

## Review Article

# Hydrogen Sulfide as an Endogenous Modulator in Mitochondria and Mitochondria Dysfunction

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Hydrogen sulfide (H<sub>2</sub>S) has historically been considered to be a toxic gas, an environmental and occupational hazard. However, with the discovery of its presence and enzymatic production through precursors of L-cysteine and homocysteine in mammalian tissues, H<sub>2</sub>S has recently received much interest as a physiological signaling molecule. H<sub>2</sub>S is a gaseous messenger molecule that has been implicated in various physiological and pathological processes in mammals, including vascular relaxation, angiogenesis, and the function of ion channels, ischemia/reperfusion (I/R), and heart injury. H<sub>2</sub>S is an endogenous neuromodulator and present studies show that physiological concentrations of H<sub>2</sub>S enhance NMDA receptor-mediated responses and aid in the induction of hippocampal long-term potentiation. Moreover, in the field of neuronal protection, physiological concentrations of H<sub>2</sub>S in mitochondria have many favorable effects on cytoprotection.

## 1. Introduction

Hydrogen sulfide (H<sub>2</sub>S) is well known as a transparent, toxic gas with the characteristic strong smell of rotten eggs [1]. In nature, H<sub>2</sub>S is produced primarily by the decomposition of organic matter and is also found in natural gas, petroleum, and volcanic and sulfur-spring emissions [2]. H<sub>2</sub>S is a small molecule that can travel through cell membranes without using specific transporters. The majority are metabolized to sulfate and thiosulfate via oxidative metabolism in mitochondria, while only low levels of H<sub>2</sub>S can be converted into less toxic compounds by the cytosolic detoxification pathway [3, 4]. These metabolic products are then expelled within 24 hours via the kidneys, intestinal tract, and lungs, to maintain balanced H<sub>2</sub>S levels [5]. Under normal circumstances, H<sub>2</sub>S does not accumulate, which means that under physiological conditions, endogenous H<sub>2</sub>S is not toxic to cells.

Recent evidence clearly indicates that mammalian tissues can also produce H<sub>2</sub>S through an endogenous synthetic system, that consists primarily of two enzymes, cystathionine

$\beta$ -synthase (CBS; EC 4.2.1.22) and cystathionine  $\gamma$ -lyase (CSE; EC 4.4.1.1) [6, 7]. The amino acid L-cysteine is a major substrate for H<sub>2</sub>S synthesis. Recent studies in humans show that H<sub>2</sub>S can also be synthesized from endogenous substrates in the gastrointestinal tract [8]. Measurement of H<sub>2</sub>S synthesis in the rat and mouse gastrointestinal tract has illustrated that CSE is expressed in all tissues with the highest level of expression in the liver. CBS is also expressed in all tissues but highest levels of CBS expression are found in the brain [6, 9].

In mitochondria, H<sub>2</sub>S acts as a cytoprotective factor by inhibiting the activity of cytochrome oxidase following ischemia/reperfusion (I/R), upregulating the level of superoxide dismutase (SOD), and downregulating levels of reactive oxygen species (ROS). H<sub>2</sub>S also acts as both a neuroprotectant by increasing the production of glutathione (GSH) and by modulating CSE translocation to mitochondria and the supply of ATP during hypoxia. Mitochondria play a key role in cell death pathways [10], and H<sub>2</sub>S is involved in regulating apoptosis [11]. Although various types

of proapoptotic signals trigger the cell-death cascade, they may all converge in mitochondria.

The physiological regulation of H<sub>2</sub>S as a gasotransmitter and modulator in both central and peripheral systems will be discussed below, along with the unique role that H<sub>2</sub>S plays in mitochondria.

## 2. Basal H<sub>2</sub>S Generation and Metabolism, and Its Physiological and Pathophysiological Functions

**2.1. Enzymes That Produce Endogenous H<sub>2</sub>S.** Three enzymes have been identified that produce endogenous H<sub>2</sub>S: cystathionine  $\beta$ -synthase (CBS), cystathionine  $\gamma$ -lyase (CSE), and 3-mercaptopyruvate sulfur transferase (3MST). These three enzymes all produce H<sub>2</sub>S from cysteine. CBS and CSE are expressed in many tissues, including the kidney and liver. However, in the human brain CBS is the main producer of H<sub>2</sub>S, while in thoracic aorta, ileum, portal vein, and uterus, CSE is predominant. 3-MST is also expressed in the brain, but most of the H<sub>2</sub>S produced by 3-MST are bound in the form of sulfane sulfur, one of the forms in which endogenous H<sub>2</sub>S is stored [12].

Understanding the distinct expression patterns of the three enzymes is helpful for drug design. Each enzyme may be a possible target for modulating endogenous H<sub>2</sub>S, while a lack of cysteine may lead to a nonspecific decrease of H<sub>2</sub>S.

CBS is a pyridoxal-5'-phosphate- (PLP-)dependent enzyme. Using northern blot assays, CBS was shown to be expressed in the hippocampus, cerebellum, cerebrum, and brainstem [13]. Besides producing H<sub>2</sub>S from cysteine, CBS also catalyzes the condensation reaction of homocysteine, which CSE cannot do. CBS is mainly localized in cerebellar Bergmann glia and astrocytes [14]. An *in vitro* study showed that the H<sub>2</sub>S level in cultured astrocytes was more than sevenfold higher than that of microglial cells [15]. H<sub>2</sub>S levels drop when CBS inhibitors such as hydroxyl amine and aminoxyacetate are induced. Inflammatory activation of astrocytes and microglia can also decrease the expression of CBS, leading to a decrease in H<sub>2</sub>S in the brain. These findings indicate that endogenous H<sub>2</sub>S in the brain is mainly produced by CBS, and that modulation of the expression of CBS can change the level of H<sub>2</sub>S. This has great pharmacological potential for the treatment of central nervous system disorders.

Several endogenous and exogenous compounds such as epidermal growth factor (EGF), transforming growth factor- $\alpha$  (TGF- $\alpha$ ) and cyclic adenosine monophosphate (cAMP), can upregulate the expression of CBS mRNA or the transcription of CBS [7]. CBS expression is abnormal in several diseases. CBS expression levels in the brains of Down's syndrome patients were found to be three times higher than normal levels, while low expression levels of CBS alleles were found in children with a high IQ [7]. This observation suggests that overexpression of CBS may have a negative influence on cognitive function. However, the absence of CBS causes severe diseases, such as homocysteinemia.

CSE is also a pyridoxal-5'-phosphate- (PLP-)dependent enzyme. CSE is mainly localized in the liver and kidney and in both vascular and nonvascular smooth muscle. Low levels of CSE are also detectable in the small intestine and stomach of rodents [16]. The expression levels of CSE in vascular smooth muscle can be ranked as artery > aorta > tail artery > mesenteric artery [16]. Regulation of CSE is less well understood than the regulation of CBS. CSE is upregulated by S-nitroso-N-acetylpenicillamine (SNAP), which is a type of NO donor. Sodium nitroprusside (SNP), another nitric oxide (NO) donor, increases the activity of CSE. Intriguingly, H<sub>2</sub>S interacts with and can act synergistically with NO in vasorelaxation, suggesting that H<sub>2</sub>S production in the cardiovascular system may be involved in the vasorelaxation effect of NO [17–19].

3MST and cysteine aminotransferase (CAT) are recently identified enzymes that can produce H<sub>2</sub>S from cysteine in the brain [10]. In brain homogenates of CBS knockout mice, H<sub>2</sub>S can still be detected, which suggests the existence of another H<sub>2</sub>S producing enzyme [20]. The activity of this enzyme requires components from both the mitochondria and cytosol. 3MST and CAT are located in the mitochondria and can act as the synaptosome, while  $\alpha$ -ketoglutarate is the cytosolic compound [7, 20, 21]. However, 3MST and CAT exert their enzymatic activity at pH 7.4, which is relatively alkaline, and the intermediate of CAT catalysis, 3-mercaptopyruvate (3MP), is an unstable molecule that affects the production of 3MST, which suggests that this pathway cannot produce H<sub>2</sub>S under physiological conditions [7, 20]. Aspartate is another substrate for CAT, that can competitively combine with CAT and suppress H<sub>2</sub>S production. There are several important differences between 3MST and CBS. First, CBS is mainly localized in astrocytes while 3MST is mostly detected in neurons. Second, 3MST produces bound sulfane sulfur more efficiently than CBS. Third, 3MST carries sulfur from H<sub>2</sub>S to bound sulfane sulfur, while such activity in CBS is weak [7]. 3MST can also be found in the thoracic aorta. 3MST, CAT, and  $\alpha$ -ketoglutarate can all be found in endothelium, which suggests that H<sub>2</sub>S can be produced in the endothelium.

**2.2. Storage and Release of H<sub>2</sub>S.** The major cellular sources of H<sub>2</sub>S and the mechanism of H<sub>2</sub>S release remain unknown, although several possibilities have been proposed. Two forms of sulfur that can release H<sub>2</sub>S have been detected and methods have been developed to measure the levels of free H<sub>2</sub>S.

Basal levels of free H<sub>2</sub>S must be kept low because frequent exposure to relatively high concentrations of H<sub>2</sub>S leads to desensitization of the response to H<sub>2</sub>S. Some endogenous H<sub>2</sub>S is likely to be released immediately after it is produced, but the majority are stored and released following stimulation. Two forms in which endogenous H<sub>2</sub>S can be stored are acid-labile sulfur and bound sulfane sulfur. However, many unanswered questions regarding the stored form of endogenous H<sub>2</sub>S still remain [7].

Acid-labile sulfur is mainly localized in the iron-sulfur center of mitochondrial enzymes. However, it can only

release H<sub>2</sub>S at approximately pH 5.4, which suggests that it is not a physiological source of H<sub>2</sub>S [22]. Besides acid conditions, acid-labile sulfur also releases H<sub>2</sub>S when the enzymes are treated with detergents and protein denaturants, because iron-sulfur complexes are unstable and readily release H<sub>2</sub>S when detached from enzymes.

In contrast to acid-labile sulfur, bound sulfane sulfur releases H<sub>2</sub>S under reducing conditions. Bound sulfane sulfur consists of divalent sulfur bound only to other sulfur atoms, in the forms of polysulfide, elemental sulfur, and persulfide. Under reducing conditions, approximately pH 8.4 is needed for cells to release H<sub>2</sub>S under physiological concentrations of glutathione and cysteine [22]. Cells that express 3MST and CAT show a nearly two-fold increase in bound sulfane sulfur levels compared to cells without 3MST and CAT. This suggests that the H<sub>2</sub>S produced by 3MST/CAT is mainly stored as bound sulfane sulfur. Most exogenously applied H<sub>2</sub>S are also stored as bound sulfane sulfur [7].

Several methods have been developed to measure free H<sub>2</sub>S under various conditions with relatively high accuracy [23]. Monobromobimane binds to thiols when mixed with brain homogenates, making it possible to determine H<sub>2</sub>S levels by using mass spectroscopy to measure the amount of monobromobimane bound to H<sub>2</sub>S [7]. Using this method, it is possible to determine the free H<sub>2</sub>S concentration in specific tissues. Furthermore, if brain homogenates are mixed with phosphate buffer, H<sub>2</sub>S stored in acid-labile sulfur form can be released and measured in addition to free H<sub>2</sub>S. A method for measuring H<sub>2</sub>S derived mainly from acid-labile sulfur has been widely applied. For this method, homogenates are treated with N,N-dimethyl-p-phenylenediamine sulfate and FeCl<sub>3</sub> in high concentrations of HCl, resulting in the production of methylene blue, which can subsequently be measured. In this method, only H<sub>2</sub>S trapped in tissues, which cannot evaporate into the air and which can be released under acid conditions, are measured [7]. Bound sulfane sulfur releases H<sub>2</sub>S under reducing conditions, so cells treated with dithiothreitol (DTT) will easily release H<sub>2</sub>S from this source.

**2.3. Peripheral Functions of H<sub>2</sub>S.** Some studies claim that H<sub>2</sub>S inhibits human recombinant Ca<sup>2+</sup>-activated K<sup>+</sup> channels (BK<sub>Ca</sub>) and native BK<sub>Ca</sub> channels expressed in the carotid body in rats. In addition, these channels are widely distributed in the central nervous system and vasculature. The inhibition of BK<sub>Ca</sub> channels by H<sub>2</sub>S is of fundamental physiological importance to carotid body function. However, another report has indicated that H<sub>2</sub>S increased the activity of BK<sub>Ca</sub> channels expressed in a rat pituitary cell line, leading to hyperpolarization and relaxation of smooth muscle cells (SMCs) [24, 25].

T-type Ca<sup>2+</sup> channels are a unique class of voltage-gated Ca<sup>2+</sup> channel. Regulation of T-type Ca<sup>2+</sup> channels is an important feature of both acute and chronic pain sensations. H<sub>2</sub>S can activate or sensitize the channels in primary afferent and spinal sensory neurons. This may, in part, account for hyperalgesia and chronic pain, because hyperalgesia and allodynia can be prevented by CSE inhibitors as well

as by a T-type channel inhibitor. Hyperalgesia can also be suppressed by blocking endogenous H<sub>2</sub>S production. However, no detailed electrophysiological investigation of the modulation of T-type Ca<sup>2+</sup> channels by H<sub>2</sub>S has been performed [24].

H<sub>2</sub>S produced via CSE has been shown to relax vascular smooth muscle via the opening of ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels, which may contribute significantly to the regulation of blood pressure. H<sub>2</sub>S is a major endothelium-derived hyperpolarizing factor (EDHF) that causes vascular endothelial and smooth muscle cell hyperpolarization and vasorelaxation by activating the ATP-sensitive, intermediate conductance, and small conductance K<sup>+</sup> channels through cysteine S-sulfhydration [20]. Most importantly, the vasodilation induced by H<sub>2</sub>S is attributable, at least in part, to activation of K<sub>ATP</sub>. Besides, a major component of endothelium-derived relaxing factor (EDRF) activity derives from hyperpolarization. Glibenclamide markedly reduces the H<sub>2</sub>S precursor sodium hydrogen sulfide (NaHS), elicited vasorelaxation and hyperpolarization, which indicates that H<sub>2</sub>S acts primarily through K<sub>ATP</sub>. Mustafa et al. have confirmed that the H<sub>2</sub>S-induced vasorelaxation through K<sub>ATP</sub> reflects direct effects on vascular smooth muscle, as NaHS relaxation is abolished by certain concentrations of glibenclamide and KCl in endothelium-denuded mesenteric artery [26].

The effects on cell hyperpolarization in intact and endothelium-denuded mesenteric arteries are not mediated by K<sub>ATP</sub>, but by the combination of intermediate- and small-conductance Ca<sup>2+</sup> activated K<sup>+</sup> channels (IK<sub>Ca</sub>/SK<sub>Ca</sub> channels), as hyperpolarization is completely blocked by selective IK<sub>Ca</sub> and SK<sub>Ca</sub> channel inhibitors such as charybdotoxin and apamin [24, 26]. The combination of glibenclamide and charybdotoxin/apamin markedly abolishes all H<sub>2</sub>S-mediated vasorelaxation and hyperpolarization in rat arteries.

The cardiovascular effects of H<sub>2</sub>S include relaxing vascular smooth muscle *in vitro* and inhibiting vascular smooth muscle proliferation and transient hypotension [27–29]. CSE is expressed in peripheral vascular systems, including the aorta, tail artery, mesenteric artery, pulmonary artery, and portal vein in rats, while CBS is undetectable in these blood vessels [29]. H<sub>2</sub>S can be produced by 3MST/CAT pathway in vascular endothelium. Both 3MST and CAT were localized to endothelium [30]. The vascular effects of H<sub>2</sub>S are extremely complex with great species and strain differences.

H<sub>2</sub>S has an inhibitory effect on L-type Ca<sup>2+</sup> currents in normotensive and spontaneously hypertensive rat strains, and it is speculated that this important modulatory effect of H<sub>2</sub>S may contribute not only to a reduction in blood pressure, but also to longer term protective effects [31]. The T-type and L-type Ca<sup>2+</sup> channels seem to be the target of H<sub>2</sub>S regulation, while there is evidence that H<sub>2</sub>S raises the intracellular calcium concentration via the activation of L-type Ca<sup>2+</sup> channels [25].

Kubo et al. investigated the inhibitory role of H<sub>2</sub>S on endothelial NO synthase, using sodium hydrogen sulfide (NaHS) as a H<sub>2</sub>S donor and glibenclamide as a K<sub>ATP</sub> channel inhibitor [32]. They showed that low concentrations of H<sub>2</sub>S that caused contractions and high concentrations cause

relaxation in the rat aorta, suggesting that there are two mechanisms for vasorelaxation:  $K_{ATP}$  channel dependent and  $K_{ATP}$  channel independent. The secondary effect of  $H_2S$ -induced vasorelaxation was a decline in blood pressure, which was antagonized by glibenclamide. This study also indicated that NaHS at 30–3000  $\mu M$  directly inhibits endothelial nitric oxide synthase (eNOS) activity in a concentration-dependent manner, causing increased vascular tension [33]. Thus,  $H_2S$  functions appear to be closely related to those of NO [34].

$H_2S$  is also an important endogenous vasorelaxant factor [35]. A study by Zhao et al. on rat aortic tissues both *in vivo* and *in vitro* demonstrated that intravenous injection of  $H_2S$  could provoke a transient but significant decrease in mean arterial blood pressure [9]. This indicates that  $H_2S$  may act as a hyperpolarizing factor, the effect of which was amplified in the endothelium. The direct effect of  $H_2S$  on  $K_{ATP}$  channel currents and membrane potential was magnified in isolated vascular SMCs. A  $H_2S$ -induced increase in  $K_{ATP}$  channel currents would lead to membrane hyperpolarization, resulting in smooth muscle relaxation. The widely accepted hypothesis is that endogenous sources of  $H_2S$  are present in vascular tissues. Furthermore, NO has been implicated in angiogenesis by many reports, and it can trigger the generation of  $H_2S$  by upregulating the expression of CSE. Therefore, NO appears to be a physiological modulator of the endogenous production of  $H_2S$  by raising CSE expression and stimulating CSE activity in vascular tissues.

NO donors upregulate expression and activity of CSE in vascular tissues and cultured aortic SMCs. NO inhibition and subsequent vascular tension are magnified by endogenous  $H_2S$ , which may contribute to circulatory regulation under physiological conditions [9]. The vasorelaxation induced by  $H_2S$  comprises a minor endothelium-dependent effect and a major direct effect on smooth muscles. This differs from the effects of NO, which only acts on smooth muscles. Conceivably, these two gases may function as a molecular switch for regulating vascular tone. This may be of therapeutic interest for various types of heart disease [9, 32].

Current evidence suggests that  $H_2S$  plays an important role in brain functions. It plays a neuromodulatory role in maintaining the balance of excitation and inhibition by a series of ion channel and receptor-mediated effects, which results in upregulation the  $\gamma$ -amino butyric acid (GABA) B receptor (GABABR) and increased  $K^+$  conductance. It has also been shown to be important for fine-tuning inhibitory neurotransmission.

Moreover, possible physiological functions of  $H_2S$  include long-term potentiation through activation of NMDA receptors, regulating the redox status, and inhibiting oxidative damage through scavenging free radicals and reactive species. Together, this indicates that  $H_2S$  has a positive impact on protecting neurons from oxidative stress in both extracellular and intracellular microenvironments. It can also fine-tune the inhibitory impact on hyperpolarizing neurons by increasing  $K^+$  efflux via  $K_{ATP}$  channels or through stimulation of the postsynaptic receptors that generate long-lasting inhibitory postsynaptic potentials [36].

Endogenous  $H_2S$  is a novel neuromodulator and transmitter in the brain.  $H_2S$  is also involved in pathologies of the central nervous system such as stroke and Alzheimer's disease. In stroke,  $H_2S$  appears to act as a mediator of ischemic injuries and thus inhibition of its production has been suggested to be a potential therapeutic approach.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used anti-inflammatory drugs but they have significant side effects, such as gastrointestinal ulceration and bleeding, allergy, and coagulation disorder. NSAIDs are, therefore, limited in their application.

There is an emerging evidence that physiological concentrations of  $H_2S$  can modulate inflammatory processes or even exert a range of anti-inflammatory effects and accelerate healing by downregulating inflammatory responses [37]. In addition,  $H_2S$  donors have been shown to reduce edema formation and leukocyte adherence to the vascular endothelium and to inhibit proinflammatory cytokine synthesis.  $H_2S$  donors can also increase the resistance of the gastric mucosa to injury and accelerate repair. The  $H_2S$  generating enzymes are constitutively expressed in many tissues and their expression can be upregulated in a variety of conditions, including at the site of injury. Several studies have demonstrated that physiological concentrations of  $H_2S$  produce anti-inflammatory effects, whereas higher concentrations, which can be produced endogenously in certain circumstances, exert pro-inflammatory effects. However, these inhibitory effects can be reversed by glibenclamide, suggesting the actions are mediated through  $K_{ATP}$  channels. In rats,  $H_2S$  donors can suppress leukocyte adherence to the vascular endothelium induced by superfusion of mesenteric venules with the pro-inflammatory peptide, formyl-methionyl-leucyl-phenylalanine (fMLP) [38, 39].

### 3. Mitochondrial Function in Diseases

Mitochondrial dysfunction plays a vital role in many human disease because of the important roles of mitochondria in cellular metabolism. DNA mutation, hypoperfusion, and generation of ROS may be key factors in the induction of mitochondrial damage and dysfunction [40–42]. Mitochondrial diseases include neurological disorders, myopathy, diabetes, and multiple endocrinopathy [43]. Diseases caused by mtDNA mutation, including Kearns-Sayre syndrome, MELAS syndrome, and Leber's hereditary optic neuropathy, are always passed down from the mother because of the mtDNA in the ovum [35]. Diseases such as Kearns-Sayre syndrome, Pearson's syndrome, and progressive external ophthalmoplegia are caused by large-scale rearrangement of mtDNA, while diseases such as MELAS syndrome, Leber's hereditary optic neuropathy, and myoclonic epilepsy with ragged red fibers are caused by point mutations in mtDNA [43].

In many diseases such as Friedreich's ataxia, hereditary spastic paraplegia, and Wilson's disease, genetic defects lead to dysfunction of mitochondrial proteins [44]. These diseases are always dominantly inherited. In some other diseases, such as coenzyme  $Q_{10}$  deficiency and Barth syndrome, oxidative

phosphorylation enzymes are mutated [43]. In addition, environmental factors have also been reported to cause mitochondrial diseases [45].

Many seemingly unrelated diseases such as Alzheimer's disease, Parkinson's disease, stroke, cardiovascular disease, and diabetes mellitus may be caused by a common factor: ROS [46–49]. Mitochondrially mediated oxidative stress plays an important role in cardiomyopathy induced by type 2 diabetes, including in fatty acid-induced mitochondrial uncoupling, mitochondrial ROS production, mitochondrial proteomic remodeling, impaired mitochondrial calcium handling, and altered mitochondrial biogenesis [36].

Comparing with exogenous antioxidants, endogenous antioxidants like  $\gamma$ -glutamylcysteinyl GSH are much more promising, because they are our systematic scavengers with no more additional side effects. Nowadays, endogenous messaging molecules including carbon monoxide (CO), NO, and H<sub>2</sub>S are attracting more and more attention worldwide. Taking hydrogen sulfide for instance, it itself can function as an antioxidant while it regulates the dynamic equilibrium between GSH and glutathione disulfide by enhancing GSH production at the same time increase GSH uptake [50]. Besides, low concentration of hydrogen sulfide will activate the protection effect of NO via other pathways. With deeper investigation of these signaling molecules, practical and harmless methods for scavenging ROS will appear in the future.

#### 4. The Role of H<sub>2</sub>S in Mitochondrial Function

ATP, which contains high-energy phosphate bonds, is produced in mitochondria and the cytosol via glycolysis, substrate-level phosphorylation, and oxidative phosphorylation. With hydrolysis of the phosphate bond, energy is released. Many photoautotrophic and chemoautotrophic bacteria and certain animals use sulfide as an energy substrate. H<sub>2</sub>S can improve mitochondrial ATP production in SMCs with impaired ATP production, especially following hypoxia [51]. It has been demonstrated that H<sub>2</sub>S can drastically reduce metabolic demand, meaning that the metabolism of H<sub>2</sub>S in mitochondria may serve as a means for energy supplementation. H<sub>2</sub>S may function as an energy substrate to sustain ATP production under stress conditions. In other words, in conjunction with hypoxia, H<sub>2</sub>S may help to produce more ATP.

**4.1. Mitochondrial Metabolism.** Under resting conditions, CSE is localized only in the cytosol, but not in the mitochondria of SMCs. Cysteine levels inside mitochondria are approximately three times higher than in the cytosol. However, in response to hypoxia CSE can translocate from the cytosol to mitochondria to confer resistance by increasing ATP synthesis. The promotion of CSE translocation is promoted by increased intracellular calcium levels via the calcium ionophore. Tissue metabolism relying on oxygen supply and oxidative phosphorylation or H<sub>2</sub>S production is greatly dependent on CSE, such as in vascular SMCs. Therefore, the stimuli for CSE translocation to mitochondria

to sustain ATP production under stress conditions may be diverse. Translocation of CSE to mitochondria metabolizes cysteine, produces H<sub>2</sub>S inside mitochondria, and increases ATP production.

**4.2. Inhibition of Cytochrome Oxidase.** Mitochondria are the major source of oxidative stress. Acute oxidative stress causes serious damage to tissues, and persistent oxidative stress is one of the causes of the aging process and of many common diseases, such as cancer [52]. Mitochondria are central to oxidative phosphorylation and are also involved in various aspects of apoptosis. Mitochondrial dysfunction contributes to a wide range of human pathologies. Perturbation of mitochondrial function causes loss of the mitochondrial transmembrane potential and the release of apoptogenic factors. Excessive oxidative damage is a major factor in many cases of mitochondrial dysfunction, because the mitochondrial respiratory chain is a significant source of damage. H<sub>2</sub>S metabolism occurs through three pathways: oxidation, methylation, and reaction with cytochrome C and other metalloproteins or disulfide-containing proteins. The major metabolic pathway for H<sub>2</sub>S is the rapid multistep hepatic oxidation of sulfide to sulfate and the subsequent elimination of sulfate in the urine. Tissues with high oxygen demand, such as the brain and heart, are especially sensitive to disruption of oxidative metabolism by H<sub>2</sub>S. The primary mechanism for the toxic action of H<sub>2</sub>S is direct inhibition of cytochrome oxidase, a critical enzyme for mitochondrial respiration. Human exposure to H<sub>2</sub>S results in concentration-dependent toxicity in the respiratory, cardiovascular, and nervous systems. Inhibition of cytochrome oxidase is the primary biochemical effect associated with lethal H<sub>2</sub>S exposure.

Acute human exposure to relatively low concentrations of H<sub>2</sub>S results in ocular and respiratory mucous membrane irritation leading to nasal congestion, pulmonary edema, and a syndrome known as gas eye, which is characterized by corneal inflammation. Acute human exposure to high concentrations of H<sub>2</sub>S leads to rapid onset of respiratory paralysis and unconsciousness that can result in death within minutes. Persistent sequelae of H<sub>2</sub>S poisoning are often related to the olfactory system and may include hyposmia, dysosmia, and phantosmia. In animals, the olfactory system is especially sensitive to H<sub>2</sub>S inhalation. Acute exposure to moderately high concentrations of H<sub>2</sub>S in rats resulted in regeneration of the nasal respiratory mucosa and full thickness necrosis of the olfactory mucosa.

The release of cytochrome C into the cytosol is an apoptogenic factor that induces cell death. Dorman et al. evaluated the relationship between the sulfide concentrations and cytochrome oxidase activity in target tissues following acute exposure to sublethal concentrations of inhaled H<sub>2</sub>S and examined the toxicokinetics of H<sub>2</sub>S in rats following acute exposure to sublethal concentrations of the gas [53]. Depression of lung cytochrome oxidase activity was observed following exposure to 30 ppm H<sub>2</sub>S, while hind-brain cytochrome oxidase activity was unaffected by H<sub>2</sub>S inhalation. Significant cytochrome oxidase inhibition in the olfactory epithelium occurred after repeated exposure to H<sub>2</sub>S

over five days. Subchronic exposure to 80 ppm H<sub>2</sub>S resulted in reduced cytochrome oxidase activity in the lung but not in the hindbrain. However, lung sulfide concentrations increased during exposure to 400 ppm H<sub>2</sub>S. Lung sulfide concentrations rapidly returned to preexposure levels within minutes after the end of a three-hour period of exposure, suggesting that rapid pulmonary elimination or metabolism of sulfide occurs. Exposure of rats to a low concentration (10 ppm) of H<sub>2</sub>S caused no significant changes in the activities of lung mitochondrial enzymes. However, exposure to sublethal concentrations of H<sub>2</sub>S (50–400 ppm) produced marked and highly significant depressions in the activities of cytochrome oxidase and succinate oxidase complexes of the respiratory chain. Acute exposure to low concentrations (>30 ppm) of H<sub>2</sub>S is associated with cytochrome oxidase inhibition in the lung. Inhibition of cytochrome oxidase often occurs in the absence of elevated H<sub>2</sub>S levels in tissue [53, 54].

**4.3. H<sub>2</sub>S as a Brain Neuroprotectant.** H<sub>2</sub>S protects neurons from oxidative stress by increasing the levels of GSH, a major intracellular antioxidant [10]. In oxidative glutamate toxicity, when extracellular concentrations of glutamate are increased, the import of cystine in exchange for glutamate by the cystine/glutamate antiporter is decreased. Since cystine is reduced to cysteine in cells for the synthesis of GSH, a decline in cystine import leads to a depression in the synthesis of GSH. H<sub>2</sub>S protects cells from oxidative stress by three mechanisms: by enhancing the production of GSH, by raising the levels of cystine/cysteine transporters, and by redistributing the localization of GSH to mitochondria [10].

Since H<sub>2</sub>S is a reducing substance and cysteine is present in plasma and blood at certain concentrations, H<sub>2</sub>S may inhibit the reaction of reducing cystine into cysteine in the extracellular space and increase the transmembrane transport of cysteine into cells for GSH production. Increased cysteine transport contributes to a greater extent to the synthesis of GSH. Increased GSH production by H<sub>2</sub>S is prominent under conditions of oxidative stress caused by glutamate. H<sub>2</sub>S increases the production of GSH and its redistribution to mitochondria. Also, its production in mitochondria may result in suppressing oxidative stress.

To determine whether the protective effect of H<sub>2</sub>S is effective, one should not only examine for glutamate toxicity but also for other markers of oxidative stress. In cerebral tissues, glutamate is not solely responsible for producing neuronal damage. The effect of H<sub>2</sub>O<sub>2</sub>-induced oxidative stress should not be neglected. H<sub>2</sub>S recovers the levels of GSH suppressed by H<sub>2</sub>O<sub>2</sub>, indicating that H<sub>2</sub>S protects cells from a range of oxidative stress stimuli. H<sub>2</sub>S can also reinstate GSH levels in the embryonic brain that have been decreased by ischemia/reperfusion and cystine import suppressed by glutamate.

In summary, H<sub>2</sub>S increases intracellular GSH concentrations by increasing the transport of cysteine to a greater extent than that of cystine. In addition, H<sub>2</sub>S increases the redistribution of GSH into mitochondria. Moreover, H<sub>2</sub>S produced in mitochondria may also contribute to the protection of cells from oxidative stress [10].

**4.4. Decreased Production of ROS following Ischemia/Reperfusion (I/R).** Many studies have shown that the physiological actions of H<sub>2</sub>S make this gas ideally suited to protect the heart, brain, liver, kidney, and lungs against injury during ischemia/reperfusion (I/R) [55]. In the cardiovascular system, numerous roles for H<sub>2</sub>S have been identified, including vasorelaxant and antiapoptotic properties by opening K<sub>ATP</sub> channels, modulating leukocyte-mediated inflammation, upregulating antioxidant signaling, and involvement in cytoprotection through the preservation of mitochondrial function. Endogenous H<sub>2</sub>S and administration of exogenous H<sub>2</sub>S have now been demonstrated to be cytoprotective in various organ systems through diverse signaling mechanisms. There is a significant decrease in H<sub>2</sub>S and an increase in plasma creatine levels in rats subjected to ischemia, indicating that H<sub>2</sub>S levels drop along with kidney function during ischemia.

The life of a cell is partly dependent on the degree of mitochondrial functionality. During I/R, mitochondria are subjected to oxygen deprivation, ROS overproduction, and mitochondrial membrane potential depolarization. Mitochondria are central to oxidative phosphorylation and most metabolic processes and are also involved in many aspects of cell death. ROS is one of the major causes of acute and chronic diseases. H<sub>2</sub>S at high levels can induce a state of hypothermia in mice by inhibiting cytochrome oxidase, which decreases their metabolic rate and core body temperature. This effect of suspension can prevent ischemic damage to cells. During myocardial ischemia, the production of ROS is accelerated and all cellular antioxidants become depleted. H<sub>2</sub>S is a cytochrome oxidase inhibitor and therefore inhibits respiration. Inhibition of respiration has been shown to decrease the production of ROS. We are only just beginning to understand the role of H<sub>2</sub>S in I/R injury. In addition, H<sub>2</sub>S can decrease the production of ROS and preserve mitochondrial function at low concentrations. Therefore, H<sub>2</sub>S acts to preserve mitochondrial function, thereby imparting cytoprotection. Under physiological conditions, ROSs are generated in cells, and increased ROS levels induce I/R damage in cardiomyocytes. The regulation of ROS levels during I/R is associated with the cardioprotection of H<sub>2</sub>S by inhibiting oxidative stress [55, 56].

The mitochondrial respiratory chain is the main source of ROS during energy metabolism. The production of ROS increases during pathological conditions, such as I/R injury to the heart. However, excessive ROSs have a pivotal role in the pathogenesis of myocardial I/R injury [55, 57]. In addition to pathways that generate ROS, the pathways that scavenge ROS, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), have an important role in regulating the levels of ROS in cardiomyocytes [58]. In the ROS scavenging pathways, superoxide is converted to H<sub>2</sub>O<sub>2</sub> by SOD, and H<sub>2</sub>O<sub>2</sub> is subsequently reduced to H<sub>2</sub>O and O<sub>2</sub> by CAT and GPx [59–62]. SOD can be activated in cardiomyocytes treated with H<sub>2</sub>S [63, 64]. However, both CAT and GPx are not activated by H<sub>2</sub>S. When the levels of ROS were decreased by H<sub>2</sub>S in mitochondria under I/R, mitochondrial cytochrome oxidase activity was inhibited and the activities of superoxide dismutases (SODs)

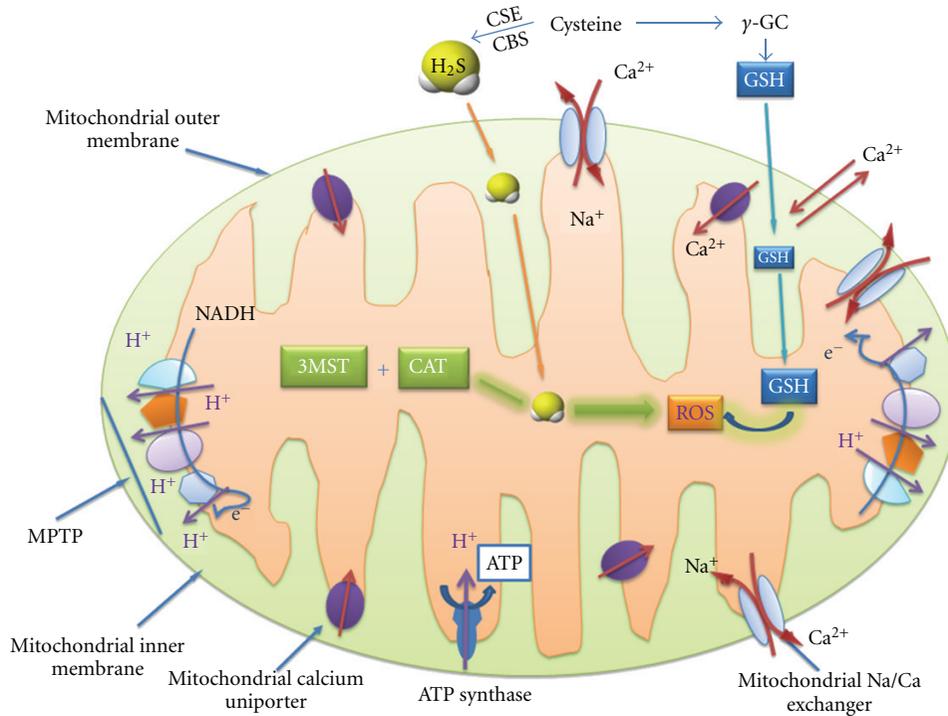


FIGURE 1: H<sub>2</sub>S can improve mitochondrial ATP productions that have impaired ATP production. The enhancement of GSH production by H<sub>2</sub>S is prominent under conditions of oxidative stress caused by glutamate. H<sub>2</sub>S increases the production of GSH and its redistribution to mitochondria.

were increased [65]. By regulating pathways that generate and scavenge ROS, H<sub>2</sub>S decreases ROS levels to protect cardiomyocytes during cardiac I/R [55]. These results suggest that H<sub>2</sub>S can also inhibit electron transport, thus reducing harmful ROS generation. Besides its regulatory role, H<sub>2</sub>S also inhibits mitochondrial cytochrome oxidase and activates SOD to decrease the levels of ROS in cardiomyocytes during I/R [65, 66].

## 5. Conclusion

The activation of K<sub>ATP</sub> channels, which are found in mitochondrial as well as plasmalemmal membranes, contributes to myocardial protection against I/R injury. H<sub>2</sub>S effects include control of respiratory chain ROS release, control of apoptosis, and promotion of GSH availability in mitochondria (Figure 1). H<sub>2</sub>S causes vasorelaxation and inhibits oxidative damage and acts as an endogenous modulator in various tissues. Although several different roles of H<sub>2</sub>S under physiological conditions have been indicated, most mechanisms of H<sub>2</sub>S activity are yet to be fully understood.

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