

## Review

# Potential for therapeutic use of hydrogen sulfide in oxidative stress-induced neurodegenerative diseases

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## Abstract

Oxidative phosphorylation is a source of energy production by which many cells satisfy their energy requirements. Endogenous reactive oxygen species (ROS) are by-products of oxidative phosphorylation. ROS are formed due to the inefficiency of oxidative phosphorylation, and lead to oxidative stress that affects mitochondrial metabolism. Chronic oxidative stress contributes to the onset of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). The immediate consequences of oxidative stress include lipid peroxidation, protein oxidation, and mitochondrial deoxyribonucleic acid (mtDNA) mutation, which induce neuronal cell death. Mitochondrial binding of amyloid- $\beta$  (A $\beta$ ) protein has been identified as a contributing factor in AD. In PD and HD, respectively,  $\alpha$ -synuclein ( $\alpha$ -syn) and huntingtin (Htt) gene mutations have been reported to exacerbate the effects of oxidative stress. Similarly, abnormalities in mitochondrial dynamics and the respiratory chain occur in ALS due to dysregulation of mitochondrial complexes II and IV. However, oxidative stress-induced dysfunctions in neurodegenerative diseases can be mitigated by the antioxidant function of hydrogen sulfide (H<sub>2</sub>S), which also acts through the potassium (K<sub>ATP</sub>/K<sup>+</sup>) ion channel and calcium (Ca<sup>2+</sup>) ion channels to increase glutathione (GSH) levels. The pharmacological activity of H<sub>2</sub>S is exerted by both inorganic and organic compounds. GSH, glutathione peroxidase (Gpx), and superoxide dismutase (SOD) neutralize H<sub>2</sub>O<sub>2</sub>-induced oxidative damage in mitochondria. The main purpose of this review is to discuss specific causes and effects of mitochondrial oxidative stress in neurodegenerative diseases, and how these are impacted by the antioxidant functions of H<sub>2</sub>S to support the development of advancements in neurodegenerative disease treatment.

Key words: central nervous system; hydrogen sulphide; mitochondrial dysfunction; neurodegenerative diseases; oxidative stress

## 1. Introduction

Oxygen consumption is essential for cell survival. However, oxygen consumption can cause cell dysfunction and cell death, due to the production of free radicals in mitochondria. Neurodegenerative diseases are caused by excessive free radical generation within neurons, which leads to neuronal cell death in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Oxidative stress

in mitochondria negatively impacts cellular function, as lipids, proteins, and nucleic acids are oxidized by reactive oxygen species (ROS), by-products of the electron transport chain (ETC), and subsequently aggregate in a destructive manner [1]. Additionally, there is an absence of protective histone molecules to protect against ROS because they are routinely generated in the inner mitochondrial membrane (IMM) [2]. Thus, mitochondrial deoxyribonucleic acid

(mtDNA) mutations are caused by excessive ROS formation.

ROS produced in mitochondria comprise hydrogen peroxide ( $H_2O_2$ ), super oxide ( $O_2^{\cdot-}$ ) and hydroxyl ion ( $\cdot OH$ ). In general, oxidative stress occurs when ROS are produced at rates higher than those at which the body can efficiently neutralize reactive metabolites [3]. It has been reported that neurodegenerative diseases may occur as a result of mitochondrial dysfunction [3], such as abnormalities in mitochondrial fusion and fission, increased level of cytoplasmic  $Ca^{2+}$ , DNA mutation, and mitochondrial membrane depolarization. Excessive ROS formation also triggers the accumulation of abnormal proteins that cause neurodegeneration [4]. For instance, oxidative changes in mitochondria may cause protein misfolding in the amyloid- $\beta$  ( $A\beta$ ) protein in AD, which results in a wide variety of pathological symptoms [5]. Oxidative stress has been linked to PD; mitochondrial fusion is inhibited by the accumulation of  $\alpha$ -synuclein ( $\alpha$ -syn) protein in PD patients [6]. In addition, the mitochondrial proteins, PTEN-induced putative kinase 1 (PINK1) and parkin, are both critical for quality control in mitochondria, and are negatively impacted in patients with PD. An expanded level of polyglutamate in huntingtin (Htt) is the major source of oxidative damage in HD [7,8]; mtDNA mutations and structural deformities in the mitochondrial genome are responsible for the pathology of ALS. A mutation in superoxide dismutase 1 (SOD1) leads to overproduction of ROS through overexpression of nitric oxide synthase (NOS), as well as abnormal gliosis involving microglial cells; these changes contribute to the pathology of ALS [9].

Notably, the therapeutic effects of hydrogen sulfide ( $H_2S$ ) can reduce the detrimental impacts of oxidative stress. The antioxidant functions of  $H_2S$  are exerted by its modifications of enzyme activities, including those of glutathione peroxidase (Gpx), SOD, and catalase (CAT) [10]. Gpx acts as intracellular enzyme that converts  $H_2O_2$  to lipid peroxide in mitochondria. Gpx is often referred to as selenocysteine peroxide, and has a key regulatory function in the inhibition of lipid peroxidation; therefore, it protects cells from oxidative stress. In humans, eight enzymes, Gpx1–Gpx8, have been identified; among these, Gpx1 is the most abundant, and Gpx enzymes are tetrameric in nature. The antioxidant properties of all Gpx enzymes can be hindered by low expression, and deficiencies of Gpx enzymes have been associated with oxidative stress [11]. SOD is a very common antioxidant that catalyzes the dismutation of  $O_2^{\cdot-}$  to molecular oxygen ( $O_2$ ) and

increases production of  $H_2O_2$ . Eventually,  $H_2O_2$  decomposes to  $H_2O$  and  $O_2$  [12]. When oxidative stress increases, the SOD concentration also increases. Notably, there are multiple SODs; these include the metalloenzymes, iron (Fe)SOD (homodimer and tetramer forms) and manganese (Mn)SOD (homodimer and homotetramer forms) [13]. Simultaneously, CAT reacts efficiently with hydrogen donors, such as phenols or peroxides, to limit the  $H_2O_2$  concentration in cells; CAT acts as a first-line antioxidant enzyme by mediating the breakdown of millions of  $H_2O_2$  molecules. A high concentration of  $H_2O_2$  is reportedly deleterious to cells [14]. The principal focus of this review is to describe mitochondrial oxidative stress, oxidative stress-induced mitochondrial dysfunctions that are linked to the onset of age-associated neurodegenerative diseases, and advanced regulatory functions of  $H_2S$  against oxidative stress.

## 2. Oxidative stress and mitochondrial dysfunction

Mitochondrial dysregulation was first associated with increased ROS formation in a living organism in 1954 [15]. ROS generation has been related to the onset of age-associated neurodegenerative maladies and cell signaling pathways [16]. Although the presence of a moderate level of ROS is advantageous for cellular function, excessive ROS generation leads to oxidative damage to cellular functions and underlying molecular mechanisms (Figure 1) [15]. Mitochondria are sources of intracellular ROS, which are formed by mitochondrial complexes I and III of the respiratory chain [17]. The metabolic activities of mitochondrial complexes generate oxidative stress by the production of  $O_2^{\cdot-}$  and  $H_2O_2$  (Figure 1). Inhibition or absence of complex I in the respiratory chain causes neuronal apoptosis [18]. For example, mitochondrial complex I is inhibited by 1-methyl-4-phenylpyridinium, a metabolite of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine, which causes cytotoxicity in dopamine neurons [19]. Mitochondrial components also show altered function under oxidative stress. Oxidative stress-induced mutations in mtDNA have harmful effects on mitochondrial function over time. mtDNA mutations result in abnormalities in the oxidative phosphorylation process, which manifests as mitochondrial dysfunction through the loss of cellular function and eventual apoptosis [20]. In addition, 8-hydroxy-2'-deoxyguanosine is a biomarker of oxidative damage and DNA damage due to free radical attack; this indicates defective mitochondrial respiration and impaired antioxidant

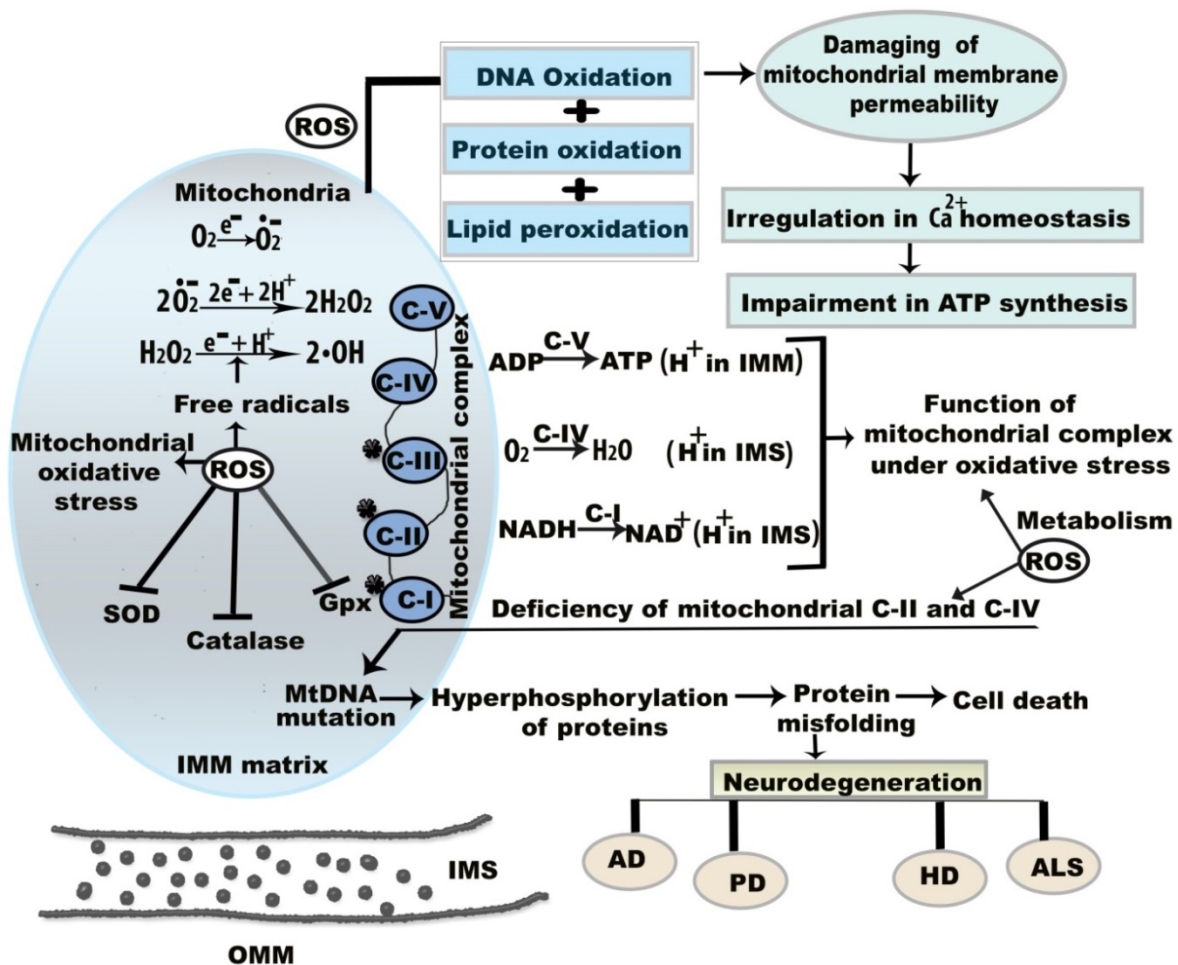
enzymes, and suggests that apoptotic cell death is likely to occur [21,22].

During aging, oxidative stress and mitochondrial dysfunction are associated through the erythroid nuclear factor-related factor 2-antioxidant response element (Nrf2-ARE) pathway. Nrf2-ARE is the master regulatory pathway for redox homeostasis [23]. In the presence of oxidative stress, Nrf2 binds to the ARE. Nrf2 deficiency impacts antioxidant enzymes, thereby causing impaired regeneration in aged skeletal muscle [24]. Coleman *et al.* described that muscle fibers of UCP1-transgenic mice showed impaired mitochondrial respiration. Aged Nrf2 knockout mice reportedly showed increased ROS and 4-hydroxynonenal (4-HNE) in muscle; however, this finding is controversial, as another study reported an altered redox balance due to an increased level of oxidative stress, and stated that there were no clear adverse effects of Nrf2 deficiency [25]. Mitochondrial Bcl-2 family proteins and apoptotic Bax proteins also

play key roles in extrinsic and intrinsic cell death pathways. Cytochrome *c* releases the Bax protein, which results in apoptosis [26].

### 3. Mitochondrial oxidative stress and neurodegenerative disease

Central nervous system (CNS) functions are related to mitochondrial function. Notably, changes in the mitochondrial genome, abnormalities in mitochondrial dynamics, excessive production of ROS, and accumulation of misfolded protein all might contribute to the onset of neurodegenerative diseases [27]. Abnormalities in mitochondrial dynamics and accumulation of metals have been shown to synergistically produce ROS [27]. In particular, AD, PD, HD, ALS, and other neurodegenerative diseases are reported to result from ROS-induced mutations in mtDNA [28].



**Figure 1. Molecular mechanism of mitochondrial oxidative stress and dysfunctions.** Oxidative stress and resultant components incite neurodegeneration through three noteworthy causes including (a) mitochondrial dysregulation, excitotoxicity, and protein aggregation (b) mtDNA mutation, and (c) energy depletion. ROS is generated that reduces membrane permeability between OMM and IMM. This permeability difference disturbs ATP synthesis and calcium (Ca<sup>2+</sup>) homeostasis between the membranes. Under oxidative stress condition, superoxide (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl (•OH) radicals are formed in IMM. Here, the star mark on mitochondrial complex I, II, III indicates that they are more prone to generate free radicals. Abbreviations: ETC, electron transport chain; IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; GPx, glutathione peroxidase, SOD, super oxide dismutase; CAT, catalase. ROS is generated that reduces membrane permeability between OMM and IMM.

Age-regulated genes may impact biological function by either increasing production of ROS or reducing the availability of ATP, which is fundamental for mitochondrial repair; in addition, the absence of ATP can cause cellular apoptosis [29]. Maharjan *et al.* reported that mitochondria act as an important regulator of cellular apoptosis with respect to neurodegeneration. Defects in the mitochondrial ETC system, deficiency in cytochrome oxidase *c*, and differences in mitochondrial membrane potential can cause disruption of energy metabolism and subsequent apoptosis [30]. For instance, inhibition of mitochondrial complex I in PD and ALS, complexes II and III in HD, and complexes II and IV in AD stimulate disorganized oxidative phosphorylation and result in apoptosis [31]. Furthermore, apoptotic pathways are initiated by caspase activity; caspases are a group of cysteine proteases that regulate apoptosis: caspase-3 was reported to participate in A $\beta$ 1-42-induced apoptosis in SH-SY5Y neuronal cells, based on oxidative stress via metallic reaction [32]. Normally, oxidative damage to cellular components results in altered catalyst function and protein structure [33].

PD is the most prominent neurodegenerative disorder. At the cellular level, PD is associated with an abundance of ROS that results in modified catecholamine digestion due to either altered mitochondrial ETC function or increased iron deposition in the substantia nigra part compacta (SNpc). Apoptosis then occurs because dopamine neurons experience increased vulnerability [6]. Moreover, O<sub>2</sub><sup>-</sup> radicals are formed as a result of insufficient oxidative phosphorylation in mitochondria which is the principal cause of ROS formation.

In HD, the underlying reason for oxidative damage is the presence of mutant Htt, which contributes to ROS production in both neuronal and non-neuronal cells [34]. Iron disorders may underlie oxidative stress in affected cells; these disorders include increased accumulation of ferritin, which is the main form of cellular iron, due to altered iron homeostasis [35]. In HD, mutant Htt binds to p53; subsequently, increased levels of p53 and associated transcriptional factors cause increased depolarization of mitochondrial membrane potential [36]. SOD1 has generally been identified as a cytoplasmic protein and is located in the outer mitochondrial membrane, intermembrane space, and IMM; SOD1 mutations are suspected to constitute the oxidative stress-induced factor in the onset of ALS. Notably, mutant SOD1 was proposed to result from increased levels of O<sub>2</sub><sup>-</sup> which can cause oxide to deliver peroxyxynitrite; this negative

feedback system suppresses SOD1 functional capacity [37]. SOD1 has generally been identified as a cytoplasmic protein and it is located in the outer membrane of mitochondria (OMM), IMS, and IMM, where SOD1 mutation is considered as the oxidative stress-induced factor in ALS.

H<sub>2</sub>S neutralizes ROS and ROS-induced mitochondrial damage in neurodegenerative diseases, and could be harnessed to achieve progressive therapeutic outcomes for oxidative stress-affected neurons, as described in the following sections.

## 4. H<sub>2</sub>S

### 4.1 Synthetic precursors and metabolism of H<sub>2</sub>S

H<sub>2</sub>S is endogenously produced from pyridoxal phosphate (PLP)-dependent enzymes in mammalian tissues and the normal level of H<sub>2</sub>S for both plasma and tissue is 50–160  $\mu$ M [38]. The H<sub>2</sub>S-producing enzymes are cystathionine  $\beta$  synthase (CBS), cystathionine  $\gamma$  lyase (CSE), cysteine aminotransferase, and a zinc-dependent enzyme, 3-mercaptopyruvate sulfurtransferase (3MST) [39]. Among these enzymes, CBS is highly expressed in the hippocampus and cerebellum, which are components of the CNS. CBS is a precursor protein, which is regulated by transforming growth factor  $\alpha$  and cyclic adenosine monophosphate [40]. CSE is generally considered to be present in endothelial cells, but has recently been observed in microglial cells, cerebellar granular neurons, and spinal cord [38]. CSE produces H<sub>2</sub>S, as well as pyruvate and ammonia byproducts, by catalyzing L-cysteine. Chiku *et al.* reported that CSE-mediated  $\alpha$  and  $\beta$ -elimination of L-cysteine produced a yield of 70% of the physiological level of H<sub>2</sub>S [41]. However, approximately 90% of the physiological level of H<sub>2</sub>S is derived from  $\alpha$ ,  $\gamma$ -elimination of homocysteine. In the presence of PLP, CSE activity is reduced because of increased Ca<sup>2+</sup> concentration [42]. An additional source of H<sub>2</sub>S is bound sulfane sulfur, where intracellular sulfur is stored in the absence of GSH and cysteine [43]. Bound sulfur is produced by 3MST; L-cysteine and  $\alpha$ -ketoglutarate combine to serve as the source of 3MST [43].

H<sub>2</sub>S metabolism occurs during mitochondrial oxidation. Sulfide is oxidized to elemental sulfur in the presence of quinone oxidoreductases (SQRs). During reduction of cysteine disulfides, SQRs produce cysteine disulfides and persulfide groups [44]. Each persulfide is oxidized by sulfur deoxygenase (SDO), thus producing sulfite (H<sub>2</sub>SO<sub>3</sub>) [44]. Oxygen consumption is necessary during H<sub>2</sub>S metabolism (Table 1) and one mole of oxygen is

consumed for each mole of H<sub>2</sub>S oxidized in the ETC system [45].

In contrast to CBS and CSE, 3MST is primarily present in kidney, liver, and cardiac cells, where it is mainly located in mitochondria; H<sub>2</sub>S is also produced in mitochondria. Recent studies have shown that, in the presence of 3MST, brain homogenates of CBS knockout mice produced levels of H<sub>2</sub>S similar to those of wild-type mice.

**Table 1: Synthetic precursors and metabolites of hydrogen sulfide (H<sub>2</sub>S)**

H <sub>2</sub> S produced enzymes	Substrates	Synthesized products
<b>H<sub>2</sub>S synthesis</b>		
CSE	L-Cysteine	Pyruvate, H <sub>2</sub> S, ammonia
CSE	L-Cystathionine	L-Cysteine, α-ketobutyrate, ammonia
CSE	L-Homocysteine	α-ketobutyrate, H <sub>2</sub> S, ammonia
CBS	L-Homocysteine, L-cysteine	L-Cystathionine, H <sub>2</sub> O
CBS, CSE	L-Homocysteine, L-cysteine	L-Cystathionine, H <sub>2</sub> S
CBS, CSE	L-Cysteine,	L-lanthionine, H <sub>2</sub> S
CBS, CSE	L-Homocysteine	L-Homolanthionine, H <sub>2</sub> S
CAT	L-Cysteine, glutamate	3-Mercaptopyruvate, α-ketoglutarate
3MST	3-mercaptopyruvate	3-Mercaptopyruvate
DAO	D-cysteine	Pyruvate, H <sub>2</sub> S
<b>H<sub>2</sub>S Metabolism</b>		
SQR	H <sub>2</sub> S	SQR persulfide
Rhodanese	Oxidized GSH, SO <sub>3</sub> <sup>2-</sup>	GSH, SSO <sub>3</sub> <sup>2-</sup>
Thiosulfate reductase	SSO <sub>3</sub> <sup>2-</sup> , GSH	SO <sub>3</sub> <sup>2-</sup> , H <sub>2</sub> S
Sulfite oxidase	SO <sub>3</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>

CSE, cystathionine γ lyase; CBS, cystathionine β synthase; CAT, cysteine aminotransferase; 3MST, 3-mercaptopyruvate sulfurtransferase; DAO, diamine oxidase; SQR, sulfide quinone oxidoreductase; GSH, glutathione.

## 4.2 Antioxidant and antiapoptotic functions of H<sub>2</sub>S

H<sub>2</sub>S provides enzymatic antioxidant function by mediating the activities of Gpx, SOD, and CAT. Gpx is the most common H<sub>2</sub>S-mediated antioxidant derivative, which acts through reduction of peroxides [46]. The antioxidant function of Gpx involves production of non-biological thiols when •OH radicals are present; these are less likely to cause oxidative damage than H<sub>2</sub>O<sub>2</sub>, which is highly reactive and has deleterious effects [47,48].

SODs play major antioxidant roles, especially against O<sub>2</sub><sup>•-</sup>. Generally, SODs exhibit three isoforms: cytoplasmic copper (Cu)/zinc (Zn) SOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular Cu/Zn SOD (SOD3). O<sub>2</sub><sup>•-</sup> is modified by SOD enzymes during cell signaling [49]. The importance of each SOD as an antioxidative agent is illustrated by the pathophysiology of CNS degenerative diseases. Initially, SOD converts O<sub>2</sub><sup>•-</sup> to H<sub>2</sub>O<sub>2</sub>; then, H<sub>2</sub>O<sub>2</sub> is

converted to H<sub>2</sub>O by CAT or Gpx (Figure 2). Increased SOD1 activity elevates H<sub>2</sub>O<sub>2</sub> levels, such that they become toxic [49]. The catalytic activities of SOD1 involve reduction and reoxidation of Cu and Mn at the active site of the enzyme; these comprise regulators of O<sub>2</sub><sup>•-</sup> proportion [50]. SOD1 and SOD2 both reduce the incidence of H<sub>2</sub>O<sub>2</sub>-induced oxidative damage [51].

CAT catalyzes H<sub>2</sub>O<sub>2</sub> to O<sub>2</sub> and H<sub>2</sub>O. H<sub>2</sub>O<sub>2</sub> participates in H<sub>2</sub>S metabolism in hypoxia, suggesting that H<sub>2</sub>O<sub>2</sub> is an effective electron receptor in this reaction [52]. Generally, CAT generates H<sub>2</sub>S from carbonyl sulfide, cysteine, GSH, or oxidized GSH, and serves as a sulfur oxidase or sulfur reductase. In the presence of the CAT inhibitor, sodium azide (NaN<sub>3</sub>), H<sub>2</sub>O<sub>2</sub> significantly expedites H<sub>2</sub>S metabolism (Figure 2). Apoptotic signals by caspase-1 and caspase-3 are sequentially activated in SOD1 mutant mice: caspase-1 is active at an early stage and caspase-3 is active in the final stage of cell death.

## 5. H<sub>2</sub>S functions in Ca<sup>2+</sup> and K<sub>ATP</sub>ion channels

In the CNS, intracellular Ca<sup>2+</sup> plays key roles in both normal and pathological signaling. H<sub>2</sub>S has been found to promote increased Ca<sup>2+</sup> levels in neurons, astrocytes, and microglial cells. In serotonergic neurons, a biphasic response is produced by H<sub>2</sub>S during depolarization [39]. In addition, plasma membrane voltage-gated channels are activated by H<sub>2</sub>S, including T-type channels, whereas L-type Ca<sup>2+</sup> channels are expressed in neurons and secrete both neurohormones and neurotransmitters [53]. The action of H<sub>2</sub>S on L-type Ca<sup>2+</sup> channels were demonstrated through a study of the effects of the L-type channel-specific blocker, nifedipine, in rat cerebellar granule neurons [38]. Recently, H<sub>2</sub>S was discovered to enhance stimulation of Ca<sup>2+</sup> entry via L-type channels; this Ca<sup>2+</sup> was shown to participate in neurotransmitter release and gene expression. Furthermore, T-type channels have a role in somatic pain; they act against high-voltage gated channels or have a low activation threshold. T-type Ca<sup>2+</sup> channels are present in hippocampal CA1 cells, thalamic neurons, and Purkinje cells in the cerebellum [54]. Additionally, H<sub>2</sub>S activates the Ca<sub>v</sub>3.2T-type channel isoform, which regulates rhythmic neuronal function and neuronal differentiation [55]. Furthermore, physiological concentrations of H<sub>2</sub>S mobilize intracellular Ca<sup>2+</sup> storage in various cells (Figure 3). Intracellular Ca<sup>2+</sup> storage participates in long-term potentiation in neurons and facilitates the release of glutamate from presynaptic terminals [55].

K<sub>ATP</sub> channels are considered primary molecular

targets for H<sub>2</sub>S. Generally, K<sub>ATP</sub> channels aid in neurotransmitter release from presynaptic neurons, control seizures, and provide neuroprotection in hypoxic conditions [56]. H<sub>2</sub>S hyperpolarizes neurons in the CA1 by K<sup>+</sup> efflux through ATP-dependent K<sub>ATP</sub> channels, which are opened as a result of oxidative glutamate toxicity [57]. By opening K<sub>ATP</sub> channels, H<sub>2</sub>S increases GSH levels. H<sub>2</sub>S is also present in immortalized mouse hippocampal cells, where it facilitates the opening of ATP-dependent K<sub>ATP</sub> channels [58]. Overall, Ca<sup>2+</sup> channels and K<sub>ATP</sub> channels contribute to H<sub>2</sub>S-mediated cell signaling.

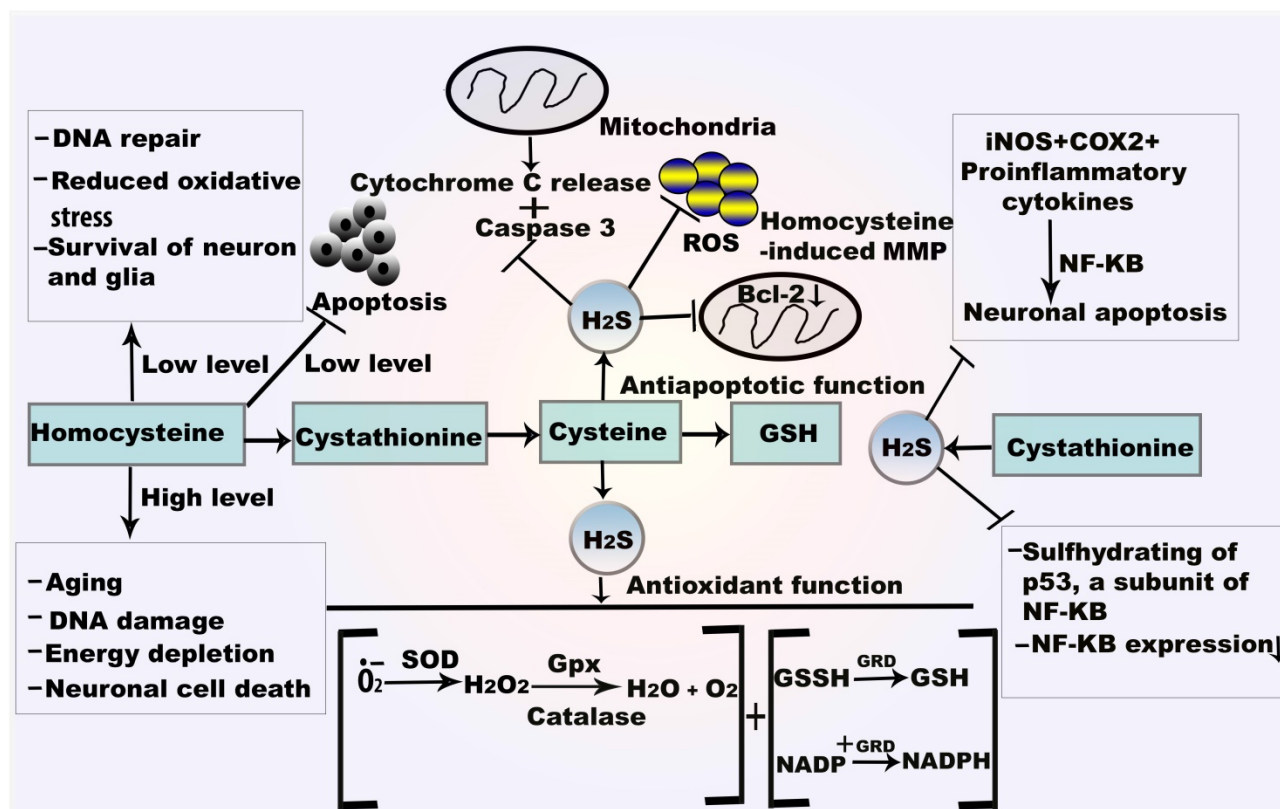
## 6. Neuroprotective potential of hydrogen sulfide as antioxidant:

Progressive loss of neurons is responsible for neurodegenerative disease. H<sub>2</sub>S acts as an effective antioxidant to fight against oxidative stress in neurodegenerative diseases, through the action of H<sub>2</sub>S donors or enzymatic antioxidant mechanisms (Figure 4).

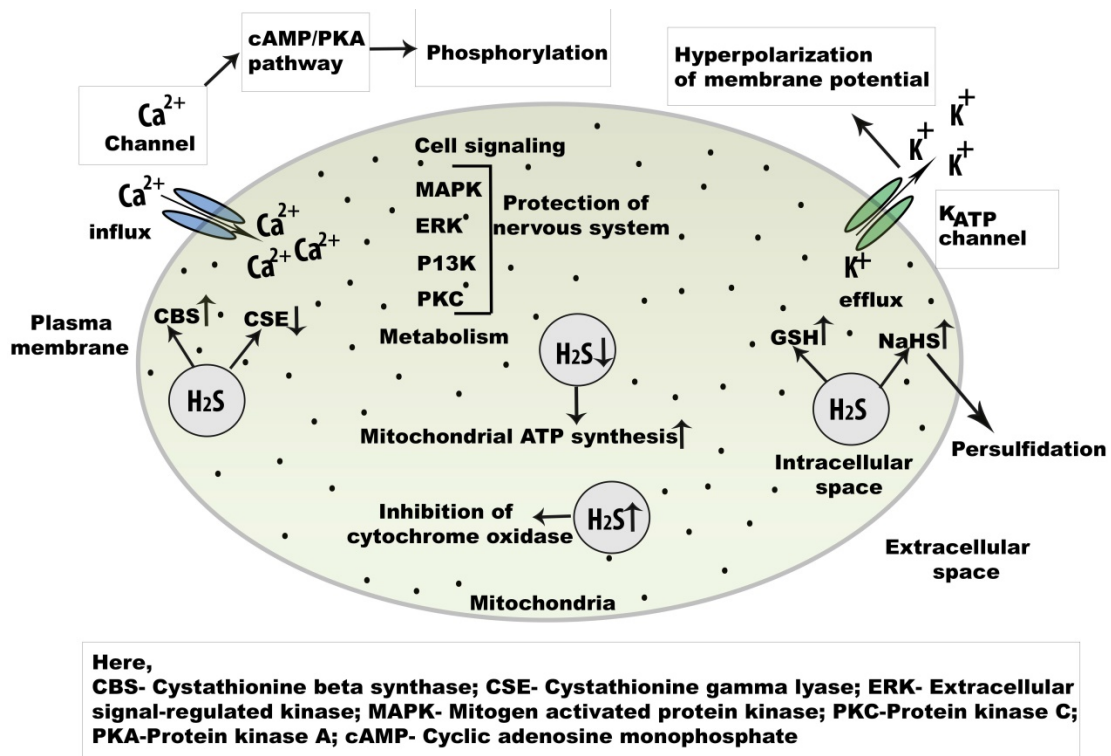
### 6.1 AD

As a gas transmitter, the antioxidant function of H<sub>2</sub>S in AD is vital. General hallmarks of AD include the mutation of amyloid precursor protein (APP) and

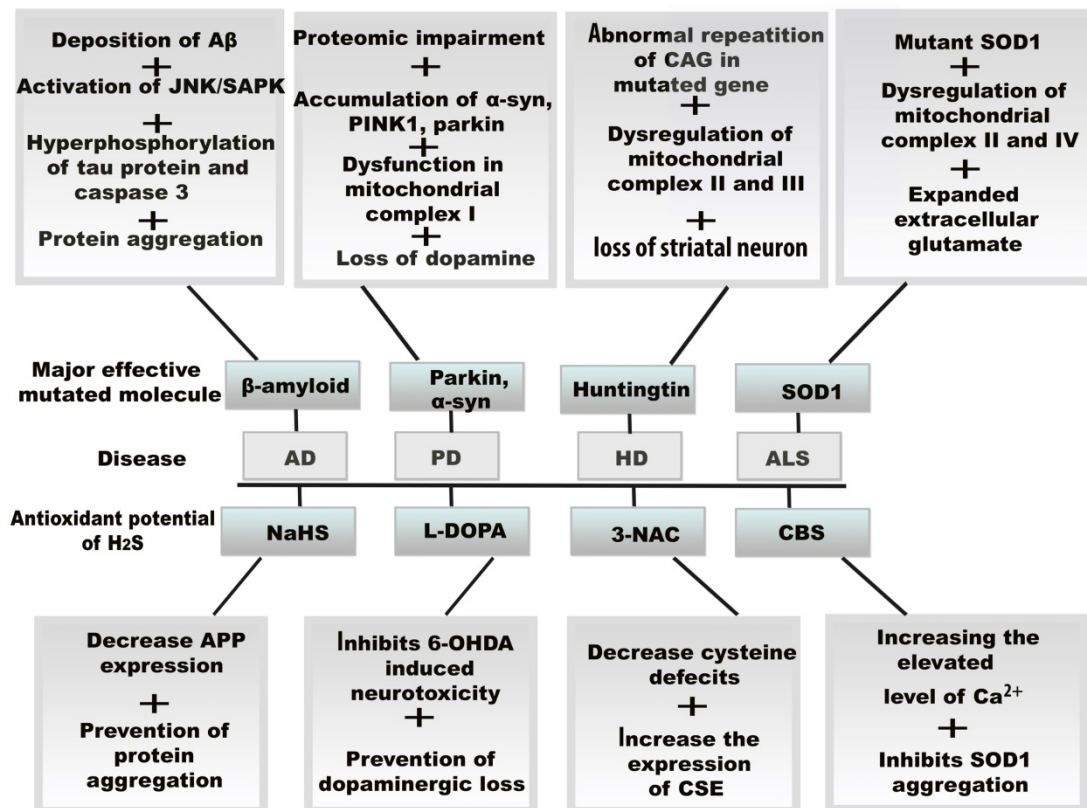
aggregation of both A $\beta$  and tau proteins. According to a clinical study, elevated homocysteine levels were decreased and excitatory amino acid transporter 3 (EAAT3/EAAC1) inhibited the GSH level [59]. Increased expression of H<sub>2</sub>S through Nrf2 indicates that MDA and 4-HNE are generated as a result of reduced homocysteine. Here, Nrf2 is the central mediator of redox balance. In addition, intraperitoneal injection of sodium hydrosulfide (NaHS) in experimental APP/PS1 mice causes downregulation of beta-secretase 1 (BACE1) through the p13/Akt pathway; notably, BACE1 is responsible for the production of A $\beta$  peptides. NaHS is an H<sub>2</sub>S donor that has been shown to decrease A $\beta$  plaques and increase spatial memory [60]. Moreover, NaHS reduces phosphorylation of APP and tau proteins at critical sites and diminishes morphological damage, including damage mediated by neuronal death [61]. NaHS acts against homocysteine-induced cognitive dysfunction [62]. Parker *et al.* showed that mitochondrial complexes II and IV were deficient in hippocampal neurons of AD patients. When H<sub>2</sub>O<sub>2</sub> causes mitochondrial membrane damage and excess Ca<sup>2+</sup>, cellular GSH regulates intramitochondrial protein thiols and selective membrane permeability [63].



**Figure 2. Mechanism of H<sub>2</sub>S in autoxidation and antiapoptosis.** GSH reacts with oxygen free radical which directly form the thiol radical and later GSSH. SOD catalyses the dismutation of O<sub>2</sub><sup>•-</sup> and converted to H<sub>2</sub>O and O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> is also attenuated by the catalysis of CAT and Gpx. H<sub>2</sub>S also provides antiapoptotic function by NF- $\kappa$ B and caspase 3. H<sub>2</sub>S from CSE plays role in sulfhydrating the p65 subunit of NF- $\kappa$ B at cysteine 38. Abbreviations: GSSH, oxidized glutathione; GRD, glutathione reductase; NF- $\kappa$ B, nuclear factor kappa B.



**Figure 3. Cell signaling regulation of endogenous H<sub>2</sub>S in the central nervous system.** Physiologically, H<sub>2</sub>S is an important signaling molecule and regulate L and T type Ca<sup>2+</sup> channel. As a key regulator of Ca<sup>2+</sup> signaling in neuron, the concentration of NaHS is increased. The activation of cAMP/PKA may open the Ca<sup>2+</sup> channel initiates phosphorylation which helps to open several Ca<sup>2+</sup> channels. Besides, cAMP/PKA pathway, cell signaling is mediated by MAPK, ERK, PI3K, and PKC pathways. *Abbreviations:* NaHS, sodium hydrogen sulphide; cAMP, cyclic adenosine monophosphate; MAPK, mitogen activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; ERK, extracellular regulatory kinase.



**Figure 4. Resultant effects of mitochondrial oxidative stress and therapeutic potential of H<sub>2</sub>S in neurodegenerative diseases.** The vital role of H<sub>2</sub>S against oxidative stress, the amplifying H<sub>2</sub>S level induces several molecular changes in neurodegenerative diseases by the increasing and decreasing the enzymes including CBS, CSE, and 3-MST. H<sub>2</sub>S also exerts its antioxidant function by binding drug molecule and activating protein precursors. *Abbreviations:* Aβ, amyloid β; AD, Alzheimer’s disease; ALS, amyotrophic lateral sclerosis; CBS, cystathionine β synthase; CSE, cystathionine γ lyase; 3-MST, 3-mercaptopyruvate sulfurtransferase; HD, Huntington disease; PD, Parkinson’s disease; L-DOPA, levodopa; 6-OHDA, 6-hydroxydopamine.

CBS in the CNS and CSE in the cardiovascular system are sources of endogenous H<sub>2</sub>S generation. In the brain, 3MST is also a significant source of H<sub>2</sub>S. Reduced expression levels of CBS and 3MST have been observed in neurons, such as rat PC12 cells, upon exposure to NaN<sub>3</sub>; conversely, H<sub>2</sub>S suppresses NaN<sub>3</sub>-induced oxidative stress [64]. Moreover, dysfunction of CBS in the trans-sulfuration pathway may reduce H<sub>2</sub>S generation in AD. Furthermore, S-adenosyl-L-methionine, an activator of CBS, is lower in AD brains than in those of normal individuals.

## 6.2 PD

H<sub>2</sub>S has also a potential role in the neuromodulation of PD. To eliminate oxidative elements, continuous Gpx action is needed to recycle reduced GSH to its oxidized form. Overexpression of CBS or H<sub>2</sub>S donors provides neuroprotection against 6-hydroxydopamine-induced neurotoxicity [62]. H<sub>2</sub>S signaling is affected by the E3-ubiquitin ligase, parkin, which is a misfolded protein in PD. The main targets for sulfhydration on parkin are cys95, cys59, and cys182 [62]. Importantly, 6-hydroxydopamine is widely regarded as the factor responsible for the death of dopaminergic neurons through dopamine uptake transporters. Two H<sub>2</sub>S donors, ACS84 and ACS50, have the greatest contributions as antioxidants. ACS84 exerts L-3,4-dihydroxyphenylalanine (L-DOPA)-mediated effects in PD, such that it can penetrate the blood brain barrier (BBB) and release H<sub>2</sub>S [65]. Because homocysteine is a precursor of H<sub>2</sub>S, the plasma level of homocysteine can be used to assess the effects of H<sub>2</sub>S in PD in the context of a particular drug treatment. L-DOPA is a potent anti-PD medication that alleviates symptoms by maintaining the dopamine concentration at the synapse and reducing motor fluctuations [66]. Approximately 15–20% of patients do not respond to L-DOPA therapy and may show adverse profiles after long-term therapy [67]. According to a clinical study by Obeid et al., 87 patients showed high levels of total homocysteine (t-homocysteine) with increased levels of APP and  $\alpha$ -synuclein [68]. A case-control study from Nigeria described 80 individuals, 40 of whom were healthy controls, while the remaining 40 were PD patients of the same age group with high levels of homocysteine who received L-DOPA mediated treatment [69]. L-DOPA mediated changes in homocysteine have revealed key regulatory functions in oxidative stress-induced neurological damage [70].

## 6.3 HD

Polyglutamate repeats in the Htt protein cause transcriptional dysfunction in motor neurons in the

HD mouse model and human HD brain during cysteine metabolism when CSE is depleted in cell culture. Reduced CSE expression causes lower levels of cysteine; as a result, H<sub>2</sub>S levels are reduced and ROS generation is increased in mitochondria (Figure 4) [62,71].

CBS might be a useful target for the treatment of neurodegeneration in HD. In a recent study, hyperhomocystinuria was observed in HD patients, as compared to controls, because the mutated Htt protein modulates homocystinuria-induced CBS activity. Moreover, HD patients are affected by both cardiovascular and cerebrovascular diseases [72]. Andrich et al. reported the concentration (17.7  $\mu$ mol/l) of homocysteine in 34 HD patients treated with antidepressants, neuroleptics, benzodiazepines, and/or tetrabenazine, compared to the concentrations in untreated HD patients (12.6  $\mu$ mol/l) and 73 healthy controls (13.3  $\mu$ mol/l). In that study, untreated HD patients were less severely affected and had shorter disease duration than the treated patients, which indicates a positive correlation between the plasma level of homocysteine and untreated HD [73]. In HD, cytosolic CSE is depleted at the transcriptional level and could reflect the translocation of CSE to insoluble aggregates. In Q111 cells, CSE was depleted to a similar extent in both supernatant and particulate fractions. Generally, striatal Q111 cells showed greater susceptibility to H<sub>2</sub>O<sub>2</sub> stress. mHtt also reportedly binds to and inhibits specific protein 1 (SP1); CSE depletion in HD seems to reflect inhibition of Sp1 by mHtt, leading to reduced CSE transcription [74].

## 6.4 ALS

H<sub>2</sub>S can counteract oxidative modification through insoluble SOD1 aggregation, which is a common feature of ALS. Free cysteine in SOD, specifically at Cys111, is responsible for SOD1 mutation in ALS (Figure 4). However, H<sub>2</sub>S provides an antioxidant function through elevation of CBS [62]. The G93A (fALS) mouse model reportedly exhibited increased H<sub>2</sub>S generation in tissues and spinal cord, along with increased intracellular Ca<sup>2+</sup> levels. In addition, elevated H<sub>2</sub>S was also identified in the CSF fluid of ALS patients, which suggests gasotransmitter signaling in ALS [62]. Posttranslational modification of SOD1 may enable formation of toxic aggregates. In a phase III clinical trial of ALS patients, ceftriaxone upregulated the GLT-1 (EAAT-2) glutamate transporter, this may have corrected glutamate levels. Another phase III clinical trial reported that high doses of methylcobalamin (vitamin B-12) reduced homocysteine levels in ALS patients [75].

An investigation of the levels of CBS-containing



lanthionine (a thioether analogue of cysteine) in ALS showed that LanCL1 levels were elevated by three-fold in SOD1<sup>G93A</sup> mice. In contrast, immunoblot analysis of spinal cord lysates from mice overexpressing wild-type human SOD1 indicated altered LanCL1 expression [76]. Therefore, CBS-targeting treatment in ALS is not yet clearly defined as a therapeutic approach. Further investigation is necessary regarding CBS-targeting treatment in ALS.

In summary, H<sub>2</sub>S exhibits protective effects in neurodegenerative diseases through antioxidant functioning. Although H<sub>2</sub>S neutralizes harmful oxidative modification in neurodegenerative diseases, additional *in vivo* studies are needed to elucidate molecular mechanisms in oxidative stress.

## 7. Pharmacological effects of H<sub>2</sub>S

The pharmacological effects of H<sub>2</sub>S are exerted by inhibition of H<sub>2</sub>S/H<sub>2</sub>S donors or augmentation of endogenous H<sub>2</sub>S; many experimental models have demonstrated the protective effects of H<sub>2</sub>S or potential targets of H<sub>2</sub>S donors in neuromodulation, hypertension, and inflammation [44]. Although some experimental studies show harmful effects of H<sub>2</sub>S, these are controversial. For instance, sulfide salts comprise donors of H<sub>2</sub>S that may have H<sub>2</sub>S-independent effects. In contrast, lower H<sub>2</sub>S levels may lead to reduced expression levels of CBS and CSE inhibitors, known as genetic inhibition. CBS and CSE inhibitors may also cause H<sub>2</sub>S-independent effects through genetic inhibition, such as cysteine deficiency due to hyperhomocysteinemia and enhanced GSH synthesis. Finally, abnormalities have been observed in mice in which CBS, CSE, or 3MST have been knocked out [77].

Sulforaphane (SF) is a derivative of H<sub>2</sub>S, synthesized from isothiocyanate, which causes enhanced expression of CBS and CSE [78,79]. Moreover, *in vivo* experiments have shown that cell signaling pathways, such as p38 MAPK and JNK, are activated by SF. After absorption, SF is conjugated with GSH by glutathione s-transferase [79]. In terms of bioavailability, the plasma concentration and metabolic components increased and reached the highest levels after 1 and 3 hours, respectively. The urinary excretion of SF drugs within 12–14 hours reflects rapid elimination [80]. Experimental studies have shown that SF-Cys and SF-N-acetyl cysteine (NAC) also exert some bioactivity. In neurodegenerative disorders, SF is observed as combined metabolites (e.g., SF-GSH, SF-Cys, and SF-NAC). SF has also shown poor ability to cross the BBB, but reaches the CNS very rapidly [79].

Among cysteine derivatives, S-propyl-cysteine (SPC), S-allyl-cysteine (SAC), and S-propargylcysteine (SPRC) are good substrates from which CBS and CSE can produce H<sub>2</sub>S. SPC, SAC, and SPRC are administered to reduce lipid peroxidation and increase the activation of GSH, SOD, and Gpx [81]. SPRC reduces NF-κB activity, decreases ROS production, and inhibits the TNF-α-induced inflammatory response [82]. According to Wang *et al.*, SPC, SAC, and SPRC all increased H<sub>2</sub>S generation by at least two-fold at the carbon terminal, as measured in homogenized rat ventricles. H<sub>2</sub>S increased in the hippocampus of lipopolysaccharide-treated rats in a dose-dependent manner [44]. A major pathway by which H<sub>2</sub>S protects against cellular damage is the Nrf2-dependent signaling pathway [83].

The pharmacological activity of H<sub>2</sub>S-releasing drugs in cell signaling has been assessed by *in vitro* studies. Studies of H<sub>2</sub>S-releasing drug *in vivo* are more difficult than *in vitro* studies due to physiological and pathological conditions. To determine more fully the pharmacological effects of H<sub>2</sub>S-releasing drugs, further research is necessary.

## 8. Conclusion

Neurons have the capacity for cell-cell communication. When this communication fails, symptoms of neurodegenerative diseases occur. As discussed above, mitochondrial damage is connected to the pathogenesis of neurodegenerative diseases. Protein damage, DNA mutations, and membrane permeability are vulnerable to oxidative damage, which plays a pathogenic role in AD, PD, HD, and ALS. Generally, mitochondrial homeostasis is maintained by various protein structures and functions are not identical among proteins. However, it remains unclear how the harmful effects of oxidative stress are mediated in specific neuronal diseases. Identification of specific disease-related proteins, to discern relationships between specific proteins and mitochondrial oxidative stress, can be achieved through further broad studies.

Mitochondrial dysfunction due to ROS formation is a prominent feature of neurodegenerative diseases, dysfunctional characteristics should be mitigated through the protective effects of the H<sub>2</sub>S gasotransmitter. Furthermore, the details of cellular responses of H<sub>2</sub>S to ROS-mediated oxidative stress must be explored. To identify the therapeutic potentials of H<sub>2</sub>S, particular enzyme inhibitors are needed, based on their abilities to augment gasotransmitter synthesis. The cytoprotective effect of H<sub>2</sub>S as a signaling molecule against ROS, as well as cell-specific enzymatic activities (e.g., CBS, CSE, and 3MST), may

add further protection against neurodegenerative diseases.

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## Competing Interests

The authors have declared that no competing interest exists.

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