

# Modulation of hydrogen sulfide by vascular hypoxia

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**Abstract:** Hydrogen sulfide (H<sub>2</sub>S) 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<sub>2</sub>S plays a role under physiological conditions, it has become clear in recent years that hypoxia modulates the production and action of H<sub>2</sub>S. Furthermore, there is growing evidence that H<sub>2</sub>S is cytoprotective in the face of hypoxic insults. This review focuses on the synthesis and signaling of H<sub>2</sub>S in hypoxic conditions in the vasculature, and highlights recent studies providing evidence that H<sub>2</sub>S is a potential therapy for preventing tissue damage in hypoxic conditions.

**Keywords:** H<sub>2</sub>S, cystathionine  $\gamma$ -lyase, vascular smooth muscle, endothelium

## Introduction

It has long been known that hydrogen sulfide (H<sub>2</sub>S) is endogenously produced by multiple enzymes, including cystathionine  $\beta$ -synthase (CBS), cystathionine  $\gamma$ -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<sub>2</sub>S 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<sub>2</sub>S regulates the function of smooth muscle and endothelial cells and can be produced by enzymes in the endothelium, media, and the perivascular fat.<sup>1-3</sup> It regulates vascular smooth muscle cell (VSMC) proliferation<sup>4-8</sup> and contraction,<sup>9-12</sup> and endothelial cell proliferation,<sup>13-16</sup> adhesion,<sup>17-19</sup> and release of dilators.<sup>20</sup> Thus, H<sub>2</sub>S 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<sub>2</sub>S is synthesized under physiological conditions and plays an important role in vascular function (reviewed in Wang<sup>21</sup> and Mancardi et al<sup>22</sup>). For example, global deletion of CSE, the predominant vascular H<sub>2</sub>S-synthesizing enzyme, results in hypertension and impaired endothelium-dependent vasorelaxation.<sup>23</sup> Additionally, H<sub>2</sub>S constrains vascular tone under normoxic conditions. However, the cellular response to hypoxia induces changes in H<sub>2</sub>S 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<sub>2</sub>S regulates vascular function under normoxic and hypoxic conditions.

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## Hypoxia and H<sub>2</sub>S synthesis

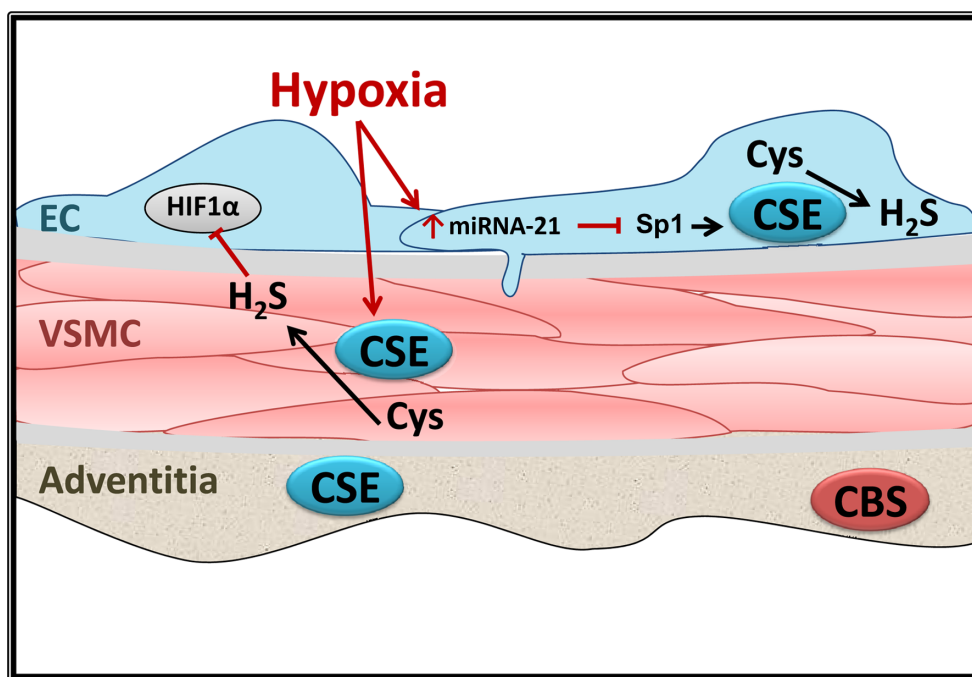
H<sub>2</sub>S is produced endogenously by desulfhydration of the amino acid cysteine by the pyridoxyl 5' phosphate-dependent enzymes CSE and CBS or 3-MST.<sup>24</sup> Although other H<sub>2</sub>S synthesis pathways have been described,<sup>25</sup> the majority of endogenous H<sub>2</sub>S synthesis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

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<sup>30</sup>). In differentiated human aortic smooth muscle cells, Sp1 stimulates increased *CTH* expression.<sup>31</sup> Sp1 levels, and in turn CSE expression, are decreased by microRNA-21 (miR-21),<sup>32</sup> which is elevated in hypoxic conditions.<sup>33</sup> 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,<sup>23</sup> Ca<sup>2+</sup>-calmodulin may acutely activate CSE in vitro. Separately, neither Ca<sup>2+</sup> nor calmodulin affects CSE activity but together causes a significant increase in enzyme generation of H<sub>2</sub>S. The concentration of Ca<sup>2+</sup> used in these studies, however, was supraphysiological at 1–2 mM, and further studies are required to determine the role of Ca<sup>2+</sup>-calmodulin in CSE regulation under physiological conditions in the vasculature. Mikami et al<sup>34</sup> assessed Ca<sup>2+</sup> regulation of H<sub>2</sub>S production by CSE that was purified from rat liver. In solution, CSE produced H<sub>2</sub>S at Ca<sup>2+</sup> concentrations from 0–100 nM, but when the Ca<sup>2+</sup> concentration was increased to 300 nM, H<sub>2</sub>S production decreased. Furthermore, adding calmodulin or a calmodulin inhibitor did not affect H<sub>2</sub>S production. These results are not in alignment with the findings from Yang et al<sup>23</sup> in intact tissues, underscoring the lack of understanding of regulation of CSE by Ca<sup>2+</sup> or other physiological regulators.

Direct evaluation of *CTH* expression and CSE activity, and H<sub>2</sub>S 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<sub>2</sub>S synthesis are not well defined, hypoxia-induced effects on H<sub>2</sub>S 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.

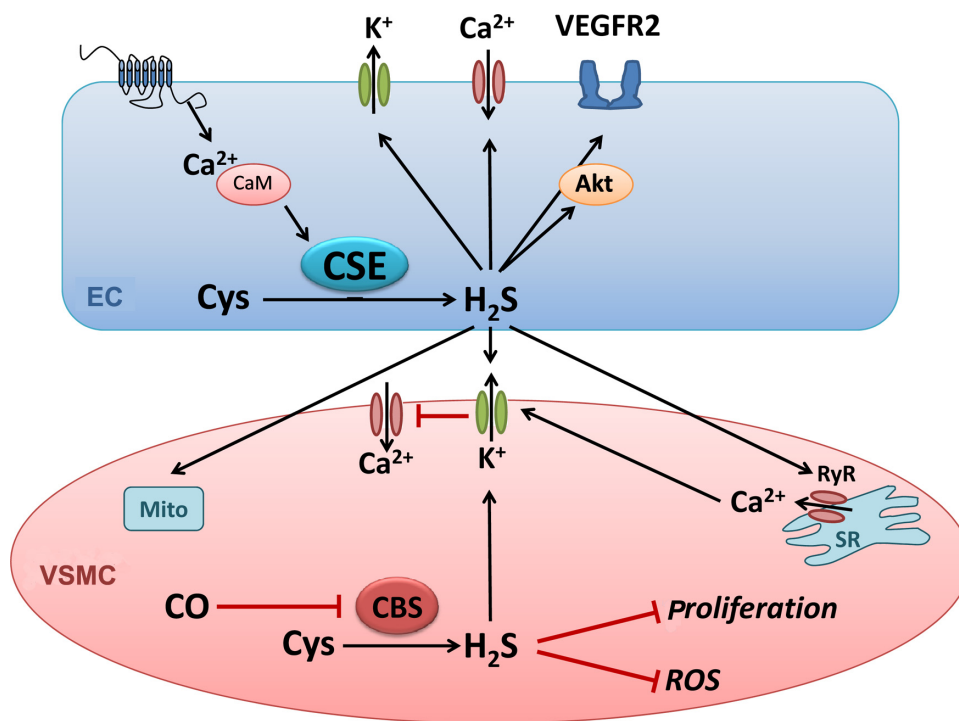
**Abbreviations:** H<sub>2</sub>S, hydrogen sulfide; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; VSMC, vascular smooth muscle cell; EC, endothelial cell; HIF1 α, hypoxia inducible factor 1α; miR-21, microRNA-21; Sp1, specificity protein 1; Cys, cysteine.

types,<sup>35–37</sup> with H<sub>2</sub>S in turn promoting protective cellular responses to hypoxia.<sup>14,38,39</sup> 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.<sup>40</sup> 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.<sup>40</sup> 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<sub>2</sub>S 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<sup>41</sup>). Studies have assessed the impact of hypoxia on H<sub>2</sub>S regulation of mitochondrial function and have shown both beneficial and deleterious effects of the gasotransmitter, depending on the concentration of H<sub>2</sub>S. Fu et al<sup>42</sup> 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<sup>2+</sup> ionophore A23187 to mimic hypoxic cellular stress led to mitochondrial CSE expression.

Translocation of CSE to mitochondria under these conditions resulted in mitochondrial H<sub>2</sub>S production and enhanced ATP production. Furthermore, the H<sub>2</sub>S 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<sub>2</sub>S 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<sup>33</sup> 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,<sup>32</sup> 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<sub>2</sub>S can activate multiple signaling pathways in both endothelial and smooth muscle cells. These pathways increase proliferation and protect from oxidative stress.

**Abbreviations:** H<sub>2</sub>S, 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<sub>2</sub>S production via a miR-21-dependent decrease in CSE expression.

Rats exposed to 3 weeks of intermittent hypoxia have decreased plasma H<sub>2</sub>S levels.<sup>43</sup> Furthermore, *CTH* mRNA expression and H<sub>2</sub>S production in lung homogenates are decreased in the hypoxic group. This decrease in CSE expression and H<sub>2</sub>S availability is associated with an increase in pulmonary artery pressure. Administration of exogenous H<sub>2</sub>S prevents the hypoxia-induced increase in pressure. Taken together, these findings suggest that intermittent hypoxia exposure decreases H<sub>2</sub>S production, and the loss of H<sub>2</sub>S 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<sub>2</sub>S production is decreased in a miR-21- and Sp-1-dependent manner.

Although the predominant pathway for H<sub>2</sub>S 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<sub>2</sub>S. 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<sup>44</sup>). H<sub>2</sub>S therapy may be a beneficial treatment for the deleterious vascular effects of hyperhomocysteinemia. For example, Sen et al<sup>45</sup> demonstrated that H<sub>2</sub>S 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.<sup>46</sup> 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<sub>2</sub>S in the endothelium

The H<sub>2</sub>S-synthesizing enzymes CSE and 3-MST are expressed in endothelial cells (Figure 1),<sup>47,48</sup> and H<sub>2</sub>S has

been implicated in a number of endothelial processes, including angiogenesis, endothelium-dependent vasodilation, and regulation of inflammation (Figure 2).<sup>48-52</sup> However, little is known about how H<sub>2</sub>S might mediate these physiological processes. In a human embryonic kidney expression system, H<sub>2</sub>S was shown to exert at least a portion of its effects through sulphydration of cysteine residues on target proteins.<sup>53</sup> Furthermore, H<sub>2</sub>S has been shown to act as both an autocrine and a paracrine factor, suggesting that in the endothelium, locally synthesized H<sub>2</sub>S might regulate vasodilation and angiogenesis via activation of Ca<sup>2+</sup>-activated K<sup>+</sup> channels, vascular endothelial growth factor (VEGF) receptor 2, or other endothelial targets.<sup>50,51,54</sup>

In the endothelium, H<sub>2</sub>S promotes angiogenesis under normoxic conditions. As reported by Cai et al,<sup>49</sup> NaHS-induced angiogenesis occurs through Akt signaling and does not involve enhanced VEGF or NO levels. There are conflicting results regarding H<sub>2</sub>S regulation of hypoxia-induced angiogenesis. A study by Wu et al<sup>55</sup> suggested that H<sub>2</sub>S 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 1 $\alpha$  (HIF1 $\alpha$ ) in endothelial cells during hypoxia has been shown to blunt hypoxia-driven increases in VEGF. This occurs through suppression of HIF1 $\alpha$  translation and may be a mechanism by which high concentrations of H<sub>2</sub>S can limit angiogenesis.

Using a rodent model of unilateral hind limb ischemia, Wang et al<sup>56</sup> provided evidence that H<sub>2</sub>S 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<sub>2</sub>S donors for patients with peripheral artery disease.

H<sub>2</sub>S has been well described as a vasodilator in the systemic circulation, but the mechanism by which H<sub>2</sub>S elicits dilation appears to vary by vascular bed and is described in greater detail below. In at least some vascular beds, H<sub>2</sub>S-mediated vasodilation requires the endothelium,<sup>48,50,51</sup> but there is evidence in other vascular beds and especially in larger arteries that the endothelium is not required.<sup>57,58</sup> Additionally, endothelial CSE-derived H<sub>2</sub>S has been shown to contribute

to cholinergic-mediated vasorelaxation of mesenteric arteries since loss of endothelial *CTH* expression blunts relaxation to methacholine.<sup>23</sup> In addition to the evidence that H<sub>2</sub>S 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,<sup>36</sup> hypoxia and exogenous H<sub>2</sub>S elicit similar relaxation responses in a rat's thoracic aorta. Preventing H<sub>2</sub>S synthesis with the CSE inhibitor propargyl glycine (PAG) almost completely blocks the relaxation response to hypoxia, bolstering the conclusion that CSE-derived H<sub>2</sub>S is required for hypoxia-mediated aortic relaxation.

Where and how hypoxia regulates H<sub>2</sub>S signaling in the vasculature is not clear. In the cerebral circulation, there is evidence that hypoxia-induced dilation requires H<sub>2</sub>S but also involves carbon monoxide (CO). In cerebral arterioles, endothelial-derived CO increases vasoconstrictor tone.<sup>59</sup> Under normoxic conditions, heme oxygenase-2 (HO-2) produces CO, which binds the heme of astrocytic CBS and inhibits CBS activity.<sup>60</sup> As demonstrated by Morikawa et al<sup>61</sup> 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<sub>2</sub>S is required for hypoxia-induced vasodilation in cerebral arterioles, but that hypoxia removes an inhibitor of H<sub>2</sub>S production rather than directly activating H<sub>2</sub>S 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<sub>2</sub>S, including an increase in both *CTH* mRNA and protein in hypoxia-exposed VSMC.<sup>40</sup>

Another potential but controversial role of H<sub>2</sub>S is modulation of inflammation. The cumulative data to date suggest that H<sub>2</sub>S is pro-inflammatory at high concentrations and anti-inflammatory at low concentrations.<sup>52</sup> Zanardo et al<sup>62</sup> demonstrated that exogenous H<sub>2</sub>S inhibits leukocyte adhesion to postcapillary mesenteric venule endothelial cells, whereas inhibiting endogenous H<sub>2</sub>S production increases leukocyte adherence to the endothelium. These compelling findings provide evidence that H<sub>2</sub>S 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<sup>63</sup> investigated the role of H<sub>2</sub>S 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<sub>2</sub>S 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<sub>2</sub>S are an intriguing area for future investigation.

In clinical studies, there is evidence that H<sub>2</sub>S 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<sub>2</sub>S protects against atherosclerosis.<sup>64</sup> Furthermore, administration of a slow-releasing H<sub>2</sub>S compound, GYY4137, decreases aortic plaque formation in apoE knockout mice.<sup>65</sup> As with many of the other conditions described above, these results underscore that H<sub>2</sub>S treatment may limit the deleterious effects of atherosclerosis and other hypoxic conditions in humans.

A final consideration regarding the effects of hypoxia on H<sub>2</sub>S in the endothelium is the interaction between H<sub>2</sub>S and NO during hypoxia. The interaction between these gases has been evaluated under normoxic conditions, with conflicting results that include both H<sub>2</sub>S inhibition of NO synthase (NOS)<sup>66</sup> and H<sub>2</sub>S activation of NOS,<sup>67</sup> as well as formation of a nitrosothiol compound from the gases.<sup>68</sup> Bir et al<sup>69</sup> evaluated the interaction between NO and H<sub>2</sub>S in a rodent model of unilateral hind-limb ischemia and reported that H<sub>2</sub>S promotes ischemic vascular remodeling in an NO-dependent manner. Specifically, H<sub>2</sub>S 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<sub>2</sub>S treatment. These results suggest a positive interaction between H<sub>2</sub>S and NO and provide more evidence of potential therapeutic benefit of H<sub>2</sub>S therapy in peripheral artery disease.

## Hypoxia and H<sub>2</sub>S in myocytes

As illustrated in Figure 2, H<sub>2</sub>S can signal through multiple pathways. In VSMC, H<sub>2</sub>S has been shown to cause hyperpolarization and relaxation,<sup>48,57,70</sup> and much attention has been given to the mechanism by which this occurs. It is of interest that most studies of H<sub>2</sub>S-induced hyperpolarization of VSMC have implicated at least one K<sup>+</sup> channel, even if results do not agree on the particular type of channel involved or the concentration of H<sub>2</sub>S required for activation. In 2001, Zhao et al<sup>2</sup> demonstrated that intravenous administration of H<sub>2</sub>S lowers blood pressure in rats and relaxes precontracted 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.<sup>50</sup> 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  $K_{ATP}$ .<sup>48,51,71</sup> Thus, concentrations of  $H_2S$  above 10  $\mu M$  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<sup>48,51</sup> 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<sup>36</sup> 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.<sup>72</sup>

Further support for a role for  $H_2S$  in HPV is provided in a recent study by Madden et al,<sup>73</sup> 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<sup>74</sup> 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).<sup>74</sup> Similarly, human pulmonary VSMC exposed to  $CoCl_2$ , to mimic hypoxia, had decreased  $H_2S$  levels.<sup>7</sup> 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.<sup>7</sup> In a study of VSMC from rats exposed to intermittent rather than sustained hypoxia, Hongfang et al<sup>4</sup> 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*<sup>+/-</sup>) do appear to have a defective angiogenic response to ischemia.<sup>75</sup> The impaired formation of collateral vessels was attributed to loss of HIF-1 $\alpha$  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,<sup>76</sup> 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<sup>61</sup> reported that cerebrovascular vasodilation following hypoxia

is diminished in *CBS*<sup>-/-</sup> compared with wild-type mice. However, it is unclear whether this is a direct vascular effect or whether it is mediated by H<sub>2</sub>S 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<sub>2</sub>S.

In human keratinocytes, cell death in response to CoCl<sub>2</sub>, a surrogate for hypoxia, was ameliorated by treatment with H<sub>2</sub>S donors. Compared with cells exposed to CoCl<sub>2</sub> alone, cells pretreated with NaHS had lower levels of ROS generation and decreased NFκB activation upstream of cyclooxygenase induction, the source of ROS.<sup>77</sup> Thus, H<sub>2</sub>S 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<sub>2</sub> triggered the induction of HIF1α-regulated genes, but H<sub>2</sub>S decreased the induction of these genes through destabilization of HIF1α protein.<sup>78</sup> H<sub>2</sub>S 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<sup>55</sup> also found that exposing cells to high levels of a H<sub>2</sub>S 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<sub>2</sub>S might regulate HIF1α protein stability by multiple mechanisms depending on the conditions and cell types in which hypoxia occurs.

H<sub>2</sub>S donors have been administered chronically in vivo to prevent hypoxia-induced morbidity. Treating rats for 3 weeks with daily injections of an H<sub>2</sub>S donor (2–200 mg/kg) protected cerebral arteries from hypoxia-induced damage.<sup>79</sup> In a rat model of myocardial ischemia/reperfusion injury, H<sub>2</sub>S administration at the time of reperfusion decreased myocardial infarct size, inflammation, and cardiomyocyte apoptosis and preserved mitochondrial function.<sup>80</sup> 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<sub>2</sub>S, 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.<sup>81</sup> Treatment with H<sub>2</sub>S 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<sub>2</sub>S signaling system provides important vascular protection against ischemia in many parts of the circulation.

## H<sub>2</sub>S and redox regulation

Oxidative stress is increased in hypoxic environments, and many studies show that H<sub>2</sub>S is protective in conditions in which oxidative stress is elevated. Bryan et al<sup>74</sup> 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<sub>2</sub>S regulates redox signaling under basal and hypoxic conditions. There is some controversy over H<sub>2</sub>S's role in regulation of redox homeostasis, with studies suggesting both direct and indirect antioxidant function for H<sub>2</sub>S. Results from a recent study by Hamar et al<sup>82</sup> indicate that H<sub>2</sub>S 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<sup>83</sup> identified nuclear factor E2-related factor (Nrf2) signaling as one of the antioxidant pathways triggered by H<sub>2</sub>S. Pretreatment with exogenous H<sub>2</sub>S 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<sub>2</sub>S administration to limit oxidative damage in hypoxic conditions. How oxidative stress may affect endogenous H<sub>2</sub>S signaling, however, is less clear. Streeter et al<sup>84</sup> 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<sub>2</sub>S production to combat redox imbalance.

## H<sub>2</sub>S in the human population

In comparison with the many studies in rodents, much less is known about the distribution and function of H<sub>2</sub>S in humans. Studies of circulating levels of H<sub>2</sub>S have shown that both increased<sup>85</sup> and decreased<sup>86</sup> levels of H<sub>2</sub>S are associated with vascular disease.

In a study comparing tissues and plasma from 14 preeclamptic women and 14 healthy controls, Wang et al<sup>86</sup>

found lower circulating H<sub>2</sub>S and lower levels of placental CSE in women with preeclampsia compared with control patients. The decrease in CSE and H<sub>2</sub>S 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<sub>2</sub>S donor. Thus, H<sub>2</sub>S 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<sup>87</sup> reported that in patients with atherosclerosis, there is an inverse correlation between plasma levels of low-density lipoprotein and H<sub>2</sub>S. In this study, higher H<sub>2</sub>S was associated with less plaque development. Studies in diabetic patients also found that low levels of H<sub>2</sub>S are associated with worse outcomes in patients that have concurrent sleep apnea,<sup>88</sup> and that renal decline progresses more rapidly in chronic kidney disease patients with low serum H<sub>2</sub>S.<sup>89</sup> In the latter study, plasma H<sub>2</sub>S levels correlated with the rate of decline in glomerular filtration rate independently of all other predictors. Thus, declines in H<sub>2</sub>S production may contribute to multiple vascular diseases in the human population.

In contrast, a study by Peter et al<sup>85</sup> found that plasma levels of H<sub>2</sub>S 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<sub>2</sub>S levels may not be as simple as that in animal models. Additional studies defining variables that affect circulating H<sub>2</sub>S levels in humans are needed, as are studies defining how circulating H<sub>2</sub>S modifies vascular responses to disease.

## Summary

It has become apparent in recent years that H<sub>2</sub>S 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<sub>2</sub>S 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 gas transmitter 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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