# Two's company, three's a crowd: can $H_2S$ be the third endogenous gaseous transmitter?

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ABSTRACT **Bearing the public image of a deadly "gas of rotten eggs," hydrogen sulfide ($H_2S$) can be generated in many types of mammalian cells. Functionally, $H_2S$ has been implicated in the induction of hippocampal long-term potentiation, brain development, and blood pressure regulation. By acting specifically on $K_{ATP}$ channels, $H_2S$ can hyperpolarize cell membranes, relax smooth muscle cells, or decrease neuronal excitability. The endogenous metabolism and physiological functions of $H_2S$ position this gas well in the novel family of endogenous gaseous transmitters, termed "gasotransmitters." It is hypothesized that $H_2S$ is the third endogenous signaling gasotransmitter, besides nitric oxide and carbon monoxide. This positioning of $H_2S$ will open an exciting field—$H_2S$ physiology—encompassing realization of the interaction of $H_2S$ and other gasotransmitters, sulfurating modification of proteins, and the functional role of $H_2S$ in multiple systems. It may shed light on the pathogenesis of many diseases related to the abnormal metabolism of $H_2S$.—Wang, R. Two's company, three's a crowd: can $H_2S$ be the third endogenous gaseous transmitter?** ***FASEB J.*** **16, 1792–1798 (2002)**

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THE CELLULAR SIGNALING process is usually initiated by the binding of neurotransmitters or humoral factors to receptors located on the plasma membrane. The ligand–receptor interaction generates intracellular second messengers that relay and direct the extracellular signals to different intracellular destinations, resulting in modulated cellular activity. The discovery of nitric oxide (NO) elucidates more than just the nature of the endothelium-derived relaxing factor (1). It presents a membrane receptor-independent signaling mechanism, emphasizing the necessity to modify the conventional doctrine about cellular signal transduction. The subsequent resurgence of carbon monoxide (CO) as another important endogenous signaling gas is embraced by researchers in almost every field of life sciences (2). To distinguish NO and CO from the classical neurotransmitters and humoral factors while acknowledging the common nature of these two gases, an effort has been made to classify these endogenous gaseous transmitters against several criteria (**Table 1**). I would recommend designating these gaseous transmitters as gasotransmitters. NO and CO are the first two identified gasotransmitters. In this hypothesis study, arguments are made to entitle hydrogen sulfide ($H_2S$) as the third gasotransmitter. Important implications of this identification are explained.

## Physical and chemical properties of $H_2S$

$H_2S$ is a colorless gas with a strong odor of rotten eggs. The detectable level of this gas by the human nose is at a concentration 400-fold lower than the toxic level. Oxidation of $H_2S$ yields elemental sulfur, sulfur oxide ($SO_2$), and sulfates such as sulfuric acid. $H_2S$ can be hydrolyzed to hydrosulfide and sulfide ions in the following sequential reactions: $H_2S \Leftrightarrow H^+ + HS^- \Leftrightarrow 2H^+ + S^{2-}$. Even in an aqueous solution, about one-third of $H_2S$ remains undissociated at pH 7.4. $H_2S$ is permeable to plasma membranes as its solubility in lipophilic solvents is ~ fivefold greater than in water.

## Endogenous generation and metabolism of $H_2S$

The biological production and utilization of $H_2S$ have been best known for certain bacteria and archae (3). A sobering fact is that mammalian cells also produce $H_2S$. The $H_2S$ concentration of rat serum is ~ 46 μM (4). Aside from circulating $H_2S$, a significant amount of $H_2S$ is produced in various tissues. For instance, the physiological concentration of $H_2S$ in brain tissue has been reported to be 50–160 μM (5, 6). Recent studies have shown that vascular tissues generate measurable amounts of $H_2S$ (4, 5).

Two pyridoxal-5′-phosphate-dependent enzymes—cystathionine β-synthase or CBS (EC 4.2.1.22) and cystathionine γ-lyase or CSE (EC 4.4.1.1)—are responsible for the majority of the endogenous production of $H_2S$ in mammalian tissues that use L-cysteine as the main substrate (7–9). In some tissues CBS and CSE are both needed for generation of $H_2S$, whereas in others one enzyme suffices (**Fig. 1**). Thus, it comes as no surprise that the expression of CBS and/or CSE is tissue specific. The expression of CBS (5, 10) and CSE (11–14) has been identified in many human and other mammalian cells, including those from liver, kidney,

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TABLE 1. *Classification of gasotransmitters (gaseous transmitters)*

(1) They are small molecules of gas, like nitric oxide (NO) and carbon monoxide (CO).
(2) They are freely permeable to membrane. As such, their effects will not rely on cognate membrane receptors.
(3) They are endogenously and enzymatically generated and their generation is regulated.
(4) They have well-defined specific functions at physiologically relevant concentrations. For instance, NO and CO both participate in vasorelaxation and synaptic transmission in the central nervous system.
(5) Their cellular effects may or may not be mediated by second messengers, but should have specific cellular and molecular targets. For instance, NO and CO activate $K_{Ca}$ channels in plasma membrane either directly or mediated by the cGMP pathway.

brain, skin fibroblasts, and blood lymphocytes. As the end product of CBS- and CSE-catalyzed cysteine metabolism, $H_2S$ exerts a negative feedback effect on the activity of these enzymes. Elevated $H_2S$ level inhibited CSE activity (15) and the rate of gluconeogenesis from cysteine (16). Another less important endogenous source of $H_2S$ is the nonenzymatic reduction of elemental sulfur to $H_2S$ using reducing equivalents obtained from the oxidation of glucose (17) (**Fig. 2**). All essential components of this nonenzymatic pathway are present in vivo, including the supply of reducible sulfur. The presence of millimolar concentration of sulfur in blood circulation has been reported in humans (18) or mice (19).

$H_2S$ in vivo is metabolized by oxidation in mitochondria or by methylation in cytosol (Fig. 1). $H_2S$ can be scavenged by methemoglobin (20) or metallo- or disulfide-containing molecules such as oxidized glutathione (21). $H_2S$ is excreted mainly by the kidney as free or conjugated sulfate (20). The interaction of hemoglobin and $H_2S$ calls for special attention. Hemoglobin may be the common "sink" for CO in forming scarlet carboxyhemoglobin (22), for NO in forming nitrosyl hemoglobin, and for $H_2S$ in forming green sulfhemoglobin (23). If this sink is filled with one gas, the binding of other gases would be affected and their individual availability to act on targeted cells would be altered. A

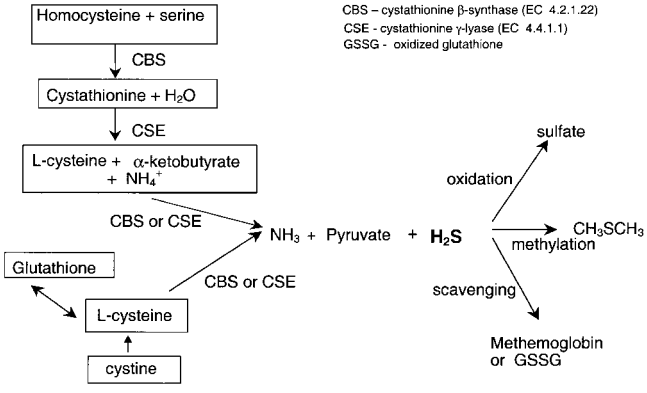


**Figure 1.** Endogenous enzymatic production and metabolism of $H_2S$.

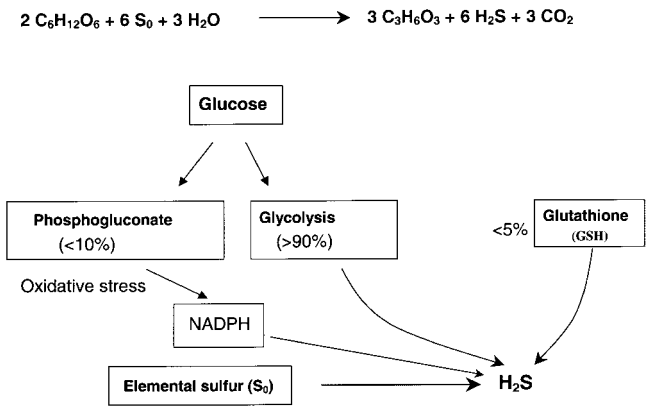


**Figure 2.** Endogenous nonenzymatic production of $H_2S$.

case in point is the observation that after pretreatment of human erythrocytes with CO to saturate the hemoglobin sink, the accumulated amount of endogenous $H_2S$ was significantly enhanced (17).

## Physiological effects of $H_2S$ and the underlying mechanisms

The physiological functions of endogenous $H_2S$ may be multifaceted. In liver and kidney, activities of the $H_2S$-generating enzymes have been studied in great detail (8, 9, 24, 25). To be succinct, a discussion of this study focuses on the physiological role of $H_2S$ in nervous and cardiovascular systems.

## Physiological effects of $H_2S$ on the nervous system

The first and most important evidence for the physiological role of $H_2S$ was obtained in 1989 when endogenous sulfide levels in rat brain tissues (1.6 μg/g) (26) and in normal human postmortem brainstem (0.7 μg/g) were reported (26, 27). Endogenous sulfide level in mice brain (28) was similar to that of rats, but threefold lower than that of bovine cerebral cortex (29). The study by Awata et al. in 1995 (30) provided the enzymatic mechanisms for this endogenous $H_2S$ in rat brain, in which activities of CBS and CSE in six different brain regions were detected though the activity of CBS was > 30-fold greater than that of CSE. Brain activities of CBS and CSE gradually increased after birth and reached adult level at 2–4 wk. The transcriptional expression of CBS in rat brain (hippocampus, cerebellum, cerebral cortex, and brainstem) was later confirmed using Northern blot analysis but no CSE mRNA was detected (6). The reduced $H_2S$ production after the inhibition of CBS further pinpointed CBS to be the major endogenous enzyme for $H_2S$ production in brain (6).

The functional role of $H_2S$ at physiologically relevant concentrations in brain was gradually uncovered in early 1990s. Chronic exposure of neonatal rats to $H_2S$ altered the release of neurotransmitters in brain with increased serotonin and norepinephrine levels in rat

cerebellum and frontal cortex (31, 32). Application of NaHS, which generates $H_2S$ once in solution, to rat hypothalamic explants in vitro did not affect the basal secretion of corticotropin-releasing hormone (CRH), but consistently reduced KCl-stimulated CRH release from the explants (33). This effect of exogenous $H_2S$ was consistent with the observation that the intramuscular application of S-adenosyl-L-methionine, an endogenous precursor of $H_2S$, to conscious rats reduced the hypothermia-induced increase in serum level of corticosterone (33).

Voltage-dependent and TTX-sensitive $Na^+$ channels may be targeted by $H_2S$ in neurons. In cultured neuroblastoma cells, NaHS or taurine alone did not alter $Na^+$ channel currents. After pretreatment of these cells with NaHS, taurine dramatically inhibited $Na^+$ channels in a reversible fashion (34). This effect of NaHS was mimicked by disulfide-reducing agents dithiothreitol and β-mercaptoethanol. A reduction of disulfide bonds between $Na^+$ channel subunits by $H_2S$ was thus suggested. Since taurine is an inhibitory neurotransmitter and a short exposure to NaHS (<2 min) resulted in a twofold increase in taurine levels in brainstem (35), the interaction between NaHS and taurine suggests that certain neuronal effects of $H_2S$ could be mediated by the alteration in taurine levels. However, the physiological importance of this study is limited since the concentration used for NaHS (10 mM) was far outside the physiological range.

NaHS induced a concentration-dependent (27–200 μM) hyperpolarization and reduced input resistance of CA1 neurons or dorsal raphe neurons (36). This concentration range is physiologically relevant in the brain (6). Changes in $K^+$ conductance were identified to be the main ionic basis for these effects, since the presence of extracellular barium or intracellular cesium abolished the NaHS-induced membrane hyperpolarization. NaHS-induced neuronal hyperpolarization was blocked by a high concentration of TEA (50 mM) but not by a low concentration of TEA (10 mM) or 4-aminopyridine (1 mM). Thus, the involvement of either calcium-activated $K^+$ channels or voltage-dependent $K^+$ channels in NaHS effect was not supported. Activation of ATP-sensitive $K^+$ ($K_{ATP}$) channels by NaHS was proposed in these experiments as the consequence of ATP depletion due to the inhibition by sulfide of the oxidative phosphorylation (36). This hypothesis was not without ambiguity, since in the same experiments manipulation of intracellular ATP concentrations did not affect the NaHS-induced membrane hyperpolarization and no $K_{ATP}$ channel currents were directly examined. Electrophysiological measurement of $K^+$ channel currents in neurons with tight control of intracellular ATP levels in the presence of NaHS/$H_2S$ would help clarify the interaction of $H_2S$ and neuronal $K_{ATP}$ channels.

In addition to $K_{ATP}$ channels, NMDA receptors may be the target of $H_2S$. In the presence of a weak tetanic stimulation, NaHS at 10–130 μM facilitated the induction of hippocampal long-term potentiation (LTP) in rat hippocampal slices by enhancing the NMDA-induced inward current (6). Interaction of $H_2S$ and NMDA receptors was possibly mediated by the activation of a cAMP-dependent protein kinase pathway. NaHS (1–100 μM) increased cAMP production in primarily cultured rat cerebral and cerebellar neurons or in selected rat brain neuronal and glial cell lines (37). By enhancing the production of cAMP, NaHS increased the sensitivity to NMDA stimulation of NMDA receptors expressed in oocytes (37).

### Physiological effects of $H_2S$ on the cardiovascular system

It has been a conventional view that $H_2S$ interferes with cardiovascular function as a result of the secondary anoxia rather than a direct action of the gas on cardiac myocytes or vascular smooth muscle cells (SMCs) (36). However, this doctrine has started to become shaky in light of two aspects of development. The location of the $H_2S$-generating enzymes as well as the detection of endogenous levels of $H_2S$ in cardiovascular system provides the endogenous sources of $H_2S$. In-depth study of the whole animal and at tissue and cellular levels defines the functional role of $H_2S$ in the cardiovascular system.

Chen et al. (38) found no activity or expression of CBS in human atrium and ventricle tissues. The activity and/or expression of CBS were also lacking in human internal mammary arteries, saphenous veins, coronary arteries, or aortic arteries (38, 39). Thus, CBS does not appear to play a major role in generating $H_2S$ in cardiovascular tissues under physiological conditions. On the other hand, expression of CSE and the endogenous production of $H_2S$ have been shown in rat portal vein and thoracic aorta (5). In rat mesenteric artery and other vascular tissues, CSE is the only $H_2S$-generating enzyme that has been identified, cloned, and sequenced (4). mRNA of this enzyme was expressed solely in vascular SMCs as detected by RT-PCR and in situ hybridization (4). No transcript of CSE was found in the endothelium layers of intact vascular tissues or cultured endothelial cells (4). Expression levels of CSE mRNA varied in different types of vascular tissues, with an intensity rank of pulmonary artery > aorta > tail artery > mesenteric artery (4). Endogenous production of $H_2S$ depends on the types of vascular tissues. For instance, the homogenates of thoracic aortas yielded more $H_2S$ than that of portal vein of rats (5).

The physiological function of $H_2S$ in the cardiovascular system has been studied recently. An intravenous bolus injection of $H_2S$ transiently decreased blood pressure of rats by 12–30 mmHg, an effect mimicked by pinacidil (a $K_{ATP}$ channel opener) and antagonized by glibenclamide (a $K_{ATP}$ channel blocker) (4). At the tissue level, $H_2S$ at physiologically relevant concentrations ($IC_{50}$, 125 μM) induced in vitro relaxation of aorta and portal vein of rats (4, 5). Whether this vasorelaxant effect was due to a direct action of $H_2S$ on vascular SMCs has been questioned. Zhao et al. (4) showed that the $H_2S$-induced relaxation of rat aortic tissues was due mainly to a direct interaction of $H_2S$ and SMCs, based on the failure of denervation of vascular

tissues in vitro to alter $H_2S$ effects and on the observation that $H_2S$ still significantly relaxed vascular tissues after endothelium removal. Zhao et al. (4) showed that a small portion of the $H_2S$-induced vasorelaxation was attenuated by either removal of the endothelium or the application of L-NAME (an inhibitor of NO synthase) in the presence of the endothelium. This endothelium-dependent effect of $H_2S$ could be explained by the release of endothelium-derived vasorelaxant factors in response to $H_2S$ stimulation. The presence of an intact endothelium might serve as a buffer to retain $H_2S$ in the blood vessel wall so that its vasorelaxant effect can be potentiated and prolonged. Another interesting observation was that the coapplication of apamin and charybdotoxin, a protocol to block the effect of endothelium-derived hyperpolarizing factor (EDHF) (40), to the endothelium-intact rat aortic tissues reduced the vasorelaxant effect of $H_2S$. It seems that $H_2S$ might release EDHF from vascular endothelium. It should be borne in mind that endothelium dependency of the vascular effects of $H_2S$ has been controversial. One study concluded that the vasorelaxant effect of $H_2S$ was independent of endothelium, even though no experimental data were shown to support this conclusion (5).

Mechanisms for the direct effect of $H_2S$ on vascular SMCs have been explored. Unlike NO or CO, $H_2S$ relaxed vascular tissues independent of the activation of cGMP pathway. Whereas the vasorelaxation induced by NO was virtually abolished by ODQ, a specific inhibitor of soluble guanylyl cyclase, the $H_2S$-induced vasorelaxation was not inhibited by ODQ (4). In fact, ODQ even potentiated the vasorelaxant effect of $H_2S$. The synergistic actions of $H_2S$ and ODQ cannot be fully understood yet. Hypothetically, the interaction between ODQ and $H_2S$ may have generated vasorelaxant free radicals, which further relaxed vascular tissues.

The most recent significant advance in our understanding of the vascular effects of $H_2S$ was the identification of $K_{ATP}$ channels in vascular SMCs as the target protein of $H_2S$. When isolated rat aortic tissues were precontracted with 20 or 100 mM KCl, the maximum vascular relaxation induced by $H_2S$ was $\sim$ 90% or 19%, respectively (4). This difference in relaxation potency of $H_2S$ represents the portion of relaxation possibly mediated by potassium conductance. Furthermore, $H_2S$-induced relaxation of the aortic tissues precontracted with phenylephrine was mimicked by a $K_{ATP}$ channel opener pinacidil but concentration-dependently inhibited by glibenclamide. Results from these tissue contractility studies were substantiated in isolated single SMCs. $K_{ATP}$ channel currents in rat aortic SMCs were significantly and reversibly increased by either $H_2S$ or pinacidil. A direct action of $H_2S$ on $K_{ATP}$ channel proteins, rather than the interfered ATP metabolism by $H_2S$, was proposed based on three lines of evidence. First, intracellular ATP concentration in these studies was clamped at a fixed level (e.g., 0.5 mM) by dialyzing cells with the pipette solution. Second, the effect of $H_2S$ on $K_{ATP}$ channels was quickly reversed on washing out $H_2S$ from the bath solution. Third, intentionally varying ATP concentrations inside the cell (from 0.2 to 3 mM) did not change the excitatory effect of $H_2S$ on $K_{ATP}$ channels. Together, these results demonstrate that $H_2S$ is an important endogenous vasoactive factor and is the first identified gaseous opener of $K_{ATP}$ channels in vascular SMCs.

### Physiological vs. toxicological effects of $H_2S$

The toxicity of $H_2S$ has been known for $\sim$ 300 years. The major lethal consequence of $H_2S$ intoxication is the loss of central respiratory drive due to biochemical lesions of the respiratory centers of the brainstem (41). For a complete toxicological profile of $H_2S$, readers are redirected to two excellent reviews by Beauchamp et al. (20) and Reiffenstein et al. (36). Note first that the endogenously generated $H_2S$ under physiological conditions is hardly accumulated or toxic to cells due to the balanced cellular metabolism of the gas (Fig. 1). In the presence of $> 30$ μM $HS^-$, no apparent disturbance in oxidative phosphorylation could be observed likely due to the rapid oxidation of $H_2S$ in mitochondria (42, 43). Second, the line between toxicological and physiological effects of $H_2S$ is very thin. The reported toxic level of $H_2S$ is $<$ twofold greater than its endogenous level in rat brain tissues (26). Intoxication of mice with NaSH only elevated the sulfide concentration from the endogenous level by 57%, 18%, and 64% in brain, liver, and kidney, respectively (28). It is thus reasoned that the dose-response relationship of $H_2S$ at the physiological concentration range must be very steep before the physiological effect of $H_2S$ sharply transforms into a highly toxic effect (4). Moreover, mammalian cells must possess a delicate regulatory mechanism to control the endogenous $H_2S$ level within the physiological range.

### Interaction of $H_2S$ with other gasotransmitters

Given that $H_2S$, NO, and CO can all be gasotransmitters, they are not redundant (**Table 2**). For example, $H_2S$, NO, and CO facilitate the induction of hippocampal LTP. This effect of $H_2S$ depends on the activation of NMDA receptors (6) whereas that of NO and CO does not. NO can act as a reactive oxygen species by impairing the reduced glutathione/oxidized glutathione balance and/or by inhibiting enzymes and ion channels through S-nitrosylation processes. $H_2S$ may also be involved in the reduction of thiols, whereas CO is not directly involved in redox reactions. Gasotransmitters may interact with each other. As discussed above, competition for the common hemoglobin sink by one gasotransmitter would potentiate or unmask the biological effect of other gasotransmitters.

Published data have shown that the endogenous production of $H_2S$ from rat aortic tissues is enhanced by NO donor treatment (4). The NO donor also enhances the expression level of CSE in cultured vascular SMCs. Similar to the release of NO by acetyl-

TABLE 2. *Metabolism and function of gasotransmitters*[a]

| | $H_2S$ | CO | NO |
|---|---|---|---|
| Main substrates | L-cysteine | Heme | L-arginine |
| Generating enzymes | CBS, CSE | Heme oxygenases | NO synthases |
| Inducer | NO | Free radicals | Acetylcholin, endotoxin |
| Scavenger | Hemoglobin | Hemoglobin | Hemoglobin |
| Inhibitor | D,L-propargylglycerine | Zinc-PPIX | L-NAME |
| Protein targets | $K_{ATP}$ channel, cAMP (?) | cGMP, $K_{Ca}$ channel | cGMP, $K_{Ca}$ channel |
| Amino acid targets | ? | Histidine | Cysteine |
| Half-life in solution | Minutes | Minutes | Seconds |
| Production tissue source | SMC, not in EC | EC < SMC | EC > SMC |

[a] Only examples, not a complete list, are given. SMC, smooth muscle cell; EC, endothelial cell; zinc-PPIX, zinc protoporphyrin-IX; L-NAME, $N^G$-nitro-L-arginine methyl ester.

choline, release of $H_2S$ by NO adds a line of essential evidence for the physiological role of $H_2S$.

Finally, the integrated vascular effect of $H_2S$ and NO may not be a simple algebraic summation of their individual actions. Hosoki et al. (5) observed that the vasorelaxant effect of sodium nitroprusside (SNP), a NO donor, was enhanced by incubating rat aortic tissues with 30 μM NaHS. On the contrary, pretreating aortic tissues in another study with 60 μM $H_2S$ inhibited the vasorelaxant effect of SNP. This discrepancy may be partially explained by the experimental conditions of these studies, including differences in tissue preparations and tension development before the application of $H_2S$. The putative interactions of NO and $H_2S$ are hypothetically presented in **Fig. 3**.

## CONCLUDING REMARKS AND PERSPECTIVES

In keeping with the criteria listed in Table 1, $H_2S$ might be classified as the third gasotransmitter besides NO and CO. This gas is endogenously generated and manifests significant effects at physiologically relevant concentrations. The effect of $H_2S$ on $K_{ATP}$ channels may represent an important endogenous mechanism in vascular SMCs, neurons, and other excitable cells to couple cellular metabolism to excitability. By demonstrating the role of NO as an inducer or as a molecular switch for endogenous $H_2S$ production, we can begin to understand how the interaction between $H_2S$ and NO provides an integrated regulation of vascular tone. These advances in $H_2S$ research may revolutionize many conventional doctrines. For example, hyperhomocystinemia is a disease with a deficient expression of CBS. The role of a low level of endogenous $H_2S$ in the pathogenesis of this disease has been largely overlooked or simply neglected (13), yet it may be an important cause of atherosclerosis and thrombotic complications associated with hyperhomocystinemia. We still have a long way to go before a complete understanding of cellular metabolism and functions of $H_2S$ is achieved. The following future studies of $H_2S$ physiology serve only as examples.

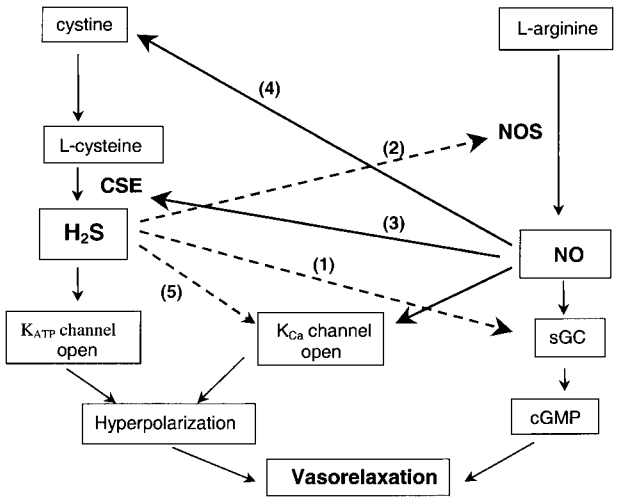


**Figure 3.** Hypothesized scheme of the interaction of $H_2S$ and NO in vascular tissues. The solid lines indicate the stimulatory inputs and the dashed lines, inhibitory inputs. (*1*) $H_2S$ may decrease the sensitivity of the cGMP pathway to NO (27). (*2*) $H_2S$ may reduce the expression level of NO synthase (NOS). (*3*) NO may increase the expression of CSE. (*4*) NO may increase the cellular uptake of cystine. (*5*) $H_2S$ may modify $K_{Ca}$ channels to decrease their sensitivity to NO.

*1)* Molecular mechanisms of the interaction of $H_2S$ and $K_{ATP}$ channels should be further investigated. As expression of different $K_{ATP}$ channel subunits is tissue-type specific, whether $H_2S$ stimulates $K_{ATP}$ channels in other tissues (e.g., lungs, kidney, pancreas) as it does in vascular SMCs and neurons may be a key to the differential effects of $H_2S$ on different tissues. Direct evidence, including single channel recording on heterologously expressed $K_{ATP}$ channels in the presence of $H_2S$, should be collected. $H_2S$ may interact with membrane and/or cytosol proteins to form reactive and unstable persulfides (44). These persulfides may take different forms, including protein-SSH, thiotaurine, thiocysteine, thiocystine, or mercaptopyruvate (45). The persulfide-related sulfuration and structural changes of the targeted proteins are recognized mechanisms for the biological effects of sulfide donors. This mechanism may underlie the interaction of $H_2S$ and $K_{ATP}$ channel proteins.

*2)* $H_2S$ may alter cellular redox status. $H_2S$ in an aqueous solution is a weak reducing agent. Vasorelaxation induced by $H_2S$ was not mimicked by the disulfide bond-reducing agents (5) but the $H_2S$-induced modulation of $Na^+$ channels in neurons was (34). This controversy

supports, rather than denounces, the importance of the reducing capability of $H_2S$. Quite likely, manifestation of the reducing effect of $H_2S$ depends on the tissue-specific targets and the tissue-specific redox environment. Does $H_2S$ have an oxidative potential? This is unsettled given the reported yield of free radicals from $H_2S$. In the presence of peroxidase and $H_2O_2$, $H_2S$ produced thiyl free radicals ($SH^{\bullet}$ and $S^{\bullet}$) (46). More vigorous studies are needed to investigate the physiological effects of $H_2S$ in the presence of different antioxidants, especially the scavengers for thiyl free radicals.

*3)* The endogenous inhibitors and stimulators for $H_2S$ production should be explored. Since CBS is a heme-containing protein (10) and heme-containing proteins are common targets of NO and CO, the activity of CBS might be under the influence of both CO and NO (47). CSE activity is increased by L-cysteine (48), but this substance is not stable and may have neurotoxicity. Steroid hormones are putative modulators of CBS functions; one such example is the testosterone-induced increase in the activity of CBS (49). The expression of CBS is also inducible. Although no CBS protein could be detected in freshly isolated human aortic tissues, primarily cultured human aortic SMCs within five passages exhibited clear CBS activity and protein expression (38). This may imply a regulatory role of endogenous $H_2S$ in the proliferation of vascular SMCs, which are normally quiescent.

*4)* Pharmacological or genomic manipulation of $H_2S$ production is an underdeveloped area with great potential. Enhancement of CBS activity by S-adenosyl-methionine (6, 9, 50) may find novel applications in dealing with some brain disorders. However, S-adenosyl-methionine may have other effects unrelated to the endogenous generation of $H_2S$ due to its methyl donor role. Specific activators of CSE, which is uniquely expressed in vascular tissues, are not available at present, but these agents can be important tools in the regulation of abnormal cardiovascular functions related to the altered endogenous $H_2S$ metabolism. Most if not all of the currently available inhibitors for different types of the $H_2S$-generating enzymes are not membrane permeable, which significantly impedes their applications under physiological conditions. A heterozygous deficiency of CBS mice has been established (51). The transgenic animal model with CSE deletion will be needed to establish the contribution of this enzyme to endogenous $H_2S$ levels in vascular tissues.

*5)* Investigations should begin to look into the pathological role of endogenous $H_2S$. Deficiency in CBS expression causes hyperhomocystinemia, which leads to premature peripheral and cerebral occlusive arterial disease (52). The pathogenic role of low levels of $H_2S$ in this disease has not been explored. Similarly, homocystinuria is an autosomal recessively inherited disorder (53) that may be closely related to the low endogenous production of $H_2S$. On the other hand, Down syndrome with elevated CBS expression, low plasma homocysteine, and significantly increased thiosulfate urinary excretion (54) may couple to abnormally high $H_2S$ levels. These observations have led to the hypothesis that the accumulation of $H_2S$ in the brain could cause the metabolic intoxication (55). Sudden infant death syndrome may be related to the abnormally higher taurine levels induced by $H_2S$ (34). The development of vascular diseases after heart transplantation is accompanied by increased total plasma homocysteine concentrations (56). In this case and other vasculopathy circumstances, a potentially lower endogenous level of $H_2S$ may be an important pathogenic factor.

Now that the role of $H_2S$ has been identified as sharing metabolic mechanisms and cellular effects similar to NO and CO, it is the time to call the family of gasotransmitters to 'please stand up.' It is expected that the gasotransmitter family will be expanded to include other yet undefined endogenous gaseous molecules. FJ

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