ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 2, 2011

DOI: 10.1089/ars.2010.3525

Free and Acid-Labile Hydrogen Sulfide Concentrations in Mouse Tissues: Anomalously High Free Hydrogen Sulfide in Aortic Tissue

Michael D. Levitt,[1,2] Mohamed Saber Abdel-Rehim,[1] and Julie Furne[1]

Abstract

Endogenously produced hydrogen sulfide is thought to function as an intracellular messenger. There is, however, little information on tissue concentrations of free hydrogen sulfide, the putative messenger form of this molecule, *versus* that of the bound (acid-labile) form. The present report describes the application of a novel technique to measure free and acid-labile hydrogen sulfide in mouse tissues. Very low free hydrogen sulfide concentrations ($<0.050\,\mu$mol/kg) were observed in brain, liver, blood, heart, kidney, striated muscle, and esophagus. Aortic concentrations of free hydrogen sulfide were 20 to 100 times greater than that of the other tissues. Acid-labile hydrogen sulfide concentrations were multiple orders of magnitude greater than that of the free form in every tissue other than aorta. Previous reports of tissue hydrogen sulfide concentrations of 30 to $>100\,\mu$mol/kg measured bound rather than free hydrogen sulfide, the observation that aorta contains anomalously high free hydrogen sulfide concentrations lends support for a vasodilator function for this molecule, and the very low free hydrogen sulfide concentrations in most tissues seemingly requires intermediation of a yet to be described receptor-like mechanism if this molecule is to serve as a gasotransmitter. *Antioxid. Redox Signal.* 15, 373–378.

Introduction

Until recently the biologic importance of hydrogen sulfide was simply that of an environmentally produced, highly toxic, malodorous gas. However, mammalian enzymes cystathionine β-synthase (EC 4.2.22) and cystathionine γ-lyase (CSE; EC 4.4.1.1) have been shown to catalyze the release of hydrogen sulfide from cysteine (23, 24). In the case of cystathionine β-synthase this hydrogen sulfide release occurs *via* condensation of cysteine and homocysteine (22). In addition, it has been proposed that under physiological conditions free hydrogen sulfide can be released from a bound form in the brain (12). Free hydrogen sulfide has been postulated to serve as an endogenously produced gaseous mediator of multiple cellular functions, including neurotransmission (1, 13), smooth muscle function (11, 25), intestinal secretion (21), and inhibition of cellular proliferation (29).

Evidence that hydrogen sulfide acts as an intracellular messenger rests, in part, on a multiplicity of *in vitro* studies showing that tissue function is altered by exposure to NaHS in the tissue bath. At physiological pH, free H_2S is in equilibrium with HS^- (pKa = 6.9) with about 2/3 existing as HS^- and 1/3 as H_2S. In this article, the sum of both molecular forms will be referred to as hydrogen sulfide, whereas the term H_2S will be reserved for the diprotonated, volatile form. In virtually all the *in vitro* studies, a tissue bath hydrogen sulfide concentration of at least 100 μM has been required to produce major changes in tissue function (1, 11, 13, 21, 25, 29). This relatively high hydrogen sulfide concentration has been considered to be physiologic, with multiple reports cited to support the concept that tissue hydrogen sulfide concentrations normally range from 30 to $>100\,\mu M$ (5, 8–10, 15, 18, 20).

Hydrogen sulfide exists in tissue in a free form ($H_2S \rightleftharpoons HS^-$) or in a variety of bound forms, which may be acid-labile or acid-stable. The acid-labile forms include persulfides and iron–sulfur clusters (26). If H_2S is to serve as a gasotransmitter, the concentration of free hydrogen sulfide, as opposed to the bound acid-labile hydrogen sulfide, must be of sufficient magnitude to alter tissue function. Thus, the distinction between bound and free hydrogen sulfide has important physiological implications. In the present report, we describe the application of a new technique to obtain measurements of both free and acid-labile hydrogen sulfide concentrations in single, small tissue specimens obtained from multiple organs of the mouse.

[1]Research Service, Veterans Affairs Medical Center, Minneapolis, Minnesota.
[2]Department of Medicine, University of Minnesota, Minneapolis, Minnesota.

Materials and Methods

Animal Studies were approved by the Animal Care Subcommittee of the Minneapolis Veterans Administration Medical Center. Measurements were carried out on tissues obtained from mice anesthetized with pentobarbital. The tissues and the number of animals studied were as follows: skeletal muscle from the thigh, 4; liver, 4; heart, 4; aorta, 10; kidney, 7; brain, 4; esophagus, 4; and whole blood, 6. (We elected to study the esophagus because this segment of gut was likely to be free of H_2S producing organisms.) No more than four tissue samples were obtained from an individual mouse. The tissues were removed in such an order that the mouse was alive for as long as possible before tissue removal. Thus, samples of muscle, liver, kidney, and esophagus were obtained first followed by tissues whose removal resulted in death (*i.e.*, heart, brain, and aorta). Blood was obtained by cardiac puncture. Tissues were removed within 2 min of death. All manipulations of tissue were carried out in polypropylene containers, and all reagents and containers (tissue grinder, syringes, *etc.*) were precooled to ice water temperature. Immediately after removal, each individual tissue sample was submerged in 0.6 ml of 50 m*M* glycine-NaOH buffer (pH 9.3) contained in a preweighed 1.6 ml micro-centrifuge tube, and the tube was reweighed to obtain the weight of tissue. Tissue weights ranged from 2.0 to 50 mg. The tissues were immediately homogenized in the glycine-NaOH buffer solution using a polypropylene pestle attached to a pellet pestle motor. The homogenate had a pH of 9.0 to 9.1. Two 0.25 ml aliquots of the homogenate were then transferred to 5 ml polypropylene syringes fitted with stopcocks. The syringes were flushed with N_2, and 4.75 ml of N_2 was added to the syringes, which were sealed. The pH of the homogenate was then reduced to 5.8–5.9 *via* addition of 0.25 ml of a 100 m*M* sodium hydrogen phosphate solution (pH 5.5) to the homogenate-containing syringes. The sealed syringes were vigorously mixed for 10 s *via* application to a vortex stirrer, and a 0.3 ml aliquot of the gas space was immediately removed and analyzed for H_2S *via* gas chromatography to determine free hydrogen sulfide concentration. Then, 0.5 ml of 50% trichloroacetic acid was added to the syringe, which was sealed and again vigorously stirred for 10 s, and the H_2S concentration of a 0.3 ml aliquot of gas was assayed to determine the acid-labile hydrogen sulfide concentration present in tissue. Preliminary studies showed that incubation and continued stirring for an additional 2 min after each acidification did not increase the H_2S concentration over that observed at the initial measurement. The blanks for these reactions consisted of 0.25 ml of the glycine-NaOH buffer (without tissue) treated exactly as described for the tissue-containing solution. The concentration of H_2S in the gas space over the tissue-free blank was subtracted from the value observed with tissue to obtain the gas space H_2S attributable to tissue.

Gas chromatography and protein analyses

The concentration of H_2S was determined using a gas chromatograph (Hewlett-Packard) equipped with a chemiluminescence sulfur detector (Sievers, Model 355, Boulder, CO). A 0.3 ml gas sample was injected onto a 2.4-m-long, 3.1-mm-diameter Teflon column packed with Chromosil 330, maintained at 80°C. The carrier gas was nitrogen at a flow rate of 25 ml/min. The concentration of H_2S was determined by comparison to a calibration curve generated with known concentrations of H_2S. As shown in Figure 1A, H_2S elutes more rapidly than do other sulfur gases, and the sensitivity of the detector is such that standards containing H_2S concentrations of 10 parts per billion (ppb) and 2 ppb, respectively, yield peaks roughly 15 and three times that of background noise (Fig. 1B, C). The linearity of the detector response to H_2S falls off by about 20% over a concentration range of 2 to 100 ppb (see Fig. 2). Tissue protein concentration was determined by the Coomassie protein assay (Pierce Chemical Co., Rockford, IL) using bovine serum albumin as a standard.

Calculations

The hydrogen sulfide ($H_2S + HS^-$) that existed in tissue at each of the two pH's of 5.8 and <1 (trichloracetic acid treated) was determined from the volume of H_2S in the gas phase plus the hydrogen sulfide calculated to be dissolved in tissue, which was calculated as follows. The aqueous solubility of H_2S at 4°C is about 4.0 ml of H_2S/ml water/760 torr H_2S, and this value is independent of the pH of the solution (2). The concentration of HS^- in solution for a given concentration of dissolved H_2S was calculated from the pH of the solution and a pKa for $H_2S \leftrightarrow HS^-$ of 6.9 (3). At pH 5.9, the pH of the

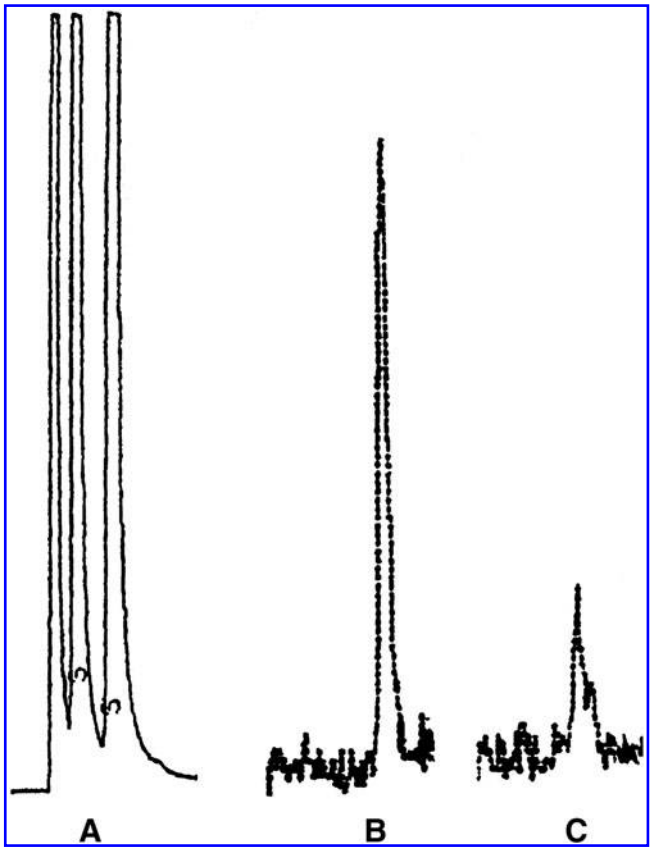

FIG. 1. Gas chromatography tracings of authentic sulfide compounds. (A) shows the separation of volatile sulfur compounds that are thought to exist *in vivo*. From left to right the peaks are H_2S, methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3). H_2S elutes with a retention time of ~30 s. **(B, C)**, respectively, show the peaks obtained with 10 and 2 parts per billion H_2S.

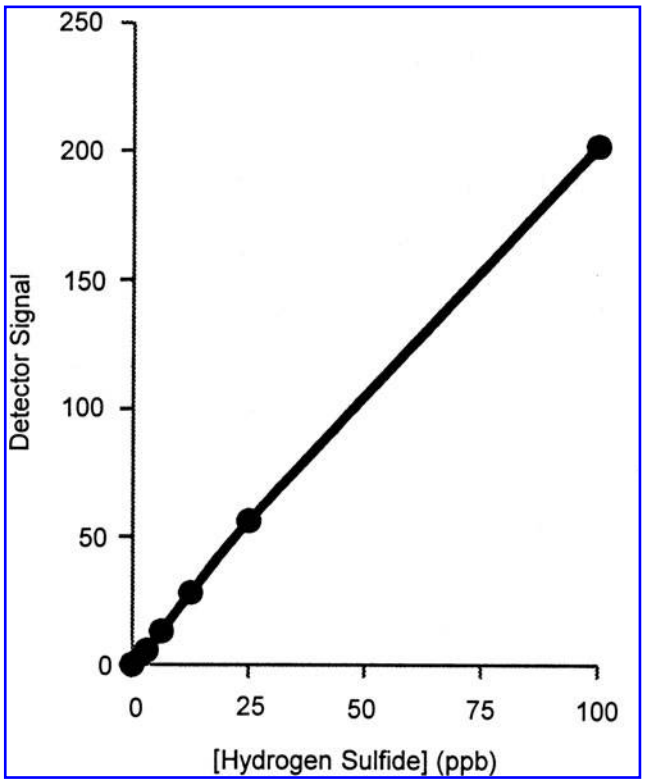

FIG. 2. Linearity of the detector response to H_2S standards ranging from 2 to 100 parts per billion.

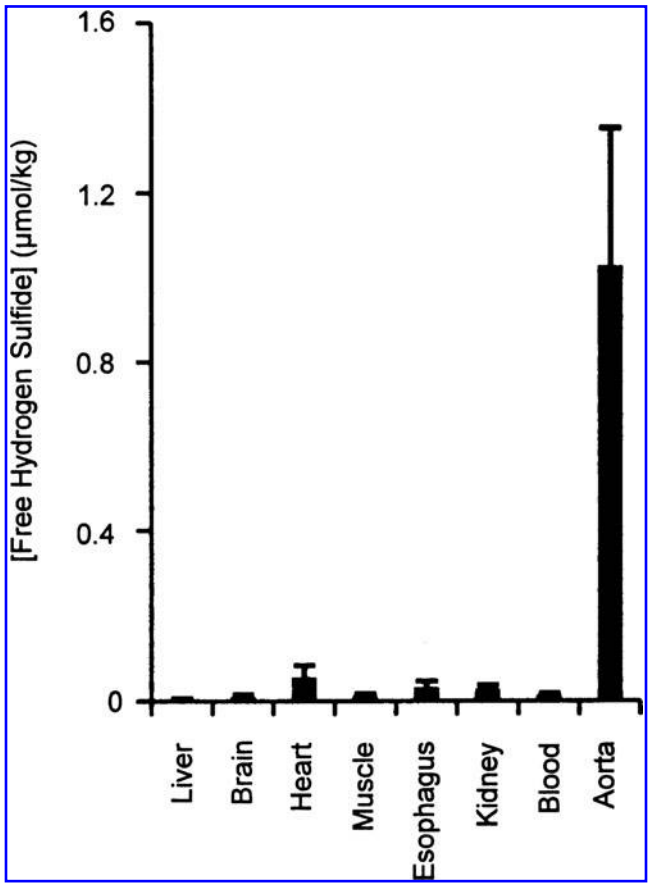

FIG. 3. Free hydrogen sulfide concentration (μmol/kg) in various tissues of the mouse. Data are presented as mean ± standard error of the mean.

mixture of the glycine-NaOH buffer containing homogenate and the phosphate solution, the ratio of H_2S to HS^- in solution is about 9:1. At the very acidic pH of the trichloroacetic acid sample, virtually all dissolved hydrogen sulfide is present as H_2S. Assuming that no acid-labile H_2S is released at pH 5.9 (12), the total free hydrogen sulfide that would exist in tissue under physiological conditions was determined from tissue-derived H_2S at pH 5.9. Acid-labile sulfide was similarly calculated from the H_2S in the gas space after acidification with trichloroacetic acid. These quantities of hydrogen sulfide were normalized for the weight or protein concentration of tissue, and the concentrations of hydrogen sulfide were expressed as μmol/kg tissue or μmol/kg protein. To determine the recovery of a known quantity of free hydrogen sulfide using the above methodology, 0.25 ml of a solution containing 0.030 μM NaHS in glycine-NaOH buffer (50 m*M*, pH 9.3) was transferred into the syringes and the analytical process was then carried out exactly as described for the tissue samples. Quadruplicate analyses showed that the recovery of free hydrogen sulfide averaged 88% ± 5%.

Results

The mean ± standard error of the mean concentrations of free hydrogen sulfide (μmol/kg) in the various tissues are shown in Figure 3, and the acid-labile concentrations are shown in Figure 4. Table 1 presents these data in units of μmol/kg protein. The mean concentrations of free hydrogen sulfide in liver, blood, kidney, esophagus, brain, and heart were all very low, ranging from a low of 0.004 ± 0.002 μmol/kg for liver to 0.055 ± 0.027 μmol/kg for heart. The concentrations in the aorta were quite variable but averaged 1.0 ± 0.30 μmol/kg, 20 to 200 times greater than that of the other tissues. Acid-labile hydrogen sulfide concentrations of the various tissues (see Fig. 4) were several orders of magnitude greater than the free hydrogen sulfide concentrations, ranging from a low of 2.5 ± 1.0 μmol/kg in whole blood to 304 ± 152 μmol/kg in cardiac tissue. When normalized for protein concentration (Table 1), the relative concentrations of hydrogen sulfide of various tissues were similar to data normalized for weight; however, the absolute values were roughly 10-fold higher.

Discussion

In a previous publication (6), we assessed free hydrogen sulfide in mouse brain and liver *via* measurements of the H_2S concentration in the gas space over liver and brain tissue macerated in sealed syringes. The goal of the present study was to measure both the free and the acid-labile hydrogen sulfide concentrations of tissues that could not be macerated (*e.g.*, muscle containing tissues), and therefore required tissue homogenization.

Several factors can influence the accuracy of assays that purport to measure the free hydrogen sulfide concentration of tissue. First, H_2S rapidly diffuses from the biopsied tissue sample into the atmosphere. To eliminate such diffusion, tissue samples were immediately immersed in and homogenized in

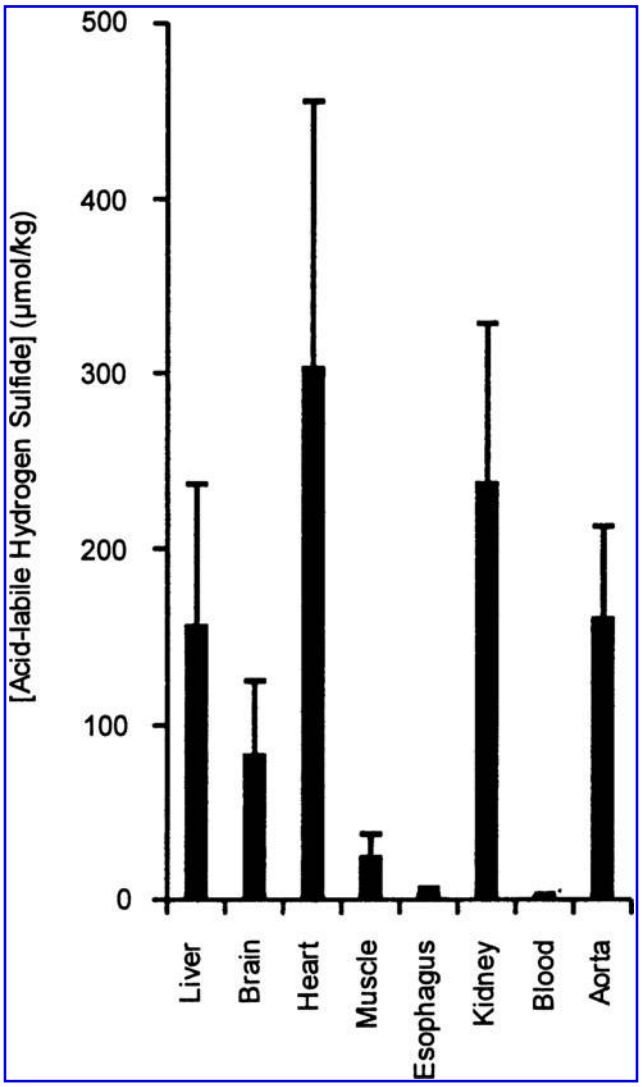

FIG. 4. Acid-labile hydrogen sulfide concentration (μmol/kg) in various tissues of the mouse. Data are presented as mean ± standard error of the mean.

Table 1. Mean ± Standard Error of the Mean of Free and Acid-Labile Hydrogen Sulfide Concentrations of Tissues Normalized for Protein Content

Tissue	*Free hydrogen sulfide (μmol/kg protein)*	*Acid-labile hydrogen sulfide (μmol/kg protein)*
Liver	0.03 ± 0.02	1090 ± 544
Brain	0.12 ± 0.06	916 ± 458
Heart	0.39 ± 0.20	2160 ± 1078
Skeletal muscle	0.09 ± 0.04	192 ± 96.8
Esophagus	0.28 ± 0.14	42.1 ± 21.0
Kidney	0.24 ± 0.09	2120 ± 808
Blood	0.07 ± 0.03	12.6 ± 5.15
Aorta	9.20 ± 2.9	1460 ± 461

a basic solution such that H_2S diffusing from the tissue would be trapped as HS^-. Second, tissues have the capability of enzymatically producing (23, 24) and catabolizing (7) free H_2S, with the balance between these two reactions determining the tissue concentration of free hydrogen sulfide. Alteration of this balance during the analytical technique influences the accuracy of the estimation of the free hydrogen sulfide concentration that existed *in vivo*. To minimize such alterations, the entire analytical process for free hydrogen sulfide was carried out rapidly (over a several minute period) at 4°C, a temperature that should reduce both the production and consumption rates. Third, hydrogen sulfide tends to rapidly react with glass and metal; thus, all procedures were carried out in vessels made of polypropylene, which we have shown to be relatively unreactive with hydrogen sulfide (14). Lastly, free hydrogen sulfide must be differentiated from the bound forms of this compound. In the present study, H_2S measurements of the gas space over the homogenate were first carried out at pH 5.9, a pH at which the majority of free hydrogen sulfide exists as H_2S but at which acid-labile hydrogen sulfide remains intact (12). Subsequent strong acidification of the homogenate then released acid-labile hydrogen sulfide, making possible measurement of the acid-labile hydrogen sulfide concentrations in the same tissue sample.

Using a somewhat different technique than was employed in the present study, we previously found that the free hydrogen sulfide concentrations of liver and brain were only about 0.015 μmol/kg (6). In addition, the nearly unmeasurable concentrations of H_2S we found in human expired air suggested that the free H_2S concentration of human blood similarly must be very low. In the present study, we once again observed very low free hydrogen sulfide concentrations (mean values <0.011 μmol/kg) in liver, brain, and blood (see Fig. 3). Low concentrations also were observed in cardiac and striated muscle, kidney, and esophagus with the highest value (0.055 μmol/kg) observed in cardiac muscle. Although there was appreciable variation between animals, the mean hydrogen sulfide concentration of aortic tissue (1.02 ± 0.32 μmol/kg) was 20 to 100 times greater than that observed for the other tissues.

In contrast to the nanomolar concentrations of free hydrogen sulfide found in most tissues, the concentrations of the bound, acid-labile form of this molecule was present in micromolar concentrations, ranging from 2.3 μmol/kg in blood to 300 μmol/kg in cardiac tissue (Fig. 4). In all tissues other than aorta, bound exceeded free hydrogen sulfide concentration by at least three orders of magnitude. Thus, it seems likely that the claims that the messenger form of H_2S in various tissues ranges in concentration from 30 to >100 μ*M* actually reflect measurements of bound, acid-labile hydrogen sulfide. In addition, H_2S synthesis by tissue commonly is measured using a technique in which trichloroacetic acid is used to stop the reaction at the end of the incubation period (19, 27). The addition of trichloroacetic acid releases the acid-labile sulfide in the tissue and, thus, erroneously elevates the quantity of free H_2S calculated to have been synthesized during the incubation.

A novel observation of the present study was the finding of relatively high free hydrogen sulfide concentrations in aortic tissue relative to that of other tissues. The very low concentrations of hydrogen sulfide in blood exclude the possibility

that blood contamination accounted for these high aortic concentrations. Although it seems likely that smooth muscle, the predominant component of the aorta, contained the hydrogen sulfide, further studies will be required to evaluate this supposition. The uniquely high free hydrogen sulfide concentration of aortic tissue suggests that this molecule may play a role in the function of this tissue, presumably as a signaling molecule. Such a messenger role for hydrogen sulfide in vascular tissue is supported by a variety of *in vitro* studies, indicating that hydrogen sulfide induces vasodilatation and an *in vivo* study demonstrating that administration of cysteine (a substrate for H_2S synthesis by CSE) causes vasodilatation, whereas proparglycine (an inhibitor of H_2S synthesis by CSE) causes vasoconstriction (4). In addition, Yang *et al.* (30) provided strong support for a physiological role for H_2S by demonstrating that hypertension developed in mice with a targeted deletion of the CSE encoding gene, a deletion that should reduce H_2S production from cysteine. In support of the concept that hydrogen sulfide production was decreased in their knockout mice, the authors reported that wild-type mice had a blood H_2S level of $\sim 40\,\mu M$, with concentrations of ~ 32 and $19\,\mu M$ observed in $CSE^{-/+}$ and $CSE^{-/-}$ mice. However, these blood concentrations are >1000-fold higher than the $0.013\,\mu$mol/kg concentration we measured for free hydrogen sulfide concentration in blood and exceed the value ($2.5\,\mu$mol/kg) we observed for acid-labile hydrogen sulfide concentration in blood. This discrepancy presumably is attributable to methodological differences in that Yang *et al.* employed an ion selective electrode to measure hydrogen sulfide. A priori, it appears that this electrode was assessing something other than free hydrogen sulfide since the odor of a solution containing $40\,\mu M$ free hydrogen sulfide would be clearly discernible by the human nose (6), and we are unaware of reports of blood possessing a strong H_2S odor.

Although most *in vitro* studies have required at least a $100\,\mu M$ hydrogen sulfide concentration to induce alterations in tissue function, it has been demonstrated that a $1\,\mu M$ concentration of hydrogen sulfide is sufficient to partially inhibit cytochrome C oxidase (17). Thus, there is evidence that the *in vivo* concentration of hydrogen sulfide we observed in aortic tissue ($1\,\mu$mol/kg) could directly influence tissue metabolism and function. We are unaware of data showing that the very low concentrations of free hydrogen sulfide (<0.05 μmol/kg) observed in all tissues other than aorta can directly influence metabolic pathways, which raises a question as to the potential for this molecule to serve as a gasotransmitter in these tissues. If hydrogen sulfide is to serve as an intracellular messenger in nonaortic tissue, it seems necessary to postulate that free H_2S/HS^- be released in proximity to some sort of receptor mechanism, which in turn activates biological activity. In this situation, hydrogen sulfide could produce a signal, while tissue catabolism would maintain the overall tissue hydrogen sulfide concentrations at very low levels, such as is the case with acetylcholine (16, 28). Confirmation of this postulate awaits the demonstration of a receptor-like mechanism for hydrogen sulfide.

Author Disclosure Statement

No competing financial interests exist.

References

1. Abe K and Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* 16: 1066–1071, 1996.
2. Carroll JJ and Mather AE. The solubility of hydrogen sulfide in water from 0 to 90°C and pressures to 1 Mpa. *Geochim Cosmochim Acta* 53: 1163–1170, 1989.
3. Cotton FA and Wilkinson G. *Advanced Inorganic Chemistry*, 5th edition. New York: Wiley, 1988, p. 1455.
4. d'Emmananuele di Villa Bianca R, Soorentine R, and Maffia P. Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation. *Proc Nat Acad Sci USA* 106: 4513–4518, 2009.
5. Dorman DC, Moulin FJ, McManus BE, Mahle KC, James RA, and Struve MF. Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. *Toxicol Sci* 65: 18–25, 2002.
6. Furne J, Saeed A, and Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am J Physiol* 295: R1479–R1485, 2008.
7. Furne J, Springfield J, Koenig T, DeMaster E, and Levitt MD. Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized function of the colonic mucosa. *Biochem Pharmacol* 62: 255–259, 2001.
8. Goodwin LR, Francom D, Dieken FP, Taylor JD, and Warenycia MW. Determination of sulfide in brain tissue by gas dialysis/ion chromatography: postmortem studies and two case reports. *J Anal Toxicol* 13: 105–109, 1989.
9. Hammerstad U, Margheri S, and Sorbo B. A sensitive gas chromatographic method for determination of protein-associated sulfur. *Anal Biochem* 178: 394–398, 1989.
10. Han Y, Oin J, Chang X, Yang Z, and Du J. Hydrogen sulfide and carbon monoxide are in synergy with each other in the pathogenesis of recurrent febrile seizures. *Cell Mol Neurobiol* 26: 101–107, 2006.
11. Huang SM, Chowdhury JU, Kobayashi K, and Tomita T. Inhibitory effects of cyanide on mechanical and electrical activities in the circular muscle of gastric antrum of guinea pig stomach. *Jpn J Physiol* 43: 229–238, 1993.
12. Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, and Kimura H. A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid Redox Signal* 11: 205–214, 2009.
13. Kombian SB, Reiffenstein RJ, and Colmers WF. The actions of hydrogen sulfide on dorsal raphe serotonergic neurons *in vitro*. *J Neuorphysiol* 70: 81–96, 1993.
14. Levitt MD, Furne J, Springfield J, Suarez F, and DeMaster E. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. *J Clin Invest* 104: 1107–1114, 1999.
15. Mitchell TW, Savage JC, and Gould DH. High-performance liquid chromatography detection of sulfide in tissues from sulfide-treated mice. *J Appl Toxicol* 13: 389–394, 1993.
16. Nelson ME, Kuryatov A, Choi CH, Shou Y, and Lindstrom J. Alternate stoichiometries of alpha4beta2 nicotinic acetylcholine receptors. *Mol Pharmacol* 63: 332–341, 2003.
17. Nicholson RA, Roth SH, Zhang A, Zheng J, Brookes J, Skrajny B, and Bennington R. Inhibition of respiratory and bioenergetic mechanisms by hydrogen sulfide in mammalian brain. *J Toxicol Environ Health* 54: 491–507, 1998.
18. Ogasawara Y, Isoda S, and Tanabe S. Tissue and subcellular distribution of bound and acid-labile sulfur, and the enzymic capacity for sulfide production in the rat. *Biol Pharm Bull* 17: 1535–1542, 1994.

19. Qu K, Chen CPLH, Halliwell B, Moore PK, and Wong PTH. Hydrogen sulfide is a mediator of cerebral ischemic damage. *Stroke* 37: 889–893, 2006.
20. Savage JC and Gould DH. Determination of sulfide in brain tissue and rumen fluid by ion-interaction, reverse-phase, high-performance, liquid chromatography. *J Chromatogr* 526: 540–545, 1990.
21. Schicho R, Krueger D, Zeller F, Von Weyhern CW, Frieling T, Kimura H, Ishii I, De Giorgio R, Campi B, and Schemann M. Hydrogen sulfide is a novel prosecretory neuromodulator in the guinea-pig and human colon. *Gastroenterology* 131: 1542–1552, 2006.
22. Singh S, Padovani D, Leslie RA, Chiku T, and Banerjee R. The relative contributions of cystathionine beta-synthase and gamma-cystathionase to H_2S biogenesis via alternative transsulfuration reactions. *J Biol Chem* 284: 22457–22466, 2009.
23. Stipanuk MH and Beck PW. Characterization of the enzymic capacity for L-cysteine desulphydration in liver and kidney of the rat. *Biochem J* 206: 267–277, 1982.
24. Stipanuk MH, Londono M, Lee JI, Hu M, and Yu AF. Enzymes and metabolites of cysteine metabolism in non-hepatic tissues of rats show little response to changes in dietary protein or sulfur amino acid levels. *J Nutr* 132: 3369–3378, 2002.
25. Tang G, Wu L, Liang W, and Wang R. Direction stimulation of KATP channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. *Mol Pharmacol* 68: 1757–1764, 2005.
26. Ubuka T. Assay methods and biological roles of labile sulfur in animal tissues. *J Chromatogr B* 781: 227–249, 2002.
27. Wallace JL, Vong L, McKnight W, Dicay M, and Martin GR. Endogenous and exogenous hydrogen sulfide promotes resolution of colitis in rats. *Gastroenterology* 137: 569–578, 2009.
28. Yamada H, Otsuka M, Fujimoto K, Kawashima K, and Yoshida M. Determination of acetylcholine concentration in cerebrospinal fluid of patients with neurologic diseases. *Acta Neurol Scand* 93: 76–78, 2009.
29. Yang G, Cao K, Wu L, and Wang R. Cystathionine γ-lyase overexpression inhibits cell proliferation via a H_2S-dependent modulation of ERK1/2 phosphorylation and p21Cip/WAK-1. *J Biol Chem* 279: 49199–49205, 2004.
30. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, and Wang R. H_2S as a physiological vasorelaxant: hypertension in mice with deletion of cystathionine γ-lyase. *Science* 322: 587–590, 2008.

Address correspondence to:
Dr. Michael D. Levitt
Research Service
Veterans Affairs Medical Center
VAMC/111D
1 Veterans Drive
Minneapolis, MN 55417

E-mail: michael.levitt@va.gov

Date of first submission to ARS Central, July 25, 2010; date of acceptance, September 2, 2010.

Abbreviations Used

CSE = cystathionine γ-lyase
EC = enzyme commission
pKa = acid dissociation constant
ppb = parts per billion

This article has been cited by:

1. Alessandra F. Perna, Diego IngrossoHomocysteine and Hydrogen Sulfide, Two Opposing Aspects in the Pathobiology of Sulfur Compounds in Chronic Renal Failure 109-123. [CrossRef]
2. Bindu D. Paul, Solomon H. Snyder. 2012. H2S signalling through protein sulfhydration and beyond. *Nature Reviews Molecular Cell Biology* . [CrossRef]
3. Yi-Hong Liu , Ming Lu , Li-Fang Hu , Peter T.-H. Wong , George D. Webb , Jin-Song Bian . 2012. Hydrogen Sulfide in the Mammalian Cardiovascular System. *Antioxidants & Redox Signaling* **17**:1, 141-185. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Victor Vitvitsky , Omer Kabil , Ruma Banerjee . 2012. High Turnover Rates for Hydrogen Sulfide Allow for Rapid Regulation of Its Tissue Concentrations. *Antioxidants & Redox Signaling* **17**:1, 22-31. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
5. Csaba Szabo . 2012. Roles of Hydrogen Sulfide in the Pathogenesis of Diabetes Mellitus and Its Complications. *Antioxidants & Redox Signaling* **17**:1, 68-80. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Jerzy Be#towski , Anna Jamroz-Wi#niewska . 2012. Modulation of H2S Metabolism by Statins: A New Aspect of Cardiovascular Pharmacology. *Antioxidants & Redox Signaling* **17**:1, 81-94. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Kenneth R. Olson . 2012. A Practical Look at the Chemistry and Biology of Hydrogen Sulfide. *Antioxidants & Redox Signaling* **17**:1, 32-44. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Hideo Kimura , Norihiro Shibuya , Yuka Kimura . 2012. Hydrogen Sulfide Is a Signaling Molecule and a Cytoprotectant. *Antioxidants & Redox Signaling* **17**:1, 45-57. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
9. Adam Faccenda, Jingyuan Wang, Bulent Mutus. 2012. Polydimethylsiloxane Permeability-Based Method for the Continuous and Specific Detection of Hydrogen Sulfide. *Analytical Chemistry* 120605083950009. [CrossRef]
10. Xinggui Shen, Elvis A. Peter, Shyamal Bir, Rui Wang, Christopher G. Kevil. 2012. Analytical measurement of discrete hydrogen sulfide pools in biological specimens. *Free Radical Biology and Medicine* **52**:11-12, 2276-2283. [CrossRef]
11. A. F. Perna, D. Ingrosso. 2012. Low hydrogen sulphide and chronic kidney disease: a dangerous liaison. *Nephrology Dialysis Transplantation* **27**:2, 486-493. [CrossRef]
12. Guangdong Yang, Hongzhu Li, Guanghua Tang, Lingyun Wu, Kexin Zhao, Qiuhui Cao, Changqing Xu, Rui Wang. 2011. Increased neointimal formation in cystathionine gamma-lyase deficient mice: Role of hydrogen sulfide in #5#1-integrin and matrix metalloproteinase-2 expression in smooth muscle cells. *Journal of Molecular and Cellular Cardiology* . [CrossRef]
13. Sangita Singh, Ruma Banerjee. 2011. PLP-dependent H2S biogenesis. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1814**:11, 1518-1527. [CrossRef]
14. Philippe Haouzi, Harold Bell, Andry Van de Louw. 2011. Hypoxia-induced arterial chemoreceptor stimulation and Hydrogen sulfide: Too much or too little?. *Respiratory Physiology & Neurobiology* . [CrossRef]
15. Eric R. DeLeon, Gilbrian F. Stoy, Kenneth R. Olson. 2011. Passive loss of hydrogen sulfide in biological experiments. *Analytical Biochemistry* . [CrossRef]
16. D. R. Linden, J. Furne, G. J. Stoltz, M. S. Abdel-Rehim, M. D. Levitt, J. H. Szurszewski. 2011. Sulfide quinone reductase contributes to hydrogen sulfide metabolism in murine peripheral tissues but not in the central nervous system. *British Journal of Pharmacology* no-no. [CrossRef]
17. Philippe Haouzi, Harold Bell, Maeve Philmon. 2011. Hydrogen sulfide oxidation and the arterial chemoreflex: Effect of methemoglobin. *Respiratory Physiology & Neurobiology* **177**:3, 273-283. [CrossRef]
18. Ruma Banerjee . 2011. Hydrogen Sulfide: Redox Metabolism and Signaling. *Antioxidants & Redox Signaling* **15**:2, 339-341. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
19. Erzsébet Zavaczki, Viktória Jeney, Anupam Agarwal, Abolfazl Zarjou, Melinda Oros, Mónika Katkó, Zsuzsa Varga, György Balla, József Balla. 2011. Hydrogen sulfide inhibits the calcification and osteoblastic differentiation of vascular smooth muscle cells. *Kidney International* . [CrossRef]