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Mini Review

Redox biology of hydrogen sulfide: Implications for physiology, pathophysiology, and pharmacology

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ABSTRACT

Hydrogen sulfide (H_2S) has emerged as a critical mediator of multiple physiological processes in mammalian systems. The pathways involved in the production, consumption, and mechanism of action of H_2S appear to be sensitive to alterations in the cellular redox state and O_2 tension. Indeed, the catabolism of H_2S through a putative oxidation pathway, the sulfide quinone oxido-reductase system, is highly dependent on O_2 tension. Dysregulation of H_2S homeostasis has also been implicated in numerous pathological conditions and diseases. In this review, the chemistry and the main physiological actions of H_2S are presented. Some examples highlighting the cytoprotective actions of H_2S within the context of cardiovascular disease are also reported. Elucidation of the redox biology of H_2S will enable the development of new pharmacological agents based on this intriguing new redox cellular signal.

Contents

Abbreviations: ARE, Antioxidant response element; CO, Carbon monoxide; CBS, Cystathionine-β-synthase; CGL, Cystathionine-γ-lyase; CcO, Cytochrome c oxidase; GSH, Glutathione; HSP, Heat shock protein; H_2S, Hydrogen sulfide; HIF, Hypoxic inducible factor; IL-1β, Interleukin 1 beta; IL-6, Interleukin 6; 3-MST, 3-mercaptopyruvate S-transferase; NO, Nitric oxide; NF-κB, Nuclear factor light chain enhancer of activated B cells; oxLDL, Oxidized low density lipoprotein; PAG, Propargylglycine; PGE2, Prostaglandin E2; NaHS, Sodium hydrosulfide; Na_2S, Sodium sulfide; SQR, Sulfide quinone oxido-reductase; TNF-α, Tumor necrosis factor alpha; VEGF, Vascular endothelial growth factor; VSMC, Vascular smooth muscle cells

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1. Introduction

Hydrogen sulfide (H_2S); a toxic gas, is endogenously produced, bioactive, and contributes to numerous physiological functions in mammalian systems. Studies support the possibility that H_2S has therapeutic potential for treating multiple diseases including cardiovascular diseases. For example, experimental animal studies show that H_2S may be effective in treating atherosclerosis and protecting against ischemia-reperfusion injury [1–3]. Interest in the cytoprotective actions of H_2S has grown since the discovery that it can induce a hypometabolic state characterized by decreased O_2 consumption, heart rate, and body temperature in non-hibernating rodents [4]. Although not discussed in this review, H_2S-dependent hypometabolism is an O_2-dependent phenomenon [5]. The proposed mitochondrial and signaling actions of H_2S make this molecule an attractive intervention for preventing and treating diseases and trauma-associated injuries. In this review article, we provide an overview of H_2S redox biology as it relates to the biological and pharmacological actions of this interesting new signaling molecule in mammalian systems.

2. Historical benefits of H_2S

The ancient Greeks, Egyptians, and Romans regularly bathed in natural sulfur springs as treatments for disease [6]. Depending on the microbiota and oxygen content, sulfur springs typically contain H_2S concentrations ranging from 1 to 500 μM [7] with anti-inflammatory, anti-bacterial, vasodilatory, and anti-fungal properties attributed to the sulfur-containing water [8]. Epidemiological studies report that a diet rich in organosulfur species is associated with longevity and decreased morbidity [9]. Members of the *Allium* genus (garlic and onions), which contain organosulfur compounds have a well-documented history of health benefits [10]. Indeed, garlic-derived compounds such as diallyl trisulfide release H_2S in the presence of cellular reductants like glutathione (GSH) [11]. Populations that consume garlic regularly have low blood pressure, low cholesterol, and less vascular disease [12]. While administration of exogenous sulfur-containing compounds shows strong promise as therapies, H_2S is also endogenously produced in many different human tissues.

3. Endogenous production of H_2S

In the early 1990s, it was discovered that H_2S is enzymatically produced by two cytosolic enzymes; cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL) [13,14]. Seminal work of Abe and Kimura showed, for the first time, that H_2S enhances long-term potentiation in the hippocampus [15]. Specifically, they demonstrated that H_2S was produced by CBS and that exogenous H_2S enhanced NMDA receptor-mediated responses. Since then many studies have shown that CBS and CGL are expressed in human tissues with H_2S contributing to physiological and pathophysiological processes (Table 1). In addition to CBS and CGL, there are other enzymes that produce H_2S with several utilizing cysteine as a substrate. The enzyme 3-mercaptopyruvate S-transferase (3-MST) is found in mitochondria and cytosol and produces H2S [16]. Several H2S producing enzymes are pyridoxal-5′-phosphate (PLP) dependent enzymes [17]. Moreover, other sulfur-containing amino acids, such as cystine and homocysteine, can be metabolized to generate H_2S. The enzymatic mechanisms of H_2S production are shown in Fig. 1. Many of these enzymes participate in the cellular sulfur cycle and have multiple enzymatic activities, including H_2S generation. Because the concentration of reduced sulfur species has an effect on many cellular processes [18], the activity of these enzymes is tightly regulated. Much of this regulation is linked to substrate availability [19]. Moreover, these are redox-sensitive enzymes, which exhibit increased activity under oxidative conditions [20]. Considering that H_2S; a reductant, is a product of these enzymes, it is conceivable that enzymatic activity may also be subject to negative feedback regulation. Finally, work by Wang et al. suggests that under certain conditions, such as oxidative stress, H_2S-producing enzymes translocate from the cytosol to mitochondria [21]. This dynamic regulation bolsters the argument that H_2S may function as a redox signaling molecule.

4. Catabolism of H_2S

Several regulated and unregulated non-enzymatic processes participate in H_2S catabolism. These pathways maintain *in vivo* H_2S concentrations, most likely, in the nM to low μM range. H_2S can react with heme proteins in mitochondria and therefore H_2S can function as a mitochondrial respiratory toxicant [22,23]. Fatal industrial accidents have been documented in individuals exposed to high concentrations of H_2S gas (e.g., >1000 pm) [24]. Therefore, the toxicological profile of H_2S has been well-studied and documented [24]. The mechanism of toxicity is through the binding of H_2S to cytochrome *c* oxidase (CcO) mediating respiratory inhibition [22]. However, this interaction is complex and poorly understood because H_2S can act as both an inhibitor and an electron donor for CcO [25]. H_2S binds to the oxidized states of the heme a-a_3 binuclear center, resulting in the reduction of the heme molecules [26]. Excess H_2S can also reduce Cu_B [27]. While the stoichiometry may vary, Cooper and Brown reported that 3 molecules of H_2S bind per inhibited CcO [28]. In this inhibitory reaction, H_2S is oxidized to sulfane sulfur and this is coupled to consumption of molecular O_2 [28]. Unlike nitric oxide ($^{\bullet}NO$), the inhibition of CcO by H_2S is noncompetitive with O_2 [27,29]. In addition, H_2S can also directly reduce the electron carrier cytochrome *c* producing the one electron oxidation product, the thiyl radical ($^{\bullet}SH$) [28].

Rhodanese, a mitochondrial sulfur transferase enzyme, catalyzes the oxidation of H_2S [30]. It is one part of three enzymatic activities characterized as a major pathway for H_2S catabolism. This pathway consists of a sulfide quinone oxido-reductase (SQR), a sulfur dioxygenase, and the sulfur transferase enzyme rhodanese (Figs. 2 and 3). H_2S reduces the external disulfide on the SQR to form a thiol (RSH) and a perthiol (RSSH). This two electron oxidation of H_2S reduces the FAD prosthetic group, which uses ubiquinone (Q) as an electron acceptor [31] The second sulfur

Table 1
Biological and therapeutic actions of H_2S are O_2-dependent.

Biological context	Low O_2	High O_2
Inflammation	Anti-inflammatory: IL-10; ↓ IL-6, ICAM	Pro-inflammatory: ↑ NF-κB, TNF-α
Vasoactivity	Vasodilatory: ↑ K_{ATP} channel conductance	Vasoconstrictive
Angiogenesis	Pro-angiogenic: VEGF, Hif1-α	No effect
Respiratory inhibition	Higher [H_2S] results in more inhibition	O_2 acts as an H_2S antagonist: ↑SOx
Ischemia-reperfusion	Ischemic tissue has higher [H_2S]: mito K_{ATP}, ARE genes	Unknown

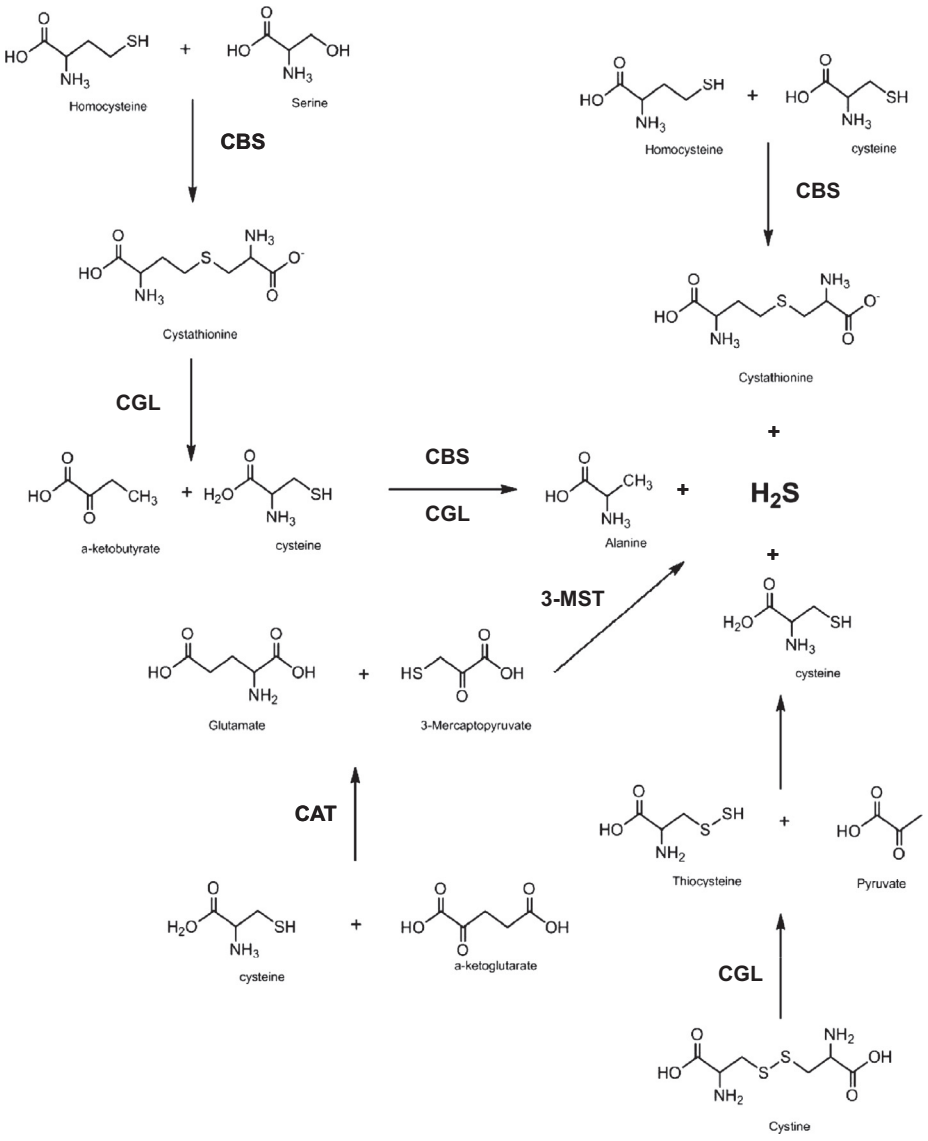

Fig. 1. The enzymatic production of H_2S. The two primary enzymes responsible for H_2S production, cystathionine-γ-lyase (CGL) and cystathionine-β-synthase (CBS), are found in the cytosol. CBS catalyzes the first step in H_2S production through the transsulfuration of homocysteine to cystathionine. CGL in an elimination reaction catalyzes the formation of cysteine and α-ketobutyrate. Cysteine is the substrate from which H_2S is directly produced either through elimination (CGL) or β-replacement (CBS). Cysteine amino transferase (CAT) catalyzes the formation of 3-mercaptopyruvate, a substrate for the mitochondrial enzyme 3-mercaptopyruvate-*S*-transferase (3-MST). 3-MST can directly produce H_2S, albeit at lower levels than CBS and CGL, in mitochondria.

atom on the perthiol is a reactive sulfane (S^0), which is oxidized by a sulfur dioxygenase enzyme encoded by the gene *ETHE1*, consuming O_2 and H_2O to form sulfite (SO_3^{-2}). While the protein responsible for this enzymatic activity is not known, the *ETHE1* gene encoding the protein has been identified. Mutations in this gene cause a buildup of H_2S leading to ethylmalonic encephalopathy [32,33]. Rhodanese then transfers a sulfane sulfur to sulfite to form thiosulfate ($S_2O_3^{-2}$) [34]. This proposed oxidation pathway, in close proximity to CcO, functions as a major clearance pathway of cellular H_2S. H_2S can also be oxidized by non-mitochondrial heme proteins such as hemoglobin (Hb) and myoglobin [35]. H_2S will reduce the ferric iron in met-Hb, restoring the oxygen binding abilities of the protein [36]. At high concentrations of H_2S, sulf-Hb can also be formed from oxy-Hb [37]. While displaying very weak affinity for O_2, sulf-Hb can still deliver O_2, albeit with no cooperativity [38]. As a result, the bioavailability of H_2S, whether in the context of steady state *in vivo* concentrations or exogenously administered, is dictated by the O_2 concentration. Therefore, O_2 can be considered an H_2S antagonist, accelerating its oxidation and attenuating its biological actions. The effect of O_2 on H_2S concentration is both direct and indirect. The spontaneous reaction of H_2S with O_2, while slow, can cause an appreciable decrease in the H_2S concentration. Thus, tissues with relatively high O_2 concentrations (e.g., alveolar epithelium) may have less H_2S compared to tissues that are in a lower O_2 environment (e.g., centrilobular region of liver). This has implications in pathological states of hypoxia such as ischemia-reperfusion, where the availability, and thus the signaling effects of H_2S may be augmented. Furthermore, O_2 concentration can indirectly affect H_2S concentration through changes in the redox state of heme proteins. Proteins such as Hb will react with H_2S at different rates depending on the redox status of the hemes. For example, H_2S will react more rapidly with met-Hb (Fe^{+3}) than with deoxy-Hb (Fe^{+2}) [36].

Because H_2S is a nucleophile, it can also react with electrophilic lipids [39]; and the thiolate anion, HS^-, can also reduce disulfide bonds (Fig. 2) [40]. Indeed, the exfoliation of skin cells in hot sulfur springs is due to H_2S reducing the structural disulfide

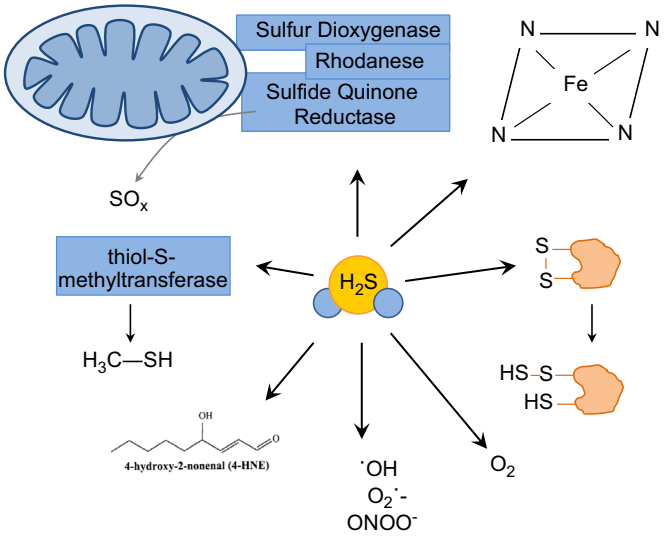

Fig. 2. Proposed pathways of H_2S removal in mammalian cells. The physiological steady-state concentration of H_2S *in vivo* is believed to be maintained in the sub-micromolar range. This steady state concentration is established by the production pathways shown in Fig. 1 and the proposed consumption pathways shown within this figure. H_2S will react non-enzymatically with many biomolecules such as reactive oxygen and nitrogen species, electrophilic lipids like 4-hydroxy-2-nonenal, free heme, and disulfide bonds to form a thiol and perthiol. The catabolism of H_2S can also be catalyzed enzymatically by the sulfide quinone oxido-reductase system (SQR) comprised by sulfur dioxygenase, rhodanese, and sulfur quinone reductase.

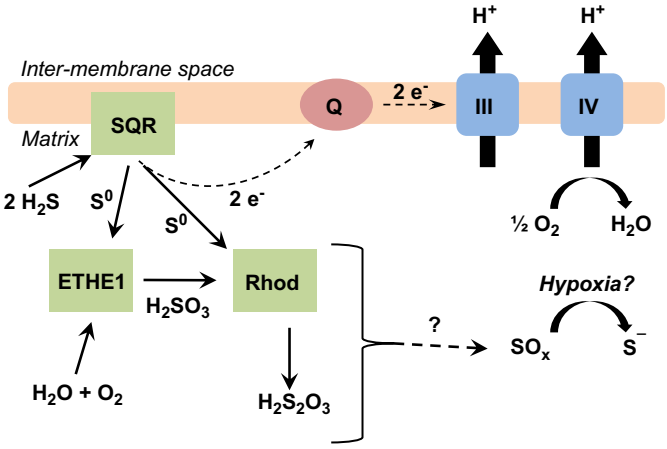

Fig. 3. The oxidation of H_2S by the sulfide quinone oxido-reductase system in mitochondria. H_2S reduces the disulfide composed of the vicinal thiols on the sulfide quinone reductase (SQR) forming a thiol and a perthiol. The second sulfur atom on the perthiol, the sulfane sulfur (S^0), is the substrate for both the sulfur transferase enzyme, rhodanese, and the sulfur dioxygenase enzyme encoded by the gene ETHE1. Rhodanese catalyzes the formation of thiosulfate ($S_2O_3^{-2}$) from sulfite (SO_3^{-2}) and S^0. ETHE1 catalyzes the formation of SO_3^{-2}. The reduced SQR can then transfer electrons into the ubiquinone (Q) pool, thus coupling the oxidation of H_2S to electron transfer, H^+ pumping, and ultimately ATP synthesis.

bonds of cellular junctions in keratinocytes [8]. While this can be harmful at high concentrations, the reduction of external disulfide bonds by H_2S may, in some instances, reverse a deleterious post-translational protein modification. Although still contentious, the *S*-sulfhydration of cysteine residues may represent an important sink for free H_2S [41]. In theory, H_2S can also reduce higher thiol oxidation states such as *S*-nitrosothiols and sulfenic acids [42]. H_2S can also be methylated by the cytosolic enzyme thiol-*S*-methyltransferase to form methane thiol. As with virtually all molecules, H_2S can react with other free radical species, as well as, a number of non-radical reactive oxygen (ROS) and nitrogen (RNS) species (Fig. 2) [43]. Many of the oxidized sulfur species as well as sulfur-centered radicals formed are less reactive than their oxygen-containing counterparts [44]. One of the most important oxidants responsible for the catabolism of H_2S is O_2. In the presence of molecular O_2 and redox active metals, H_2S will spontaneously oxidize [45]. In an oxygenated biological medium, metalloproteins catalyze H_2S oxidation. This makes O_2 tension a critical methodological consideration when conducting biologically relevant experiments.

5. Measurement of H_2S in mammalian samples

As the field of H_2S continues to grow, accurate measurement of H_2S in biological samples is critical for proper understanding of its biochemistry and identifying its key physiological roles. Early studies reported endogenous H_2S concentrations in mammalian tissues to be approximately 160 μM [46]. However, when considering that the K_i of CcO for H_2S is 0.2 μM [28] it is unlikely that free H_2S exists at high μM concentrations. Older methods relied on inducing large pH shifts during sample preparation followed by capturing the released sulfide anions with metals such as silver or zinc [47] and then measuring these complexes by spectrophotometry or chromatography [46]. One problem with these methods is that they liberate multiple acid-labile sulfur species, including those in Fe–S centers, giving artificially high values. Recently, the mono-bromobimane assay has been refined to exclude sulfur species other than free H_2S [47]. As techniques improve, the reported concentrations of H_2S in mammalian samples have decreased from the 100–200 μM range to less than 500 nM [48]. Other techniques, including polarography, have measured H_2S concentrations in mouse blood in the sub-μM range (Stein et al., unpublished data). These lower values are more plausible when considering the inhibitory potential of H_2S for heme-containing proteins, in addition to, parallels between H_2S and other signaling molecules such as ·NO and carbon monoxide (CO). It is also important to note that whole tissue or cell lysate measurements of H_2S overlook compartmental differences in H_2S concentrations and thus likely underestimate the effective localized concentrations of H_2S *in vivo*. Because the technology required to accurately measure H_2S on the sub-cellular level is unavailable, it is important to recognize these limitations when considering physiologically relevant levels and reactions of H_2S.

6. Biological roles of H_2S

Like ·NO and CO, H_2S is produced in many different cell types and can easily diffuse without the need for transporters. Therefore, it is not surprising that H_2S has diverse biological actions (Fig. 4). Important factors that determine the biological actions of H_2S include, but are not limited to, differences in the solubility of H_2S in aqueous vs. lipid phases, proximity of the target to H_2S-detoxifying enzymes, heme redox state, and inter- and intracellular differences in O_2 tension.

7. Vasoactivity

One of the first physiological roles that prompted investigators to regard H_2S as the "third gaseous signaling molecule" was vasodilation. In 2001, Zhao et al. showed that H_2S decreased blood pressure in rats *in vivo* and caused vascular smooth muscle cell (VSMC) relaxation *in vitro* [49]. H_2S-mediated vasodilation has also been shown in the ileum of the gastrointestinal tract and the *vas deferens* [50]. Additionally, others have shown that transgenic mice deficient in CBS are

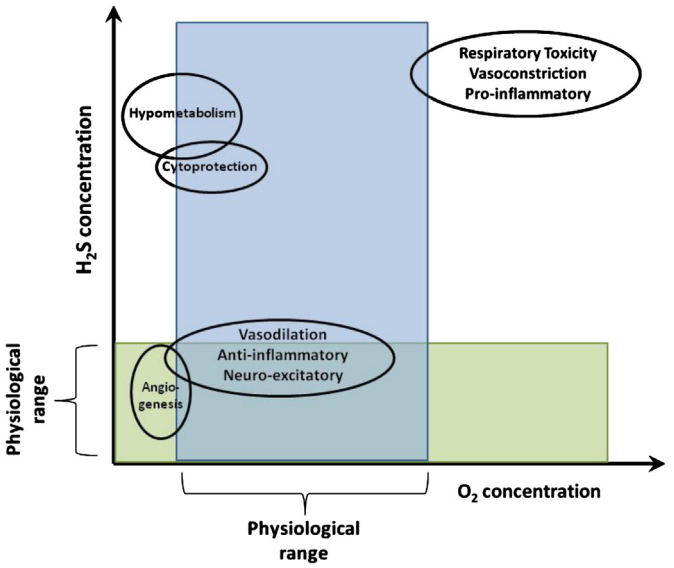

Fig. 4. Interaction of O_2 and H_2S on physiological outcomes. H_2S plays a role in many physiological processes. Additionally, it is capable of acting as a therapeutic agent. The concentration of H_2S, whether endogenously produced or exogenously administered, will dictate the outcome. This can be beneficial at low and intermediate concentrations or harmful at high concentrations. High O_2 can reverse many of the beneficial roles of H_2S seen at lower O_2 concentrations, resulting in, for example, vasoconstriction rather than vasodilation. Additionally, under hypoxic and normoxic conditions, H_2S promotes angiogenesis. However, at higher concentrations of both O_2 and H_2S, an inhibition of cellular proliferation is seen. H_2S has a narrow therapeutic window within which it is cytoprotective. At high concentrations it can be pro-apoptotic and pro-inflammatory. Finally, the larger doses of H_2S necessary to induce a hypometabolic effect, can, if pushed further, result in cardiac and respiratory toxicity.

chronically hypertensive [51]. H_2S is thought to induce vasodilation by increasing the conductance of adenosine triphosphate (ATP) sensitive potassium channels (K_{ATP}). Furthermore, the specific molecular targets of H_2S were shown to be cysteine 6 and 26 of the extracellular portion of the rvSUR1 subunit of the K_{ATP} channel complex [52]. These vicinal thiols form a disulfide bond, which H_2S reduces, increasing channel conductance. However, the vasodilatory effect of H_2S is highly O_2-dependent as supra-physiological levels of O_2 (200 μM) cause H_2S-induced vasoconstriction [53]. Recently, conflicting reports have emerged showing that the contribution of the K_{ATP} channels to H_2S-induced vasodilation is minimal and that vasodilation is due to metabolic inhibition (i.e., decrease in ATP), intracellular pH changes, and modulation of Cl^-/HCO_3^- channels [54]. There are several methodological differences including O_2 tension, pre-contraction agent, vessel type, and the type of sulfide-based chemical used, which may account for discrepancies among experimental studies. There are also indications that H_2S may act through $^{\bullet}NO$ to stimulate vasodilation. Studies suggest that H_2S liberates $^{\bullet}NO$ from *S*-nitrosothiols [55]. Others show that endothelial denudation and nitric oxide synthase (NOS) inhibitors shift the dose–response curve to the right [56]. However, H_2S increases eNOS phosphorylation and subsequent $^{\bullet}NO$ production in an Akt-dependent manner [57]. While the mechanism(s) responsible for vasodilation remain unclear, the role of H_2S as a vasodilator is accepted and there is great interest in employing H_2S-releasing agents as therapies to treat hypertension.

8. Angiogenesis

H_2S can cause cell proliferation and migration [58,59]; however, there appears to be a narrow concentration range of the proliferative effect, below which no effect is seen and above which there is anti-proliferation and H_2S cytotoxicity [60]. In cell culture experiments, low micromolar concentrations of H_2S increase endothelial cell number, proto-vessel formation, and cell migration [58]. Chicken chorioallantoic membranes, an *in vivo* model of angiogenesis, display increased branching and lengthening of blood vessels in response to 48 h incubation with H_2S [59]. Additionally, aortic tissue isolated from transgenic mice lacking CSE, the primary H_2S-producing enzyme in the endothelium, exhibit marked decreases in angiogenesis [59].

The mechanism of H_2S-induced angiogenesis operates through several pathways, including activation of ATP-sensitive potassium (K_{ATP}) channels [49]. Papapetropoulos et al. showed that treatment of endothelial cells with the K_{ATP} channel inhibitor glibenclamide reduced cell migration, which was accompanied by decreased H_2S-induced p38 and heat shock protein 27 (Hsp27) phosphorylation [59]. Additionally, H_2S can stimulate angiogenesis through phosphatidylinositol 3-kinase (PI3K) and Akt activation [61]. H_2S can also activate hypoxia inducible factor-1α (HIF-1α) and thus increase expression of vascular endothelial growth factor (VEGF) [62]. Conversely, VEGF-stimulated angiogenesis is suppressed in CSE knockout mice [58].

Endogenous H_2S production is known to be upregulated during wound healing [63]. Topically applied H_2S accelerates wound closure and healing [59]. Angiogenesis is very important in both acute and chronic ischemia as poorly vascularized tissue will lose function and possibly become necrotic. In models of chronic hind limb ischemia, sodium hydrosulfide (NaHS) increased capillary formation and blood flow [64]. Similar results were found in chronically ischemic hearts with improvements in cardiac function following H_2S treatment [65]. These studies indicate that endogenous H_2S is crucial in physiological angiogenesis and that those capabilities can be employed in disease treatment.

9. Inflammation

Several studies report that H_2S is a mediator of inflammation [66–69], while others report that H_2S ameliorates inflammatory sequelae [70–72]. These studies are likely in conflict because different models of inflammation, H_2S production inhibitors, H_2S "donors", and different O_2 tensions were used for experiments. Using NaHS, several groups have shown that H_2S augments neutrophil migration and adhesion, nociception, and increases endotoxemia [66,67,73]. This is likely because upon application NaHS "releases" all its H_2S instantly, which may be pro-inflammatory in some cases. This is in contrast to the much slower kinetics and magnitude of endogenous H_2S production. Moreover, a burst of H_2S may alter $^{\bullet}NO$ homeostasis as well, decreasing its bioavailability through direct reactions and through inhibition of NOS [74]. Regarding endogenous production, some groups report that CSE is upregulated during lipopolysaccharide (LPS)-induced endotoxemia and that pharmacological inhibition of CSE attenuated inflammation [68,69]. Whiteman et al. highlighted differences between using rapid release of high concentrations of H_2S vs. using slow-releasing donor compounds, which more closely mimic endogenous production. They showed that a slow-releasing H_2S donor dose-dependently inhibited LPS-induced inflammation by decreasing the pro-inflammatory mediators IL-1β, IL-6, TNF-α, and PGE_2 [74]. Additionally, they showed an increase in the anti-inflammatory cytokine IL-10 in an NFκB-dependent mechanism [75]. This report is similar to others that show H_2S-dependent inhibition of $^{\bullet}NO$ and TNF-α through p38 inhibition; a known downstream target of H_2S [76]. In contrast to [68], which used a non-specific PLP-dependent enzyme inhibitor, propargylglycine (PAG), others have used the CSE inhibitor

β-cyanoalanine [70]. Treatment with β-cyanoalanine increased leukocyte adhesion to the endothelium, indicating some off-target effects of PAG and that endogenous H_2S is anti-inflammatory [70]. Like vasodilation, the anti-inflammatory effects of H_2S appear to be K_{ATP} channel-dependent with glibenclamide reversing the inhibitory effects of H_2S on leukocyte adhesion [71]. Although there are conflicting reports regarding the role of H_2S in inflammation, common themes have emerged that shed light on the reasons for these disparate results. H_2S donor compounds with slow release kinetics that mimic endogenous production appear to ameliorate inflammation. This is contrasted against the apparent pro-inflammatory potential of low purity sulfide salts, which immediately dissociate in solution to rapidly deliver large doses of H_2S. As with vasoactivity, the role of H_2S in inflammation is redox sensitive and this becomes important when impure sulfide salts are used in experiments. In addition to sulfide, these salts contain oxidized sulfur species such as sulfates, sulfenic acid, and sulfonates. In contrast to H_2S, these species can have proinflammatory properties [75]. Additionally, experiments conducted at supra-physiological O_2 tensions may yield misleading results, as high O_2 concentrations increase production of proinflammatory oxidized sulfur species [53,75]. In order to move H_2S from the "bench to the bedside", comprehensive pharmacological studies are needed, which focus on routes of administration, type of donor compounds used, kinetics and magnitude of H_2S release, timing of the intervention, physiologically and pathologically relevant O_2 tensions, and the type of inflammatory condition being addressed.

10. Hydrogen sulfide and cytoprotection

As mentioned in earlier sections, the therapeutic window for effective H_2S treatment is likely very narrow because H_2S is a potent inhibitor of mitochondrial respiration. Therefore, several pharmacological factors including dose, route of administration, and timing of H_2S exposure must be carefully considered when using H_2S as a therapy. In the following paragraphs, select examples from the literature are presented showing cytoprotective effects of H_2S and other sulfide-based compounds in models of cardiovascular disease.

11. Cardiovascular disease

Studies show decreased plasma levels of H_2S in atherosclerosis models [51,77]. Furthermore, endogenous production of H_2S in sclerotic aortic tissues is impaired due to decreased expression of CGL in VSMCs [78]. Conversely, in cases of H_2S over-production (e.g., Trisomy 21) progression of atherosclerosis is slower [79]. H_2S may slow disease progression of atherosclerosis by inhibiting several elements of foam cell formation. Macrophages incubated with oxidized lipoprotein (oxLDL) show decreased intracellular lipid accumulation when treated with H_2S, whereas inhibiting endogenous H_2S production exacerbates lipid accumulation [80]. Expression of scavenger receptors responsible for oxLDL accumulation was also decreased with H_2S treatment [80]. Using apolipoprotein E knockout mice, H_2S decreases expression of adhesion molecules, thus preventing the recruitment of macrophages into the vascular intima, which is accompanied by a reduction in pro-inflammatory cytokines [2]. In atherosclerotic plaques, VSMCs proliferate and assume a fibrogenic phenotype, contributing to vessel occlusion. H_2S, at high concentrations, prevents VSMC proliferation [81]. Together, these results support the concept that sulfide-based therapies attenuate some of the early metabolic changes that contribute to cardiovascular disease. What is not known is whether H_2S is efficacious in end stage cardiovascular disease.

Considerable work has also focused on the impact sulfide-based therapies have in ameliorating cardiac ischemia-reperfusion injury. Lefer and colleagues have shown that pretreatment with sodium sulfide (Na_2S) before cardiac ischemia-reperfusion reduces infarct size and improves overall cardiac function [3,82,83]. There are several proposed mechanisms underlying this protective effect. First, H_2S up-regulates anti-oxidant response element (ARE) genes by inducing the translocation of Nrf-2 to the nucleus [84]. This increases GSH and expression of antioxidant proteins such as heme oxygenase-1, glutathione S-transferase, and thioredoxin-1 [84]. Second, H_2S acts through Akt-dependent pro-survival pathways to prevent caspase 3 activation and association of pro-apoptotic proteins to mitochondria that are necessary for mitochondrial permeability pore formation and cytochrome *c* release [85]. The effects of H_2S on the mitochondrion are likely central to overall cardiomyocyte protection [86], which may also involve activation of K_{ATP} channels [87]. While some groups attribute the cytoprotection to mitochondrial K_{ATP} channels, others using organelle-specific K_{ATP} channel blockers show that cytoprotection is due to effects on sarcolemmal channels [88].

Interestingly, daily H_2S administration for several months prevented perivascular fibrosis and subsequent arteriole occlusion in spontaneously hypertensive rats. [89], suggesting that H_2S administration may prevent pathogenic vascular remodeling. H_2S prevents thickening of damaged arterial intima and medial thickening of intramyocardial coronary arterioles [90]. Finally, H_2S induces neovascularization in models of chronic ischemia [64]. This molecular action of H_2S may be highly important following acute cardiac ischemia, as maintenance of a highly vascularized myocardium is required for proper organ function. These select findings, when taken together, demonstrate the promise of H_2S as a treatment for chronic cardiovascular diseases, as well as, in the emergency room for patients suffering from cardiac arrest.

12. Summary

The actions of H_2S described in this review highlight its ability to function in redox biology and cell signaling. With a pK_a of 6.9, both protonated and anion forms of H_2S are present at physiological pH. This allows for the free diffusion of H_2S to all cellular compartments. Because H_2S-producing enzymes play a diverse role in the larger sulfur cycle of the cell, the production of H_2S is dependent on cellular redox status. During conditions of oxidative stress the amount of reduced substrates like cysteine available for H_2S production may be limited. Moreover, steady state H_2S concentrations will be determined by H_2S consumption pathways that are redox sensitive as well. Importantly, O_2 being an antagonist of H_2S will also accelerate H_2S oxidation and inhalation of 100% O_2 is, in fact, a treatment for H_2S gas inhalation. Also highlighted herein, is the impact of O_2 tension on the biological actions and outcomes of H_2S. For example, H_2S-mediated vasodilation is observed at physiological O_2 concentrations, whereas at vasoconstriction occurs at hyperoxia (Fig. 4). These same patterns can also be extended to other biological processes such as inflammation and angiogenesis (Fig. 4). Furthermore, many of the cytoprotective actions of H_2S are triggered by and act to attenuate oxidative stress as exemplified by the ability of H_2S to induce the expression of antioxidant enzymes during myocardial ischemia-reperfusion. In conclusion, investigation of the redox biology of H_2S will not only increase understanding of its role in human physiology, but its potential therapeutic role in pathologies where oxidative stress is central to the disease process.

Authors contributions

AS and SMB wrote the manuscript.

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