Hypoxia

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REVIEW

Modulation of hydrogen sulfide by vascular hypoxia

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Abstract: Hydrogen sulfide (H_2S) has emerged as a key regulator of cardiovascular function. This gasotransmitter is produced in the vasculature and is involved in numerous processes that promote vascular homeostasis, including vasodilation and endothelial cell proliferation. Although H_2S plays a role under physiological conditions, it has become clear in recent years that hypoxia modulates the production and action of H_2S. Furthermore, there is growing evidence that H_2S is cytoprotective in the face of hypoxic insults. This review focuses on the synthesis and signaling of H_2S in hypoxic conditions in the vasculature, and highlights recent studies providing evidence that H_2S is a potential therapy for preventing tissue damage in hypoxic conditions.

Keywords: H_2S, cystathionine γ-lyase, vascular smooth muscle, endothelium

Introduction

It has long been known that hydrogen sulfide (H_2S) is endogenously produced by multiple enzymes, including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST). This small polar molecule with a structure very similar to water was thought for many years to be a toxic metabolite with no physiological function, but recent studies have demonstrated that H_2S acts in mammalian tissues as a second messenger, an antioxidant, and a sulfhydrating agent. It has important functions in regulating neurotransmission, transcription, angiogenesis, and vascular tone, and both the production and mechanism of action can be modified by exposure to hypoxia.

Within the vasculature, H_2S regulates the function of smooth muscle and endothelial cells and can be produced by enzymes in the endothelium, media, and the perivascular fat.[1–3] It regulates vascular smooth muscle cell (VSMC) proliferation[4–8] and contraction,[9–12] and endothelial cell proliferation,[13–16] adhesion,[17–19] and release of dilators.[20] Thus, H_2S causes both vasodilation and inhibition of vascular wall remodeling, similar to effects of the well-known gasotransmitter nitric oxide (NO).

It has become clear in recent years that H_2S is synthesized under physiological conditions and plays an important role in vascular function (reviewed in Wang[21] and Mancardi et al[22]). For example, global deletion of CSE, the predominant vascular H_2S-synthesizing enzyme, results in hypertension and impaired endothelium-dependent vasorelaxation.[23] Additionally, H_2S constrains vascular tone under normoxic conditions. However, the cellular response to hypoxia induces changes in H_2S synthesis and signaling. Within the vascular wall, multiple responses have been attributed to this newest member of the gasotransmitter family, and this review will focus on how H_2S regulates vascular function under normoxic and hypoxic conditions.

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Hypoxia and H_2S synthesis

H_2S is produced endogenously by desulfhydration of the amino acid cysteine by the pyridodoxyl 5′phosphate-dependent enzymes CSE and CBS or 3-MST.[24] Although other H_2S synthesis pathways have been described,[25] the majority of endogenous H_2S synthesis occurs via CSE or CBS.[24] Tissue distribution of these enzymes varies, with CBS producing H_2S in the central nervous system[26,27] and CSE being largely responsible for H_2S production in peripheral tissues, including the vasculature.[2,23,28,29] Because of the tissue distribution of H_2S-generating enzymes and the vascular focus of this review, more attention will be given to the effects of hypoxia on CSE generation of H_2S.

Transcriptional and posttranscriptional regulation of CSE under physiological conditions is currently under investigation. One known transcriptional regulator of the CSE gene, *CTH*, is the ubiquitously expressed transcription factor specificity protein 1 (Sp1). Sp1 represses or activates thousands of genes involved in a variety of processes and is regulated by phosphorylation (reviewed in Tan and Khachigian[30]). In differentiated human aortic smooth muscle cells, Sp1 stimulates increased *CTH* expression.[31] Sp1 levels, and in turn CSE expression, are decreased by microRNA-21 (miR-21),[32] which is elevated in hypoxic conditions.[33] Thus, it appears that hypoxia decreases CSE expression in the vascular wall by miR-21-induced suppression of Sp-1-dependent transcription (Figure 1).

As reported by Yang et al,[23] Ca^{2+}-calmodulin may acutely activate CSE in vitro. Separately, neither Ca^{2+} nor calmodulin affects CSE activity but together causes a significant increase in enzyme generation of H_2S. The concentration of Ca^{2+} used in these studies, however, was supraphysiological at 1–2 mM, and further studies are required to determine the role of Ca^{2+}-calmodulin in CSE regulation under physiological conditions in the vasculature. Mikami et al[34] assessed Ca^{2+} regulation of H_2S production by CSE that was purified from rat liver. In solution, CSE produced H_2S at Ca^{2+} concentrations from 0–100 nM, but when the Ca^{2+} concentration was increased to 300 nM, H_2S production decreased. Furthermore, adding calmodulin or a calmodulin inhibitor did not affect H_2S production. These results are not in alignment with the findings from Yang et al[23] in intact tissues, underscoring the lack of understanding of regulation of CSE by Ca^{2+} or other physiological regulators.

Direct evaluation of *CTH* expression and CSE activity, and H_2S synthesis in vivo, has also yielded conflicting results, and evaluation of these parameters in response to hypoxia is limited. Although the mechanisms of hypoxia's regulation of H_2S synthesis are not well defined, hypoxia-induced effects on H_2S synthesis have been observed in many cell

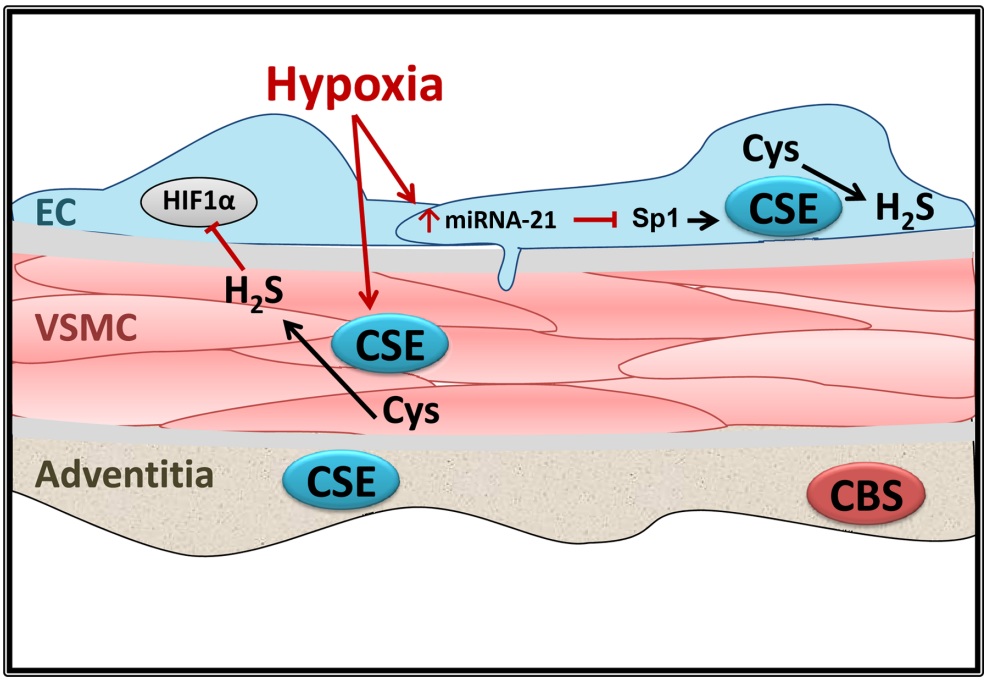

Figure 1 Effects of hypoxia on CSE expression in the vascular wall.
Note: Hypoxia has been shown to decrease CSE expression but direct effects of hypoxia on CSE activity are less defined.
Abbreviations: H_2S, hydrogen sulfide; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; VSMC, vascular smooth muscle cell; EC, endothelial cell; HIF1α, hypoxia inducible factor 1α; miR-21, microRNA-21; Sp1, specificity protein 1; Cys, cysteine.

types,[35–37] with H_2S in turn promoting protective cellular responses to hypoxia.[14,38,39] In a study using multiple cell culture lines, in vitro measurements of *CTH* promoter activity revealed a decrease in promoter activity after 2 hours of hypoxia.[40] A decrease in *CTH* messenger RNA (mRNA) also occurred very quickly after the onset of exposure to hypoxia, but this repression was followed by a recovery of *CTH* mRNA and protein levels within 2 hours.[40] It is not clear whether miR-21 provides a sustained inhibition of CSE expression in vivo, and additional study is needed to better understand this regulation.

As illustrated in Figure 2, H_2S is involved in regulating multiple endothelial processes. It has been shown to regulate mitochondrial function under physiological conditions, acting as an electron donor at low concentrations and inhibiting mitochondrial complex IV at higher concentrations (reviewed in Szabo et al[41]). Studies have assessed the impact of hypoxia on H_2S regulation of mitochondrial function and have shown both beneficial and deleterious effects of the gasotransmitter, depending on the concentration of H_2S. Fu et al[42] investigated CSE expression and activity in mitochondria of mesenteric artery VSMC under basal and hypoxic conditions. CSE expression was not detected in mitochondria under basal conditions, but exposure to the Ca^{2+} ionophore A23187 to mimic hypoxic cellular stress led to mitochondrial CSE expression. Translocation of CSE to mitochondria under these conditions resulted in mitochondrial H_2S production and enhanced ATP production. Furthermore, the H_2S donor NaHS caused a concentration-dependent decrease in ATP production in normoxia but increased ATP production during hypoxia. These results suggest that enhanced VSMC mitochondrial CSE expression and activity, triggered by cellular stressors such as hypoxia, may be one mechanism by which H_2S is protective during hypoxic insults in the vasculature.

Evidence that CSE expression is decreased in the vasculature in conditions associated with hypoxia is growing. For example, Cindrova-Davies et al[33] demonstrated that CSE mRNA and protein expression is decreased in placentas from women with intrauterine growth restriction or preeclampsia with diminished umbilical artery blood flow, two conditions associated with placental hypoxia. CSE expression is not decreased if the preeclampsia is not accompanied by diminished umbilical artery flow, suggesting that it is the functional hypoxia rather than other preeclamptic factors that decrease placental CSE expression. Expression of miR-21, which can indirectly suppress CSE expression through downregulation of Sp1 as described above,[32] is also increased in intrauterine growth restriction and preeclampsia placentas. Furthermore, exposure of placental explants from healthy placentas to

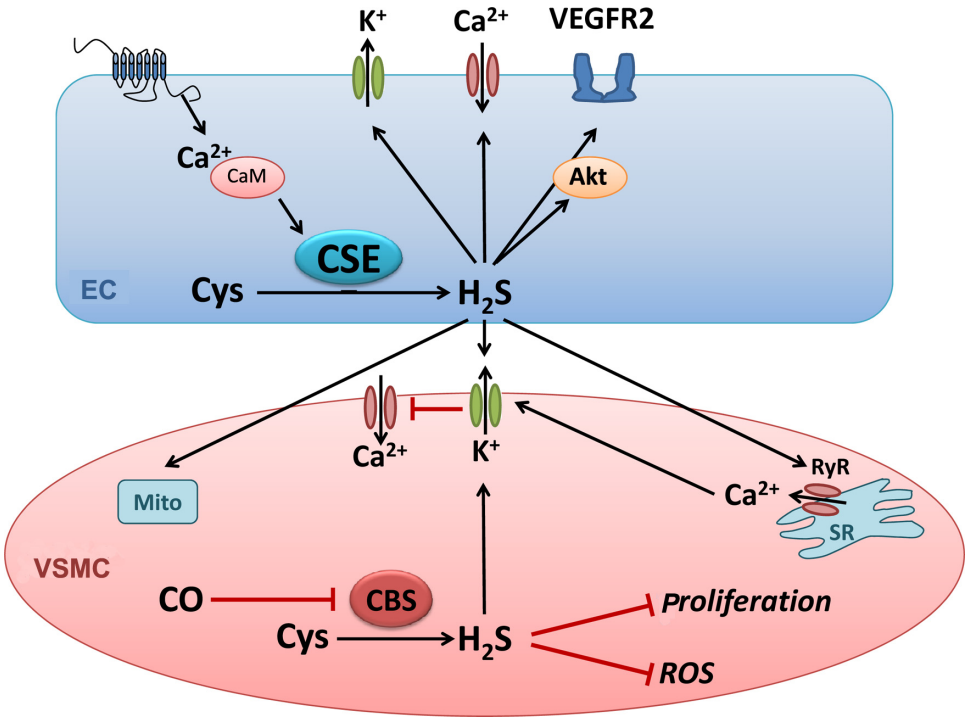

Figure 2 Hydrogen sulfide signaling in the vascular wall.
Notes: H_2S can activate multiple signaling pathways in both endothelial and smooth muscle cells. These pathways increase proliferation and protect from oxidative stress.
Abbreviations: H_2S, hydrogen sulfide; CO, carbon monoxide; Cys, cysteine; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; VEGFR2, vascular endothelial growth factor receptor 2; ROS, reactive oxygen species; VSMC, vascular smooth muscle cell; Mito, mitochondria; EC, endothelial cell; CaM, calmodulin; RyR, ryanodine receptor; SR, sarcoplasmic reticulum.

hypoxia for 3 hours, followed by reoxygenation for 20 hours, decreases CSE expression and increases miR-21 expression. These findings suggest that hypoxic conditions, at least during pregnancy, may decrease H_2S production via a miR-21-dependent decrease in CSE expression.

Rats exposed to 3 weeks of intermittent hypoxia have decreased plasma H_2S levels.[43] Furthermore, *CTH* mRNA expression and H_2S production in lung homogenates are decreased in the hypoxic group. This decrease in CSE expression and H_2S availability is associated with an increase in pulmonary artery pressure. Administration of exogenous H_2S prevents the hypoxia-induced increase in pressure. Taken together, these findings suggest that intermittent hypoxia exposure decreases H_2S production, and the loss of H_2S may be involved in the development of hypoxia-induced pulmonary hypertension. However, miR-21 and Sp1 levels have not been evaluated in this model, and it is unclear whether H_2S production is decreased in a miR-21- and Sp-1-dependent manner.

Although the predominant pathway for H_2S synthesis in the vasculature involves enzymatic activity of CSE, it is important to consider the indirect vascular effects of CBS deficiency. CBS activity regulates clearance of homocysteine by metabolizing it to H_2S. Increased plasma homocysteine levels are a risk factor for cardiovascular disease in humans. Homozygous and heterozygous deletion of CBS has been employed to model hyperhomocysteinemia experimentally, and this deletion results in vascular complications such as endothelial dysfunction, oxidative stress, and remodeling (reviewed in Steed and Tyagi[44]). H_2S therapy may be a beneficial treatment for the deleterious vascular effects of hyperhomocysteinemia. For example, Sen et al[45] demonstrated that H_2S supplementation prevents hyperhomocysteinemia-induced renal damage in mice.

Interestingly, there appears to be a link between homocysteine levels and hypoxia. Homocysteine levels were measured in patients with metabolic syndrome with and without obstructive sleep apnea. Patients with sleep apnea, who are exposed to intermittent bouts of hypoxia during sleep, have higher serum homocysteine levels than those observed in patients with metabolic syndrome alone.[46] Furthermore, higher homocysteine levels correlates with more severe sleep apnea. Whether the same is true for patients without metabolic syndrome is unclear.

Hypoxia and H_2S in the endothelium

The H_2S-synthesizing enzymes CSE and 3-MST are expressed in endothelial cells (Figure 1),[47,48] and H_2S has been implicated in a number of endothelial processes, including angiogenesis, endothelium-dependent vasodilation, and regulation of inflammation (Figure 2).[48–52] However, little is known about how H_2S might mediate these physiological processes. In a human embryonic kidney expression system, H_2S was shown to exert at least a portion of its effects through sulfhydration of cysteine residues on target proteins.[53] Furthermore, H_2S has been shown to act as both an autocrine and a paracrine factor, suggesting that in the endothelium, locally synthesized H_2S might regulate vasodilation and angiogenesis via activation of Ca^{2+}-activated K^+ channels, vascular endothelial growth factor (VEGF) receptor 2, or other endothelial targets.[50,51,54]

In the endothelium, H_2S promotes angiogenesis under normoxic conditions. As reported by Cai et al,[49] NaHS-induced angiogenesis occurs through Akt signaling and does not involve enhanced VEGF or NO levels. There are conflicting results regarding H_2S regulation of hypoxia-induced angiogenesis. A study by Wu et al[55] suggested that H_2S decreases the angiogenic response to hypoxia. The authors demonstrated that NaHS stimulates endothelial tube formation, as does hypoxia. During hypoxia, however, NaHS decreases tube formation. Furthermore, NaHS decreases expression of the transcription factor hypoxia inducible factor 1α (HIF1α) in endothelial cells during hypoxia has been shown to blunt hypoxia-driven increases in VEGF. This occurs through suppression of HIF1α translation and may be a mechanism by which high concentrations of H_2S can limit angiogenesis.

Using a rodent model of unilateral hind limb ischemia, Wang et al[56] provided evidence that H_2S promotes angiogenesis in hypoxic conditions in vivo. Daily treatment with NaHS increases collateralization, capillary density, and blood flow in rat hind limbs made ischemic by femoral artery ligation. NaHS also increases VEGF protein expression in hind-limb muscles and phosphorylation of the VEGF receptor 2 in endothelial cells from ischemic hind limbs, but does not affect plasma levels of NO metabolites. Although not in agreement with the findings described above, these results highlight the potential therapeutic benefit of H_2S donors for patients with peripheral artery disease.

H_2S has been well described as a vasodilator in the systemic circulation, but the mechanism by which H_2S elicits dilation appears to vary by vascular bed and is described in greater detail below. In at least some vascular beds, H_2S-mediated vasodilation requires the endothelium,[48,50,51] but there is evidence in other vascular beds and especially in larger arteries that the endothelium is not required.[57,58] Additionally, endothelial CSE-derived H_2S has been shown to contribute

to cholinergic-mediated vasorelaxation of mesenteric arteries since loss of endothelial *CTH* expression blunts relaxation to methacholine.[23] In addition to the evidence that H_2S contributes to vasodilation under normoxic conditions, evidence is accumulating showing this gasotransmitter is involved in hypoxia-mediated vasodilation. As described in a study by Olson et al,[36] hypoxia and exogenous H_2S elicit similar relaxation responses in a rat's thoracic aorta. Preventing H_2S synthesis with the CSE inhibitor propargyl glycine (PAG) almost completely blocks the relaxation response to hypoxia, bolstering the conclusion that CSE-derived H_2S is required for hypoxia-mediated aortic relaxation.

Where and how hypoxia regulates H_2S signaling in the vasculature is not clear. In the cerebral circulation, there is evidence that hypoxia-induced dilation requires H_2S but also involves carbon monoxide (CO). In cerebral arterioles, endothelial-derived CO increases vasoconstrictor tone.[59] Under normoxic conditions, heme oxygenase-2 (HO-2) produces CO, which binds the heme of astrocytic CBS and inhibits CBS activity.[60] As demonstrated by Morikawa et al[61] in mice, hypoxia decreases cerebral CO production and elicits HO-2- and CBS-dependent vasodilation of arterioles in the cerebral cortex. These findings suggest that H_2S is required for hypoxia-induced vasodilation in cerebral arterioles, but that hypoxia removes an inhibitor of H_2S production rather than directly activating H_2S signaling. It is unclear whether this pathway is unique to the cerebral circulation, where arterioles are in close proximity to CBS-expressing astrocytes. Indeed, the findings are somewhat confounded by evidence that hypoxia regulates expression of the enzymes that produce H_2S, including an increase in both *CTH* mRNA and protein in hypoxia-exposed VSMC.[40]

Another potential but controversial role of H_2S is modulation of inflammation. The cumulative data to date suggest that H_2S is pro-inflammatory at high concentrations and anti-inflammatory at low concentrations.[52] Zanardo et al[62] demonstrated that exogenous H_2S inhibits leukocyte adhesion to postcapillary mesenteric venule endothelial cells, whereas inhibiting endogenous H_2S production increases leukocyte adherence to the endothelium. These compelling findings provide evidence that H_2S can protect arteries from some aspects of inflammation, but little is known about the effect of hypoxia on this process in the vasculature. Fiorucci et al[63] investigated the role of H_2S in gastric mucosal injury caused by diminished blood flow, and thus hypoxia, after acetyl salicylic acid (ASA) treatment. NaHS reduced gastric injury and decreased inflammation. ASA treatment appeared to decrease endogenous H_2S generation by reducing levels of the *CTH* activator Sp1. Since Sp1 is repressed by miR-21, as discussed above, and miR-21 is upregulated by hypoxia, this may be the pathway for ASA suppression of *CTH* expression. Studies to determine whether hypoxia does indeed decrease the anti-inflammatory effects of H_2S are an intriguing area for future investigation.

In clinical studies, there is evidence that H_2S signaling is impaired in atherosclerosis, a condition involving inflammation of the endothelium and potentially leading to peripheral artery disease and thus, limb ischemia and hypoxia. A recent study demonstrated that knockout of *CTH* in mice exacerbates the effects of an atherogenic diet, suggesting that endogenous H_2S protects against atherosclerosis.[64] Furthermore, administration of a slow-releasing H_2S compound, GYY4137, decreases aortic plaque formation in apoE knockout mice.[65] As with many of the other conditions described above, these results underscore that H_2S treatment may limit the deleterious effects of atherosclerosis and other hypoxic conditions in humans.

A final consideration regarding the effects of hypoxia on H_2S in the endothelium is the interaction between H_2S and NO during hypoxia. The interaction between these gases has been evaluated under normoxic conditions, with conflicting results that include both H_2S inhibition of NO synthase (NOS)[66] and H_2S activation of NOS,[67] as well as formation of a nitrosothiol compound from the gases.[68] Bir et al[69] evaluated the interaction between NO and H_2S in a rodent model of unilateral hind-limb ischemia and reported that H_2S promotes ischemic vascular remodeling in an NO-dependent manner. Specifically, H_2S therapy increases NO metabolites in plasma and hind-limb muscles, and scavenging NO blunts the improvement in hind-limb blood flow and prevents the enhanced angiogenesis conferred by H_2S treatment. These results suggest a positive interaction between H_2S and NO and provide more evidence of potential therapeutic benefit of H_2S therapy in peripheral artery disease.

Hypoxia and H_2S in myocytes

As illustrated in Figure 2, H_2S can signal through multiple pathways. In VSMC, H_2S has been shown to cause hyperpolarization and relaxation,[48,57,70] and much attention has been given to the mechanism by which this occurs. It is of interest that most studies of H_2S-induced hyperpolarization of VSMC have implicated at least one K^+ channel, even if results do not agree on the particular type of channel involved or the concentration of H_2S required for activation. In 2001, Zhao et al[2] demonstrated that intravenous administration of H_2S lowers blood pressure in rats and relaxes preconstricted aortic

segments, and the ATP-sensitive K^+ channel (K_{ATP}) blocker glibenclamide prevents the dilation, whereas inhibitors of other K^+ channels do not. This was followed by studies from the same group in the rat perfused mesenteric-arterial bed, in which it was observed that the dilation to exogenous H_2S was more pronounced than in the aorta, was endothelium dependent, and was only partially inhibited by glibenclamide.[50] Thus, at least within the rat, H_2S can cause dilation in some arteries through direct actions on the VSMC.

Several studies in rat mesenteric arteries, however, have observed dilation at much lower concentrations of H_2S than those required to relax aortic segments and suggest that in this bed, vasodilation requires the endothelium and is mediated by large-conductance calcium-sensitive potassium channels (BK_{Ca}) rather than K_{ATP}.[48,51,71] Thus, concentrations of H_2S above 10 μM activate K_{ATP} channels to hyperpolarize and relax VSMC, but lower concentrations stimulate K_{ATP}-insensitive relaxation of VSMC, and further studies are necessary to determine whether only one or both of these pathways are physiologically relevant. In spite of the controversy over the molecular mechanism of action of H_2S, there is little controversy that endogenous activity of CSE in the vasculature is affected by several hypoxia-inducing diseases and that H_2S can protect VSMC from hypoxia-induced damage and death.

As described above, H_2S elicits VSMC relaxation in normoxic conditions. However, hypoxia appears to diminish H_2S's ability to promote vasodilation. In a series of studies, Jackson-Weaver et al[48,51] reported that isolated mesenteric arteries from rats exposed to intermittent hypoxia have decreased expression and function of CSE that results in increased myogenic tone compared with arteries from control rats. Furthermore, in rats exposed to intermittent hypoxia, an apparent decrease in CSE expression causes VSMC depolarization and diminished activity of Ca^{2+} sparks, a vasodilatory signaling pathway. Thus, intermittent hypoxia appears to impair H_2S inhibition of myogenic tone and to promote enhanced contraction.

Evidence from Olson et al[36] suggests that H_2S may be an O_2 sensor/transducer in hypoxic responses of vertebrate arteries. In isolated bovine pulmonary artery rings, both hypoxia and H_2S (1 mM) elicit contraction when administered separately. When applied together, these agents are competitive, suggesting that they produce contraction through similar mechanisms and H_2S may mediate hypoxic pulmonary vasoconstriction (HPV). Additionally, hypoxia and H_2S cause VSMC membrane potential depolarization in bovine pulmonary arteries. The mechanism by which H_2S mediates hypoxic vasoconstriction in pulmonary arteries is not clear, but results from a subsequent study from this group suggested H_2S stimulates production of mitochondrial superoxide, which is dismutated to hydrogen peroxide to trigger downstream hypoxic vasoconstriction.[72]

Further support for a role for H_2S in HPV is provided in a recent study by Madden et al,[73] in which HPV responses were measured in intact, isolated, perfused rat lungs in conditions that promote or inhibit H_2S synthesis. Providing cysteine to enhance H_2S production augmented the hypoxia-induced increase in pulmonary artery pressure in isolated lungs. Conversely, inhibition of CSE with PAG diminished the pressure response to hypoxia. These findings highlight the contribution of H_2S to HPV responses and suggest that this gasotransmitter is crucial for an appropriate pulmonary vascular response to hypoxia.

H_2S also appears to protect VSMC from hypoxia-induced apoptosis and mitochondrial depolarization. A 2011 study by Bryan et al[74] demonstrated that SMC from CSE knockout mice were more susceptible to apoptosis, mitochondrial depolarization, and cell death after exposure to 1.0% O_2. There was also increased generation of mitochondrial reactive oxygen species (ROS).[74] Similarly, human pulmonary VSMC exposed to $CoCl_2$, to mimic hypoxia, had decreased H_2S levels.[7] However, in pulmonary VSMC, hypoxia augmented proliferation and this was ameliorated by exogenous H_2S, suggesting endogenous H_2S might blunt hypoxia-induced vascular remodeling and the development of pulmonary hypertension.[7] In a study of VSMC from rats exposed to intermittent rather than sustained hypoxia, Hongfang et al[4] reported that treatment with NaHS inhibits the hypoxia-mediated increase in pulmonary artery muscularization resulting from VSMC proliferation, as well as the increase in pulmonary artery pressure, further supporting a protective role of H_2S on VSMC proliferation in conditions of pulmonary hypoxia.

The role of CBS-generated H_2S under hypoxic conditions has not been extensively investigated. However, mice heterozygous for a mutated *CBS* gene (*CBS*$^{+/-}$) do appear to have a defective angiogenic response to ischemia.[75] The impaired formation of collateral vessels was attributed to loss of HIF-1α and VEGF signaling, suggesting that CBS generation of H_2S might act permissively to promote HIF signaling in skeletal muscle arteries. In addition, hypoxia has been shown to increase the expression of *CBS* mRNA in the cerebral circulation in a HIF-dependent manner,[76] suggesting a feedback regulation of CBS on HIF signaling. Acute regulation of CBS generation of H_2S is also apparent in the cerebrovascular circulation. Studies by Morikawa et al[61] reported that cerebrovascular vasodilation following hypoxia

is diminished in *CBS*$^{+/-}$ compared with wild-type mice. However, it is unclear whether this is a direct vascular effect or whether it is mediated by H_2S release from neurons or glial cells, since the studies were performed in mice with global knockdown of the *CBS* gene. Thus both acute and chronic responses to hypoxia have some dependence on CBS production of H_2S.

In human keratinocytes, cell death in response to $CoCl_2$, a surrogate for hypoxia, was ameliorated by treatment with H_2S donors. Compared with cells exposed to $CoCl_2$ alone, cells pretreated with NaHS had lower levels of ROS generation and decreased NFκB activation upstream of cyclooxygenase induction, the source of ROS.[77] Thus, H_2S appears to exert both direct and indirect antioxidant effects to protect VSMC and other cells from hypoxia-induced damage and cell death.

In HEP3B cells, exposure to 1.0% O_2 triggered the induction of HIF1α-regulated genes, but H_2S decreased the induction of these genes through destabilization of HIF1α protein.[78] H_2S was shown to inhibit mitochondrial oxygen consumption, leading to an increase in mitochondrial ROS generation and ubiquitination and degradation of the HIF1α protein. Wu et al[55] also found that exposing cells to high levels of a H_2S donor (10–100 μM NaHS) diminished hypoxia induction and stabilization of HIF1α protein. However, in the study by Wu et al, the effects were independent of ubiquitination, suggesting that H_2S might regulate HIF1α protein stability by multiple mechanisms depending on the conditions and cell types in which hypoxia occurs.

H_2S donors have been administered chronically in vivo to prevent hypoxia-induced morbidity. Treating rats for 3 weeks with daily injections of an H_2S donor (2–200 mg/kg) protected cerebral arteries from hypoxia-induced damage.[79] In a rat model of myocardial ischemia/reperfusion injury, H_2S administration at the time of reperfusion decreased myocardial infarct size, inflammation, and cardiomyocyte apoptosis and preserved mitochondrial function.[80] Furthermore, cardiac-specific overexpression of CSE diminished ischemia-reperfusion injury as measured by myocardial infarct size. In the kidney, ischemia markedly reduced renal production of H_2S, and CSE knockout mice had increased damage and mortality after renal ischemia/reperfusion injury, suggesting that endogenous CSE activity is an important defense against hypoxic stress and preserves vascular perfusion.[81] Treatment with H_2S donors in this study prevented ischemia-induced renal injury in the CSE knockout mice, and overexpression of CSE reduced ROS generation in isolated tubules exposed to hypoxia. It is particularly intriguing that the level of CSE expression in kidneys from individual mice positively correlated with glomerular filtration rate and that glomeruli expressed high levels of CSE. These studies further suggest that the H_2S signaling system provides important vascular protection against ischemia in many parts of the circulation.

H_2S and redox regulation

Oxidative stress is increased in hypoxic environments, and many studies show that H_2S is protective in conditions in which oxidative stress is elevated. Bryan et al[74] demonstrated that ROS levels are higher in mesenteric artery VSMC from CSE knockout mice under normoxic conditions. Hypoxia increases ROS levels in VSMC from wildtype mice but elicits an even greater increase in cells from CSE knockout mice. These findings suggest H_2S regulates redox signaling under basal and hypoxic conditions. There is some controversy over H_2S's role in regulation of redox homeostasis, with studies suggesting both direct and indirect antioxidant function for H_2S. Results from a recent study by Hamar et al[82] indicate that H_2S is not an effective antioxidant itself. On the other hand, there is ample evidence that this gasotransmitter regulates the cellular redox environment by activating antioxidant pathways.

Calvert et al[83] identified nuclear factor E2-related factor (Nrf2) signaling as one of the antioxidant pathways triggered by H_2S. Pretreatment with exogenous H_2S reduced cardiac damage following myocardial ischemia/reperfusion injury, and it increased expression of Nrf2, a transcription factor that regulates many antioxidant genes, and Nrf2's downstream targets HO-1 and thioredoxin. This study, like many others, suggested a potential therapeutic benefit of exogenous H_2S administration to limit oxidative damage in hypoxic conditions. How oxidative stress may affect endogenous H_2S signaling, however, is less clear. Streeter et al[84] reported that *CTH* mRNA expression is increased in cerebral arteries from diabetic rats compared with arteries from control rats, suggesting that oxidative stress may trigger upregulation of endogenous H_2S production to combat redox imbalance.

H_2S in the human population

In comparison with the many studies in rodents, much less is known about the distribution and function of H_2S in humans. Studies of circulating levels of H_2S have shown that both increased[85] and decreased[86] levels of H_2S are associated with vascular disease.

In a study comparing tissues and plasma from 14 preeclamptic women and 14 healthy controls, Wang et al[86]

found lower circulating H_2S and lower levels of placental CSE in women with preeclampsia compared with control patients. The decrease in CSE and H_2S levels was associated with reduced fetal size. In pregnant mice, the CSE inhibitor, PAG, caused hypertension, liver dysfunction, and fetal growth restriction that was reversed by co-administration of an H_2S donor. Thus, H_2S production in placental vessels appears to be necessary for normal placental perfusion and fetal development, however, it is unclear whether this is mediated entirely through endothelial pathways of vessel growth or is also partially dependent on changes in VSMC that lead to enhanced constrictor sensitivity.

Jain et al[87] reported that in patients with atherosclerosis, there is an inverse correlation between plasma levels of low-density lipoprotein and H_2S. In this study, higher H_2S was associated with less plaque development. Studies in diabetic patients also found that low levels of H_2S are associated with worse outcomes in patients that have concurrent sleep apnea,[88] and that renal decline progresses more rapidly in chronic kidney disease patients with low serum H_2S.[89] In the latter study, plasma H_2S levels correlated with the rate of decline in glomerular filtration rate independently of all other predictors. Thus, declines in H_2S production may contribute to multiple vascular diseases in the human population.

In contrast, a study by Peter et al[85] found that plasma levels of H_2S were higher in patients with peripheral artery disease than in a group of patients without detectable vascular disease. This study suggests the relationship between vascular disease and circulating H_2S levels may not be as simple as that in animal models. Additional studies defining variables that affect circulating H_2S levels in humans are needed, as are studies defining how circulating H_2S modifies vascular responses to disease.

Summary

It has become apparent in recent years that H_2S is produced endogenously and is involved in numerous physiological processes, including many that maintain vascular homeostasis. With a role in promoting endothelial proliferation, maintaining normal blood pressure, and eliciting vasodilation, this gas has emerged as a key regulator of vascular function. The protective role H_2S plays in hypoxic conditions in many vascular beds is also becoming apparent. As highlighted in this review, there is exciting new evidence suggesting that this newest gasotransmitter may be a beneficial therapy for patients with a variety of conditions associated with tissue hypoxia.

Disclosure

The authors report no conflicts of interest in this work.

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