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FORUM REVIEW ARTICLE

Hydrogen Sulfide: Neurophysiology and Neuropathology

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Abstract

Hydrogen sulfide (H_2S) was known to be a toxic gas and an environmental hazard for many decades. However, it is now recognized that H_2S may serve as a gaseous mediator that is endogenously produced to influence biological functions in mammalian. Together with nitric oxide and carbon monoxide, it forms the group of mediators that has been termed the "gasotransmitters." The past decade has seen an exponential growth of scientific interest in the physiological and pathological significance of H_2S especially with respect to its role in the central nervous system and the cardiovascular system. In the central nervous system, H_2S facilitates long-term potentiation and regulates intracellular calcium concentration and pH level in brain cells. Intriguingly, H_2S produces antioxidant, anti-inflammatory, and antiapoptotic effects that may have relevance to neurodegenerative disorders. Abnormal generation and metabolism of H_2S have been reported in the pathogenesis of ischemic stroke, Alzheimer's disease, Parkinson's disease, and recurrent febrile seizure. Exogenously applied H_2S is demonstrated to have value for the treatment of febrile seizure and Parkinson's disease. This article presents an overview of current knowledge of H_2S in relation to brain functions, with a special emphasis on its neuroprotective effects and the underlying cellular and molecular mechanisms. *Antioxid. Redox Signal.* 15, 405–419.

Introduction

Hydrogen sulfide (H_2S, molecular weight 34.08) is a colorless, water-soluble gas with a characteristic smell of rotten eggs. At high concentrations (>500 ppm), H_2S rapidly causes loss of consciousness and respiratory failure; thus, H_2S poisoning is often associated with fatalities (58). Nevertheless, the notoriety of H_2S as a toxic gas is experiencing a transformation, with increasing number of literature describing that it regulates a range of physiological and pathological processes in mammals. Thus, H_2S is a physiologically important molecule and it has been referred to as the third gaseous mediator alongside nitric oxide (NO) and carbon monoxide (CO). H_2S is largely produced from L-cysteine (Cys) and homocysteine (Hcy) by the actions of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). In general, CBS appears to be predominant in the central nervous system (CNS), whereas CSE is mainly expressed in the cardiovascular system (see the Biosynthesis and Metabolism of H_2S in CNS section). H_2S protects the heart against ischemia-reperfusion injury (17, 33) and elicits an antihypertensive effect by modulating vascular tone (26) and renin activity (50). It also induces a state of suspended animation in mouse (6), inhibits insulin release, and plays both pro- and anti-inflammatory roles in different systems and diseases [see (45) for review]. Further, H_2S is revealed to be a novel neuromodulator (1). The actions of H_2S in the CNS were first reported in 1996 by Abe and Kimura. These authors reported that H_2S at concentrations below 130 μM selectively enhances N-Methyl-D-aspartate (NMDA) receptor-mediated response and facilitates the induction of long-term potentiation (LTP), whereas at higher concentrations (>320 μM), it inhibits synaptic transmission in the hippocampus (1). Subsequently, more and more physiological and pathological functions of H_2S in the CNS were uncovered. In our previous reviews, we have focused on discussing the neurochemistry (56) and signaling properties of H_2S (65) in the CNS. In this article, we present current knowledge of H_2S to facilitate better understanding of its brain functions in both health and disease, with a special emphasis on its neuroprotective effects and the underlying cellular and molecular mechanisms involved.

Chemical Properties of H_2S

The solubility of H_2S in water ranges from 5.3 g/L at 10°C to 3.2 g/L at 30°C (54). It is weakly acidic because it dissociates into H^+ and HS^- in solution. According to a standard Henderson-Hasselbach calculation, at 20°C and pH 7.4, H_2S exists as ~30%–33% H_2S and 67%–70% HS^-, with negligible S^{2-} due to the high pK_{a2} (>12). However, at 37°C and pH 7.4, <20% of H_2S exists as the undissociated form (H_2S) (16). The term "free H_2S" is often used to refer to the sum of H_2S, HS^-, and S^{2-}, as it is used in this article. Sometimes, the sum of H_2S and HS^- is denoted as total sulfide, but this may be

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misleading as this term may also be used for acid-labile sulfide and dithiothreitol-labile sulfide.

The alkali salts of H_2S, sodium hydrosulfide (NaHS), and sodium sulfide (Na_2S) (or their hydrous forms) are widely used as exogenous sources of H_2S in laboratories. In aqueous solution, both can give a rapid bolus of H_2S at consistent concentrations, and thus their use is unlikely to reflect the biosynthesis and release of H_2S *in vivo*. To date, a small number of slow-releasing H_2S compounds such as GYY4137 and S-diclofenac are available and more novel compounds are under development (46, 47).

Biosynthesis and Metabolism of H_2S in CNS

The desulfhydration of Cys is proposed to be the major source of H_2S in mammals. This process is catalyzed *via* two pyridoxal-5′-phosphate (PLP)-dependent enzymes CBS and CSE. In the transsulfuration pathway, Cys is derived from Hcy with CBS catalyzing the β-replacement reaction of Hcy to yield cystathionine, which is then lyzed by CSE into Cys and α-ketobutyrate (Fig. 1). CBS can efficiently produce H_2S *via* a β-replacement reaction in which Cys is condensed with Hcy to form cystathionine and H_2S, and this reaction is far more efficient when compared to β-elimination of Cys (9). Detailed kinetic analysis performed by Banerjee's group demonstrated that CBS produces H_2S overwhelmingly from Cys+Hcy (96%) under simulated physiological conditions, whereas Cys and Cys+Cys accounts for only 1%–3% (63). Therefore, cysteine and Hcy are the preferred substrates of CBS for H_2S biosynthesis. On the other hand, CSE produces H_2S from Cys (70%) or Hcy (γ-elimination, 29%) under normal conditions (10 μM Hcy). When the concentration of Hcy was increased from 10 to 40 and 200 μM to simulate mild and severe hyperhomocysteinemia, the contribution from Hcy increased from 29% to 63% and 90%, respectively, whereas contribution from Cys decreased correspondingly to 37% and 10%, respectively (63). As Vmax for the γ-elimination of Hcy is twice that for the β-elimination of Cys, this shift may represent a marked increase in the generation of H_2S under hyperhomocysteinemic conditions. Therefore, H_2S production derived from CSE is sensitive to Hcy (11). Basically, under normal conditions (10 μM Hcy) CSE represents ~32% of the H_2S generation by the transsulfuration pathway, but it increases to ~45% and ~74% under moderate and severe hyperhomocysteinemia conditions (63). In contrast, CBS is not sensitive to Hcy concentrations with Cys+Hcy as the predominant substrates. For this reason, in homocystinurics with CBS deficiency, CSE may be the major enzyme to produce H_2S. Moreover, the level of H_2S produced by CSE is predicted to be higher due to the enhanced accumulation of Hcy (11).

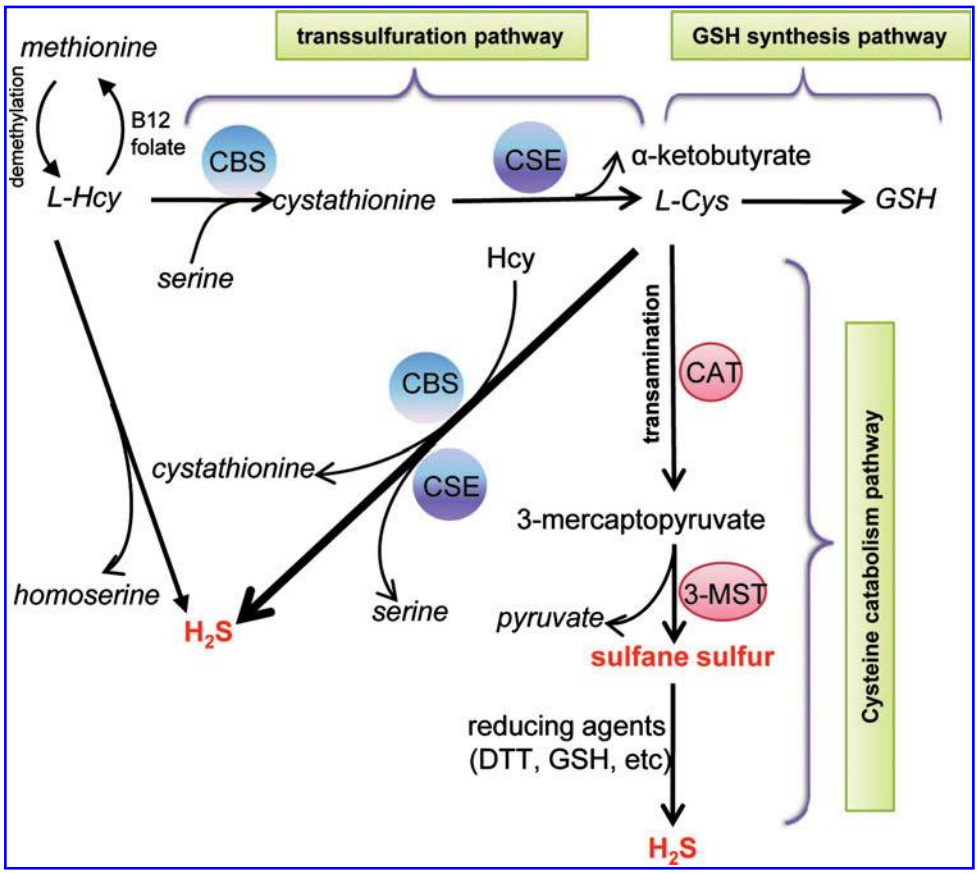

FIG. 1. Endogenous source of hydrogen sulfide (H_2S) in mammalian. H_2S is endogenously produced by the action of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) in the transsulfuration pathway. By kinetic simulation, it is found that CBS generates H_2S most efficiently from L-cysteine (Cys)+ homocysteine (Hcy), with cystathionine as a byproduct. This reaction contributes >95% of the net H_2S production by CBS. On the contrary, the preferred substrates for CSE are Cys and Hcy. Together they contribute well over 90% of the net H_2S production by CSE. In addition, the cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3-MST) are components of the Cys catabolism pathway. CAT catalyzes the transamination of Cys to yield 3-mercaptopyruvate, a substrate of 3-MST to produce pyruvate and sulfane sulfur, which may liberate H_2S in the presence of reductants such as dithiothreitol (DTT) and glutathione (GSH). The transsulfuration pathway is critical for creating Cys from the essential amino acid methionine, which is first converted to Hcy by demethylation. CBS condenses serine and Hcy to produce cystathionine, which is converted to Cys and α-ketobutyrate by CSE. The synthesis of GSH is regulated at the substrate level by Cys. Thus, the transsulfuration pathway also links to the GSH homeostasis in brain. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

CBS is highly expressed in the brain and thus believed to be the primary physiologic source of H_2S in the CNS (1), although both CBS and CSE activities were detected in different brain regions (3, 71). CBS is a cytoplasm PLP-dependent enzyme. Human CBS has a complex structure and regulatory mechanisms. It contains the N-terminal heme-binding domain, the catalytic domain, and the C-terminal regulatory domain. Two other gaseous transmitters, CO and NO, can bind to the heme-binding domain and result in the inhibition of CBS activity (67, 68). Moreover, the S-adenosyl-L-methionine, which may bind to the C-terminal domain, can instantaneously activate CBS. At the transcriptional level, glucocorticoids can stimulate the *CBS* gene expression, whereas insulin can inhibit it (57). So far, at least 153 mutations in human *CBS* gene (see www.uchsc.edu/cbs/cbsdata/cbsmain.htm) have been identified in patients with homocystinuria, a hereditary disease characterized by an accumulation of Hcy in the serum and uria. Several polymorphisms have been identified within the *CBS* gene. Apparently, there are gain-of-function polymorphisms (699C to T and 844_845ins68) and loss-of-function polymorphism (1080C to T) (40). The two single-nucleotide polymorphisms in the *CBS* gene, 699C to T and 1080T to C, are associated with decreased risk of coronary artery disease and increased responsiveness to folic acid-induced total Hcy lowering effect.

The cellular localization of CBS is still controversial. Using immunohistochemical techniques, Robert *et al.* showed that CBS protein has a predominantly neuronal localization in most areas of the brain, especially in hippocampus and cerebellum (59). In contrast, Enokido *et al.* later demonstrated that CBS is preferentially expressed in astrocytes rather than neurons, which is verified by combined biochemical and histological examination, as well as *in situ* hybridization (18). This fits with recent findings that CBS mainly localizes to astrocytes (41). Lee *et al.* demonstrated that the basal H_2S level in unstimulated human astrocytes is $\sim 3.0\,\mu$mol/g protein, which is 7.9-fold higher than that in cultured microglia. More importantly, only astrocytes, instead of microglia, are strongly immunostained for CBS (41). However, Vitvitsky and his colleagues showed the incorporation of radiolabel from methionine into glutathione (GSH) in both cultured human astrocytes and neurons (71). Another group also showed that inhibition of CSE leads to a significant loss of GSH in adult brain slices (14). Since the only known route for the transfer of radiolabeled methionine to GSH is *via* the transsulfuration pathway involving CBS and CSE, these experiments indirectly justify the existence of CBS in both astrocytes and neurons. Nevertheless, studies consistently identified temporal expression of CBS in developing and adult mouse CNS. During the embryonic period, CBS protein level is generally low, but it dramatically increases from late prenatal to early postnatal period (18, 59).

In biomedical studies, small molecule inhibitors, such as hydroxylamine and aminooxyacetate acid (AOAA), have been used to determine the significance of the endogenously generated H_2S. These agents are able to inhibit the biosynthesis of H_2S from Cys, but they are general inhibitors of all PLP-dependent enzymes and are used quite to liberate bound PLP for quantitation (34, 64). In addition to heme and PLP, hydroxylamine also reacts with nonheme iron proteins, for example, ribonucleotide reductase and is used to inhibit cell growth. Hence, caution should be taken when interpreting results obtained from work involving these inhibitors.

In addition to CBS, there is also report showing that CSE plays an important role in human brain, despite its predominant localization in the cardiovasculature. In fact, CSE is critical for maintaining GSH homeostasis in brain, which in turn preserves mitochondrial function (14). CSE is the rate-limiting enzyme in the transsulfuration pathway for the sulfur transfer from methionine to Cys, which is a limiting reagent in the synthesis of GSH. Moreover, CSE mRNA is localized in brain and found to be predominantly present in neurons by *in situ* hybridization. The CSE activity in mouse brain was as low as 1% of the hepatic activity. However, in human brain the activity was 100 times more than that in mouse brain. Further, an intact transsulfuration pathway in the brain mediated by both CBS and CSE links to GSH homeostasis, which greatly contributes to the redox-buffering capacity in brain (71). Nevertheless, the general consensus is that CSE is the primarily physiological source of H_2S generation in the peripheral tissues. There is definitive evidence that *CSE* knockout mice developed hypertension, which establishes that H_2S is a major physiologic signaling molecule regulating vascular tone in mammals. So far, there is little knowledge about the physiologic relevance of CSE relative to CBS in brain, in addition to its role in transsulfuration pathway linking to GSH homeostasis. This issue merits further investigation.

Recently, Kimura's group reported another source of H_2S in the brain homogenates of *CBS*-knockout mice (62). They show 3-mercaptopyruvate sulfurtransferase (3-MST) in combination with Cys aminotransferase (CAT) produces H_2S from Cys. Like CBS and CSE, CAT is also a PLP-dependent enzyme that catalyzes the metabolism of Cys and α-ketoglutarate to yield 3-mercaptopyruvate as the substrate for 3-MST. 3-MST is localized to mitochondria and nerve endings. As its name implies, it belongs to the family of sulfurtransferases, which catalyze the transfer of sulfane sulfur from persulfide or thiosulfate or mercaptopyruvate to an acceptor, and liberates H_2S under certain conditions. Thus, 3-MST does not produce H_2S by itself. Instead, it produces sulfane sulfur (or bound sulfur), which, in the presence of reductants like dithiothrietol used in *in vitro* assays, liberates H_2S (34). Bound sulfur may be a source of H_2S in brain and it can immediately release H_2S in response to physiologic stimulation (31). This may explain why H_2S was not depleted in the brain homogenates of *CBS* knock mice. Presumably, H_2S is derived from this pool of sulfane sulfur under reducing conditions. However, the physiological significance of H_2S derived from this source is yet to be determined with *3-MST* knockout mice or other techniques. With respect to development, 3-MST protein expression in the mouse brain is maintained from embryonic day 16 to postnatal day 14 (P14) but downregulated between P28 and P52, and then increased slightly thereafter up to P156 (62).

However, the contributions and differences of CBS and 3-MST with respect to H_2S generation under physiological and pathological conditions are still not clearly understood. As these two enzymes have different cell-type-specific expression profiles in the brain, it is possible that they may have different functions in various pathophysiological situations. It may be speculated that CBS may relate closely to the anti-neuroinflammatory role of H_2S, whereas 3-MST may contribute more to the antioxidant action due to their different cellular localization.

In addition to biosynthesis, there are two forms of sulfur stores in mammals, acid-labile sulfur and bound sulfane sulfur (31). The former store, mainly localized to the iron–sulfur center of enzymes in mitochondria, releases H_2S under acidic conditions, whereas the latter store, primarily localized to the cytoplasm, releases H_2S under reducing conditions. The physiological importance of H_2S released by bound sulfur remains unclear. However, the general consensus is that acid-labile sulfur is not a source of H_2S under physiological conditions.

H_2S is proposed to undergo various chemical reactions during its catabolism in mammals. These include oxidation to sulfate, methylation to methanethiol, and dimethyl sulfide as well as reaction with metallo- or disulfide-containing proteins such as hemoglobin. However, its metabolic fates in cells remain elusive. *In vitro* experiments found that H_2S decays rapidly with a short life of $\sim$10 min in cell culture (28, 74). Moreover, the beneficial effect of H_2S is still there even if the NaHS is removed by wash away before the subsequent exposure to oxidative stress-inducing insults (49). It is likely that H_2S could be stored and immediately released in response to physiological stimulation (31).

Concentrations of Free H_2S in Brain

Three methods employed for H_2S measurement in the brain are methylene blue colorimetric assay, polarographic H_2S sensor, and gas/ion chromatography combined with electrochemical detection. The polarographic H_2S sensor method is used for real-time measurement of H_2S production in biological samples (15). It was reported that H_2S was produced rapidly by brain supernatants at $\sim$10.6 pmol/s/mg protein (15). The gas/ion chromatography with electrochemical detection has also been applied for measuring H_2S levels in brain (22), whereas methylene blue assay is relatively less used because it uses strong acid, which may lead to artificially elevated value due to the release of H_2S from acid-labile sulfur. Early in 1989, Warenycia *et al.* first reported that rat brain tissue contained relatively high levels of free H_2S ($\sim$54 μM) (72), and subsequent research work from various groups also showed high concentrations of H_2S in brain (ranging 50–160 μM) in a variety of mammalian species, including rat, bovine, mouse, and human (22, 60). These values are frequently cited in the literature. However, more recent estimates indicate that the concentration of H_2S in brain or plasma may be much lower, which is in the nanomolar range. Ishigami *et al.* found that H_2S in brain is undetectable using gas chromatography with a detection limit of 9.2 μM, where powdered silver was employed to absorb H_2S, and N_2 gas, thiourea, as well as H_2SO_4 were applied to release H_2S (31). Furne *et al.* reported that free H_2S level in brain is $\sim$14 nM by gas chromatography (19). In that study, the brain tissue was rapidly homogenized in a gas-tight syringe, and the concentration of H_2S initially present in tissue was calculated from the H_2S concentration in the gas space over the homogenate.

There seems to be a general consensus that the earlier measurements of 50–160 μM were almost certain to be overestimates arising from unintended conversions. These may include free H_2S, HS^-, protein-bound sulfide such as acid-labile sulfur and dithiothreitol-labile sulfide or even the total sulfide in the tissues. Strong acid used for tissue process may lead to the release of acid-labile sulfur from iron–sulfur centers. Moreover, strong base may lead to the liberation of H_2S in the presence of reductants, which is commonly applied in some protein assays. The determination of H_2S in biological samples is often influenced by a number of factors such as its instability, high volatility, great susceptibility to oxidation, and release of sulfide out of the commonly used reagent dithiothreitol. Therefore, a more reliable and validated method with high sensitivity at the nanomolar range will be of great importance for the determination of the actual value of H_2S level in the brain.

H_2S as a Neuromodulator

H_2S serves as a neuromodulator that potentiates or inhibits the transmission of nerve impulses in neurons. For example, it is known to regulate LTP in hippocampus, a synaptic model of learning and memory. It selectively stimulates NMDA receptor-mediated currents *via* activating adenylyl cyclase and the subsequent cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) cascades and thus facilitates the induction of LTP in the presence of a weak titanic stimulation (1, 36). However, H_2S alone did not induce LTP, implying that H_2S merely modulates LTP in active synapses (1). H_2S could also promote astrocytic glutamate uptake, which plays an important part in clearing excessive glutamate from synaptic clefts and maintaining normal neurotransmission between neurons. These observations indicate that H_2S plays an important modulatory role in the CNS (Fig. 2). In addition, Kombian *et al.* found that H_2S reversibly inhibited both fast and slow synaptic responses in dorsal raphe serotonergic neurons (39). In another study, NaHS was shown to upregulate expression of γ-aminobutyric acid $(GABA)_B$ receptor subunits 1 and 2, whereas hydroxylamine downregulated expression of $GABA_B$ receptor subunit 2, but not that of $GABA_B$ receptor subunit 1 (24). As GABA is the major inhibitory neurotransmitters, this may also imply that H_2S is critical in maintaining the excitatory/inhibitory balance in neurotransmission and thus support the proposal that H_2S serves as a novel neuromodulator. However, as discussed earlier, one should be cautious with such interpretation as hydroxylamine has actions other than inhibiting CBS (34, 64).

Intracellular calcium ($[Ca^{2+}]_i$) homeostasis plays an important role in regulating synaptic activity and plasticity, as well as signal transmission between neuron and glial cells. H_2S has been found to increase $[Ca^{2+}]_i$ in neurons, astrocytes, and microglia by increasing calcium influx through L- and T-type calcium channels and NMDA receptors located in plasma membrane, and calcium release from $[Ca^{2+}]_i$ stores (44, 53, 77), as represented in Figure 3. Besides, both PKA and phospholipase C/PKC pathways mediate the action of H_2S on $[Ca^{2+}]_i$ (44, 77). In view of the reciprocal interactions between glia and neuron, H_2S may thereby regulate synaptic activity by modulating the activities of both neurons and glia (53). Thus, these findings consolidate the neuromodulatory action of H_2S in the CNS. Primary cultured human astrocytes synthesize H_2S at a rate of 15 μmol/g protein/h, which is 7.5-fold higher than that in microglial cells, where H_2S exhibits anti-inflammatory and neuroprotective effects (41). Moreover, inflammatory stimulations of microglia and astrocytes cause downregulation of CBS and H_2S synthesis (41).

In addition, H_2S regulates intracellular pH (pH_i) in rat primary cultured microglia and astrocytes through modu-

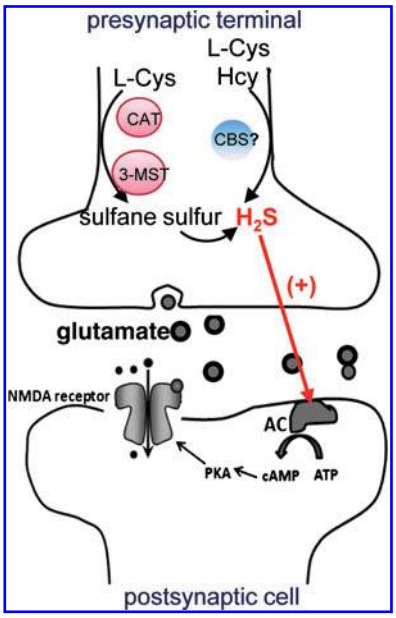

FIG. 2. Schematic diagrams showing the neuromodulatory role of H_2S in central nervous system. The H_2S produced by its generating enzyme, presumably CBS (its neuronal localization is still controversial), and the H_2S liberated by sulfane sulfur under reducing conditions may activate adenylyl cyclase (AC) and its downstream protein kinase A (PKA) pathway to modulate N-Methyl-D-aspartate (NMDA) receptor activity and thus facilitates NMDA receptor-mediated long-term potentiation formation in the hippocampus. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

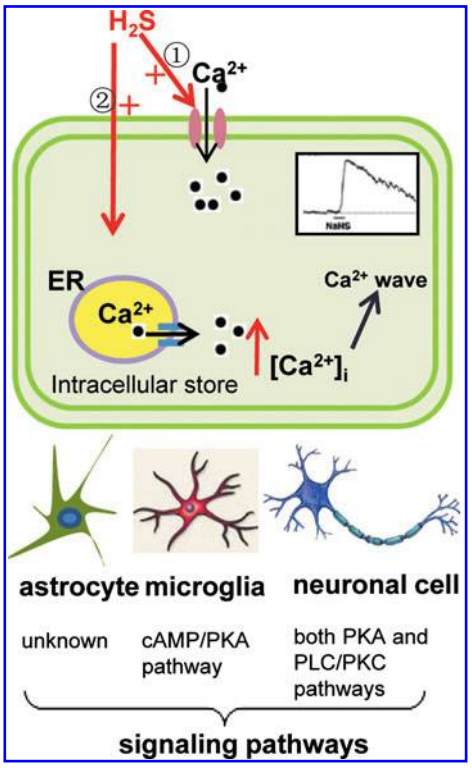

FIG. 3. Schematic diagrams showing the regulatory effects of H_2S on intracellular calcium ($[Ca^{2+}]_i$) in both neuronal and glial cells and the underlying mechanisms involved. H_2S induces Ca^{2+} waves by increasing $[Ca^{2+}]_i$ levels, which occurs *via* influx of extracellular Ca^{2+} through Ca^{2+} channels on cell membrane (labeled as ①) and release from $[Ca^{2+}]_i$ stores (*e.g.*, ER) (labeled as ②). Different signaling molecules are found to mediate these effects in different type of brain cells. For example, cAMP/PKA pathway contributes to the H_2S regulation of $[Ca^{2+}]_i$ in rat microglia in primary culture. In SH-SY5Y neuron-like cells, both PKA and phospholipase C (PLC)/PKC pathways mediate the regulatory effects of H_2S on $[Ca^{2+}]_i$ homeostasis. ER, endoplasmic reticulum; cAMP, cyclic adenosine monophosphate. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

lating the activities of Cl^-/HCO_3^- exchanger and Na^+/H^+ exchanger (Fig. 4) (48). pH_i homeostasis has an important role in the maintenance of normal cell function *via* changes in ion channel conductance, synaptic transmission as well as gap junctions. pH_i disturbance is an early event that occurs in brain under pathophysiological conditions such as hypoxia and ischemia. There is growing evidence demonstrating that the acid–base transporters contribute to the pH_i regulation in CNS. The regulatory effects of H_2S on pH_i *via* these transporters provide additional evidence that H_2S serves as a novel neuromodulator, not only under physiological conditions, but also in pathological situations.

H_2S as a Neuroprotectant

At micromolar range, H_2S has been demonstrated to show neuroprotective (antinecrotic and antiapoptotic) effects through multiple mechanisms in a series of *in vitro* studies. The following sections summarize and discuss the possible mechanisms underlying the neuroprotection offered by H_2S.

Anti-inflammation

Neuroinflammation is a complex response to brain injury involving the activation of glia, release of inflammatory mediators within the brain, and recruitment of peripheral immune cells. Neuroinflammation has emerged to be a contributing factor intricately related to the cascade of events leading to neurodegeneration. Abundant evidence from postmortem and *in vivo* studies support that neuroinflammation is closely related to the pathogenesis of degenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD).

The activation of glial cells (especially microglia), which is a rapid cellular response to microenvironmental change, for example, neuronal injury, traumatic, or infectious stimuli, triggers deleterious events such as oxidative stress and cytokine-receptor-mediated apoptosis, leading to neuronal loss and possible disease progression. Hence, inhibition of the neuroinflammatory processes is a recognized therapeutic strategy aimed at delaying or halting the progression of neurodegenerative diseases. In 2007, our group first reported that NaHS attenuates lipopolysaccharide-induced production and release of NO and tumor necrosis factor alpha in primary cultured microglia and astrocytes, and murine immortalized BV2 microglial cells (29). In consistency, Lee *et al.* later demonstrated the antineuroinflammatory role of H_2S and three H_2S-releasing compounds (anethole trithione hydroxide, S-diclofenac, and S-aspirin) (42). H_2S may exert antineuroinflammatory actions *via* inhibiting the production of

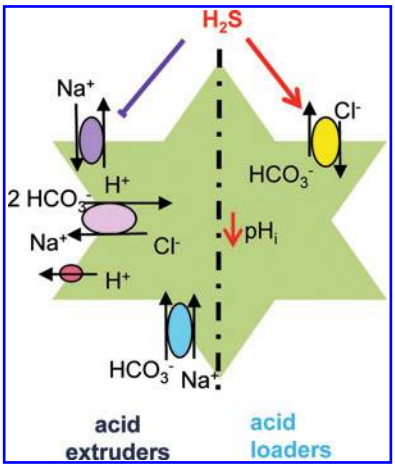

FIG. 4. Schematic illustration of the acid–base membrane transporters involved in intracellular pH regulation by H_2S in glial cells. H_2S decreases intracellular pH in glial cells *via* the stimulation of Na^+-independent Cl^-/HCO_3^- exchanger and the inhibition of Na^+/H^+ exchanger. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

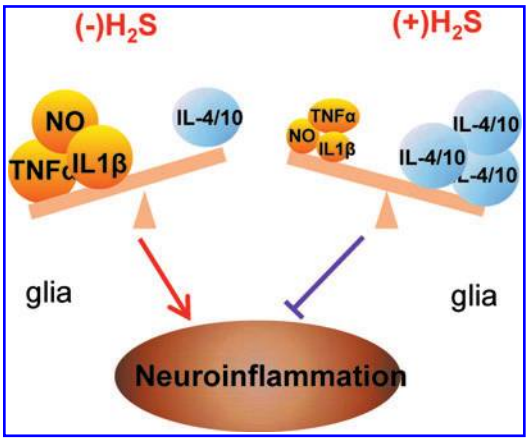

FIG. 5. Schematic diagrams illustrating the antineuroinflammatory effects of H_2S in microglia and astrocytes. Microglia and, to a lesser extent, astrocytes mediate the neuroinflammatory processes in the central nervous system. These two types of glial cells, when activated appropriately, produce proinflammatory mediators that contribute to persistent neuroinflammatory responses. However, this process could be inhibited in the presence of H_2S, which may upregulate the production of anti-inflammatory cytokines such as interleukin (IL)-4/10 and downregulate the release of proinflammatory factors, including tumor necrosis factor-alpha (TNF-α), IL-1β, and nitric oxide (NO). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

proinflammatory factors and enhancing the production of anti-inflammatory cytokines, as illustrated in Figure 5. Inhibition of p38/c-Jun-N-terminal kinase and nuclear factor kappa-light-chain-enhancer of activated B cells signaling pathways are recognized as possible mechanisms by which H_2S restrains the extent of neuroinflammation and thereby limits the extent of neuronal injury. However, the actions of H_2S on other inflammation-related intracellular molecules such as nuclear factor-erythroid 2-related factor 2, heat shock proteins, and matrix metalloproteinases still remain to be investigated. H_2S may exert indirect neuroprotective effects *via* its anti-inflammatory role by which it inhibits proinflammatory factors released during microglial activation and thus alleviates neuroinflammation-related neurotoxicity (27, 42). Hu *et al.* found that the conditioned media from rotenone (10 n*M*)-treated microglia significantly decreased the cell viability of SH-SY5Y neuronal cells; however, this effect was alleviated in the neuronal cells treated with the conditioned media from NaHS plus rotenone cotreated microglia. At such low concentration, rotenone fails to decrease cell viability in SH-SY5Y cells, but it is enough to stimulate microglia activation. Hence, the observed protective effect of H_2S, at least in part, arises from the suppression of proinflammatory factors released by rotenone-induced microglia.

Antioxidation

Oxidative stress results from an overabundance of reactive free radicals secondary to either an overproduction of reactive oxygen species or a failure of cellular antioxidant buffering mechanisms such as GSH, a major and potent intracellular antioxidant. Excessive free radicals can react with essential molecules, including proteins, lipids, and nucleic acids and thereby disrupt their normal functions. Oxidative stress is implicated in the pathogenesis of neurodegenerative disorders, including AD and PD. H_2S may act as a reducing agent. However, unlike the more abundant antioxidants (GSH present at 1–10 m*M* concentration and Cys present at ~100 μM concentration), H_2S is present at relatively low concentrations and it is also a poor reductant (redox potential of +0.17 *vs.* −0.25 V for the other two thiols) (34). Hence, the physiological relevance of the antioxidant properties by itself is an open question. However, NaHS, at the concentrations from 25 to 250 μM, is able to enhance the reducing activity in neurons and protect them against oxidative damage induced by glutamate, hydrogen peroxide, or hypochlorous acid, primarily *via* increasing GSH levels instead of directly functioning as an antioxidant (38, 73). The GSH increase involves the stimulatory effects of H_2S on the activity of γ-glutamylcysteine synthetase, cystine, and Cys transport in neurons as well as glutamate uptake in astrocytes (37, 38, 49) (as shown in Fig. 6). Cys availability is a rate-limiting factor in GSH synthesis. Extracellular Cys is easily oxidized to cystine. The transport of cystine into cells, mainly mediated by a cystine/glutamate antiporter system Xc^-, is therefore essential in providing cells with Cys as substrates for GSH synthesis. Excess extracellular glutamate may suppress cystine transportation into cells *via* Xc^-. The excessive glutamate in the synaptic cleft can be cleared by neighboring glial cells through glutamate uptake *via* the excitatory amino acid transporters (EAATs). In astrocytes, glutamate uptake is mainly mediated by two EAATs subtypes: EAAT1 and EAAT2 (also known as GLAST and GLT-1, respectively). As GSH has a high turnover in cells, the inhibition of cystine transport by excessive glutamate may lead to rapid GSH depletion, which in turn increases the vulnerability of cells to oxidative injuries and ultimate cell death. Our group recently found that NaHS at 100 μM promotes [^{3}H]glutamate uptake in astrocytes *via* enhancing the trafficking of glial glutamate transporter GLT-1 (49). This may not only lower extracellular glutamate and relieve the inhibition by glutamate on cystine transportation,

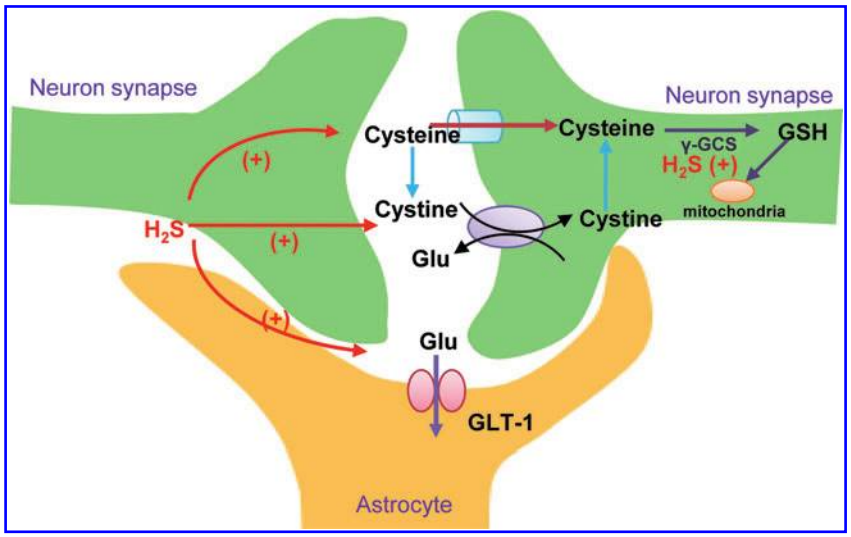

FIG. 6. Schematic paradigms illustrating the mechanisms for the elevation of GSH induced by H_2S in brain cells. H_2S enhances the transportation of cystine and Cys into cells to provide substrates for GSH synthesis in neurons. H_2S also enhances glutamate uptake *via* glutamate transporter GLT-1 in astrocytes and thus clears the excessive glutamate in synaptic cleft. This process may also relieve the inhibition by glutamate on cystine transportation and thus facilitates the cystine transport into the neuronal cell. In addition, H_2S enhances the activity of γ-glutamylcysteine synthetase (γ-GCS), a rate-limiting enzyme in GSH synthesis, and facilitates the redistribution of GSH into mitochondria and protects against oxidative stress. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

but also yield the driving force for the cystine/glutamate antiporter Xc^- function that enables the transportation of cystine into cells, and eventually results in the elevation of intracellular Cys followed by an increase in intracellular GSH, and thus protects neurons against oxidative stress. These observations provide strong evidence for the powerful antioxidative action of H_2S in CNS, and also offer evidence for its neuroprotective effects because excitotoxicity, mainly derived from excessive accumulation of glutamate in the synaptic cleft, greatly contributes to the development of stroke, traumatic brain injury, and some neurodegenerative disorders.

Antiapoptosis

Apoptosis, or programmed cell death, is a phenomenon that has been demonstrated to participate in neural development and play a role in neurodegeneration. Several pathological studies have revealed signs of apoptotic cell death in brains of PD and AD patients, although the extent is limited probably due to the slow progress of neurodegeneration. As the activation of apoptotic pathway most likely represents end-stage processes in neurodegeneration, the inhibitors of apoptosis have been proposed as potential neuroprotective agents regardless of the initial cause of neuronal loss. Mounting evidence shows that H_2S has antiapoptotic effects on neuronal cells and thereby might become a potential candidate for neuroprotection. NaHS ($<300\ \mu M$) inhibits the apoptosis of PC12 and SH-SY5Y cells induced by various toxins, including 1-methyl-4-phenylpyridine, 6-hydroxydopamine (6-OHDA), rotenone, and β amyloid (66, 70, 76), all of which are commonly used in establishing *in vivo* and *in vitro* models for PD and AD. In addition, H_2S protects hippocampal neurons against vascular dementia-induced injury *via* its antiapoptotic function (78). Most of the data reported so far indicate that the antiapoptotic effects of H_2S mainly result from the preservation of mitochondrial integrity, that is, suppression of the mitochondrial apoptotic pathway (28, 76), as shown in Figure 7. H_2S may inhibit the forming and opening of mitochondrial permeability transition pore and the subsequent release of cytochrome c from mitochondria to cytosol, as well as the activation of caspase cascades. These effects are dependent on the opening of mitochondrial ATP-

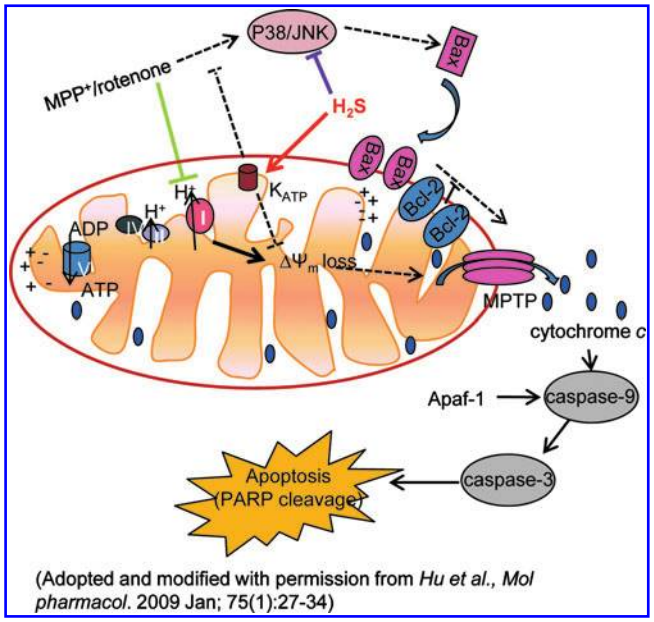

FIG. 7. Proposed signaling mechanisms for direct neuroprotection of H_2S on neurotoxin-induced apoptosis. 1-Methyl-4-phenylpyridine (MPP^+) or rotenone (complex I inhibitors) may initiate the $\Delta\Psi_m$ dissipation and induce the mitochondrial permeability transition pore (MPTP) opening. These neurotoxins may also evoke the forming and opening of MPTP by activating p38/c-Jun-N-terminal kinase (JNK), inducing the release of cytochrome *c* from mitochondria to cytosol. The subsequently formed apoptosome (cytochrome *c*, apaf-1, and pro-caspase-9 complex) leads to the activation of caspase-9/3-dependent apoptotic pathway. The mechanisms underlying the anti-apoptotic effects of H_2S may result from opening of mito-ATP-sensitive potassium channel (K_{ATP}) channels, which in turn mediates the prevention of $\Delta\Psi_m$ loss and the inhibition of p38/JNK pathway. Adopted and modified with permission from Hu *et al.* (28). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

sensitive potassium channels (K_{ATP}) (28). There is no report on the interaction between H_2S and death receptor apoptotic pathway. Brittain *et al.* recently identified the interaction of H_2S with human neuroglobin, a protein associated with mitochondria and protecting neurons from apoptotic stress (8); however, the biological significance of this interaction remains to be defined.

However, there is a study demonstrating that NaHS, at the concentration from 200 to 1000 μM, greatly induced the mature cortical neurons apoptosis through the mechanisms involving the activation of calpain proteases and lysosomal destabilization, rather than caspases activation (10). Seemingly, this report is contradictory to the previous findings for the beneficial effects of H_2S. However, NaHS, at a higher concentration up to 1000 μM, yields $\sim$333 μM H_2S. This is much higher than the physiological range of H_2S (50–160 μM) in the brain, as discussed earlier. Such high levels of H_2S may produce toxic effects on cells *via* inhibition of cytochrome c oxidase or other unidentified targets. In sum, several studies indicate that H_2S exerts divergent effects on various brain cells and different animal disease models by using NaHS at various concentrations/doses. Some of them are summarized in Table 1. It is likely that H_2S may play a neuroprotective role at physiological concentrations but may exhibit neurotoxic effects at significantly higher concentrations. In this sense, H_2S shares similar features with another gaseous transmitter NO, which is also found to be neuroprotective at physiological amounts but clearly neurotoxic at higher concentrations. Additionally, similar to NO-induced protein S-nitrosylation, protein S-sulfhydration has recently been proposed to be a mechanism for H_2S-mediated signaling (52). Nitrosylation appears to inhibit protein activity, whereas sulfhydration seems to enhance it. Sulfhydration is a post-translational modification. About 10%–25% of endogenous GAPDH, β-tubulin, and actin are basally S-sulfhydrated and this sulfhydration directly enhances actin polymerization without affecting depolymerization (52). Of interest, the same group also showed that the stimulation of K_{ATP} by H_2S also arises from this protein-sulfhydrating effect (20). Therefore, protein S-sulfhydration may be an essentially molecular mechanism for H_2S-mediated various biological effects.

All the aforementioned findings clearly indicate that H_2S shows neuroprotective effects at low concentrations through various signaling pathways acting either cooperatively or independently. Then, how does H_2S on earth exert these effects *via* a myriad of mechanisms? The answer seems to lie in the physical and chemical properties of this gaseous transmitter. H_2S is a lipophilic molecule and it readily crosses cell membrane. This enables it to rapidly switch on or S-sulfhydrate intracellular targets. H_2S not only modulates intracellular signaling molecules such as PKA and mitogen-activated protein kinases, but also regulates different ion channels such as L- and T-type calcium channel, K_{ATP} channel, and chloride channel. H_2S may react with these molecules within a few seconds, which in turn activate its downstream signaling pathways, resulting in the occurrence of biological functions of H_2S in the CNS. However, no specific target/receptor has yet been identified presently. It is therefore proposed that H_2S not only directly exerts effects in those cells where it is produced, but also acts on neighboring cells by diffusing into the surround and propagating the signal among neuron and glial cells, thus displaying a neuromodulatory role in the CNS.

H_2S in CNS Diseases

Other than the physiological roles of a neuromodulator, there is increasing evidence that H_2S is involved in the pathophysiology of CNS diseases such as epilepsy, stroke, Down's syndrome, AD, and PD, as summarized in Table 2. In addition, as mentioned above, the deficiency of CBS in humans results in homocystinuria, with increased plasma levels of Hcy and methionine but decreased levels of Cys. The clinical phenotype of these patients includes mental retardation, lens dislocation, and skeletal abnormality. This also implies the significance of H_2S in health. Kinetic analysis has demonstrated that the CSE-catalyzed H_2S production is sensitive to the level of Hcy and thus may increase in proportion to the grade of hyperhomocysteinemia (11). Hence, excessive production of H_2S may be a contributing factor to the consequences of hyperhomocysteinemia. Remarkably, hyperhomocysteinemia is an independent risk factor for stroke (35) and has also been found to be correlated to a range of neurodegenerative disorders, including AD and PD. There is a significant increase of total Hcy level in the cerebrospinal fluid in patients with AD and PD (32). As discussed earlier, Hcy is maintained at relatively low concentrations and can be converted to Cys *via* the transsulfuration pathway. Cys is an essential substrate for the synthesis of GSH, the most abundant antioxidant in mammalian. In fact, oxidative stress and reduced GSH levels are common to the pathogenesis of neurodegenerative disorders. Therefore, the elevation of Hcy level and reduction of GSH level in AD and PD suggest the deficiency of CBS and/or CSE in the transsulfuration pathway (71), which links Hcy to GSH homeostasis. The recognition that CSE is sensitive to Hcy, whereas CBS is not should indicate an important role of CSE in relation to CNS diseases despite its predominant localization in the cardiovascular system. Further, in addition to inducing neuronal apoptosis, Hcy is also able to promote the proliferation and activation of microglia (80), which is a contributing factor to the pathological progress of neurodegenerative diseases. All in all, these findings indicate a correlationship between abnormal H_2S biosynthesis and the development of the CNS diseases.

Ischemic stroke

Ischemic stroke is caused by an interruption of blood supply to the brain (global) or part of the brain (focal) either by thrombosis or embolism. In a small clinical study, Wong *et al.* found that high plasma Cys level is correlated to poor clinical outcome 3 months poststroke in acute stroke patients (75). These authors further demonstrated that Cys loading by the intraperitoneal route dose dependently increases the infarct volume in rats after middle cerebral artery occlusion. Such effect of Cys was reversed by the coadministration of AOAA (a nonspecific inhibitor of PLP-dependent aminotransferases) (75) and mimicked by NaHS (55), indicating that H_2S may be responsible for this effect. This is supported by the observation that the increased infarct caused by either Cys or NaHS was reversed by MK-801, an NMDA receptor blocker, as H_2S is known to facilitate NMDA functions (36), thus enhancing the excitotoxicity triggered by ischemia. The dose at which NaHS mimicked the Cys loading effect was 0.18 mmol/kg, which is 66% of the reported LD_{50} for NaHS (72). In other words, H_2S exacerbated tissue injuries in the rat middle cerebral artery occlusion model only at relatively high and most

Table 1. Toxic and Protective Effects of Hydrogen Sulfide in Cells and Animals

	NaHS (*μM*)	*Effects of H_2S donors*	*Ref.*
In vitro studies			
Rat hippocampal astrocytes	60–300	Increase $[Ca^{2+}]_i$ and induce calcium waves	53
Rat cortical microglia	100–500	Increase $[Ca^{2+}]_i$	44
Rat cortical neurons	30–200	Increase intracellular GSH and protect against NMDA-induced excitotoxicity	38
SH-SY5Y neuroblastoma cells	25–250	Protect against hypochlorous acid-induced decrease in cell viability	73
	1–300	Inhibit rotenone-induced apoptosis via preservation of mitochondrial function	28
	1–50	NaHS and three other S-NSAIDs exert neuroprotective effects through their anti-inflammatory actions in microglia	42
PC12 cells	50–200	Protect against $A\beta_{(25–35)}$-induced damage via scavenging ROS	66
	200–800	Inhibit MPP^+-induced damage in PC12 cells	76
Mature mouse cortical neurons	200–1000	Induce neuronal apoptosis involves the activation of calpain proteases and lysosomal destabilization	10
Rat microglia and BV-2 cells	10–300	Suppress LPS-stimulated NO and TNF-α generation	29
In vivo studies	(*μ*mol/kg)		
Ischemic stroke	180 (i.p.)	Increase infarct size induced by ischemic stroke	75
Febrile seizure	56	Alleviate hippocampal damage induced by febrile seizure	25
Parkinson's disease	30, 100 (i.p.)	Exert neuroprotection in 6-OHDA and rotenone-induced model rat	27

$[Ca^{2+}]_i$, intracellular calcium; GSH, glutathione; H_2S, hydrogen sulfide; LPS, lipopolysaccharide; MPP^+, 1-methyl-4-phenylpyridine; NMDA, N-methyl-D-aspartate; NO, nitric oxide; 6-OHDA, 6-hydroxydopamine; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha. i.p., intraperitoneal injection; NaHS, sodium hydrosulfide; NSAIDs, non-steroid anti-inflammatory drugs.

certainly not physiological concentrations. Further, the endogenous levels of H_2S and its synthesizing activity in the affected cerebral cortex were significantly increased after middle cerebral artery occlusion (55).

More importantly, inhibition of H_2S production by various known inhibitors (AOAA and hydroxylamine, β-cyanoalanine, and DL-propargylglycine) preadministered before middle cerebral artery occlusion could reduce the infarct volume, strongly indicating that increased H_2S levels contributed significantly to the tissue infarction. Among these inhibitors, AOAA, at a dose of 0.05 mmol/kg, appears to be most effective followed by hydroxylamine which produces effect at 0.5–1.0 mmol/kg, in reducing middle cerebral artery occlusion-induced ischemic infarction. However, AOAA at higher doses (up to 0.5 mmol/kg) was not effective in ameliorating ischemic infarction (55). Seemingly, the inhibition of H_2S synthesis

Table 2. Experimental Evidence for the Role of Hydrogen Sulfide in Central Nervous System Diseases

Disease	*Evidence*	*Ref.*
Ischemic stroke	Ischemic stroke increased tissue H_2S in cerebral cortex.	55
	Administration of cysteine and NaHS increases infarct size.	75
AD	Total homocysteines are increased in AD brains and serum.	12
	H_2S attenuated beta-amyloid-induced damage in PC12 cells.	66
	H_2S attenuates LPS-induced cognitive impairment in rats.	21
Febrile seizure	Increased plasma level of H_2S and expression of CBS in hippocampus of febrile seizure model rat NaHS improve hippocampal damage induced by recurrent febrile seizure.	25
HD	Plasma total homocysteine is increased in HD patients.	2
	CBS interacts with huntingtin.	7
PD	Plasma homocysteine levels are elevated in PD patients treated with L-dopa.	79
	H_2S exerts neuroprotective effects on neurotoxins-induced PD model rats.	27

AD, Alzheimer's disease; CBS, cystathionine β-synthase; HD, Huntington's disease; PD, Parkinson's disease.

would be a potential approach for stroke therapy based upon these observations. However, it is premature to draw any conclusion for a number of still unresolved issues: (i) sublethal dose of NaHS administration was employed in this study; (ii) the inhibitors used are nonspecific inhibitors and may exhibit H_2S-unrelated effects; (iii) the causality between stroke and endogenous H_2S level elevation remains to be assessed in future. Moreover, in an *in vitro* study, NaHS (10–100 μM) was shown to protect neurons against hypoxic injury *via* stimulation of K_{ATP} channels (69), but at least, these studies strongly suggest that H_2S is involved in the pathogenesis of ischemic stroke. It is highly likely that physiological levels of H_2S exert a protective effect on cells against insults such as hypoxia. In the event of a stroke, overproduction of H_2S may facilitate the cell death through enhancing excitotoxicity induced by excessive accumulation of extracellular glutamate. Whether or not inhibition of H_2S production is a viable therapeutic approach for the treatment of acute stroke remains to be investigated. Nevertheless, elevated plasma Cys level in stroke patients could be used as an index to predict the poor outcome of stroke. Since H_2S preconditioning-induced cardioprotection is well demonstrated, another important potential clinical application of H_2S in stroke is in ischemic pre/postconditioning, which is deserving of attention in the future.

Alzheimer's disease

AD is the most common age-related neurodegenerative disorder. Its etiology remains unclear, but current evidence indicates the involvement of amyloid and tau proteins. In 1996, Morrison *et al.* first reported that the brain levels of S-adenosylmethionine, a CBS activator, are severely decreased in AD patients (51). The total serum level of Hcy (a precursor of Cys when acted on by CBS followed by CSE) is accumulated and increased in AD patients (12). One possible explanation is that the transsulfuration pathway linking Hcy and GSH metabolism, mediated by CBS and CSE, is disrupted. Because CBS is an important biosynthetic source of H_2S generation in brain, although the contribution of 3-MST to H_2S formation is also reported in brain, the dysfunction of the transsulfuration pathway may lead to the reduced production of H_2S in AD, in addition to GSH. Indeed, several lines of evidence from both *in vivo* and *in vitro* studies indicate that H_2S treatment elicits neuroprotective effects against pathological progression of AD. First of all, H_2S is shown to scavenge the cytotoxic lipid oxidation product 4-hydroxynonenal (61), which is markedly increased in brains of severe AD patients. Second, H_2S was shown to ameliorate β amyloid-induced damage in PC12 cells through reducing the loss of mitochondrial membrane potential and attenuating the increase of intracellular reactive oxygen species (66). Third, H_2S-releasing compounds are capable of attenuating neuroinflammation (42), a contributing factor implicated in AD pathogenesis. Importantly, H_2S attenuates lipopolysaccharide-induced cognitive impairment in rats *via* its anti-inflammatory action (21). Additionally, garlic extracts, mainly the organosulfur-containing compounds such as S-allylcysteine and diallyl-disulfide, have been shown to reduce cerebral amyloid, inflammation, and tau conformational changes in AD transgenic model. Moreover, these garlic extracts (both fresh and boiled) not only inhibited β amyloid fibril formation but also was capable of defibrillating β amyloid preformed fibrils, thus exhibiting an antiamyloidogenic activity on amyloid-beta fibrillogenesis (23). H_2S can be formed nonenzymatically from polysulfides in garlic (5). Based upon these findings, it is logical to assume that H_2S would be beneficial for AD treatment. However, more direct evidence for the potential benefits of H_2S or its donors in AD animal models is lacking at present.

Parkinson's disease

Like that in AD, plasma Hcy levels are also found to be elevated in PD patients treated with L-3,4-dihydroxyphenylalanine (79). However, little is known about the role of H_2S in the initiation and development of PD. We recently demonstrated that H_2S levels in the substantia nigra and striatum are considerably reduced in both 6-OHDA and rotenone-induced PD-like rats (27). More importantly, these authors found that systemic administration of NaHS (30 and 100 μmol/kg) dramatically attenuated the progression of movement dysfunction and loss of tyrosine hydroxylase positive-neurons in the substantia nigra induced by either rotenone or 6-OHDA. In addition, NaHS treatment inhibited the microglial activation in the substantia nigra and the accumulation of proinflammatory factors such as tumor necrosis factor-alpha and NO in the striatum. These observations indicate a role of endogenous H_2S in the development of PD and thus its potential therapeutic value for PD treatment. This is supported by *in vitro* observations that H_2S protects PC12 and SH-SY5Y cells against various neurotoxins (6-OHDA, 1-methyl-4-phenylpyridine and rotenone) *via* antioxidative and antiapoptotic mechanisms (28, 70, 76). Besides the direct protective effects on neuronal cells, H_2S is also able to indirectly protect SH-SY5Y cells against microglia-mediated neuroinflammatory toxicity induced by rotenone (27). This shows great disease-modifying significance because neuroinflammation is now recognized to be a critical factor in the pathogenesis of PD and other degenerative disorders. H_2S exerts both direct and indirect neuroprotection on dopaminergic neurons, and thus modulating H_2S synthetic pathways may become a potential approach for the treatment of PD (shown in Fig. 8). Hence, the recently developed H_2S-releasing L-3,4-dihydroxyphenylalanine hybrid molecules, which have been demonstrated to show antioxidant, anti-inflammatory, and monoamine oxidase B inhibitory effects (43), would be of great value for PD treatment. However, the effects of H_2S on the occurrence of Lewy's body, another important pathological feature in PD, have not been explored. Further, the physiological relevance of the H_2S-generating enzymes such as CBS, CSE, CAT, as well as 3-MST in the development of PD is yet to be determined.

Recurrent febrile seizure

Febrile seizure is the most common seizure type in children, often causing hippocampal damage. Han *et al.* reported the plasma level of H_2S and expression of CBS in hippocampus were dramatically increased in the rat models of recurrent febrile seizure (25). In the same study, NaHS (56 μmol/kg) was found to lessen the hippocampal damage induced by recurrent febrile seizure, whereas hydroxylamine (12.5 mg/kg) aggravated this damage. The mechanisms by which NaHS alleviates the hippocampal damage involve its regulation of $GABA_B$ receptor function, as NaHS treatment could reverse downregulation of both $GABA_B$ receptor protein and mRNA in

the hippocampus of recurrent febrile seizure model rats (24). Therefore, H_2S plays an important role in regulation of the GABAergic system besides its well-characterized actions on the glutamatergic system. It is thus not surprising that H_2S may serve as a neuromodulator to maintain the excitatory/inhibitory neurotransmission in brain. Further, the increased H_2S concentration as well as CBS expression in recurrent febrile seizure may be a compensatory response to suppress the neuronal hyperexcitability and thus alleviate the neuronal damage in hippocampus. Endogenous H_2S may act in synergy with CO to protect against the hippocampal damage in recurrent febrile seizure because the researchers found that blockade of H_2S production could reduce the CO level and the heme oxygenase expression, whereas administration of exogenous H_2S could elevate them, and vice versa. Moreover, the different intracellular targets of these two gaseous transmitters [H_2S acts on adenylyl cyclase (36), whereas CO affects guanylyl cyclase (13)] also point out the possible synergistic effect on memory processing. However, care should be taken in interpreting these observations as the inhibitor hydroxylamine used in that study can also permanently disable heme-containing molecules. Whether or not the interplay of these two gaseous molecules exerts a critical role in regulating the development of recurrent febrile seizure is yet to be defined.

Other CNS diseases

In addition to playing a part in the pathophysiology of aforementioned CNS diseases, H_2S is also closely related to the pathogenesis of other CNS diseases such as Down syndrome and another degenerative disorder, Huntington's disease. Down syndrome is a chromosomal disorder characterized by the presence of all or part of an extra chromosome 21. A clinical study found that high level of thiosulfate—a catabolite of H_2S, exists in the urine of Down syndrome patients, compared to that of the matched control subjects (4). Further, clinical investigations have shown the overproduction of endogenous H_2S in Down syndrome patients and thus established a correlationship between Down syndrome and chronic H_2S poisoning. Excess H_2S may be responsible for many clinical features of Down syndrome such as mental retardation. In fact, overproduction of H_2S in Down syndrome is not surprising because the gene for CBS, one of the three enzymes in mammals responsible for H_2S biosynthesis, is localized on chromosome 21 (*21q22.3*). In addition, Ichinohe *et al.* reported that CBS is enriched and localized to astrocytes and those surrounding senile plaques in the brains of Down's patients with AD (30). These observations imply that the overproduction of H_2S, derived from CBS, may also be related to the formation and/or clearance of misfolded protein aggregates, which is a common pathological feature of neurodegenerative disorder. Compared to AD and PD, there is little knowledge about the role of H_2S in Huntington's disease. There is only evidence that CBS interacts with huntingtin, mutants of which cause Huntington's disease. The plasma Hcy levels are also elevated in patients of Huntington's disease (2, 7). The elevation of plasma Hcy level in patients of the most common degenerative disorders, that is, AD, PD, and Huntington's disease, implicates a correlationship between Hcy aberration and the pathogenesis of neurodegenerative disorders. This field of research has been extensively carried out, but few are related to H_2S aberration. Hence, further investigations are needed to determine the significance of H_2S in this disorder.

Challenges and Limitations

Although mounting evidence establishes the neuromodulatory function of H_2S in the CNS and thus suggests that modulating H_2S formation system may be a promising therapeutic approach for CNS diseases, researchers have come across challenges working with this gaseous molecule. First, the actual value of H_2S in brain tissues is still in

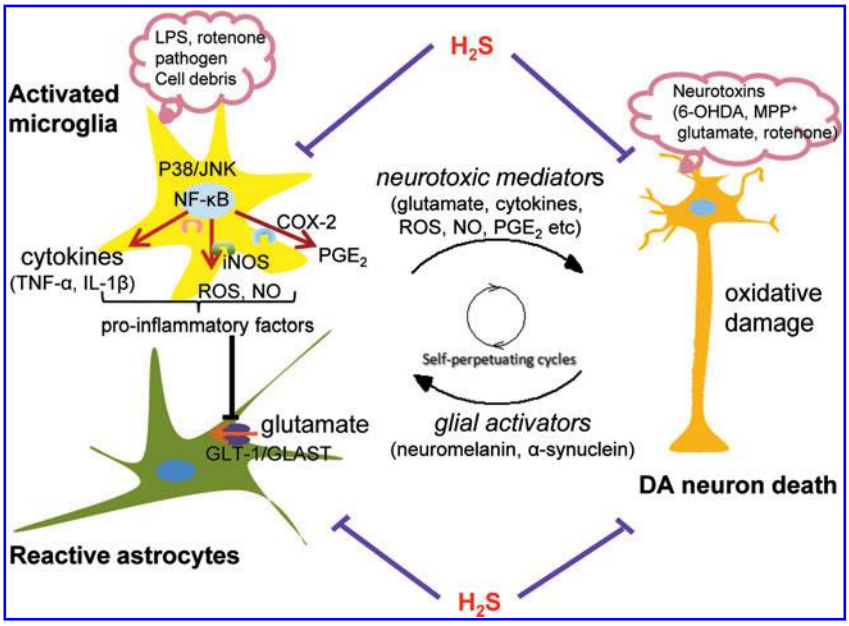

FIG. 8. Schematic paradigms illustrating the neuroprotective roles of H_2S in Parkinson's disease. H_2S shows direct protective effects on dopaminergic neurons against various neurotoxins (*e.g.*, 6-hydroxydopamine [6-OHDA], MPP^+, rotenone, and glutamate)-induced neuronal loss *via* antioxidant and antiapoptotic mechanisms. This may limit the injured dopaminergic neurons to release substances such as neuromelanin and α-synuclein, which are capable of activating glial cells in the injured site. In response to the stimuli resulting from external pathogen, neuronal injury, and/or neurotoxins, glial cells, especially microglia, are activated to release proinflammatory and neurotoxic factors such as TNF-α, IL-1β, NO, reactive oxygen species (ROS), and prostaglandin E_2 (PGE_2), which may initiate or exacerbate dopaminergic neuronal damage, leading to the progression of Parkinson's disease. Moreover, H_2S could enhance the glutamate uptake by astrocytes *via* GLT-1 and thereby reduces extracellular glutamate levels. Thus, H_2S may also exert indirect protection *via* its antineuroinflammatory role in microglia and astrocytes. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

debate due to the methodological and technical problem of H_2S determination. This affects the doses of H_2S chosen for experimental application. The H_2S levels achieved in the nigrostriatal tract is still unknown although it is reported that administration of NaHS at 1.68 and 5.6 mg/kg/day could be beneficial for rotenone/6-OHDA-induced PD model rats. Second, the therapeutic window for H_2S treatment is quite narrow since lots of studies show that H_2S produces beneficial effects at physiological concentrations (NaHS $<300\,\mu M$) but brings about harmful actions at significantly higher concentrations. Similar situation exists in the inflammation-regulatory effects by H_2S. Li *et al.* points out that physiological concentrations of H_2S produce anti-inflammatory effects, whereas higher concentrations, due to overaccumulation in already inflamed site, can exert proinflammatory effects (45). Third, several inhibitors of H_2S biosynthesis, such as AOAA and hydroxylamine, are commonly used for the exploration into the physiological relevance of endogenous H_2S. Unfortunately, none of these are specific for H_2S biosynthesis. In addition, few H_2S donors except NaHS and Na_2S are commercially available. Also, as mentioned earlier, no specific receptor/target of H_2S has been defined. Last but not the least, although $CBS^{-/-}$ mice have been developed for testing the role of H_2S in CBS predominantly expressed tissues especially in CNS, other sources of H_2S, derived from CSE or released by bound sulfane under reducing conditions, could not be ignored. These may bring difficulties for the examination of the physiological significance of endogenous H_2S in the CNS.

Further, attention should also be paid to the regimen of H_2S given (bubbling, bolus, or slow-releasing of H_2S) during experimentation. Bubbling of H_2S gas gives a sustained source of H_2S, but it also releases unpleasant smell in the working environment, which may bring about unhappiness to researchers and even animals. Moreover, NaHS gives a rapid bolus of H_2S at a constant concentration. It also releases H_2S more accurately and reproducibly than bubbling of H_2S gas in solution. Alternatively, the novel H_2S-releasing compounds such as GYY4137 and S-diclofenac slowly release H_2S and thus they may presumably reflect the enzymatic generation of H_2S. For this reason, they would be more suitable for exploring the H_2S biology in the CNS as well as other body systems. To address these abovementioned issues, more efforts should be put into the improvement for the techniques of H_2S determination and the development of more commercially available H_2S-releasing compounds. This will strengthen and enrich our knowledge of H_2S biology in the CNS and other tissues.

Conclusions

Accumulating evidence demonstrates that H_2S confers pathophysiologically regulatory effects in brain, rather than being mere environmental toxin as previously described; therefore, now it must be considered as a biologically important molecule in both health and disease. This review summarizes the current knowledge on the effects of H_2S in the CNS and discusses its roles in neuroprotection, as well as its therapeutic potential for neurodegenerative disorders. In summary, H_2S acts as a neuromodulator in the CNS and may be involved in the pathogenesis of CNS diseases. Knowledge of H_2S biology in the CNS highlighted here also raises the possibility of manipulating the H_2S system for therapeutic benefits to the patients suffering from AD, PD, and recurrent febrile seizure as well. However, in contrast to the great knowledge of the various biological functions of H_2S in peripheral tissues, especially in the cardiovascular system, the exploration of H_2S biology in the CNS is still at infancy. Great interest in unveiling this mystery is expected to be seen in the near future. Insight into the biological significance of H_2S in brain may promote the understanding of the etiologies and pathologies of these diseases, perhaps leading to new treatment approaches. Bearing this in mind, intensive and extensive studies should be conducted in the near future to achieve a more comprehensive acknowledge of this gas in CNS, helping to seek promising and more effective therapeutic agents for neurodegenerative disorders (*i.e.*, AD, PD, and Huntington's disease). However, due to the limitations mentioned above, there is still a long way to go before the mystery of H_2S biology in the CNS could be uncovered and the application of any H_2S-releasing compound could be developed for clinical uses.

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Abbreviations Used

3-MST = 3-mercaptopyruvate sulfurtransferase
6-OHDA = 6-hydroxydopamine
AC = adenylyl cyclase
AD = Alzheimer's disease
AOAA = aminooxyacetate acid
$[Ca^{2+}]_i$ = intracellular calcium
cAMP = cyclic adenosine monophosphate
CAT = cysteine aminotransferase
CBS = cystathionine β-synthase
CNS = central nervous system
CO = carbon monoxide
CSE = cystathionine γ-lyase
Cys = L-cysteine
DTT = dithiothrietol
EAATs = excitatory amino acid transporters
ER = endoplasmic reticulum
GABA = γ-aminobutyric acid
γ-GCS = γ-glutamylcysteine synthetase
GSH = glutathione
Hcy = homocysteine
HD = Huntington's disease
H_2S = hydrogen sulfide
IL = interleukin
JNK = c-Jun-N-terminal kinase
K_{ATP} = ATP-sensitive potassium channel
LPS = lipopolysaccharide
LTP = long-term potentiation
MPP^+ = 1-methyl-4-phenylpyridine
MPTP = mitochondrial permeability transition pore
NaHS = sodium hydrosulfide
NMDA = N-Methyl-D-aspartate
NO = nitric oxide
PD = Parkinson's disease
PGE_2 = prostaglandin E_2
pH_i = intracellular pH
PKA = protein kinase A
PKC = protein kinase C
PLC = phospholipase C
PLP = pyridoxal-5′-phosphate
ROS = reactive oxygen species
TNF-α = tumor necrosis factor-alpha

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