HHS Public Access

Author manuscript

Methods Enzymol. Author manuscript; available in PMC 2015 March 11.

Published in final edited form as:

Methods Enzymol. 2015 ; 555: 207–229. doi:10.1016/bs.mie.2014.11.025.

Role of Hydrogen Sulfide in Brain Synaptic Remodeling

Pradip Kumar Kamat, **Anuradha Kalani**, and **Neetu Tyagi**[1]

Department of Physiology and Biophysics, School of Medicine, University of Louisville, Louisville, Kentucky, USA

Abstract

Synapses are the functional connection between neurons which are necessary for the transfer of electric activity or chemical activity from one cell to another. Synapses are formed by the pre- and postsynaptic membrane which communicates between pre- and postneurons while a neurochemical modulator is operated in this process. H_2S has been known as a toxic gas with rotten eggs smell. However, increasing number of researches show that it regulate a variety of physiological and pathological processes in mammals. Hence, H_2S is a physiologically important molecule and has been referred to as the third gaseous molecule alongside carbon monoxide and nitric oxide. The previous era has made an exponential development in the physiological and pathological significance of H_2S. Specifically, in the central nervous system, H_2S facilitates long-term potentiation and regulates intracellular calcium concentration in brain cells. We as well as others have also shown that H_2S has antioxidant, antiapoptotic, and anti-inflammatory properties against various neurodegenerative disorders such as stroke, Alzheimer's disease, and vascular dementia. In this chapter, we highlight the current knowledge of H_2S and its neuroprotective effects with a special emphasis on synaptic remodeling.

1. INTRODUCTION

Hydrogen sulfide (H_2S) was found to be produced endogenously in various parts of the body such as the heart (Geng et al., 2004), blood (Zhao, Chen, Shen, Kahn, & Lipke, 2001), and central nervous system (CNS) (Warenycia et al., 1989). H_2S is synthesized endogenously by a variety of mammalian tissues by two pyridoxal-5′ -phosphate-dependent enzymes responsible for metabolism of L-cysteine which is a by-product of L-methionine, homocysteine, and cystathione. Cystathionine beta-synthase (CBS), cystathionine gamma-lyase (CSE), and a newly identified enzyme, 3-mercaptopyruvate sulfurtransferase (3MST) (Sen et al., 2012) are involved in generation of H_2S. The substrate of CBS and CSE can be derived from alimentary sources or can be liberated from endogenous proteins (Rezessy-Szabo et al., 2007; Zhu, Song, Li, & Dao, 2008). In the CNS, CBS was found highly expressed in the hippocampus and cerebellum (Abe & Kimura, 1996). CBS is mainly confined to astrocytes (Enokido et al., 2005; Ichinohe et al., 2005) and microglial cells. CSE is mainly expressed in the cardiovascular system, but was also found in microglial cells (Oh

[1]Corresponding author: n0tyag01@louisville.edu.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

et al., 2006), spinal cord (Distrutti et al., 2006), and cerebellar granule neurons (Garcia-Bereguiain, Samhan-Arias, Martin-Romero, & Gutierrez-Merino, 2008). However, 3MST is also an important enzyme for the synthesis of H_2S in the brain which is localized within neurons and astrocytes (Shibuya et al., 2009; Zhao, Chan, Ng, & Wong, 2013). 3-Mercaptopyruvate is converted from cysteine by the action of cysteine aminotransferase (Tanizawa, 2011) (Fig. 1). By comparing the production of H_2S in different brain cells, Lee, Kim, Kim, and Ahn (2009) found that H_2S production in astrocytes was 7.9-fold higher than in cultured microglial cells, 9.7-fold higher than in neuron-committed teratocarcinoma NT2 cell line (NT-2 cells), and 11.5-fold higher than in neuroblastoma cell line (SH-SY5Y cells) (Lee et al., 2009). These data clearly indicate that astrocytes may be the main cells that produce H_2S in the brain. The estimated physiological concentration of H_2S was recently measured to be around 14–30 μ*M*, based on measurements of the brains of mice (Furne, Saeed, & Levitt, 2008) and consistent with values reported by another group (Ishigami et al., 2009). Above information regarding H_2S in the brain indicates the impact of H_2S sulfide on neuronal function. Neural circuits are composed of mainly glutamatergic and GABAergic neurons, which communicate through synaptic connections. GABAergic synapse maturation occurs in many brain regions. In addition, changes in GABAergic output are cell wide and not target-cell specific. Chang et al. (2014) found that glutamatergic neuronal activity also determined the AMPA receptor (a non-NMDA-type ionotropic transmembrane receptor) for glutamate that mediates fast synaptic transmission in the CNS. AMPA receptor also determines the properties of synapses on the partner with GABAergic neuron. The *N*-methyl-D-aspartate (NMDA) receptor is a major type of ionotropic glutamate receptor that plays a pivotal role in the CNS under both physiological and pathological conditions. The functional diversity of NMDA receptors can be mainly attributed to their different subunit compositions that perform multiple functions in various situations. Recent reports have indicated that synaptic NMDA receptors have a distinct role in neuronal cell survival. NMDA receptors modulate the LTP and synaptic plasticity which is responsible for learning and memory function and therefore maintains synapse function. Although H_2S enhances the induction of hippocampal LTP, the mechanism by which H_2S modulates synaptic activity is still in debate. H_2S enhances the responses of neurons to glutamate in hippocampal slices, and H_2S alone induces the increase in intracellular Ca^{2+} in astrocytes (Nagai, Tsugane, Oka, & Kimura, 2004). In the presence of H_2S, the induction of LTP is enhanced at the synapse and Ca^{2+} waves are induced in the surrounding astrocytes. Ca^{2+} waves propagate and reach another synapse and may modulate it. H_2S may therefore modulate synaptic activity by enhancing the responses to glutamate in neurons and inducing Ca^{2+} waves in astrocytes that propagate and modulate the neighboring synapse. Some of the reports showed that H_2S controls the neuronal signaling. As signaling passes through neuronal synapse, it is also modulated by H_2S. Thus, understanding the role of H_2S in the brain will help in gaining a mechanism for controlling synapse function. This review presents an overview of the current evidence that H_2S probably acts as a neuromodulator and/or as an intracellular messenger and plays an important role in synaptic remodeling.

2. PHARMACOLOGICAL AND PHYSIOLOGICAL EFFECT OF H_2S

Dietary garlic has long been known for its beneficial effects on the cardiovascular system. Their components are the main source of H_2S donor naturally. The paste of garlic cloves allows the enzyme alliinase to metabolize the amino acid alliin, producing allicin (diallyl thiosulfinate), which decomposes to polysulfides, including diallyl disulfide and diallyl trisulfide (Banerjee & Maulik, 2002). When these compounds react with reduced thiol groups, including reduced glutathione, in cells, they produce H_2S. In experimental set of condition, H_2S or H_2S donors (most commonly NaHS) provide protection in many physiological systems including the cardiovascular system and central nervous system (Wallace, 2007). Most notably, H_2S mainly attenuate vasoconstriction and reduce damage via dilating blood vessels (e.g., myocardial infarct size) in several animal models of cardiovascular disease. However, in the brain, H_2S acts as anti-inflammatory, antioxidant, and finally as a neuroprotective agent which can also be designated as neuromodulator. Most researchers prefer to use pharmacological inhibitors that inhibit H_2S synthesis such as $_{DL}$-propargylglycine (PAG); a CSE inhibitor and β-cyano-$_{L}$-alanine (BCA), $_{L}$-amino ethoxyvinylglycine, aminooxyacetic acid, trifluoroalanine, and hydroxylamine. Though BCA is a selective inhibitor for CSE, it also inhibits CBS at high concentrations. In contrast, none of the compounds tested exhibited significant selectivity toward CBS. In addition, the above-mentioned compounds did not inhibit 3MST. The effects of H_2S on cells studied using H_2S donors are variable in nature. Lower (micromolar) (lower micromolar concentrations) of H_2S are generally cytoprotective, with protection often ascribed to a reduction of free radicals generation such as neutralization of reactive oxygen and nitrogen species. These effects have been reported in neurons, myoblasts, neutrophils, and macrophages. At higher concentration such as millimolar levels, H_2S is often proapoptotic or cytotoxic, via free radical and oxidant generation, glutathione depletion, and thus promotes apoptosis. In addition to that, when sulfide donors or precursors administered systemically can convert into H_2S gas, this gas can be absorbed via the lung into the circulation and after that all over the body. Rezessy-Szabo et al. (2007) have reported the capacity and potential of intravenously (i.v.) administered H_2S donors to induce exhalation of H_2S. Previously, we and others have used H_2S donors such as IK-1001 (sodium sulfide for injection), a parenteral injectable GMP formulation of H_2S, and H_2S donor (NaHS, GYY-4137) which acts as a neuroprotector and vasoprotector. This compound has recently been proven to be protective against various physiological and pathological conditions. Moreover, the effect of H_2S donors were used in many experimental studies to characterize the pharmacological effects of H_2S *in vitro* and *in vivo* (Esechie et al., 2008; Kamat et al., 2013; Mishra, Tyagi, Sen, Givvimani, & Tyagi, 2010; Qipshidze, Metreveli, Mishra, Lominadze, & Tyagi, 2012; Sen et al., 2012; Simon et al., 2008; Sodha et al., 2008; Tyagi, Vacek, Givvimani, Sen, & Tyagi, 2010).

3. EFFECT OF H_2S ON THE CNS

Several findings suggested that H_2S exists in the CNS at a nanomolar (n*M*) or very low micromolar (μ*M*) concentrations. In contrasts to previous literature, CNS concentrations of H_2S 50–160 μ*M*/l have been reported (Lopez, Prior, Reiffenstein, & Goodwin, 1989; Warenycia et al., 1989). This has significant impact on most previously published papers

which used H_2S concentrations in the range of μ*M*. Previous findings may yet be valid based on recent evidence showing that μ*M* concentrations of H_2S rapidly decays *in vitro* to undetectable levels within 30 min suggesting single μ*M* doses of H_2S might have already exerted their effects before they decay to undetectable levels and imply that the action of H_2S is likely a molecular "switch" that activates downstream pathways that persist long after H_2S decay. H_2S is usually stored as bound sulfane sulfur in neurons and astrocytes (Ishigami et al., 2009). Upon neuron excitation or other stimulation, the bound sulfane sulfur then releases free H_2S. Free H_2S is mainly oxidized to thiosulfate, sulfite, and finally sulfate by thiosulfate:cyanide sulfurtransferase in mitochondria (Lowicka & Beltowski, 2007). H_2S can also be methylated by the enzyme thiol-*S*-methyltransferase to methanethiol and dimethylsulfide or be bound to methemoglobin, an oxidized form of hemoglobin (Lowicka & Beltowski, 2007). The clearance of H_2S via transport from the brain to the clearance organs such as kidneys, lungs, or liver is less likely as concentrations of H_2S in the blood are lower than 14 n*M* (Whitfield, Kreimier, Verdial, Skovgaard, & Olson, 2008) and H_2S has a short half-life *in vitro* (Hu et al., 2009). It was proposed that the neurological and cardiovascular actions of H_2S were continuously modulated primarily by circulating sulfide rather than by endogenous production (Olson et al., 2008). This was disproved based on recent studies reporting undetectable H_2S levels in mouse and rat blood samples using sensors that can detect 14 n*M* of H_2S (Whitfield et al., 2008), implying that H_2S found in the CNS is more likely to be derived directly from the CNS than from the blood. This also supports the hypotheses put forth by recent reviews (Li et al., 2005; Rezessy-Szabo et al., 2007) that H_2S does not circulate in the plasma at measurable conditions (Whitfield et al., 2008) which is consistent with speculations that H_2S has neuromodulatory functions *in vivo* (Abe & Kimura, 1996; Kim, Lee, Jang, Han, & Kim, 2011). In the CNS, H_2S acts as a messenger in response to specific stimuli (usually noxious) such as febrile seizures (Han et al., 2005), stimuli leading to pain (Kubo, Kajiwara, & Kawabata, 2007), and cerebral ischemia (Qu, Chen, Halliwell, Moore, & Wong, 2006), which do not occur frequently. Therefore, it is clear that H_2S is present in the brain and comes from different sources and is involved in the regulation of intracellular signaling molecules, ion channel function, and the release and function of amino acid neurotransmitters (Fig. 2).

As reports suggesting that H_2S might play a role in synaptic transmission, it also maintains excitatory postsynaptic potentials (EPSPs). EPSPs are necessary for electrical and chemical stimulation in synapse. In the hippocampus field and population spikes evoked by the electrical stimulation of the Schafer collaterals in the CA1 region, there is a concentration-dependent sensitivity toward H_2S (Abe & Kimura, 1996). H_2S concentrations greater than 130 μ*M* were found to suppress both field EPSPs and population spikes. The suppression by H_2S was specific to EPSPs and the population spikes as the action potentials generated by direct stimulation of presynaptic fibers were not affected by H_2S. This indicates that the physiological concentration of H_2S is a determining factor for synapse to work properly or improperly.

4. EFFECT OF H_2S ON BRAIN CELLS (ASTROCYTE, MICROGLIA, AND OLIGODENDROCYTE)

Glial cells (astrocytes, oligodendrocytes, and microglia) are significantly abundant in the brain and are pathologically linked to neurodegenerative disorders. Large numbers of available data imply that neurodegeneration in different brain pathology is associated with the alterations in glial cells (Klegeris & McGeer, 2002; McGeer, Yasojima, & McGeer, 2002). Microglia play important roles in responses of the brain to injury and activated microglia congregate around degenerating neurons, and may produce toxins and inflammatory cytokines that contribute to the neurodegenerative process (Li et al., 2005; Rai, Kamat, Nath, & Shukla, 2013, 2014). The severe changes in glial cells and microglial cells in brain disease may promote neuronal degeneration (Jantzen et al., 2002). In addition, although synapses degenerate in vulnerable neuronal circuits, the remaining synapses may increase in size to compensate and astrocytes may play a role in this process (Murai, Nguyen, Irie, Yamaguchi, & Pasquale, 2003). Studies show that glial activation modifies long-term depression (LTD) and potentiation of synaptic transmission in the hippocampus (Albensi & Mattson, 2000). Finally, changes in the mitochondrial membrane permeability in synaptic terminals have been associated with impaired synaptic plasticity in the hippocampus (Albensi, Alasti, & Mueller, 2000). Progressively, accumulating evidence suggested that astrocytes play roles in synaptic transmission through the regulated release of synaptically active molecules including glutamate, purines (ATP and adenosine), GABA, and D-serine (Perea & Araque, 2010; Perea, Navarrete, & Araque, 2009; Shigetomi, Bowser, Sofroniew, & Khakh, 2008). Synaptic stimulation through NMDA receptors is important for learning and memory functions, but excess glutamate can over stimulate these receptors resulting in excitotoxicity and neurodegeneration (Kamat, Rai, Swarnkar, Shukla, & Nath, 2014; Michaels & Rothman, 1990). The release of such gliotransmitters occurs in response to changes in neuronal synaptic activity, which involves astrocyte excitability as reflected by increases in astrocyte Ca^{2+} and can alter neuronal excitability (Halassa, Fellin, & Haydon, 2007; Halassa, Fellin, Takano, Dong, & Haydon, 2007; Nedergaard, Ransom, & Goldman, 2003). Such evidence has given rise to the “tripartite synapse” hypothesis (Perea et al., 2009). Synaptically associated astrocytes are considered as an integral modulatory element of tripartite synapses consisting of the presynapse, the postsynapse, and the glial element (Fig. 3). Astrocytes may secrete glial binding proteins into the synaptic cleft, thus binding free neurotransmitters and thereby reducing the levels of neurotransmitters available for stimulating the postsynapse through receptors. Astrocytes also have membrane-bound receptors for neurotransmitters, and when these bind to neurotransmitters, the astrocytes upregulate the amount of binding protein secreted into the synapse. Thus, astrocytes play an important role in the formation, maintenance, and proper functioning of synapses (Christopherson et al., 2005; Ransom, Behar, & Nedergaard, 2003). Astrocytes exert a powerful influence on the synapse remodeling and pruning of the healthy adult CNS or in response to CNS disorders (Barker & Ullian, 2008). It has been reported that H_2S enhances the induction of hippocampal long-term potentiation (LTP) and induces calcium waves in astrocytes. Based on these observations, it could be strongly suggest that H_2S acts as a synaptic modulator in the brain mediated through astrocytes. Tsugane, Nagai, Kimura, Oka,

and Kimura (2007) showed that differentiated astrocytes acquire sensitivity to H_2S that is diminished by their transformation into reactive astrocytes.

5. SYNAPSE

Synapses are the structures where neurons exchange the neurotransmitter which is essential for neuronal functions and mediate signals to individual target cells. At a synapse, the plasma membrane of the signal-passing neuron (the presynaptic neuron) comes into close proximity with the membrane of the target (postsynaptic) cell. Both the presynaptic and postsynaptic sites of neurons contain extensive groups of molecular machinery that link the two membranes together and carry out the signaling process. Astrocytes also exchange information with the synaptic neurons, responding to synaptic activity and, in turn, regulating neurotransmission (Fig. 3). It is well known that the synapse plays an important role in the formation of learning and memory through molecular machinery. Memory formation process occurs by way of synaptic strengthening which is known as LTP. Memory formation is related to altered release of neurotransmitters and plasticity of synapses. The postsynaptic cell can be regulated by altering the function and number of its receptors. Changes in postsynaptic signaling are most commonly associated with *N*-methyl-$_D$-aspartic acid receptor (NMDAR)-dependent LTP and LTD, which are the most analyzed forms of plasticity at excitatory synapses. When astrocytes interact with neuronal synapses, the structure is known as tripartite synapse. Synaptic physiology is usually based on the bidirectional communication between astrocytes and neurons. Since recent evidence has demonstrated that astrocytes integrate and process synaptic information as well as control synaptic transmission and plasticity, astrocytes, being active partners in synaptic function, are cellular elements involved in the processing, transfer, and storage of information by the nervous system. As evidences suggest, H_2S is generated from astrocytes and considered as the main source. Therefore, it may affect neuronal function when it comes in contact with the synapse and may influence the synapse function.

6. GLIA AND NEURONS INTERACTIONS

Glial cells have been considered to be the nonexcitable and supportive elements in the nervous system, but they are now regarded as fundamentals for neuronal activity and modulate synaptic activity (Haydon, 2001). Glial cells, such as microglia and astrocytes, have neurotransmitter and hormone receptors and also integrate neuronal functions. The multiple interactions between neurons and glia strongly suggest that glial cells are integral parts of neurons and are referred to as modulatory elements in synaptic transmission (Araque, Parpura, Sanzgiri, & Haydon, 1999). The observation that H_2S enhances the induction of hippocampal LTP suggests that H_2S may modulate some aspects of synaptic activity. Although H_2S enhances the NMDA receptor-mediated responses to glutamate in neurons, the effects of H_2S in the absence of glutamate on brain cells are not well understood. Interactions between neurons and glia may modulate synaptic transmission, as neuronal activity can evoke glial function; may inhibit the exocytosis of glutamate or some other factor from nerve terminals when neurons are stimulated by NMDA. H_2S released in response to neuronal excitation may increase intracellular Ca^{2+} and induce Ca^{2+} waves in neighboring astrocytes. Physiological concentrations of H_2S specifically potentiate the

activity of NMDA receptors and alter the induction of LTP in the hippocampus, a synaptic model for memory (Abe & Kimura, 1996). H_2S can also regulate the release of the corticotrophin-releasing hormone from the hypothalamus (Dello Russo et al., 2000; Walsh et al., 2014). H_2S increases intracellular concentrations of Ca^{2+} in glia and induces Ca^{2+} waves, which mediate glial signal transmission (Nagai et al., 2004). Given the accumulating evidence for reciprocal interactions between glia and neurons, it has been suggested that glia modulate the synaptic transmission. H_2S may regulate the synaptic activity by modulating the activity of both neurons and glia in the brain. Based upon these observations, it has been proposed that H_2S may function as a neuromodulator (Abe & Kimura, 1996).

7. EFFECT OF H_2S ON NEURONAL REDOX STRESS

H_2S has been functioning as an endogenous neuromodulator through the activation of NMDA receptors. NMDA receptors also lead to a sustained rise of neuronal cytosolic calcium ion, and interestingly, NMDA receptors are highly sensitive to oxidative stress (Kamat et al., 2013, Kamat, Tota, Saxena, Shukla, & Nath, 2010; Rai et al., 2013). The reduction potential of H_2S is close to that of the thiol group of reduced glutathione, and H_2S, like reduced glutathione, inhibits the oxidative stress-induced damage (Tyagi, Mishra, & Tyagi, 2009; Zhou & Freed, 2005). The reports from various experiments showed protective mechanisms of H_2S against cellular stress. The protection afforded by H_2S against hydrogen peroxide-induced cellular damage (Wang et al., 2013), against protein nitration induced by peroxynitrite (Whiteman et al., 2004), and against oxidative stress-induced death of neurons (Kalani, Kamat, Chaturvedi, Tyagi, & Tyagi, 2014; Kalani, Kamat, Givvimani, et al., 2014; Kamat et al., 2013). These reports further support a role of H_2S as a significant cellular antioxidant. However, the molecular mechanisms through which H_2S can attenuate the neuronal oxidative stress are still to be settled because H_2S can act both as a sacrificial scavenger of ROS and also as an inhibitor of major ROS production in cells. In addition to the well-known inhibition by H_2S of the mitochondrial respiration (Wang, Guo, & Wang, 2012), it is to be noted that the expression of gp91phox (ROS generation regulatory protein), a plasma membrane-bound NADPH-dependent oxidase which releases superoxide anion, has been recently shown to be downregulated by H_2S level (Dong et al., 2012).

The oxidative stress plays a critical role at the early stages of apoptosis. As we and others have shown in earlier works (Kalani, Kamat, Chaturvedi, et al., 2014; Kalani, Kamat, Givvimani, et al., 2014; Kamat et al., 2010; Tyagi et al., 2009), oxidative stress is largely generated by production of superoxide anions such as reactive oxygen species and nitrogen species. Glutathione (g-glutamylcysteinyl glycine; GSH) is a tripeptide containing cysteine, glutamate, and glycine with the amine group of cysteine forming a peptide bond with the carboxyl group of the side chain found in glutamate. It can exist alone in reduced forms as glutathione or in an oxidized dimer form also known as glutathione disulfide (GSSG) (Monks, Ghersi-Egea, Philbert, Cooper, & Lock, 1999). Glutathione biosynthesis is catalyzed by the enzyme g-glutamylcysteine synthetase and glutathione synthase, while glutathione recovery from GSSG is catalyzed by GSSG reductase (Kimura & Kimura, 2004). Recent studies have also suggested that H_2S can antagonize apoptosis through inhibiting the production of ROS and thus promotes neuronal survival (Tang et al., 2013, 2011). H_2S can also protect against oxidative stress-induced neuronal damage through

increasing the level of intracellular glutathione (Kimura & Kimura, 2004; Yang et al., 2011). Previous evidences also show that the pretreatment of H_2S may attenuate neuronal injury/apoptosis through inhibition of cellular apoptosis (Biermann, Lagreze, Schallner, Schwer, & Goebel, 2011; Elrod et al., 2007). Glutathione evenly distributed throughout the brain occurs low in neuronal cells but high in astrocytes and oligodendrocytes, indicating that these glial cells may be the major source of glutathione generated from H_2S; which is also an indication of the presence of H_2S in astrocyte and microglial cells. Lastly, glutathione is formed by the H_2S in the CNS.

As a reducing agent, glutathione protects neuronal as well as non-neuronal cells from free radical species either by direct action or indirectly by promoting the regeneration of other antioxidant systems (Monks et al., 1999). H_2S was also reported to inhibit peroxynitrite-induced cytotoxicity, intracellular protein nitration, and protein oxidation in human neuroblastoma SH-SY5Y cells. These data suggest that H_2S has the potential to act as an inhibitor of peroxynitrite-mediated processes *in vivo* and the potential antioxidant action of H_2S (Whiteman et al., 2004). Similarly, H_2S inhibits cell toxicity due to oxytosis (a novel form of apoptosis), a form of oxidative glutamate toxicity independent of glutamatergic signaling at ionotropic glutamate receptors, in neuronal cells and primary cultured immature cortical neurons (Umemura & Kimura, 2007). In these cells, increased intracellular cysteine levels were observed to correlate to the glutathione levels (Fig. 3). Therefore, H_2S protects against the activity of peroxynitrite and other damages of the cells from free radicals, presumably through increased glutathione production in the neuronal cells as well as neuronal supportive cells.

8. EFFECT OF H_2S ON GLUTAMATE NEUROTRANSMISSION

NMDA receptor blockers were reported to inhibit H_2S-induced cell death in neurons (Cheung, Peng, Chen, Moore, & Whiteman, 2007) and infarct volume in an *in vivo* rat stroke model (Qu et al., 2006), suggesting that H_2S may induce cell death through the opening of NMDA receptors. In summary of the properties of H_2S-induced NMDA signaling, H_2S may promote excitation and regulate survival/death decisions of the neurons. It was reported that H_2S increased glutamate secretion in rat cerebellar granule neurons, which resulted in the neuronal cell death (Garcia-Bereguiain et al., 2008). This was demonstrated by a significant increase in extracellular concentrations of glutamate from physiological concentrations of 2–5 μ*M* (Erecinska, Dagani, Nelson, Deas, & Silver, 1991; Erecinska & Silver, 1990) to supraphysiological (and thus toxic) concentrations of 10–15 μ*M*. This observation was confirmed by blockade of H_2S-induced cell death by NMDA blocker MK-801 and glutamate antagonist DL-2-amino-5-phosphonovaleric acid. A recent paper by Whitfield et al. (2008) suggested the amount of H_2S derived from the plasma is likely to be negligible compared to endogenous production. On the other hand, H_2S is more likely to decay rapidly than persist at micromolar concentrations *in vitro* (Hu et al., 2009), and this suggests that the observed downstream effects of glutamate release by H_2S due to continuous maintenance of high H_2S concentrations (Garcia-Bereguiain et al., 2008) may be reflective of a toxicological situation. If so, a toxic exposure of the cells to glutamate due to a constant high level of H_2S may lead to excitotoxicity and neuronal cell death, thus leading

to the compromised neuronal functions, e.g., memory, in addition to neuropathic pain (Hudspith, 1997).

9. EFFECT OF H_2S ON NMDA RECEPTOR REGULATION

The NMDA type of glutamate receptor (NMDAR) plays a key role in neuronal plasticity, learning, and memory in the CNS, most of which is related to its high permeability to Ca^{2+} (Li & Tsien, 2009). Synaptic stimulation through NMDA receptors is important for the learning and memory functions, but excess glutamate can over stimulate these receptors resulting into excitotoxicity and neurodegeneration (Michaels & Rothman, 1990). Glutamate is an important excitatory amino acid that functions as a neurotransmitter in the mammalian brain. Glutamate plays a role in physiological processes including learning and memory, especially with respect to its central role in induction of LTP, perception of pain, and also in pathological processes such as excitatory neuronal injury (Hudspith & Munglani, 1998). NMDA receptors are a class of receptor-operated glutamate receptors mostly expressed in the nervous system (central and peripheral) (Laezza, Doherty, & Dingledine, 1999). Although there is no direct evidence demonstrating agonist activity of H_2S on NMDA receptors, accumulating evidence indicates that H_2S may produce physiological or pathological functions via regulating NMDA receptors. It was found that H_2S stimulates LTP via potentiation of NMDA receptors. This effect was achieved mainly by H_2S-induced activation of cAMP/PKA pathway (Kimura, 2000). Excessive NMDA receptor activation causes calcium overload in the cells leading to cell death (Gagliardi, 2000). Harris, Ganong, and Cotman (1984) reported that hippocampal LTP alone is not facilitated by H_2S alone and it needs a stimulation to activate NMDA receptors. It seems that H_2S alone does not induce any illusive currents, but significantly increases the NMDA current. The enhancing effect of H_2S on the NMDA receptor is also concentration dependent. Therefore, H_2S enhances the induction of LTP by activating NMDA receptors. Some reports also suggest that disulfide bonds play a role in modulating the function of many proteins, including NMDA receptors. It is therefore possible that H_2S interacts with disulfide bonds or free thiol group (S-H) in NMDA receptors (Fig. 3). Therefore, NMDA receptors have important roles in the brain disease (Myers, Dingledine, & Borges, 1999) and H_2S may modulate this disease progression.

10. EFFECT OF H_2S ON GABA-MEDIATED NEUROTRANSMISSION

GABA, an inhibitory neurotransmitter, is present at high concentrations in the mammalian brain, especially in the axons. Within the mammalian CNS, GABA is the major inhibitory neurotransmitter: About 20–30% of all synapses in the CNS employ GABA as their neurotransmitter (Kaila, 1994). GABA-mediated inhibition in the CNS is critical as loss of GABAergic inhibition leads to seizures and neuronal hyperexcitability. There are three types of receptors for GABA in the CNS: GABAA, GABAB, and GABAC receptors, and they produce slow, prolonged inhibitory signals and function to modulate the release of neurotransmitters (Chebib & Johnston, 1999). H_2S was known to promote amelioration of hippocampal damage induced by recurrent seizures via reversing the loss of GABABR1 and GABABR2 which is caused by febrile seizures (Han et al., 2005). This amelioration was outlined to the increased mRNA and protein levels of these GABA receptors, which may be

due to acute increases in the Ca^{2+} leading to Ca^{2+}-dependent transcription by H_2S induction (Clapham, 2007; Lipscombe, Helton, & Xu, 2004; Pietrobon, 2002). This may have an effect on the restoration of the excitation/inhibition balance perturbed and affecting slow, prolonged inhibitory signals and neurotransmitter release. It was inferred from the above study that H_2S may have therapeutic use in the treatment of excitatory diseases such as epilepsy (Han et al., 2005).

11. EFFECT OF H_2S ON CALMODULIN KINASE

Calcium/calmodulin-dependent protein kinase II (CaM kinase II or CaMKII) is a serine/threonine-specific protein kinase that is regulated by the Ca^{2+}/calmodulin complex. CaMKII is also necessary for Ca^{2+} homeostasis in the neuronal cells. On the other hand, CBS is the main enzyme for the synthesis of H_2S in the brain. In addition, it is also regulated by *S*-adenosylmethionine which acts as an allosteric activator of CBS. As CBS enzyme is a Ca^{2+} and calmodulin-dependent enzyme, the biosynthesis of H_2S is strongly controlled by the intracellular concentration of the Ca^{2+} ion. In addition to that, CBS is also regulated by *S*-adenosyl-L-methionine (SAM) and pyridoxal-5′ -phosphate. It was recently found that Ca^{2+}/calmodulin-mediated pathways are involved in the regulation of CBS activity, which acts as an allosteric activator of CBS. In neurons, H_2S stimulates the production of cAMP probably by direct activation of adenylyl cyclase and thus activates cAMP-dependent processes. Cyclic-AMP-mediated pathways may be involved in the modulation of NMDA receptors by H_2S (Kimura, 2000). Ko and Chu (2005) showed a novel regulatory mechanism for H_2S production by Ca^{2+}/calmodulin. In addition to that, they have also shown that L-glutamate, as well as electrical stimulation, enhances the production of H_2S from brain slices and that LTP is altered in CBS knockout mice. The observations by Ko and Chu (2005) also support that endogenous H_2S is produced when CBS is activated by the Ca^{2+} which occurs with neuronal excitation, and that H_2S may function as a neuromodulator or neurotransmitter (Baranano, Ferris, & Snyder, 2001). Thus, H_2S is produced in response to neuronal excitation and alters hippocampal LTP, a synaptic basis of memory (Fig. 4).

12. CONCLUSION

Recent and previous evidences suggest that H_2S plays an important role in the maintenance of physiological conditions of neurons by its neuroprotective effects. H_2S is originated from both neurons and glial cells in the CNS. It has a potential effect on neurotransmitters such as glutamate, GABA, and AMPA neuronal receptors. This effect of H_2S also has an impact on synapse signaling by interacting with neuronal receptor and thus maintains the neuronal function as well as synapse functions. Under pathological condition, H_2S acts as an anti-inflammatory and anti-oxidative molecule and hence protects neurons and synapse from abnormal pathology, thereby remodeling the neurons as well as neuronal synapse during or after pathology.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health grant HL107640-NT.

ABBREVIATIONS

3MST	3-mercaptopyruvate sulfurtransferase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
Ca^{2+}/calmodulin	calcium ion-dependent calmodulin kinase
CBS	cystathionine beta-synthase
CNS	central nervous system
CSE	cystathionine gamma-lyase
GSH	reduced form of glutathione
GSSG	oxidized form of glutathione
H_2S	hydrogen sulfide
LTD	long-term depression
LTP	long-term potentiation
NMDAR	*N*-methyl-D-aspartate receptor
PAG	DL-propargylglycine
SAM	*S*-adenosyl-L-methionine

REFERENCES

Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 1996; 16:1066–1071. [PubMed: 8558235]

Albensi BC, Alasti N, Mueller AL. Long-term potentiation in the presence of NMDA receptor antagonist arylalkylamine spider toxins. Journal of Neuroscience Research. 2000; 62:177–185. [PubMed: 11020211]

Albensi BC, Mattson MP. Evidence for the involvement of TNF and NF-kappaB in hippocampal synaptic plasticity. Synapse. 2000; 35:151–159. [PubMed: 10611641]

Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: Glia, the unacknowledged partner. Trends in Neurosciences. 1999; 22:208–215. [PubMed: 10322493]

Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: A review. Nutrition Journal. 2002; 1:4. [PubMed: 12537594]

Baranano DE, Ferris CD, Snyder SH. Atypical neural messengers. Trends in Neurosciences. 2001; 24:99–106. [PubMed: 11164940]

Barker AJ, Ullian EM. New roles for astrocytes in developing synaptic circuits. Communicative & Integrative Biology. 2008; 1:207–211. [PubMed: 19513261]

Biermann J, Lagreze WA, Schallner N, Schwer CI, Goebel U. Inhalative preconditioning with hydrogen sulfide attenuated apoptosis after retinal ischemia/reperfusion injury. Molecular Vision. 2011; 17:1275–1286. [PubMed: 21633713]

Chang LC, Jamain S, Lin CW, Rujescu D, Tseng GC, Sibille E. A conserved BDNF, glutamate- and GABA-enriched gene module related to human depression identified by coexpression meta-analysis and DNA variant genome-wide association studies. PLoS One. 2014; 9:e90980. [PubMed: 24608543]

Chebib M, Johnston GA. The 'ABC' of GABA receptors: A brief review. Clinical and Experimental Pharmacology & Physiology. 1999; 26:937–940. [PubMed: 10561820]

Cheung NS, Peng ZF, Chen MJ, Moore PK, Whiteman M. Hydrogen sulfide induced neuronal death occurs via glutamate receptor and is associated with calpain activation and lysosomal rupture in mouse primary cortical neurons. Neuropharmacology. 2007; 53:505–514. [PubMed: 17692345]

Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell. 2005; 120:421–433. [PubMed: 15707899]

Clapham DE. Calcium signaling. Cell. 2007; 131:1047–1058. [PubMed: 18083096]

Dello Russo C, Tringali G, Ragazzoni E, Maggiano N, Menini E, Vairano M, et al. Evidence that hydrogen sulphide can modulate hypothalamo-pituitary-adrenal axis function: In vitro and in vivo studies in the rat. Journal of Neuroendocrinology. 2000; 12:225–233. [PubMed: 10718918]

Distrutti E, Sediari L, Mencarelli A, Renga B, Orlandi S, Antonelli E, et al. Evidence that hydrogen sulfide exerts antinociceptive effects in the gastrointestinal tract by activating KATP channels. The Journal of Pharmacology and Experimental Therapeutics. 2006; 316:325–335. [PubMed: 16192316]

Dong XB, Yang CT, Zheng DD, Mo LQ, Wang XY, Lan AP, et al. Inhibition of ROS-activated ERK1/2 pathway contributes to the protection of H2S against chemical hypoxia-induced injury in H9c2 cells. Molecular and Cellular Biochemistry. 2012; 362:149–157. [PubMed: 22134701]

Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, et al. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:15560–15565. [PubMed: 17878306]

Enokido Y, Suzuki E, Iwasawa K, Namekata K, Okazawa H, Kimura H. Cystathionine beta-synthase, a key enzyme for homocysteine metabolism, is preferentially expressed in the radial glia/astrocyte lineage of developing mouse CNS. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2005; 19:1854–1856. [PubMed: 16160063]

Erecinska M, Dagani F, Nelson D, Deas J, Silver IA. Relations between intracellular ions and energy metabolism: A study with monensin in synaptosomes, neurons, and C6 glioma cells. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 1991; 11:2410–2421. [PubMed: 1869922]

Erecinska M, Silver IA. Metabolism and role of glutamate in mammalian brain. Progress in Neurobiology. 1990; 35:245–296. [PubMed: 1980745]

Esechie A, Kiss L, Olah G, Horvath EM, Hawkins H, Szabo C, et al. Protective effect of hydrogen sulfide in a murine model of acute lung injury induced by combined burn and smoke inhalation. Clinical Science. 2008; 115:91–97. [PubMed: 18315525]

Furne J, Saeed A, Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2008; 295:R1479–R1485.

Gagliardi RJ. Neuroprotection, excitotoxicity and NMDA antagonists. Arquivos de Neuro-Psiquiatria. 2000; 58:583–588. [PubMed: 10920427]

Garcia-Bereguiain MA, Samhan-Arias AK, Martin-Romero FJ, Gutierrez-Merino C. Hydrogen sulfide raises cytosolic calcium in neurons through activation of L-type Ca^{2+} channels. Antioxidants & Redox Signaling. 2008; 10:31–42. [PubMed: 17956188]

Geng B, Yang J, Qi Y, Zhao J, Pang Y, Du J, et al. H_2S generated by heart in rat and its effects on cardiac function. Biochemical and Biophysical Research Communications. 2004; 313:362–368. [PubMed: 14684169]

Halassa MM, Fellin T, Haydon PG. The tripartite synapse: Roles for gliotransmission in health and disease. Trends in Molecular Medicine. 2007; 13:54–63. [PubMed: 17207662]

Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG. Synaptic islands defined by the territory of a single astrocyte. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2007; 27:6473–6477. [PubMed: 17567808]

Han Y, Qin J, Chang X, Yang Z, Bu D, Du J. Modulating effect of hydrogen sulfide on gamma-aminobutyric acid B receptor in recurrent febrile seizures in rats. Neuroscience Research. 2005; 53:216–219. [PubMed: 16122826]

Harris EW, Ganong AH, Cotman CW. Long-term potentiation in the hippocampus involves activation of N-methyl-d-aspartate receptors. Brain Research. 1984; 323:132–137. [PubMed: 6151863]

Haydon PG. GLIA: Listening and talking to the synapse. Nature Reviews. Neuroscience. 2001; 2:185–193.

Hudspith MJ. Glutamate: A role in normal brain function, anaesthesia, analgesia and CNS injury. British Journal of Anaesthesia. 1997; 78:731–747. [PubMed: 9215028]

Hudspith M, Munglani R. A role for presynaptic NMDA receptors in central sensitization in the spinal cord dorsal horn? British Journal of Anaesthesia. 1998; 81:294–295. [PubMed: 9813542]

Ichinohe A, Kanaumi T, Takashima S, Enokido Y, Nagai Y, Kimura H. Cystathionine beta-synthase is enriched in the brains of Down's patients. Biochemical and Biophysical Research Communications. 2005; 338:1547–1550. [PubMed: 16274669]

Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, Kimura H. A source of hydrogen sulfide and a mechanism of its release in the brain. Antioxidants & Redox Signaling. 2009; 11:205–214. [PubMed: 18754702]

Jantzen PT, Connor KE, DiCarlo G, Wenk GL, Wallace JL, Rojiani AM, et al. Microglial activation and beta-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2002; 22:2246–2254. [PubMed: 11896164]

Kaila K. Ionic basis of GABAA receptor channel function in the nervous system. Progress in Neurobiology. 1994; 42:489–537. [PubMed: 7522334]

Kalani A, Kamat PK, Chaturvedi P, Tyagi SC, Tyagi N. Curcumin-primed exosomes mitigate endothelial cell dysfunction during hyperhomocysteinemia. Life Sciences. 2014; 107:1–7. [PubMed: 24780320]

Kalani A, Kamat PK, Givvimani S, Brown K, Metreveli N, Tyagi SC, et al. Nutri-epigenetics ameliorates blood-brain barrier damage and neurodegeneration in hyperhomocysteinemia: Role of folic acid. Journal of Molecular Neuroscience: MN. 2014; 52:202–215. [PubMed: 24122186]

Kamat PK, Kalani A, Givvimani S, Sathnur PB, Tyagi SC, Tyagi N. Hydrogen sulfide attenuates neurodegeneration and neurovascular dysfunction induced by intracerebral-administered homocysteine in mice. Neuroscience. 2013; 252:302–319. [PubMed: 23912038]

Kamat PK, Rai S, Swarnkar S, Shukla R, Nath C. Mechanism of synapse redox stress in Okadaic acid (ICV) induced memory impairment: Role of NMDA receptor. Neurochemistry International. 2014; 76:32–41. [PubMed: 24984170]

Kamat PK, Tota S, Saxena G, Shukla R, Nath C. Okadaic acid (ICV) induced memory impairment in rats: A suitable experimental model to test anti-dementia activity. Brain Research. 2010; 1309:66–74. [PubMed: 19883632]

Kim B, Lee J, Jang J, Han D, Kim KH. Prediction on the seasonal behavior of hydrogen sulfide using a neural network model. The Scientific World Journal. 2011; 11:992–1004.

Kimura H. Hydrogen sulfide induces cyclic AMP and modulates the NMDA receptor. Biochemical and Biophysical Research Communications. 2000; 267:129–133. [PubMed: 10623586]

Kimura Y, Kimura H. Hydrogen sulfide protects neurons from oxidative stress. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2004; 18:1165–1167. [PubMed: 15155563]

Klegeris A, McGeer PL. Cyclooxygenase and 5-lipoxygenase inhibitors protect against mononuclear phagocyte neurotoxicity. Neurobiology of Aging. 2002; 23:787–794. [PubMed: 12392782]

Ko TH, Chu H. Spectroscopic study on sorption of hydrogen sulfide by means of red soil. Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy. 2005; 61:2253–2259.

Kubo S, Kajiwara M, Kawabata A. Dual modulation of the tension of isolated gastric artery and gastric mucosal circulation by hydrogen sulfide in rats. Inflammopharmacology. 2007; 15:288–292. [PubMed: 17952370]

Laezza F, Doherty JJ, Dingledine R. Long-term depression in hippocampal interneurons: Joint requirement for pre- and postsynaptic events. Science. 1999; 285:1411–1414. [PubMed: 10464102]

Lee J, Kim CH, Kim DG, Ahn YS. Zinc inhibits amyloid beta production from Alzheimer's amyloid precursor protein in SH-SY5Y cells. The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology. 2009; 13:195–200.

Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, Wang ZJ, et al. Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2005; 19:1196–1198. [PubMed: 15863703]

Li F, Tsien JZ. Memory and the NMDA receptors. The New England Journal of Medicine. 2009; 361:302–303. [PubMed: 19605837]

Lipscombe D, Helton TD, Xu W. L-type calcium channels: The low down. Journal of Neurophysiology. 2004; 92:2633–2641. [PubMed: 15486420]

Lopez A, Prior MG, Reiffenstein RJ, Goodwin LR. Peracute toxic effects of inhaled hydrogen sulfide and injected sodium hydrosulfide on the lungs of rats. Fundamental and Applied Toxicology: Official Journal of the Society of Toxicology. 1989; 12:367–373. [PubMed: 2714535]

Lowicka E, Beltowski J. Hydrogen sulfide (H_2S)—The third gas of interest for pharmacologists. Pharmacological Reports: PR. 2007; 59:4–24. [PubMed: 17377202]

McGeer PL, Yasojima K, McGeer EG. Association of interleukin-1 beta polymorphisms with idiopathic Parkinson's disease. Neuroscience Letters. 2002; 326:67–69. [PubMed: 12052540]

Michaels RL, Rothman SM. Glutamate neurotoxicity in vitro: Antagonist pharmacology and intracellular calcium concentrations. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 1990; 10:283–292. [PubMed: 1967639]

Mishra PK, Tyagi N, Sen U, Givvimani S, Tyagi SC. H_2S ameliorates oxidative and proteolytic stresses and protects the heart against adverse remodeling in chronic heart failure. American Journal of Physiology. Heart and Circulatory Physiology. 2010; 298:H451–H456. [PubMed: 19933416]

Monks TJ, Ghersi-Egea JF, Philbert M, Cooper AJ, Lock EA. Symposium overview: The role of glutathione in neuroprotection and neurotoxicity. Toxicological Sciences: An Official Journal of the Society of Toxicology. 1999; 51:161–177. [PubMed: 10543018]

Murai KK, Nguyen LN, Irie F, Yamaguchi Y, Pasquale EB. Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. Nature Neuroscience. 2003; 6:153–160.

Myers SJ, Dingledine R, Borges K. Genetic regulation of glutamate receptor ion channels. Annual Review of Pharmacology and Toxicology. 1999; 39:221–241.

Nagai Y, Tsugane M, Oka J, Kimura H. Hydrogen sulfide induces calcium waves in astrocytes. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2004; 18:557–559. [PubMed: 14734631]

Nedergaard M, Ransom B, Goldman SA. New roles for astrocytes: Redefining the functional architecture of the brain. Trends in Neurosciences. 2003; 26:523–530. [PubMed: 14522144]

Oh GS, Pae HO, Lee BS, Kim BN, Kim JM, Kim HR, et al. Hydrogen sulfide inhibits nitric oxide production and nuclear factor-kappaB via heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide. Free Radical Biology & Medicine. 2006; 41:106–119. [PubMed: 16781459]

Olson KR, Healy MJ, Qin Z, Skovgaard N, Vulesevic B, Duff DW, et al. Hydrogen sulfide as an oxygen sensor in trout gill chemoreceptors. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2008; 295:R669–R680.

Perea G, Araque A. GLIA modulates synaptic transmission. Brain Research Reviews. 2010; 63:93–102. [PubMed: 19896978]

Perea G, Navarrete M, Araque A. Tripartite synapses: Astrocytes process and control synaptic information. Trends in Neurosciences. 2009; 32:421–431. [PubMed: 19615761]

Pietrobon D. Calcium channels and channelopathies of the central nervous system. Molecular Neurobiology. 2002; 25:31–50. [PubMed: 11890456]

Qipshidze N, Metreveli N, Mishra PK, Lominadze D, Tyagi SC. Hydrogen sulfide mitigates cardiac remodeling during myocardial infarction via improvement of angiogenesis. International Journal of Biological Sciences. 2012; 8:430–441. [PubMed: 22419888]

Qu K, Chen CP, Halliwell B, Moore PK, Wong PT. Hydrogen sulfide is a mediator of cerebral ischemic damage. Stroke: A Journal of Cerebral Circulation. 2006; 37:889–893. [PubMed: 16439695]

Rai S, Kamat PK, Nath C, Shukla R. A study on neuroinflammation and NMDA receptor function in STZ (ICV) induced memory impaired rats. Journal of Neuroimmunology. 2013; 254:1–9. [PubMed: 23021418]

Rai S, Kamat PK, Nath C, Shukla R. Glial activation and post-synaptic neurotoxicity: The key events in streptozotocin (ICV) induced memory impairment in rats. Pharmacology, Biochemistry, and Behavior. 2014; 117:104–117.

Ransom B, Behar T, Nedergaard M. New roles for astrocytes (stars at last). Trends in Neurosciences. 2003; 26:520–522. [PubMed: 14522143]

Rezessy-Szabo JM, Nguyen QD, Hoschke A, Braet C, Hajos G, Claeyssens M. A novel thermostable alpha-galactosidase from the thermophilic fungus Thermomyces lanuginosus CBS 395.62/b: Purification and characterization. Biochimica et Biophysica Acta. 2007; 1770:55–62. [PubMed: 17008008]

Sen U, Sathnur PB, Kundu S, Givvimani S, Coley DM, Mishra PK, et al. Increased endogenous H_2S generation by CBS, CSE, and 3MST gene therapy improves ex vivo renovascular relaxation in hyperhomocysteinemia. American Journal of Physiology. Cell Physiology. 2012; 303:C41–C51. [PubMed: 22517358]

Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, et al. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. Antioxidants & Redox Signaling. 2009; 11:703–714. [PubMed: 18855522]

Shigetomi E, Bowser DN, Sofroniew MV, Khakh BS. Two forms of astrocyte calcium excitability have distinct effects on NMDA receptor-mediated slow inward currents in pyramidal neurons. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2008; 28:6659–6663. [PubMed: 18579739]

Simon F, Giudici R, Duy CN, Schelzig H, Oter S, Groger M, et al. Hemodynamic and metabolic effects of hydrogen sulfide during porcine ischemia/reperfusion injury. Shock. 2008; 30:359–364. [PubMed: 18323742]

Sodha NR, Clements RT, Feng J, Liu Y, Bianchi C, Horvath EM, et al. The effects of therapeutic sulfide on myocardial apoptosis in response to ischemia-reperfusion injury. European Journal of Cardio-Thoracic Surgery: Official Journal of the European Association for Cardio-thoracic Surgery. 2008; 33:906–913. [PubMed: 18314343]

Tang XQ, Chen RQ, Dong L, Ren YK, Del Soldato P, Sparatore A, et al. Role of paraoxonase-1 in the protection of hydrogen sulfide-donating sildenafil (ACS6) against homocysteine-induced neurotoxicity. Journal of Molecular Neuroscience: MN. 2013; 50:70–77. [PubMed: 22843253]

Tang XQ, Chen RQ, Ren YK, Soldato PD, Sparatore A, Zhuang YY, et al. ACS6, a hydrogen sulfide-donating derivative of sildenafil, inhibits homocysteine-induced apoptosis by preservation of mitochondrial function. Medical Gas Research. 2011; 1:20. [PubMed: 22146536]

Tanizawa K. Production of H_2S by 3-mercaptopyruvate sulphurtransferase. Journal of Biochemistry. 2011; 149:357–359. [PubMed: 21324981]

Tsugane M, Nagai Y, Kimura Y, Oka J, Kimura H. Differentiated astrocytes acquire sensitivity to hydrogen sulfide that is diminished by the transformation into reactive astrocytes. Antioxidants & Redox Signaling. 2007; 9:257–269. [PubMed: 17115938]

Tyagi N, Mishra PK, Tyagi SC. Homocysteine, hydrogen sulfide (H_2S) and NMDA-receptor in heart failure. Indian Journal of Biochemistry & Biophysics. 2009; 46:441–446. [PubMed: 20361707]

Tyagi N, Vacek JC, Givvimani S, Sen U, Tyagi SC. Cardiac specific deletion of N-methyl-d-aspartate receptor 1 ameliorates mtMMP-9 mediated autophagy/mitophagy in hyperhomocysteinemia. Journal of Receptor and Signal Transduction Research. 2010; 30:78–87. [PubMed: 20170426]

Umemura K, Kimura H. Hydrogen sulfide enhances reducing activity in neurons: Neurotrophic role of H_2S in the brain? Antioxidants & Redox Signaling. 2007; 9:2035–2041. [PubMed: 17822366]

Wallace JL. Hydrogen sulfide-releasing anti-inflammatory drugs. Trends in Pharmacological Sciences. 2007; 10:501–505. [PubMed: 17884186]

Walsh JJ, Friedman AK, Sun H, Heller EA, Ku SM, Juarez B, et al. Stress and CRF gate neural activation of BDNF in the mesolimbic reward pathway. Nature Neuroscience. 2014; 17:27–29.

Wang M, Guo Z, Wang S. Cystathionine gamma-lyase expression is regulated by exogenous hydrogen peroxide in the mammalian cells. Gene Expression. 2012; 15:235–241. [PubMed: 23539901]

Wang X, Han A, Wen C, Chen M, Chen X, Yang X, et al. The effects of H_2S on the activities of CYP2B6, CYP2D6, CYP3A4, CYP2C19 and CYP2C9 in vivo in rat. International Journal of Molecular Sciences. 2013; 14:24055–24063. [PubMed: 24336065]

Warenycia MW, Goodwin LR, Benishin CG, Reiffenstein RJ, Francom DM, Taylor JD, et al. Acute hydrogen sulfide poisoning. Demonstration of selective uptake of sulfide by the brainstem by measurement of brain sulfide levels. Biochemical Pharmacology. 1989; 38:973–981. [PubMed: 2930598]

Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Cheung NS, et al. The novel neuromodulator hydrogen sulfide: An endogenous peroxynitrite 'scavenger'? Journal of Neurochemistry. 2004; 90:765–768. [PubMed: 15255956]

Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, Olson KR. Reappraisal of H_2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2008; 294:R1930–R1937.

Yang Z, Yang C, Xiao L, Liao X, Lan A, Wang X, et al. Novel insights into the role of HSP90 in cytoprotection of H_2S against chemical hypoxia-induced injury in H9c2 cardiac myocytes. International Journal of Molecular Medicine. 2011; 28:397–403. [PubMed: 21519787]

Zhao H, Chan SJ, Ng YK, Wong PT. Brain 3-mercaptopyruvate sulfurtransferase (3MST): Cellular localization and downregulation after acute stroke. PLoS One. 2013; 8:e67322. [PubMed: 23805308]

Zhao H, Chen MH, Shen ZM, Kahn PC, Lipke PN. Environmentally induced reversible conformational switching in the yeast cell adhesion protein alpha-agglutinin. Protein Science: A Publication of the Protein Society. 2001; 10:1113–1123. [PubMed: 11369849]

Zhou W, Freed CR. DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T alpha-synuclein toxicity. The Journal of Biological Chemistry. 2005; 280:43150–43158. [PubMed: 16227205]

Zhu W, Song X, Li M, Dao J. CBS gene variations and serum homocysteine level associated with congenital heart defects. Wei Sheng Yan Jiu = Journal of Hygiene Research. 2008; 37:463–467. [PubMed: 18839533]

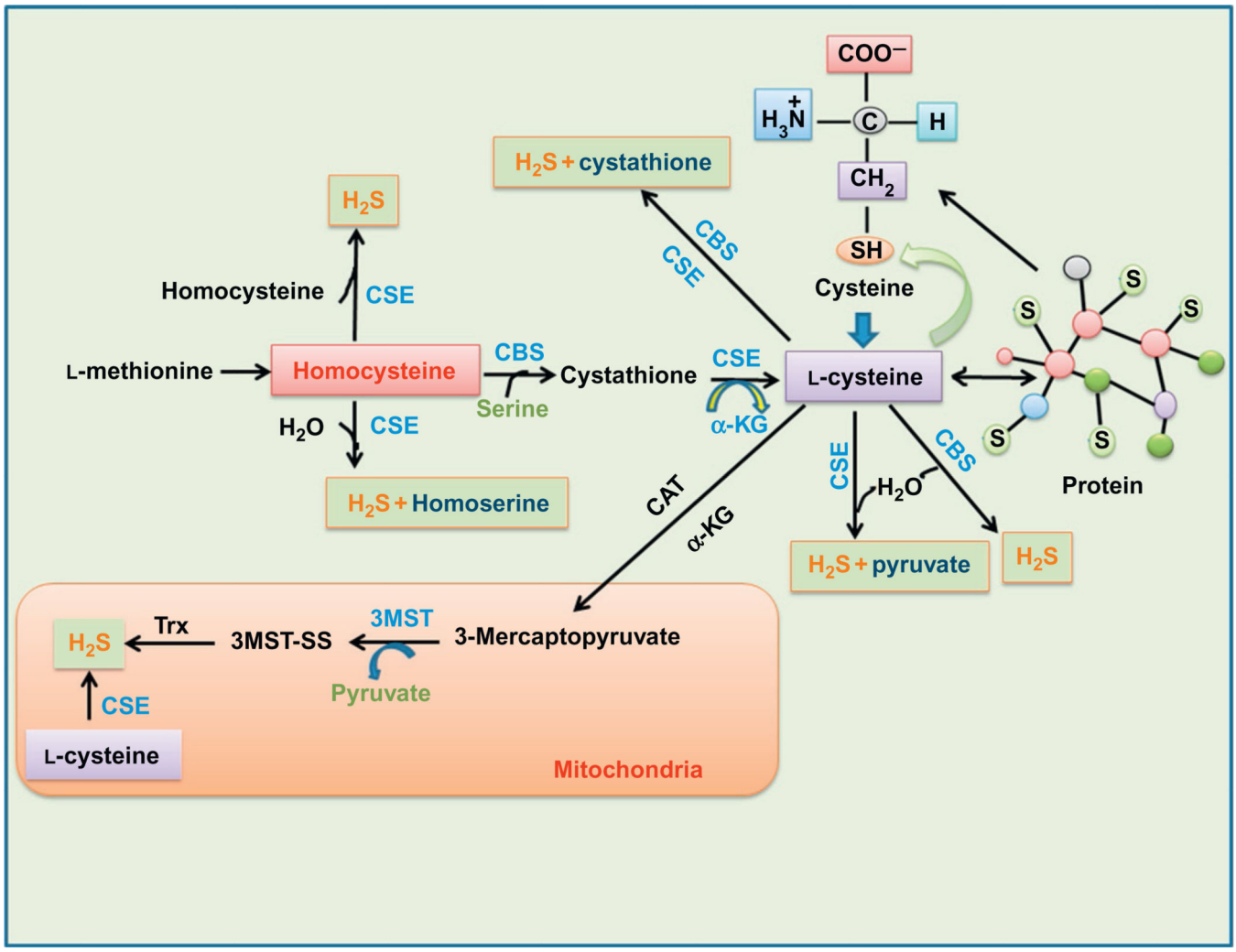

Figure 1.

H_2S formation in cytosol as well as in mitochondria. The three enzymes CBS, CSE, and 3MST are responsible for H_2S generation in cells. CBS and CSE are usually present in cytosol while 3MST is present in mitochondria. The sources of cytosolic H_2S generation are L-methionine, homocysteine, cystathione, and L-cysteine. L-Cysteine also originates from a protein and cysteine and moreover also produces pyruvate and cystathione. In mitochondria, the source of H_2S synthesis are mercaptopyruvate and L-cysteine. CBS, cystathionine β-synthase; 3MST, 3-mercaptopyruvate sulfurtransferase; CSE, cystathionine γ-lyase; α-KG, α-ketoglutarate; CAT, cysteine aminotransferase; H_2S, hydrogen sulfide; TRX, thioredoxin.

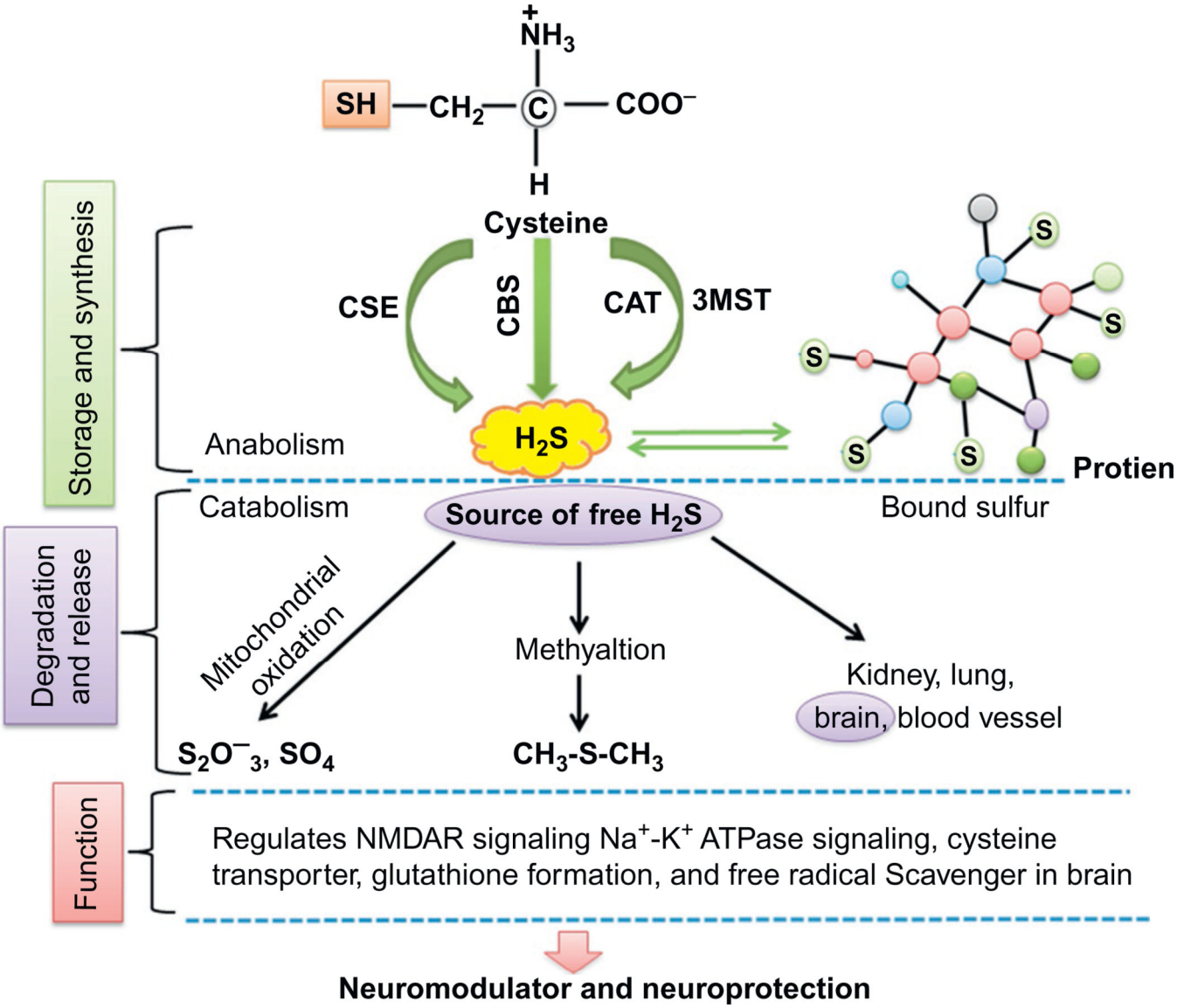

Figure 2.

Diagrammatic representation shows the sources of H_2S and moreover anabolism and catabolism of H_2S. The usual sources of H_2S are blood lung, kidney, heart, and brain. Free H_2S gets converted into sulfate and thiosulfate through mitochondrial oxidation and sulfomethane via methylation which becomes a source of H_2S. Diagram also depicts the synthesis, storage, and function of H_2S in neuronal synapse. These free H_2S involved in NMDA receptor signaling, Na^+ K^+ ATPase signaling, and glutathione formation maintains and regulates neuronal function.

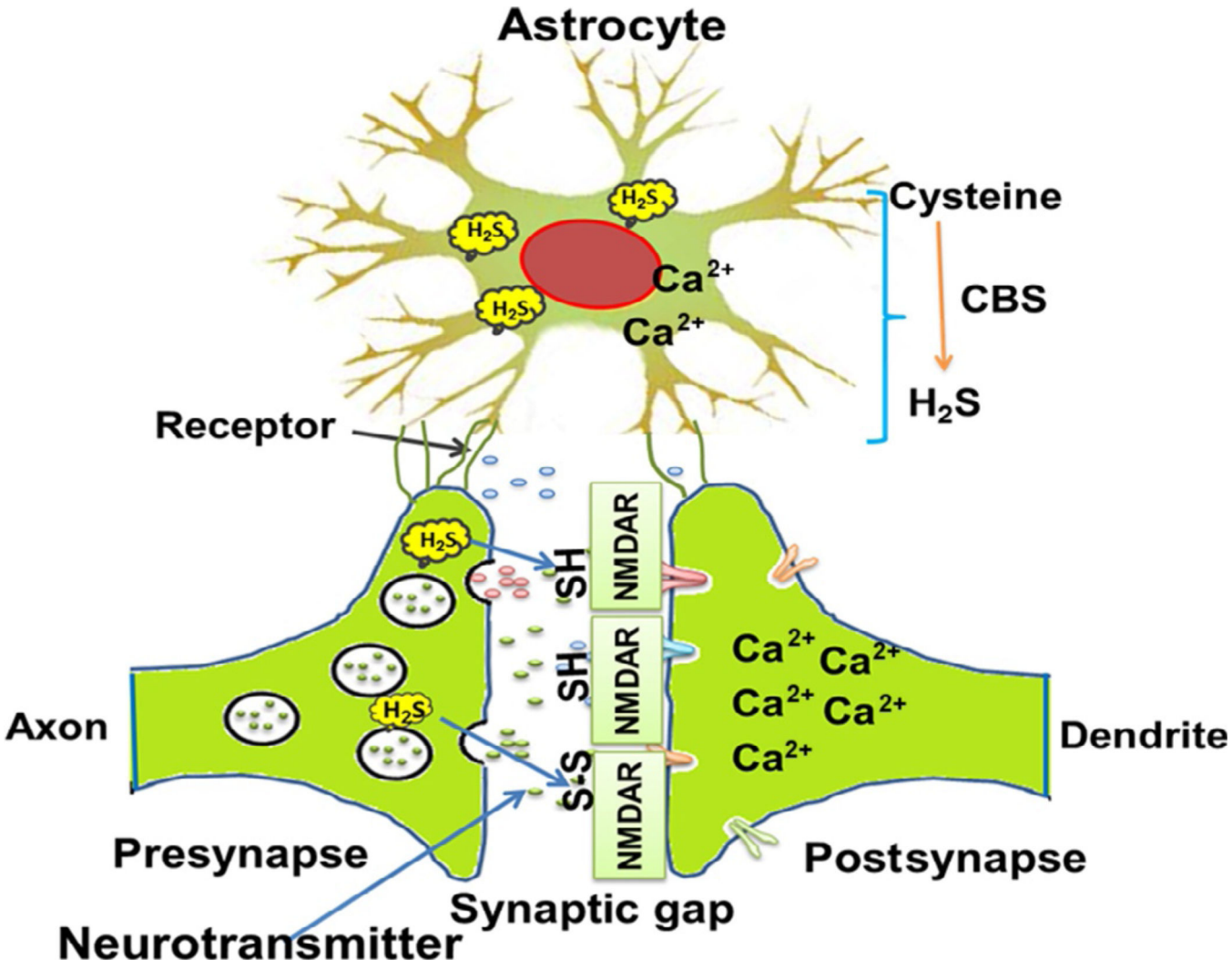

Figure 3.

Cartoon represents the structure of tripartite synapse (presynapse, postsynapse, and astrocyte). In astrocyte, H_2S interacts with H_2S and slow down the excessive glutamate and calcium ion release from astrocyte. On the other hand, in pathological condition H_2S moderates the calcium ion influx through NMDA receptor in postsynapse and prevents from excitotoxic cell death. Neuronal synapses showing the presence of H_2S in presynapse and postsynapse. Moreover, it also represents how H_2S interacts with NMDA receptors; actually, H_2S modulates the NMDA receptor and controls synapse function properly. Apart from that H_2S also associated with glutathione production in cells which acts as cellular antioxidant.

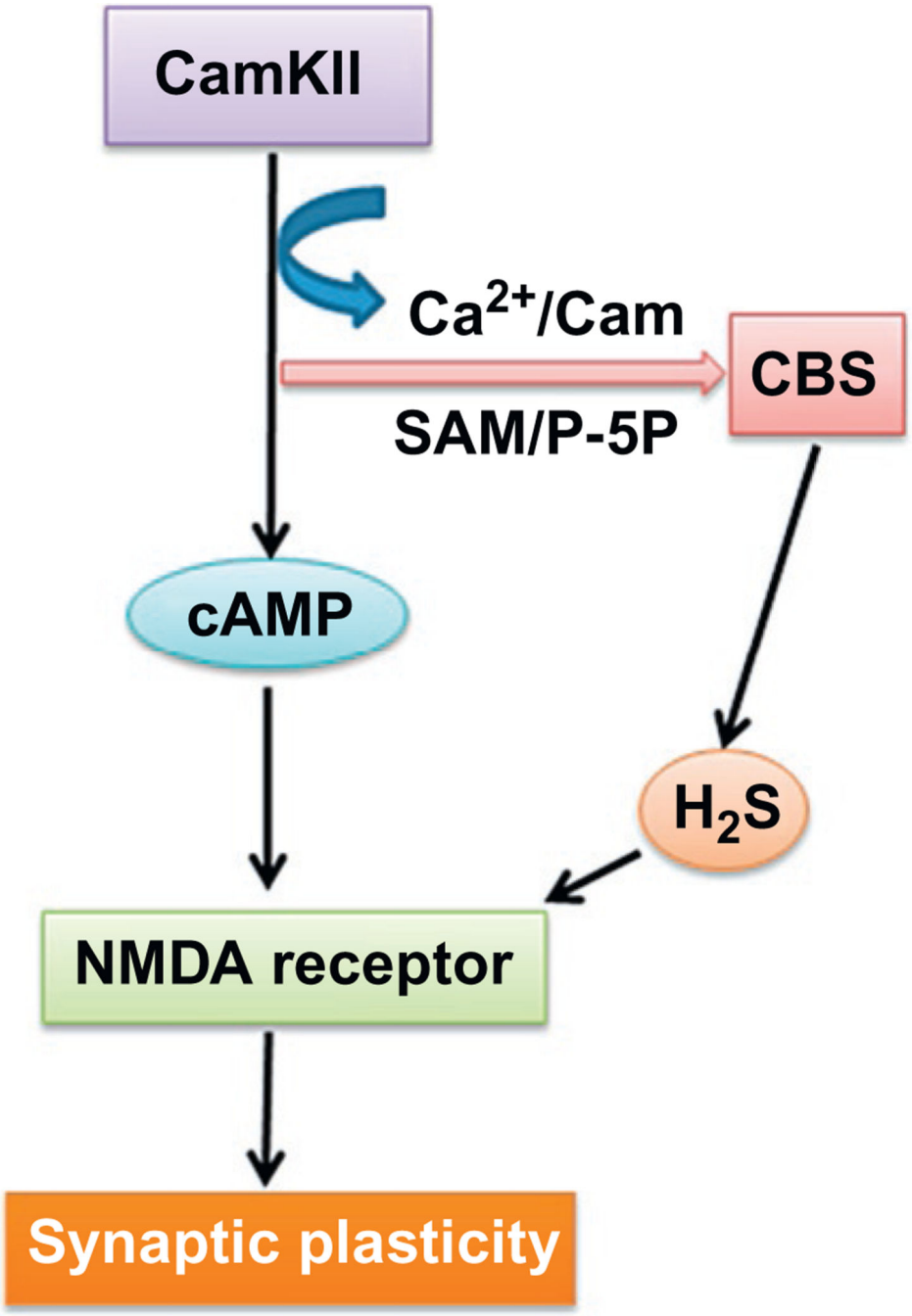

Figure 4.

Flow diagram indicates the interaction and regulation of CBS enzyme with the help of CaMKII, calcium ion, *S*-adenosyl methionine, and pyridoxal-5′-phosphate. In other ways, CaMKII also potentiate the NMDA receptor via cAMP pathways. Further, H_2S produced from different sources with the help of CBS enzyme interacts with NMDA receptor and modulates their function and thereby maintains the synaptic plasticity. P-5P, pyridoxal-5′-

phosphate; cAMP, cyclic adenosine monophosphate; CamKIIα, calmodulin kinase II alfa; SAM, *S*-adenosyl methionine; NMDA, *N*-methyl-D-aspartate.