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Signaling Molecules: Hydrogen Sulfide and Polysulfide

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Abstract

Significance: Hydrogen sulfide (H_2S) has been recognized as a signaling molecule as well as a cytoprotectant. It modulates neurotransmission, regulates vascular tone, and protects various tissues and organs, including neurons, the heart, and kidneys, from oxidative stress and ischemia-reperfusion injury. H_2S is produced from L-cysteine by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST) along with cysteine aminotransferase. ***Recent Advances:*** In addition to these enzymes, we recently identified a novel pathway to produce H_2S from D-cysteine, which involves D-amino acid oxidase (DAO) along with 3MST. These enzymes are localized in the cytoplasm, mitochondria, and peroxisomes. However, some enzymes translocate to organelles under specific conditions. Moreover, H_2S-derived potential signaling molecules such as polysulfides and HSNO have been identified. ***Critical Issues:*** The physiological stimulations, which trigger the production of H_2S and its derivatives and maintain their local levels, remain unclear. ***Future Directions:*** Understanding the regulation of the H_2S production and H_2S-derived signaling molecules and the specific stimuli that induce their release will provide new insights into the biology of H_2S and therapeutic development in diseases involving these substances. *Antioxid. Redox Signal.* 22, 362–376.

Introduction

THE PHYSIOLOGICAL ROLE of hydrogen sulfide (H_2S) was initially proposed on the basis of the finding that H_2S facilitates the induction of hippocampal long-term potentiation (LTP) by enhancing the activity of *N*-methyl D-aspartate (NMDA) receptors (1). It was later found that H_2S also relaxes vascular smooth muscle by activating adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP})-, intermediate conductance Ca^{2+}-sensitive K^+ (IK_{Ca}),- and small conductance Ca^{2+}-sensitive K^+ (SK_{Ca}) channels (25, 52, 104). The release of insulin and angiogenesis are also regulated by this molecule (8, 31, 64, 103). In addition to these activities, we found that H_2S protects neurons from oxidative stress and ischemia-reperfusion injury by recovering the levels of glutathione, a major intracellular antioxidant, as well as by directly scavenging reactive oxygen species (ROS) produced in mitochondria, and suppressing the excessive increase in intracellular Ca^{2+} concentrations (33, 34, 44, 95). These findings led to the identification of the protective effect of H_2S on various organs, including the heart and kidney, from oxidative stress and ischemia-reperfusion injury (16, 90).

H_2S is produced by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST). CBS and CSE produce H_2S from cysteine alone or from cysteine with homocysteine (1, 11, 25, 80). 3MST produces H_2S from 3-mercaptopyruvate (3MP), which is produced from cysteine and α-ketoglutarate by cysteine aminotransferase (CAT) (13, 42, 76). Since the endogenous-reducing substance required for the production of H_2S by 3MST has not been identified, the 3MST/CAT pathway has not been recognized as the H_2S-producing pathway. We recently found that thioredoxin and dihydrolipoic acid (DHLA) are the endogenous reducing substances that associate with 3MST for the production of H_2S (43). Similar results were also obtained by Banerjee *et al.* (98).

H_2S is even produced from D-cysteine *via* D-amino acid oxidase (DAO) along with 3MST (74). An achiral 3MP, which is a substrate for 3MST, is produced by DAO from D-cysteine. Although 3MST is a ubiquitous enzyme, DAO is localized only in the kidney and the brain. Therefore, the production of H_2S from D-cysteine is restricted to these organs, and it correlates well with the levels of DAO and 3MST. In the kidney, H_2S produced from D-cysteine protects the renal cortex from ischemia-reperfusion injury more efficiently than does L-cysteine. The administration of

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D-cysteine provides a novel therapeutic approach to deliver H_2S to specific tissues or organs such as the kidney and the brain.

H_2S induces Ca^{2+} influx in astrocytes by activating transient receptor potential (TRP) channels, and the EC_{50} of sodium hydrosulfide (NaHS) is 116 μM (54). Although the endogenous concentration of H_2S was initially reported to be 50–160 μM, re-evaluation revealed it to be in the range of 10 nM to 3 μM (20, 26, 97). The K_m values of H_2S-producing enzymes are greater than the endogenous concentrations of their substrate, cysteine. These observations suggest that the concentrations of H_2S are not adequate for inducing physiological responses. To compensate for the inadequate concentrations, H_2S is stored as bound sulfane sulfur, which can release H_2S when cells are stimulated. Alternatively, H_2S is oxidized to polysulfides (H_2S_n). In our recent study, we found that polysulfides, which exist in the brain, activate TRP ankyrin 1 (TRPA1) channels in astrocytes to induce Ca^{2+} influx more potently than does parental H_2S, with the EC_{50} of Na_2S_3 being 91 nM (35). Polysulfides are potential signaling molecules that modulate the activity of enzymes, channels, and receptors by inducing conformational changes through adding bound sulfane sulfur to thiols of cysteine residues, a process termed sulfhydration (sulfuration) (66, 89).

H_2S Production

H_2S is produced by CBS, CSE, and 3MST, along with CAT. CBS, CSE, and CAT are pyridoxal 5′-phosphate (PLP)-dependent enzymes that are suppressed by hydroxylamine (74).

Cystathionine β-Synthase

CBS is expressed in various tissues, including the kidney, liver, brain, ileum, uterus, placenta, and pancreatic islets (1, 31, 65, 82). CBS produces H_2S from cysteine *via* a β-elimination reaction, and more efficiently *via* a β-replacement reaction in which cysteine is condensed with homocysteine (10, 80). The production of H_2S by CBS is enhanced by *S*-adenosyl methionine, an allosteric activator of CBS (1, 72). The activity of CBS is suppressed by nitric oxide ($^{\bullet}NO$) and carbon monoxide (CO), which bind to the heme localized to the amino terminus of CBS (49, 85).

CBS also catalyzes the condensation reaction of homocysteine and serine to produce cystathionine. This reaction is preferred compared with metabolizing cysteine to produce H_2S because of the K_m values of substrates involved (50). However, a higher reaction rate does not necessarily exclude the possibility of an alternate reaction, which might depend on the availability of substrates and the subsequent metabolism of the products.

Cystathionine γ-Lyase

CSE is expressed in tissues such as the kidney, liver, thoracic aorta, ileum, portal vein, uterus, pancreatic islets, and placenta (25, 31, 65, 104). The expression of CSE is not detected in the brain, and the activity of CSE is 100 times less than that in the liver (1, 17, 28, 92). CSE produces H_2S by α, β-elimination of cysteine under normal conditions. Under conditions of high homocysteine concentrations, as in homocysteinemia, the α, γ-elimination, and γ-replacement reactions of homocysteine become dominant for H_2S production (11).

CSE is a cytoplasmic enzyme. When smooth muscle cells obtained from mesenteric arteries were exposed to a calcium ionophore—A23187—for more than 16 h, CSE translocated to the mitochondria and was involved in ATP production (19). Although the mitochondria and endoplasmic reticulum store Ca^{2+}, excessive Ca^{2+} due to long-lasting Ca^{2+} influx results in toxicity that can cause cell death. Therefore, it is necessary to determine how cells avoid toxicity due to high intracellular Ca^{2+} concentrations that result from long-lasting Ca^{2+} influx.

CSE has canonical activity that catalyzes cystathionine to produce cysteine (11). Although turnover rate of cysteine production is greater than H_2S production under normal conditions, the rate of H_2S production becomes compatible to that of cysteine production under the conditions with high homocysteine concentrations such as homocysteinemia (11, 80). The H_2S-producing activity of CSE is regulated by Ca^{2+}, and it is intriguing to know whether the cysteine production is regulated in a similar fashion (45). Therefore, understanding how CSE catalyzes the production of either H_2S or cysteine *in vivo*, or both simultaneously, is of particular interest.

3-Mercaptopyruvate Sulfurtransferase/ Cysteine Aminotransferase

3MST and CAT are ubiquitous enzymes (74–76). CAT, which is identical to aspartate aminotransferase (AAT), metabolizes cysteine and α-ketoglutarate to 3MP and glutamate. 3MP is further metabolized by 3MST to H_2S and pyruvate.

The contribution of the 3MST/CAT pathway to H_2S production is greater than that of CBS. The expression of 3MST and CAT in HEK293F cells increased the level of bound sulfane sulfur, which is a storage form of H_2S, to approximately twice as much as the control (76). In contrast, cells expressing CBS do not significantly increase the levels of bound sulfane sulfur.

3MST and CAT are localized in both the mitochondria and cytosol, and the activity of both enzymes is predicted to be suppressed under oxidative stress conditions such as those prevailing in the mitochondria (30). However, Neuro2a cells expressing both 3MST and mitochondrial CAT showed a significant resistance to oxidative stress caused by high concentrations of glutamate, whereas cells expressing CBS did not (33). These observations suggest that 3MST and mitochondrial CAT are functional in the mitochondria even under conditions of oxidative stress and that these enzymes protect cells from oxidative stress damage to a greater extent than does CBS.

3MST requires a reducing substance such as dithiothreitol (DTT) to produce H_2S. Since an endogenous-reducing substance had not been identified, 3MST had not been recognized as an H_2S-producing enzyme. Along with the previous findings that an endogenous-reducing substance, thioredoxin, exists at a concentration of $\sim$20 μM in cells and is able to interact with 3MST, our findings suggest that 3MST reacts with thioredoxin to produce H_2S (24, 43, 53). There are two forms of thioredoxin in mammals: thioredoxin 1 is localized to the cytosol, and thioredoxin 2 is localized to the mitochondria (24, 81). Since a sequence containing active-site cysteine residues is conserved among different species, we

used bacterial thioredoxin. Since thioredoxin is readily oxidized, it is necessary to reduce it by thioredoxin reductase. The mammalian thioredoxin reductase is a selenoprotein, and bacteria are not able to incorporate selenium into the protein being produced. Therefore, we added lysates of A549 human lung adenocarcinoma cells, which possess abundant thioredoxin reductase, to thioredoxin with nicotinamide adenine dinucleotide phosphate (NADPH). The reduced form of thioredoxin enhanced the production of H_2S by 3MST by ~10-fold compared with the control and 4-fold compared with DTT treatment (43).

DHLA, which is a dithiol similar to DTT, exists at ~40 μM in the brain (32, 67, 93). Since DHLA is readily oxidized to α-lipoic acid, it should be reduced with $NaBH_4$ before use (89). In the presence of 40 μM DHLA, H_2S production by 3MST was increased threefold, which is a level similar to that seen in the presence of DTT (43). Banerjee *et al.* showed thioredoxin as a potential reductant and DHLA as a less efficient one (98). They showed that the effect of thioredoxin estimated from the K_m values is ~500 times greater than that of DTT, and that of DHLA is ~1/3 of DTT. Our observations showed that the effect of the endogenous concentration 20 μM thioreidoxin is ~10 times greater than that of DTT, and the effect of 40 μM DHLA is ~3 times greater than that of DTT (43). The applied methods to measure H_2S levels are different between two studies: One measured the levels of produced lead sulfide by spectrophotometer, while the other measured H_2S by gas chromatography. These data are not contradictory (43, 98). Other endogenous-reducing substances, such as cysteine, glutathione, NADH, NADPH, and CoA, do not have any effect on H_2S production by 3MST (43). The reducing potentials of thioredoxin and the dithiols DTT and DHLA are −0.26, −0.29, and −0.33 V, respectively; those of monothiol glutathione, cysteine, and CoA are between −0.22 and −0.35 V; and those of NADH and NADPH are −0.32 V (7, 12, 23, 29). There is no correlation between the reducing potential and the ability to associate with 3MST to enhance the production of H_2S. Since thioredoxin has two cysteine residues at its active site, a dithiol may be critically required for these substances to facilitate the production of H_2S by 3MST. A possible mechanism is that 3MST receives sulfur from 3MP to produce 3MST persulfide, which is transferred to one of the two thiols of thioredoxin or DHLA to produce thioredoxin- or DHLA-persulfide. These compounds are reduced by the remaining thiol in thioredoxin or DHLA to release H_2S (43).

Regulation of CAT/3MST and CSE by Ca^{2+}

Although it is well known that CBS is regulated by *S*-adenosyl methionine (1, 72), the regulation of other H_2S-producing enzymes, such as CSE and 3MST/CAT, is not well understood. We recently found that CAT and CSE are regulated by Ca^{2+} (Fig. 1) (44, 45). Since 3MP production by CAT is regulated by Ca^{2+}, H_2S production by the 3MST/CAT pathway is regulated by Ca^{2+}. In the absence of Ca^{2+}, the production of H_2S by the 3MST/CAT pathway is maximal and is suppressed by Ca^{2+} in a concentration-dependent manner (44). The production of H_2S is ~80% of the maximal production at 10 nM Ca^{2+}, 20% at 100 nM, and completely suppressed at 3 μM Ca^{2+}. The intracellular concentration in neurons at a steady state is ~100 nM. When neurons are excited, the Ca^{2+} concentration increases to 3 μM. In retinal photoreceptor cells, Ca^{2+} concentrations are maintained at levels lower than those in other neurons. When photoreceptor cells are exposed to light, the Ca^{2+} concentration decreases to ~10 nM. It increases to 600–800 nM in darkness (40). The 3MST/CAT pathway produces H_2S at a steady state under low Ca^{2+} concentrations, but stops producing it when neurons are excited or photoreceptor cells are exposed to light. Given that 3MST and CAT are also localized to the vascular endothelium and that the 3MST/CAT pathway is a major pathway for H_2S production (75), the production of H_2S in the vascular endothelium should also be suppressed by Ca^{2+}, which is increased by stimulation with transmitters such as acetylcholine. Calmodulin or the calmodulin inhibitor W7 does not have any effect on the production of H_2S *via* the 3MST/CAT pathway, suggesting that calmodulin is not involved in this regulation by Ca^{2+} (44). The regulation of NO production is, in contrast, increased by Ca^{2+}/calmodulin (5).

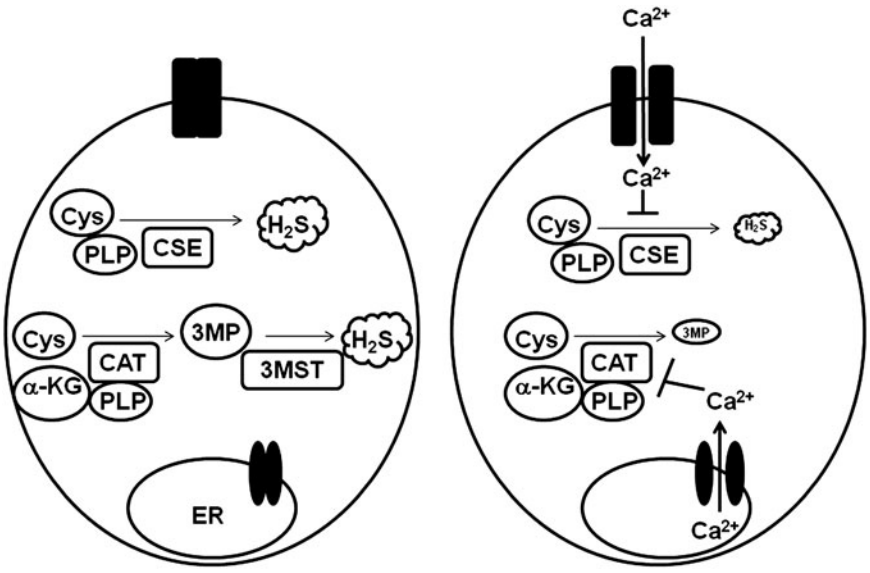

FIG. 1. Suppression of the H_2S-producing activities of CSE and the 3MST/CAT pathway by Ca^{2+}. CSE and the 3MST/CAT pathway produce H_2S in a steady state at low intracellular concentrations of Ca^{2+}. When intracellular Ca^{2+} concentrations are increased by Ca^{2+} influx or a Ca^{2+} release from the mitochondria or endoplasmic reticulum, CSE decreases the production of H_2S by 50%, and the 3MST/CAT pathway nearly ceases producing H_2S. CAT, cysteine aminotransferase; CSE, cystathionine γ-lyase; H_2S, hydrogen sulfide; 3MST, 3-mercaptopyruvate sulfurtransferase.

The activity of CSE, a PLP-dependent enzyme, is also suppressed by Ca^{2+} in the presence of PLP (Fig. 1). In the presence of 100 n*M* Ca^{2+}, the activity is maintained at the maximal level; whereas it is suppressed by ~50% with Ca^{2+} concentrations greater than 300 n*M* (45). Therefore, in the steady state with 100 n*M* Ca^{2+}, CSE efficiently produces H_2S, but production is decreased to 50% when cells are stimulated to increase their intracellular Ca^{2+} stores. PLP, which is in Schiff-base linkage with CSE, dissociates from CSE and forms a new linkage with a substrate cysteine to produce H_2S (84). The low-Ca^{2+} steady state facilitates the formation of this linkage between cysteine and PLP, leading to enhanced H_2S production; whereas linkage formation is suppressed when Ca^{2+} concentrations are increased, resulting in suppression of H_2S formation. Calmodulin is not involved in this regulation by Ca^{2+} (45). H_2S may regulate vascular tone in the steady state, and once intracellular Ca^{2+} concentrations in the endothelium are increased by stimulation, the contribution of H_2S to relaxing vascular smooth muscle is likely to be lowered.

Contradictory data exist regarding the regulation of H_2S production in vascular endothelium by Ca^{2+}/calmodulin, and the blood pressure of CSE knockout mice is higher than that of wild-type mice (101). However, these previous data were obtained in the presence of 1–2 m*M* Ca^{2+}, which are extracellular concentrations. The blood pressure was found to be normal in CSE knockout mice established by another group (27). Considering the fact that vascular endothelium produces H_2S only in the presence of α-ketoglutarate, the 3MST/CAT pathway is the major pathway that produces H_2S in the vascular endothelium (75). Although 3MST and CAT are localized to the vascular endothelium, we and Olson *et al.* have not observed CSE in the endothelium (62, 75, 104). CSE mRNA was not found in the endothelium by both RT-PCR as well as *in situ* hybridization, but the same group later found the CSE protein in the endothelium *via* immunohistochemical analysis (101, 104). Even if H_2S produced by CSE is involved in the regulation of blood pressure, the 3MST/CAT pathway may compensate for the production of H_2S in the endothelium (75).

Other contradictory data on CSE knockout mice suggests that H_2S is either protective or apoptotic in vascular smooth muscle cells. The vascular smooth muscle cells of the wild-type mice produce ATP to a much greater extent than those of CSE knockout mice, and H_2S improves mitochondrial ATP production in vascular smooth muscle cells under hypoxia, which alone decreases ATP production (19). In contrast, the cellular apoptosis was less in CSE knockout mice than in the wild type, and the apoptosis was enhanced by exogenously applied H_2S or a hypoxic insult, suggesting that H_2S causes apoptosis simply by inducing hypoxia (6, 100). The levels of toxic homocysteine are greater and those of cytoprotective glutathione are less in CSE knockout mice than in the wild type, and higher rates of cell death were reported by Ishii *et al.* (27). It is intriguing to know the mechanism for the resistance to apoptosis and the involvement of H_2S in the induction of apoptosis in CSE knockout mice.

With regard to the cell proliferation of vascular smooth muscle, cells in CSE knockout mice proliferate to a much greater extent than those in the wild-type mice, and H_2S suppresses the cell proliferation (100). In contrast, H_2S induces angiogenesis, which is more significant in the wild-type mice than in the CSE knockout mice (64). Some factors in addition to H_2S may be involved in differentiating the vascular cell proliferation from angiogenesis.

Okamoto *et al.* showed that a lack of CSE causes apoptotic beta-cell death and facilitates the development of high-fat diet-induced diabetes (60), whereas Yang *et al.* showed that CSE deficiency protects pancreatic beta cells and delays the development of streptozotocin-induced diabetes (103). In spite of the degree of damage caused in beta cells by streptozotocin being greater than the high-fat diet, CSE deficiency protects pancreatic beta cells from streptozotocin-induced toxicity. It is necessary to clarify the involvement of H_2S in beta-cell apoptosis as well as the possibility of compensation for H_2S production by other H_2S-producing enzymes such as 3MST (79).

Mice lacking CSE showed dysfunctional endothelial nitric oxide synthetase (eNOS) and diminished NO levels, resulting in exacerbated myocardial and hepatic ischemia-reperfusion injuries (36). In contrast, endogenous production of NO from neuronal NOS (nNOS) is significantly higher in the muscle layer of the mouse colon in CSE knockout mice than in the wild-type mice (71). A lack of H_2S or the increased homocysteine levels may cause an opposite effect on eNOS and nNOS. Further studies are awaited to clarify the mechanism.

A lack of H_2S does not necessarily exert the same effect with CSE knockout mice. Although H_2S is thought to be cytoprotective against systemic inflammation, the administration of propagyl glycine and CSE knockout exert anti-inflammatory in sepsis and acute liver failure (3, 79, 88). The interesting observation is that the levels of 3MST were increased in CSE knockout mice, suggesting the compensation for H_2S production by 3MST (79). To determine the involvement of H_2S in these effects, it is necessary to accurately compare the endogenous H_2S levels between CSE knockout and the wild-type mice, as only *in vitro* production of H_2S in the presence of a substrate cysteine or H_2S released from acid-labile sulfur was measured in these studies.

Production of H_2S from D-Cysteine *via* the 3MST/DAO Pathway

Mammalian enzymes generally metabolize L-amino acids, though there are a few exceptions, such as D-serine and D-asparate. Therefore, we applied D-cysteine to brain homogenates as a negative control of L-cysteine for the production of H_2S. Surprisingly, H_2S was produced from D-cysteine (74). D-Cysteine is metabolized by DAO to achiral 3MP, which is a substrate for 3MST in the production of H_2S (Fig. 2). DAO is localized to peroxisomes, whereas 3MST is found in mitochondria (21, 76). Peroxisomes and mitochondria are in close proximity to each other, are in physical contact, and exchange their metabolites between them (69). Since 3MST is a ubiquitous enzyme, DAO, which is localized to the brain and the kidney, restricts the activity of the 3MST/DAO pathway; the production of H_2S from D-cysteine occurs only in the brain and the kidney. H_2S production from D-cysteine is the greatest in the cerebellum in the brain, but the production in the kidney is seven times greater than that in the cerebellum (74).

The existence of racemase, which induces a chiral change of L-cysteine to D-cysteine, or D-cysteine-producing enzymes, is not well understood. L-amino acids are nonenzymatically

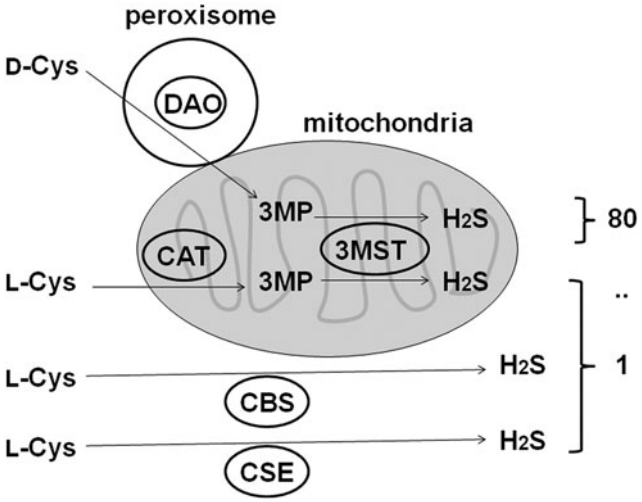

FIG. 2. Production of H_2S from D-cysteine by the 3MST/DAO pathway. D-Cysteine is metabolized by the peroxisomal enzyme DAO to 3MP, which is transferred to the mitochondria and metabolized by 3MST to H_2S. In the kidney, the production of H_2S from D-cysteine is 60 times higher than that from L-cysteine. DAO, D-amino acid oxidase; 3MP, 3-mercaptopyruvate.

racemized by the heat and alkaline conditions applied during food processing, and ~40% of L-cysteine is changed to D-cysteine by alkaline treatment; D-cysteine is easily absorbed through the gastrointestinal tract and enters the blood stream (39, 41). D-Cysteine may, thus, be mostly provided from food.

Interaction of H_2S with $^{\bullet}NO$

The smooth muscle-relaxing effect of H_2S on the thoracic aorta was rather weak compared with that on other smooth muscles such as the ileum and portal vein. We found a synergistic effect of H_2S and $^{\bullet}NO$ on the relaxation of vascular smooth muscle (Fig. 3) (25). Moore *et al.* also observed a synergistic effect between the two substances on the inhibition of the twitch responses of the ileum to electrical stimulation (86); however, this synergistic effect is controversial and was not observed in another study (104). Instead, the production of H_2S is enhanced by the $^{\bullet}NO$ donor sodium nitroprusside (SNP), and transcription of CSE is upregulated by another $^{\bullet}NO$ donor *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP). H_2S enhances the production of NO in the heart and plasma, and it has the opposite effect on NO in colon smooth muscle (36, 71).

Although the concentrations are too high to be physiological, 200 μM H_2S and 1 mM nitrite react in the presence of 30 μM Fe^{3+}-porphyrins to produce HSNO and HNO (46). It is not clear at present whether these substances have physiological roles and whether $^{\bullet}NO$ released from these compounds is the final effector molecule. In order to produce HSNO and HNO, both H_2S and $^{\bullet}NO$ have to be present at the same time. However, H_2S production *via* the 3MST/CAT pathway and by CSE is suppressed by Ca^{2+}, whereas $^{\bullet}NO$ production by NOS is enhanced by Ca^{2+} (5, 44, 45). H_2S is produced by CSE and 3MST/CAT when cells are in the steady state with low intracellular concentrations of Ca^{2+}, while $^{\bullet}NO$, which is produced by the Ca^{2+}/calmodulin-regulated enzyme NOS, is produced when intracellular Ca^{2+} concentrations are increased (5, 44, 45). Therefore, it is difficult to provide both substances simultaneously *via* enzymatic production. Another source of H_2S, bound sulfane sulfur, is present as a storage form of H_2S in cells (26). It is necessary to clarify the endogenous production of HSNO and HNO and their physiological roles.

Nitroso-cyclic guanosine monophosphate (cGMP), which is produced from nitro-cGMP by the addition of bound sulfane sulfur, has been proposed to be involved in the protection of the heart (57). Since 1 mM NaHS is required to sulfhydrate (sulfrate) nitro-cGMP to produce nitroso-cGMP, its physiological relevance needs to be re-evaluated.

Bound Sulfane Sulfur as an Intracellular Storage Form of H_2S

H_2S applied to tissue homogenates is recovered only in the presence of a reducing agent, DTT, suggesting that H_2S is stored as bound sulfane sulfur, the formation of which is

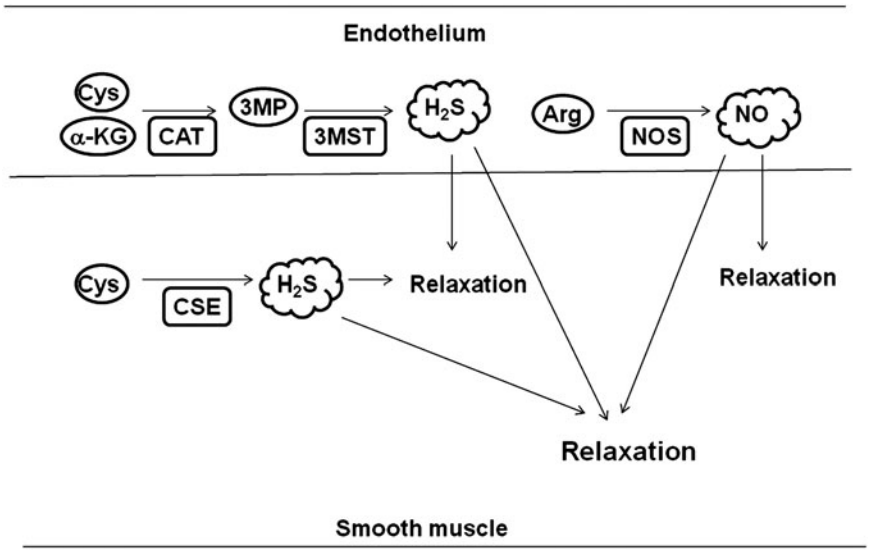

FIG. 3. Synergistic effect of H_2S and NO on vascular smooth muscle relaxation. The 3MST/CAT pathway mainly produces H_2S in the endothelium, while CSE produces H_2S in smooth muscles. There is a synergistic effect of H_2S on vascular smooth muscle relaxation with NO, which is produced by NOS from arginine. NOS, nitric oxide synthetase.

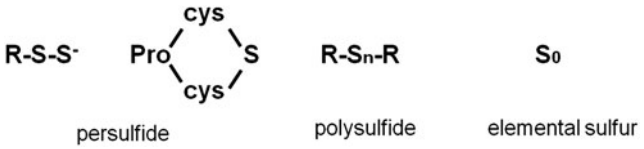

FIG. 4. Bound sulfane sulfur. The trisulfide bridge between two cysteine residues in a protein is a persulfide. Elemental sulfur attached to proteins and polysulfides release H_2S under reducing conditions.

called sulfhydration or sulfuration (Fig. 4) (26, 51, 89). As in the case of exogenously applied H_2S, endogenously produced H_2S by enzymes is also stored as bound sulfane sulfur (26). Cells that express the H_2S-producing enzymes 3MST and CAT contain twice as much of bound sulfane sulfur as control, whereas cells expressing 3MST mutants, which are not able to produce H_2S, contain levels of H_2S that are not significantly different from those of the control (76). Neurons and astrocytes release H_2S under reducing conditions, such as those with endogenous levels of the reducing substances glutathione and cysteine, at pH 8.4, which are induced in astrocytes when the surrounding neurons are excited (26). The majority of proteins contain bound sulfane sulfur, which releases H_2S under reducing conditions; $\sim 1.5\ \mu$mol H_2S/g protein is released over a period of 3 h in the brain, and 25%–50% of GAPDH, β-tubulin, and actin in the liver are sulfhydrated or sulfurated estimated by combining densitometric analysis with the modified biotin switch assay (26, 51). However, specific proteins that contain releasable bound sulfane sulfur, and physiological signals that induce the release of H_2S from the reservoir, have not been identified.

Physiological Role of H_2S and Its Product Polysulfide

Modification of synaptic transmission

The induction of hippocampal LTP requires the activation of NMDA-type glutamate receptors but not α-amino-3-hydroxy-5-methy-4 isoxazole proprionic acid (AMPA)-type receptors in neurons (1). NMDA receptors have a cysteine disulfide bond at the hinge region of the ligand-binding domain, whereas AMPA receptors do not. The reduction of the cysteine disulfide bond by a reducing substance DTT enhances the activity of NMDA receptors (2). As predicted, DTT facilitates the induction of LTP. Since DTT is a dithiol while H_2S is a monothiol, it is predicted that DTT has a greater effect on the induction of LTP than does H_2S. However, H_2S exerts a greater effect on LTP; it further facilitates the induction of LTP even after the induction by DTT (1). It is difficult to explain the difference in the effects of both substances on LTP induction based only on their reducing activity on NMDA receptors.

Our previous finding shows that H_2S activates astrocytes to induce Ca^{2+} influx, which propagates to nearby astrocytes as Ca^{2+} waves to transmit signals between astrocytes (54). The response of astrocytes to H_2S shows sensitization and desensitization depending on the applied concentrations of H_2S. Ca^{2+} signals induced by H_2S are augmented by repetitive applications of H_2S at low concentrations, whereas they are weakened at high concentrations (54). We recently found that H_2S is oxidized to polysulfides (H_2S_n) ($n = 3$–7), which induce Ca^{2+} influx in astrocytes ~ 300 times more potently than does parental H_2S, by activating TRPA1 channels (35, 55, 56, 63). The sensitivity of astrocytes to polysulfides depends on their maturity; glial fibrillary acidic protein (GFAP)-negative premature astrocytes do not respond well to polysulfides, whereas GFAP-positive mature astrocytes respond well to them (35, 91). Leukemia inhibitory factor accelerates the maturation of astrocytes and renders them sensitive to H_2S (91). Reactive astrocytes, which are induced by epidermal growth factor (EGF) and transforming growth factor alpha (TGFα) and are observed in injured brains, do not respond to H_2S and polysulfides.

Although the levels of TRPA1 channels in astrocytes are extremely low, astrocytes respond well to the TRPA1 channel-selective agonists allyl isothiocyanate and cinnamaldehyde. These observations suggest that TRPA1 channels are functional in astrocytes (35, 77, 78). Responses of astrocytes to polysulfides are suppressed by TRPA1 channel-selective inhibitors such as AP-18 and HC030031, as well as by broad-spectrum TRP channel inhibitors such as La^{3+}, Gd^{3+}, and

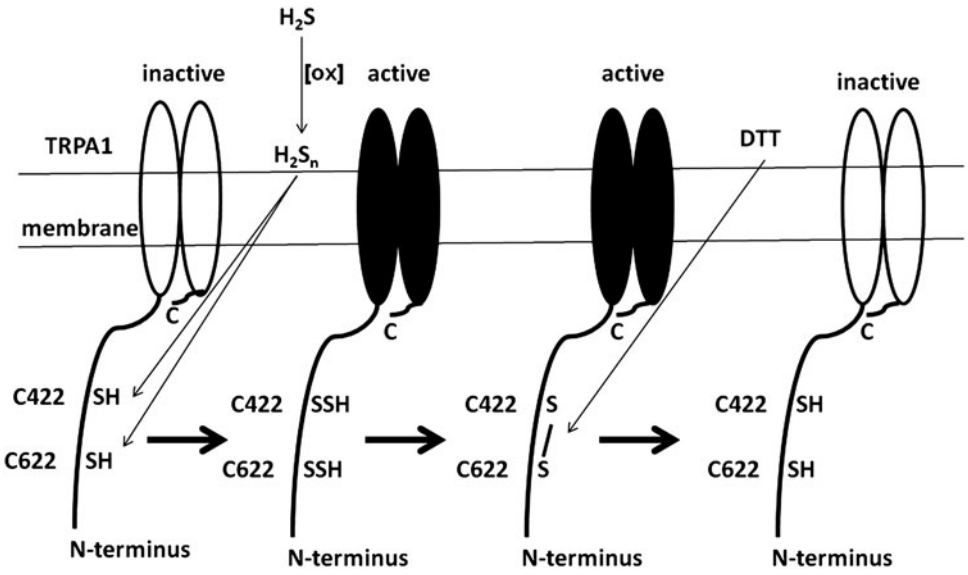

FIG. 5. Activation of TRPA1 channels through the addition of bound sulfane sulfur (sulfhydration or sulfration) by polysulfides produced from H_2S. Polysufides produced from H_2S add bound sulfane sulfur (sulfhydration or sulfuration) to the active cysteine residues located at the amino-terminus of TRPA1 channels to activate the channels. Sulfurated residues may further react with each other and produce cysteine disulfide bonds, which can be reduced back to cysteine residues by DTT, and the channels return to their inactive state. DTT, dithiothreitol; TRAP1, transient receptor potential ankyrin 1.

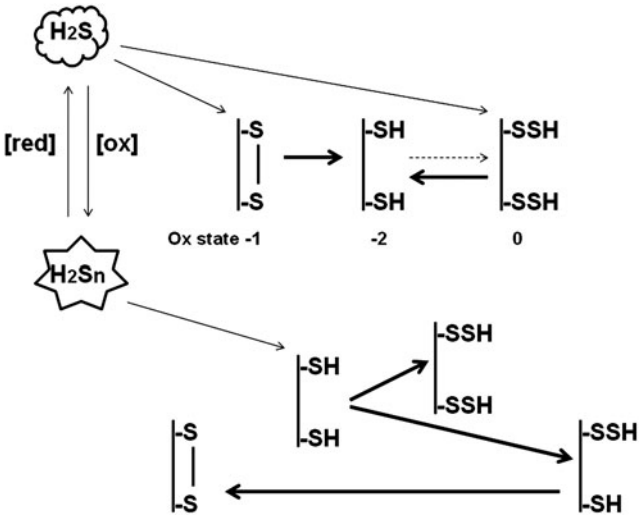

FIG. 6. Reduction of the cysteine disulfide bond by H_2S and the addition of bound sulfane sulfur (sulfhydration or sulfuration) to cysteine residues by polysulfides. H_2S (oxidation state: -2) reduces cysteine disulfide bond (oxidation state: -1) to generate two thiols of cysteine residues (oxidation state: -2). Polysulfides (oxidation state of inner sulfur: 0) add bound sulfane sulfur to cysteine residues (sulfhydration or sulfuration). A persulfurated cysteine (oxidation state of inner sulfur 0) reacts with a cysteine (thiol, oxidation state -2) to produce a cysteine disulfide bond.

ruthenium red (35). TRPA1 channel-selective siRNAs greatly suppress the levels of TRPA1 mRNA as well as responses to polysulfides. These observations suggest that polysulfides activate TRPA1 channels to induce Ca^{2+} influx in astrocytes.

TRPA1 channels were initially reported to be activated by H_2S in the urinary bladder and sensory neurons (58, 83). However, the activation was achieved by applying high concentrations of H_2S, in the range 1–10 m*M*. Therefore, these responses may be induced by polysulfides produced by the oxidation of some percentage of H_2S molecules. Ohta *et al.* identified the target of H_2S (or polysulfides) as Cys422 and Cys622 located in the amino terminus of TRPA1 channels, by showing that replacement of both these cysteine residues with serine abolished the sensitivity of the channels to H_2S (polysulfides) (Fig. 5) (58). They also showed that the application of DTT to TRPA1 channels, which have been activated by H_2S, suppresses the responses. These observations suggest that the activation of these channels is induced by the addition of bound sulfane sulfur (sulfhydration or sulfuration) to Cys422 and Cys622 by polysulfides, and the subsequent production of the cysteine disulfide bond, and their activity is suppressed when DTT removes bound sulfane sulfur or reduces the cysteine disulfide bond. Although it is not well understood whether polysulfides are actively transported into cells, polysulfides are known to readily pass through the plasma membrane (22, 35).

High-performance liquid chromatography (HPLC) analysis of brain homogenates derivatized by monobromobimane shows the existence of polysulfides in the brain at micromolar concentrations, which are sufficient to activate TRPA1 channels (35). Polysulfides are a mixture of substances with different numbers of sulfur atoms in equilibrium, and the longer the chain of sulfur, the less water soluble is the compound. The determination of the number of sulfur atoms in polysulfides by liquid chromatography tandem mass spectrometry is awaited.

The oxidation state of the S in H_2S and the thiol of a cysteine residue is -2, whereas the internal sulfurs in polysulfides are more oxidized. The exposed thiols of the two cysteine residues preferentially react with polysulfides but not with H_2S to produce bound sulfane sulfur, which induces conformational changes in the receptor to modify its activity.

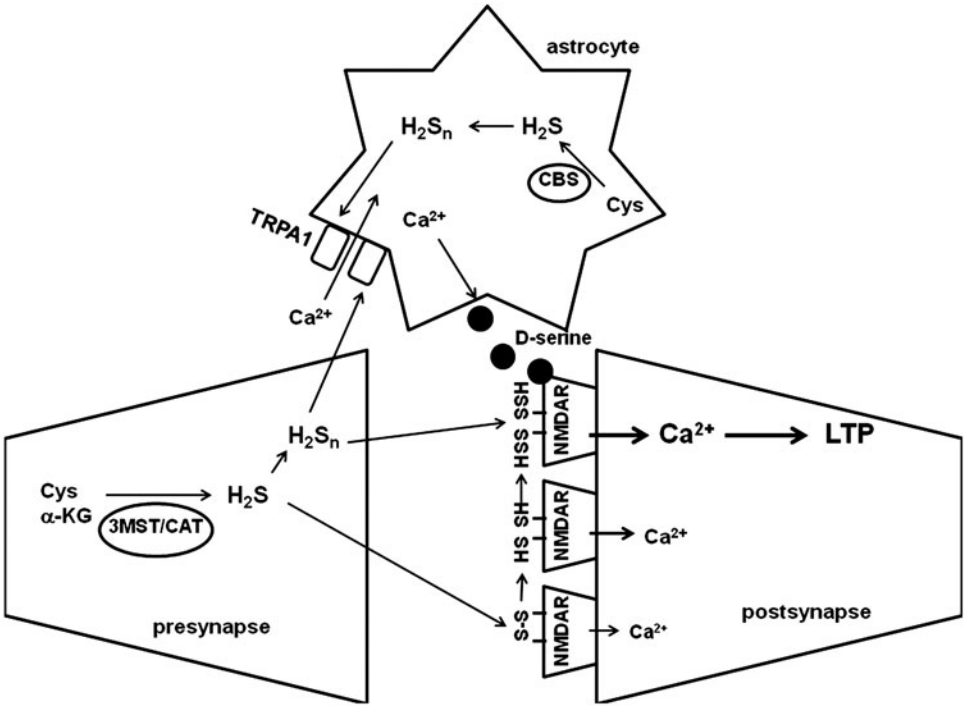

FIG. 7. Facilitation of hippocampal LTP induction by H_2S and polysulfides. H_2S reduces cysteine disulfide bond of NMDA receptors to enhance its activity. Polysulfides activate TRPA1 channels in astrocytes to induce Ca^{2+} influx, which triggers a release of the gliotransmitter D-serine that enhances the activity of NMDA receptors. Through these effects, H_2S and polysulfides facilitate the induction of LTP. LTP, long-term potentiation; NMDA, *N*-methyl D-aspartate.

Zhu *et al.* showed that H_2S reduces the cysteine disulfide bond in vascular endothelium growth factor receptor 2 (VEGFR2) to stimulate angiogenesis, but that H_2S does not add bound sulfane sulfur to thiols of cysteine residues (66, 87, 89). They observed a transient addition of bound sulfane sulfur (sulfhydration or sulfuration) to thiols of cysteine residues as an intermediate step in the course of the reduction of cysteine disulfide bond, but it disappeared immediately by a later reduction by the next H_2S molecule (Fig. 6).

As described earlier, it is difficult to explain the induction of LTP only by the redox modulation of NMDA receptor activity. Presynaptic neurons release neurotransmitters to postsynaptic neurons, and some of them come into contact with astrocytes, which have neurotransmitter receptors and respond to the transmitters. Activated astrocytes, in turn, release gliotransmitters to modulate synaptic activity. The induction of hippocampal LTP may be facilitated by the combinatorial effect of H_2S and polysulfides at the synapse by activating the surrounding astrocytes. In postsynaptic neurons, H_2S enhances the activity of NMDA receptors by reducing the cysteine disulfide bond of the receptors, and polysulfides further modify their activity by adding bound sulfane sulfur to the thiols of the cysteine residues. In astrocytes that surround synapses, polysulfides activate TRPA1 channels to induce Ca^{2+} influx, which, in turn, facilitates the release of the gliotransmitter D-serine to synapses. D-Serine enhances the activity of NMDA receptors by binding to the glycine-binding site of the receptors. The induction of LTP is enhanced by H_2S and polysulfides *via* these integrated mechanisms (Fig. 7) (1, 35, 77, 78).

Cytoprotective effect

Increase in the production of glutathione. Accumulating evidence shows that H_2S is cytoprotective in various tissues and organs. We found that H_2S protects neurons from oxidative stress by increasing the levels of glutathione, a major intracellular antioxidant (34). This finding led to the identification of a cytoprotective effect on various organs, including the heart and kidney (16, 90). In bacteria as well, inactivation of the bacterial homologs of CBS, CSE, and 3MST decreases the production of H_2S, causing vulnerability of bacteria for antibiotics, suggesting that the cytoprotective effect of H_2S is considered a universal defense mechanism that functions from bacteria to mammals (73).

In the extracellular space, cysteine exists its oxidized form cystine, which is reduced to cysteine in cells. H_2S enhances the activity of cystine/glutamate antiporter to increase the transport of cystine into cells, even in the presence of high concentrations of extracellular glutamate (34). Although the concentration is less than that of cystine, $\sim 20\,\mu M$ cysteine was found in blood (68), H_2S also facilitates the transport of cysteine into cells. Glutathione is produced by two enzymes, glutamate cysteine ligase (GCL) (γ-glutamylcysteine synthase [γ-GCS]), which is a rate-limiting enzyme in the production of γ-glutamyl cysteine from glutamate and cysteine, and glutathione synthetase (GS), which produces glutathione by adding glycine to γ-glutamyl cysteine. H_2S has no effect on GS, whereas it enhances the activity of GCL. Although the application of H_2S to cells increases the intracellular concentrations of γ-glutamyl cysteine, a product of GCL, H_2S does not induce the effect when it is applied to cell lysates. H_2S may activate some cell surface receptor to induce the intracellular signaling that activates GCL (34). By these effects, H_2S increases the production of glutathione (34). *In vivo,* the increase in glutathione levels is also induced by H_2S. Intraperitoneal application of NaHS to pregnant rats protects fetal brains from ischemia-reperfusion injury by reinstating the glutathione levels decreased by ischemia-reperfusion (33).

ROS scavenger. H_2S also scavenges ROS. 3MST and CAT are mainly localized to the mitochondria, where the respiratory chain produces ROS. Cells expressing 3MST and CAT show significant resistance to oxidative stress (33). H_2S also scavenges peroxynitrite ($ONOO^-$), which is formed from the interaction of $^{\bullet}NO$ with superoxide (O_2^-), and hypochlorous acid (HOCl), and has been shown to protect the glial cell line SH-SY5Y from its toxicity (95, 96). However, the contribution of scavenging effect of H_2S should be minor compared to that of glutathione when its production is increased by H_2S, because the intracellular concentrations of glutathione are between 1 and 10 m*M*; whereas those of H_2S are between 10 n*M* and 3 μM (20, 26, 97). In addition to reducing substances to suppress oxidative stress, glutathione may also exert radical scavenging activity by neutralizing free radicals through its electron donating ability (18). Suppression of oxidative stress by increased levels of glutathione should be more effective than ROS scavenging by H_2S itself (Fig. 8).

Upregulation of antioxidant genes *via* Nrf2. H_2S attenuates myocardial ischemia-reperfusion injury by preserving mitochondrial structure and function (16). It also suppresses the apoptotic cell death of cardiomyocytes, as shown by suppressed caspase-3 activity as well as a decreased number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive nuclei. An increase in the production of H_2S in cardiac muscle by overexpressing CSE significantly limits the extent of injury. Based on these observations, Lefer *et al.* suggest that either the administration of H_2S or the modulation of endogenous H_2S production may have clinical benefits for ischemic disorders. They further clarified the mechanism of the cardioprotective effect of H_2S. In the early phase after administration, H_2S facilitates the nuclear localization of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that binds to the antioxidant responsive element, and in the late phase, antioxidant gene products such as thioredoxin and heme oxygenase 1 are upregulated (9). H_2S also increases the phosphorylation of protein kinase Cε and signal transducers and activators of transcription 3 (STAT3), leading to an increase in the expression of Bcl-2 and cyclooxygenase-2 as well as the inactivation of the proapoptogen Bad (9).

Under steady-state conditions, Kelch ECH-associating protein 1 (Keap1) binds to Nrf2 and retains it in the cytoplasm, leading to ubiquitination and degradation of the complex in the proteosome. Several cysteine residues in Keap1 serve as the sensor for stress signals, and their modification causes conformational changes in Keap1, resulting in the release of Nrf2, enabling it to translocate into the nucleus (94). H_2S has been proposed to be involved in the interaction between Nrf2 and Keap1 (102). H_2S adds bound sulfane sulfur to cysteine residues (sulfhydration or sulfration) in

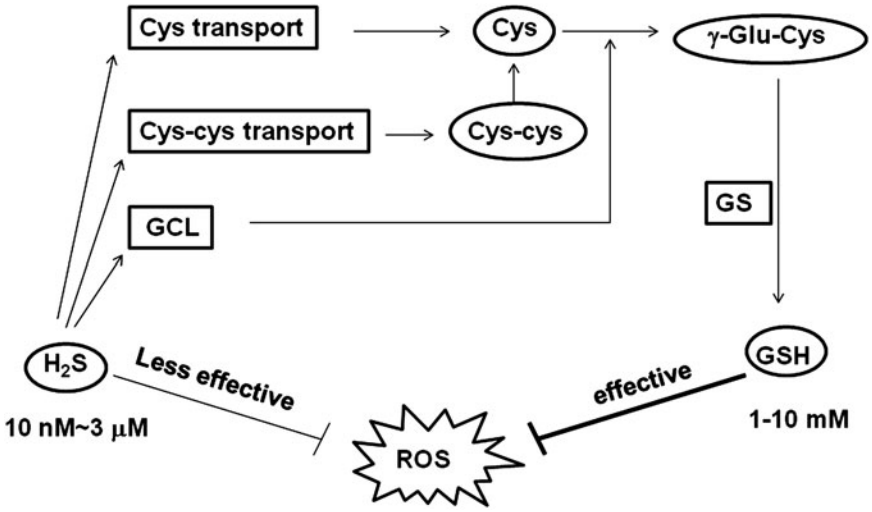

FIG. 8. Effective ROS scavenging by GSH, while scavenging by H_2S is less efficient. H_2S enhances the activity of cysteine transporter and cysteine/glutamate antiporter to increase cysteine intracellular concentrations. Through this effect and by enhancing the activity of GCL (γ-GCS), H_2S increases glutathione production. Since the intracellular concentrations of H_2S and glutathione range from 10 n*M* to 3 μ*M* and from 1 to 10 m*M*, respectively, glutathione scavenges ROS more efficiently than H_2S. GCL, glutamate cysteine ligase; γ-GCS, γ-glutamylcysteine synthetase; ROS, reactive oxygen species.

Keap1, causing Keap1 to release Nrf2 to translocate into the nucleus. Nrf2 upregulates glutathione-producing enzymes, resulting in the increased production of glutathione. *Via* this mechanism, H_2S protects embryonic fibroblast cells from oxidative stress and premature senescence. Since H_2S is not able to add bound sulfane sulfur to cysteine residues (sulfhydration or sulfration) as described earlier, H_2S may be oxidized to polysulfide, which adds bound sulfane sulfur (sulfhydrates or sulfurates) to the cysteine residues of Keap1. This alternative mechanism is suggested by the following study.

Polysulfide efficiently adds bound sulfane sulfur (sulfhydrate or sulfrate) to cysteine residues of Keap1 to produce Keap1 homodimers or heterodimers with another protein that leads to the release of Nrf2 to translocate into the nucleus (Fig. 9) (37). Polysulfide also activates PI3 kinase to phosphorylate Akt, which, in turn, phosphorylates Nrf2 to further facilitate the translocation of Nrf2 to the nucleus. Glutathione levels in Neuro2A cells are increased twice as much as in the control at 12 h after the application of polysulfide (37).

Cytoprotective effect of H_2S. The kidney is one of the main organs protected from ischemia-reperfusion injury by H_2S. H_2S administration attenuates the renal dysfuction and injury caused by ischemia-reperfusion (4, 90). Increased creatinine levels and acute tubular necrosis as well as decreased urinary flow after ischemia-reperfusion are attenuated by H_2S administration. Changes in the expression of apoptotic genes, such as an increase in caspase 3 and a decrease in Bid and Bcl-2, that accompany injury, changes in the phosphorylation of inflammatory factors such as mitogen-activated protein kinases, are attenuated by H_2S (4, 90). H_2S protects the kidney from ischemia-reperfusion injury through its anti-apoptotic and anti-inflammatory effects. The anti-apoptotic effect of H_2S is also induced through the regulation of NF-κB. However, the effect of H_2S on NF-κB is unclear.

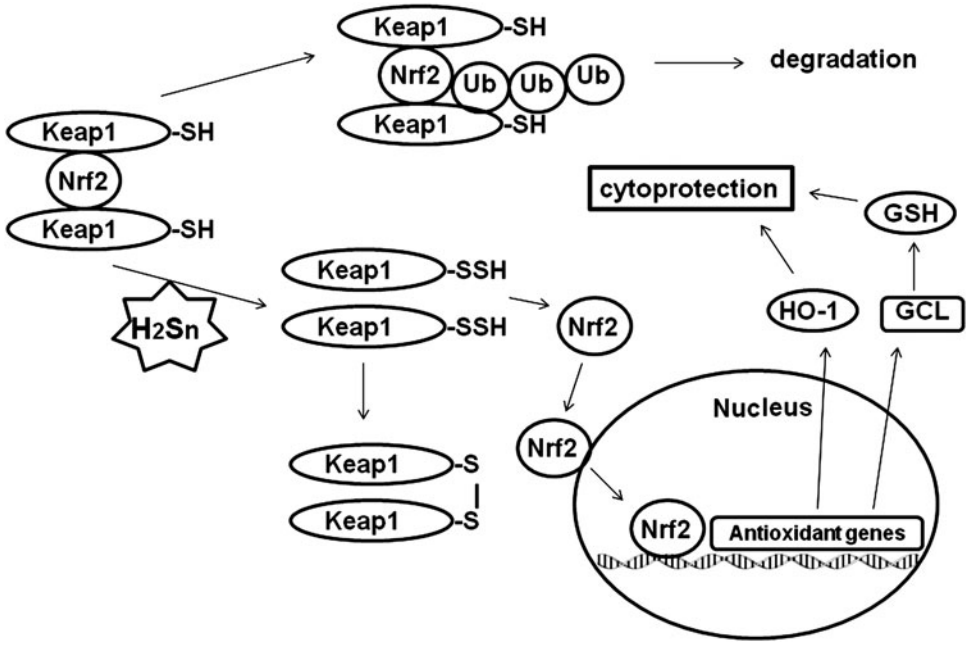

FIG. 9. Nrf2 translocation by detachment from Keap1 through the addition of bound sulfane sulfur by polysulfides. A transcription factor, Nrf2, binds to Keap1, and this complex is ubiquitinated and subsequently degraded. Polysulfides add bound sulfane sulfur (sulfhydration or sulfuration) to cysteine residues in Keap1 that causes conformational changes in Keap1, resulting in the release of Nrf2. Nrf2 translocates to the nucleus to upregulate antioxidant genes such as *HO-1* and *GCL*, resulting in the protection of neurons from oxidative stress. *HO-1*, heme oxygenase 1; Keap1, Kelch ECH-associating protein 1; Nrf2, nuclear factor erythroid 2-related factor 2.

In some studies, H_2S inhibits the activity of NF-κB; while in other studies, it activates NF-κB (15, 59, 70, 90). The application of H_2S inhibited the translocation of NF-κB p65 from the cytosol to the nucleus and suppressed the transcription of inducible nitric oxide synthase, cyclo-oxygenase-2, and intercellular adhesion molecule-1 in kidneys injured by ischemia-reperfusion (90). A similar inhibitory effect of H_2S on NF-κB was observed in macrophages in which H_2S suppressed the phosphorylation of I-κB, resulting in the inhibition of NF-κB translocation to the nucleus and its DNA-binding activity (15, 59). In contrast, TNF-α induces I-κB degradation to facilitate the translocation of NF-κB to the nucleus, and stimulates CSE transcription to increase the production of H_2S. H_2S sulfhydrates NF-κB, which upregulates the transcription of anti-apoptotic genes such as *TRAF*, *cIAP2*, *Bcl-XL*, *A20*, and *XIAP* (70).

The application of H_2S is promising for renal transplantation as well (105). Supplementation of preservation solutions with H_2S for prolonged cold storage of the kidney and subsequent transplantation preserved the structure and function of the kidney well, compared with the standard preservation solution without H_2S (105). Although the protection of the kidney from warm ischemia-reperfusion by H_2S was successful only for shorter periods, it was recently demonstrated that H_2S supplementation significantly preserved the kidney against injury due to clinically relevant, prolonged, warm ischemia-reperfusion. Based on these findings, it is proposed that supplementation with H_2S may have clinical benefits for donation after cardiac death in renal transplantation, and for surgeries which require the clamping of the renal pedicle (105).

Since CBS, CSE, 3MST, CAT, and DAO are localized in the renal cortex, the application of L- or D-cysteine provides safe and effective delivery of H_2S to the kidney. The production of H_2S from D-cysteine is 60 times as high as that from L-cysteine in the kidney (74). The oral administration of D-cysteine increases the levels of bound sulfane sulfur, which is a cellular storage form of H_2S, and these levels are observed to be twice as high as those in the control at 30 min after administration. The levels gradually decrease and return to control levels after 12 h. The administration of D-cysteine preserved the structure of renal cortex well. Although L-cysteine administration also increases the levels of bound sulfane sulfur, the levels are less than those achieved by D-cysteine, and returned to the control level at 3 h after administration. Preservation of the kidney with L-cysteine is not as effective as with D-cysteine (74). The administration of D-cysteine will provide a new therapeutic approach that efficiently delivers H_2S to specific tissues, especially the kidney.

H_2S protects cardiac muscles from ischemia-reperfusion injury by increasing the production of NO (36). The following observations were made in CSE knockout mice: (i) the levels of H_2S and bound sulfane sulfur in tissues and blood were decreased; (ii) the levels of peroxidized lipid and oxidized protein were increased; (iii) the activity of eNOS was suppressed; (iv) the levels of NO metabolites (nitrite and nitrosylated proteins) were decreased; (v) administration of H_2S activated eNOS and increased NO availability; and (vi) ischemia-reperfusion injury was rescued by H_2S. In addition to these observations in CSE knockout mice, the administration of H_2S failed to protect the cardiac muscle from ischemia-reperfusion injury in eNOS defective mutant mice (36). Similar results were also obtained in a mouse model of pressure overload-induced heart failure, which suggests that H_2S protects the heart by upregulating eNOS (38, 47).

H_2S protects pancreatic beta cells from glucotoxicity induced by a high-fat diet and prevents the development of type 2 diabetes (60). Blood glucose levels, the number of beta cells with apoptotic death, and the expression of thioredoxin-binding protein-2 (TBP-2) were higher in CSE knockout mice fed a high-fat diet than in the wild-type mice. A high level of glucose induces the expression of TBP-2, which binds to thioredoxin and reduces its activity, resulting in an increase in oxidative stress that causes beta-cell death. High glucose levels also increase the expression of CSE, the product of which (H_2S) suppresses the expression of TBP-2. Therefore, the absence of CSE causes progressive beta-cell failure, leading to the development of type 2 diabetes. Opposite results were obtained in the streptozotocin-induced diabetic model in a previous study (99).

CSE knockout mice, which show acute skeletal muscle atrophy (myopathy) and reduced levels of glutathione, are vulnerable to oxidative stress that is more conspicuous in mice fed a low cysteine diet (27). Embryonic fibroblasts obtained from CSE knockout mice, which show lower levels of glutathione, are sensitive to ER stress-induced apoptosis (14). These observations suggest that H_2S enhances the production of glutathione to make cells resistant to ER-stress-induced apoptosis.

Conclusions and Perspectives

Since the discovery of the neuroprotective effect of H_2S from oxidative stress, the cytoprotective effect of this molecule has been confirmed in various organs and tissues under oxidative stress and ischemia-reperfusion injury. In addition to CBS, CSE, and the 3MST/CAT pathway, a novel pathway to produce H_2S from D-cysteine, the 3MST/DAO pathway was recently discovered. It produces H_2S from D-cysteine much more efficiently than from L-cysteine in the kidney, and it protects the kidney from ischemia-reperfusion injury more efficiently than does L-cysteine. D-Cysteine is less toxic than L-cysteine (48, 61), and it promises novel therapeutic and clinical applications. Although D-cysteine is provided from food, whether racemase, which transforms L-cysteine to D-cysteine, and/or other pathways that produce D-cysteine, exist in mammals and their physiological roles remain intriguing.

Polysulfide is another potential signaling molecule derived from H_2S. Polysulfides activate TRPA1 channels much more potently than the parental molecule H_2S, and they regulate Keap1 to release Nrf2, which, in turn, upregulates antioxidant genes such as HO-1 and GCL and modifies the activity of phosphatase and tensin homolog (PTEN), a lipid phosphatase (22, 35, 37, 55, 63). Since TRPA1 channels are involved in the regulation of inflammation, pain, cardiovascular system, gastrointestinal tract, Nrf2 in anti-oxidation, and PTEN in cancer, the physiological roles of polysulfides should be robust.

H_2S reduces the cysteine disulfide bond to expose two thiols, and transiently adds bound sulfane sulfur (sulfhydrate or sulfurate), but this is immediately removed by another molecule of H_2S to return the molecule to the thiol state (Fig. 6) (87). Polysulfides preferentially add bound sulfane sulfur to thiols to produce persulfide. If only one of the thiols is persulfurated, another thiol may react with it to produce a

cysteine disulfide bond. Alternatively, both thiols may be evenly persulfurated. H_2S is a reducing substance (oxidation state: -2), while polysulfides are relatively oxidizing substances (oxidation state of inner sulfur: 0). Therefore, polysulfides rather than H_2S add bound sulfane sulfur (sulfhydrate or sulfurate) to cysteine residues.

The regulation of H_2S-producing enzymes is not well understood. Although the production of H_2S by CBS is enhanced by *S*-adenosyl methionine, the physiological stimuli that induce the changes in the levels of *S*-adenosyl methionine are not clarified. Since CSE and the 3MST/CAT pathway produce H_2S at steady-state low intracellular concentrations of Ca^{2+}, the H_2S produced by these enzymes may play a role mainly in the steady state. H_2S can also be released from bound sulfane sulfur from astrocytes when nearby neurons are excited, though the release has not been successfully measured. It is necessary to identify the physiological stimulation that triggers the release of H_2S, and to measure the levels of H_2S at the time of stimulation. Moreover, the process of polysulfide production, its cellular and tissue localization, the physiological stimulation that induces its release, and its concentrations at the time of stimulation are intriguing. H_2S is involved in responses such as the reducing cysteine disulfide bond, while polysulfides are involved in the addition of bound sulfane sulfur (sulfhydrate or sulfurate) to cysteine residues to modify the structure of target proteins. It is necessary to determine how cells differentially utilize H_2S and polysulfides at the appropriate time and location. Understanding the production and function of both H_2S and polysulfides will provide new insights into the biology of H_2S and its therapeutic applications.

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Author Disclosure Statement

No conflicts of interest 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. Aizenman E, Lipton DA, and Loring RH. Selective modulation of NMDA responses by reduction and oxidation. *Neuron* 2: 1257–1263, 1989.
3. Ang SF, Moochhala SM, and Bhatia M. Hydrogen sulfide promotes transient receptor potential vanilloid 1-mediated neurogenic inflammation in polymicrobial sepsis. *Crit Care Med* 38: 619–628, 2010.
4. Bos EM, Leuvenink HGD, Snijder PM, Koosterhuis NJ, Hillebrands J-L, Leemans JC, Florquin S, and Goor H. Hydrogen sulfide-induced hypometabolism prevents renal ischemia/reperfusion injury. *J Am Soc Nephrol* 20: 1901–1905, 2009.
5. Bredt DA and Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci U S A* 87: 682–685, 1990.
6. Bryan S, Yang G, Wang R, and Khaper N. Cystathionine gamma-lyase-deficnet smooth muscle cells exhibit redox imbalance and apoptosis under hypoxic stress conditions. *Exp Clin Cardiol* 16: e36–e41, 2011.
7. Burton K and Wilson TH. The free-energy changes for the reduction of diphosphopyridine nucleotide and the dehydrogenation of L-malate and L-glycerol 1-phosphate. *Biochem J* 54: 86–94, 1953.
8. Cai WJ, Wang MJ, Moore PK, Jin HM, Yao T, and Zhu YC. The novel proangiogenic effect of hydrogen sulfide is dependent on Akt phosphorylation. *Cardiovasc Re*s 76: 29–40, 2007.
9. Calvert JW, Jha S, Gundewar S, Elrod JW, Ramachandran A, Pattillo CB, Kevil CG, and Lefer DJ. Hydrogen sulfide mediates cardioprotection through Nrf2 signaling. *Circ Res* 105, 365–374, 2009.
10. Chen X, Jhee KH, and Kruger WD. Production of the neuromodulator H_2S by cystathionine beta-synthase *via* the condensation of cysteine and homocystein. *J Biol Chem* 279: 52082–52086, 2004.
11. Chiku T, Padovani D, Zhu W, Singh S, Vitvitsky V, and Banerjee R. H_2S biogenesis by human cystathionine γ–lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia. *J Biol Chem* 284: 11601–11612, 2009.
12. Cleland WW. Dithiothreitol, a new protective reagent for SH groups. *Biochemistry* 3: 480–482, 1964.
13. Cooper AJL. Biochemistry of sulfur-containing amino acids. *Annu Rev Biochem* 52: 187–222, 1983.
14. Dickhout JG, Carlisle RE, Jerom DE, Mohammed-Ali Z, Jiang H, Yang G, Mani S, Garg SK, Banerjee R, Kaufman RJ, Maclean KN, Wang R, and Austin RC. Integrated stress response modulates cellular redox state *via* induction of cystathionine γ-lyase. *J Biol Chem* 287: 7603–7614, 2012.
15. Du J, Huang Y, Yan H, Zhang Q, Zhao M, Zhu M, Liu J, Chen SX, Bu D, Tang C, and Jin H. Hydrogen sulfide suppresses ox-LDO-stimulated monocyte chemoattractant protein-1 generation from macrophages *via* NF-κB pathway. *J Biol Chem* 289: 9741–9753, 2014.
16. Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabo C, *et al.* Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci U S A* 104: 15560–15565, 2007.
17. Finkelstein JD. Methionine metabolism in mammalos. *J Nutr Biochem* 1: 228–237, 1990.
18. Fiser B, Jojart B, Csizmadia IG, and Viskolcz B. Glutathione-hydroxyl radical interaction: a theoretical study on radical recognition process. *PLoS One* 8: e73652, 2013.
19. Fu M, Zhang W, Wu L, Yang G, Li H, and Wang R. Hydrogen sulfide (H_2S) metabolism in mitochondria and its regulatory role in energy production. *Proc Natl Acad Sci U S A* 109, 2943–2948, 2012.
20. Furne J, Saeed A, and Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am J Physiol Regul Integr Comp Physiol* 295: R1479–R1498, 2008.
21. Gould SJ, Keller GA, and Subramani S. Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. *J Cell Biol* 107: 897–905, 1988.
22. Greiner R, Palinkas Z, Basell K, Becher D, Antelmann H, Nagy P, and Dick TP. Polysulfides link H_2S to protein thiol oxidation. *Antioxid Redox Signal* 19: 1749–1765, 2013.

23. Holmgren A. Thioredoxin. 6. The amino acid sequence of the protein from *Escherichia coli* B. *Eur J Biochem* 6: 475–484, 1968.
24. Holmgren A. The thioredoxin system. In *Redox Biochemistry*, edited by Banerjee R. Hoboken, NJ: John Wiley & Sons, 2008, pp. 68–74.
25. Hosoki R, Matsuki N, and Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 237: 527–531, 1997.
26. 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.
27. Ishii I, Akahoshi N, Yamada H, Nakano S, Izumi T, and Suematsu M. Cystathionine γ-lyase-deficient mice require dietary cysteine to protect against acute lethal myopathy and oxidative injury. *J Biol Chem* 285: 26358–26368, 2010.
28. Ishii I, Akahoshi N, Yu X-N, Kobayashi Y, Namekata K, Komaki G, and Kimura H. Murine cystathionine γ-lyase: complete cDNA and genomic sequences, promoter activity, tissue distribution and developmental expression. *Biochem J* 381:113–123, 2004.
29. Jocelyn PC. The standard redox potential of cysteine-cystine from the thiol-disulphide exchange reaction with glutathione and lipoic acid. *Eur J Biochem* 2: 327–331, 1967.
30. Kabil O and Banerjee R. Enzymology of H_2S biogenesis, decay and signaling. *Antioxid Redox Signal* 20: 770–782, 2014.
31. Kaneko Y, Kimura Y, Kimura H, and Niki I. L-cysteine inhibits insulin release from the pancreatic beta-cell: possible involvement of metabolic production of hydrogen sulfide, a novel gasotransmitter. *Diabetes* 55: 1391–1397, 2006.
32. Kataoka H, Hirabayashi N, and Makita M. Analysis of lipoic acid in biological samples by gas chromatography with flame photometric detection. *J Chromatogr* 615: 197–202, 1993.
33. Kimura Y, Goto Y-I, and Kimura H. Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxid Redox Signal* 12: 1–13, 2010.
34. Kimura Y and Kimura H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J* 18: 1165–1167, 2004.
35. Kimura Y, Mikami Y, Osumi K, Tsugane M, Oka J-I, and Kimura H. Polysulfides are possible H_2S-derived signaling molecules in rat brain. *FASEB J* 27: 2451–2457, 2013.
36. King AL, Polhemus D, Bhushan S, Otsuka H, Kondo K, Nicholson CK, Bradley JM, Islam KN, Calvert JW, Tao Y-X, Dugas TR, Kelley EE, Elrod JW, Huang PL, Wang R, and Lefer DJ. Hydrogen sulfide cytoprotective signaling is endothelial nitric oxide synthase-nitric oxide dependent. *Proc Natl Acad Sci U S A* 111: 3182–3187, 2014.
37. Koike S, Ogasawara Y, Shibuya N, Kimura H, and Ishii K. Polysulfide exerts a protective effect against cytotoxicity cuased by t-buthylhydroperoxide through Nrf2 signaling in neuroblastoma cells. *FEBS Lett* 587: 3548–3555, 2013.
38. Kondo K, Bhushan S, King AL, Prabhu SD, Hamid T, Koenig S, Murohara T, Predmore BL, Gojon GS, Gojon GJ, Wang R, Karusula N, Nicholson CK, Calvert JW, and Lefer DJ. H_2S protects against pressure overload-induced heart failure *via* upregulation of endothelial nitric oxide synthase. *Circulation* 127: 1116–1127, 2013.
39. Krijgsheld KR, Glazenburg EJ, Scholtens E, and Mulder GJ. The oxidation of L- and D-cysteine to inorganic sulfate and taurine in the rat. *Biochim Biophys Acta* 677: 7–12, 1981.
40. Krizaj D and Copenhagen DR. Calcium regulation in photoreceptors. *Front Biosci* 7: d2023–d2044, 2002.
41. Liardon R and Ledermann S. Racemization kinetics of free and protein-bound amino acids under moderate alkaline treatment. *J Agric Food Chem* 34: 557–565, 1986.
42. Meister A, Fraser PE, and Tice SV. Enzymatic desulfuration of β-mercaptopyruvate to pyruvate. *J Biol Chem* 206: 561–575, 1954.
43. Mikami Y, Shibuya N, Kimura Y, Nagahara N, Ogasawara Y, and Kimura H. Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. *Biochem J* 439: 479–485, 2011.
44. Mikami Y, Shibuya N, Kimura Y, Nagahara N, Yamada M, and Kimura H. Hydrogen sulfide protects the retina from light-induced degeneration by the modulation of Ca^{2+} influx. *J Biol Chem* 286: 39379–39386, 2011.
45. Mikami Y, Shibuya N, Ogasawara Y, and Kimura H. Hydrogen sulfide is produced by cystathionine γ-lyase at the steady-state low intracellular Ca^{2+} concentrations. *Biochem Biophys Res Commun* 431: 131–135, 2013.
46. Miljkovic JL, Kenkel I, Ivanovic-Burmazovic I, and Filipovic MR. Generation of HNO and HSNO from nitrite by heme-iron-catalyzed metabolism with H_2S. *Angew Chem Int Ed* 52: 12061–12064, 2013.
47. Minamishima S, Bougaki M, Sips PY, Yu JD, Minamishima YA, Elrod JW, Lefer DJ, Bloch KD, and Ichinose F. Hydrogen sulfide improves survival after cardiac arrest and cardiopulmonary resuscitation *via* a nitric oxide synthase 3-dependent mechanism in mice. *Circulation* 120: 888–896, 2009.
48. Misra CH. Is a certain amount of cysteine prerequisite to produce brain damage in neonatal rats? *Neurochem Res* 14: 253–257, 1989.
49. Morikawa T, Kajimura M, Nakamura T, Hishiki T, Nakanishi T, Yukutake Y, Nagahata Y, Ishikawa M, Hattori K, Takenouchi T, Takahashi T, Ishii I, Matsubara K, Kabe Y, Uchiyama S, Nagata E, Gadalla MM, Snyder SH, and Suematsu M. Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc Natl Acad Sci U S A* 109: 1293–1298, 2012.
50. Mudd SH, Levy HL, and Kraus JP. Disorders of transsulfuration. In *The Metabolic and Molecular Bases of Inherited Disease*, edited by Scriver CR, *et al.* New York: McGraw-Hill, 2001, pp. 2007–2056.
51. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, and Snyder SH. H_2S signals through protein S-sulfhydration. *Sci Signal* 2: ra72, 2009.
52. Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, Barodka VM, Gazi FK, Barrow RK, Wang R, Amzel LM, Berkowitz DE, and Snyder SH. Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. *Circ Res* 109: 1259–1268, 2011.
53. Nagahara N, Yoshii T, Abe Y, and Matsumura T. Thioredoxin-dependent enzymatic activation of mercaptopyruvate sulfurtransferase. An intersubunit disulfide bond serves as a redox switch for activation. *J Biol Chem* 282: 1561–1569, 2007.
54. Nagai Y, Tsugane M, Oka J, and Kimura H. Hydrogen sulfide induces calcium waves in astrocytes. *FASEB J* 18: 557–559, 2004.

55. Nagai Y, Tsugane M, Oka J-I, and Kimura H. Polysulfides induce calcium waves in rat hippocampal astrocytes. *J Pharmacol Sci* 100: 200, 2006.
56. Nagy P and Winterbourn CC. Rapid reaction of hydrogen sulfide with the neutrophil oxidant hypochlorous acid to generate polysulfides. *Chem Res Toxicol* 23: 1541–1543, 2010.
57. Nishida M, Sawa T, Kitajima N, Ono K, Inoue H, Ihara H, Motohashi H, Yamamoto M, Suematsu M, Kurose H, Vliet A, Freeman BA, Shibata T, Uchida K, Kumagai Y, and Akaike T. Hydrogen sulfide anion regulates redox signaling *via* electrophile sulfhydration. *Nat Chem Biol* 8: 714–724, 2012.
58. Ogawa H, Takahashi K, Miura S, Imagawa T, Saito S, Tominaga M, and Ohta T. H_2S functions as a nociceptive messenger throughtransient receptor potential ankyrin 1 (TRPA1) activation. *Neuroscience* 218: 335–343, 2012.
59. Oh G-S, Pae H-O, Lee B-S, Kim B-N, Kim J-M, Kim H-R, Jeon SB, Jeon WK, Chae H-J, and Chung H-T. Hydrogen sulfide inhibits nitric oxide production and nuclear factor-κB *via* heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide. *Free Rad Biol Med* 41: 106–119, 2006.
60. Okamoto M, Yamaoka M, Takei M, Ando T, Taniguchi S, Ishii I, Tohya K, Ishizaki T, Niki I, and Kimura T. Endogenous hydrogen sulfide protects pancreatic beta-cells from a high-fat diet-induced glucotoxicity and prevents the development of type 2 diabetes. *Biochem Biophys Res Commun* 442: 227–233, 2013.
61. Olney JW, Zorumski C, Price MT, and Labruyere J. L-cysteine, a bicarbonate-sensitive endogenous excitotoxin. *Science* 248: 596–599, 1990.
62. Olson KR, Whitfield NL, Bearden SE, Leger JS, Nilson E, Gao Y, and Maddeen JA. Hypoxic pulmonary vasodilation: a paradigm shift with a hydrogen sulfide mechanism. *Am J Physiol Regal Integr Comp Physiol* 298: R51–R60, 2010.
63. Oosumi K, Tsugane M, Ishigami M, Nagai Y, Iwai T, Oka J-I, and Kimura H. Polysulfide activates TRP channels and increases intracellular Ca^{2+} in astrocytes. *Bull Jpn Soc Neurochem* 49: 517, 2010.
64. Papapetropoulos A, Pyriochou A, Altaany Z, Yang G, Marazioti A, Zhou Z, Jeschke MG, Branski LK, Herndon DN, Wang R, and Szabo C. Hydrogen sulfide is an endogenous stimulator of angiogenesis. *Proc Natl Acad Sci U S A* 106: 21972–21977, 2009.
65. Patel P, Vatish M, Heptinstall J, Wang R, and Carson RJ. The endogenous production of hydrogen sulphide in intrauterine tissues. *Reprod Biol Endocrinol* 7: 10, 2009.
66. Paul BD and Snyder SH. H_2S signaling through protein sulfhydration and beyond. *Nat Rev Mol Cell Biol* 13: 499–507, 2012.
67. Petersen Shay K, Moreau R, Smith E, and Hagen T. Is α–lipoic acid a scavenger of reactive oxygen species *in vivo*? Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity. *IUBMB Life* 60: 362–367, 2008.
68. Richie JP and Lang CA. The determination of glutathione, cyst(e)ine, and other thiols and disulfides in biological samples using high-performance liquid chromatography with dual electrochemical detection. *Anal Biochem* 163: 9–15, 1987.
69. Schumann U and Subramani S. Special delivery from mitochondria to peroxisomes. *Trends Cell Biol* 18: 253–256, 2008.
70. Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, Kim S, and Snyder SH. Hydroen sulfide-linked sulfhydration of NF-κB mediates its intiapoptotic actions. *Mol Cell* 45: 13–24, 2012.
71. Sha L, Linden DR, Farrugia G, and Szurszewski JH. Effect of endogenous hydrogen sulfide on the transwall gradient of the mouse colon circular smooth muscle. *J Physiol* 592: 1077–1089, 2014.
72. Shan X, Dunbrack RLJ, Christopher SA, and Kruger WD. Mutation in the regulatory domain of cystathionine β–synthase can functionally suppress patient-derived mutations in cis. *Hum Mol Genet* 10: 635–643, 2001.
73. Shatalin K, Shatalina E, Mironov A, and Nudler E. H_2S: A universal defense against antibiotics in bacteria. *Science* 334: 986–990, 2011.
74. Shibuya N, Koike S, Tanaka M, Ishigami-Yuasa M, Kimura Y, Ogasawara Y, Fukui K, Nagahara N, and Kimura H. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. *Nat Commun* 4: 1366, 2013.
75. Shibuya N, Mikami Y, Kimura Y, Nagahara N, and Kimura H. Vascular endothelium exresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J Biochem* 146: 623–626, 2009.
76. Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, and Kimura H. 3-Mercaptopyruvate sulfurtransferease produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal* 11: 703–714, 2009.
77. Shigetomi E, Jackson-Weaver O, Huckstepp RT, O'Dell TJ, and Khakh BS. TRPA1 channels are regulators of astrocyte basal calcium levels and long-term potentiation *via* constitutive D-serine release. *J Neurosci* 33: 10143–10153, 2013.
78. Shigetomi E, Tong X, Kwan KY, Corey DP, and Khakh BS. TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. *Nat Neurosci* 15: 70–80, 2012.
79. Shirozu K, Tokuda K, Marutani E, Lefer D, Wang R, and Ichinose F. Cystathionine γ-lyase deficiency protects mice from galactosamine/lipopolysaccharide-induced acute liver failure. *Antioxid Red Signal* 20: 204–216, 2014.
80. Singh S, Padovani D, Leslie RA, Chiku T, and Banerjee R. Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H_2S biogenesis *via* alternative transsulfuration reactions. *J Biol Chem* 284: 22457–22466, 2009.
81. Spyrou G, Enmark E, Miranda-Vizuete A, and Gustafsson JA. Cloning and expression of a novel mammalian thioredoxin. *J Biol Chem* 272: 2936–2941, 1997.
82. Stipanuk MH and Beck PW. Characterization of the enzymic capacity for cysteine desulphhydration in liver and kidney of the rat. *Biochem J* 206: 267–277, 1982.
83. Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, Andersson KE, Hogestatt ED, and Zygmunt PM. Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. *Eur Urol* 53: 391–399, 2008.
84. Sun Q, Collins R, Huang S, Holmberg-Schiavone L, Anand GS, Tan CH, van-den-Berg S, Deng LW, Moore PK, Karlberg T, and Sivaraman J. Structural basis for the inhibition mechanism of human cystathionine gamma-lyase, an enzyme responsible for the production of H_2S. *J Biol Chem* 284: 3076–3085, 2009.
85. Taoka S and Banerjee R. Characterization of NO binding to human cystathionine beta-synthase: Possible implications of the effects of CO and NO binding to the human enzyme. *J Inorg Biochem* 87: 245–251, 2001.

86. Teague B, Asiedu S, and Moore PK The smooth muscle relaxant effect of hydrogen sulphide *in vitro*: evidence for a physiological role to control intestinal contractility. *Br J Pharmacol* 137: 139–145, 2002.
87. Toa BB, Liu SY, Zhang CC, Fu W, Cai WJ, Wang Y, Shen Q, Wang MJ, Chen Y, Zhang LJ, and Zhu YZ. VEGFR2 functions as an H2S-targeting receptor protein kinase with its novel Cys1045-Cys1024 disulfide bond serving as a specific molecular switch for hydrogen sulfide actions in vascular endothelial cells. *Antioxid Redox Signal* 19: 448–464, 2013.
88. Tokuda K, Kida K, Marutani E, Crimi E, Bougaki M, Khatri A, Kimura H, and Ichinose F. Inhaled hydrogen sulfide prevents endotoxin-induced systemic inflammation and improves survival by altering sulfide metabolism in mice. *Antioxid Redox signal* 17: 11–21, 2012.
89. Toohey JI. Sulfur signaling: is the agent sulfide or sulfane? *Anal Biochem* 413: 1–7, 2011.
90. Tripatara P, Patel NSA, Collino M, Gallicchio M, Kieswich J, Castiglia S, Benetti E, Stewart KN, Brown PAJ, Yaqoob MM, Fantozzi R, and Thiemermann C. Generation of endogenous hydrogen sulfide by cystathionine γ-lyase limits renal ischemia/reperfusion injury and dysfunction. *Lab Invest* 88: 1038–1048, 2008.
91. Tsugane M, Nagai Y, Kimura Y, Oka J-I, and Kimura H. Differentiated astrocytes acquire sensitivity to hydrogen sulfide that is diminished by the transformation into reactive astrocytes. *Antioxid Redox Signal* 9: 257–269, 2007.
92. Vitvitsky V, Kabil O, and Banerjee R. High turnover rates for hydrogen sulfide allow for rapid regulation of its tissue concentrations. *Antioxid Red Signal* 17: 22–31, 2012.
93. Volini M and Westley J. The mechanism of the rhodanese-catalyzed thiosulfate-lipoate reaction. Kinetic analysis. *J Biol Chem* 241: 5168–5176, 1966.
94. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, and Talalay P. Protection against electrophile and oxidant stress by induction of the phase 2 response:fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci U S A* 101: 2040–2045, 2004.
95. Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Hheung NS, Halliwell B, and Moore PK. The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger'? *J Neurochem* 90: 765–768, 2004.
96. Whiteman M, Cheung NS, Zhu YZ, Chu SH, Siau JL, Wong BS, Armstrong JS, and Moore PK. Hydrogen sulphide: a novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain? *Biochem Biophys Res Commun* 326: 794–798, 2005.
97. Wintner EA, Deckwerth TL, Langston W, Bengtsson A, Leviten D, Hill P, Insko MA, Dumpit R, VandenEkart E, Toombs CF, and Szabo C. A monobromobimane-based assay to measure the pharmacokinetic profile of reactive sulphide species in blood. *Br J Pharmacol* 160: 941–957, 2010.
98. Yadav PK, Yamada K, Chiku T, Koutmos M, and Banerjee R. Structure and kinetic analysis of H2S production by human mercaptopyruvate sulfurtransferase. *J Biol Chem* 288: 20002–20013, 2013.
99. Yang G, Pei Y, Teng H, Cao Q, and Wang R. Specificity protein-1 as a critical regulator of human cystathionine γ-lyase in smooth muscle cells. *J Biol Chem* 286: 26450–26460, 2011.
100. Yang G, Wu L, Bryan S, Khaper N, Mani S, and Wang R. Cystathionine gamma-lyase deficiency and overproliferation of smooth muscle cells. *Cardiovascular Res* 86: 487–495, 2010.
101. 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 physiologic vasorelaxant: Hypertension in mice with deletion of cystathionine γ-lyase. *Science* 322: 587–590, 2008.
102. Yang G, Zhao K, Ju Y, Mani S, Cao Q, Puukila S, Khaper N, Wu L, and Wang R. Hydrogen sulfide protects against cellular senescence *via* S-sulfhydration of Keap1 and activation of Nrf2. *Antioxid Redox Signal* 18: 1906–1919, 2013.
103. Yang W, Yang G, Jia X, Wu L, and Wang R. Activation of KATP channels by H_2S in rat insulin-secreting cells and the underlying mechanisms. *J Physiol* 569: 519–531, 2005.
104. Zhao W, Zhang J, Lu Y, and Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. *EMBO J* 20: 6008–6016, 2001.
105. Zhu JXG, Kalbfleisch M, Yang YX, Bihari R, Lobb I, Davison M, Mok A, Cepinskas G, Lawendy A-R, and Sener A. Detrimental effects of prolonged warm renal ischaemia-reperfusion injury are abrogated by supplemental hydrogen sulphide: an analysis using real-time intravital microscopy and polymerase chain reaction. *Br J Urol Int* 110: E1218–E1227, 2012.

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Abbreviations Used

AAT = aspartate aminotransferase
AMPA = α-amino-3-hydroxy-5-methy-4 isoxazole proprionic acid
ATP = adenosine triphosphate
CAT = cysteine aminotransferase
CBS = cystathionine β-synthase
cGMP = cyclic guanosine monophosphate
CO = carbon monoxide
CSE = cystathionine γ-lyase
DAO = D-amino acid oxidase
DHLA = dihydrolipoic acid
DTT = dithiothreitol
EGF = epidermal growth factor
eNOS = endothelial nitric oxide synthetase
GAPDH = glyceraldehydes-3-phosphate dehydrogenase
GCL = glutamate cysteine ligase
γGCS = γ-glutamylcysteine synthetase
GS = glutathione synthetase
GFAP = glial fibrillary acidic protein

Abbreviations Used (Cont.)

HPLC = high-performance liquid chromatography
HOCl = hypochlorous acid
H_2S = hydrogen sulfide
H_2S_n = polysulfide
IK_{Ca} = intermediate conductance Ca^{2+}-sensitive K^+
K_{ATP} = ATP-sensitive K^+
Keap1 = Kelch ECH-associating protein 1
LTP = long-term potentiation
3MP = 3-mercaptopyruvate
3MST = 3-mercaptopyruvate sulfurtransferase
NADH (NAD) = nicotinamide adenine dinucleotide
NADPH = nicotinamide adenine dinucleotide phosphate
NaHS = sodium hydrosulfide
NMDA = *N*-methyl D-aspartate
NO = nitric oxide
NOS = nitric oxide synthetase
Nrf2 = nuclear factor erythroid 2-related factor 2
nNOS = neuronal NOS
$ONOO^-$ = peroxynitrite
PI3 = phosphoinositide-3
PLP = pyridoxal 5′-phosphate
PTEN = phosphatase and tensin homolog
ROS = reactive oxygen species
RT-PCR = real-time polymerase chain reaction
SK_{Ca} = small conductance Ca^{2+}-sensitive K^+
SNAP = *S*-nitroso-*N*-acetyl-D,L-penicillamine
SNP = sodium nitroprusside
STAT = signal transducers and activators of transcription
TBP-2 = thioredoxin binding protein-2
TGFα = transforming growth factor alpha
TRPA1 = transient receptor potential ankyrin 1
TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling
VEGFR2 = vascular endothelium growth factor receptor 2