

SPECIAL REPORTS AND REVIEWS

The Emerging Roles of Hydrogen Sulfide in the Gastrointestinal Tract and Liver

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Hydrogen sulfide, like nitric oxide, was best known as a toxic pollutant before becoming recognized as a key regulator of several physiologic processes. In recent years, evidence has accumulated to suggest important roles for hydrogen sulfide as a mediator of several aspects of gastrointestinal and liver function. Moreover, alterations in hydrogen sulfide production could contribute to disorders of the gastrointestinal tract and liver. For example, nonsteroidal anti-inflammatory drugs can reduce production of hydrogen sulfide in the stomach, and this has been shown to contribute to the generation of mucosal injury. Hydrogen sulfide has also been shown to play a key role in modulation of visceral hyperalgesia. Inhibitors of hydrogen sulfide synthesis and drugs that can generate safe levels of hydrogen sulfide in vivo have been developed and are permitting interventional studies in experimental models and, in the near future, humans.

Small molecular weight gases have been among the most studied biologic mediators over the past 20 years. Nitric oxide is now recognized as one of the most important such mediators in the human body, mediating blood flow, neurotransmission, immune reactions, and muscle contraction. The importance of nitric oxide (NO) in this regard was recognized by the award of a Nobel Prize to Furchgott, Murad, and Ignarro in 1998. Hydrogen sulfide (H₂S) is the latest gas to be recognized as an important endogenous mediator. As was initially the case for NO, the toxic effects of H₂S were recognized well before its physiologic roles became apparent. Also similar to the case of NO, H₂S is being implicated both as an agent preventing tissue damage and inflammation and as an agent causing tissue damage and inflammation. Undoubtedly, the parallels to NO will continue to unfold, and it will become clear that the roles played by H₂S in various tissues and in various circumstances largely depend on its local concentration. In the context of the digestive system, roles for hydrogen sulfide in maintenance of mucosal integrity, regulation of blood flow, and

modulation of inflammatory reactions are emerging. In this report, we review the endogenous production of H₂S and its regulation and the evidence suggesting roles of this mediator in various disorders. In some cases, administration of H₂S-releasing agents may have therapeutic benefit, whereas, in others, inhibition of the synthesis of this gas shows some promise in experimental models.

Chemistry, Toxicity, and Synthesis

Hydrogen sulfide is a colorless gas with a strong odor that has been widely studied in the context of water and industrial air pollution. Its production is mainly associated with the pulp and paper industry, petroleum refineries, tanneries, and mining. As described in detail later in this article, H₂S can perform many physiologic functions and is produced in many tissues of the body. Toxicity of H₂S is seen at concentrations well above those produced endogenously and is usually associated with the presence of those high concentrations in the lung. At concentrations in excess of 250 ppm, H₂S can cause pulmonary edema, and, with exposure to concentrations in excess of 1000 ppm, coma and death can ensue.¹

H₂S is a weak diprotic acid that dissociates in 2 steps:



In aqueous solution, approximately one third of the gas is in the nondissociated form. H₂S is permeable to plasma membranes because its solubility in lipophilic solvents is 5-fold greater than in water.^{2–4}

Abbreviations used in this paper: ASC, alanine, serine, and cysteine-preferring; CBS, cystathionine β-synthase; CSE, cystathionine-γ-lyase; COX, cyclooxygenase; NSAID, nonsteroidal anti-inflammatory drug; PLP, pyridoxal 5'-phosphate; SAM, S-adenosylmethionine.

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0016-5085/06/\$32.00

doi:10.1053/j.gastro.2006.02.033

Table 1. Concentrations and Rates of Generation of H₂S in Various Tissues and Cells

Species	Tissue	Concentration	Generation	Reference
Rat	Whole brain	87.2 nmol/g		54
	Brainstem	68.3 nmol/g		108
	Cerebellum	87.8 nmol/g		109
	Hippocampus	97.8 nmol/g		109
	Striatum	102.8 nmol/g		109
	Ileum		6.5 nmol/min/g	6
	Liver	26.0 nmol/g		110
	Kidney	39.9 nmol/g		110
	Heart	129.3 nmol/g		110
	Spleen	12.5 nmol/g		110
	Lung	30.0 nmol/g		111
	Plasma	0.4 nmol/g		108
	Erythrocytes	0.2 nmol/g		108
	Mesenteric aorta		3 nmol/min/g	6
	Tail artery		8 nmol/min/g	6
	Aorta		3 nmol/min/g	6
Man	Serum	46 μmol/L		6
	Brainstem	38.3 nmol/g		54
	Serum	1.2 μmol/L		112

NOTE: A variety of different analytical methods were used to detect H₂S in the cited studies.

Aside from that present in the circulation, a significant amount of H₂S is produced in various tissues (Table 1). Sulfur is a chemically and biologically active element. Sulfur compounds in tissues can be present in stable and labile forms. Compounds such as methionine, cysteine, and taurine are stable sulfur compounds, as opposed to acid-labile sulfur and sulfane sulfur compounds.⁵ One of the major sources of labile sulfur in mammalian tissue is cysteine. Cysteine is a substrate for 2 pyridoxal-5'-phosphate-dependent enzymes: cystathionine β-synthase (CBS; EC 4.2.1.22) and cystathionine γ-lyase (CSE; EC 4.4.1.1).³ These enzymes are responsible for the majority of the endogenous production of H₂S in mammalian tissues. Deficiency of CBS is the major cause of inherited homocystinuria. Another source of H₂S may be the erythrocyte because it has been shown that washed human erythrocytes incubated with glucose and elemental sulfur produce H₂S at a fairly constant rate.⁴ Thus, because labile sulfur and glucose are both available in circulating blood, it is feasible that this pathway could play a role in producing H₂S through nonenzymatic reactions.⁵

H₂S is present in micromolar concentrations in blood.^{6–8} Given the potential toxicity of this gas, efficient systems exist to metabolize and scavenge H₂S in vivo. H₂S is metabolized by oxidation in mitochondria or by methylation in cytosol. It can be scavenged by methemoglobin or by oxidized glutathione. H₂S is excreted mainly by the kidney as free or conjugated sulfate.³ The fact that H₂S interacts with hemoglobin, as do 2 other key gaseous transmitters, suggests a possible interplay among these 3 mediators (discussed in more detail be-

low). Interestingly, it has been shown that H₂S poisoning in animals is prevented by nitrite administration, suggesting that nitrite-induced methemoglobinemia has an antidotal effect against H₂S intoxication. This procedure has been successfully used in the resuscitation of several victims of H₂S poisoning.^{9,10}

Sulfur Amino Acid Metabolism

Sulfur-containing amino acids are the key precursors for endogenous H₂S synthesis. They are also important factors for cellular stability—through their antioxidant and scavenging properties—and have many metabolic functions. Because of their relevance as regulatory intermediates in key homeostatic functions, altered sulfur amino acid metabolism is increasingly recognized as a causative factor in a number of disorders.^{11,12} The factors that influence sulfur amino acid metabolism can also affect the production of H₂S. Sulfur amino acid metabolism in mammals is regulated through the balance of production and disposal of cellular homocysteine and cysteine (Figure 1).

The transsulfuration pathway is only one source of cysteine. Other important sources are dietary intake and protein degradation. In extracellular fluids, cysteine is predominantly found in a dimerized state (cystine), whereas, inside the cell, it is rapidly converted into the monosulfhydryl form.³ Both cysteine and cystine have specific transport systems. Cysteine is transported mainly by the alanine, serine, and cysteine-preferring (ASC) system,¹³ a Na⁺-dependent transporter that is specific for neutral amino acids with short- to intermediate-length side chains. The ASC system mediates both inward and

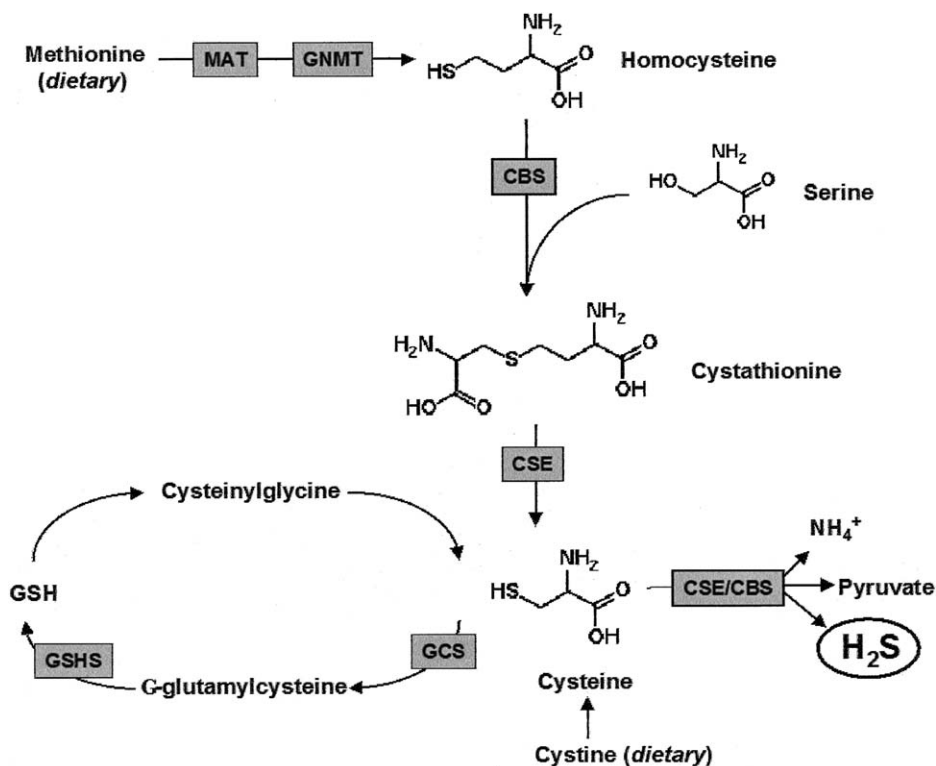


Figure 1. Endogenous pathways of hydrogen sulfide production. Methionine, an essential amino acid, is obtained by dietary intake and transported to the liver mainly by the System L.¹³ Methionine adenosyltransferase (MAT) catalyzes the ATP-dependent conversion of methionine to S-adenosylmethionine. S-adenosylmethionine is converted to S-adenosylhomocysteine by the glycine N-methyltransferase (GNMT) and then hydrolyzed to homocysteine. CBS (cystathionine β-synthase), which is regulated by S-adenosylmethionine,^{16,107} catalyzes the production of cystathionine by adding serine to homocysteine. Cystathionine can then be converted to cysteine via CSE (cystathionine-γ-lyase). Cysteine, which can also be produced via reduction of dietary cystine, can then be converted to ammonium, pyruvate, and H₂S via the actions of either CSE or CBS. GSH, glutathione; GSHS, glutathione synthetase; GCS, γ-glutamylcystine synthetase.

outward flows of its substrate amino acids and is subject to *trans*-stimulation.¹³ Therefore, intracellular cysteine concentrations depend on the intracellular and extracellular levels of not only cysteine but also of other system ASC amino acids. Elevated levels of extracellular cysteine will raise its intracellular level. However, elevated extracellular levels of other system ASC amino acids will competitively inhibit the influx of cysteine (*cis*-inhibition) and stimulate the efflux of cysteine (*trans*-stimulation). Another transport system for cysteine disposal is System Xc⁻.¹³ This transporter is also regulated by extra- and intracellular gradients of glutamate and, in normal conditions, is not very active. The intracellular concentrations of cysteine are relatively low because normally it is stored as glutathione. Glutathione releases cysteine continuously to maintain constant intracellular levels (Figure 1). Several pathologic states might alter the rate of this conversion: oxidative stress, which leads to depletion of glutathione levels, causes a reduction of cysteine and its precursors. The mutual conversion of glutathione and cysteine represents the γ-glutamyl cycle, from the name of the enzyme responsible for cysteine storage: γ-glutamylcystine synthetase. Intracellular cysteine is handled by different pathways. It might be used for the synthesis of proteins and taurine or for the production of H₂S (Figure 1).¹⁴ This reaction is regulated by several cofactors, including S-adenosylmethionine

(SAM), NO, the Ca/calmodulin system, several hormones, and glucocorticoids.^{15,16}

Molecular Biology of CBS and CSE

CBS and CSE are important for the metabolism of sulfur-containing amino acids, as well as for the production of H₂S, ammonium, and pyruvate from L-cysteine. CBS is the predominant H₂S-generating enzyme in the brain and nervous system and is highly expressed in liver and kidney.³ CSE is mainly expressed in the liver and in vascular and nonvascular smooth muscle. A low level of expression of the CSE transcript, protein, and enzymatic activity is also detectable in the small intestine and stomach of rodents. The 2-kilobase (kb) transcripts have been detected in rat and mouse brain and in mouse heart, lung, and adipose tissue. However, no expression of CSE protein or activity was detected in these tissues.³

CBS

CBS catalyzes the pyridoxal 5'-phosphate (PLP)-dependent β-replacement reaction in which the thiolate of L-homocysteine replaces the hydroxyl group of L-serine. Human CBS is an especially interesting PLP enzyme because it has a complex domain structure and regulatory mechanism.

The allosteric activator SAM increases CBS activity approximately 3-fold,¹⁷ and likely binds to the C-termi-

nal regulatory domain.¹⁸ CBS from higher eukaryotes contains a unique heme moiety of unknown function,^{19–21} which is not found in CBS from yeast (*Saccharomyces cerevisiae*)^{22,23} or from the protozoan hemoflagellate *Trypanosoma cruzi*.²⁴

The human CBS gene spans over 30 kb and consists of 23 exons, ranging in size from 42 to 209 base pair (bp).²⁵ The CBS polypeptide is encoded by exons 1–14 and exon 16.²⁵ The human CBS gene encodes multiple mRNA differing in their 5' untranslated regions, resulting from the use of 5 alternative noncoding exons, designated CBS -1a, -1b, -1c, -1d, and -1e.^{26,27} Transcripts containing the CBS -1a and -1b exons appear to be most abundant and are found in an assortment of adult and fetal tissues. In contrast, usage of exons CBS -1c, -1d, and -1e seems to be rare.²⁷ There are at least 2 GC-rich TATA-less promoters immediately upstream of exons CBS -1a and -1b, containing numerous putative transcription elements specificity protein-1, activator proteins-1 and -2, upstream stimulatory factor 1, and both nuclear factor Y and 1.^{25,28–30} Specificity protein-1-like proteins and Kruppel-like factors are highly related redox-sensitive zinc-finger proteins that are important components of the eukaryotic cellular transcriptional machinery.

Expression of the gene encoding human CBS is tissue specific and is also regulated via a redox-sensitive mechanism that is linked to the proliferation status of the cell.³⁰ Transcriptional and posttranslational redox regulation of mammalian CBS is consistent with the metabolic connection between L-homocysteine and glutathione, a key cellular redox compound derived from L-cysteine.

The CBS gene product is a polypeptide of 63 kilodaltons that contains 3 domains: the heme-binding domain, the catalytic core, and the regulatory domain, comprising the N-terminal ~70 residues, the central ~340 residues, and the C-terminal ~140 residues, respectively.³¹ The catalytic form of CBS is a tetrameric protein that is dependent on pyridoxal phosphate. Human CBS is also allosterically regulated by SAM, a major product of L-methionine metabolism.

A large number of mutations in different regions of the human CBS has been found in patients with homocystinuria, a human hereditary disease that is characterized by very high plasma levels of the toxic amino acid L-homocysteine.^{32,33} Crystal structures of truncated forms of the human enzyme have revealed the structure of the catalytic domain and of the N-terminal heme-binding site.^{31,34} The location of homocystinuria-causing mutations in the 3-dimensional structure of human CBS is of interest, although the roles of the mutated residues are not fully understood.^{31,35} Deficiency of CBS activity

leads to elevated levels of homocysteine and methionine in plasma and urine, and decreased levels of compounds metabolically distal to the block at CBS, such as cystathionine and cysteine. Homocystinuria because of CBS deficiency is a recessively inherited inborn error of sulfur amino acid metabolism. CBS catalyzes the condensation of homocysteine and serine to form cystathionine, a precursor of cysteine. The clinical phenotype of patients with homocystinuria includes mental retardation, lens dislocation, skeletal abnormalities, and vascular disease.³³

CSE

The mammalian CSE genes and proteins (43 kilodaltons) share substantial identity (47%–61%) with those of *C elegans* and yeast, indicating the evolutionary conservation of this enzyme.^{36,37} In the mouse gene, a core promoter exists in the 5'-flanking region proximal to the transcriptional start site that contains several putative transcriptional factor-binding sites,³⁷ although factors that regulate basal activity are not identified and appear to be cell type specific.

Myeloid zinc finger-1 and specificity protein-1 seem to play major roles in the regulation of basal transcriptional activity.³⁷ Recently, we have demonstrated that CSE expression is induced by bacterial endotoxin in a specificity protein-1-dependent fashion.³⁸ Regulatory mechanisms for CSE transcription remain to be determined. Interestingly, promoter analyses of the human CBS gene revealed that the binding of myeloid zinc finger-1, specificity protein-1, and upstream stimulatory factor-1 to their consensus sequences within the CBS core promoters is important for the transcriptional activity.^{28,29} Little is known about the cellular consequences of an elevated CSE expression or about the associated increase in endogenously produced H₂S. CSE-derived H₂S inhibits cell proliferation^{39,40} and induces cell death predominantly by an apoptotic mechanism in polymorphonuclear cells.⁴¹ H₂S treatment has also been shown to lead to nasal lesions and olfactory epithelial necrosis.⁴² On the other hand, H₂S induces serum-independent cell-cycle entry in rat intestinal epithelial cells and increases the fraction of colonic mucosa cells in the S phase.⁴³

Deficiency of CSE activity in humans is presumed to cause cystathioninemia (cystathioninuria), an autosomal recessive inborn error probably with no consistent clinical consequence.⁴⁴ Multiple mutations in the human CSE gene have recently been identified in patients with cystathioninemia.⁴⁵ In rats, CSE expression is restricted to specific tissues; CSE is highly expressed in liver and kidney with very low expression in brain.^{46,47} Using DL-propargylglycine, an irreversible CSE inhibitor,⁴⁸

CSE has been shown to be essential in rat liver, kidney, and cultured hepatocytes for an adequate supply of cysteine to synthesize glutathione,^{49–51} a major intracellular antioxidant that protects cells from oxidative stress.

Interactions of H₂S and NO

One of the first indications that there may be important interactions between H₂S and NO was the observation that low concentrations of H₂S markedly increased the vasorelaxation induced by an NO donor, sodium nitroprusside.⁷ The mechanism underlying this effect was not clear, but, when it was subsequently shown that H₂S promotes the release of NO from vascular endothelium,⁵² that sodium nitroprusside increased the conversion of L-cysteine to H₂S,⁶ and that this NO donor also increased expression of CSE in vascular smooth muscle cells,⁶ it became clear that an H₂S-NO interaction was quite complex. In fact, carbon monoxide may be another factor capable of interacting with H₂S and NO, given that all 3 bind avidly to heme. Hemoglobin has been referred to as a common “sink” for these 3 gaseous mediators, and saturation with one could lead to enhanced plasma levels, and biologic effects, of the others.³ Indeed, saturation of erythrocytes with carbon monoxide results in elevated plasma H₂S levels.⁴

NO may regulate H₂S levels in a number of ways, other than through displacement from binding to hemoglobin.³ NO can increase CSE activity, possibly through nitrosylation of 1 or more of the 12 cysteines in that molecule. NO also appears to be able to increase CSE expression, the uptake of cystine by cells, and the activity of cGMP-dependent protein kinase, which is a stimulant of CSE activity.³

The interaction of NO and H₂S is evident in settings of diminished NO synthesis. Suppression of NO synthase activity for a prolonged period of time results in elevated systemic blood pressure. Zhong et al⁵³ demonstrated that this was accompanied by a marked decrease in CSE activity and messenger RNA (mRNA) expression. Although treatment with an H₂S donor reduced the systemic blood pressure, it did not restore NO synthesis.

Physiologic and Pathophysiologic Actions of H₂S: Neuromodulation

Relatively high concentrations of H₂S (50–160 μmol/L) can be detected in the brain of several mammalian species, including mouse, rat, human, and bovine,^{54,55} and recent data have demonstrated that H₂S is also produced in spinal cord tissue.⁵⁶ Awata et al have found expression and activity of CBS and CSE in 6 different rat brain regions, although the activity of CBS

was >30-fold greater than that of CSE.⁵⁷ The reduced H₂S production after inhibition of CBS and the fact that CSE inhibitors do not suppress H₂S production in the central nervous system (CNS) further pinpointed CBS to be the major endogenous enzyme for H₂S production in the brain.⁸ H₂S has been implicated in several neuronal functions, including the induction of the release of the corticotrophin-releasing hormone from the hypothalamus⁵⁸ and induction of hippocampal long-term potentiation, a synaptic model of learning, memory, and hyperalgesia.⁸ Moreover, H₂S promotes glutamate-mediated transmission that may have implications in the pathogenesis of neurodegenerative diseases in which excessive activation of N-methyl-D-aspartate receptors has been reported.^{59,60} Indeed, H₂S levels were found to be decreased by ~55% in the brains of 13 patients with Alzheimer's disease, compared with controls,⁶¹ and, in another study, urinary thiosulfate (a metabolite of H₂S) and erythrocyte sulfhemoglobin levels were both significantly increased in subjects with Down syndrome.⁶²

In the context of the gastrointestinal tract, little is known about the effects of H₂S in the enteric nervous system. However, it has recently been shown that H₂S-releasing agents exerted antinociceptive effects in a model of colorectal distention-induced pain. This was observed both in healthy rats and in rats with colitis. The latter effect appears to be mediated via activation of K_{ATP} channels and to be NO dependent.⁵⁶ These studies point to a potential therapeutic application of H₂S-releasing drugs.

The mechanisms through which H₂S modulates neuronal functions are partially known. H₂S increases cAMP levels in neuronal and glial cell lines and primary neuron cultures and also hyperpolarizes dorsal raphe neurons, probably by activating the K_{ATP} channels.⁶³ Moreover, it facilitates hippocampal long-term potentiation by increasing the sensitivity of N-methyl-D-aspartate (NMDA) receptors by a cAMP-mediated pathway.⁵⁹ Finally, there is evidence that sodium hydrogen sulfide (NaHS), which releases H₂S when in aqueous solution, increases intracellular Ca²⁺ concentrations in primary cultures of astrocytes and hippocampal slices,⁶⁴ but the molecular mechanism of this effect is still unknown.

Smooth Muscle

Aside from the possibility of modulating smooth muscle function through its neuromodulatory effects, H₂S can also directly alter smooth muscle tone.⁷ An initial indication of this possibility came from studies demonstrating that low molecular weight S-nitrosothiol intermediates could induce relaxation of coronary smooth

muscle and carotid and cerebral arteries.^{65–67} The actions of H₂S itself have been studied in vascular tissues,^{6,7,52} and uterine strips from pregnant rats,^{68,69} in which the predominant effect was relaxation. Similarly, it has been recently demonstrated that NaHS induces a dose-dependent relaxation of the rabbit ileum and rat vas deferens,⁷⁰ and, even in species of fresh and saltwater trout, H₂S was shown to elicit dose-dependent relaxation of isolated, precontracted branchial arteries.⁷¹ In rat studies, bolus injection of H₂S produced a transient decrease in mean arterial blood pressure,⁶ whereas intraperitoneal administration of NaHS induced a significant increase in colonic compliance during colorectal distention.⁵⁶

The mechanism through which H₂S exerts its relaxant properties is not fully understood, although it is likely mediated by the opening of K⁺_{ATP} channels. These channels couple cellular electrical activity to metabolism in a variety of tissues.⁷² In vascular smooth muscle cells, opening of K⁺_{ATP} channels hyperpolarizes the cell membrane and inactivates voltage-dependent L-type Ca²⁺ channels, leading to relaxation and blood vessel dilation by virtue of the reduced intracellular free Ca²⁺ concentrations.⁷³ For this reason, in conditions such as diabetes and hypertension, K⁺_{ATP} channels are important therapeutic targets. H₂S exerts a relaxant effect on rat aortic tissue and induces a transient reduction of blood pressure through a direct stimulation of K⁺_{ATP} channels and subsequent hyperpolarization of rat aortic vascular smooth muscle cells.⁶ Thus, glibenclamide, a K⁺_{ATP} channel antagonist, reversed these actions, whereas pinacidil, a K⁺_{ATP} channel opener, mimicked the H₂S-induced relaxation. These results have been recently confirmed by Tang et al.⁷⁴ Using a sophisticated whole-cell and single-cell patch-clamp technique, they demonstrated that exogenous and endogenous H₂S increases the K⁺_{ATP} currents and hyperpolarizes membrane potentials of rat mesenteric artery smooth muscle cells through a direct stimulation of K⁺_{ATP} channels. Finally, H₂S also relaxes smooth muscle in the colon by acting on the K⁺_{ATP} channels. Once again, glibenclamide reversed, whereas pinacidil mimicked, the H₂S-induced relaxation of colonic smooth muscle.⁵⁶

Other potential targets of action of H₂S on vascular smooth muscle include voltage-dependent Ca²⁺ channels and Ca²⁺-dependent K⁺ channels.⁵² Also, as discussed previously in this article, at low concentrations, H₂S enhances the smooth muscle relaxant effect of NO, suggesting a cross talk between these 2 gases, producing synergistic effects.⁷

Although the major reported effect of H₂S in the context of smooth muscle is relaxation, it is noteworthy that, in some tissues and in some species, H₂S can exert

powerful contractile responses. For example, H₂S produces excitatory motor responses in the rat urinary bladder by activating capsaicin-sensitive primary afferent neurons.⁷⁵ The concentration-dependent contractile response of H₂S was unaffected by the vanilloid receptor 1 antagonists capsazepine and SB366791, whereas the non-selective cation channel blocker ruthenium red completely abolished the NaHS-induced bladder contractions. This suggests that H₂S can act on an unidentified vanilloid-like channel on sensory neurons.⁷⁶

Immune and Inflammatory Processes

In recent years, several papers have been published on H₂S in the context of immune and inflammatory reactions. Some papers suggest an anti-inflammatory role of H₂S (Figure 2), whereas others point to a contribution of H₂S to tissue injury and inflammation. H₂S is an extremely potent inhibitor of leukocyte adherence to the vascular endothelium in rat stimulated by intragastric aspirin. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) stimulate leukocyte adherence^{77–79} largely via inhibition of cyclooxygenase (COX)-2.⁸⁰ Doses of H₂S as low as 10 μmol/kg (intraperitoneally) significantly suppressed aspirin-induced leukocyte adherence.³⁸ This effect could be reversed by glibenclamide, which is a selective antagonist of K⁺_{ATP} channels.³⁸ H₂S also suppressed leukocyte adherence in rat mesenteric venules initiated by exposure to a formylated peptide (unpublished observation, December 2005).

H₂S might interfere with inflammatory processes by diminishing the tissue injury induced by neutrophils. One such mechanism that has been suggested is the induction of apoptosis in neutrophils by H₂S.⁴¹ H₂S also appears to be a potent inhibitor of tissue injury mediated via neutrophil-derived hypochlorous acid. Hypochlorous acid is produced by the enzyme myeloperoxidase, which is found in all cells of myeloid origin but in particularly high concentrations in neutrophils. Whiteman et al⁸¹ demonstrated that H₂S can interfere with brain tissue injury caused by hydrochlorous acid and, in cultured neuroblastoma cells, could inhibit hypochlorous acid-mediated cytotoxicity, intracellular protein oxidation, and lipid peroxidation. The same group also reported that H₂S interfered with peroxynitrite-mediated cytotoxicity and tyrosine nitration.⁸²

As described in more detail below, H₂S appears to exert analgesic effects that are mediated via K⁺_{ATP} channels.⁵⁶ Interestingly, activation of K⁺_{ATP} channels has

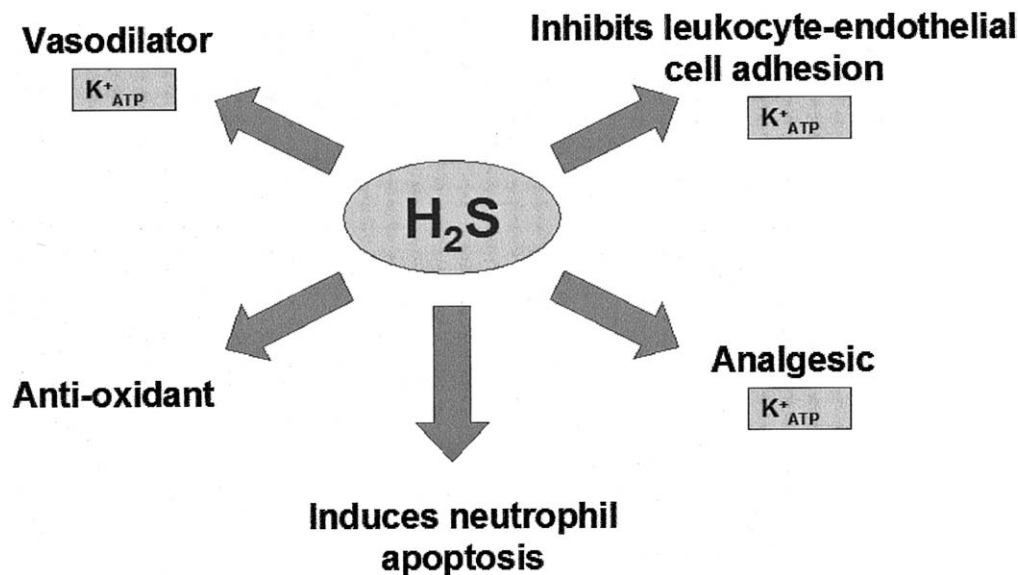


Figure 2. Anti-inflammatory actions of hydrogen sulfide. H₂S exerts many actions that can reduce the severity of inflammation or tissue injury, several of which are mediated via activation of K⁺_{ATP} channels. Antioxidant activities include the ability of H₂S to reduce tissue injury mediated by the neutrophil product, hypochlorous acid, or peroxynitrite.

been suggested to be a mechanism through which NSAIDs produce their analgesic effects.⁸³

As mentioned above, some recent studies suggest that H₂S can contribute to inflammatory processes. For example, Li et al⁸⁴ reported that endotoxin administration to mice resulted in inflammation in the lung, liver, and kidney that was accompanied by increased H₂S synthetic activity in those tissues. Administration of an H₂S donor (NaHS) similarly resulted in inflammatory changes in those tissues. These authors also reported elevated H₂S levels in the plasma of septic shock patients.⁸⁴ Bhatia et al⁸⁵ used the carrageenan-induced hindpaw inflammation model in rats to examine a potential role for H₂S. They observed increased H₂S synthetic activity in the paw following carrageenan administration, accompanied by increased granulocyte infiltration (myeloperoxidase activity). Pretreatment with an inhibitor of CSE diminished the H₂S synthetic activity and the myeloperoxidase activity. Although there is some incongruence in terms of the dose dependency of inhibition of H₂S synthetic activity and levels of myeloperoxidase,⁸⁵ these results nevertheless are consistent with a contribution of H₂S to acute inflammation.

Gastric Mucosal Integrity

Injury to the upper gastrointestinal tract is a common complication of NSAID (including acetylsalicylic) therapy.⁸⁶ Owing largely to the inhibition of COX isoenzymes, NSAIDs reduce the ability of the mucosa to resist injury. Inhibition of generation of COX-1- and COX-2-derived eicosanoids results in altered gastric mucosal blood flow and increased leukocyte-endothelial adhesive interactions in the gastric microcirculation, im-

portant events in the process of gastric injury caused by NSAIDs.^{87,88} Human and animal studies have highlighted the role of gaseous mediators, particularly NO, in maintaining gastric mucosal integrity.⁸⁹ Thus, by modulating the expression/activity of adhesion molecules at the leukocyte-endothelium interface and by maintaining gastric mucosal blood flow, NO compensates for depressed generation of protective eicosanoids.^{88,89} Recent studies suggest that H₂S may similarly contribute to mucosal defense. The gastric mucosa expresses both CSE and CBS and has the ability to generate H₂S.³⁸ Similarly to other nonneuronal tissues, CSE seems to be the main enzyme involved in the H₂S generation by the gastric mucosa. This view is supported by the observation that generation of H₂S by the gastric mucosa depends on the presence of pyridoxal-5'-phosphate, but not calmodulin, an essential cofactor for CBS. Moreover, DL-propargylglycine, an irreversible inhibitor of CSE, inhibits gastric H₂S formation.³⁸

H₂S appears to play a significant role in regulating gastric mucosal blood flow. Using laser-Doppler flowmetry, we have shown that exposure to H₂S (100 μmol/L) increased gastric mucosal blood flow by ~25%, while reducing systemic blood pressure by ~10 mm Hg.³⁸ Gastric vasodilation elicited by H₂S was reversed by glibenclamide, suggesting, as for several other H₂S-mediated activities, an involvement of K_{ATP} channels.

In addition to its role in regulating gastric mucosal blood flow, H₂S is involved in maintenance of gastric mucosal integrity in rodents exposed to NSAIDs (Figure 3). Thus, NaHS prevents the reduction of mucosal blood flow caused by aspirin and prevents the adherence of leukocytes to the vascular endothelium (as described previously).

Effects of NSAIDs on mucosal H₂S generation may contribute to the ability of these drugs to cause injury. Administration of NSAIDs results in a significant decrease in H₂S synthesis.³⁸ This is attributable to the ability of these drugs to modulate CSE expression (they do not affect CBS expression).³⁸ The effect on H₂S production was most profound with indomethacin, a non-selective COX inhibitor, which reduced CSE activity/expression by approximately 80%, but was also observed with aspirin, ketoprofen, and diclofenac.³⁸ The reason for the down-regulation of CSE gene expression following NSAID administration is likely related to a peculiar interaction of these drugs with the gene for CSE. Recent studies have highlighted that the core promoter of the CSE gene, located in the 5'-flanking region proximal to the transcriptional start site, contains putative transcriptional factor-binding sites, including myeloid zinc finger-1 and specificity protein-1. Specificity protein-1, a member of the Sp/Kruppel-like factor family,⁹⁰ is a ubiquitously expressed transcription factor that recognizes GC-rich sequences present in regulatory regions of numerous housekeeping genes and is a known target for NSAIDs.⁹¹ Previous studies have shown that specificity protein-1 DNA-binding activity is attenuated by NSAID treatment through a mechanism that involves inhibition of extracellular signal-regulated kinase (ERK) phosphorylation.⁹¹ Using HEK-293 cells transfected with a CSE promoter containing the specificity protein-1 binding site, we found that NSAIDs suppress CSE expression via inhibition of the ERK/Sp1-signalling pathway. The ability of NSAIDs to regulate specificity protein-1 phosphorylation might also help to explain COX-independent activities of NSAIDs. Abnormal specificity protein-1 expression and activation have been observed in several human cancers including human hepatocellular carcinoma,⁹² gastric carcinoma,⁹³⁻⁹⁵ and pancreatic adenocarcinoma.⁹² Elevated specificity protein-1 expression correlated with malignancy and reduced survival of patients with gastric cancer.^{95,96} Because the CSE/H₂S pathway is also involved in regulating neoplastic growth,⁹⁷ these observations raise the possibility that reduction of CSE expression might contribute to the putative antineoplastic activity of NSAIDs. However, there is no direct evidence to support this notion at present.

Intestinal Motility and Perception

The possibility that H₂S is an important mediator of gastrointestinal motility has not yet been explored in any detail. There are, however, some data to suggest such a role. Hosoki et al demonstrated that the rat ileum

expressed both CBS and CSE mRNA and could synthesize H₂S.⁷ Moreover, ileum precontracted by acetylcholine was relaxed by NaHS in a dose-dependent manner. These data were then confirmed in the ileum of other species. Teague et al demonstrated that NaHS produced dose-related inhibition of the spontaneous contractions in the isolated rabbit ileum, whereas, in the guinea pig ileum, it did not have any effect on the resting tone, but reduced the contractile response to exogenous acetylcholine and electrical field stimulation.⁷⁰ Moreover, an NaHS-related reduction of the contractile response of the electrical field-stimulated rat ileum was also observed.⁷⁰

We recently reported that both CSE and CBS are expressed in the rat colon.⁵⁶ Furthermore, intraperitoneal administration of NaHS or L-cysteine dose dependently relaxed the colon *in vivo*. The relaxant action was completely blocked by pretreating the rats with glibenclamide and was reproduced by parenteral administration of pinacidil, a K_{ATP} channel activator. Interestingly, inhibition of NO synthesis reversed the effects of NaHS, consistent with the interactions of NO and H₂S that were discussed above.⁵⁶

Apart from these relaxant effects, it seems that H₂S may have also exerted antinociceptive effects because NaHS dose dependently reduced the pain induced by colorectal distention in healthy rats and in rats with colitis. These effects were independent from the relaxant activity of H₂S because they were observed at doses of NaHS that did not increase colonic compliance. Moreover, the H₂S-induced antinociceptive effect was prevented by blocking both K_{ATP} channels and NO pathways, indicating that the integrity of these 2 systems is required.⁵⁶

Hepatic Circulation

Portal hypertension is a multifactorial syndrome characterized by increased hepatic vascular resistance (because of a high hepatic vascular tone) and increased splanchnic blood flow (because of a pronounced splanchnic vasodilatation). A growing body of evidence suggests that the elevated microvascular tone within the cirrhotic liver is a consequence of mechanical factors (disruption of the hepatic vascular bed, scarring, and nodule formation) and vasculogenic processes at the sinusoidal endothelial cells. Moreover, the vascular component of portal hypertension is the result of insufficient production and/or availability of vasodilators within the hepatic vascular bed.^{98,99} A deficiency of NO may be particularly significant. The insufficient hepatic availability of NO is mostly due to decreased endothelial NO synthase activity, rather than to decreased expression of the enzyme.

This is caused by, among other factors, increased caveolin binding and decreased phosphorylation of endothelial NO synthase. Moreover, the dynamic, variable, and reversible nature of intrahepatic vascular tone in cirrhotic liver emphasizes the importance of the interplay between vasoconstrictive and vasodilatory mediators.^{98,99} Of relevance, not only is the production of vasoconstricting agents (eg, endothelins) increased, the “sensitivity” of the hepatic vascular smooth muscle to these agents is increased in cirrhotic states. In addition, hepatic stellate cells exhibit an impaired response to vasodilators and an enhanced response to vasoconstrictors.^{98,99} Because of the pathophysiologic importance of these events, vasoactive substances have been an area of investigation in both experimental systems and in human liver disease. In this context, we have recently examined the role of H₂S in regulating the microcirculation in normal and cirrhotic rat liver.¹⁰⁰ H₂S attenuated vasoconstriction induced by norepinephrine in normal and cirrhotic rat liver. This effect was blocked by glibenclamide. In normal liver, a similar reduction of intrahepatic resistance could be elicited by administration of L-cysteine as was observed with administration of H₂S. This observation suggests that H₂S is an important endogenous modulator of the hepatic microcirculation. The vasodilating effect of H₂S was not affected by pretreatment with an NO synthase inhibitor. Increasing the shear stress in the hepatic microcirculation, which triggers release of NO, did not stimulate the release of H₂S. A detailed analysis of the H₂S-generating enzymes in subpopulations of hepatic cells revealed that CSE and CBS are not expressed in the vascular endothelium of the hepatic sinusoids. This suggests that vasorelaxation induced by endogenous H₂S is largely, or completely, endothelium independent. In contrast, CSE mRNA and protein were detected in hepatic stellate cells. These fibroblast-like cells can generate H₂S from L-cysteine and relax in response to H₂S. In aggregate, these data suggest that hepatic NO and H₂S are released by different cellular sources and that their hemodynamic effects involve different cellular targets.

Human studies have demonstrated that methionine metabolism is altered in cirrhosis and that impairment of liver function is associated with inhibition of the transsulfuration pathway, leading to elevated plasma levels of homocysteine. This defect might be ascribed, at least partially, to a reduction in the expression/activity of CBS and CSE, which are involved in homocysteine metabolism.^{101–105}

Studies in animals have suggested that the mechanism underlying the block of the transsulfuration pathway in cirrhosis is an impaired activity of the CBS/CSE system.^{98,99} This results in reduced production of H₂S in the

cirrhotic liver. Moreover, the reduced activity of CSE in cirrhotic hepatic stellate cells favors enhanced contraction around the sinusoids, thereby contributing to increased intrahepatic resistance and portal hypertension. In addition, the activated hepatic stellate cells not only express reduced CSE activity but exhibit a reduced expression of CSE. This may be due to a transcriptional down-regulation of the gene during hepatic stellate cells *trans*-differentiation and proliferation, although the factors involved in its regulation remain to be elucidated. Collectively, these observations suggest that H₂S acts as a vasodilator that regulates intrahepatic blood flow in normal and cirrhotic liver and may play an important role in the pathogenesis of portal hypertension. Furthermore, because the vasodilator activity of H₂S is largely NO independent and the 2 systems are expressed by different cell subtypes, H₂S and NO may regulate hepatic and portal blood flow in a cooperative manner.

Conclusions and Future Directions

As was the case for NO 2 decades ago, there is emerging evidence for H₂S in many aspects of gastrointestinal and liver function. H₂S appears to be an important mediator of mucosal and hepatic blood flow and an important contributor to mucosal defense against injury. Deficiencies of H₂S synthesis may contribute to the pathogenesis of several gastrointestinal and liver disorders. Roles for H₂S in inflammation and immunity have been identified but not yet completely defined. Some studies suggest anti-inflammatory effects of H₂S, whereas others suggest a contribution of this gas to immune-mediated tissue injury. These controversies will likely only be clarified when more highly selective inhibitors of H₂S synthesis and simpler methods for quantification of H₂S production become available.

The observation that NSAIDs, including aspirin, can suppress the expression of one of the key enzymes required for H₂S synthesis suggests that this could be another mechanism contributing to the gastrointestinal damage produced by this widely used class of drugs. These observations also raise the possibility of H₂S-releasing agents being utilized for increasing the resistance to mucosal injury and possibly for accelerating repair of mucosal injury.

A possible role of H₂S in the colon has long been the subject of speculation. This has largely focused on the potentially harmful effects of bacterially derived H₂S because H₂S can easily permeate membranes and therefore affect cell functions in the host. There is little direct evidence to support this notion. One recent study raised the possibility that low levels of circulating SAM may

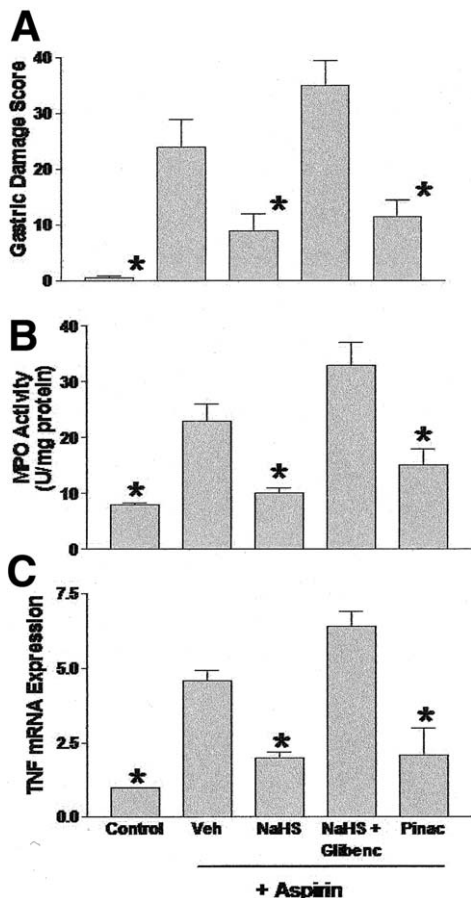


Figure 3. Reduction of aspirin-induced gastric damage and inflammation by hydrogen sulfide. Aspirin (30 mg/kg, orally) induced extensive gastric damage in the rat stomach (A). This was accompanied by infiltration of granulocytes into gastric tissue, as indicated by elevated myeloperoxidase (MPO) activity (B) and increased expression of TNF- α mRNA (C). Pretreatment with an H₂S donor (NaHS; 100 μ mol/kg) reduced gastric damage and inflammation. This was reversed by the K⁺_{ATP} channel antagonist glibenclamide and mimicked by the K⁺_{ATP} channel agonist pinacidil. The TNF- α mRNA data are shown as the fold-change from control (no aspirin) levels. **P* < .05 versus the group treated with vehicle + aspirin. This graph was constructed using previously published data.³⁸

contribute to disease severity in inflammatory bowel disease.¹⁰⁶ They observed an inverse relationship between SAM levels and disease activity. They suggested that, because SAM is important in detoxification of constantly produced hydrogen sulfide in the colon, a deficiency of it could lead to accumulation of H₂S in the lumen, which might exacerbate tissue injury. However, SAM also regulates enzymes such as CSE,^{16,107} which is largely responsible for H₂S production in the gut. Thus, one might also postulate that low levels of SAM might result in low H₂S production by colonic tissue, which, as shown in the gastric mucosa, renders the tissue more susceptible to injury and inflammation. Clearly, further studies are needed to clarify any role of H₂S in the colon with respect

to mucosal injury and inflammation. As was the case for NO, the emerging role of H₂S in the gastrointestinal tract, liver, and other organs may open new avenues for development of therapeutics for a variety of disorders.

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Received December 12, 2005. Accepted February 15, 2006.

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Supported by grants from the Canadian Institutes of Health Research (to J.L.W.).

J.L.W. is an Alberta Heritage Foundation for Medical Research Senior Scientist and holds a Canada Research Chair in Inflammation Research.

The authors thank Drs Elisabetta Antonelli, Giovanni Rizzo, and Stefano Orlandi for their critical comments and suggestions.