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Hydrogen Sulfide: Redox Metabolism and Signaling

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Abstract

The recognition of hydrogen sulfide (H_2S) as an endogenously produced gas with signaling potential has stimulated research on a multitude of physiological effects mediated in the cardiovascular, immune, gastrointestinal, genitourinary, endocrine, and central nervous systems. The heightened activity in the area of H_2S biology led to convening of the first international conference on H_2S in Shanghai in the summer of 2009 and to two Forum issues published in 2010 by *Antioxidants & Redox Signaling* on the physiological effects of H_2S. Yet, fundamental questions regarding the biogenesis and regulation of H_2S, the bioenergetics of its catabolism, its tissue concentrations, and elucidation of its molecular targets remain. Some of these issues are the subject of the current Forum on H_2S. *Antioxid. Redox Signal.* 15, 339–341.

Reactions in the sulfur metabolic pathway involving the amino acids cysteine and homocysteine are potential sources of hydrogen sulfide (H_2S) (4, 28). These include the two enzymes in the transsulfuration pathway, cystathionine β-synthase (CBS), and γ-cystathionase (CSE), which successively convert serine and homocysteine to cystathionine and cystathionine, to cysteine. Lax substrate specificity creates the potential for a multitude of alternative reactions to be catalyzed by both enzymes leading to H_2S generation from either cysteine or homocysteine or both (6, 7, 27). A third potential route for H_2S generation is *via* the combined actions of cysteine aminotransferase and mercaptopyruvate sulfurtransferase (26), enzymes involved in cysteine catabolism. However, the product of coupling these two enzymes is persulfide rather than H_2S, which can be liberated in the presence of reductant (15).

An efficient mitochondrial pathway for H_2S oxidation exists (10) and is important for holding steady-state tissue levels of H_2S at very low values. This has led to the hypothesis that H_2S might function as an oxygen sensor since, under conditions of hypoxia, the catabolic removal of H_2S would be impeded, leading to augmented H_2S levels and activation of its signaling responses (23). Similarities between the effects of H_2S and hypoxia and enhancement and abrogation of the hypoxic responses by H_2S precursors and H_2S synthesis inhibitors, respectively, support the proposal that H_2S might be involved in oxygen sensing (23).

The discovery of H_2S as a physiological mediator was made by Abe and Kimura, who first reported its neuormodulatory effects (1) and soon thereafter, its vasorelaxant effect on smooth muscle (11). Today, a plethora of physiological effects are associated with H_2S with some of the major links being to the cardiovascular and central nervous systems and to inflammation. H_2S modulates a range of cardiovascular effects, including cardioprotection against ischemic and myocardial reperfusion injury and vascular contraction or relaxation (9, 23, 25, 29). The endothelial-derived relaxing factor activity of H_2S in mice lacking the CSE gene is controversial, with two groups reporting conflicting results. Thus, Wang and coworkers reported marked age-dependent hypertension (30), whereas Ishii and coworkers reported a normotensive phenotype in the CSE knockout mice (14). H_2S stimulates opening of K_{ATP} channels, suggesting a mechanism for its vasorelaxant effects in addition to its reported involvement in the Nrf2 and ERK and PI3K/Akt signaling pathways (5, 13, 31). In brain, H_2S has varied effects ranging from enhancing hippocampal long-term potentiation (1), to triggering calcium waves and increasing intracellular calcium levels in astrocytes (22), to increased neuroprotection by stimulating glutathione synthesis (19, 20). A possible mechanism for neuromodulation by H_2S involves induction of cyclic AMP synthesis and activating N-methyl D-aspartate-receptor-mediated excitatory postsynaptic currents (18). Both pro- and anti-inflammatory effects have been ascribed to H_2S and efforts are underway to develop the therapeutic potential of H_2S-releasing drugs to counter inflammatory diseases.

Large discrepancies have been reported in the literature on tissue concentrations of H_2S and H_2S production rates in the presence of exogenous substrate. These stem in part from the technical difficulties associated with handling H_2S, which is redox sensitive, and the considerably larger tissue stores of sulfane sulfur and acid-labile sulfur that can be released if adequate precautions are not taken during sample preparation. Additionally, the complexity in utilization of cysteine

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and/or homocysteine for H_2S generation by CBS and CSE leads to a significant underestimation of H_2S production rates and bias against CBS when only cysteine is applied as substrate, which is commonly the case in the field. Finally, the use of compounds such as hydroxylamine and aminooxyacetate that inhibit many pyridoxal phosphate enzymes and are not specific for CBS or CSE produces results that are not readily interpretable. Two articles in this Forum address these quantitative issues surrounding H_2S determination. In one, Levitt and coworkers report that steady-state tissue levels of H_2S are negligibly low with the exception of aorta, which exhibits 20–100 times higher H_2S concentrations (21). In the other article, the author's group uses quantitative Western blot analyses to determine the absolute protein levels of CBS and CSE in liver and kidney and reports the tissue H_2S production capacity in the presence of saturating concentrations of cysteine and homocysteine used alone or in combination as substrates (16). These studies reveal the differential importance of CBS versus CSE in various tissues. From simulations of the liver data adjusted for the difference in protein levels of CBS and CSE, it is estimated that CSE accounts for ~96% of H_2S production at physiologically relevant concentrations of substrate.

Bouillaud and coworkers discuss sulfide bioenergetics in the context of the mitochondrial catabolic pathway and the similarities between cyanide and H_2S inhibition of cytochrome c oxidase (3). They also discuss potential mechanisms for neutralizing sulfide particularly as it pertains to the biology of colonocytes, cells that are routinely exposed to high sulfide concentrations, and to the invertebrates living in sulfide-rich habitats. The first enzyme in the H_2S oxidation pathway, sulfide quinone reductase, has a very high affinity for sulfide, and oxidation activity can be detected at intracellular sulfide concentrations $\geq$10–20 nM, thus protecting cytochrome c oxidase from inhibition. These data also argue against significant steady-state H_2S levels in tissues under normoxic conditions. However, when oxygen concentrations are limiting, H_2S levels are expected to rise leading to inhibition of cytochrome c oxidase concomitant with enhanced mitochondrial reactive oxygen species generation, which is in fact observed under hypoxic conditions.

Tiranti and coworkers discuss the effects of chronic sulfide exposure as seen in the inherited disorder, ethylmalonic encephalophathy, on degradation of cytochrome c oxidase (8). Ethylmalonic encephalopathy results from mutations in *Ethe*1, the gene encoding the second enzyme in the mitochondrial sulfide oxidation pathway, sulfur dioxygenase. Using tissue-specific conditional *Ethe*1 knockouts, the authors demonstrate that the cytochrome c oxidase deficiency is limited to the tissues targeted for ablation. Hence, H_2S acts locally in tissue with disrupted *Ethe*1, leading to *heme a* inhibition and enhanced degradation of cytochrome c oxidase subunits.

López-Garriga and co-workers (24) discuss hemeproteins such as cyctochrome c oxidase, myoglobin, and hemoglobin as targets for H_2S. Inhibition of cytochrome c oxidase is responsible for decreasing metabolic activity and inducing a hibernation-like state (2). The basis for the differential reactivity of H_2S with hemeproteins that results in formation of stable hexacoordinate low spin Fe^{III}-SH_2 complex versus iron reduction and conversion to an unligated Fe^{II} heme and the influence of the iron oxidation state are discussed.

The remaining two articles focus on the neurophysiological effects of H_2S. One, a comprehensive review by Jin-Song and colleagues (12), covers the current state of our understanding of the neurophysiology and neuropathology of H_2S, discussing the underlying cellular mechanisms and the neuroprotective effects of this gaseous mediator. The original research article by Ichinose and coworkers examines the protective effects of H_2S on a murine model of Parkinson's disease induced by the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (17). They demonstrate that the neuroprotective effects of H_2S are correlated with induction of antioxidant genes, including glutamate cysteine ligase and heme oxygenase-1.

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Abbreviations Used

CBS = cystathionine β-synthase
CSE = cystathionase
H_2S = hydrogen sulfide