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Regulation of vascular tone homeostasis by NO and H_2S: Implications in hypertension

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Abstract

Nitric oxide (NO) and hydrogen sulfide (H_2S) are two gasotransmitters that are produced in the vasculature and contribute to the regulation of vascular tone. NO and H_2S are synthesized in both vascular smooth muscle and endothelial cells; NO functions primarily through the sGC/cGMP pathway, and H_2S mainly through activation of the ATP-dependent potassium channels; both leading to relaxation of vascular smooth muscle cells. A deficit in the NO/H_2S homeostasis is involved in the pathogenesis of various cardiovascular diseases, especially hypertension. It is now becoming increasingly clear that there are important interactions between NO and H_2S and that have a profound impact on vascular tone and this may provide insights into the new therapeutic interventions. The aim of this review is to provide a better understanding of individual and interactive roles of NO and H_2S in vascular biology. Overall, available data indicate that both NO and H_2S contribute in vascular (patho)physiology and in regulating blood pressure. In addition, boosting NO and H_2S using various dietary sources or donors could be a hopeful therapeutic strategy in the management of hypertension.

Graphical abstract

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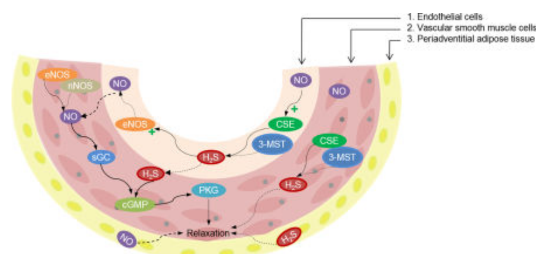
Conflicts of interest

None

Author contribution

AG and KK formulated the general concept of this review. SG, SJ, KK, and AG did the literature review and each contributed to the writing of the manuscript. The submission was approved by all four co-authors.



Keywords

Nitric oxide; hydrogen sulfide; hypertension; vascular tone; cell signaling; protein modification

1. Introduction

Nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) constitute gaseous transmitters (gasotransmitters) [1]. Traditionally, all three have been considered to be toxic gases, however, we now know that they are generated in the human body and exert physiological effects in many different tissues and are particularly active in the cardiovascular system [1–6].

H_2S concentration in the aorta is about 20–200 times greater than that in other tissues, indicating its vasoregulatory functions [7]. Mice that are deficient in one of the H_2S-generating enzymes are hypertensive [8] and in rats [9, 10] as well as in mice [8], H_2S administration decreases arterial blood pressure indicating a physiological role for H_2S in regulating blood pressure [11]. In addition, H_2S-induced vasorelaxation in mesenteric arteries of genetically modified mice is more potent compared to that observed in the wild type mice, indicating super sensitivity associated with decreased endogenous production of H_2S [8]. It has been suggested that deficiency in H_2S-generating enzymes is an important factor that predisposes to induction of hypertension; thus, H_2S enhancement may be an alternative approach for treating hypertension [8] (see [12] for review).

Both NO and H_2S contribute to vascular physiology and pathophysiology [1]. They have similar biological functions with some cross talk between the enzymes that are involved in their generation. There also appears to be some interactions between the pathways that these two gasotransmitters affect (reviewed in [13, 14]). In this review, endothelium-dependent and - independent mechanisms underlying H_2S-induced vasorelaxation or vasoconstriction have been discussed, we have also delved into potential interactions between H_2S and NO that affect vascular function. Although, most effects of H_2S are very similar to what is seen with NO [13], it should be stressed that the role of NO in vascular physiology and signaling is well entrenched and appreciated whereas in comparison, H_2S is relatively a new player on the scene and much is to be learned about its function in various settings. Thus, H_2S and its role in physiology is currently a very active area of research and development.

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2. NO and H_2S in the pathogenesis of hypertension

Hypertension is a multifactorial disease that leads to fatal cardiovascular events as a silent killer due to chronic arterial remodeling and dysfunction [15]. Of the world's adult population, 26.4% that is about 1 billion people had hypertension in the year 2000 and it has been estimated that the prevalence will increase to 29% or 1.5 billion people worldwide by the year 2025 [16]. Alteration of vascular structure and function through various pathways, including oxidative stress, inflammation, and endothelial dysfunction contribute to the pathogenesis of hypertension (reviewed in [17]). The endothelium forms an important part of the vasculature and plays a key role in regulation of vascular tone by releasing of NO [18] and H_2S [19]. Endothelial dysfunction in hypertension is associated with a decreased availability of NO and H_2S as reviewed previously ([20, 21]).

2.1. NO and the pathogenesis of hypertension

NO, which was first identified as endothelium-derived relaxing factor (EDRF) is produced by the NO-synthase (NOS) family in endothelial cells [22]. NO has a key regulatory role in maintaining proper blood pressure and its deficiency contributes to the pathogenesis of hypertension (see reviews [23–26]). In patients with essential hypertension, impairment of NO-mediated vasodilatation has been reported [27]. Recently, results of a population-based study of a cohort population of European ancestry indicates that a genetic predisposition to enhanced NO signaling (higher expression of the endothelial NOS (eNOS) and soluble guanylyl cyclase (sGC) genes) is associated with reduced levels of blood pressure, as well as lower risks of both coronary heart and peripheral arterial diseases [28]. In addition, inactivation of NO signaling is associated with higher systolic and diastolic blood pressures and a higher risk of coronary heart disease [28].

In animal models, deletion of eNOS gene or chronic inhibition of NO synthesis leads to the development of hypertension [23]. In addition, deficiency in the release of NO by the endothelium in hypertension has been reported in clinical studies [26]. Quenching of NO or its increased degradation by superoxide anions to form peroxynitrite may lead to NO deficiency, endothelial dysfunction, and hypertension (reviewed in [27, 29]). It has been shown that reduced NO bioavailability due to hemolysis causes pulmonary arterial hypertension and sildenafil, which potentiates NO-dependent signaling, rescues hemolysis-associated pulmonary arterial hypertension in mice [30].

NO donors, synthetic or natural from various plants/vegetables/fruits by increasing NO bioavailability and compensating for the NO-disrupted pathways have a therapeutic role in hypertension (as reviewed in [31]). For example, beetroot due to its high inorganic nitrate content is considered as an adjuvant for the treatment of hypertension [32, 33]. In different animal models of hypertension, treatment with nitrate and/or nitrite, which boost NO levels, is effective in reducing elevated blood pressure [34–37]. In addition, acute and short-term hypotensive effects of inorganic nitrates have been reported in several clinical studies [38–41] as well as in meta-analyses [42, 43]. Results from a population-based study strongly suggested that higher dietary nitrite intake correlated with reduced risk of hypertension [44]. Also, higher intake of dietary nitrate has shown to be effective against development of

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hypertension [45]. Thus, the antihypertensive effects of NO-donors have implications for nutritionally based preventive and therapeutic strategies against cardiovascular disease [46].

2.2. H_2S and the pathogenesis of hypertension

H_2S has been shown to be involved in blood pressure regulation [19] and its deficiency contributes to the pathogenesis of hypertension [8, 47]. Decreases in cystathionine γ-lyase (CSE) activity, a specific enzyme for H_2S production in the vasculature, decreases H_2S production and its circulating level and leads to hypertension [47]. Deletion of CSE has a hypertensive effect [8]. Circulating levels of H_2S can be a predictor for the development of hypertension [48] with lower plasma concentrations being associated with higher blood pressure [49].

Administration of H_2S donors such as sodium sulfide (Na_2S) and sodium hydrosulfide (NaSH) reduces blood pressure in different animal models of hypertension [50–55]. In addition, increased garlic consumption, a putative H_2S donor, is associated with lower incidence of hypertension in some populations as reviewed in [56]. Indeed, garlic due to its high content of organosulfur compounds decreases blood pressure, which in part is due to H_2S production [57, 58]. Development of NO- and H_2S-releasing agents either alone or in combination, is currently an active area of research for various clinical applications including cardiovascular disease [59–61]. To sum up, reduced bioavailability of both NO and H_2S are involved in pathogenesis of hypertension.

3. H_2S and NO synthesis in vasculature

3.1. H_2S synthesis in vascular smooth muscle and endothelial cells

H_2S is generated by enzymatic and non-enzymatic pathways. Non-enzymatically, H_2S is produced by reducing elemental sulfur or organic polysulfides via glucose-supported and thiol-dependent as well as glutathione-dependent cellular reactions [57, 62, 63]. Although non-enzymatic production of H_2S has been described in erythrocytes and has also been suggested to occur in the vasculature [57], it is not a major source.

Most, if not all, of the biosynthesis of H_2S has been attributed to three enzymes [12, 64, 65], cystathionine β-synthase (CBS, EC 4.2.1.22), CSE (CGL or CTH, EC 4.4.1.1), and the tandem enzymes cysteine aminotransferase (CAT, EC 2.6.1.3) and 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2), (Figure 1). Among these enzymes, CBS and CSE [2, 64] are cytosolic, 3MST and CAT are found in both cytosol and mitochondria [66, 67]. All H_2S-producing enzymes are expressed in the vasculature [64], however, CBS may play a negligible role in H_2S production within cardiovascular tissues [68]. CSE, which is regulated by calcium/calmodulin [11], along with 3-MST are mainly responsible for H_2S biosynthesis in the cardiovascular system [3, 68–72]. These two enzymes are localized in both vascular smooth muscle (VSM) and endothelial cells [11, 64]. Because the concentration of cysteine in the mitochondria is much higher than that in the cytoplasm, it is conceivable that most of the H_2S produced by 3-MST occurs within the mitochondria [63].

Although reports are conflicting, it seems that CSE is the major H_2S-producing enzyme in the VSM cells [9, 11]. L-cysteine, a substrate of CSE, when added to cultured rat aorta

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smooth muscle cells increased H_2S production rate thus suggesting it to be the major source for H_2S production in VSM cells [73]. Similar to NO production [74, 75], elevated H_2S levels in a negative feedback manner, can inhibit H_2S production by VSM cells [73]; in the liver, increased H_2S concentration by inhibiting CSE activity, decreases H_2S production [76]. CAT mRNA is expressed in both VSM and endothelial cells [77], however, immunohistochemistry of rat thoracic aorta showed that CAT was localized only to endothelial cells [78]. In contrast to earlier reports that endothelial cells are not a source of H_2S [9], expression of CSE has now been reported in the endothelial cells of mice, bovine, and humans [8, 11, 12]. It seems that at least in rats, 3MST along with CAT are the major enzyme for producing H_2S in endothelium [11]. In endothelial cells, H_2S production is highly dependent on α-ketoglutarate and 3-MST, which along with CAT contribute more than CSE in H_2S production [11, 78]. Both mitochondrial and cytosolic forms of CAT are found within the endothelial cells [78].

3.2. H_2S generation by periadventitial adipose tissue

H_2S is also generated by periadventitial adipose tissue (PAT), which consist the major part of adventitia and contributes in vascular relaxation [79]. In rat, although both CBS and CSE are expressed in the PAT, H_2S appears to be mainly generated by CSE-catalyzed L-Cysteine [79]. This is because CSE inhibitors (propargylglycine or β-cyano-L-alanine) reduces H_2S production by more than 70% [79]. CSE–H_2S pathway in the PAT is upregulated by phenylephrine, serotonin, or angiotensin II [79]. Moreover, in hypertensive rats where the hypertension was induced by constriction of the abdominal aorta, H_2S synthesis and CSE expression was increased in isolated periaortic adipose tissue. Thus, the CSE–H_2S pathway in the PAT may act as a back-up vasodilatory system in hypertension to compensate for the elevated blood pressure in response to both the acute effects of vasoconstrictors and chronic hypertension. [80]. In addition, the role of PAT-derived H_2S may be species-specific and vary between different vascular beds, as CSE expression is much lower in mice PAT than in rat [81, 82].

3.3. Rate of H_2S production in vasculature

Using a polarographic H_2S sensor, rate of H_2S production in rat liver, brain, aorta, and heart has been reported to be 12.3 ± 4.6, 10.6 ± 3.2, 5.8 ± 1.7, and 1.1 ± 0.3 pmol/s/mg protein, respectively [73]. Tissue generation of H_2S and in particular the liver contributes to circulating levels of H_2S [83]. CSE expression in vascular tissues is different and can be ranked as pulmonary artery > aorta > tail artery > mesenteric artery [9]. H_2S production rates are 3.6 ± 1.3, 8.7 ± 2.7, and 3.4 ± 0.7 nmol/min/g tissue for the aorta, tail artery, and mesenteric artery, respectively [84]; in addition, H_2S concentration in smooth muscle cells can be ~100 μM in certain blood vessels [84].

3.4. NO synthesis in vasculature

As shown in Figure 1, NO is produced in all tissues [85–88] by NOS-dependent (L-arginine-NO pathway) and independent (nitrate-nitrite-NO pathway) pathways [89, 90]. NO is mostly produced from L-arginine by the enzymes known as NOS (reviewed in [91]). There are three isoforms of NOS including constitutive neuronal (nNOS/NOS1), inducible (iNOS/NOS2), and constitutive eNOS (NOS3) [91]. The existence of a mitochondrial NOS has also been

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reported [92], although this is a debatable issue as there is always a small percentage (1–4%) of contamination in mitochondrial preparations which may contain NOS from extra-mitochondrial sources [87, 93]. In any case, even if such a NOS does exist, its physiological relevance is not immediately apparent. The largest source of eNOS and nNOS are the endothelium and skeletal muscle, respectively [94]. NO production from nitrate-nitrite-NO pathway may contribute to the therapeutic effects of nitrate/nitrite in various disorders including hypertension [95, 96] (reviewed in [97] and [89]).

All NOS isoforms are expressed in VSM [98] and endothelial [99] cells and eNOS is the major isoform regulating vascular function [50]. Expression of NOS isoforms in elastic type vessels like aorta is lower than the muscular type arteries and arterioles; in addition, it seems that nNOS is the major and eNOS is the minor NOS isoform in VSM cells [98]. While it has been reported that both VSM and endothelial cells generate NO, the production of NO by the VSM cells is however not sufficient to relax endothelium-deprived vascular preparations of bovine pulmonary arteries [100]. However, other studies have suggested that NO production by the VSM cells is sufficient to modulate vascular contractility in bovine carotid and human renal arteries [101] and rat aorta [102]. This apparent discrepancy may stem from the different types of blood vessels investigated as in VSM cells, the expression pattern of NOS isoforms varies in different types of blood vessel [98]. In the blood vessel walls, NO is mainly produced from the L-arginine-eNOS pathway [103].

In addition to NO production by the endothelial and VSM cells, all blood cells also produce NO (monocytes > neutrophils > lymphocytes > red blood cells (RBCs) > platelets) [104] (reviewed in [105]), which may contribute to regulation of vascular homeostasis [104]. Platelet-derived NO, inhibits platelet activity and can modulate thrombosis [105]. In hypertensive patients, platelet-derived NO decreases which suggests a link between altered platelets function and hypertension [106]. Under physiological states, eNOS-derived NO from blood cells decreases blood pressure [107] and RBC eNOS expression and activity are compromised in patients with coronary artery disease [104]. RBCs play an important role in regulation of vascular tone by regulating local H_2S levels. H_2S, whether generated in RBCs by 3-MST or diffused into RBCs from blood, binds to hemoglobin and is oxidized to thiosulfate and hydropolysulfides [108]; myoglobin shows a similar capacity for H_2S oxidation [109].

3.5. Rate of NO production

In mice, total production of NO is about 0.2 mmol/kg/day of which approximately 70% is derived from eNOS [110]. The largest source of eNOS is the endothelium [94]. The rate of NO formation in Wistar rats and humans has been reported to be 0.33–0.85 μmol/kg/h and about 0.9 μmol/kg/h, respectively [111–114] with the vascular endothelium being the main site of NO synthesis [115].

To summarize, CSE is the major H_2S-producing enzyme in VSM cells and eNOS is the main source for NO generation in vasculature. In addition, blood cells produce both NO and H_2S that may contribute to the regulation of vascular tone.

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4. Major types of ion channels in VSM and endothelial cells

Ion channels constitute one major group of target proteins regulated by H_2S [70]. VSM cells express many ion channels (Table 1) and amongst them voltage-dependent calcium channels (VDCC), K^+ channels, endoplasmic reticulum/sarcoplasmic reticulum (ER/SR) release channels, and transient receptor potential (TRP) channels have a more important role in regulating VSM tone (reviewed in [116]).

4.1. K^+-channels

VSM cells express a wide range of potassium channels including multiple types of voltage-gated K^+ (K_v) channels, Ca^{2+}-activated K^+ (K_{Ca}), inward rectifier K^+ (K_{ir}) channels, and two-pore K^+ (K_{2P}) channels [116]. ATP-dependent K^+ channels (K_{ATP}) are a combination of inward rectifier K^+ channels (Kir6.x), as pore-forming subunits, and sulfonylurea receptors (SUR.x), as regulatory subunits [68, 117]. The predominant K_{ATP} current in VSM cells is Kir6.1/SUR2B [7, 118]. Principal voltage-gated K^+ channels in VSM cells include $K_v1.x$, $K_v2.1$, $K_v9.x$, $K_v3.1$, $K_v4.x$, and $K_v7.x$ [116]. Among K_v7 channels, Kv7.4 is abundantly expressed in the VSM [3]. Among Ca^{2+}-activated K^+ channels, big conductance Ca^{2+}-sensitive K^+ (BK_{Ca}) or $K_{Ca}1.1$ (also named BK, Maxi K, or Slo1) channels are more important in VSM cells [119, 120]. The effects of H_2S on K^+ channels have been described below in sections 7.1.1, 7.1.2, and 7.1.3.

4.2. Ca^{2+}-channels

The principal VDCC in arterial smooth muscle cells is L-type $Ca_v1.2$ [116, 121]. Ryanodine receptors (RyR_s) and inositol-3-phosphate receptor (IP_3R) form Ca^{2+}-release channels in ER/SR membrane [122–125]. All three RyR identified isoforms (RyR_1, RyR_2 and RyR_3) [122] are expressed in VSM cells, although RyR_2 and RyR_3 are predominant isoforms [126–129]. All three isoforms of IP_3R (IP_3R_1, IP_3R_2 and IP_3R_3) [130] are also expressed in VSM cells, IP_3R_1 is however the predominant isoform [125]. RyR_s have been found in macrovascular but not microvascular endothelial cells [131–134]. All three isoforms of IP_3R are expressed in endothelial cells and IP_3R_2 and IP_3R_3 are largely restricted to the endothelium [135].

TRP channels are non-selectively permeable to cations [136]. Activation of most TRP channels results in a rise in $[Ca^{2+}]_i$, and leads to various vascular responses, including changes in vascular tone [137]. At least 19 TRP channels have been found in vascular endothelial cells [137]. In VSM cells, TRPC3, -C6, and -M4 contribute to membrane depolarization [137, 138] and TRPV4 contributes to membrane hyperpolarization by stimulating RyR and BK_{Ca} channels [137–139]. The effects of H_2S on Ca^{2+}-release channels in ER/SR membrane have been described in section 7.1.4.

5. Mechanism of vascular smooth muscle cell contraction

A rise in $[Ca^{2+}]_i$ triggers the contraction of smooth muscle cells [142] and is a key step in VSM cell contraction [143]. $[Ca^{2+}]_i$ is the primary determinant of the contractile tone within smooth muscles [144]. Ca^{2+} binds to calmodulin (CaM) and Ca^{2+}-CaM complex exposes the catalytic site and activates myosin light chain kinase (MLCK), which phosphorylates Ser19

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on the 20 KDa myosin regulatory light chain (RLC_{20}) and causes actin-myosin interaction and contraction of the smooth muscle cell [142, 143, 145] (Figure 3). The effects of H_2S on this pathway have been described in sections 6 and 7.

Increases in $[Ca^{2+}]_i$ and contraction of (vascular) smooth muscle is initiated in different ways [143, 145]: (1) vasoconstrictor-mediated response (activation of G-protein-coupled receptors (GPCRs) by extracellular ligands like norepinephrine); (2) myogenic response (e.g. strain-mediated signaling in VSM cells); (3) membrane depolarization by nerves. Vasoconstrictor agonists such as norepinephrine, epinephrine, and angiotensin II bind to their GPCRs and through Gq/11 heterotrimeric G-protein activates phospholipase Cβ (PLCβ), which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) to IP_3 and diacylglycerol (DAG) [143, 146]. Binding of IP_3 to IP_3R on SR, increases $[Ca^{2+}]_i$ and causes contraction [143, 146]. Decrease in $[Ca^{2+}]_i$ and RLC_{20} dephosphorylation by myosin light chain phosphatase (MLCP) cause relaxation [142, 143, 146]. DAG activates protein kinase C (PKC), which phosphorylates downstream targets that are involved in contraction including MLCK and Rho kinase (ROK), a Ser/Thr kinase [143, 145]. The effects of H_2S and NO on this pathway have been described in sections 13.2 and 9.1.1, respectively.

5.1. Ca^{2+} sensitization

It has been shown that agonist-induced force is greater than depolarization-induced force at similar or lower $[Ca^{2+}]_i$, a phenomenon that is due to agonist-induced Ca^{2+}-sensitization of the contractile/regulatory apparatus [145, 147]. PKC and ROK (Rho's primary effector) signaling pathways contribute in Ca^{2+} sensitization [143]. Activation of Rho, which is a small monomeric G-protein, promotes ROK-dependent phosphorylation of Thr695 and Thr850 on myosin-binding subunit (MBS), also named myosin phosphatase target subunit (MYPT1) in MLCP and increases contraction [143, 145]. Inhibition of MLCP by Rho causes Ca^{2+} sensitization and over activity of Rho-ROK pathway is implicated in hypertension [142]. In addition to Gq/11, vasoconstrictor receptors are coupled to G12/13, which activates Rho guanine nucleotide exchange factors (GEFs) and then ROK [142, 143]. Both ROK and PKC phosphorylate and activate PKC-potentiated inhibitor protein (CPI-17), which inhibits MLCP [143]. Ratio of MLCK activity-to MLCP activity is a major determinant of Ca^{2+} sensitivity in VSM [147, 148] (Figure 3).

6. H_2S-induced vasorelaxation

H_2S-induced vasorelaxation was first reported by Hosoki et al. (1997) in portal vein and thoracic aorta [149]. Zhao et al. (2001) showed that H_2S induces a dose-dependent relaxation of phenylephrine-precontracted rat aorta (IC_{50}, 125 ± 14 μM) with a threshold concentration of 60 μM; they also showed that H_2S acts through opening of K_{ATP} channels [9]. Relaxing response to H_2S on VSM has been observed in large vessels [3] but it has more of an effect on small resistance rather than large conduit arteries [3, 64, 69, 150]. For example, H_2S is 5-fold more potent in relaxing rat mesenteric artery (EC_{50}, 25.2 ± 3.6 μM) than rat aorta (EC_{50}, 125 ± 14 μM) [151]. Unlike H_2S, NO primarily acts on larger vessels [83]. In addition, there is heterogeneity in vascular response to H_2S [64] and vascular actions of H_2S is different between species and strains [4]. These differences may be related to

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different oxygen sensitivity of blood vessels to H_2S and also different sensitivities of contractile proteins to H_2S and intracellular calcium levels (reviewed in [152]). At physiological O_2 levels, H_2S causes vasorelaxation, this effect is promoted at below physiological O_2 levels while it induces vasoconstriction at higher than physiological O_2 levels; oxygen consumption rate at small peripheral vessels due to the high content of VSM cells is high and these vessels have lower oxygen partial pressure [153]. Different distribution of the molecular targets of H_2S like K_{ATP} channels in tissues has also been suggested [152]. A recent review has summarized the effects of H_2S on vascular tone in different experimental settings [154].

7. Mechanisms of H_2S-induced vasorelaxation

It seems that H_2S acts through different pathways for relaxation of vascular beds [7, 64] (Figure 2). H_2S-induced vasorelaxation involves mostly endothelium-independent (i.e. a direct effect on VSM cells) and to a lesser extent endothelium-dependent mechanisms [4, 7, 9, 155]. Endothelium-independent mechanisms by which H_2S exerts its vasorelaxatory effects include activation of K^+ channels [3, 4, 7, 9, 116], decrease in intracellular pH [7, 155, 156], and metabolic inhibition [155, 157]. Endothelium-dependent mechanisms include release of NO and/or endothelium-derived hyperpolarizing factor (EDHF) [158].

7.1. Endothelium-independent mechanisms

7.1.1. Effects of H_2S on K_{ATP} channels—Activation of potassium channels and in particular K_{ATP} channels is one of the primary mechanisms underlying H_2S-induced vasorelaxation [3, 9, 69, 70, 151] (Figure 2). H_2S stimulates (opens) K_{ATP} channels on VSM and hyperpolarizes membrane potential [3, 68, 70, 158], which closes VDCC, reduces intracellular Ca^{2+} and results in smooth muscle relaxation [7, 68, 70]. SUR_S are sites of modulation by H_2S as K^+ currents generated by K_{ir} subunits are insensitive to H_2S [70]. In addition, H_2S increases open probability of K_{ATP} channels independent of ATP levels [70]. H_2S interacts with Cys6 and Cys26 residues on extracellular N-terminal of rat vascular SUR1 (rvSUR1) subunit of K_{ATP} channels and possibly modifies it through *S*-sulfhydration (section 13 for more details) thus opening the channel [68]. It should be noted that the vasorelaxatory effects of H_2S are not exert through K_{ATP} channels only [155, 156] as it has been reported that glibenclamide only partially prevents H_2S-induced vasorelaxation [3, 69, 155] or even has no effect [4, 155]. In addition, contribution of K_{ATP} channels in H_2S-induced vasorelaxation are different among various vascular tissues [7] and the effects are minimal in rat coronary arteries [7], where K_v contributes to this function [7]. In rats, activation of K_{ATP} channels by H_2S is strong in mesenteric and aorta but is weak in coronary arteries [7].

7.1.2. Effects of H_2S on K_v channels—H_2S, as an adipocyte-derived relaxing factor (ADRF), which is released by PAT (Figure 2), activates $K_v7.x$ channels in VSM and produces relaxation [7, 116], this pathway is known as the ADRF-K_v pathway [150]. K_v7 channels activate at ~ –60 mV and keep resting membrane voltage far from threshold for activation of voltage-gated Ca^{2+} channels (~ –40 mV), this effect prevents vasoconstriction [3]. Four-aminopiridine (4-AP), which blocks many classes of K_v channels other than K_v7,

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did not affect H_2S-induced vasorelaxation while linopiridine, a rather selective K_v7 blocker, significantly reduced H_2S-induced vasorelaxation in the rat thoracic aorta [3]. The molecular target for PAT-derived H_2S in smooth muscle cells varies depending on the vessel size (reviewed in [159]). PAT-derived H_2S induces hyperpolarization of adjacent VSM cells in large conduit and small resistance arteries by activating K_{ATP} and K_v7.x channels, respectively [160].

7.1.3. Effects of H_2S on K_{Ca} channels—Reducing the contractile response to excitatory stimuli following an increase in intracellular Ca^{2+} concentrations is a major physiologic function of BK_{Ca} channels in VSM cells [120]. Ca^{2+} sparks, local transient spatially and temporally limited elevations of intracellular Ca^{2+} caused by the opening of RyRs in the SR membrane [161, 162], increase local concentrations of Ca^{2+} in subsarcolemmal space and therefore increases the open probability of BK_{Ca} channels [161], which hyperpolarize the membrane and reduces the open probability of VDCC and reduction in global $[Ca^{2+}]_i$ [161]. In rat mesenteric arteries, inhibition of CSE by β-cyano-L-alanine reduced Ca^{2+} spark frequency and adding exogenous H_2S in the form of NaSH increased Ca^{2+} spark frequency in endothelium-intact rat mesenteric arteries. These findings indicate that endogenous H_2S activates Ca^{2+} sparks, which activate BK_{Ca} channels and produce hyperpolarization and therefore VSM relaxation [161]. It has however been reported that BK_{Ca} channels, found in VSM cells, do not contribute to the H_2S-induced vasorelaxation [158].

Effects of H_2S on BK_{Ca} appear to be tissue-specific as it has been reported that H_2S activates the channel in small endothelium-intact mesenteric arteries [69], endothelial cells of small mesenteric arteries [161], and other tissues [163, 164] and inhibits the channel in some other tissues [161, 165]. The effects of H_2S on BK_{Ca} channel is dose-dependent; Sitdikova et al. reported two concentration ranges where H_2S affects BK_{Ca} channels; a high range had a Hill coefficient of 47.9 and an EC_{50} of 2000 μM for NaSH, while a low range had a Hill coefficient of 0.67 and an EC_{50} of 169 μM [164].

7.1.4. Effects of H_2S on ER/SR calcium release channels—RyR_s participate in Ca^{2+} mobilization in smooth muscle [166]. H_2S donors (Na_2S and NaSH) increase Ca^{2+} spark frequency and decrease $[Ca^{2+}]_i$ in VSM cells of piglet cerebral and rat mesenteric artery, respectively [161, 167]. IP_3R activity is regulated by IP_3 phosphorylation (via protein kinase A (PKA) and PKC) and Ca^{2+} concentrations; $[Ca^{2+}]_i$ up to 300 nM increases and $>$ 300 nM inhibits the effectiveness of IP_3 to release Ca^{2+} [130, 168]. H_2S inhibits intracellular Ca^{2+} release from IP_3R in smooth muscle cell of isolated aortic rings by inhibiting phosphodiesterase 5 (PDE_5) activity and then activating the cyclic guanosine monophosphate/protein kinase G (cGMP–PKG) pathway [169–171], (Figure 2).

7.1.5. Changes in intracellular pH—Intracellular acidification causes vasorelaxation while alkalinization causes vasoconstriction [7, 156, 162]. Intracellular pH (pH_i) in VSM cells (7.1–7.2) is maintained by buffering systems (HCO_3^-/CO_2 and proteins) and also ionic exchangers (sodium-hydrogen exchanger (NHE), anion exchanger (AE), and Ca^{2+}-ATPase) [7, 156]. H_2S activates chloride-bicarbonate exchanger (CBE), which decreases pH_i and activates K_{ATP} channels [7], (Figure 2).

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NaSH decreases pH_i in embryonic rat aortic smooth muscle cells (A7r5 cells) in a dose-dependent manner, an effect that is significantly attenuated by inhibition of CBE [156]. In fact, NaSH increases the CBE activity by ~ 60% in A7r5 cells, this effect may be due to PKC or PKA activation [156]. In this study, DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid disodium salt hydrate), which is a CBE inhibitor, abolished NaSH-induced relaxation in KCl precontracted aortic rings of male Sprague-Dawley young rats [156]. It should be noted that H_2S is a weak acid (pK_{a1} value of 6.76 and pK_{a2} >12 at 37°C [7, 13, 65], i.e. only 18.5% of H_2S remains undissociated at physiologic pH (7.4)) [7] and this in fact could decrease the pH_i, thus limiting the findings reported [156]. Inhibition of CBE by DIDS completely prevents H_2S-induced vasorelaxation on precontracted thoracic aorta in rats [155]. Therefore, it appears that H_2S decreases pH_i in a concentration-dependent manner [7] and this at least in part may contribute to the H_2S-induced vasorelaxation [156]. Supporting this notion are reports that vascular K_{ATP} channels are pH-sensitive and intracellular acidification activates K_{ATP} channels [155, 162, 172] and decreases Ca^{2+} influx through VDCC [162].

It has however been reported that high doses of Na_2S (300 μM and 1 mM) induces an alkaline shift in pH (7.4 to 7.54 and 7.75, respectively) [167] and that this could potentially activate RyR in smooth muscle cells of piglet cerebral arteriole [173, 174].

7.1.5.1 Net acid flux by H_2S: The transport of CO_2 from the peripheral tissues to the lungs is facilitated by the production of carbonic acid (H_2CO_3) in RBC in a cyclic reaction called the Jacobs-Stewart cycle [175]. In this cycle, CO_2 diffuses into the RBC and is hydrated to H_2CO_3 by the enzyme carbonic anhydrase, which is then dissociated to a proton (H^+) and bicarbonate ion (HCO_3^-). The H^+ is buffered by hemoglobin and the HCO_3^- is exchanged across the membrane by Cl^-/HCO_3^- exchanger (anion-exchange transporter, AE1) [176, 177]. Thus, one complete cycle results in the net efflux of 1 H^+ (net acid efflux) from peripheral tissue to the RBCs [177, 178].

H_2S and HS^- can act in a Jacobs-Stewart cycle to transport H^+ across the RBCs [178]. H_2S diffuses into RBCs from H_2S-producing tissues and is rapidly converted to HS^- and H^+ [178]. H^+ is buffered by hemoglobin and HS^- exchanges with Cl^- and enters the plasma where it is rapidly protonated to H_2S, which can then start another cycle [178, 179]. The net acid efflux by H_2S/HS^- acting analogously by CO_2/HCO_3^- with more efficiency because inter-conversion of H_2S/HS^- is rapid and does not require a hydration step [178, 179]. There is no report to address the effects of H_2S on vascular tone through Jacobs-Stewart cycle, however, Jenning et al. have reported that under physiological conditions, this cycle is very unlikely to affect pH_i in most cells [178] and have proposed that the vasorelaxatory effect of exogenous H_2S at least in part is due to H_2S/HS^- transport in a Jacobs-Stewart cycle [178].

7.1.6. Metabolic inhibition—In vitro and in vivo studies have shown that H_2S and NO inhibit cytochrome c oxidase, the terminal enzyme in the mitochondrial respiratory chain [157, 180]. NO inhibits cytochrome oxidase in vitro, however, this issue is still disputable as to whether NO is an in vivo inhibitor of the enzyme [157]. At physiological O_2 levels, sGC is ~50-fold more sensitive to NO than to cytochrome oxidase [157] and inhibition of cytochrome oxidase by NO is unlikely to participate in vasorelaxation.

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In vivo, H_2S at 50 ppm, inhibits cytochrome c oxidase in lung mitochondrion of rats [180]. In vitro, H_2S dose-dependently and in a noncompetitive manner inhibits cytochrome c oxidase ($IC_{50} = 1.2 \pm 0.3$ μM) in lung mitochondrion of rats [180]. In rat thoracic aortic rings, H_2S causes ~35% decrease in ATP levels in about 10 sec [155]. Inhibition of cytochrome oxidase by H_2S may contribute to a decrease in cellular ATP levels and activation of K_{ATP} channels [157]. However, H_2S could activate K_{ATP} channels in presence of elevated levels of ATP and mediate vasorelaxation independent of decrease in cellular ATP levels [153]. In intact cells, respiration of mitochondria is half inhibited at 30 μM of H_2S [157]. As H_2S is a substrate for mitochondrial respiratory chain, it increases O_2 consumption at low concentrations and inhibits O_2 consumption at high concentrations (>20 μM) [157]. Like NO, there is no firm evidence that H_2S is a significant inhibitor of respiration in vivo [157].

7.2. Endothelium-dependent mechanisms

An increase in IC_{50} of H_2S-induced relaxation of phenylephrine-precontracted aorta has been reported following removal of endothelium with saponin (135.5 ± 14 to 273 ± 16 μM), indicating that H_2S-induced vasorelaxation is facilitated by the endothelium [84]; in addition, inhibition of NO production increases the IC_{50} of H_2S-induced relaxation [84]. The same results have been reported for mesenteric artery, where endothelium removal reduced H_2S-induced relaxation by 6.4-fold (EC_{50} of H_2S were 25.2 ± 3.6 vs. 160.8 ± 8.6 μM in presence and absence of endothelium, respectively) [151].

7.2.1. Endothelium-dependent activation of Ca^{2+} sparks—Ca^{2+}-activated K^+ channels including BK_{Ca}, $K_{Ca}2.3$ (also named SK3) and $K_{Ca}3.1$ (also named IK) channels are present in vascular endothelium [69, 161]. H_2S activates BK_{Ca} [69, 161, 181] and IK_{Ca}/SK_{Ca} [3, 83, 158, 181] channels on the endothelium of rat mesenteric arteries; this hyperpolarizes the endothelial cell membranes, which enhances calcium influx through non-voltage-gated calcium channels, leading to increases in endothelial cell $[Ca^{2+}]_i$ and activation of cytochrome P_{450} epoxygenase and TRP channels to increase Ca^{2+} sparks in VSM cells causing vasorelaxation [161, 181]. Half of the resting BK_{Ca} current in endothelial cells has been attributed to the endogenous H_2S activation of BK_{Ca} channels [161]. Unlike VSM cells, hyperpolarization-induced by activation of Ca^{2+}-activated K^+ channels in endothelial cells does not reduce calcium influx due to absence of VDCC in the endothelium [182]. Although controversial, this hyperpolarization increases calcium entry [161, 182] through TRP channels by increasing electrochemical driving force for Ca^{2+} [182]; this may be the mechanism by which H_2S increases $[Ca^{2+}]$ in the endothelial cells [161], (Figure 2).

7.2.2. H_2S as an EDHF candidate—Depending on vascular beds and species, EDHF is an amalgam of different mechanisms and compounds [182]. Putative EDHFs include prostaglandin I_2 (PGI_2), epoxyeicosatrienoic acid, H_2O_2, potassium ions, C-type natriuretic peptide and NO [83]. H_2S also acts as an EDHF candidate [83, 158]. Classical endothelium-dependent hyperpolarization response is initiated in the endothelium following increases in intracellular Ca^{2+} and opening of the SK_{Ca} and IK_{Ca} channels (reviewed in [183]), both of which are activated by H_2S [3, 158, 181]. H_2S hyperpolarizes VSM cells by ~7 mV through Ca^{2+} spark activation [161]. Electrotonic conduction of hyperpolarization between

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endothelial and VSM cells may contribute to the effects of EDHF [184]. Myoendothelial gap junctions (MEGJ) may contribute in diffusion of factors involved in endothelium-derived relaxation including NO, PGI_2, and EDHF [184]. MEGJ are present between the inner layer of media and endothelial cells in many arteries and are more abundant in smaller resistance arteries [184, 185]; thus EDHF activity is more important in smaller arteries [184, 185]. Hyperpolarization of endothelial cells is transmitted to the adjacent VSM cells via MEGJ or it increases extracellular K^+; increased extracellular K^+ by IK_{Ca} activates Na^+-K^+-ATPase and by SK_{Ca} activates K_{ir} [183], both of which hyperpolarize VSM cells [183]. H_2S may diffuse from endothelial to VSM cell through MEGJ [83] (Figure 2).

Collectively, H_2S-induced vasorelaxation is mostly endothelium-independent and is mostly achieved through modulation of ion channels and in particular K_{ATP} channels; other mechanisms including acidification of intracellular fluid may also play a role.

8. H_2S-induced VSM cell contraction

In addition to relaxation, which seems to be the primary action of H_2S on the vasculature [64], H_2S can also exert VSM contraction under some conditions [155]. H_2S exerts biphasic effect on vascular tissue and produces vasoconstriction at lower concentrations (10–100 μM of NaSH yields ~3–30 μM H_2S) in rat aorta [10, 186] or 30–100 μM of H_2S in rat and mouse aortae [4] and vasorelaxation at higher concentrations, i.e. > 60 μM [7, 158, 186]; in physiological solutions, about one-third of NaSH exists as H_2S [186]. It has been argued that contractile activity of H_2S on blood vessels, which needs lower concentrations, is physiologically more important than its relaxant activity, which needs higher concentrations [187]. H_2S-induced vasoconstriction may be due to NO quenching [7] or inactivation [158]/ inhibition of eNOS [7], and NO-independent mechanisms [94] including lowering cyclic adenosine monophosphate (cAMP) levels in VSM cells at high concentrations [94].

8.1. Inhibition of NO by H_2S

Contractile activity of H_2S in phenylephrine-precontracted rat aorta disappeared after endothelium removal, NOS inhibition, or sGC inhibition [4]. Contractile effect of H_2S is endothelium-dependent [10] and involves the NO-cGMP pathway [187]. In addition, H_2S reduces acetylcholine-induced relaxation but not sodium nitroprusside (SNP)-induced relaxation [187]. H_2S at 30–3000 μM inhibits eNOS activity in a concentration-dependent manner, which contributes to its vasoconstrictor activity [187]. However, H_2S also quenches endogenous NO release from phenylephrine -precontracted endothelium of intact aortic rings [10]. H_2S stimulates AE_2, the predominant isoform of anion exchangers in VSM cells [188]. Of note, an influx of bicarbonate and efflux of superoxide anions has been associated with NO inactivation and vasoconstriction [3]. H_2S-induced stimulation of AE_2 causes extracellular accumulation of superoxide anions, which may react with NO [188]. In addition, Tiron (a superoxide scavenger) increases H_2S-induced vasorelaxation at high doses and decreases H_2S-induced vasoconstriction at low doses [188]. The suppression effect of H_2S on NO production may therefore be involved in the increased activity of AE_2 as DIDS reversed the NO lowering effect of H_2S and abolished the vasoconstrictor effects of low

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concentrations of H_2S [188]. In addition, when HCO_3^- was added to the HEPES buffer, H_2S-induced vasorelaxation in KCl-precontracted rat aorta was reduced [188].

8.2. Reduction in cAMP levels

cAMP is produced from ATP by adenylyl cyclase (AC), of which nine isoforms (ACI-IX) has been identified; isoforms III, IV, V, VI, and VIII are expressed in vascular vessels [186]. Decreased cAMP levels contribute to H_2S-induced vasoconstriction as low concentrations of NaSH (50–100 μM) and causes vasoconstriction in isoprenaline, salbutamol, and forskolin (an activator of AC)-relaxed aortic rings in rat [186]. Low concentrations of NaSH also suppress forskolin-induced cAMP accumulation in aortic smooth muscle cell line (A7r5 cells) [186].

8.3. Variation in oxygen pressure

Oxygen tension is an important factor for controlling vascular responses to H_2S [153]. H_2S-induced vasorelaxation is more prominent in absence of oxygen rather than in its presence [155]. EC_{50} for H_2S-induced relaxation in phenylephrine-precontrated rat aorta at 40 μM O_2 is about 17-fold lower than that at 200 μM O_2 (14.6 μM *vs.* 256 μM) [153]. Under physiological O_2 conditions, H_2S relaxes vessels but it causes contraction of aortic smooth muscle at high O_2 levels [11]. Low concentrations of H_2S in presence of high oxygen (95%), under which H_2S is rapidly oxidized and less available, produce vasoconstriction [153, 155]; this vasoconstriction may be due to H_2S oxidation products [153]. H_2S may compete with oxygen at the level of cytochrome c, and therefore the vasorelaxation by H_2S is less pronounced in the presence of higher oxygen concentration [155].

To sum up, H_2S in addition to having vasorelaxatory effects, it could also produce vasoconstriction; this effect is highly dose dependent however, its underlying mechanism(s) has not been completely elucidated; effects on NO production and oxygen availability may be involved in this dual action.

9. NO-induced vasorelaxation

NO is the most potent endogenous vasorelaxant [189]. Vascular NO production is mostly due to eNOS activity in endothelium; eNOS-derived NO reaches its targets on the endothelial cells or diffuse into VSM cells [13]. Actions of NO could be cGMP-dependent as well as cGMP-independent (mostly reactive nitrogen species-mediated) [190], (Figure 3).

9.1. cGMP-dependent actions

Activation GC, generation of cGMP and activation of PKG is the most important physiologic signaling pathway activated by NO (Figure 3) [90, 97, 169, 191, 192]. Low nM concentrations of NO reversibly activate GC [157, 192, 193], which converts guanosine triphosphate to cGMP [193]. GC has two isoforms: soluble (cytosolic) and membrane (particulate); the receptor for NO is sGC [193], which is a heterodimer of α and β subunits [193] and contains a ferrous heme prosthetic group on histidine 105 residue of the β subunit [193]. When NO binds to the ferrous heme iron, histidine 105 is disrupted and inhibition of the catalytic activity of sGC by the heme is overcomed yielding an increase in V_{max} and a

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decrease in K_m of the enzyme (reviewed in [193]). cGMP-dependent PKG, cGMP-gated cation channels, and cGMP-specific PDE_5 are among the targets of cGMP [194, 195]. H_2S via sulfhydration of PDE_5, inhibits its activity and rises cGMP level in vascular tissues [196]. PKG is a serine/threonine kinase and exists as a homodimer [195]. Two isoforms of PKG have been found in mammalian cells, viz. PKGI and PKGII [197]; PKGI activates PDE_5 and provides a negative-feedback loop [195]. Vascular cGMP is degraded by PDE_5 [13]. Activation of PKG by cGMP is responsible for NO-induced vasorelaxation [13], (Figure 3).

9.1.1. Vasodilatory mechanisms of NO/cGMP/PKG pathway—PKGI family is more commonly involved in NO/cGMP/PKG signaling pathway [148], which exerts its vasodilatory effects in VSM cells via a decrease in both $[Ca^{2+}]_i$ [198] and Ca^{2+} sensitivity [143].

Decreases in $[Ca^{2+}]_i$ is achieved thorough three different mechanisms: (1) decreased IP_3-mediated Ca^{2+} release from SR by phosphorylation of IP_3R_1-associated cGMP kinase substrate at Ser696 and decreased generation of IP_3 by phosphorylation of G-protein-activated PLCβ3 at Ser26 and Ser1105 [103, 148, 199, 200]; (2) increased Ca^{2+}-sequestration by phosphorylation of phospholamban at Ser16 and increased sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activation [201]; (3) decreased Ca^{2+} entry induced by hyperpolarization, which is generated by activation of K^+ channels ($K_v2.1$, K_{ATP}, Kir2.1 and BK_{Ca} channels) [198, 202–206], and also decreased activity of Ca^{2+} channels ($Ca_v1.2$, TRPC1,3,6) [137, 138, 207–212].

An NO-induced decrease in Ca^{2+} sensitivity is achieved thorough decreasing activity of Rho/ROK pathway [143]. PKGI phosphorylates Rho at Ser188 and MBS of MLCP at Ser695, and therefore decreases inhibitory effect of ROK on the MLCP and causes vasorelaxation [148, 213–215]. PKGI also phosphorylates and translocates Rho from plasma membrane to the cytosol, where is inactive [148, 213]. In addition, PKGI increases activity of regulator of G-protein signaling 2 (RGS2), which decreases both $[Ca^{2+}]_i$ and Ca^{2+} sensitivity [143].

9.2. cGMP-independent actions

NO reduces $[Ca^{2+}]_i$ in rabbit carotid artery by increasing Ca^{2+} uptake by SR; this effect is mostly cGMP-independent and occurs via SERCA activation [144, 216]. NO activates SERCA in VSM cells and decreases $[Ca^{2+}]_i$, this causes refilling of intracellular Ca^{2+} stores and inhibits store-operated Ca^{2+} entry and relaxation [144]. In fact, under physiological conditions, NO can react with superoxide anion to produce peroxynitrite ($ONOO^-$), which in turn activates SERCA by reversible S-glutathionation (GSS-) that forms GSS-SERCA at cys674 [216] (Figure 3). Low concentrations of $ONOO^-$ (10–50 μM) increases SERCA activity in aortic homogenates while higher concentrations (>100 μM) inhibits it [216]. Using rabbit aortae, it has also been shown that NO can directly interact with BK_{Ca} channels thus increasing channel activity [217]. In addition, NO increases the frequency of Ca^{2+} sparks [218] by S-nitrosylation and activates RyRs [219] and in turn the activity of BK_{Ca} channels in rat cerebral artery (reviewed in [218]).

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10. Interaction between H_2S and NO

H_2S and NO influence each other at the level of biosynthesis (Figure 1) and biological responses as reviewed in [220] (Figure 2). Hosoki et al. initially reported that NaSH at a concentration that does not produce vascular relaxation (30 μM), potentiates the vasorelaxatory effects of SNP, with decreasing its EC_{50} by ~13-fold [149]. It has been suggested that physiological role of H_2S is regulation of local concentrations of NO [10].

10.1. Effect of H_2S on NO production and bioavailability

Effects of H_2S on NO production/bioavailability are complex and include effects on NOS enzymes, L-arginine transport, and NO release from its storage forms. H_2S inhibits L-arginine transport in human umblical vein endothelial cells [7] and increases NO release from NO storage pools (e.g. nitrite) [13], which in part is catalyzed by xanthine oxidase [13].

Effect of H_2S on activity, mRNA expression, and protein expression of NOS isoforms is controversial. It has been reported that H_2S increases activity of eNOS [13, 68, 221], has no effect on iNOS and nNOS activities [7], or inhibits all NOS isoforms [187]. These differences may be related at least in part to the time of NOS activity measurement after H_2S exposure as stimulatory effects of H_2S on eNOS activity is transient [222]. In addition, L-cysteine at low concentrations increases the activity of nNOS and iNOS but not that of eNOS, and at high concentrations, it inhibits the activity of all NOS isoforms [187]. The biological interactions of NO and H_2S are complicated, and these discrepancies may be due to various experimental conditions, vascular bed, species, cell type, and H_2S dose [63, 149, 223]; so further elucidations are needed to improve our understanding as to how H_2S and NO can reciprocally regulate their synthetic enzymatic pathways. eNOS is mostly expressed in the endothelium [224] but CSE is present in both the endothelium and VSM cells [9]; so when endothelium is impaired and NO is reduced, H_2S is still produced by the VSM cells and can represent a backup system within the vasculature in pathological conditions [224].

The stimulatory effects of H_2S on eNOS activity is partly due to an increase in $[Ca^{2+}]_i$ within the endothelial cells [1, 13, 68, 83, 189, 225, 226]; increased intracellular calcium is a rapid and potent activator of eNOS [13], causing phosphorylation of S1177 on human eNOS [64, 189, 227], and inhibiting the S-nitrosylation of eNOS [228]. In addition, H_2S prevents oxidized low-density lipoprotein mediated proteosome dependent eNOS degradation [227] and stabilizes the dimeric active form of eNOS [64] through sulfhydration of Cys443 [13, 228].

Elevation of endothelial $[Ca^{2+}]_i$ by ligands such as acetylcholine induces relaxation of adjacent VSM cells through endothelium-derived relaxing factors including NO, PGI_2, and EDHF [13, 182]. In primary cultures of human endothelial cells derived from saphenous veins, NaSH increased $[Ca^{2+}]_i$ at concentrations ≥ 50 μM independently of extracellular Ca^{2+}, indicating the importance of intracellular Ca^{2+} pools as well as IP_3R and RyR [225]. In bovine aortic endothelial cells, NaSH increased NO production in a dose-dependent manner through the Ca^{2+}/CaM pathway [189]. This effect was independent of extracellular Ca^{2+} and was a result of Ca^{2+} release from ER through IP_3R and RyR [189]. In human

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umblical vein-derived EA.hy926 cells, NaSH increased intracellular Ca^{2+} with a peak at 30 μM; this effect was independent of both extracellular Ca^{2+} and store-operated calcium entry and required IP_3-dependent intracellular Ca^{2+} mobilization mostly from ER [226]. H_2S, probably via S-sulfhydration, stimulates PLC and increases IP_3 production [226]. On the other hand, in endothelial cells of aortic rings from Wistar rats, it has been demonstrated that NaSH increases $[Ca^{2+}]_i$ independently from ER stores; this effect is driven by the reverse mode (3Na^+ out: 1 Ca^{2+} in) of Na^+-Ca^{2+} exchanger (NCX) and by K_{ATP} channels indicating requirement of Ca^{2+} entry from extracellular fluid for NaSH-induced Ca^{2+} signaling [1]. NaSH increases intracellular Ca^{2+} levels in intact endothelium of rat aorta by activating non-selective cation channels, which their nature is yet to be elucidated [1]. This effect causes local Na^+ accumulation under plasma membrane (called LNat [229]) and switches NCX to its reverse mode, which increases $[Ca^{2+}]_i$ [1]. NaSH stimulates K_{ATP} in endothelial cells and increases K^+ efflux, which increases Ca^{2+} entry by increasing the driving force for calcium entry [1]. To sum up, as shown in Figure 2, H_2S-induced increases in endothelial $[Ca^{2+}]_i$ is achieved by increasing IP_3-dependent intracellular Ca^{2+} mobilization, activating K_{ATP} channels, and favoring the reverse mode of NCX [1, 13, 68]. Molecular mechanism underlying endothelial Ca^{2+} mobilization are however different depending on species and vessel type [1, 226]. Administration of NaSH (56 μmol/kg/day for 20 week) to hypertensive rats improves the endothelium-dependent relaxation; this effect was shown to be due to inhibition of the renin–angiotensin system and activation of eNOS by phosphorylation [230].

It has also been reported that NaSH inhibits eNOS in a concentration-dependent manner with an IC_{50} of 170 μM [4]. Inhibitory effects of H_2S on eNOS are due to inhibition of tetrahydrobiopterin (BH_4) functions [187] and reducing the phospho-eNOS (serine 1177) [231]. BH_4 is needed to stabilize the active dimeric form of all NOS isoforms [99]. Proper dimerization (coupling) is important for NOS activity [232] and NOS monomers can not catalyze NO production [94]. Mammalian NOS catalyze oxidation of L-arginine to NO and L-citrulline [233] using molecular oxygen and reduced nicotinamide-adenine-dinucleotide phosphate as co-substrates, and flavin mononucleotide, flavin adenine dinucleotide, and BH_4 as cofactors [193, 234], from which BH_4 is essential and rate-limiting [87]. In cultured mouse aortic endothelial cells, H_2S by reducing the phospho-eNOS (serine 1177) levels inhibits eNOS activity [231]. Consistent with these reports, in isolated rat aorta and cultured human umbilical vein endothelial cells, administration of H_2S decreased the activity of the L-arginine/NOS/NO pathway [235].

10.2. Effect of NO on H_2S production

Effects of NO on H_2S production are also not straightforward. In vascular tissues, NO increases activity and expression of CSE enhancing H_2S production [9, 11, 70, 181, 187, 223], (Figure 1). Zhao et al. showed that incubating homogenized rat vascular tissues with SNP increases H_2S production; in this study, another NO donor, s-nitroso-N-acetylpenicillamine (SNAP), increased the transcriptional level of CSE in VSM cells [9]. Furoxan, another NO donor, increases the level of H_2S production induced by S-propyl-L-cysteine, an H_2S donor, in isolated rat aortic ring [236]. NO-induced H_2S production is mediated by cGMP as it is decreased by inhibition of sGC; thus cGMP activates H_2S-producing enzymes [237]. In addition, CSE is the target of *S*-nitrosylation at its multiple

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reactive cysteine residues [152]. Heme-containing proteins are targets of NO, thus, the activity of CBS might be affected by NO [29]. However, another report suggests that NO could not increase the expression of H_2S producing enzymes or levels of H_2S in endothelial cells [238]. In addition, NO directly inhibits CSE activity in vitro with an IC_{50} of about 100 nM [83].

10.3. Interaction between NO and H_2S on the NO/sGC/cGMP pathway

H_2S is considered to be an enhancer of NO/cGMP/sGC/PKG pathway [13] and potentiates the vasorelaxant effects of NO [181]. H_2S increases NO-responsive form of sGC [64] and inhibits PDE_5, which decreases cGMP degradation [13]. Reaction of H_2S and cGMP produces 8-SH-cGMP, which is active and less sensitive to degradation by PDE_5 [13]. Inhibition of sGC and endothelial NO synthesis inhibits both H_2S-induced and NO-induced angiogenesis indicating the requirement of NO for angiogenesis by H_2S [169]. In addition, NO-induced angiogenesis requires H_2S [223], (Figure 3).

10.4. Effects of NO and H_2S intermediates/metabolites on vascular tone

As shown in Figure 4, interaction between NO and H_2S and their respective metabolites can produce intermediates that have distinct physiological functions as compared to both NO and H_2S [220, 239–241]. These interactions produce different products including nitrosopersulfide ($SSNO^-$) [220, 242–245]. S-nitrosothiols (RSNOs) are produced when NO reacts with thiol groups; and the reaction between RSNO and H_2S produces thionitrous acid (HSNO) [241, 242, 244, 246]. HSNO acid could freely diffuse through the membranes, acting as an NO carrier [244, 246]. HSNO acid could also react with hydrogen persulfide (HSSH) to produce $SSNO^-$, which also can act as a NO carrier, leading to relaxation of VSM cells upon NO release [220, 243, 244]. In addition, HSNO is relatively unstable and in the presence of H_2S and HSSH can produce nitroxyl (HNO) [220, 243, 244], which exerts vasorelaxatory effects by activation of cGMP-dependent [247] and independent [248] pathways.

To sum up, interaction between NO and H_2S is seen at the level of biosynthesis, signaling pathways, and production of some intermediates with vasoactive effects.

11. Role of platelets in vascular tone regulation: Effects of NO and H_2S

The expression and function of two NOS isoforms, eNOS and iNOS, have been reported in platelets; however, their activity and regulation are controversial (reviewed in [249]). NO produced by endothelial cells or by platelets, inhibits platelet adhesion, aggregation, recruitment, and formation of leukocyte-platelet aggregates. NO inhibits platelet activation by both cGMP-dependent and cGMP-independent pathways. In platelets, NO activates sGC and increases cGMP levels; cGMP activates PKG and inhibits almost all agonist-induced events, including increases in intracellular calcium levels [250], integrin activation [251], cytoskeletal reorganization, and platelet granule secretion (reviewed in [252]). Most of the NO donors exert their inhibitory effects on platelet activation in a cGMP-independent manner [253–255]. Furthermore, activation of platelet ADP-ribosyltransferase [256] and

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inhibition of platelet Ca^{2+} mobilization by NO in a cGMP-independent manner have also been reported [257].

Both CBS and CSE are expressed in platelets and generate detectable amounts of H_2S [258]. Deficiency in H_2S production stimulates platelets aggregation in a methionine-induced hyperhomocysteinemia rat model [259]. H_2S inhibits platelet activity both in vivo and in vitro [259–266], although controversial [258].

Inhibition of platelet aggregation by H_2S is mediated through upregulation of NOS [261, 262], increased cAMP levels, suppression of cytosolic Ca^{2+} mobilization [264], antioxidant activity [265], and vasodilation [266] amongst others. However, it has also been reported that H_2S-induced inhibition of platelet aggregation is not dependent on cAMP/cGMP generation, NO production, or potassium-channels opening [260]. In contrast, a recent report showed that NaSH and L-cysteine dose-dependently enhanced platelet aggregation in hyperhomocysteinemia [258]. This discrepancy may be related to the relative doses of H_2S employed, which has ranged from mostly high (1–10 mM) to significantly lower doses (0.1–100 μM) [258].

12. NO, H_2S and inflammation

Depending on the concentration and source of production, NO is implicated as a pro-inflammatory or an anti-inflammatory agent (reviewed in [267, 268]). Under non-inflammatory conditions, iNOS is not expressed in endothelial cells, but in presence of pro-inflammatory stimuli, high levels of NO are produced by iNOS that contribute to inflammation [269]. The expression of iNOS in response to cytokines is also increased in VSM cells [270], where it can prevent pathologic vaso-occlusion triggered by other local mediators or increase VSM proliferation (reviewed in [271]). On the other hand, eNOS-derived NO may serve as an anti-inflammatory agent by regulating expression of pro-inflammatory molecules such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and cyclooxygenase-2 [272, 273] as well as preventing the leukocyte adhesion [274] and rolling [275] in the microvasculature. In addition, nNOS, which is predominantly expressed in the nucleus of endothelial cells, exerts an anti-inflammatory role [276].

H_2S has also both pro- and anti-inflammatory effects depending on its concentration as well as its rate of generation; physiological concentrations of H_2S can exert anti-inflammatory effects, whereas higher concentrations are pro-inflammatory [277]. Thus, decreased H_2S concentrations in the blood may contribute to vascular inflammation [278].

Different mechanisms are involved in the anti-inflammatory effects of H_2S, including inhibition of NF-κB and cyclooxygenase-2 [279]; inhibition of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α [277]; inhibition of leukocyte adherence to the vascular endothelium [280, 281]; inhibition of iNOS expression [282]; decreased expression of monocyte chemoattractant protein-1 (ICAM-1) [283–285], and vascular cell adhesion molecule-1 (VCAM-1) [285]; scavenging of ROS [286], and upregulation of heme oxygenase-1 [285]. Inhibitory effect of H_2S on leukocyte adhesion and infiltration is mediated through activation of K_{ATP} [280].

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Hypertension by stimulating genes involved in the recruitment of inflammatory cells into the arterial wall exerts pro-inflammatory effects. It seems that the anti-hypertensive effects of H_2S, at least in part, is mediated through suppression of inflammation in the endothelial.

13. Post-translational protein modifications by NO and H_2S

Post-translational modifications of proteins are central molecular mechanisms that mediate signal transductions. One such modification is *S*-nitrosylation, which involves the addition of NO to a cysteine thiol to form an *S*-nitroso-protein (SNO-protein) [287]. To put this in perspective, *S*-nitrosylation may be comparable to that of phosphorylation and ubiquitylation, which are critical cellular regulatory mechanisms [51]. *S*-sulfhydration or more correctly sulfuration, is transfer of a sulfhydryl group (-SH) to a cysteine residue of a protein to form a hydropersulfid moiety (-SSH) [68, 70] or persulfide group [64]. Most of H_2S signaling is done through protein *S*-sulfhydration [64].

13.1. *S*-nitrosylation

Protein *S*-nitrosylation is a reversible modification that can activate or inhibit protein function and be beneficial or detrimental (reviewed in [288, 289]). All mammalian cells have low levels of nitrosylated proteins, named constitutive nitrosylation [290]. *S*-nitrosylation is specific and only some proteins are nitrosylated despite presence of cysteine residues on almost all proteins and production of NO by most cells [291]; it depends on the presence of metal ions (Mg^{2+} or Ca^{2+}), local pH, and the acid-base motifs [228]. Peroxynitrite and NO act as specific protein posttranslational modifiers [288].

S-nitrosylation contributes to vascular tone regulation through different mechanisms including effects on cell signaling pathways and activities of PDE_5, eNOS, and calcium channels. *S*-nitrosylation is involved in long-lasting inhibitory effect of NO on arterial tone; this effect is mediated by persistent increase in NO, cGMP, and *S*-nitrosylated cysteine residues in arteries [292]. *S*-nitrosylaion at Cys181 of PDE_5 negatively regulates its activity and contribute to the regulation of cGMP signaling in VSM cells [293]. In addition, *S*-nitrosylation decreases eNOS activity by increasing its monomer formation [228]; *S*-nitrosylation also desensitizes sGC and decreases its response to NO in aortic smooth muscle cells [294]; these effects can justify negative feedback on NO production.

S-nitrosylation of PKC decreases its activity and subsequently inhibits PKC-dependent contractile responses in VSM cells of mouse aorta [295]. *S*-nitrosoglutathione inhibits α_1-adrenergic receptor-mediated pulmonary vasoconstriction and ligand binding likely via *S*-nitrosylation of its GPCR system [296]. In addition, NO donors can *S*-nitrosylate bradykinin receptors in aortic endothelial cells [297] and muscarinic receptors in rat atria [90] which disrupt their coupling to G proteins. In HEK-293 cells, NO by *S*-nitrosylation decreases binding affinity of angiotensin II type 1 receptor [298] and conductance of L-type Ca^{2+} channels [299]. *S*-nitrosylation of connexin43 on Cys271 also regulates gap junction communication between endothelium and smooth muscle [300].

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13.2. *S*-sulfhydration

S-sulfhydration contributes to vascular tone regulation by affecting DNA repair, modulation of ion channels activities, and NO production. The integrity and function of endothelial cells due to DNA damage is impaired with aging and results in age-associated cardiovascular disorders [41]. In human endothelial cells, *S*-sulfhydration activates mitogen-activated ERK (extracellular regulated kinase) kinase (MEK1) at cysteine 341 which causes phosphorylation and translocation of extracellular regulated kinases 1 and 2 (ERK1/2) into nucleus; ERK1/2 stimulates poly(ADP-ribose)ation polymerases (PARPs) activity through direct interaction which subsequently improves DNA damage repair and cellular senescence [40]. Regulation of ion channels via *S*-sulfhydration has been reported in endothelial and VSM cells. For example, *S*-sulfhydration of K_{ATP} channel activates it by decreasing ATP binding and increasing PIP_2 binding, which leads to hyperpolarization of VSM cells and therefore vasorelaxation; *S*-sulfhydration also activates IK_{Ca} and SK_{Ca} channels in vascular endothelial cells [83] and leads to vasorelaxation. In addition, *S*-sulfhydration of IP_3R inhibits Ca^{2+} release from ER and causes smooth muscle relaxation [64].

Inhibition of ROK activity by *S*-sulfhydration following NaSH administration has been reported in colonic smooth muscle [301]; which can give insight into the mechanism of action of H_2S on Rho kinase activity in VSM. H_2S promotes PDE_5 sulfhydration, inhibits its dimerization and activity, giving rise to cGMP levels in vascular tissues [196]. Unlike *S*-nitrosylation which inhibits eNOS activity, *S*-sulfhydration of eNOS increases its activity [228] and subsequently activation of NO/sGC/PKG pathway, which causes VSM cells hyperpolarization and vasorelaxation [89]. *S*-sulfhydration and *S*-nitrosylation competitively modify the same cysteine residue of eNOS; *S*-sulfhydration of eNOS decreases its *S*-nitrosylation, while *S*-nitrosylation of eNOS does not affect its *S*-sulfhydration. Indeed, sulfhydrated cysteine is required for eNOS dimer stability [228]. H_2S also regulates platelet activity by protein *S*-sulfhydration, as exposure to H_2S increases the *S*-sulfhydration of platelet proteins and reduces the exocytosis of platelet granules and prolongs thrombus formation, therefore decreases expression of prothrombotic adhesion molecules on activated platelets [266].

14. Conclusion and perspectives

All H_2S-producing enzymes are found in vasculature: 3-MST has more of a contribution in endothelial cells [11] and CSE is the major enzyme in both VSM cells [9, 11] and PAT [79]. All NO-producing enzymes are also expressed in vasculature [98, 99] and eNOS [103] and nNOS [98] are the major isoforms in endothelial and VSM cells, respectively [103]. Both NO and H_2S have roles in vascular (patho)physiology [103] and in regulating blood pressure [8–11, 13]. H_2S causes both vasorelaxation and vasoconstriction; while mechanisms underlying H_2S-induced vasorelaxation have been identified in more details, H_2S-induced vascular contraction needs to be more investigated as it has been suggested that H_2S-induced vasoconstriction is physiologically more important than its relaxant activity, which needs higher concentrations [4]. Production of H_2S and NO by blood cells may also contribute in regulation of vascular tone; this topic needs further research. Regarding effects of gasotranmitters on vascular tone, translational work in this area requires a deep

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understanding of the biology and pharmacology of H_2S and NO, as well as an ability to integrate this scientific knowledge with the principles of drug development [14]. It should be noted that most data presented in these studies are from animals and in particular rodents, and although vascular regulation is generally similar in humans and rodents [83, 302] and that these data may be extrapolated to humans, we must still be cautious when doing so. In addition, it should be emphasized that while the vasorelaxatory effects of NO has been established, and has withstood the test of time, H_2S is the youngest member of the gasotransmitter family and as such it certainly needs further work to fully elucidate its vascular and other effects.

Another area that warrants attention is the cardiovascular effect of H_2S. This opens possibilities for a nutritionally based interventional framework. For example, it has been reported that the major beneficial effects of garlic rich diets are mediated by H_2S production [57]. According to experimental, preclinical, and clinical studies [19, 303, 304], elevation of H_2S by using dietary sources or donors could be a hopeful therapeutic strategy in addressing/managing hypertension [19]. Many antihypertensive drugs exert their protective effects at least in part by increasing NO bioavailability [15]. Deficiencies in H_2S/NO may contribute to the development of hypertension, and in this context, combination therapy may prove fruitful in managing hypertension [8, 89, 243, 305].

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Abbreviation

ADRF	Adipocyte-derived relaxing factor
AE	Anion exchanger
ATP	Adenosine triphosphate
BH_4	Tetrahydrobiopterin
BK_{Ca}	Big conductance Ca^{2+}-sensitive K^+ channel
CaM	Calmodulin
cAMP	Cyclic adenosine monophosphate
CAT	Cysteine aminotransferase
CBE	Chloride-bicarbonate exchanger
CBS	Cystathionine β-synthase
cGMP	Cyclic guanosine monophosphate
CO	Carbon monoxide
CPI-17	PKC-potentiated inhibitor protein

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CSE	Cystathionine γ-lyase
DAG	Diacylglycerol
DIDS	4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid disodium salt hydrate
EDHF	Endothelium-derived hyperpolarizing factor
EDRF	Endothelium-derived relaxing factor
GEF	Guanine nucleotide exchange factor
eNOS	Endothelial NOS
ER	Endoplasmic reticulum
ERK	Extracellular regulated kinase
GPCR	G-protein-coupled receptors
HNO	Nitroxyl
HSNO	Thionitrous acid
HSSH	Hydrogen persulfide
H_2S	Hydrogen sulfide
iNOS	Inducible NOS
IL-1β	Interleukin-1β
IP_3R	Inositol-3-phosphate receptor
K_{ATP}	ATP-dependent K^+ channels
K_{Ca}	Ca^{2+}-activated K^+ channel
K_{ir}	Inward rectifier K^+ channel
K_{2P}	Two-pore K^+ channel
K_v	Voltage-gated K^+ channel
MBS	Myosin-binding subunit
MEGJ	Myoendothelial gap junctions
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MYPT	Myosin phosphatase target subunit
NaSH	Sodium hydrosulfide
Na_2S	Sodium sulfide

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NCX	Na^+-Ca^{2+} exchanger
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHE	Sodium-hydrogen exchanger
nNOS	Neuronal NOS
NO	Nitric oxide
NOS	NO synthase
$ONOO^-$	Peroxynitrite
PARP	Poly(ADP-ribose) polymerases
PAT	Periadventitial adipose tissue
PDE_5	Phosphodiesterase 5
PGI_2	Prostaglandin I_2
pH_i	Intracellular pH
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein kinase G
PLCβ	Phospholipase C-β
RBC	Red blood cell
RLC_{20}	20 KDa myosin regulatory light chain
ROK	Rho kinase
RSNO	S-nitrosothiol
RyR	Ryanodine receptor
SERCA	Sarco/endoplasmic reticulum Ca^{2+}-ATPase
sGS	Soluble guanylyl cyclase
-SH	Sulfhydryl group
SNP	Sodium nitroprusside
$SSNO^-$	Nitrosopersulfide
SR	Sarcoplasmic reticulum
SUR	Sulfonylurea receptor

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TRP	Transient receptor potential
TNF-α	Tumor necrosis factor-α
VCAM-1	Vascular cell adhesion molecule-1
VDCC	Voltage-dependent calcium channels
VSM	Vascular smooth muscle
3-MST	3-mercaptopyruvate sulfurtransferase

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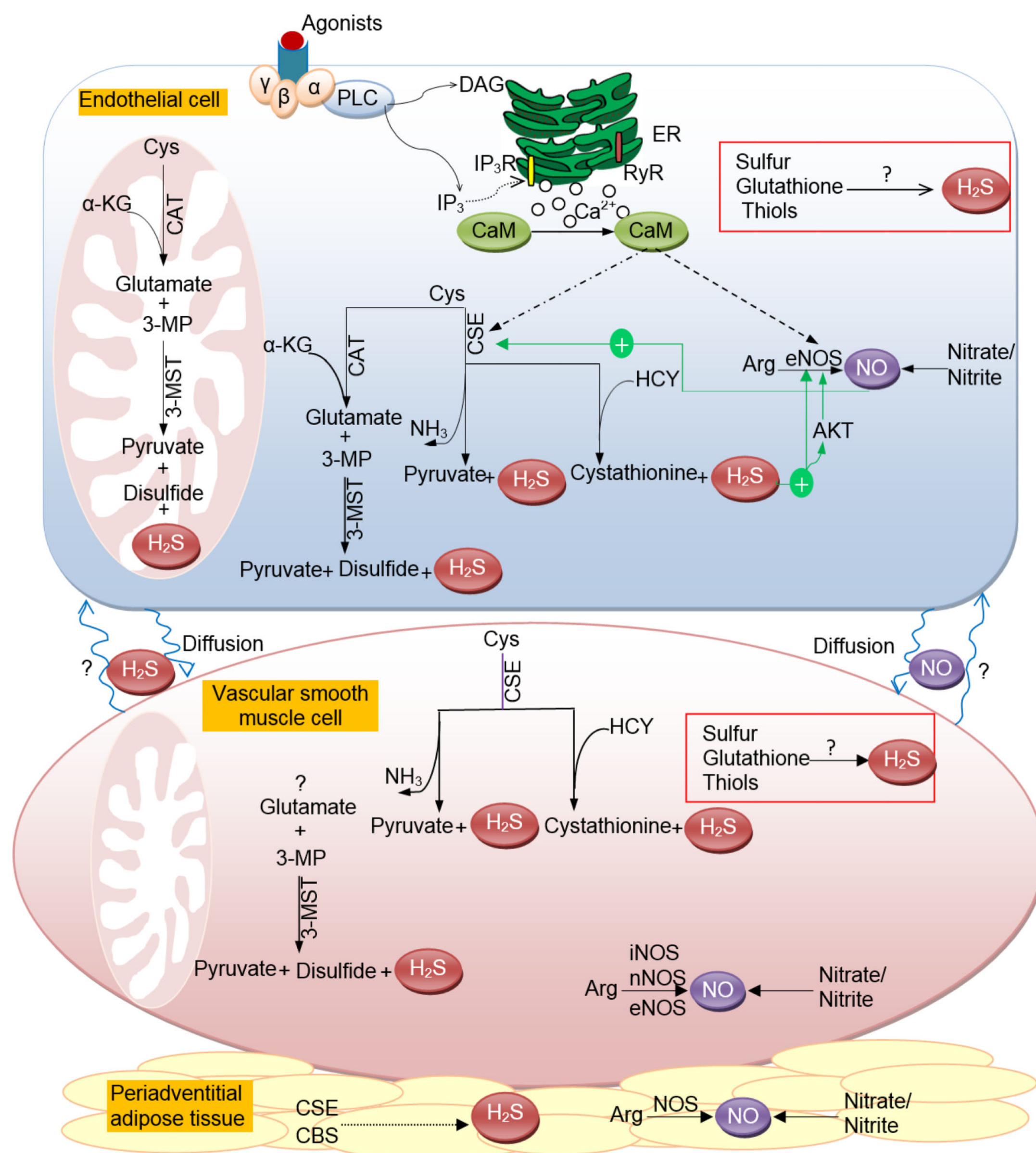

Figure 1. Hydrogen sulfide and nitric oxide biosynthetic pathways in vessels

Hydrogen sulfide (H_2S) and nitric oxide (NO) are generated by enzymatic and non-enzymatic pathways. Non-enzymatic production of H_2S in the vessels, shown in the boxes, has not yet been fully understood. NO is produced by nitrate/nitrite pathway which can be enzymatic or non-enzymatic. In endothelial cells, vasoconstrictor agonists through phospholipase Cβ (PLCβ)/ inositol-3-phosphate (IP_3) and diacylglycerol (DAG) pathways increase $[Ca^{2+}]_i$ and cause formation of calcium-calmodulin (CaM). CaM stimulates both endothelial NO synthase (eNOS) and cystathionine γ-lyase (CSE) that generate NO and H_2S, respectively. H_2S can increase eNOS activity and therefore NO production directly or

through Akt activation. NO increases CSE activity and expression and enhances H_2S production. H_2S is also produced in periadventitial adipose tissue (PAT) by CSE and cystathionine-beta-synthase (CBS). Cys, Cysteine; HCY, homocysteine; NH_3, ammonia; 3-MP, 3-mercaptopyruvate; 3-MST, 3-mercaptopyruvate sulfuretransferase; CAT, cysteine aminotransferase; α-KG, α-ketoglutarate.

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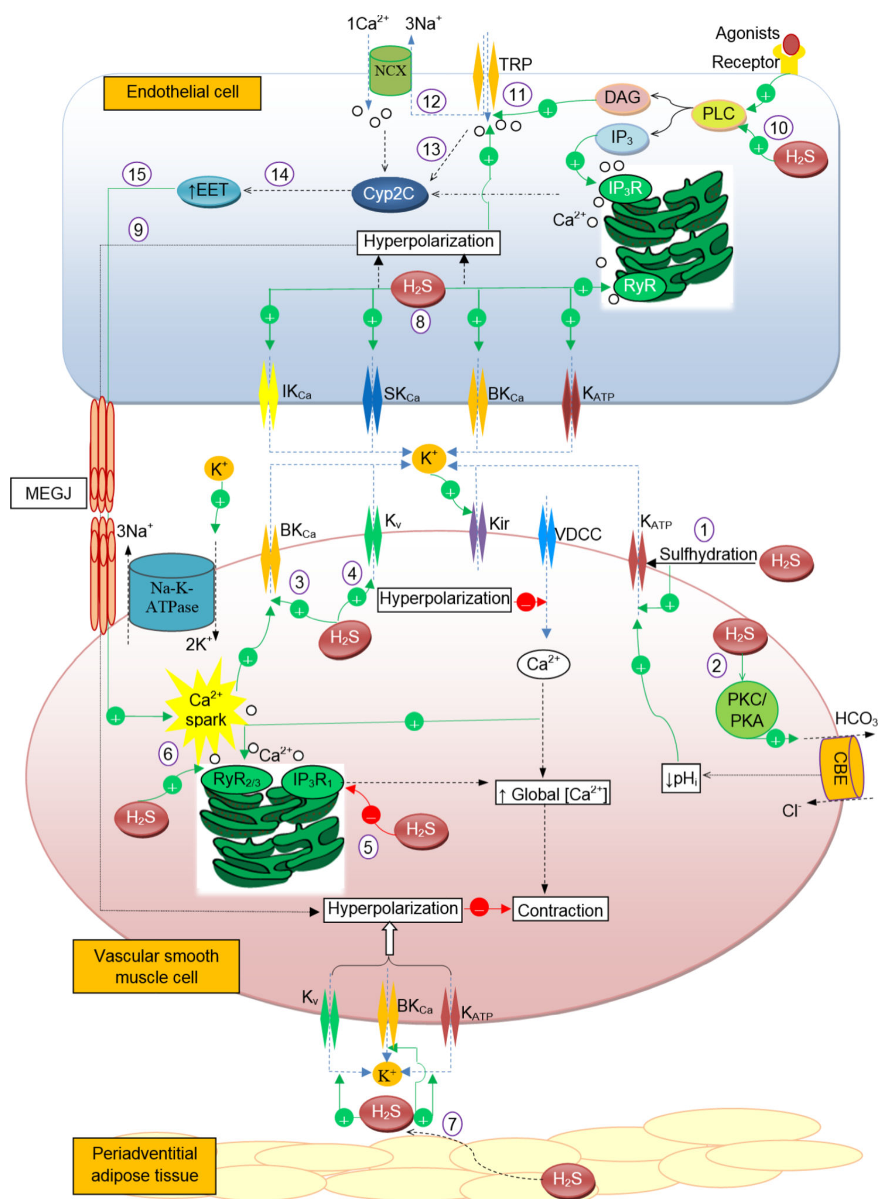

Figure 2. Mechanisms of hydrogen sulfide-induced vasorelaxation

In vascular smooth muscle (VSM) cell, H_2S causes hyperpolarization, which closes voltage-dependent Ca^{2+}-channels (VDCC) and therefore relaxes smooth muscle from different pathways: (1) activation of K_{ATP} channels, (2) protein kinase C (PKC)/protein kinase A (PKA)-dependent activation of chloride/bicarbonate exchanger (CBE), (3) activation of big conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels and (4) activation of voltage-dependent K^+ (K_v) channels. In addition, H_2S inhibits Ca^{2+} release from inositol triphosphate receptors (IP_3) channels (5) and activates Ca^{2+} sparks (6). Diffused H_2S from periadventitial adipose tissue (PAT) activates K_{ATP}, BK_{Ca}, and K_v channels on VSM and causes hyperpolarization

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(7). In endothelium, H_2S causes hyperpolarization through activation of SK_{Ca}, IK_{Ca}, and BK_{Ca} channels and also K_{ATP} channels (8); hyperpolarization transfer from endothelial to VSM cells via myoepithelial gap junctions (MEGJ) (9). In addition, H_2S increases intracellular Ca^{2+} concentration by acting on phospholipase Cβ (PLCβ) (10), and transient receptor potential (TRP) channels (11), which favor reverse mode of Na^+-Ca^{2+} exchanger (NCX) (12), and activates cytochrome P450 epoxygenase (Cyp2C) (13) and production of epoxyeicosatrienoic acid (EET) (14); EET diffuse to VSM and activates Ca^{2+} sparks (15), which leads to relaxation. ER, endoplasmic reticulum; SR, sarcoplasmic reticulum; DAG, diacylglycerol; RyRs, ryanodine receptors.

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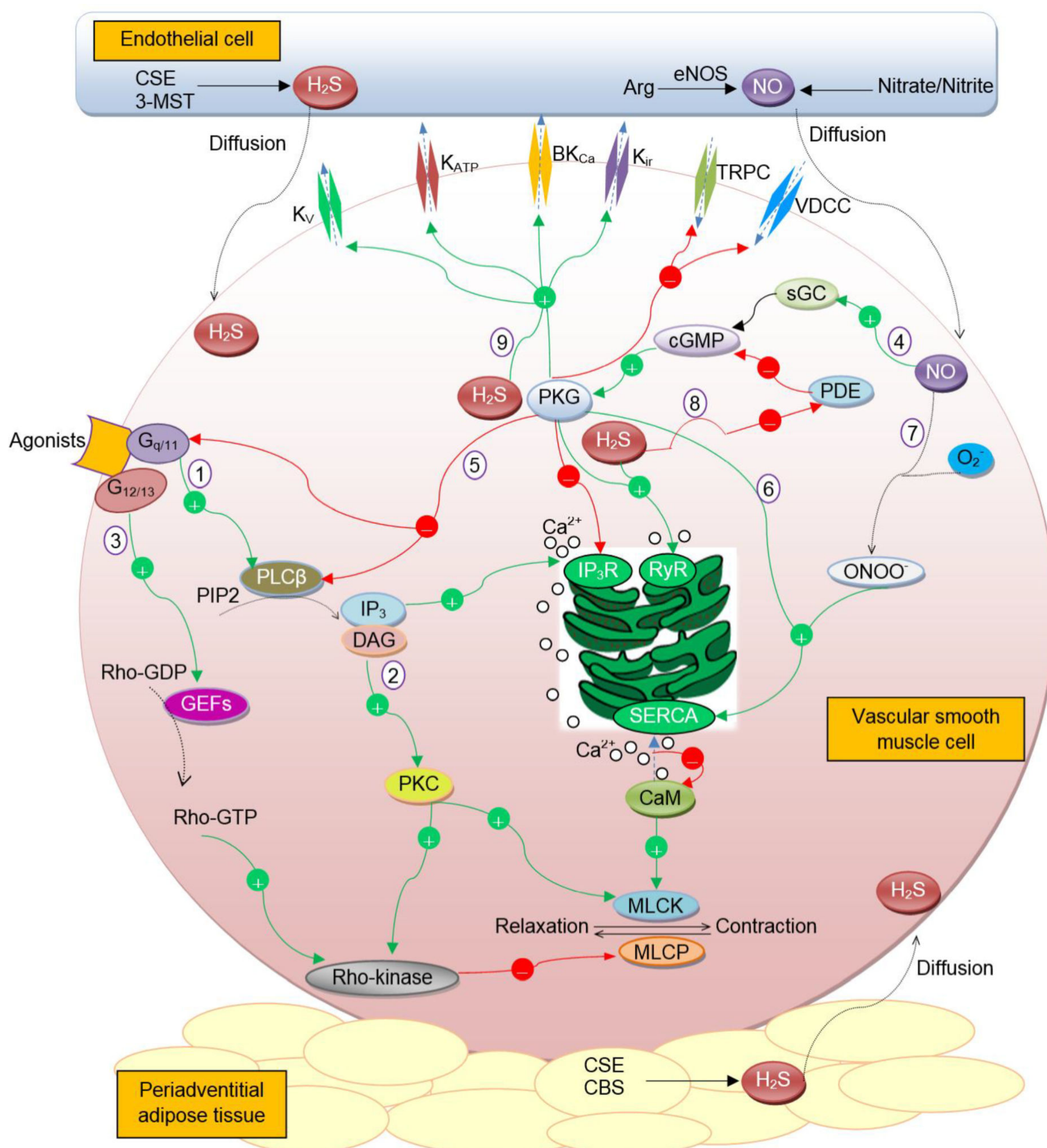

Figure 3. Interaction between hydrogen sulfide and nitric oxide signaling pathways in vessels

Vasoconstrictor agonists through Gq/11/phospholipase Cβ (PLCβ) increases $[Ca^{2+}]_i$ and causes contraction through calcium-calmodulin (CaM)/myosin light-chain kinase (MLCK) pathway (1). Diacylglycerol (DAG) activates protein kinase C (PKC), which phosphorylates MLCK and Rho kinase (2). Vasoconstrictor receptors are also coupled to G12/13, which activates Rho guanine nucleotide exchange factors (GEFs) and then Rho-kinase, which inhibits myosin light-chain phosphatase (MLCP) (3). NO through sGC/cGMP/PKG pathway (4) inhibits transient receptor potential type C (TRPC) channels, voltage-dependent calcium channels (VDCC), and inositol-3-phosphate (IP_3) receptor (IP_3R), as well as activates

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inward-rectifier K^+ channels (K_{ir}), big conductance Ca^{2+} activated K^+ channels (BK_{Ca}), voltage-dependent K^+ channels (K_v), ATP-sensitive K^+ channel (K_{ATP}), and ryanodine receptors (RyR) in vascular smooth muscle (VSM) cells. PKG inhibits Gq/11 and PLCβ (5) as well as activates sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (6) and decreases $[Ca^{2+}]_i$ and therefore causes VSM relaxation. NO reacts with superoxide anion (O_2^-) to produce $ONOO^-$, which activates SERCA (7). H_2S inhibits phosphodiesterase (PDE) and activates the cGMP–PKG pathway (8). In addition, H_2S activates K_{ir}, BK_{Ca}, K_v, K_{ATP}, and RyR independent of cGMP–PKG pathway (9). PIP_2, phosphatidylinositol 4,5-bisphosphate; sGC, soluble guanylyl cyclase.

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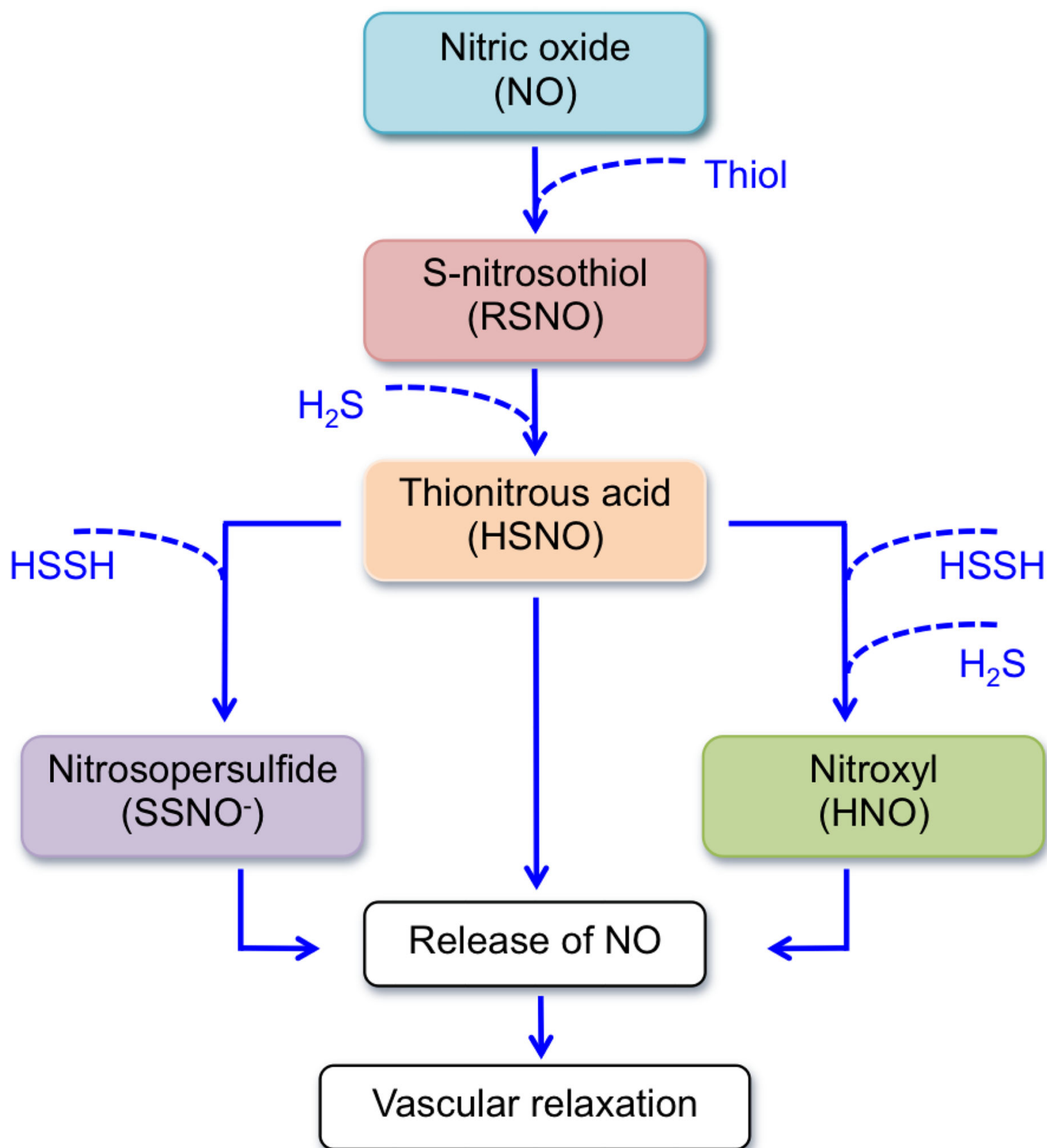

Figure 4. Intermediates produced from reaction between hydrogen sulfide and nitric oxide and their vasoregulatory effects

HSSH, hydrogen persulfide.

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Table 1

Major types of ion channels affected by hydrogen sulfide and/or nitric oxide in vascular smooth muscle and endothelial cells [116, 121, 122, 125–135, 137, 140, 141]

Location of channels	Channel Type	VSM cells	Endothelial cells
Plasma membrane	Potassium	K_{ir}6.x (K_{ATP})	K_{ir}? (K_{ATP})
		K_{Ca}1.1 (BK_{Ca})	K_{Ca}1.1 (BK_{Ca}), K_{Ca}3.1 (IK_{Ca}), K_{Ca}2.3 (SK_{Ca}3)
		K_v7.x	NR
	Calcium	Ca_v1.2	NR
		TRPC1, -C3, -C6	TRP?
Endoplasmic/sarcoplasmic reticulum membrane	RyR	RyR_2, RyR_3	RYR?
	IP_3R	IP_3R_1	IP_3R_2, IP_3R_3

K_{ir}, inward-rectifier K^+ channels; K_{ATP}, ATP-dependent K^+ channels; K_{Ca}, Ca^{2+}-activated K^+ channels; BK_{Ca}, big conductance K_{Ca}; IK_{Ca}, intermediate conductance K_{Ca}; SK_{Ca}, small conducatance K_{Ca}; K_V, voltage-dependent K^+ channels; Ca_V, voltage-dependent Ca^{2+} channels; TRP, transient receptor potential channels; RyR, ryanodine receptors; IP_3R, inositol-3- phosphate receptor;

NR, not reported; ?, the subtype of the channel has not yet been identified.