Exploring mitochondrial hydrogen sulfide signalling for therapeutic interventions in vascular diseases

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Highlights

1. H_2S is a powerful signalling molecule with a diverse physiological role.

2. As a positive modulator of mitochondrial electron transport chain, H_2S interact with many redox reactions and induce post translational modification to proteins via sulfhydration.

3. With the growing interest and research in the H_2S donors and pro-drugs hold a promising future for treating vascular diseases.

Abstract

Hydrogen sulfide (H_2S), a gaseous signalling molecule, is important in numerous physiological and pathophysiological processes. Despite its initial identification as an environmental toxin, H_2S is now well described as an essential physiological molecule that is finely balanced to maintain cellular functions, especially in modulating mitochondrial activity. Mitochondria are responsible for the oxidation of H_2S and its safe elimination while maintaining mitochondrial biogenesis. H_2S oxidation in mitochondria generates various reactive sulfur species that could post-translationally modify proteins by sulfhydration. Sulfhydrated proteins participate in many regulatory activities either by direct interactions in the electron transport chain or indirect regulation by epigenetics. These investigations explain the importance of research of H_2S as a therapeutic molecule beyond the traditional understanding as a protective role through its anti-inflammatory and antioxidant properties. This review focuses on highlighting the significant involvement of the H_2S pathway in vascular diseases and current H_2S donors for therapeutic use under development.

1. Introduction

The endothelium is formed by a monolayer of endothelial cells located in the inner layer of the vascular wall and continuously exposed to hemodynamic shearing forces, circulating molecules in the blood and mediators released from underlying smooth muscle cells. Disturbances to the physiological status of the endothelium lead to activated endothelial cells that culminate in vascular dysfunction [1]. Dysfunctional endothelium contribute to a pro-inflammatory and pro-thrombotic phenotype, which causes disturbances to endothelial homeostasis and impair vasodilation-vasocontraction [1, 2]. Consequently, endothelial dysfunction has been described as a hallmark for developing several pathophysiological conditions, including atherosclerosis, diabetes, stroke and ageing-related diseases [1, 3, 4]. Endothelial dysfunction appears to be a consequence, at least in part, of increased production of highly reactive oxygen and nitrogen species, which are byproducts of normal metabolism of oxygen. Endothelial cells are exposed to the highest oxygen levels in the blood and mainly generate ATP via aerobic glycolysis [5]. Therefore, endothelium is less dependent on mitochondrial oxidative phosphorylation than most cells using the tricarboxylic acid cycle, aiding cells to survive in highly oxygenated environment with a controlled production of mitochondrial reactive oxygen species (ROS). In this regulated environment, endothelium maintain the regulation of vascular relaxation and vascular smooth muscle dilation by sustained nitric oxide (NO) production. In addition to NO, there are two more gases: carbon monoxide (CO) [6] and hydrogen sulfide (H_2S) have been subsequently identified as gasotransmitters. Many evidence suggest that the vasoprotective effect of H_2S either as a direct antioxidant or via indirect mechanisms. The study of H_2S as a physiological mediator began with discovering its endogenous production and bioactive properties in mammals [7-9]. Further developments in later years led to the re-evaluation of H_2S from a toxic molecule to a protective gaseous signalling mediator when present at low concentrations [10], putting it on a par with the NO and CO [11, 12].

H_2S can be synthesized by both endothelial cells and vascular smooth muscle cells within the vascular wall. The production of H_2S has shown to 100-1000 fold higher in smooth muscle (nanomolar) as compared to endothelial cell lines (picomolar) allowing H_2S to act as a smooth muscle relaxant [13]. In mammals, the desulfhydration of the amino acid cysteine is considered as a major source of H_2S through the transsulfuration pathway mediated by two cytosolic enzymes, cystathionine-β-synthase (CBS), cystathionine γ-lyase (CSE) and the mitochondrial enzyme, 3-mercaptopyruvate sulfurtransferase (3-MST). Once generated, H_2S is believed to function as a secondary messenger, and its signalling functions have been explored, including its ability to reduce free radicals, interactions with metal centers in the active sites of proteins and post-translational modification of thiol groups in cysteine residues [14, 15]. Whilst the involvement of H_2S has been described in almost every cell and tissue type, its ability to relieve endothelial dysfunction has gained much interest in physiology and pathophysiology [16-18].

2. Biogenesis and metabolism of H_2S

2.2 Biogenesis of H_2S

The intracellular H_2S concentration is maintained at a low steady-state through a balance between its biogenesis and metabolism [19]. In mammals, endogenous H_2S production is

catalysed primarily by the transsulfuration pathway (Figure 1A) [19]. While the pyridoxal-5´-phosphate-dependent CBS and CSE are mainly located in the cytoplasm, the pyridoxal-5´-phosphate-independent 3-MST enzyme resides in both mitochondria and cytoplasmic compartments [20], and utilizes a different enzymatic reactions to produce H_2S. In addition, enzymatic activity of CAT produces 3-mercaptopyruvate, which is subsequently used by mitochondrial 3-MST for H_2S production.

In mammals, the activity of CBS is commonly found in the brain and central nervous system [21, 22]; preferentially expressed in the glia and astrocytes [23]. While CSE is the main H_2S -producing enzyme in the cardiovascular system, H_2S-generating 3-MST enzyme is ubiquitously expressed in all tissues [24]. Unlike CBS and CSE, 3-MST is regulated in a redox-state dependent-manner rather than by transcriptional regulation [25]. 3-MST contains a redox-sensitive cysteine in its active site (Cys^{247}) that is modified to cysteine sulfenate, resulting activity loss under oxidative stress [26]. This also works in favor of restoring cellular redox state by increasing the availability of cysteine to produce cellular antioxidants such as thioredoxin (Trx) and glutathione (GSH), contributing for the cellular redox homeostasis. MST exhibits its activity in combination with another mitochondrial enzyme, cysteine aminotransferase (CAT), and there is a growing interest in the CAT: MST axis for H2S synthesis and cell metabolic rewiring [14, 15]. Especially, H2S synthesis through this pathway is shown to highly dependent on glutathione (GSH) levels [16] interconnecting cysteine bioavailability for protein synthesis and cellular redox status. The potential role of 3-MST and CAT on endothelial cell bioenergetics and metabolism has been recently reported through 3-MST inhibition studies [27]. Silencing 3-MST suppressed the angiogenesis by decreasing mitochondrial respiration, mitochondrial adenosine triphosphate (ATP) production and perturbed the entire endothelial cell metabolome [27].

Although there is an apparent overlap in tissue localization, the activity of these enzymes can vary under specific conditions. For instance, CSE can be translocated to the mitochondria under the regulation of Tom20 in mitochondrial membrane [28]. Since the cysteine level inside mitochondria is about three times that in the cytosol, mitochondrial translocation of CSE increases mitochondrial H_2S production and ATP production [28]. The accumulation of CBS in mitochondria increased H_2S, which prevented cytochrome c release from mitochondria and decreased hypoxia-induced ROS generation [29]. Therefore, endogenous H_2S was identified as an important regulator of energy production in mammalian cells when the oxygen supply is limited, such as in hypoxic conditions. This action of H_2S also observed in hibernating animals where metabolic depression is apparent. In addition to being formed enzymatically from the substrate cysteine, hibernating animals regenerate H_2S from its oxidation products, including thiosulfate and polysulfides [30, 31]. This protective mechanism not only facilitated ATP production during hibernation, but also preserved free cysteine for synthesis of a major antioxidant, glutathione (GSH) [30].

In addition to the above-mentioned enzymes, recent reports show a fourth enzymatic pathway regulated by peroxisomal enzyme, D-amino acid oxidase (DAO) that uses D-cysteine instead of L-cysteine as a substrate to produce 3-mercaptopyruvate (3MP), which is a substrate for 3-MST [24]. Since the expression of DAO is organ specific, the usage of D-cystine to produce H_2S is high in tissues such as kidney and the cerebellum [24, 25]. For example, the production of

H_2S from D-cysteine in the kidney is 60 times higher than that from L-cysteine [25]. Therefore, administration of D-cysteine has been suggested as a therapeutic approach to deliver H_2S to specific tissues [32].

The level of endogenous H_2S production is variable depending on both the tissue and experimental conditions used. Endogenous levels of H_2S have been measured as; 10-15 nM in murine brain, but higher concentrations of 1 µM were observed in the murine aorta [33]. In vascular tissue, the endogenous production in rat aorta and mesenteric artery are inferior compared to the rat tail artery and ileum [34]. In healthy humans, plasma levels were found to be between 70 -125 µM [35]. These variations in the H_2S content suggest that specific tissues demand higher production, which is associated with effective metabolic machinery to avoid the accumulation of the gaseous molecule and ultimately toxicity [36].

2.3 Metabolism and oxidation of H_2S

Mitochondrial enzymes play a central role in the catabolism and oxidation of H_2S, which regulates its steady-state levels. The oxidation of H_2S begins in the mitochondrial matrix and is completed in the inter-mitochondrial membrane space through tightly regulated mitochondrial sulfide oxidation pathway (Figure 1B) [37]. In the mitochondrial matrix, H_2S is oxidised by a cluster of mitochondrial enzymes, otherwise known as sulfide oxidation unit (SOU) that consists of sulfide quinone oxidoreductase (SQR), ethylmalonic encephalopathy 1 (ETHE1 or persulfide dioxygenase, PDO), Thiosulfate sulfurtransferase (TST) and sulfite oxidase (SO) [38, 39]. H_2S is oxidised by SQR to generate a sulfane sulfur (S^0) by forming a persulfide (R-SSH). This reaction causes the release of two electrons, which are transferred to the electron transport chain (ETC) via coenzyme Q (CoQ). Persulfide is then oxidised by ETHE1 to produce sulfite and further oxidised to SO_4^{2-} by SO or to $S_2O_3^{2-}$ by TST [40].

Apart from oxidation, H_2S can also be catabolized through methylation, a cytosolic process that yields methanethiol that can be further methylated to dimethyl sulfide (non-toxic compound) via thiol S-methyltransferase (TSMT) [41]. Metabolically, dimethyl sulfide serves as a substrate for rhodanese forming thiocyanate (SCN^-) and sulfate, which is the major end-product of H_2S clearance [42]. Another H_2S catabolism pathway includes H_2S-scavenging by metalloproteins such as methemoglobin, forming sulfhemoglobin or forming disulfides such as oxidised glutathione (GSSG) [43]. H_2S biosynthesis and oxidation pathways are also connected to other metabolic pathways such as serine biosynthesis, the folate cycle, and the nucleotides metabolism [44]. Therefore, fine-tuned H_2S synthesis and oxidation is important not only to maintain cellular bioenergetics but also for cellular metabolic profile.

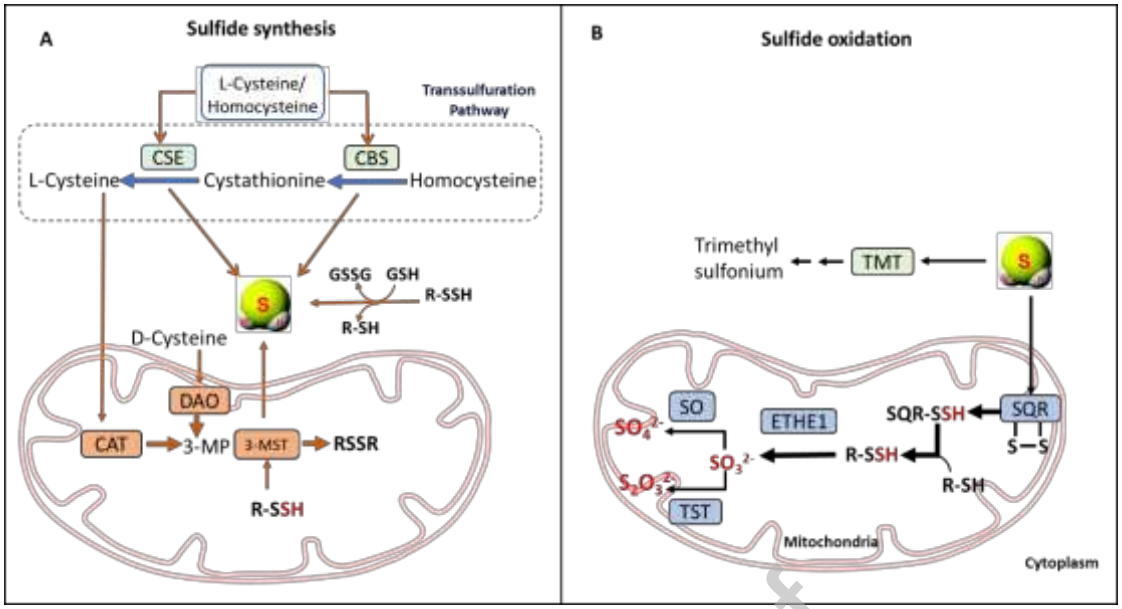

Figure 1: Cellular pathways leading to H_2S synthesis and oxidation. A) CBS catalyses the first step in transsulfuration by converting homocysteine to cystathione, which is subsequently cleaved into cysteine and α-ketobutyrate by CSE. Mitochondrial 3-MST generates H_2S via cysteine aminotransferase (CAT), using L-cysteine and requires the transfer of sulfur from a persulfide (RSSH). Persulfides can also act as a source of H_2S with the aid of reductants such as glutathione (GSH). B) H_2S oxidation occurs in mitochondria. H_2S-reduced SQR generates persulfide by accepting a thiol (R-SH). This persulfide is then oxidised to short-lived sulfite (SO_3^{2-}) via ETHE1 and rapidly convert to thiosulfate ($S_2O_3^{2-}$) and sulfate (SO_4^{2-}) by thiosulfate sulfurtransferase (TST) and SO, respectively.

3. Implications of H_2S on redox balance

It is now evident that there are a number of mechanisms whereby H_2S can potentially influence redox balance. While some reactions occur over a relatively short timescale such as free radical scavenge and interactions with the electron transport chain, other H_2S induced changes might operate on a longer timescale. Long-term effectors of H_2S includes oxygen-sensitive regulation of mitochondrial functions by translocating CSE or CBS under hypoxic conditions [29]. Rapid and long term effectors of H_2S-mediated O_2 sensing in cells and tissue is extensively reviewed in Olson, 2015 [45]. In addition, H_2S-mediated post translational modification of cysteine thiol groups in target proteins via S-sulfhydration (also termed as persulfidation) will be described below.

3.1 H_2S as an antioxidant

The regulatory mechanisms of H_2S on endothelial cells as an antioxidant could be via several pathways; it can quench free radicals as a chemical reductant, increase intracellular antioxidants such as GSH and redoxins or scavenge free radicals by increasing the expression of enzymatic antioxidants such as nuclear factor erythroid 2-related factor 2 (Nrf2) , superoxide dismutase (SOD), CAT or glutathione peroxidase (GPx) [16]. Depending on the local concentration, H_2S can acts as an antioxidant toward ROS and RNS such as hypochlorous acid, hydrogen peroxide, lipid hydroperoxides, superoxide and peroxynitrite [46]. Even though the

calculated rate constants for the reactions between HS- and ROS/ RNS are highly favorable, very low physiological concentrations [47] suggest that H_2S has little importance as a direct antioxidant in humans.

In addition to be a precursor for H_2S, cysteine is also the source of GSH production. H_2S in extracellular space has been shown to increase GSH production by inducing a reduction of cystine into cysteine [48]. H_2S treatments is suggested to enhance γ-glutamyl cysteine synthetase (γ-GCS) activity to increase cellular GSH synthesis and augments the localization of GSH to mitochondria. Since GSH is not synthesised in mitochondria, authors suggested an important role of H_2S by enhancing cystine/cysteine transporters and redistribution GSH to mitochondria [48]. As described before, H_2S induced GSH production also observed in other mammals during hibernation as a protective mechanism against oxidative stress [30].

H_2S administrations has shown to increase other antioxidant proteins such as Trx-1 via Nrf-2 pathway. H_2S-dependent cardioprotection was observed in a mouse model of ischemia-induced heart failure, where Trx-1 is upregulated at both gene and protein levels [49]. H_2S is able to regulate the activity of several members of the sirtuin (SIRT) family that catalyses post-translational modifications of both histone and nonhistone proteins. H_2S increased the expression of SIRT 1 in an oxidative stress induced cardiomyocytes, endothelial cells [50, 51] and induced cardio protection in diabetic rat model [52]. Cardiomyocytes treated with the SIRT1 inhibitors reverted the antioxidant protection induced by H_2S indicating the importance of SIRT pathway in cells [51]. H_2S also shown to induce other members of SIRT family such as SIRT3; a major regulator of mitochondrial function [53] and nuclear located SIRT6 [54], to exert either physiological or pathophysiological effects.

3.2 H_2S and mitochondrial respiratory oxidation

The influence on mitochondrial respiratory chain is one of the main pathways that H_2S helps to maintain cellular redox balance. The absence or reduced ability of O_2 to act as the terminal electron acceptor such as in hypoxic conditions could prevent electron flow along the respiratory chain followed by accumulation of H_2S in mitochondria. A recent report described a genetic defect in SQR gene in patients with Leigh syndrome. Patients presented with abrogated SQR enzyme activity and decreased mitochondrial complex IV activity with intermittent accumulation of H_2S [55]. Excess accumulation of H_2S was also reported in patients with pathogenic variants in ETHE1 [56]. Both CoQ deficient *in vitro* and *in vivo* models caused a reduction in SQR levels and activity leading to an impairment of H_2S oxidation with accumulation of H_2S and depletion of the glutathione system [57]. However, the regulation and interplay between these pathways is still a developing field. Recent studies suggest CoQ supplementation increases SQR expression, thus inducing the H_2S oxidation pathway whilst down-regulating CSE and CBS in the transsulfuration pathway [44].

The pathological consequences of H_2S accumulation are associated with its capacity to exhibit toxicity at high concentrations by binding the copper center and blocking cytochrome oxidase (complex IV), compromising the functioning of the mitochondrial electron transport chain (ETC) [36]. H_2S interacts with ETC via two ways: firstly by transferring sulfide-derived electrons at the level of complex III via the reduced quinone and into the complex IV via reduced

cytochrome c (Cyt c) [58]; secondly by transferring electrons to ETC via Cyt c that bypass the complex III to increase ATP synthesis [59]. During the process of H_2S mediated Cyt c reduction, generation of a mixture of reactive sulfur species (RSS) including $SO_3^{\bullet-}$, $SO_2^{\bullet-}$, $H_2S_2^{\bullet-}$ was observed [58]. Due to their highly reactive nature, these products readily reduce O_2 to form superoxide and then H_2O_2. In turn, H_2O_2 and reactive sulfur species reoxidised Cyt c to its original state for further H_2S removal [58].

3.3 H_2S-Induced protein sulfhydration

Owing to its unique sulfur containing functional -SH, cysteine residues play a ubiquitous role in protein structure and function. Cysteine thiol groups react with ROS, RNS or reactive sulfur species (RSS) to produce various post translational modifications on proteins [60, 61]. While oxidative modifications such as S-nitrosylation (-SNO), S-sulfenylation (-SOH) are reversible modifications, higher modifications (sulfonic acids) result protein or enzyme deactivation [62]. Sulfhydration is a reversible post-translational modification resulting from the conversion of a thiol group (R-SH) to a persulfides (R-SSH) [63]. However, as reductants, H_2S or HS- cannot directly react with protein thiols. Sulfhydration can occur by nucleophilic attack of an HS^- anion on the sulfur atoms of disulfides, -SNOs or -SOH, as well as by transsulfation reactions. These reactive sulfur species (RSS) generated as a result of H_2S oxidation can react with protein thiols to generate protein sulfhydrates (persulfidates). Sulfhydration could change the original function of proteins, serving as important switchers or regulators in many physiological and pathophysiological processes.

3.3.1 Sulfhydration of cytosolic proteins

Research now demonstrates that RSS could sulfhydrate proteins under physiological conditions, accounting for 10-25% of liver proteins, including actin, tubulin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [14]. This highly abundant post-translational modification has shown to affect a variety of cellular signaling and biological pathways (Table 1). Sulfhydration can modify enzymatic activity of proteins, activity of ion channels, nuclear localization of proteins [10]. For example, endothelial nitric oxide synthase (eNOS), which causes vasodilation, can sulfhydrate the same cystine residue (Cys 443) that undergoes nitrosylation. H_2S releasing donors increased eNOS activity by eNOS dimerization via sulfhydration dependent mechanism [64]. Another protein that loses its nitosylation site to sulfhydration in the presence of H_2S is the phosphatase and tensin homolog deleted on chromosome ten (PTEN). PTEN is a phosphatase that suppresses the activity of the class I phosphoinositide 3-kinase/AKT signalling pathway. Sulfhydration of PTEN has shown to inhibit its enzymatic activity under physiological conditions [65]. The regulation of ion channels by means of post-translational modification enables cells to respond to changing environments. Sulfhydration activated ATP-sensitive potassium (K_{ATP}) ion channels that control membrane potential and cellular excitability [66] and Ca^{2+} flux via multiple Ca^{2+} transient receptor potential (TRP) ion channels [67]. H2S-induced sulfhydration has been reviewed previously in relations to its diverse roles in pathophysiological processes [68].

3.3.2 Sulfhydration of mitochondrial proteins

Change to mitochondrial activity were either observed as direct sulfhydration of mitochondrial proteins or through indirect actions of sulfhydrated cytosolic proteins. Módis and collaborators found that sulfhydration of alpha subunit of ATP synthase (ATP5A1) at Cys^{244} and Cys^{294} in response to H_2S exposure [69], where the modification increased ATP synthase activity and stimulated mitochondrial bioenergetics in HEK293 and HepG2 cells. This observation was also confirmed by reduced levels of sulfhydrated ATP5A1 and ATP synthase activity in the livers of CSE knockout mice [69]. While this is an interesting observation, it remains to be examined whether S-sulfhydration of ATP synthase occurs in other cell types and their exact functional role. S-sulfhydration of interferon regulatory factor 1 (IRF-1), a mitochondrial transcriptional factor that is involved in mitochondrial biogenesis pathway, enhances mitochondrial DNA replication and cellular bioenergetics via modification of TFAM promoter [70]. Thereby sulfhydration mediates epigenetic regulation of TFAM which leads to promotion of methylation and maintenance of mitochondrial DNA. SIRTs enzymes and PARPs can utilize NAD^+, a cofactor required for mitochondrial function that is upregulated by H_2S in the vascular endothelium [71]. Sulfhydration of SIRT3 enhance its catalytic activity leading to reduction of mitochondria dysfunction, improvement of mitochondrial ETC performance and ATP production in the vasculature [72] [73]. The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a key regulator of calcium signaling in health and disease. Treatment with H_2S inducer drugs, S-propyl-L-cysteine (SPRC) or sodium hydrosulfide (NaHS), attenuated the development of heart failure in animals and preserved mitochondrial function via sulfhydration of CaMKII at Cys^6 [74]. The intrinsic apoptotic pathway is initiated by the release of Cyt c, a mitochondrial intermembrane space protein, to the cytoplasm. H_2S in known to regulate Cyt c release by maintaining mitochondrial membrane potential ($\Delta\Psi m$) [75]. Vitvitsky et al, 2018 showed that reduction of Cyt c by H_2S increased the formation of RSS allowing the proteins in close proximity, such as procaspase 9 to be sulfhydrated [58].

Nonetheless, it is important to maintain intracellular sulfanesulfur homeostasis. In this context, redox systems such as thioredoxin (Trx)/ Trx reductase (TrxR1) and glutaredoxin (Grx)/glutathione reductase (GR)/GSH play a vital role. Elevated levels of protein sulfhydration was observed in mouse livers where hepatocytes lack both TrxR1 and GR suggesting the importance of reducing systems [76]. The increased Trx levels is associated with HIV-1 infection and these patients had significantly lower total sulfanesulfur levels compared to patients treated with antiretroviral therapy. This evidence suggests a role for the mitochondrial Trx system as regulator of protein sulfhydration [77].

Thus, by enhancing mitochondrial cellular function and bioenergetics via target protein sulfhydration, H_2S helps to protect mitochondria against a variety of stressors. The advent of H_2S releasing drugs, especially mitochondrial-targeted, should permit considerable insight into this field.

General Function	Protein Sulfhydrated	Modified Cysteine	Cellular Effect	Reference
Cell Signalling	eNOS	443	Promotes eNOS phosphorylation and dimerization	[64]
	GAPDH	150, 156, 152	Inhibition of GAPDH catalytic activity and enhanced nuclear translocation	[14] [78]
	PC	265	Promotes gluconeogenesis	[79]
	PTEN	71, 124	Inhibits s-nitrosylation of PTEN	[65]
	PTP1B	215	Inhibits PTP1B activity and thereby promotes PERK activity under ER stress	[80]
	PP1c	127	Promote eIFα phosphorylation resulting in inhibition of general protein synthesis	[81]
	PPARγ	139	Increases PPARγ nuclear accumulation, induce adipogenesis	[82]
	RAGE	259, 301	Prevents RAGE-mediated oxidant pathological effects	[83]
	Sp1	68, 75	Enhances VEGFR2 expression and maintain vascular homeostasis	[84]
	SUR1 subunit of K_{ATP}	6, 26	Activates K_{ATP} channel	[66]
	SUR2B subunit of K_{ATP}	24, 1455	Reduces the tyrosine nitration of Kir6.1	[85]
	TRPV6	172, 329	Induces Ca^{2+} influx and maintains bone homeostasis	[67]
	Hrd1	115	Regulates VAMP3 ubiquitylation and prevents CD36 translocation	[86]
Transcription Factors	SIRT1	NR	Increases its deacetylase activity, reduced its degradation and endothelial inflammation	[87]
	GATA3	84/182, 84/248	Inhibits GATA3 nuclear translocation and differentiation of splenocytes	[88]
	Keap1	151	Activates Nrf2-target signalling and ameliorates cellular oxidative stress	[89-91]
Apoptosis and Cell proliferation	Kir6.1 subunit of K_{ATP} channel	43	Stimulates vasorelaxation and hyperpolarization	[92]
	MEK1	341	Stimulates nuclear translocation of phosphorylated ERK leading to PARP-1 activation and repair DNA damage	[93]
	AR	611, 614	Inhibits AR-DNA binding activity and AR dimerization, which suppress cancer proliferation	[94]
	Akt	77	Contributes to activation of protein kinase GSK3β	[95]
	Caspase 3	163	Inhibits protein activity	[96]
	p65 subunit of NF-KB	38	Anti-apoptotic actions in liver and inhibits ox-LDL-induced macrophage inflammation	[97, 98]
	Runx2	123, 132	Promotes osteoblast differentiation and maturation	[99]
Mitochondrial Function	ATP5A1	244, 294	Stimulates ATP synthase activity	[69]
	DJ-1	106	Prevents irreversible oxidation of DJ-1 to maintain mitochondrial redox balance	[100]
	IRF-1	53	Increases TFAM expression to maintain mitochondrial DNA replication	[70]
	LDHA	163	Stimulates LDH activity by increasing NAD+ generation and enhances mitochondrial bioenergetics	[101]
	PP2A	NR	Inhibits PP2A activity, which leads to AMP kinase activation	[102]
	p66Shc	59	Prevents mitochondrial reactive oxygen species production	[103]

	Parkin	59, 95, 182	Activates E3-ubiquitin ligase activity of parkin, which induces degradation of misfolded proteins and reduces neuron cell death	[104]
	PPRC	NR	Promotes murine mitochondrial biogenesis	[105]
	PGC-1α	NR	Stimulates mitochondrial biogenesis	[105]
	SIRT3	256, 259, 280, 283	Increases deacetylase activity and improves mitochondrial function	[53]
	USP8	NR	Increases association of parkin with USP8, which promotes mitophagy	[106]

Table 1. Sulfhydrated proteins involved in a range of cellular functions. Effects of sulfhydration on the activity of identified proteins involved and its cellular and subcellular function.

4. Therapeutic strategies for H_2S donors

Considering the mounting evidence of potential therapeutic option in a range of conditions, a wide range of commercially available H_2S donors including sulfide-containing salts, H_2S-releasing prodrugs and natural products containing sulfur have been investigated [107]. Common H_2S donors and their specific vascular effects at the subcellular level are summarised in **Table 2.**

4.1 General H_2S donors

Sulfide-containing salts such as sodium hydrogen sulfide (NaHS) and disodium sulfide (Na_2S) are common fast releasing H_2S donors used *in vitro* and *in vitro*. These donors have displayed cytoprotective actions in vascular models through direct modulation of mitochondrial biogenesis and function [105, 108, 109]. Lawesson's reagent has been widely used to generate H_2S-releasing compounds. For example, commonly used GYY4137 (morpholin-4-ium-4-methoxyphenyl phosphinodithioate) is synthesised upon reaction with morpholine [110]. GYY4137 is water soluble and is believed to release H_2S at a controlled rate under physiological conditions. Furthermore, H_2S release from GYY4137 is pH and temperature dependent, with a greater release at acidic pH's and higher temperatures [110]. In vascular endothelium, GYY4137 can protect mitochondria and endothelial cells from oxidative stress [111].

Allium vegetables, such as garlic and onion are known to contain H_2S generating molecules. Garlic contains γ-glutamyl-S-allyl-L-cysteine and S-allyl-L-cysteine sulfoxides able to generate H_2S by chemical transformation [112]. Diallyl disulfide (DADS) and diallyl trisulfide (DATS) are both derived from garlic and act as H_2S donors when they react with biological thiols including GSH. However, whereas cell treatment with DADS observed impaired mitochondrial function [113], DATS reduced mitochondrial ROS production [114].

A novel H_2S prodrug, SG1002 has been observed to promote H_2S bioavailability in heart failure patients [115]. In a murine model with induced transverse aortic constriction, SG1002 preserved cardiac function following activation of a VEGF-Akt-eNOS-NO-cGMP signalling pathway in cardiomyocytes. Furthermore, mitochondrial functions were preserved, oxidative stress was attenuated, and increased myocardial vascular density were observed following the administration of SG1002. Studies demonstrate that H_2S treatments could stimulates endothelial cell proliferation, migration and tube formation *in vitro* [116]. H_2S interventions

were associated with an increase in vascular endothelial growth factor (VEGF) expression and activation of its receptor, stimulating angiogenesis *in vivo* [117].

Previous work by Wang and colleagues demonstrated that the inhibition of CSE results in the increased production of antiangiogenic factors, such as soluble Fms-Like Tyrosine Kinase-1 (sFlt-1) and soluble endoglin (sEng) and the administration of H_2S donors had been shown to suppress sFlt-1 and sEng in endothelial cells [118]. Antiangiogenic factors such as sFlt-1 could inhibit the mitochondrial respiration and promote mitochondrial-specific superoxide production in endothelial cells [119]. Recently we described how H_2S produced by CSE pathway maintains endothelial mitochondrial bioenergetics and loss of CSE increases the production of mitochondrial-specific superoxide [120]. MZe786, the ADTOH H_2S donor linked with aspirin, has been explored and observed to improve outcomes in mice with a compromised CSE pathway and/or an increased expression of sFlt-1. MZe786 was employed in both HO-1 haploid deficient (Hmox1+/−) pregnant mice in a high sFlt-1 environment and a refined reduced uterine perfusion pressure (RUPP) model to mimic preeclampsia in C57Bl/6 J mice [121, 122]. MZe786 was found to correct the induced preeclampsia state by reducing blood pressure and renal damage. Additionally, the overexpression of sFlt-1 has been observed to inhibit cardiac mitochondrial activity in the same Hmox1+/− mouse model [123]. Moreover, MZe786 was found to rescue mitochondrial activity by stimulating cardiac mitochondrial biogenesis and antioxidant defence in Hmox1−/− mice and in Hmox1+/− mice exposed to a high sFlt-1 environment thus improving outcomes.

Sodium thiosulfate in its role as a H_2S producer shows beneficial properties and is currently being investigated in phase I trial for potential benefits in patients with acute coronary syndrome and undergoing coronary angiography [124]. Furthermore, anti-inflammatory and antioxidant effects of thiosulfate are linked to mitochondrial thiosulfate sulfotransferase induced sulfur transfer to GSH and thioredoxin, thus promoting thiol-dependent antioxidative mechanisms [125]. In addition to these H_2S generators, currently available endogenous inducers of H_2S-generating enzymes include S-adenosylmethionine (SAM), epidermal growth factors (EGF), S-nitroso-N-acetylpenicillamine (SNAP) and microRNAs-21 [21, 23, 126, 127]. Recently, it was reported that administration of SAM could potentially inhibit vascular endothelial growth factor-A-related diseases induced by adverse effects of long-term treatment of levodopa (L-dopa) [128].

4.2 Mitochondrial targeted H_2S donors

More recently, special attention has been given to the mitochondrial targeted drugs. Mitochondrial-targeted H_2S donors, including AP39 and AP123, improved mitochondrial function in glucose oxidase-induced oxidative stress in endothelial cells and restored vascular homeostasis [129]. AP39 treatment attenuated endothelial senescence and protects against oxidative stress in acute renal injury and hyperglycemic injury [130, 131]. Both AP39 and AP123 have been observed to decrease hyperpolarisation of the mitochondrial membrane and inhibit mitochondrial oxidant production whilst increasing the electron transport at respiratory complex III thus improving cellular metabolism [132]. Therefore, investigating the role of H_2S on mitochondrial activity in endothelial cells should increase our understanding of future drug targets.

Compound	Model	Target	Outcome	References
Na_2S (10-500 μg kg^{-1}) (1-50 μM)	Mice cardiomyocytes	Mitochondrial ETC, Mitochondrial Complex II	↑ mitochondria function/appearance ↓myocardial infarct size, inflammation, apoptosis	[108]
GYY4137 (12.5-100 μM)	EA.hy926 cells	Redox steady state, SIRT3 expression, MAPK phosphorylation, Antioxidant enzymes	↑endothelial function, mitochondria respiratory capacity/membrane potential ↓oxidative stress, apoptosis	[90]
(100 μM)	HPAEC cells	PI3K pathway and Redox steady state	↑ alveolar network formation, mitochondrial membrane potential ↓oxidative stress, apoptosis	[133]
(0.25 mg kg^{-1} 250 μM)	Mice glomerular endothelial cells	NMDA-R1 subunit, mPTP channel Ca^{2+} channel Mitochondrial cyclophilin D	↓ renal injury, oxidative stress Mitigate Ca^{2+} channel expression Prevent mPTP opening	[134]
NaHS (100-300 μM)	Rat cardiomyocytes	Redox steady state, Antioxidant enzymes, Mitochondrial Complex IV	↓ ischemia/reperfusion injury, oxidative stress	[135]
(50-300 μM)	b.End3 microvascular endothelial cell diabetic rats	Cellular DNA and PARP pathway, Mitochondrial membrane potential, Redox steady state	↑ mitochondrial and cellular function	[109]
(10 μM)	HEK293 cell CSE KO mice	ATP5A1 sulfhydration	↑ mitochondrial bioenergetics	[69]
(30 μM)	CSE KO mice hepatocytes	Mitochondrial DNA and Complex V, Nrf2/1- transcription factors, PPRC, PGC-1β, Tfam expressions/ activity	↑ mitochondrial biogenesis ↓ tissue damage	[105]
(100 μM)	Rat aortic endothelial cells	Redox steady state, Antioxidant enzymes, Apoptosis pathway, Mitochondrial fission/fussion pathway, PINK1/Parkin signalling	↑ mitochondrial membrane potential, mitophagy ↓ oxidative stress, apoptosis	[136]
AP39 (30-300 nM)	b.End3 mice microvascular endothelial cells	Mitochondrial ETC activity and complexes II/III, Redox steady state, LDH pathway	↑ mitochondria activity ↓ oxidative stress, hyperglycemic injury	[130], [129]
(1 μmol kg^{-1})	Sprague Dawley rats	Akt/ eNOS phosphorylation, PTP channel	↓ infarct size, renal inflammation ↑ post-ischaemic recovery	[137]
(10 ng/ml)	HUVECs	SRSF2 and CDKN2A genetic expression, Cytokines expression	↓ inflammation, senescence	[131]
AP123 (30-300 nM)	b.End3 mice microvascular endothelial cells	Mitochondrial ETC activity and complexes II/III, Redox steady state, LDH pathway	↑ mitochondria activity ↓ oxidative stress, hyperglycemic injury	[130]
DADS (1 mM)	CD-1 mice cells	Redox steady state, Antioxidant enzymes	↑lipid peroxidation, mitochondria membrane depolarization, oxidative stress	[113]
50 mg kg^{-1} 25 μM	Sprague-Dawley rats H9c2 cells	PGC1α/Tfam/Nrf2/eNOS expressions, Apoptosis pathway, Antioxidant enzymes, α-skeletan actin expression, Citric acid cycle	↑mitochondrial function/apoptosis/ biogenesis, NO bioavailability ↓oxidative stress, cardiac hypertrophy	[138]
DATS (100 μM)	HUVECs	Redox steady state, Apoptosis pathway, AMPK pathway, mitochondrial dynamic and membrane potential	↑ mitochondria function ↓ mitochondrial apoptosis × mitochondrial fission	[114]
SG-1002 20 mg kg^{-1}	CSE KO mice	Akt/eNOS phosphorylation, VEGF/NOX4/HO-1 expression	↓ oxidative stress, fibrosis, cardiac enlargement, mitochondria function	[139]

20 mg kg^{-1}	CSE KO mice H9c2 cardiomyocytes	AMPK phosphorylation, mitochondrial DNA, PGC-1α pathway	↑ mitochondria respiration/biogenesis/ content	[102]
ATB-337 (10-50 µmol kg^{-1})	Wistar rats	COXs/ TNF-α activity	↓ inflammation. Platelet aggregation, intestinal tissue damage, gastric mucosa injury	[140]
ATB-429 (25-130 mg kg^{-1})	Mice	Cytokine expression	↓ inflammation, severity of colitis	[141]
ATB-346 (16 mg kg^{-1})	Mice	COX-2 activity	↓ intestinal manipulation.	[142]
ATB-352 (4.6-46 mg kg^{-1})	Mice	Involvement of the endogenous cannabinoid system	↓ gastrointestinal toxicity and nociceptive response to noxious stimuli	[143]
SG-1002 (20-40 mg kg-1 day-1)	Mice	VEGF-Akt-eNOS-NO-cGMP signalling pathway	↑ cardiac function	[144]
GIC-1001 (30-60 mg kg-1)	Mice	Activation of peripheral opioids receptors	↓ nociceptive response to noxious stimuli	[145]
MZe786 (20 mg kg-1)	Mice	Rescuing mitochondrial activity	↓ hypertension and renal damage ↑ cardiac mitochondrial biogenesis and antioxidant defence	[121, 146, 147]
Sodium thiosulphate (0.15 g/250 ml over 15 min)	Humans	Anti-inflammatory and antioxidant effects are linked to its reaction with mitochondrial thiosulfate sulfurtransferase	↓ systolic blood pressure an hour after administration ↑ cardio protection	[148]
ACS94 (20 mg kg-1)	Sprague-Dawley male rats	Metabolic pathways relating to Hcys, Cys, GSH (by TSP pathway)	↑ GSH and circulating H_2S ↓ Homocysteine	[149]
ACS6 (10pM-1µM)	PAECs	Adenylyl cyclase-PKA pathway	↑ NADPH, cAMP ↓Superoxide formation and phosphodiesterase type 5 activity	[150]
ACS67 (5 µl of 4 nM solution	RGC-5	Attenuates the process of oxidative-induced RGC-5 cell death	↑ GSH ↓ H_2O_2-induced toxicity	[151]
ACS84 (0.25-10 µM)	BV-2, neurons isolated from hippocampus of Sprague Dawley rats	Phosphorylation of p38- and JNK	↑ Mitochondria function ↓ cytotoxicity and inflammation	[152]

Table 2: H_2S donors. Summary of H_2S donors and their effects on cellular function with a focus on mitochondrial activity. ↑ Denotes activation or increase, ↓ Denotes attenuation or decrease

5. Involvement of H_2S in vascular diseases

Endothelial dysfunction is ubiquitously observed in both arteries and small vessels in multiple vascular disorders. It is known that H_2S contributes to vascular protection, specifically, reduced levels of plasma H_2S have been observed in many pathological conditions such as stroke [153], diabetes [154], pulmonary hypertension [155], portal hypertension [156], chronic obstructive

pulmonary disease [157] and age-related diseases [158]. Evidence on the roles of H_2S in these pathologies is also accumulating (Figure 2), as discussed below.

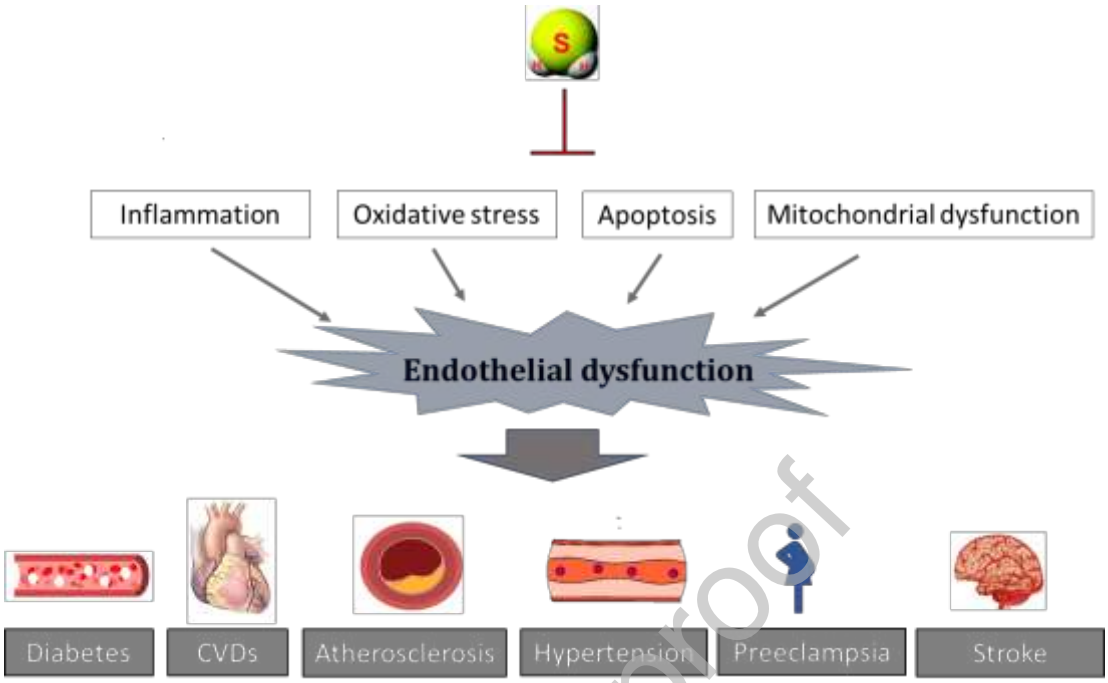

Figure 2: The disease association with endothelial dysfunction and H_2S. Endothelial cell dysfunction is characterized by disturbed redox balance and increased inflammatory reactions within the blood vessel wall. Other contributing factors such as induction of apoptotic pathways and disrupted mitochondrial function further exacerbate changes to vascular tone. Endothelial dysfunction reflects a phenotype that is attributed to many vascular and cardiovascular diseases

5.1 Cardiovascular Diseases (CVDs)

CVDs have been the leading cause of mortalities and are accounted for majority of all deaths worldwide [159]. Over the last decade, increasing evidence has demonstrated that H_2S plays important roles in maintaining cardiovascular homeostasis [4, 41]. *In vivo* studies demonstrated that disruption of CSE pathway in mice, the major H_2S producing enzyme in cardiovascular system, leads to, enhanced atherosclerosis [160] and endothelial dysfunction [2]. Clinical evidence showed that significant changes in CSE/ H_2S pathway are associated with heart failure, atherosclerosis, myocardial ischemia and diabetes [161], suggesting that dysregulation of CSE/H_2S pathway is implicated in the pathogenesis of CVDs. Furthermore, administration of H_2S donor or manipulation of endogenous H_2S production improves cardiovascular function and disease outcomes in a range of animal CVD models, including atherosclerosis, heart failure and myocardial infarction [162] [108]. The beneficial effects of H_2S are mediated through its roles of anti-inflammation, anti-apoptosis, anti-oxidative stress and proangiogenic, highlighting the therapeutic potentials of H_2S in CVDs.

5.2 Atherosclerosis

Atherosclerosis is a leading risk factor for cardiovascular diseases, leading to narrowing and hardening of arteries as a consequence of fatty plaque formation in the artery wall; consequently H_2S has been investigated as an attractive therapeutic option against atherosclerosis [163]. Mani et al. demonstrated that CSE knockout mice fed with a high-fat diet developed an endothelial dysfunction–related atherosclerosis phenotype which included oxidative stress and excessive expression of adhesion molecules [164]. In an ApoE $^{-/-}$ murine atherosclerotic experimental model, lower H_2S levels and lower CSE expression were reported. These *in vivo* disturbances were significantly abolished by exogenous H_2S administration [165]. Administration of H_2S or overexpression of CSE decreased inflammatory markers through NF-κB downregulation that ameliorated the atherosclerotic lesion [166] and reduced pro-inflammatory cytokine production [152]. In addition, oxidative stress-induced mitochondrial dysfunction in vascular cells is considered to play a role in the pathogenesis of atherosclerosis [167]. The excessive superoxide production by mitochondria can trigger a pro-inflammatory response in the vascular wall that ultimately leads to atherosclerosis development [168, 169]. H_2S releasing compound, diallyl trisulfide treatment reduced mitochondrial oxidative stress and improved vasculature offering therapeutic route against atherosclerosis [170]. Alternatively, treatment with H_2S might protect the vascular wall by increasing NO bioavailability [160].

5.3 Diabetes

Endothelial dysfunction plays an important role in the pathogenesis of diabetic complications such as vascular dysfunction, nephropathy, retinopathy, neuropathy, and cardiomyopathy. Plasma levels of H_2S were found to be significantly decreased in patients with type 2 diabetes (~100μM) when compared with age-matched normal control subjects (~130μM) [154]. The same study also reported a reduced H_2S levels in streptozotocin (STZ)-treated diabetic rats compared with control Sprague–Dawley rats. Supplementation with H_2S or L-cysteine prevented secretion of inflammatory cytokines ,IL-8 and MCP-1 in high-glucose–treated human U937 monocytes [154]. In contrary, some studies reported that the expression levels and the activities of CSE and CBS in the pancreas beta cells, as well as plasma H_2S concentrations are increased in STZ-treated rats [171]. The local increase of H_2S levels prolonged pancreatic beta cell survival against high glucose induced cytotoxicity [171]. Therefore, more studies exploring this association are warranted. The potential of exogenous treatment with H_2S donors has been explored, showing protection against hyperglycemic stress in both *in vitro* and *in vivo* models [154, 172, 173]. In these models, H_2S improved diabetes-related complications by inhibiting mitochondrial oxidative stress [109].

5.4 Hypertension

Lower circulating levels of H_2S have also been reported in other forms of hypertensive disorders, such as pulmonary hypertension [155] and portal hypertension [156]. These findings are further supported by evidence showing reduced endogenous H_2S pathways in hypertensive patients, as demonstrated by Greaney et al. who reported reduced expression of CSE and 3-MST in cutaneous tissue in hypertensive patients [174]. As CSE is the most active H_2S-producing enzyme

in the vasculature, it is possible that a defective CSE/H_2S pathway is responsible for reduced H_2S production and therefore, the key culprit in the onset of endothelial dysfunction leading to hypertension. Many authors have explored the potential for H_2S donors to prevent/treat hypertension. In this regard, H_2S donors have effectively shown to ameliorate hypertensive phenotype in many animal models. Intraperitoneal injections of GYY4137 reversed pulmonary hypertension in rats and demonstrated that H_2S suppresses the oxidative stress by enhancing the activity of the intracellular antioxidants; catalase and mitochondrial SOD [175]. Furthermore, H_2S protected against the production of mitochondrial reactive oxygen species and apoptosis in pulmonary artery smooth muscle cells exposed to hypoxia [175]. A high-salt diet, a contributing factor to clinical hypertension, results in vascular apoptosis. The molecular events associated with an enhanced mitochondrial protection in the presence of exogenous H_2S were also demonstrated in HUVEC exposed to a high salt environment. This study showed that exogenous H_2S inhibited apoptosis, reduced superoxide production, cytochrome c release, and caspases expression, as well as restoring mitochondrial membrane potential [176].

5.5 Stroke

Sharing a common defective pathway with other vascular disorders, ischemic stroke has been associated with high blood pressure and endothelial dysfunction [177]. Interestingly, the level of endogenous H_2S in the brain has been estimated at 50 to 160 µM/L [178] and some early studies reported correlations with high levels of cysteine in serum and poor clinical outcomes in acute stroke patients [179, 180]. Moreover, Sun et al. demonstrated that plasma H_2S was significantly lower in patients with stroke, accompanied by elevated blood pressure and hyperhomocysteine [181]. Studies performed in stroke-prone spontaneously hypertensive rats, showed that oxidative stress correlated with lower CBS activity and these events were prevented by the administration of H_2S [182]. In terms of the potential mechanisms involved, it has been reported that the protective effect of H_2S links to NO activity (eNOS/ NO signaling pathway) to protect against ischemic brain injury by attenuating oxidative stress and promoting mitochondrial homeostasis [183]. Apart from endothelial dysfunction, several other pathological processes contribute to this complex disease. Therefore, H_2S may play different roles in the nervous system to the vascular system and further investigations might be needed to get a better understanding of H_2S interactions [184]. These investigations emphasize the dual role of H_2S and attention to fine balance of H_2S for therapeutic use.

5.6 Preeclampsia

Preeclampsia clinically manifests as *de novo* hypertension often accompanied by proteinuria after the 20th week of gestation [185]. While the pathogenesis of preeclampsia is still not clear, some molecular mechanisms such as dysregulation of vascular factors such as sFlt-1 and placental growth factor (PIGF) are considered key events in the development and progression of preeclampsia [186]. It has been proposed that the carbon monoxide/heme oxygenase-1 pathway together with H_2S-CSE pathway are protective routes in pregnancy that when defective may result in preeclampsia pathogenesis [187]. Evidence provided by Wang and colleagues

showed that the expression of CSE was reduced in placentas from preeclamptic women [118]. In addition, the inhibition of CSE resulted in elevated circulating levels of sFlt-1 in mice, while GYY4137 abrogated these effects [118]. Exogenous H_2S has also shown beneficial effects in protecting against sFlt-1-induced vascular damage. In a study by Holwerda et al., H_2S restored sFlt-1-induced hypertension and proteinuria via induction of VEGF expression in rats [17]. The potential of H_2S to modulate the angiogenic balance in models of preeclampsia has also been demonstrated by others [188, 189]. A link between preeclampsia, H_2S and mitochondria was demonstrated by Covarrubias *et al*., who observed that the mitochondrial H_2S donor AP39 prevented antiangiogenic factors and oxidative stress response in trophoblasts exposed to hypoxia. These effects were credited to H_2S-mediated protection of mitochondrial cytochrome c oxidase activity, reduction of superoxide, as well as sFlt-1 levels [190]. Recently, our lab demonstrated that CSE-derived H_2S sustains mitochondrial bioenergetics and modulates the generation of mitochondrial reactive oxygen species in endothelial cells. In addition, using AP39 as exogenous H_2S donor, we showed that H_2S was metabolized at SQR in mitochondria, contributes to the regulation of sFlt-1 levels [120].

6. Conclusion

It is evident that as a gasotransmitter, H_2S plays a fundamental role in vascular function and redox biology. However, the field of H_2S biology is still a developing area with many unanswered questions from H_2S biochemistry to its biological functions. H_2S can act as a substrate of and an inhibitor within biological pathways depending on concentration. Most research reports on the overall effect of H_2S but overlook many intermediatory reactive sulfur species that may result during specific pathways. For example, the exact mechanism of the sulfhydration process (via H_2S itself, or, more likely, via intermediary persulfide reactions) remains to be characterised. Given the key involvement of mitochondria in H_2S oxidation pathway, further research is needed to understand the fine balance between H_2S and ROS. The majority of studies focused on CSE knockout experiments to explore the H_2S pathway but the roles of other H_2S generating enzymes: CBS, 3-MST or DAO are less well defined in relation to mitochondrial function.

Recently, there is increasing interest in using H_2S as a therapeutic molecule and a drive to investigate better H_2S pro-drugs with some specially designed to target mitochondria. These are very promising research avenues for treating many vascular, cardiac, and neurodegenerative diseases. Without a doubt, H_2S-mitochondrial research will present interesting and important new findings in near future and these works will benefit understanding of biology and for future therapeutic targets.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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List of abbreviations

AR, androgen receptor; *AMPK, 5'-adenosine monophosphate-activated protein kinase; Akt, protein kinase B; ATP5A1, α-subunit of ATP synthase; ATP, adenosine triphosphate; b.End3 cells, brain-derived microvascular endothelial cells; Bax, apoptosis regulator BAX; Bcl-2, B-cell lymphoma 2; Ca2+, calcium; CDKN2A, cyclin-dependent kinase inhibitor 2A; CAT, catalase; CSE KO, cystathionine γ-lyase knockout; COX, cyclooxygenase;* Cys, cysteine residues; *DADS, diallyl disulfide; DATS, diallyl trisulfide; DNA, deoxyribonucleic acid; DJ-1, known as Parkinson disease protein 7 (PARK7); Drfp1, dynamin related protein 1; eNOS, endothelial nitric oxide synthase; EA.hy926 cells, human umbilical vein cell line; Fis1, mitochondrial fission 1 protein;* GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GATA3, GATA binding protein 3; *GSH, glutathione; GYY4137, mor- pholin-4-ium 4-methoxyphenyl-morpholino-phosphinodithioate; HEK293 cells, human embryonic kidney 293 cells; HUVECs, human umbilical vein endothelial cells; HO-1, heme oxygenase-1; ILs, interleukins; IFNγ, interferon gamma; IRF-1, interferon regulatory factor-1; Keap1, Kelch-like ECH-associated protein 1*; *KATP ATP sensitive potassium channel;* KLF5, krüppel-like factor 5; *LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; Mn-SOD, manganese-dependent superoxide dismutase; MEK1, map kinase-1; MitoROS, mitochondrial reactive oxygen species; mPTP, mitochondrial permeability transition pore; Na2S, sodium sulfide; NaHS, sodium hydrosulfide; Nrf1, nuclear respiratory factor 1; Nrf2, nuclear factor erythroid 2-related factor 2; NMDAR1 subunit, N-methyl-D-aspartate receptor; NOX4, NADPH oxidase 4; NF-KB, nuclear factor-k betta cells; NR, not reported;* ox-LDL, oxidised low-density lipoprotein; *PARP, Poly (ADP-ribose) polymerase; PPARγ, peroxisome proliferator-activated receptor-γ; PI3K, phosphoinositide 3-kinase; PINK1, PTEN-induced kinase 1; PGC, peroxisome proliferator-activated receptor-γ coactivator; PP1c, protein phosphatase-1; PC, pyruvate carboxylase; PTEN, phosphatase and tensin homolog deleted on chromosome ten; PTP1B, protein tyrosine phosphatase 1B; PP2A, protein phosphatase 2A; PPRC, peroxisome*

proliferator-activated receptor-γ coactivator-related protein; RAGE ,receptor for advanced glycation end-products; Runx2, runt-related transcription factor 2; Sp-1, specificity protein 1; VEGFR, receptor of vascular endothelial growth factor; *TRPV6, transient receptor potential cation channel subfamily V member 6; BMMSC, bone marrow mesenchymal*; *SIRT1, sirtuin 1; SIRT3, sirtuin 3; USP8, ubiquitin specific peptidase 8 USP8.*

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