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Review

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Hydrogen Sulfide: A Neuromodulator and Neuroprotectant in the Central Nervous System

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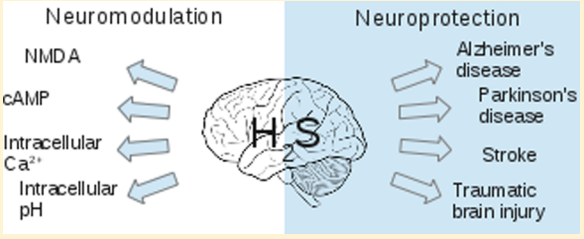
ABSTRACT: Hydrogen sulfide (H_2S) used to be known as a toxic gas. However, in the last two decades, accumulating evidence has revealed its role as a bioactive molecule in the biological systems. H_2S has relatively high expression in the brain, exerting multiple functions in both health and diseases. It modulates neurotransmission by influencing behaviors of NMDA receptors and second messenger systems including intracellular Ca^{2+} concentration and intracellular cAMP levels and so forth. H_2S shows potential therapeutic value in several CNS diseases including Alzheimer's disease, Parkinson's disease, ischemic stroke, and traumatic brain injury. As a neuroprotectant, H_2S produces antioxidant, anti-inflammatory, and antiapoptotic effects in pathological situations. Sulfhydration of target proteins is an important mechanism underlying these effects. This Review summarizes the current understanding of H_2S in the central nervous system, with emphasis on its role as a neuromodulator and a neuroprotectant.



KEYWORDS: *Hydrogen sulfide, neuromodulation, neuroprotectant, neurodegeneration, Alzheimer's disease, Parkinson's disease, stroke*

1. INTRODUCTION

Hydrogen sulfide (H_2S) was known as a toxic gas. However, as increasing knowledge about the biological functions of H_2S has been unfolding in the last two decades, it has now been recognized as another gaseous signal molecule after nitric oxide and carbon monoxide. Extensive functions of H_2S have been revealed in the cardiovascular, central nervous, and other biological systems under both physiological and pathological conditions. H_2S is expressed at a relatively high concentration in the brain. It facilitates hippocampal long-term potentiation (LTP) by enhancing NMDA receptor-mediated responses.[1] It also modulates intracellular Ca^{2+} and pH homeostasis in neurons, microglial cells, and astrocytes.[2−5] Impaired metabolism of H_2S was found in several CNS disorders such as Alzheimer's disease and Parkinson's disease. The plasma H_2S level was reported to be correlated with long-term clinical outcome in stroke patients.[6] These findings suggest that H_2S may be involved in the initiation or progression of these diseases. More importantly, extensive studies have demonstrated the potential therapeutic value of H_2S in several CNS diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), traumatic brain injury, and perhaps ischemic stroke as well. In these diseases, H_2S may act as a neuroprotectant via its antioxidant, anti-inflammatory, and antiapoptotic effects. Although it is still not clear how H_2S exerts these functions at the molecular level, a series of studies suggest that H_2S modifies specific Cys residues in proteins through the formation of a persulfide (−SSH) bond, and this modification has been termed as protein sulfhydration. Sulfhydration may change the conformation and consequently the activities of target proteins, although it is still uncertain how much this mechanism can explain the multiple biological effects of H_2S. In this Review, we will try to summarize the current knowledge about H_2S in the central nervous system, with an emphasis on its roles in neuromodulation and neuroprotection.

2. CHEMICAL PROPERTIES

H_2S is a colorless and poisonous gas with an odor of rotten eggs. It is slightly soluble in water and acts as a weak acid, dissociating into H^+ and the hydrosulfide ion HS^-. At body temperature and neutral pH, less than 20% of H_2S exists as undissociated form in solution.[7]

Due to the inconvenience to use the gas in laboratories, H_2S salts, sodium hydrosulfide (NaHS) and sodium sulfide (Na_2S), are often used as donors in research. H_2S is produced rapidly in their aqueous solutions. To further mimic the real synthesis and release process of H_2S in vivo, slow-releasing H_2S compounds (such as GYY4137, ACS compounds, AP39, etc.) are synthesized.[8−10]

3. METABOLISM

Three sources of H_2S biosynthesis have been identified. H_2S is synthesized by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) or cooperation of 3-mercaptopyruvate sulfurtransferase (3-MST) together with cysteine aminotransferase (CAT). CBS catalyzes the β-replacement reaction where Cys reacts with Hcy and produces cystathionine and H_2S. CSE catalyzes the reaction of Cys, producing H_2S and serine as a byproduct. 3-MST and CAT mediate the catabolism of Cys and produce sulfane sulfur, which releases H_2S when reduced by

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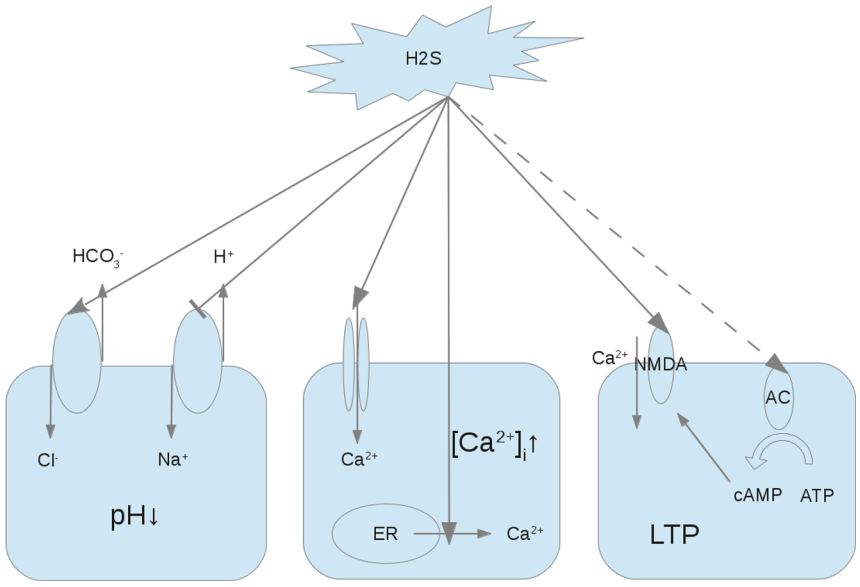

Figure 1. Schematic diagram showing the neuromodulatory roles of H_2S by regulation of second messenger systems. H_2S decreases intracellular pH in primary cultured rat microglia and astrocytes, possibly via enhancing the activity of Cl^-/HCO_3^- exchanger and inhibiting that of Na^+/H^+ exchanger.[5] H_2S increases intracellular Ca^{2+} in various cell types such as neurons,[4] astrocytes,[3] and microglial cells,[2] which is likely mediated by both extracellular Ca^{2+} influx and Ca^{2+} release from intracellular store. H_2S facilitates hippocampal long-term potentiation by enhancing NMDA receptor-mediated responses, which is possibly mediated by the cAMP/PKA pathway and sulfhydration of NMDA.[1,16]

physiological reducing reagents like thioredoxin. In the central nervous system, the CBS pathway is considered to be the predominant source of endogenous H_2S.[7] However, in CBS knockout mice, a similar level of H_2S was still found in the brain tissue even in the absence of pyridoxal-5′-phosphate (PLP). These data suggest that 3-MST is also an important PLP-independent pathway to produce endogenous H_2S in brain.

The metabolic fate of H_2S in cells is not fully understood yet. It is proposed that H_2S's catabolism may involve chemical reactions such as oxidation to sulfate,[11] methylation to methanethiol and dimethyl sulfide, and reactions with metallo- or cysteine-containing proteins.[7] H_2S can also be stored in tissues as bound sulfane sulfur, including polysulfides and persulfides, and released in response to physiological/pathological stimulations.[12,13]

4. H_2S AS A NEUROMODULATOR

With increasing knowledge about its role in the central nervous system in the last decades, H_2S has been recognized as a neuromodular (Figure 1). In 1996, Kimura's group first reported that H_2S facilitated hippocampal long-term potentiation by enhancing NMDA receptor-mediated responses.[1] Later, they reported that this effect was possibly mediated by the cAMP/PKA pathway.[14] According to these results, the NMDA receptor was activated when its subunits were directly phosphorylated by protein kinase A (PKA) at specific sites, which mediated excitatory postsynaptic currents. H_2S activated adenylyl cyclase and increased cAMP production, which may boost the activity of PKA and consequently NMDA phosphrylation. Another hypothesis was also described by Kimura. Since the activity of NMDA receptors was enhanced when its cysteine disulfide bonds were reduced,[15] H_2S may contribute to NMDA activation via reducing the cysteine disulfide and sulfhydrating the cysteine residues.[16] However, further investigation is needed to confirm this hypothesis.

H_2S also regulated intracellular Ca^{2+} level in various cell types in the brain. Kimura's group showed that H_2S increased intracellular Ca^{2+} and induced Ca^{2+} waves in primary cultured astrocytes and hippocampal slices.[3] H_2S increased Ca^{2+} concentration by inducing Ca^{2+} influx from extracellular space and, to a lesser extent, releasing Ca^{2+} from its intracellular stores. Similarly, our group also found that H_2S induced elevation of intracellular Ca^{2+} concentration in microglial cells[2] and neuronal SH-SY5Y cells.[4] It seems that both cAMP/PKA and PLC/PKC pathways contributed to the calcium regulatory effect of H_2S in neuronal cells.[2,4] As intracellular calcium acts as a universal second messenger in cells, H_2S's effects on Ca^{2+} may exert deep and extensive influence on a variety of intracellular processes. However, the biological significance and detailed mechanisms are still open to further investigation.

H_2S was reported to modulate adenylyl cyclase activity and influence cAMP level in cells. Kimura showed that H_2S increased the production of cAMP in the primary cultured brain cells, neuronal and glial cell lines, and *Xenopus* oocytes.[14] This effect was proposed as the mechanism for the effect of H_2S on NMDA receptors and the induction of hippocampal LTP. Interestingly, our group found that H_2S treatment significantly reversed forskolin-induced cAMP elevation in cardiac myocytes,[17] vascular smooth muscle cells,[18] As4.1 cells,[19] and SH-SY5Y cells,[20] and opioid withdrawal-induced cAMP rebound in SH-SY5Y cells.[21] These data suggest that H_2S may produce dual effects on cAMP production in brain cells. On the one hand, H_2S may stimulate AC at its resting status by modification of AC structure directly or secondary to other signaling pathways. On the other hand, H_2S may also block the association between forskolin and AC and therefore inhibit the effect of forskolin. This is probably mediated by sulfhydration of cysteine residues in the forskolin binding site in AC. More experiments are warranted to confirm this hypothesis and to study the underlying molecular mechanisms.

H_2S also regulates intracellular pH in primary cultures of rat microglia and astrocytes. NaHS in the range from 10 to 200 μM decreased intracellular pH in a concentration-dependent manner. NaHS significantly increased the activity of Cl^-/

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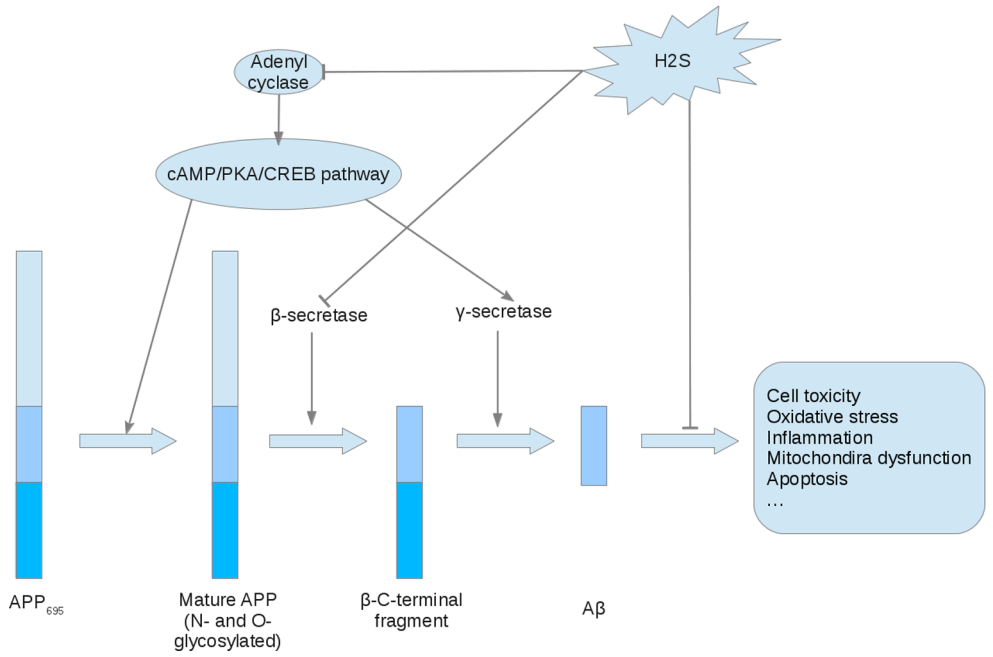

Figure 2. Schematic illustration showing the effect of H_2S on Aβ formation and toxicity. H_2S suppresses Aβ production and prevents its cytotoxicity. H_2S decreases Aβ production by reducing the expression of β-secretase 1 (BACE1),[40] and APP maturation and γ-secretase activity. This is mediated by inhibition of adenyl cyclase/cAMP/PKA/CREB pathway.[20]

HCO_3^- exchanger but inhibited that of Na^+/H^+ exchanger. As intracellular pH homeostasis is an important basis for the normal physiological functions of cells and a critical factor in pathological conditions such as hypoxia and ischemia, it is reasonable to speculate that the regulatory effects of H_2S on intracellular pH may have significant roles in both health and diseases.

5. H_2S AS A NEUROPROTECTANT

5.1. Therapeutic Effects of H_2S in CNS Diseases.

5.1.1. AD. AD is the most prevalent neurodegenerative disease and the leading cause of senile dementia.[22] The prevalence of AD is as high as almost 50% among those with age above 85 years.[23] It is not only a threat to human health, but also a source of many economic, political, and social problems. It is a progressive age-dependent neurodegenerative disease which ultimately leads to cognitive dysfunction. Neurofibrillary tangles and β-amyloid (Aβ) plaques in the cortex and hippocampus are the hallmarks of AD.[24] Regardless of intensive investigations about this disease, its cause and progression are still not well understood.

H_2S has been speculated to be involved in AD for a long time. In as early as 1996, Morrison et al. showed that the endogenous H_2S synthesis was permutated in AD patients, as indicated by the fact that the brain levels of *S*-adenosylmethionine, a CBS activator, were severely decreased in AD patients.[25] What is more, the total serum Hcy (a precursor of Cys when acted on by CBS followed by CSE) was found to be accumulated in AD patients, which was proposed to be a risk factor of AD.[26] It is reasonable to speculate that Hcy accumulation is caused by decreased CBS activity. On the other hand, it was also reported that neurotoxicity of elevated Hcy contributed to the down-regulation of both expression and activity of CBS in PC12 cells.[27] Regardless of the interplay between high plasma Hcy and CBS dysfunction in AD, it is clear that the CBS mediated Hcy transsulfuration pathway is involved in AD. Not surprisingly, H_2S generation is hampered due to the disturbance of the CBS mediated Hcy transsulfuration pathway in AD patients.[28] Actually, Liu et al. reported that levels of H_2S were decreased in AD patients and the change in H_2S level may be related to the severity of AD.[29] Although it remains a question of how much of a role H_2S plays in the pathogenesis of AD, intensive investigations have been inspired by these findings.

Both in vitro and in vivo experiments have shown that H_2S plays a protective role in AD. Oxidative stress is considered as an important player in AD pathogenesis and progression. A series of investigations showed that H_2S had a strong antioxidant capacity to resist AD-related oxidative stress factors such as HOCl, Aβ, MDA, and 4-HNE.[30−33] Whiteman et al. reported that NaHS significantly inhibited HOCl-induced cytotoxicity, intracellular protein oxidation, and lipid peroxidation in SH-SY5Y cells, which were increased in the temporal and frontal cortex of AD brains.[30] It was also reported that NaHS ameliorated Aβ-induced damage in PC12 cells, possibly by attenuating mitochondrial membrane potential ($\Delta\Psi_m$) loss and inhibiting the intracellular reactive oxygen species (ROS) increase.[31] It has been demonstrated that H_2S reduced MDA levels in human umbilical vein endothelial cells exposed to hydrogen peroxide and destroyed lipid hydroperoxides in oxidized low-density lipoprotein.[32,33] Schreier et al. demonstrated that H_2S protected neuronal cells (SH-SY5Y) from the cytotoxic lipid oxidation product 4-hydroxynonenal (HNE),[32] which was markedly elevated in the brains of severe AD patients.

A number of in vivo studies also confirmed the protective role of H_2S in AD. Xuan et al. reported that NaHS pretreatment ameliorated learning and memory deficits in a rat AD model induced by $A\beta_{1-40}$.[34] Giuliani et al. found that both short-term and long-term administration of NaHS significantly improved learning and memory in several AD models, including rat models induced by $A\beta_{1-40}$ or

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streptozotocin, and a mouse model harboring human transgenes APPSwe, PS1M146 V, and tauP301L (3xTg-AD mice).[35] It is also shown that both H_2S[36] and *S*-propargyl-cysteine,[37] a novel H_2S-modulated agent, attenuated lipopolysaccharide-induced cognitive deficits in rats by modulating neuroinflammation.

Taken together, the findings from both in vivo and in vitro studies show that H_2S acts as a potential therapeutic compound for AD in several models, possibly by interfering with multiple targets. Among these, amyloid β (Aβ) is an important target for H_2S. Aβ peptide is the major component of senile plaques in the brains of AD patients.[38] It is generated through sequential cleavage of APP by β- and γ-secretases.[39] A series of studies showed that H_2S influenced Aβ formation[20,40] and toxicity[31] in multiple ways. Zhang et al. reported that H_2S decreased Aβ42 production in rat PC12 cells, via down-regulating the expression of β-site amyloid precursor protein cleaving enzyme 1 (BACE-1) expression,[40] which is the rate limiting enzyme of APP proteolytic cleavage. We recently found that H_2S suppressed HENECA (a selective A2A receptor agonist)-induced Aβ42 formation in SH-SY5Y cells via inhibiting APP glycosylation and γ-secretase.[20] These data suggest that H_2S may suppress Aβ formation by inhibition of different catalyzing steps (Figure 2). In addition to inhibition of Aβ formation, H_2S may also protect cells against Aβ-induced cell injury.[31] This effect is mediated by the inhibitory effect of H_2S on Aβ-induced cell cycle re-entry, inflammation, and mitochondrial member potential ($\Delta\Psi$m) loss.

5.1.2. PD. The endogenous H_2S level was found to be markedly decreased in the substantia nigra (SN) in a 6-hydroxydopamine (6-OHDA)-induced PD rat model.[41] How the decreased endogenous H_2S production contributes to PD pathogenesis still remains elusive. Evidence for the therapeutic effects of H_2S was collected from exogenous application of H_2S releasing compounds. Our group first reported that NaHS dramatically reversed PD progression and alleviated Parkinsonian symptoms and dopaminergic neuron degeneration in the SN using rat PD models induced by 6-OHDA or rotenone.[41] Consistently, Ichinose and his colleagues showed that H_2S prevented the movement disorder, the degeneration and apoptosis of tyrosine hydroxylase (TH)-containing neurons, and gliosis in nigrostriatal region in an MPTP-induced mouse model.[42] Furthermore, we also confirmed that H_2S protected dopaminergic neurons against MPTP-induced degeneration in mice.[43] What is more, the therapeutic effect of H_2S was also confirmed with ACS84, an H_2S-releasing L-Dopa derivative, in the 6-OHDA-induced PD rat model.[44]

The belief in the therapeutic effect of H_2S on PD is further reinforced by a series of in vitro study using several toxins widely used to create PD models. Tang et al. showed that NaHS protected PC12 cells against MPP(+)-induced cytotoxicity and apoptosis by attenuating the loss of mitochondrial membrane potential and intracellular ROS increase.[45] Our group reported that H_2S inhibited rotenone-induced apoptosis via preserving mitochondrial function in SH-SY5Y cells,[46] and protected SH-SY5Y cells against 6-OHDA-induced cell injury[47] as well as endoplasmic reticulum stress.[48]

Multiple mechanisms about H_2S's therapeutic role in PD have been speculated. Parkin protein is known to protect neurons and facilitate protein degradation. Loss of its function leads to dopaminergic cell death. And mutations in this gene may cause a familial form of PD. Snyder's group reported that sulfhydration of parkin was associated with its activity and function in PD. Precisely, parkin sulfhydration was markedly depleted in the brains of PD patients, while H_2S could restore its sulfhydration and enhance its catalytic activity, thus excerting neuroprotective effects in PD.[49] Apart from the direct sulfhydration of parkin, other mechanisms are reported. We found that H_2S specifically inhibited 6-OHDA-induced NADPH oxidase activation in rats.[41] On the other hand, Ichinose F showed that H_2S upregulated expression of genes encoding antioxidant proteins, including heme oxygenase-1 and glutamate-cysteine ligase in mice after MPTP administration.[42] Similarly, our recent work revealed that H_2S enhanced UCP2-mediated antioxidation and subsequently suppressed ROS-triggered endoplasmic reticulum stress in a mouse model using MPTP.[43] It can be inferred from these studies that the neuroprotective effect of H_2S in PD is probably also mediated by its antioxidant effect. Additionally, it was also found that microglial activation in the SN and proinflammatory factors (e.g., TNF-alpha and nitric oxide) in the striatum were inhibited by H_2S in a rat model using rotenone,[41] suggesting the involvement of the anti-inflammatory functions of H_2S.

5.1.3. Ischemic Stroke. Conflicting results have been reported about the role of H_2S in the ischemic stroke. Chen and his colleagues showed in a clinical study that early high plasma cyst(e)ine level (within 24 h of stroke onset) may predict poor clinical outcome in patients with acute stroke.[6] Precisely, early plasma cyst(e)ine levels were significantly correlated with long-term clinical outcome (assessed at 3 months) as well as early stroke deterioration. They confirmed this relation in a rat stroke model, showing that administration of cysteine increased the infarct volume in a dose-dependent manner. This effect of cysteine was abolished by a CBS inhibitor, aminooxyacetic acid, indicating that H_2S may be involved in ischemic brain damage. In another study, Wong's group showed that H_2S production by 3-mercaptopyruvate sulfurtransferase (3MST) was downregulated after stroke in astrocytes, suggesting that the elevated H_2S level was mainly mediated by CBS.[50] The detrimental effect of H_2S was further confirmed by Wong's group using a permanent occlusion of the middle cerebral artery model (pMCAO),[51] which is a classical in vivo model for ischemic stroke. According to their discovery, MCAO increased H_2S level and H_2S synthesizing activity in the cortex. And MCAO-induced infarct volume was reduced dose dependently by four different inhibitors of H_2S synthesis enzymes, but significantly increased by administration of NaHS.

However, these findings have been challenged by more recent discoveries. Our group found that H_2S could protect neurons against hypoxic injury.[52] It was also shown that H_2S-induced long-term (2 days) hypothermia reduced infarct volume in aged rats after focal ischemia caused by reversible occlusion of the right middle cerebral artery.[53] Similarly, Gheibi et al. reported that treatment with H_2S reduced brain injuries and postischemic cerebral edema in a dose-dependent manner in a transient model of focal cerebral ischemia likely via blocking programmed cell death.[54] Additionally, Wang et al. reported that H_2S protected blood-brain barrier integrity after focal cerebral ischemia followed by reperfusion.[55] The beneficial effects of H_2S in global cerebral ischemia were also reported. For example, Yin and his colleagues showed that exogenous H_2S protected against global cerebral ischemia/reperfusion injury in rats via multiple mechanisms, including its antioxidative, anti-inflammatory, and antiapoptotic effects.[56] H_2S was also found to attenuate cognitive deficits by improving the survival rate of hippocampal neurons in a four-vessel

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occlusion model in rats.[57] In addition to these discoveries focusing on the neuroprotective effects of H_2S during or shortly after cerebral ischemia/reperfusion, it was also reported that treatment with H_2S augmented angiogenesis in the peri-infarct area, and significantly improved functional outcomes after 2 weeks in a rat MCAO model,[58] suggesting its potential value in regenerative recovery after stroke.

Different hypotheses have been proposed to resolve the seemingly conflict results. In a review paper,[7] we pointed out a few unresolved issues about the early work done by Wong and Chen, which supported the detrimental effects of H_2S in stroke, including that (i) sublethal dose of NaHS was employed; (ii) nonspecific CBS inhibitors were used; and (iii) the causality between stroke and endogenous H_2S level elevation remains to be assessed in the future. It seems that the first issue has been answered by Li and his colleagues, who reported dual effects of H_2S on focal cerebral ischemic injury, where low-dose (2.8 mg/kg) NaHS ameliorated MCAO-induced injury while high-dose (11.2 mg/kg) aggravated it.[59] These concentrations seem to be in line with the different concentrations used in experiments giving rise to conflicting results, though it is still uncertain whether the conflicts can be fully answered by this discovery. Since stroke is a complex disease where several lines of pathological processes occur and interact with each other in a complex way, it is possible that H_2S may play different roles in this dynamic process, and more investigations are required to gain a better understanding of it.

5.1.4. Trauma Injury. Traumatic brain injury (TBI) occurs when the brain is injured by an external force traumatically. It is a serious public health problem worldwide, especially in children, young adults, and elderly people.[60] TBI can cause a series of physical, cognitive, social, emotional, and behavioral effects, and outcome can range from complete recovery to permanent disability or death. In addition to the damage caused at the moment of injury, brain trauma causes secondary injury, which consists of a series of events including blood-brain barrier damage, proinflammatory factors release, free radical overload, excitotoxicity, influx of cations into neurons, mitochondria dysfunction, changes in the blood flow to the brain, raised intracranial pressure, and so on.[61] Secondary injury takes place in minutes and days following the initial injury, and contributes substantially to the damage from the primary injury, thus becoming an important target for therapeutic intervention.

H_2S is proposed to be involved in and play beneficial effects in TBI. Both CBS expression and H_2S levels experienced dynamic changes in plasma, brain cortex, and hippocampus after experimental TBI in mice.[60] This was reflected by a slow decrease, reaching the lowest level at 1 day after TBI, followed by a gradual elevation. Treatment with NaHS reduced surgical trauma-induced inflammatory response and cognitive deficits in mice.[62,63] H_2S may ameliorate TBI-induced increase of blood-brain barrier permeability, brain edema, and lesion volume, as well as neurologic dysfunction. This effect was mediated by activation of ATP sensitive mitochondrial K^+ channels, reduction of oxidative stress,[64] mitigating apoptosis, and autophagy.[63] Thus, these discoveries suggest the potential therapeutic value of H_2S in trauma injury.

5.2. Mechanisms of H_2S-Induced Neuroprotection. Although the roles of H_2S in different CNS disorders may vary, some common mechanisms of action are shared. A wide range of H_2S's beneficial effects are mediated by its anti-inflammatary, antioxidant, anti-ER stress, and antiapoptosis effects. With increasingly deeper insights into these effects, it seems that many of them can be explained by the sulfhydration of target proteins by H_2S.

5.2.1. Antioxidation. H_2S has been found to protect cells from oxidative injury induced by different agents such as glutamate,[65,66] hydrogen peroxide,[67,68] and hypochlorous acid.[30] H_2S is a reductive substance and can act as an antioxidative agent in vivo. However, its physiological concentration is low compared to more abundant antioxidants such as glutathione (1−10 mM). Additionally, H_2S is also a poor reductant with a redox potential of +0.17 V compared to −0.25 V of GSH.[69] Thus, H_2S is not likely to play the antioxidant effect by itself. Kimura and colleagues showed that H_2S increased GSH levels in neurons.[65,66] For this reason, H_2S probably upregulates GSH production by increasing cysteine availability. Cystine (cysteine) is the rate-limiting substrate of glutathione synthesis. H_2S elevated glutathione level by enhancing the activity of γ-glutamylcysteine synthetase (the first enzyme of the cellular GSH biosynthesis) and upregulating cystine and cysteine transport. What is more, high extracellular glutamate concentration inhibits cystine transport and consequently exacerbates GSH depletion, while our group found that H_2S promoted glutamate uptake by astrocytes via enhancing glial glutamate transporter GLT-1 function,[67] thus contributing to elevation of the antioxidant GSH level in cells.

In addition to enriching physiological antioxidants, H_2S also protects against oxidative stress via reducing production of reactive oxygen species (ROS) (Figure 3). NADPH oxidase

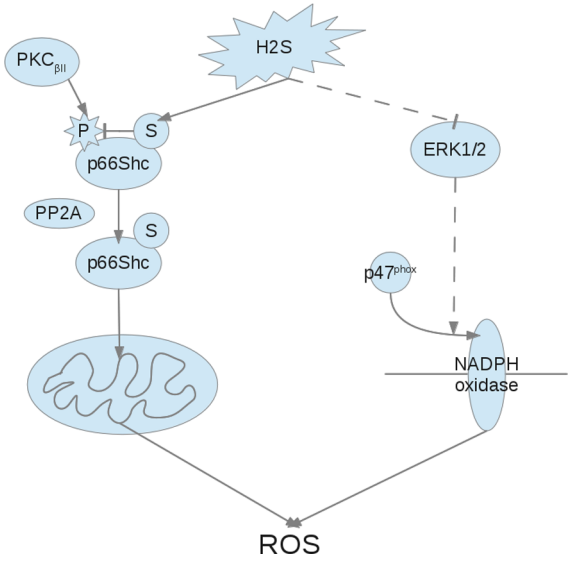

Figure 3. Schematic diagram showing the inhibitory effect of H_2S on ROS production. Via sulfhydration of p66Shc, H_2S inhibits phosphorylation of p66Shc by $PKC_{\beta II}$ and thus suppresses its translocation to mitochondria and consequent ROS production.[68] On the other hand, H_2S may also inhibit NADPH oxidase activation by preventing its cytosolic subunit $p47^{phox}$ from translocating to the plasma membrane, which perhaps involved the ERK1/2 pathway.[41]

and mitochondriol respiration chain are the two major sources of intracellular ROS. H_2S decreased 6-OHDA-induced ROS production in rats, possibly by suppressing NADPH oxidase activation in an ERK1/2-dependent manner.[41] On the other hand, we recently reported that H_2S exerted antioxidant effect by decreasing mitochondrial ROS production.[68] The protein p66Shc acts as an activator of mitochondrial redox signaling. We found that H_2S decreased mitochondrial ROS production

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under stress by sulfhydration of p66Shc. Precisely, application of exogenous H_2S with NaHS or overexpression of CBS induced sulfhydration and inhibited phosphorylation of p66Shc caused by H_2O_2/D-galactose in SH-SY5Y cells or in the mice cortex. Site-directed mutation showed that cystein-59 of p66Shc was involved in its phosphorylation, possibly by influencing its interaction with PP2A. Actually, C59S mutant attenuated the effects of H_2S on p66Shc phosphorylation and mitochondrial ROS production, suggesting that theses effects were dependent on p66Shc sulfhydration. Thus, H_2S may play its antioxidant role in multiple ways, by regulating endogenous antioxidants as well as by decreasing ROS production.

5.2.2. Anti-Inflammation. H_2S has also been found to play both pro- and anti-inflammatory effects. The proinflammatory effects were reported in some pathological situations such as pancreatitis and septic/endotoxic and hemorrhagic shock.[70−72] The anti-inflammatory effect of H_2S was first reported by our group.[73] We found that both endogenous and exogenous H_2S could attenuate lipopolysacchrides-induced NO release and TNF-alpha production in primary cultured microglia as well as immortalized BV-2 microglial cells. This effect was mimicked by SB 203580, a specific p38 mitogen-activated protein kinase (MAPK) inhibitor, suggesting that H_2S may regulate neuroinflammation via the MAPK pathway.[73] Lee et al. also reported similar anti-inflammatory effect of both H_2S and three H_2S-releasing NSAIDs (anethole trithione hydroxide, *S*-diclofenac, and *S*-aspirin).[74] In addition to TNF-α and NO, H_2S also decreased the release of the proinflammatory cytokine interleukin-1β, and upregulated the anti-inflammatory cytokines such as iterleukin-4/10. The anti-inflammatory effect of H_2S was suggested to be mediated by modulating NF-κB pathways in activated B cells. However, more comprehensive investigation is needed.[7]

5.2.3. Anti-ER Stress. The endoplasmic reticulum (ER) plays an important role in the regulation of the post-translational folding and modification of proteins. The folding capacity of ER is fine-tuned in response to both the external and internal environmental status of cells. However, when it is exceeded, excessive unfolded proteins can result in a condition termed as ER stress, and triggers a series of cellular and molecular processes known as ER stress response.[75] If cells fail to respond to ER stress properly, which can be caused by aging, environmental factors, or genetic mutations, pathological conditions may occur and lead to diseases such as diabetes, AD, and PD.[76]

H_2S may modulate ER stress response via sulfhydration of the phosphatase PTP1B.[75] Specifically, the phosphatase PTP1B could be reversibly sulfhydrated by endogenous H_2S in response to ER stress. Sulfhydration of PTP1B resulted in inhibition of its phosphatase activity, and protected pTyr 619 of its substrate PERK [protein kinase-like endoplasmic reticulum (ER) kinase] from dephosphorylation by PTP1B, leaving PERK in the phosphorylated active status. As PERK could phosphorylate the eukaryotic translational initiation factor 2 (eIF2α) and lead to attenuation of protein translation, its activation contributes to amelioration of ER stress. This study provides a good explanation about the effect of H_2S on ER stress at the molecular level. On the other hand, we also showed that H_2S was able to protect SH-SY5Y cells against 6-OHDA-induced ER stress, suggesting a potential mechanism by which it modulates PD.[48] However, more investigations are needed to fully establish the relationship between H_2S-induced anti-ER stress effect and CNS diseases.

5.2.4. Antiapoptosis. Accumulating evidence shows that H_2S plays antiapoptotic effect on neuronal cells at relatively low concentrations. Several groups have independently reported that H_2S protected PC12 and SH-SY5Y cells from apoptosis induced by a variety of toxins, such as 1-methyl-4-phenylpyridine, rotenone, and β amyloid.[45,46,77] Similar antiapoptotic effects are also found in animal models of PD, AD, and vascular dementia.[34,41,44,78] Most of these studies suggest that the antiapoptotic effect of H_2S is probably mediated by inhibiting the mitochondrial apoptotic pathway, that is, by preserving mitochondrial integrity, preventing cytochrome *c* release, and the consequent activation of caspase cascades.

6. OUTLOOK AND CONCLUSIONS

The last two decades have seen a rapid increase in the knowledge on the biological functions of H_2S. The neuromodulatory effects of H_2S have almost reached a consensus. The potential therapeutic value of H_2S in several CNS diseases have been widely accepted. These discoveries have inspired interests in the unresolved problems about H_2S. Currently, considerable efforts are still devoted to uncovering the molecular mechanisms of H_2S. Increasing numbers of sulfhydration targets are identified, shedding new light upon known effects of H_2S by providing deeper insights into its biological functions.

To exploit the therapeutic effects of H_2S, several groups are working on H_2S slow releasing compounds, with the hope of finding better "druglike" compounds. These attempts include GYY4137,[8] ACS14,[79] and so on. Their therapeutic value have been demonstrated in vitro or in animal models.[21,80] However, more investigation is still needed to study the potential toxic effects after long-term treatment with these H_2S-releasing compounds.

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Notes

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REFERENCES

(1) Abe, K., and Kimura, H. (1996) The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci. 16*, 1066−1071.

(2) Lee, S. W., Hu, Y. S., Hu, L. F., Lu, Q., Dawe, G. S., Moore, P. K., Wong, P. T., and Bian, J. S. (2006) Hydrogen sulphide regulates calcium homeostasis in microglial cells. *Glia 54*, 116−124.

(3) Nagai, Y., Tsugane, M., Oka, J., and Kimura, H. (2004) Hydrogen sulfide induces calcium waves in astrocytes. *FASEB J. 18*, 557−559.

(4) Yong, Q. C., Choo, C. H., Tan, B. H., Low, C. M., and Bian, J. S. (2010) Effect of hydrogen sulfide on intracellular calcium homeostasis in neuronal cells. *Neurochem. Int. 56*, 508−515.

(5) Lu, M., Choo, C. H., Hu, L. F., Tan, B. H., Hu, G., and Bian, J. S. (2010) Hydrogen sulfide regulates intracellular pH in rat primary cultured glia cells. *Neurosci. Res. 66*, 92−98.

(6) Wong, P. T., Qu, K., Chimon, G. N., Seah, A. B., Chang, H. M., Wong, M. C., Ng, Y. K., Rumpel, H., Halliwell, B., and Chen, C. P. (2006) High plasma cyst(e)ine level may indicate poor clinical outcome in patients with acute stroke: Possible involvement of hydrogen sulfide. *J. Neuropathol. Exp. Neurol. 65*, 109−115.

(7) Hu, L. F., Lu, M., Hong Wong, P. T., and Bian, J. S. (2011) Hydrogen sulfide: neurophysiology and neuropathology. *Antioxid. Redox Signal. 15*, 405−419.
(8) Robinson, H., and Wray, S. (2012) A new slow releasing, H(2)S generating compound, GYY4137 relaxes spontaneous and oxytocin-stimulated contractions of human and rat pregnant myometrium. *PLoS One 7*, e46278.
(9) Sparatore, A., Santus, G., Giustarini, D., Rossi, R., and Del Soldato, P. (2011) Therapeutic potential of new hydrogen sulfide-releasing hybrids. *Expert Rev. Clin. Pharmacol. 4*, 109−121.
(10) Szczesny, B., Modis, K., Yanagi, K., Coletta, C., Le Trionnaire, S., Perry, A., Wood, M. E., Whiteman, M., and Szabo, C. (2014) AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells in vitro. *Nitric Oxide 41*, 120−130.
(11) Kabil, O., and Banerjee, R. (2014) Enzymology of H2S biogenesis, decay and signaling. *Antioxid. Redox Signaling 20*, 770−782.
(12) Ishigami, M., Hiraki, K., Umemura, K., Ogasawara, Y., Ishii, K., and Kimura, H. (2009) A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid. Redox Signaling 11*, 205−214.
(13) Kimura, Y., Mikami, Y., Osumi, K., Tsugane, M., Oka, J., and Kimura, H. (2013) Polysulfides are possible H2S-derived signaling molecules in rat brain. *FASEB J. 27*, 2451−2457.
(14) Kimura, H. (2000) Hydrogen sulfide induces cyclic AMP and modulates the NMDA receptor. *Biochem. Biophys. Res. Commun. 267*, 129−133.
(15) Aizenman, E., Lipton, S. A., and Loring, R. H. (1989) Selective modulation of NMDA responses by reduction and oxidation. *Neuron 2*, 1257−1263.
(16) Kimura, H. (2013) Physiological role of hydrogen sulfide and polysulfide in the central nervous system. *Neurochem. Int. 63*, 492−497.
(17) Yong, Q. C., Pan, T. T., Hu, L. F., and Bian, J. S. (2008) Negative regulation of beta-adrenergic function by hydrogen sulphide in the rat hearts. *J. Mol. Cell. Cardiol. 44*, 701−710.
(18) Lim, J. J., Liu, Y. H., Khin, E. S., and Bian, J. S. (2008) Vasoconstrictive effect of hydrogen sulfide involves downregulation of cAMP in vascular smooth muscle cells. *Am. J. Physiol.: Cell Physiol. 295*, C1261−1270.
(19) Lu, M., Liu, Y. H., Ho, C. Y., Tiong, C. X., and Bian, J. S. (2012) Hydrogen sulfide regulates cAMP homeostasis and renin degranulation in As4.1 and rat renin-rich kidney cells. *Am. J. Physiol.: Cell Physiol. 302*, C59−66.
(20) Nagpure, B. V., and Bian, J. S. (2014) Hydrogen sulfide inhibits A2A adenosine receptor agonist induced beta-amyloid production in SH-SY5Y neuroblastoma cells via a cAMP dependent pathway. *PLoS One 9*, e88508.
(21) Yang, H. Y., Wu, Z. Y., Wood, M., Whiteman, M., and Bian, J. S. (2014) Hydrogen sulfide attenuates opioid dependence by suppression of adenylate cyclase/cAMP pathway. *Antioxid. Redox Signaling 20*, 31−41.
(22) Reitz, C., Brayne, C., and Mayeux, R. (2011) Epidemiology of Alzheimer disease. *Nat. Rev. Neurol. 7*, 137−152.
(23) Castellani, R. J., Rolston, R. K., and Smith, M. A. (2010) Alzheimer disease. *DM, Dis.-Mon. 56*, 484−546.
(24) Tanzi, R. E., and Bertram, L. (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: A genetic perspective. *Cell 120*, 545−555.
(25) Morrison, L. D., Smith, D. D., and Kish, S. J. (1996) Brain S-adenosylmethionine levels are severely decreased in Alzheimer's disease. *J. Neurochem. 67*, 1328−1331.
(26) Clarke, R., Smith, A. D., Jobst, K. A., Refsum, H., Sutton, L., and Ueland, P. M. (1998) Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch. Neurol. 55*, 1449−1455.
(27) Tang, X. Q., Shen, X. T., Huang, Y. E., Chen, R. Q., Ren, Y. K., Fang, H. R., Zhuang, Y. Y., and Wang, C. Y. (2011) Inhibition of endogenous hydrogen sulfide generation is associated with homocysteine-induced neurotoxicity: Role of ERK1/2 activation. *J. Mol. Neurosci. 45*, 60−67.
(28) Dwyer, B. E., Raina, A. K., Perry, G., and Smith, M. A. (2004) Homocysteine and Alzheimer's disease: A modifiable risk? *Free Radical Biol. Med. 36*, 1471−1475.
(29) Liu, X. Q., Liu, X. Q., Jiang, P., Huang, H., and Yan, Y. (2008) [Plasma levels of endogenous hydrogen sulfide and homocysteine in patients with Alzheimer's disease and vascular dementia and the significance thereof]. *Zhonghua Yixue Zazhi 88*, 2246−2249.
(30) Whiteman, M., Cheung, N. S., Zhu, Y. Z., Chu, S. H., Siau, J. L., Wong, B. S., Armstrong, J. S., and Moore, P. K. (2005) Hydrogen sulphide: a novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain? *Biochem. Biophys. Res. Commun. 326*, 794−798.
(31) Liu, Y. Y., and Bian, J. S. (2010) Hydrogen sulfide protects amyloid-beta induced cell toxicity in microglia. *J. Alzheimer's Dis. 22*, 1189−1200.
(32) Schreier, S. M., Muellner, M. K., Steinkellner, H., Hermann, M., Esterbauer, H., Exner, M., Gmeiner, B. M., Kapiotis, S., and Laggner, H. (2010) Hydrogen sulfide scavenges the cytotoxic lipid oxidation product 4-HNE. *Neurotoxic. Res. 17*, 249−256.
(33) Wen, Y. D., Wang, H., Kho, S. H., Rinkiko, S., Sheng, X., Shen, H. M., and Zhu, Y. Z. (2013) Hydrogen sulfide protects HUVECs against hydrogen peroxide induced mitochondrial dysfunction and oxidative stress. *PLoS One 8*, e53147.
(34) Xuan, A., Long, D., Li, J., Ji, W., Zhang, M., Hong, L., and Liu, J. (2012) Hydrogen sulfide attenuates spatial memory impairment and hippocampal neuroinflammation in beta-amyloid rat model of Alzheimer's disease. *J. Neuroinflammation 9*, 202.
(35) Giuliani, D., Ottani, A., Zaffe, D., Galantucci, M., Strinati, F., Lodi, R., and Guarini, S. (2013) Hydrogen sulfide slows down progression of experimental Alzheimer's disease by targeting multiple pathophysiological mechanisms. *Neurobiol. Learn. Mem. 104*, 82−91.
(36) Gong, Q. H., Wang, Q., Pan, L. L., Liu, X. H., Huang, H., and Zhu, Y. Z. (2010) Hydrogen sulfide attenuates lipopolysaccharide-induced cognitive impairment: a pro-inflammatory pathway in rats. *Pharmacol., Biochem. Behav. 96*, 52−58.
(37) Gong, Q. H., Wang, Q., Pan, L. L., Liu, X. H., Xin, H., and Zhu, Y. Z. (2011) S-Propargyl-cysteine, a novel hydrogen sulfide-modulated agent, attenuates lipopolysaccharide-induced spatial learning and memory impairment: Involvement of TNF signaling and NF-kappaB pathway in rats. *Brain. Behav. Immun. 25*, 110−119.
(38) Selkoe, D. J. (2002) Alzheimer's disease is a synaptic failure. *Science 298*, 789−791.
(39) Selkoe, D. J. (1991) Amyloid protein and Alzheimer's disease. *Sci. Am. 265*, 68−71 74−66, 78..
(40) Zhang, H., Gao, Y., Zhao, F., Dai, Z., Meng, T., Tu, S., and Yan, Y. (2011) Hydrogen sulfide reduces mRNA and protein levels of beta-site amyloid precursor protein cleaving enzyme 1 in PC12 cells. *Neurochem. Int. 58*, 169−175.
(41) Hu, L. F., Lu, M., Tiong, C. X., Dawe, G. S., Hu, G., and Bian, J. S. (2010) Neuroprotective effects of hydrogen sulfide on Parkinson's disease rat models. *Aging Cell 9*, 135−146.
(42) Kida, K., Yamada, M., Tokuda, K., Marutani, E., Kakinohana, M., Kaneki, M., and Ichinose, F. (2011) Inhaled hydrogen sulfide prevents neurodegeneration and movement disorder in a mouse model of Parkinson's disease. *Antioxid. Redox Signaling 15*, 343−352.
(43) Lu, M., Zhao, F. F., Tang, J. J., Su, C. J., Fan, Y., Ding, J. H., Bian, J. S., and Hu, G. (2012) The neuroprotection of hydrogen sulfide against MPTP-induced dopaminergic neuron degeneration involves uncoupling protein 2 rather than ATP-sensitive potassium channels. *Antioxid. Redox Signaling 17*, 849−859.
(44) Xie, L., Hu, L. F., Teo, X. Q., Tiong, C. X., Tazzari, V., Sparatore, A., Del Soldato, P., Dawe, G. S., and Bian, J. S. (2013) Therapeutic effect of hydrogen sulfide-releasing L-Dopa derivative ACS84 on 6-OHDA-induced Parkinson's disease rat model. *PLoS One 8*, e60200.
(45) Yin, W. L., He, J. Q., Hu, B., Jiang, Z. S., and Tang, X. Q. (2009) Hydrogen sulfide inhibits MPP(+)-induced apoptosis in PC12 cells. *Life Sci. 85*, 269−275.

(46) Hu, L. F., Lu, M., Wu, Z. Y., Wong, P. T., and Bian, J. S. (2009) Hydrogen sulfide inhibits rotenone-induced apoptosis via preservation of mitochondrial function. *Mol. Pharmacol. 75*, 27−34.
(47) Tiong, C. X., Lu, M., and Bian, J. S. (2010) Protective effect of hydrogen sulphide against 6-OHDA-induced cell injury in SH-SY5Y cells involves PKC/PI3K/Akt pathway. *Br. J. Pharmacol. 161*, 467−480.
(48) Xie, L., Tiong, C. X., and Bian, J. S. (2012) Hydrogen sulfide protects SH-SY5Y cells against 6-hydroxydopamine-induced endoplasmic reticulum stress. *Am. J. Physiol.: Cell Physiol. 303*, C81−91.
(49) Vandiver, M. S., Paul, B. D., Xu, R., Karuppagounder, S., Rao, F., Snowman, A. M., Ko, H. S., Lee, Y. I., Dawson, V. L., Dawson, T. M., Sen, N., and Snyder, S. H. (2013) Sulfhydration mediates neuroprotective actions of parkin. *Nat. Commun. 4*, 1626.
(50) Zhao, H., Chan, S. J., Ng, Y. K., and Wong, P. T. (2013) Brain 3-Mercaptopyruvate Sulfurtransferase (3MST): Cellular Localization and Downregulation after Acute Stroke. *PLoS One 8*, e67322.
(51) Qu, K., Chen, C. P., Halliwell, B., Moore, P. K., and Wong, P. T. (2006) Hydrogen sulfide is a mediator of cerebral ischemic damage. *Stroke 37*, 889−893.
(52) Tay, A. S., Hu, L. F., Lu, M., Wong, P. T., and Bian, J. S. (2010) Hydrogen sulfide protects neurons against hypoxic injury via stimulation of ATP-sensitive potassium channel/protein kinase C/extracellular signal-regulated kinase/heat shock protein 90 pathway. *Neuroscience 167*, 277−286.
(53) Florian, B., Vintilescu, R., Balseanu, A. T., Buga, A. M., Grisk, O., Walker, L. C., Kessler, C., and Popa-Wagner, A. (2008) Long-term hypothermia reduces infarct volume in aged rats after focal ischemia. *Neurosci. Lett. 438*, 180−185.
(54) Gheibi, S., Aboutaleb, N., Khaksari, M., Kalalian-Moghaddam, H., Vakili, A., Asadi, Y., Mehrjerdi, F. Z., and Gheibi, A. (2014) Hydrogen Sulfide Protects the Brain Against Ischemic Reperfusion Injury in a Transient Model of Focal Cerebral Ischemia. *J. Mol. Neurosci. 54*, 264−270.
(55) Wang, Y., Jia, J., Ao, G., Hu, L., Liu, H., Xiao, Y., Du, H., Alkayed, N. J., Liu, C. F., and Cheng, J. (2014) Hydrogen sulfide protects blood-brain barrier integrity following cerebral ischemia. *J. Neurochem. 129*, 827−838.
(56) Yin, J., Tu, C., Zhao, J., Ou, D., Chen, G., Liu, Y., and Xiao, X. (2013) Exogenous hydrogen sulfide protects against global cerebral ischemia/reperfusion injury via its anti-oxidative, anti-inflammatory and anti-apoptotic effects in rats. *Brain Res. 1491*, 188−196.
(57) Wen, X., Qi, D., Sun, Y., Huang, X., Zhang, F., Wu, J., Fu, Y., Ma, K., Du, Y., Dong, H., Liu, Y., Liu, H., and Song, Y. (2014) H(2)S attenuates cognitive deficits through Akt1/JNK3 signaling pathway in ischemic stroke. *Behav. Brain Res. 269*, 6−14.
(58) Jang, H., Oh, M. Y., Kim, Y. J., Choi, I. Y., Yang, H. S., Ryu, W. S., Lee, S. H., and Yoon, B. W. (2014) Hydrogen sulfide treatment induces angiogenesis after cerebral ischemia. *J. Neurosci. Res. 92*, 1520−1528.
(59) Li, G. F., Luo, H. K., Li, L. F., Zhang, Q. Z., Xie, L. J., Jiang, H., Li, L. P., Hao, N., Wang, W. W., and Zhang, J. X. (2012) Dual effects of hydrogen sulphide on focal cerebral ischaemic injury via modulation of oxidative stress-induced apoptosis. *Clin. Exp. Pharmacol. Physiol. 39*, 765−771.
(60) Zhang, M., Shan, H., Wang, T., Liu, W., Wang, Y., Wang, L., Zhang, L., Chang, P., Dong, W., Chen, X., and Tao, L. (2013) Dynamic change of hydrogen sulfide after traumatic brain injury and its effect in mice. *Neurochem. Res. 38*, 714−725.
(61) Park, E., Bell, J. D., and Baker, A. J. (2008) Traumatic brain injury: Can the consequences be stopped? *CMAJ. 178*, 1163−1170.
(62) Chu, Q. J., He, L., Zhang, W., Liu, C. L., Ai, Y. Q., and Zhang, Q. (2013) Hydrogen sulfide attenuates surgical trauma-induced inflammatory response and cognitive deficits in mice. *J. Surg. Res. 183*, 330−336.
(63) Zhang, M., Shan, H., Chang, P., Wang, T., Dong, W., Chen, X., and Tao, L. (2014) Hydrogen sulfide offers neuroprotection on traumatic brain injury in parallel with reduced apoptosis and autophagy in mice. *PLoS One 9*, e87241.
(64) Jiang, X., Huang, Y., Lin, W., Gao, D., and Fei, Z. (2013) Protective effects of hydrogen sulfide in a rat model of traumatic brain injury via activation of mitochondrial adenosine triphosphate-sensitive potassium channels and reduction of oxidative stress. *J. Surg. Res. 184*, e27−35.
(65) Kimura, Y., Goto, Y., and Kimura, H. (2010) Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxid. Redox Signaling 12*, 1−13.
(66) Kimura, Y., and Kimura, H. (2004) Hydrogen sulfide protects neurons from oxidative stress. *FASEB J. 18*, 1165−1167.
(67) Lu, M., Hu, L. F., Hu, G., and Bian, J. S. (2008) Hydrogen sulfide protects astrocytes against H(2)O(2)-induced neural injury via enhancing glutamate uptake. *Free Radical Biol. Med. 45*, 1705−1713.
(68) Xie, Z. Z., Shi, M. M., Xie, L., Wu, Z. Y., Li, G., Hua, F., and Bian, J. S. (2014) Sulfhydration of p66Shc at Cysteine59 Mediates the Antioxidant Effect of Hydrogen Sulfide. *Antioxid. Redox Signaling*, DOI: 10.1089/ars.2013.5604.
(69) Kabil, O., and Banerjee, R. (2010) Redox biochemistry of hydrogen sulfide. *J. Biol. Chem. 285*, 21903−21907.
(70) Mok, Y. Y., Atan, M. S., Yoke Ping, C., Zhong Jing, W., Bhatia, M., Moochhala, S., and Moore, P. K. (2004) Role of hydrogen sulphide in haemorrhagic shock in the rat: Protective effect of inhibitors of hydrogen sulphide biosynthesis. *Br. J. Pharmacol. 143*, 881−889.
(71) Bhatia, M., Wong, F. L., Fu, D., Lau, H. Y., Moochhala, S. M., and Moore, P. K. (2005) Role of hydrogen sulfide in acute pancreatitis and associated lung injury. *FASEB J. 19*, 623−625.
(72) Zhang, H., Zhi, L., Moore, P. K., and Bhatia, M. (2006) Role of hydrogen sulfide in cecal ligation and puncture-induced sepsis in the mouse. *Am. J. Physiol.: Lung Cell. Mol. Physiol. 290*, L1193−1201.
(73) Hu, L. F., Wong, P. T., Moore, P. K., and Bian, J. S. (2007) Hydrogen sulfide attenuates lipopolysaccharide-induced inflammation by inhibition of p38 mitogen-activated protein kinase in microglia. *J. Neurochem. 100*, 1121−1128.
(74) Lee, M., Sparatore, A., Del Soldato, P., McGeer, E., and McGeer, P. L. (2010) Hydrogen sulfide-releasing NSAIDs attenuate neuroinflammation induced by microglial and astrocytic activation. *Glia 58*, 103−113.
(75) Krishnan, N., Fu, C., Pappin, D. J., and Tonks, N. K. (2011) H2S-Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci. Signaling 4*, ra86.
(76) Yoshida, H. (2007) ER stress and diseases. *FEBS J. 274*, 630−658.
(77) Tang, X. Q., Yang, C. T., Chen, J., Yin, W. L., Tian, S. W., Hu, B., Feng, J. Q., and Li, Y. J. (2008) Effect of hydrogen sulphide on beta-amyloid-induced damage in PC12 cells. *Clin. Exp. Pharmacol. Physiol. 35*, 180−186.
(78) Zhang, L. M., Jiang, C. X., and Liu, D. W. (2009) Hydrogen sulfide attenuates neuronal injury induced by vascular dementia via inhibiting apoptosis in rats. *Neurochem. Res. 34*, 1984−1992.
(79) Sparatore, A., Perrino, E., Tazzari, V., Giustarini, D., Rossi, R., Rossoni, G., Erdmann, K., Schroder, H., and Del Soldato, P. (2009) Pharmacological profile of a novel H(2)S-releasing aspirin. *Free Radical Biol. Med. 46*, 586−592.
(80) Osborne, N. N., Ji, D., Majid, A. S., Del Soldata, P., and Sparatore, A. (2012) Glutamate oxidative injury to RGC-5 cells in culture is necrostatin sensitive and blunted by a hydrogen sulfide (H2S)-releasing derivative of aspirin (ACS14). *Neurochem. Int. 60*, 365−378.