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Review Article

Hydrogen Sulfide as an Endogenous Modulator in Mitochondria and Mitochondria Dysfunction

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Hydrogen sulfide (H_2S) 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_2S has recently received much interest as a physiological signaling molecule. H_2S 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_2S is an endogenous neuromodulator and present studies show that physiological concentrations of H_2S 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_2S in mitochondria have many favorable effects on cytoprotection.

1. Introduction

Hydrogen sulfide (H_2S) is well known as a transparent, toxic gas with the characteristic strong smell of rotten eggs [1]. In nature, H_2S 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_2S 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_2S 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_2S levels [5]. Under normal circumstances, H_2S does not accumulate, which means that under physiological conditions, endogenous H_2S is not toxic to cells.

Recent evidence clearly indicates that mammalian tissues can also produce H_2S through an endogenous synthetic system, that consists primarily of two enzymes, cystathionine β-synthase (CBS; EC 4.2.1.22) and cystathionine γ-lyase (CSE; EC 4.4.1.1) [6, 7]. The amino acid L-cysteine is a major substrate for H_2S synthesis. Recent studies in humans show that H_2S can also be synthesized from endogenous substrates in the gastrointestinal tract [8]. Measurement of H_2S 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_2S 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_2S 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_2S 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_2S as a gasotransmitter and modulator in both central and peripheral systems will be discussed below, along with the unique role that H_2S plays in mitochondria.

2. Basal H_2S Generation and Metabolism, and Its Physiological and Pathophysiological Functions

2.1. Enzymes That Produce Endogenous H_2S. Three enzymes have been identified that produce endogenous H_2S: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfur transferase (3MST). These three enzymes all produce H_2S 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_2S, 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_2S produced by 3-MST are bound in the form of sulfane sulfur, one of the forms in which endogenous H_2S 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_2S, while a lack of cysteine may lead to a nonspecific decrease of H_2S.

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_2S 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_2S level in cultured astrocytes was more than sevenfold higher than that of microglial cells [15]. H_2S levels drop when CBS inhibitors such as hydroxyl amine and aminooxyacetate are induced. Inflammatory activation of astrocytes and microglia can also decrease the expression of CBS, leading to a decrease in H_2S in the brain. These findings indicate that endogenous H_2S in the brain is mainly produced by CBS, and that modulation of the expression of CBS can change the level of H_2S. 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-α (TGF-α) 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_2S interacts with and can act synergistically with NO in vasorelaxation, suggesting that H_2S 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_2S from cysteine in the brain [10]. In brain homogenates of CBS knockout mice, H_2S can still be detected, which suggests the existence of another H_2S 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 α-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_2S under physiological conditions [7, 20]. Aspartate is another substrate for CAT, that can competitively combine with CAT and suppress H_2S 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_2S to bound sulfane sulfur, while such activity in CBS is weak [7]. 3MST can also be found in the thoracic aorta. 3MST, CAT, and α-ketoglutarate can all be found in endothelium, which suggests that H_2S can be produced in the endothelium.

2.2. Storage and Release of H_2S. The major cellular sources of H_2S and the mechanism of H_2S release remain unknown, although several possibilities have been proposed. Two forms of sulfur that can release H_2S have been detected and methods have been developed to measure the levels of free H_2S.

Basal levels of free H_2S must be kept low because frequent exposure to relatively high concentrations of H_2S leads to desensitization of the response to H_2S. Some endogenous H_2S 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_2S can be stored are acid-labile sulfur and bound sulfane sulfur. However, many unanswered questions regarding the stored form of endogenous H_2S still remain [7].

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

release H_2S at approximately pH 5.4, which suggests that it is not a physiological source of H_2S [22]. Besides acid conditions, acid-labile sulfur also releases H_2S when the enzymes are treated with detergents and protein denaturants, because iron-sulfur complexes are unstable and readily release H_2S when detached from enzymes.

In contrast to acid-labile sulfur, bound sulfane sulfur releases H_2S 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_2S 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_2S produced by 3MST/CAT is mainly stored as bound sulfane sulfur. Most exogenously applied H_2S are also stored as bound sulfane sulfur [7].

Several methods have been developed to measure free H_2S under various conditions with relatively high accuracy [23]. Monobromobimane binds to thiols when mixed with brain homogenates, making it possible to determine H_2S levels by using mass spectroscopy to measure the amount of monobromobimane bound to H_2S [7]. Using this method, it is possible to determine the free H_2S concentration in specific tissues. Furthermore, if brain homogenates are mixed with phosphate buffer, H_2S stored in acid-labile sulfur form can be released and measured in addition to free H_2S. A method for measuring H_2S derived mainly from acid-labile sulfur has been widely applied. For this method, homogenates are treated with N,N-dimethyl-p-phenylenediamine sulfate and $FeCl_3$ in high concentrations of HCl, resulting in the production of methylene blue, which can subsequently be measured. In this method, only H_2S 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_2S under reducing conditions, so cells treated with dithiothreitol (DTT) will easily release H_2S from this source.

2.3. Peripheral Functions of H_2S. Some studies claim that H_2S inhibits human recombinant Ca^{2+}-activated K^+ channels (BK_{Ca}) and native BK_{Ca} 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_{Ca} channels by H_2S is of fundamental physiological importance to carotid body function. However, another report has indicated that H_2S increased the activity of BK_{Ca} channels expressed in a rat pituitary cell line, leading to hyperpolarization and relaxation of smooth muscle cells (SMCs) [24, 25].

T-type Ca^{2+} channels are a unique class of voltage-gated Ca^{2+} channel. Regulation of T-type Ca^{2+} channels is an important feature of both acute and chronic pain sensations. H_2S 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_2S production. However, no detailed electrophysiological investigation of the modulation of T-type Ca^{2+} channels by H_2S has been performed [24].

H_2S produced via CSE has been shown to relax vascular smooth muscle via the opening of ATP-sensitive K^+ (K_{ATP}) channels, which may contribute significantly to the regulation of blood pressure. H_2S 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^+ channels through cysteine S-sulfhydration [20]. Most importantly, the vasodilation induced by H_2S is attributable, at least in part, to activation of K_{ATP}. Besides, a major component of endothelium-derived relaxing factor (EDRF) activity derives from hyperpolarization. Glibenclamide markedly reduces the H_2S precursor sodium hydrogen sulfide (NaHS), elicited vasorelaxation and hyperpolarization, which indicates that H_2S acts primarily through K_{ATP}. Mustafa et al. have confirmed that the H_2S-induced vasorelaxation through K_{ATP} 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_{ATP}, but by the combination of intermediate- and small-conductance Ca^{2+} activated K^+ channels (IK_{Ca}/SK_{Ca} channels), as hyperpolarization is completely blocked by selective IK_{Ca} and SK_{Ca} channel inhibitors such as charybdotoxin and apamin [24, 26]. The combination of glibenclamide and charybdotoxin/apamin markedly abolishes all H_2S-mediated vasorelaxation and hyperpolarization in rat arteries.

The cardiovascular effects of H_2S 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_2S can be produced by 3MST/CAT pathway in vascular endothelium. Both 3MST and CAT were localized to endothelium [30]. The vascular effects of H_2S are extremely complex with great species and strain differences.

H_2S has an inhibitory effect on L-type Ca^{2+} currents in normotensive and spontaneously hypertensive rat strains, and it is speculated that this important modulatory effect of H_2S 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^{2+} channels seem to be the target of H_2S regulation, while there is evidence that H_2S raises the intracellular calcium concentration via the activation of L-type Ca^{2+} channels [25].

Kubo et al. investigated the inhibitory role of H_2S on endothelial NO synthase, using sodium hydrogen sulfide (NaHS) as a H_2S donor and glibenclamide as a K_{ATP} channel inhibitor [32]. They showed that low concentrations of H_2S 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 μM 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 γ-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 γ-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_2S 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_2S 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_2S can improve mitochondrial ATP production in SMCs with impaired ATP production, especially following hypoxia [51]. It has been demonstrated that H_2S can drastically reduce metabolic demand, meaning that the metabolism of H_2S in mitochondria may serve as a means for energy supplementation. H_2S may function as an energy substrate to sustain ATP production under stress conditions. In other words, in conjunction with hypoxia, H_2S 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_2S 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_2S 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_2S 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_2S 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_2S. The primary mechanism for the toxic action of H_2S is direct inhibition of cytochrome oxidase, a critical enzyme for mitochondrial respiration. Human exposure to H_2S 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_2S exposure.

Acute human exposure to relatively low concentrations of H_2S 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_2S leads to rapid onset of respiratory paralysis and unconsciousness that can result in death within minutes. Persistent sequelae of H_2S 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_2S inhalation. Acute exposure to moderately high concentrations of H_2S 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_2S and examined the toxicokinetics of H_2S 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_2S, while hindbrain cytochrome oxidase activity was unaffected by H_2S inhalation. Significant cytochrome oxidase inhibition in the olfactory epithelium occurred after repeated exposure to H_2S

over five days. Subchronic exposure to 80 ppm H_2S 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_2S. 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_2S caused no significant changes in the activities of lung mitochondrial enzymes. However, exposure to sublethal concentrations of H_2S (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_2S is associated with cytochrome oxidase inhibition in the lung. Inhibition of cytochrome oxidase often occurs in the absence of elevated H_2S levels in tissue [53, 54].

4.3. H_2S as a Brain Neuroprotectant. H_2S 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_2S 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_2S is a reducing substance and cysteine is present in plasma and blood at certain concentrations, H_2S 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_2S is prominent under conditions of oxidative stress caused by glutamate. H_2S 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_2S 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_2O_2-induced oxidative stress should not be neglected. H_2S recovers the levels of GSH suppressed by H_2O_2, indicating that H_2S protects cells from a range of oxidative stress stimuli. H_2S 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_2S increases intracellular GSH concentrations by increasing the transport of cysteine to a greater extent than that of cystine. In addition, H_2S increases the redistribution of GSH into mitochondria. Moreover, H_2S 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_2S 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_2S have been identified, including vasorelaxant and antiapoptotic properties by opening K_{ATP} channels, modulating leukocyte-mediated inflammation, upregulating antioxidant signaling, and involvement in cytoprotection through the preservation of mitochondrial function. Endogenous H_2S and administration of exogenous H_2S have now been demonstrated to be cytoprotective in various organ systems through diverse signaling mechanisms. There is a significant decrease in H_2S and an increase in plasma creatine levels in rats subjected to ischemia, indicating that H_2S 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_2S 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_2S 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_2S in I/R injury. In addition, H_2S can decrease the production of ROS and preserve mitochondrial function at low concentrations. Therefore, H_2S 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_2S 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_2O_2 by SOD, and H_2O_2 is subsequently reduced to H_2O and O_2 by CAT and GPx [59–62]. SOD can be activated in cardiomyocytes treated with H_2S [63, 64]. However, both CAT and GPx are not activated by H_2S. When the levels of ROS were decreased by H_2S in mitochondria under I/R, mitochondrial cytochrome oxidase activity was inhibited and the activities of superoxide dismutases (SODs)

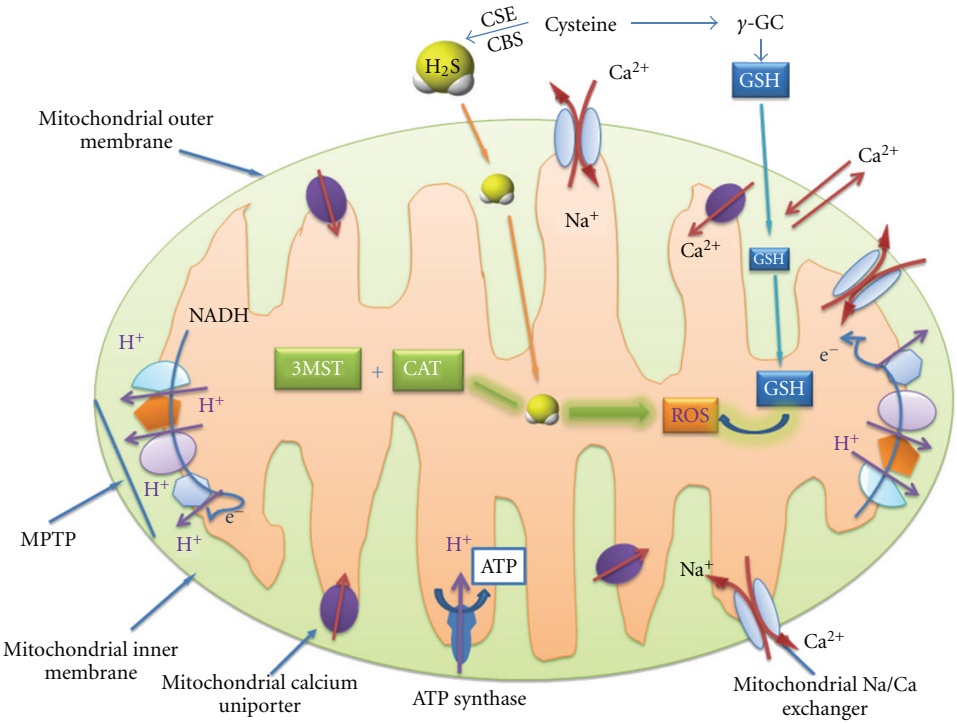

FIGURE 1: H_2S can improve mitochondrial ATP productions that have impaired ATP production. The enhancement of GSH production by H_2S is prominent under conditions of oxidative stress caused by glutamate. H_2S increases the production of GSH and its redistribution to mitochondria.

were increased [65]. By regulating pathways that generate and scavenge ROS, H_2S decreases ROS levels to protect cardiomyocytes during cardiac I/R [55]. These results suggest that H_2S can also inhibit electron transport, thus reducing harmful ROS generation. Besides its regulatory role, H_2S 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_{ATP} channels, which are found in mitochondrial as well as plasmalemmal membranes, contributes to myocardial protection against I/R injury. H_2S effects include control of respiratory chain ROS release, control of apoptosis, and promotion of GSH availability in mitochondria (Figure 1). H_2S causes vasorelaxation and inhibits oxidative damage and acts as an endogenous modulator in various tissues. Although several different roles of H_2S under physiological conditions have been indicated, most mechanisms of H_2S activity are yet to be fully understood.

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