Physiology: Regulatory, Integrative and Comparative Physiology website.). Typically, the earlier studies anecdotally and experimentally support rapid consumption of sulfide by blood in vivo and in vitro and when directly measured show that plasma (or blood) sulfide is 2 μM or less (see supplemental information for details). In pioneering studies, Haggard (11) showed that quickly injecting 10 ml of 77 mM Na₂S in a dog was rapidly lethal, whereas injecting five times that dose over 20 min did not harm the animal, leaving Haggard to conclude that sulfide was rapidly metabolized. Prior et al. (28) found in rats an inhaled LC₅₀ (concentration at which 50% of the subjects died) of 335 ppm over 6 h of exposure to H₂S, while the LC₁₀ over the same time was 299 ppm. Assuming that H₂S readily equilibrates across the alveolar membranes, this would produce sulfide values in the plasma of 157 and 143 μM, respectively (see supplemental information for calculations). Prior et al. (28) suggested that the steepness of the dose-response curve was due to an overload of the sulfide detoxification system, implying that the majority of the lower dose is being continuously detoxified. Given this capacity to metabolize sulfide, it is difficult to imagine that there would be any opportunity for sulfide to accumulate under resting conditions. It is also clear that H₂S can leave the blood across the alveolar membrane, since intravenously injected NaHS is partially recoverable as H₂S in exhaled air (7). If 10 μM total sulfide in plasma equilibrated with alveolar gas, H₂S should be readily detectable in exhaled air at a concentration of 22 ppm. However, reported levels of H₂S in human exhaled (24) and end-expiratory air (34) are only ~50 ppb, >400-fold less than predicted. In fact, 22 ppm is within the range of H₂S concentrations in the typical human flatus (18) and slightly higher than the Occupational Safety and Health Administration Permissible Exposure Limit (4).

Methodological consideration of recent sulfide measurements. With only one exception, all studies after 2000 reported that plasma (or blood) sulfide was between 10 and 300 μM. The exception is an anecdotal report of ≤5 μM in rat blood measured with a polarographic H₂S sensor similar to ours (16). There are a number of factors that could contribute to the elevated sulfide values in recent studies. These include modifications to the original methods without proper verification of their accuracy, use of impure NaHS as a standard and nonlinearity of standard curves at low [sulfide]. Artificial generation of sulfide from proteins is also quite likely when using the strong alkaline AOB associated with the ion selective sulfide electrode, as clearly shown in Fig. 2. In fact, Khan et al. (15) also observed that directly mixing blood and AOB results in protein desulfuration. These points are considered in greater detail in the supplemental information.

In summary, our studies with the polarographic H₂S sensor indicate that circulating free sulfide levels are considerably lower than previously reported, and it is unlikely H₂S or sulfide functions as a signaling molecule in the circulation. However, the inverse coupling of myocardial sulfide and PO₂ in trout hearts is evidence of the initial oxygen sensing mechanism in ischemic preconditioning and supports a paracrine/autocrine mode of sulfide signaling.

Perspectives

H₂S is one of the most ancient gases on earth and has accompanied living organisms throughout the course of their evolution. Not surprisingly, this association has often been exploited to the advantage of the organism, and our perception of sulfide biology has changed from viewing it as a toxic gas to a biologically relevant “gastrotansmitter.” The precedent for this was originally established by studies on nitric oxide (NO), and the potential of H₂S as a mediator, like NO, of a variety of physiological and pathological processes has caught the attention of basic scientists and clinicians alike. In the present experiments, we provide additional support for the role of sulfide metabolism as an oxygen “sensor” that may provide the long-sought-after couple in hypoxia-mediated excitation and activation in a variety of cells. However, as we also show, we must temper our exuberance for this novel signaling system while the methodology is refined.

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GRANTS

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