H_2S and Blood Vessels: An Overview

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Abstract

The physiological and biomedical importance of hydrogen sulfide (H_2S) has been fully recognized in the cardiovascular system as well as in the rest of the body. In blood vessels, cystathionine γ-lyase (CSE) is a major H_2S-producing enzyme expressed in both smooth muscle and endothelium as well as periadventitial adipose tissues. Regulation of H_2S production from CSE is controlled by a complex integration of transcriptional, posttranscriptional, and posttranslational mechanisms in blood vessels. In smooth muscle cells, H_2S regulates cell apoptosis, phenotypic switch, relaxation and contraction, and calcification. In endothelial cells, H_2S controls cell proliferation, cellular senescence, oxidative stress, inflammation, etc. H_2S interacts with nitric oxide and acts as an endothelium-derived relaxing factor and an endothelium-derived hyperpolarizing factor. H_2S generated from periadventitial adipose tissues acts as an adipocyte-derived relaxing factor and modulates the vascular tone. Extensive evidence has demonstrated the beneficial roles of the CSE/H_2S system in various blood vessel diseases, such as hypertension, atherosclerosis, and aortic aneurysm. The important roles signaling in the cardiovascular system merit further intensive and extensive investigation. H_2S-releasing agents and CSE activators will find their great applications in the prevention and treatment of blood vessel-related disorders.

Keywords

Hydrogen sulfide • Cystathionine γ-lyase • Blood vessel • Smooth muscle cells • Endothelial cells • Periadventitial adipose tissues • Blood vessel-related disorders

1 Hydrogen Sulfide Is a Gasotransmitter

The physiological and biomedical importance of hydrogen sulfide (H_2S) has been fully recognized in the cardiovascular system as well as in the rest of the body. The gasotransmitter identity of H_2S is validated against six criteria of gasotransmitters (Wang 2002, 2014):

1. H_2S is a small gas molecule. Once produced inside eukaryotes, it is partially dissolved in biological milieu in its free or bounded forms and partially dissociated to HS^-. It is occasionally squabbled over whether H_2S or nitric oxide (NO) in eukaryotes is still *gas*. Oxygen in the blood is a gas and this does not need verification by seeing oxygen gas bubbles. This is the same case with gasotransmitters. NO, carbon monoxide (CO), H_2S, and NH_3 are gas molecules in our bodies.
2. H_2S is freely permeable to the plasma membrane and intracellular organelle membranes. The same signal relay sequence for neurotransmitters is not required for H_2S signal since there will be no synaptic barrier or cognate

membrane receptors for the transmembrane movement of this gas molecule. The idea of a "gas channel" has been around for a while. No experimental evidence exists to date, however, which shows the reliance of transmembrane movement of H_2S on such "channels". Furthermore, one has to distinguish the transmembrane movement of undissociated gas molecule from that of its dissociated ionic forms. Whereas ammonia gas (NH_3) freely permeates cell membranes, ammonium ion (NH_4^+) passes the membrane through ion channels.

3. H_2S is endogenously generated in mammalian cells under both physiological and pathophysiological conditions. L-cysteine and homocysteine are the most important substrates of enzymatic H_2S production. Yes. H_2S is a metabolic product of reverse-transsulfuration pathway. But the production of H_2S is more than a metabolic need, more than a sulfur transfer phenomenon, and more than the degradation of cysteine or homocysteine. It interacts with different protein targets, alters the directions of multiple signaling pathways, and forms signaling webs and nets intracellularly and intercellularly. And H_2S does all these in response to the functional regulation as well as the metabolic needs of the body.
4. H_2S has well-defined specific functions at physiologically relevant concentrations. The effects of H_2S on the cardiovascular system, neuronal system, respiratory system, and gastrointestinal system, to name a few, have been extensively and convincingly documented. These effects of H_2S are realized at its physiologically relevant concentrations because decreasing endogenous H_2S level (the knockout or knockdown of H_2S-generating enzymes from the whole animal to tissue to cell levels) or increasing it (overexpression or knock-in of these enzymes) has been clearly correlated to the correspondingly functional changes in different systems.
5. The application of H_2S donors (fast releasing or slow releasing) has shown the similar effects as endogenous H_2S on different biological systems.
6. H_2S is involved in signal transduction and has specific cellular and molecular targets. It induces *S*-sulfhydration of numerous proteins. It regulates the levels and activities of traditional second messengers, such as cGMP, cAMP, and intracellular calcium. Its action and production are sensitive to cellular oxygen levels. Mitochondrial bioenergetics, endoplasmic reticulum stress, and gene transcription and translation in the nucleus are within the impact radius of H_2S.

It is relevant here to comment on the contextual connection of neurotransmitter and gasotransmitter to the conventional denotation of "transmitter". A biological transmitter usually refers to a biological molecule that is generated in response to the homeostatic need and serves for "communication" to meet this need. Neurotransmitter and gasotransmitter both conduct the "communication" between the homeostatic needs and functional changes. The former does this via second messengers, and the latter directly interacts with its downstream signaling webs and nets.

2 Endogenous Production of H_2S in Blood Vessels

Different enzymes are involved in the production of H_2S from both vascular smooth muscle cells (SMCs) and endothelial cells (ECs). Cystathionine β-synthase (CBS) is critical for the transsulfuration of homocysteine to generate cystathionine and then to H_2S. Similar to CBS, cystathionine γ-lyase (CSE) is also a pyridoxal-5′-phosphate-dependent H_2S-generating enzyme. In the cardiovascular system, the transformation of L-cysteine to H_2S is mainly catalyzed by CSE with ammonium and pyruvate as two coproducts.

CSE gene in the cardiovascular system was cloned for the first time in 2001 (Zhao et al. 2001). This vascular CSE gene cloned from rat mesenteric artery tissues (GenBank #AB052882) shares the same sequence with that of rat liver CSE gene (GenBank #AY032875). Hosoki et al. (1997) detected CSE mRNA in rat thoracic aorta and portal vein (Hosoki et al. 1997). Zhao et al. showed CSE mRNA in rat mesenteric artery, tail artery, and pulmonary arteries (Zhao et al. 2001). The first Western blot study on CSE protein expression in the cardiovascular system was reported in 2006 (Yang et al. 2006) when CSE proteins were observed in human aorta vascular SMCs. In 2008, the expression of CSE proteins in vascular endothelium was reported (Yang et al. 2008). Following studies have demonstrated endothelial expression of CSE in mice, rats, and humans (Altaany et al. 2013; Papapetropoulosa et al. 2009). To date, CSE proteins and/or mRNA have been detected in newborn pig cerebral microvessels (Leffler et al. 2011), mouse renal artery SMCs (Sen et al. 2012b), rat pulmonary artery SMCs and ECs (Sun et al. 2011; Chen et al. 2009), mouse pulmonary artery SMCs and ECs (Wang et al. 2011), human pulmonary artery SMCs (Kiss et al. 2008), bovine pulmonary artery SMCs and sea lion resistance pulmonary arteries (Olson et al. 2010), rat hepatic artery and portal vein (Siebert et al. 2008), and human internal mammary artery (Webb et al. 2008).

The detection of CBS proteins in blood vessels has been reported in hepatic artery and pulmonary artery. CBS proteins were observed in rat pulmonary artery rings (Sun et al. 2011) and bovine pulmonary artery endothelial cells (Olson et al. 2010). In rat hepatic artery and portal vein, both CSE and CBS proteins were localized, but interestingly the terminal branches of the hepatic afferent vessels only expressed CSE (Siebert et al. 2008).

Cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (MST) are other two enzymes involved in H_2S production in the cardiovascular system. Whereas CAT uses PLP as its cofactor, zinc is the cofactor of MST. The sequential reactions catalyzed by CAT and MST lead to the transformation of cysteine to 3-mercaptopyruvate (3-MP) to sulfane sulfur. This bound sulfur will need to be released or reduced to free H_2S (Wang 2012b). CAT protein was found in vascular ECs, and MST protein was localized in both ECs and SMCs of rat thoracic aortae (Shibuya et al. 2009). MST protein was also localized in bovine pulmonary artery SMCs and sea lion resistance pulmonary arteries (Olson et al. 2011).

3 Regulation of CSE-Mediated Vascular Production of H_2S

H_2S production through CSE is both tonic under resting conditions and phasic upon specific stimulations. The activation of muscarinic cholinergic receptor by acetylcholine in vascular ECs leads to the elevation of intracellular calcium. Subsequently, calcium-activated calmodulin stimulates CSE to produce H_2S in ECs (Yang et al. 2008). Testosterone is another endogenous CSE stimulator in vascular system (Bucci et al. 2009). Its vasorelaxant effect on rat aortic rings in vitro was inhibited by DL-propargylglycine (PPG) and β-cyano-L-alanine (BCA), two inhibitors of CSE. As well, H_2S production was increased by testosterone but inhibited by PPG and BCA (Bucci et al. 2009).

It has been shown that vascular endothelial growth factor (VEGF) stimulated H_2S production in cultured human umbilical vein endothelial cells (HUVECs) (Papapetropoulos et al. 2009). This effect of VEGF can be attributed to increased intracellular calcium and calcium-activated calmodulin in ECs. Whether VEGF affects CSE gene expression is unknown. Hassan et al. showed that platelet-derived growth factor-BB (PDGF-BB) upregulated CSE mRNA and protein levels in mesangial cells (Hassan et al. 2012). Hypoxia is another factor that leads to increased H_2S production in human placenta and rat liver, uterus, and fetal membranes (Patel et al. 2009). CSE gene expression in the vascular system may also be inhibited by insulin (Wang 2004).

CSE activities in vascular SMCs were increased by NO (Zhao et al. 2001). After incubating rat aortic tissue homogenates with a NO donor for 90 min, H_2S generation from the homogenates was significantly increased. One of the underlying mechanisms for this effect of NO is the upregulation of CSE expression, which was confirmed 6 h after incubating cultured vascular SMCs with a NO donor (Zhao et al. 2001). Similar observations were made by Patel et al. that NO donors increased both the expression and activity of CSE proteins in rat fetal membranes (Patel et al. 2009).

The regulation of CSE gene expression mostly occurs at the CSE promotor site. The specific protein 1 (Sp1) transcription factor, nuclear factor erythroid-2-related factor-2 (Nrf2), and farnesoid X receptor (FXR) ligand can all bind to the CSE promotor, hence stimulating CSE transcription (Zhang et al. 2011; Yang et al. 2011; Hassan et al. 2012; Renga et al. 2009). This transcriptional regulation mechanism can explain the inhibitory effect of microRNA 21 (miR-21) on CSE expression since miR-21 directly repressed the expression of Sp1 (Yang et al. 2012a, b). In contrast, increased CSE expression by TNF-α resulted from TNF-α-stimulated Sp1 binding to the CSE promoter (Sen et al. 2012a). In the case of Nrf2, it binds to an antioxidant-responsive element (ARE) to mediate the targeted gene transcription after Nrf2 is translocated into the nucleus. The CSE promotor contains an ARE sequence and this provides an oxidative stress-sensitive mechanism for regulating CSE expression. Hassan et al. showed that PDGF-BB-induced CSE expression in mesangial cells is inhibited by co-treatment with antioxidants or by Nrf2 knockout. Furthermore, stabilization of Nrf2 protein upregulated CSE protein expression (Hassan et al. 2012). The evidence for the interaction of FXR with the CSE

promotor was derived from HepG2 cells. The human CSE gene contains an FXR-responsive element in its 5′-flanking region. Treatment of HepG2 cells with an FXR ligand increased CSE expression and mutation of FXR blocks FXR ligand-induced CSE expression (Renga et al. 2009).

4 H_2S and SMCs

4.1 H_2S and SMC Apoptosis, Proliferation, and Migration

Abnormal SMC proliferation and apoptosis are among the causative factors for vascular remodeling. H_2S regulation of SMC proliferation and apoptosis has been extensively studied (Fig. 1). We previously demonstrated that exogenously applied H_2S or endogenous H_2S derived from overexpressed CSE gene inhibits proliferation and induces apoptosis of human aorta SMCs by activating ERK and caspase 3 (Yang et al. 2004, 2006). Du et al. also found that H_2S dose-dependently suppressed the proliferation of rat SMCs through the MAPK pathway (Du et al. 2004). Region-specific chromatin remodeling of MAPK signaling pathway-associated genes such as Ntf3, Pcna, and Pdgfα can be regulated by H_2S, and Brg1 acts as a switch to turn these genes "on" in a spatially and temporally specific manner to inhibit SMC proliferation (Li et al. 2013). The hypoxia-induced proliferation of pulmonary artery SMCs is the main cause of pulmonary arterial hypertension, and H_2S was demonstrated to inhibit $CoCl_2$-induced pulmonary arterial SMC proliferation by the upregulation of cyclooxygenase-2 and prostacyclin (Li et al. 2014). Indeed, SMCs from CSE knockout mice displayed an increased proliferation rate in vitro and in vivo, and these cells were more susceptible to apoptosis induced by an oxidative stress inducer (H_2O_2) or a high dose of H_2S (100 uM) (Yang et al. 2010). CSE knockout mice exhibited decreased endogenous H_2S level in the cardiovascular system and impaired endothelium-dependent vasorelaxation and age-dependent hypertension (Yang et al. 2008). The altered SMC proliferation in CSE knockout mice provides new insight into the pathogenesis of hypertension and underscores the importance of H_2S in homeostatic control of vascular integrity. Li et al. further found that the proliferation of cultured vascular SMCs isolated from wild-type mice was inhibited, but that from CSE gene knockout mice increased, by estrogen treatments, indicative of the interaction of H_2S and estrogen in regulating SMC proliferation (Li et al. 2012). In a rat model of pulmonary hypertension and pulmonary artery structural remodeling induced by high pulmonary blood flow, the inhibition of endogenous H_2S production by PPG markedly decreased the rate of pulmonary artery SMC apoptosis but supplements of H_2S donor increased pulmonary artery SMC apoptosis, as demonstrated by positive TUNEL staining (Li et al. 2009). Consistent with these in vivo findings, Baskar et al. observed that S-diclofenac, a novel molecule containing an H_2S-releasing dithiolthione moiety, stabilized p53 and induced the expressions of downstream proteins, such as p21, p53AIP1, and Bax, to repress SMC proliferation (Baskar et al. 2008).

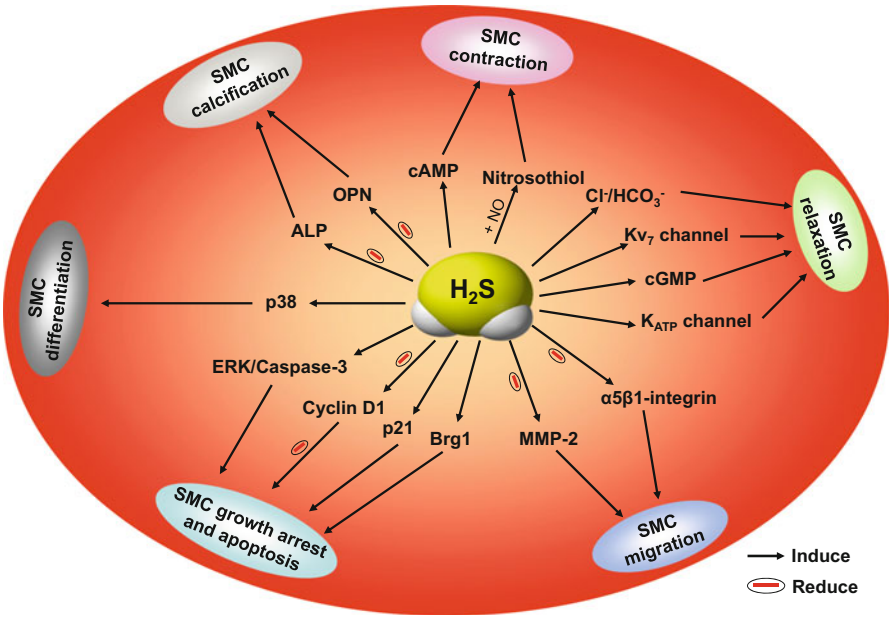

Fig. 1 H_2S signaling in SMC functions. H_2S maintains SMC differentiation by inducing p38 MAPK. H_2S stimulates SMC growth arrest and induces SMC apoptosis by activating ERK/caspase 3, p21, and/or Brg1 pathways but inactivating cyclin D1. H_2S also attenuates SMC migration by inhibiting the expressions of MMP-2 and α5β1-integrin. By activating Cl^-/HCO_3^-, Kv_7 channel, cGMP, and/or K_{ATP} channel, H_2S induces SMC relaxation, while H_2S mediates SMC contraction by enhancing cAMP production and interacting with NO to form nitrosothiol. H_2S also reduces SMC calcification by inhibiting the activities of ALP and OPN. Abbreviation used in this figure: ALP, alkaline phosphatase; Brg1, Brahma-related gene 1; cAMP, adenosine 3′,5′-cyclic monophosphate; cGMP, cyclic guanosine monophosphate; ERK, extracellular-signal-regulated kinase; H_2S, hydrogen sulfide; K_{ATP} channel, ATP-sensitive potassium channel; Kv_7 channel, voltage-gated potassium channel subunit K_v7; MMP-2, matrix metallopeptidase 2; NO, nitric oxide; OPN, osteopontin; SMC, smooth muscle cell

Altered SMC proliferation and apoptosis have been considered as key events in vascular injury in diseases such as atherosclerosis and restenosis after invasive intervention. CSE knockout mice fed with atherogenic diet developed early fatty streak lesions in the aortic root and increased aortic intimal proliferation (Mani et al. 2013). Treatment of CSE knockout mice with NaHS inhibited the accelerated atherosclerosis development. By using a rat model of vascular remodeling induced by balloon injury, Meng et al. found that CSE expression and H_2S production are significantly reduced in the blood vessel during the development of neointimal formation after balloon injury and the administration of NaHS attenuated the development of neointimal hyperplasia by inhibiting SMC proliferation (Meng et al. 2007). NaHS induced a significant reduction in cell proliferation in the neointima. We also noticed that CSE deficiency in mice led to increased neointima formation in carotid arteries 4 weeks after ligation, which were attenuated by NaHS

administration (Yang et al. 2012a, b). All these data suggest that endogenous H_2S is critical for the inhibition of SMC proliferation during neointimal formation. In response to vascular injury, SMCs will first migrate from the tunica media to the intima, where they contribute to neointima formation (Thyberg 1998). Exogenously applied NaHS significantly inhibited SMC migration, and SMCs and aortic explants isolated from CSE knockout mice exhibited more migration and outgrowth compared with that from wild-type mice. SMCs became more elongated and spread in the absence of CSE. The interactions among α5β1-integrin, fibronectin, and MMP-2 promoted CSE deficiency-enhanced SMC adhesion and migration (Yang et al. 2012a, b). These studies provide further insight into the pathogenesis of proliferative cardiovascular disorders and underscore the protective effects of H_2S in maintaining vascular homeostasis.

4.2 H_2S and SMC Phenotype Modulation

Vascular SMCs are highly specialized cells whose contractile status regulates blood vessel tone, blood pressure, and blood flow distribution. In contrast to cardiac and skeletal muscle cells which exit cell cycle and undergo final differentiation, vascular SMCs are highly plastic and can switch their phenotypes between contractile and synthetic phenotypes in response to extracellular stimuli or damage (Owens 2007). SMC phenotypic switching is believed to play a key role in many cardiovascular diseases, such as hypertension, atherosclerosis, coronary heart diseases, postangioplasty restenosis, and transplantation arteriopathy (Thyberg 1998). The H_2S-regulated SMC phenotypic switch has been explored (Fig. 1). CSE expression and H_2S production were reduced in proliferated SMCs compared with differentiated SMCs in a Sp1-dependent manner (Yang et al. 2011). In the presence of 10 % serum, H_2S strikingly induced the expressions of SMC differentiation maker genes in proliferative human aorta SMCs. In addition, H_2S-stimulated SMC differentiation maker gene expressions were reversed by co-treatment of the cells with SB203580, a p38 MAPK inhibitor. In the absence of serum, exogenously applied H_2S did not change the expressions of SM-MHC and calponin in SMCs. However, the inhibition of endogenous H_2S production by PPG significantly repressed the expressions of SM myosin heavy chain and calponin (Yang et al. 2011). Induction of SMC differentiation maker gene expressions by H_2S in the presence of, but not absence, serum suggests that H_2S may coordinate the expression of proliferative and contractile proteins to induce differentiated SMC phenotype. All these data indicate that CSE/H_2S system is essential for the maintenance of SMC differentiation.

4.3 H_2S and SMC Relaxation and Contraction

H_2S-induced vasorelaxation is a well-known vascular event, and the most widely characterized cellular target for H_2S in SMCs is the ATP-sensitive K^+ (K_{ATP})

channels (Fig. 1). In the vascular system, specifically in SMCs, K_{ATP} channels contribute significantly toward vasodilation in response to various vasoactive substances. The opening of K_{ATP} channels hyperpolarizes cell membrane and inactivates voltage-dependent L-type Ca^{2+} channels, leading to cell relaxation and blood vessel dilation by reducing intracellular free Ca^{2+} concentration (Wang 2012a, b). It has been previously shown that H_2S at physiologically relevant concentrations induced the relaxation of rat aortic tissue and transient reduction of blood pressure, and these vascular effects of H_2S were mediated by a direct stimulation of K_{ATP} channels and subsequent hyperpolarization of aortic SMCs (Zhao et al. 2001; Cheng et al. 2004; Wang 2014; Sun et al. 2011; Siebert et al. 2008). Using the whole-cell and single-channel patch-clamp technique, Tang et al. demonstrated that H_2S activates K_{ATP} channels and hyperpolarized cell membrane in rat mesenteric artery SMCs (Tang et al. 2005, 2010). H_2S enhanced the amplitude of whole-cell K_{ATP} currents and increased the open probability of single K_{ATP} channels. Furthermore, inhibition of endogenous H_2S production with PPG reduced whole-cell K_{ATP} currents. H_2S also causes the relaxation of human airway SMCs via stimulating sarcolemmal K_{ATP} channels (Fitzgerald et al. 2014). Other studies proved that H_2S interacts with the SUR subunits of the K_{ATP} channel complex to cause the channel to open, and the sulfhydryl groups located on the extracellular surface of the SUR subunits are potential targets for H_2S *S*-sulfhydration (Jiang et al. 2010). The deletion of extracellular cysteine 6 or 26 of SUR1 subunits caused the loss of channel sensitivity to H_2S. It appears that H_2S firstly breaks the disulfide bond between cysteine 6 and cysteine 26 and then caused their *S*-sulfhydrations. In addition to K_{ATP} channels, H_2S is reported to activate Kv7 voltage-gated potassium channels (particularly the Kv7.4 subtype) in SMCs, and the activation of Kv7 channel mediates a significant part of the vasorelaxing effects of H_2S (Martelli et al. 2013).

H_2S may induce SMC relaxation by altering intracellular pH. H_2S has been shown to decrease intracellular pH in a dose-dependent manner. Ionic exchangers, including Na^+/H^+ and Cl^-/HCO^{3-} and Ca^{2+} ATPase, maintain the resting pH in SMCs between 7.1 and 7.2. Preincubation of SMCs with a selective inhibitor of Cl^-/HCO_3^-, but not Na^+/H^+ exchanger inhibitor, prevented the drop of intracellular pH and vasorelaxation caused by H_2S, suggesting that Cl^-/HCO^{3-} exchanger is involved in H_2S-induced relaxation in SMCs (Lee et al. 2007). Intracellular acidosis could activate K_{ATP} channel in SMCs and decrease vascular tone. As such, H_2S-induced opening of K_{ATP} channels may be partially triggered by intracellular acidification (Liu et al. 2011).

H_2S may also cause vasorelaxation by increasing cGMP level in SMCs. Incubation of cultured rat aortic SMCs with NaHS led to a concentration-dependent increase in cGMP levels. The NaHS-induced rise in cGMP was evident as early as 1 min, reached a maximum at 3 min, and remained elevated for at least 10 min. Blockade of CSE activity by PPG or BCA or knockdown of CSE mRNA by siRNA resulted in a significant reduction of cGMP accumulation (Bucci et al. 2010). In contrast, overexpression of CSE elevated intracellular cGMP level. Vascular tissue levels of cGMP in CSE knockout mice were lower than those in wild-type control

mice. Intracellular cGMP levels reflect the balance between the rate of cGMP synthesis via guanylyl cyclases and breakdown by phosphodiesterases. It appears that H_2S does not activate soluble guanylate cyclase, because H_2S-induced vasorelaxation is not inhibited by a soluble guanylate cyclase inhibitor (Coletta et al. 2012). Further studies showed that H_2S acts as an endogenous inhibitor of phosphodiesterase. In a cell-free assay, Bucci et al. demonstrated that NaHS at 10–30 nM significantly inhibits phosphodiesterase activity and causes a reduction in the breakdown of 5′-GMP (Bucci et al. 2012). H_2S also ameliorated the reduction in cGMP levels brought about by overexpression of phosphodiesterase 5A (Bucci et al. 2010, 2012). Because phosphodiesterases are involved in the degradation of both cAMP and cGMP, the researchers did not test the cellular effects of H_2S on cAMP level in SMCs. In contrast, another study by Lim et al. proved that NaHS significantly reverses forskolin-induced cAMP accumulation in SMCs (Lim et al. 2008). cAMP plays important roles in the regulation of mature contractile phenotype in SMCs. The effect of H_2S on SMC relaxation is actually biphasic depending on its concentration (Kubo et al. 2007; Liu et al. 2011). At higher level, H_2S produces vasorelaxation effect, while it induces vasoconstriction at lower concentration (Ali et al. 2006). To this end, the researchers observed that NaHS at a concentration range of 10–100 μM concentration-dependently reverses the vasodilation caused by isoprenaline and salbutamol (two β-adrenoceptor agonists) and forskolin (a selective adenylyl cyclase activator) in phenylephrine-precontracted rat aortic rings (Lim et al. 2008). Therefore, the authors proposed that the contractile effect of H_2S observed in isolated rat aorta is, at least partially, associated with reducing cAMP level in SMCs. In addition, H_2S may react with NO to form a compound, probably nitrosothiol, which leads to less NO bioavailability (Whiteman et al. 2006).

4.4 H_2S and SMC Calcification

Vascular calcification is implicated in the pathogenesis of various vascular diseases and resulted from passive precipitation of calcium and phosphate. Vascular calcification is now considered to be an active, regulative process similar to osteogenesis (McCarty and DiNicolantonio 2014). Calcified vessels have decreased capacity for vasodilatation and increased stiffness and promote a form of thrombus and atherosclerotic plaque rupture. Osteoblastic differentiation of SMCs is involved in the pathogenesis of vascular calcification. H_2S has been shown to ameliorate SMC calcification. In a rat vascular calcification model induced by the administration of vitamin D3 plus nicotine, aortic CSE expression and H_2S content were significantly reduced (Wu et al. 2006). Supplement of NaHS significantly reduced aortic calcium mineral deposits, OPN mRNA expression, and ALP activity, pointing to a regulatory role of CSE/H_2S pathway in the pathogenesis of vascular calcification. By using cultured SMCs, Zavaczki et al. explored the roles of H_2S in phosphate-induced osteoblastic transformation and mineralization (Zavaczki et al. 2011). H_2S inhibited calcium deposition in the extracellular matrix and suppressed the

induction of the genes involved in osteoblastic transformation of SMCs, including alkaline phosphatase, osteocalcin, and Cbfa1. H_2S also prevented phosphate uptake and phosphate-triggered upregulation of the sodium-dependent phosphate cotransporter. H_2S, regardless of its exogenous or endogenous origin, is a potent inhibitor of phosphate-induced calcification and osteoblastic differentiation of SMCs. In contrast, silencing CSE by siRNA and inhibition of CSE activity by PPG attenuated receptor activator of nuclear factor κ-B ligand-induced tartrate-resistant acid phosphatase type 5 activities and pit formation in RAW264.7 cells. Moreover, knockdown of CSE suppressed the expression of osteoclast differentiation markers. A large-scale proteomics study also identified that CSE acts in early stages of osteoclastogenesis (Itou et al. 2014). These results suggest that CSE is a potent inducer of calcium resorption in inflammatory cells.

5 H_2S and ECs

5.1 H_2S and EC Proliferation and Angiogenesis

H_2S significantly stimulates endothelial cell growth and angiogenesis (Wang 2012a, b; Polhemus and Lefer 2014; Liu et al. 2011) (Fig. 2). Several groups have demonstrated that H_2S significantly promotes cell growth and capillary-like structure formation of cultured ECs (Altaany et al. 2013; Papapetropoulosa et al. 2009; Liu et al. 2011). Supplement of exogenous H_2S increased cellular infiltration and neovascularization in mouse Matrigel, enhanced the length of vascular network in the chick chorioallantoic membrane model, and promoted the formation of collateral vessels in ischemic hind limbs in rats (Papapetropoulosa et al. 2009; Köhn et al. 2012a; Hoefer 2007). A delayed wound healing was found in CSE knockout mice when compared with wild-type littermates (Papapetropoulosa et al. 2009). Endothelial progenitor cells are a population of rare cells that circulate in the blood with the ability to differentiate into ECs. H_2S was shown to improve endothelial progenitor cell function in diabetic wound healing of type 2 diabetic mice. Conversely, PPG treatment reduced progenitor cell function and delayed wound healing (Liu et al. 2014). Under hypoxic condition, H_2S induced endothelial proliferation and migration by promoting VEGF and HIF-1a expression and increasing HIF-1a-binding activity (Liu et al. 2010). In contrast, H_2S decreased cell proliferation and capillary tube formation of EA.hy926 cells under hypoxia by inhibiting the expression of VEGF and HIF-1a (Wu et al. 2012). The discrepancy of the aforementioned observations may be due to different hypoxic models, H_2S concentrations, and cell types. Pupo et al. showed that NaHS at 10 uM did not exert any effect on cell migration and proliferation of normal human microvascular ECs (HMVECs) (Pupo et al. 2011). This apparent discrepancy may be ascribed to tissue specificity. Different from HMVECs, H_2S donors failed to induce cell proliferation but promote their migration of ECs obtained from human breast carcinoma (B-TEC). B-TECs pretreated with PPG showed drastically reduced migration

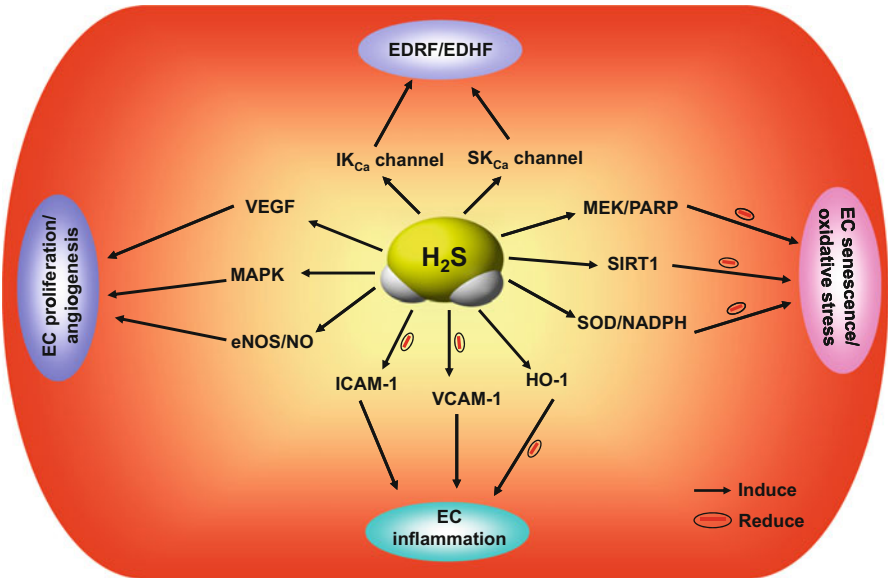

Fig. 2 H_2S signaling in EC functions. H_2S stimulates EC proliferation and angiogenesis by activating VEGF, MAPK, and/or eNOS/NO pathways. H_2S attenuates EC inflammation by inhibiting the expressions of ICAM-1 and VCAM-1 but stimulating HO-1. H_2S lowers EC senescence and oxidative stress by activating MEK/PARP, SIRT1, and/or SOD/NADPH pathways. H_2S acts as EDRF and EDHF at least through IK_{Ca} channel and SK_{Ca} channel. Abbreviation used in this figure: EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; eNOS, endothelial nitric oxide synthase; HO-1, heme oxygenase-1; ICAM-1, intercellular adhesion molecule 1; IKCa channel, intermediate-conductance, calcium-activated potassium channel; MAPK, mitogen-activated protein kinase; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; NO, nitric oxide; PARP, poly (ADP-ribose) polymerase; SIRT1, sirtuin-1; SK_{Ca} channel, small-conductance calcium-activated potassium channel; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor

induced by VEGF (Pupo et al. 2011). The authors concluded that H_2S plays a role in proangiogenic signaling of tumor-derived but not normal human ECs.

In addition to the direct stimulatory effect on EC growth and angiogenesis, H_2S was also reported to ameliorate stress-induced cell death of ECs. Exposure of primary human umbilical vein endothelium cells (HEVECs) to a high concentration of glucose (25 mM) resulted in the induction of apoptosis, but pretreatment with NaHS (50 μM) for 30 min attenuated the pro-apoptotic effect of 25 mM glucose. Further investigation of the apoptotic mechanisms in the cells demonstrated that high glucose upregulated the ratio of Bax/Bcl-2, activated caspase-3, increased the levels of reactive oxygen species and malondialdehyde, and suppressed superoxide dismutase activity (Guan et al. 2012). All these effects of glucose could be prevented by pretreatment with 50 μM NaHS. Pretreatment with NaHS (0.05-0.1 mM) attenuated methionine- or oxidized low-density lipoprotein-induced oxidative stress and cell death of ECs (Tyagi et al. 2009; Muellner et al. 2009).

Exposure of bEnd3 microvascular ECs to elevated extracellular glucose induced mitochondrial formation of ROS, and H_2S replacement protected against ROS formation, improved endothelial metabolic state, and maintained normal endothelial function (Suzuki et al. 2011). H_2S was also found to preserve the activities and protein levels of the antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase in H_2O_2-exposed ECs (Wen et al. 2013). Zofenoprilat is a sulfhydryl-containing angiotensin-converting enzyme inhibitor, which can enhance CSE-dependent availability of H_2S. Zofenoprilat was shown to exert a protective effect on doxorubicin-induced endothelial damage without affecting its antitumor efficacy (Monti et al. 2013).

5.2 H_2S and EC Senescence and Oxidative Stress

Growing evidence shows that the progress of vascular aging alters cardiovascular function and subsequently increases the risk of cardiovascular diseases. Vascular aging has been largely associated with senescence of the vascular endothelium. Several lines of evidence point to the implication of H_2S signaling in the process of endothelial senescence (Fig. 2). Calorie restriction is reported to decelerate biological aging process, resulting in longer maintenance of youthful health and an increase in both median and maximum life span. Calorie restriction maintained normal H_2S level in vascular tissues from rats during aging, suggesting a protective role of H_2S in vascular aging (Predmore et al. 2010). Oxidative stress is a driving factor for vascular aging. H_2O_2 treatments of HUVECs lead to high rate of senescent cells, which was attenuated by NaHS incubation putatively through the modulation of SIRT1 activity (Suo et al. 2013). H_2S also improved the function of senescent HUVECs. Zhao et al. found that H_2S attenuates cellular senescence and DNA damage in HUVECs by MEK1 *S*-sulfhydration and PARP-1 activation (Zhao et al. 2014). In the presence of H_2S, activated PARP-1 recruits XRCC1 and DNA ligase III to DNA breaks to mediate DNA damage repair. AP39, a mitochondria-targeted H_2S donor, was shown to exert a concentration-dependent effect on mitochondrial activity in bEnd.3 murine microvascular ECs, as evidenced by the stimulation of mitochondrial electron transport and cellular bioenergetic function. Furthermore, AP39 pretreatment protected against glucose oxidase-induced mitochondrial DNA damage (Szczesny et al. 2014). A recent study showed that H_2S increases the life span of *Caenorhabditis elegans*. The life span-prolonging and health-promoting effects of H_2S in *C. elegans* are likely due to the antioxidant action (Qabazard et al. 2014). Similarly, compared with the lower passage of ECs, the higher passage of ECs had lower SOD activity and higher H_2O_2 level, whereas NaHS pretreatment reversed the changes of SOD activity and H_2O_2 level, indicating that H_2S delays senescence of HUVECs through lessening oxidative stress (Qi et al. 2012; Muellner et al. 2009). By using porcine pulmonary arterial ECs, Muzaffar et al. demonstrated that H_2S inhibited superoxide formation and upregulation of NADPH oxidase through the adenylyl cyclase-PKA pathway

(Muzaffar et al. 2008). In spite of these findings, the in vivo role of H_2S in regulating EC senescence and vascular aging remains unclear.

5.3 H_2S and EC Inflammation

The regulatory role of H_2S in inflammation involves the endothelium and its interaction with leukocytes (Whiteman and Winyard 2011; Zanardo et al. 2006). H_2S donors (NaHS and Na_2S) inhibited but PPG promoted aspirin-induced leukocyte adherence in mesenteric venules via the activation of K_{ATP} channels (Zanardo et al. 2006). H_2S also suppressed leukocyte infiltration in an air pouch model and carrageenan-induced paw edema, implicating a protective role of H_2S in acute inflammation by acting at the leukocyte–endothelium interface (Perna et al. 2013). Further study demonstrated that H_2S treatment of ECs decreased the expression of MCP-1, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) at the mRNA and protein levels. In an in vitro model entailing monocyte adhesion to an endothelial monolayer, H_2S prevented the increase in monocyte adhesion induced by tumor necrosis factor-α (TNF-α). Pan et al. also showed that H_2S dose-dependently suppressed TNF-α-induced mRNA and protein expressions of ICAM-1 and VCAM-1 in HUVECs, possibly through the upregulation of HO-1 (Pan et al. 2011). By using another H_2S donor, SPRC, the same group found that H_2S exerts anti-inflammatory effects on TNF-α-stimulated ECs through scavenging ROS, inhibiting JNK1/2/NF-κB pathways, and attenuating adhesion molecule expression (Pan et al. 2012). NaHS was shown to reduce oxLDL-induced foam cell formation in macrophages and TNF-α-stimulated ICAM-1 expression in HUVECs (Zhao et al. 2011; Wang et al. 2013b). In contrast, Choi et al. showed that Korean red ginseng extracts inhibit the expression of inflammatory mediators, including IL-8 and IL-6, via reduced CSE expression and H_2S production in ECs (Choi et al. 2012). Supplement of exogenously applied H_2S reversed the Korean red ginseng extract-improved inflammation status in ECs.

H_2S protects vascular tissues from atherogenic damage by inhibiting adhesion molecule expression and suppressing monocyte adhesion to the activated endothelium (Wang et al. 2009). In spontaneously hypertensive rat (SHR), the expression of ICAM-1 and NF-kappaB p65 protein in aortic ECs was significantly higher, and NaHS treatment reduced blood pressure in SHR rats and downregulated the expressions of ICAM-1 and NF-kappaB p65 in aortic ECs. On the other hand, inhibition of H_2S production enhanced the expressions of ICAM-1 and NF-kappaB p65 protein in aortic ECs (Jin et al. 2008), suggesting that H_2S might attenuate the development of hypertension by suppressing endothelial inflammation reactions.

5.4 Interplay Between H_2S and NO in ECs

Both H_2S and NO are major gasotransmitters produced in ECs, and growing evidence showed that the "cross talk" between NO and H_2S mediates the

cardioprotective effect of H_2S (Jamroz-Wiśniewska et al. 2014). Predmore et al. first showed that H_2S stimulated eNOS phosphorylation and NO production in ECs, and pharmacological inhibition of Akt, the kinase responsible for eNOS Ser 1177 phosphorylation, attenuated the stimulatory effect of H_2S on NO production (Predmore et al. 2011). Al Tanny et al. confirmed that H_2S promotes NO production in ECs via the activation of a cascade of phosphorylation events, starting from p38 MAPK to Akt to eNOS (Altaany et al. 2013). Deficiencies in H_2S signaling can directly impact on processes regulated by NO (Coletta et al. 2012). H_2S promotes EC tube formation, proliferation, and angiogenesis by both NO-dependent and NO-independent mechanisms. H_2S also modulates eNOS via *S*-sulfhydration and prevents eNOS coupling collapse and thus increases NO and decreases $ONOO^-$ and O_2 levels in ECs (Al Taany et al. 2014). Exposure of ECs to H_2S increased intracellular cGMP in a PI3K/Akt and NO-dependent manner, and NO and H_2S are mutually required for the physiological control of vascular function (Coletta et al. 2012). H_2S may stimulate NO production by different mechanisms. Kida et al. showed that NaHS dose-dependently increased NO production in cultured ECs by releasing calcium from the intracellular store in endoplasmic reticulum. NaHS-induced eNOS phosphorylation and NO production were abolished by the ryanodine receptor inhibitor, inositol 1,4,5-triphosphate receptor inhibitor, and calcium chelator but not by the PI3K/Akt inhibitor and the absence of extracellular calcium (Kida et al. 2013). NaHS was also shown to increase eNOS expression and NO production in rat corpus, suggesting H_2S could exert its pro-erectile effects by augmenting NO pathway. In contrast, Na_2S, another H_2S donor, was shown to reduce the level of phospho-eNOS (serine 1177) and inhibit eNOS activity in cultured mouse aortic ECs (Chai et al. 2014). The discrepancy may be due to the difference of cell type and H_2S dose used. Further elucidation of the H_2S–NO relationship in the vascular biology would provide more insight into the vasodilator function of H_2S and improve our understanding of the pathogenic mechanisms for cardiovascular diseases.

5.5 H_2S Acts as an Endothelium-Derived Relaxing Factor (EDRF) and an Endothelium-Derived Hyperpolarizing Factor (EDHF)

In response to a variety of chemical and physical stimuli, ECs produce and release various vasoactive factors, including EDRF and EDHF. Both EDRF and EDHF relax vascular SMCs, causing blood vessel to expand in diameter (Wang 2012a, b). NO is a well-described EDRF, but other EDRFs are also produced and released from the endothelium. H_2S shares many features with NO. Accumulating evidence supports the concept that H_2S acts as both EDRF and EDHF (Baragatti et al. 2013; Han et al. 2013; Skovgaard et al. 2011; Wang 2009) (Fig. 2). Endogenously generated H_2S induces vasorelaxation in part through an endothelium-dependent mechanism (Yang et al. 2008; Zhao et al. 2001). The vasorelaxation elicited by H_2S is greater in small resistance arteries as compared with that in larger conduit arteries such as the aorta (Tang et al. 2013). Knocking out CSE expression attenuated

acetylcholine-induced membrane hyperpolarization of the isolated ECs and led to membrane depolarization of the whole vascular tissues. Different from EDRFs, the unique property of EDHF is its sole effect on hyperpolarizing vascular SMCs so as to close voltage-dependent calcium channels (Wang 2012a, b). The effect of EDHF is mainly mediated by the opening of small-conductance and/or intermediate-conductance K_{Ca} channels (Mustafa et al. 2011; Wang 2012a, b). Using electrophysiological microelectrode technique, Tang et al. demonstrated that the unique EC dependence of H_2S-induced SMC hyperpolarization was linked to the opening of IK_{Ca} and/or SK_{Ca} channels (Tang et al. 2013). The identification of H_2S as an EDRF/EDHF will not only help better understand the mechanisms underlying endothelium-dependent vasorelaxation of different types of vascular tissues but also shed light on devising novel therapeutic agents to deal with specific cardiovascular diseases.

6 H_2S and Periadventitial Adipose Tissue (PAT)

Adventitia is the outermost connective tissue covering a blood vessel, also called the tunica adventitia or the tunica externa. PAT, the major part of adventitia and defined as the accumulation of adipocytes around vascular structures, can be found in the proximity of virtually all blood vessels (Gollasch 2012). PAT is composed of various cells such as adipocytes, fibroblasts, and macrophages and can release various active agents, e.g., adipocyte-derived relaxing factor (ADRF), which play important roles in modulating the vascular tone. Recent studies have shown that H_2S can be generated from PAT and act as an ADRF, contributing to vascular relaxation (Fang et al. 2009). Immunohistochemical staining revealed the presence of CSE protein in PAT. In isolated PAT, H_2S production was reduced in an age-dependent manner. The CSE/H_2S pathway was upregulated in PAT as a compensatory mechanism against the elevated blood pressure in hypertension (Bełtowski 2013). In consistent with this study, Köhn et al. showed that the inhibition of CSE activity by PPG reversed the anti-contractile effect of PAT in rat aorta (Köhn et al. 2012a, b). Statins are well-known drugs to reduce plasma LDL cholesterol, improve endothelial function, ameliorate oxidative stress, and maintain coagulation–fibrinolysis balance. It was recently found that atorvastatin, one kind of statins, increased H_2S production in PAT and H_2S mediated the protective role of statins in the cardiovascular system. Atorvastatin augmented the anti-contractile effect of PAT, most likely in an H_2S- and K_{ATP} channel-dependent manner, because its effect was abolished by CSE inhibitor PPG and K_{ATP} channel blocker glibenclamide (Wójcicka et al. 2011). A statin-induced increase in H_2S production may also contribute to the antiatherogenic effect of statins since H_2S inhibits platelet aggregation, vascular SMC proliferation, LDL oxidation, and local inflammatory reaction.

7 H_2S and Blood Vessel-Related Disorders

Blood vessel diseases are also called peripheral vascular diseases or artery diseases, including hypertension, atherosclerosis, aortic aneurysm, etc. H_2S plays an important protective role in various blood vessel diseases (Wang 2012a, b; Yang 2011).

7.1 H_2S and Hypertension

Hypertension or high blood pressure is a chronic medical condition in which the blood pressure is elevated. Hypertension results from a complex interaction of vasoactive factors with various types of cells in blood vessel walls. Recent studies have shown that CSE/H_2S system produces antihypertensive effects in different hypertensive models. An acute intravenous bolus injection of NaHS caused a transient fall or mean arterial blood pressure in anesthetized rats (Zhao et al. 2001). Exogenous H_2S administration lowers blood pressure and prevents the hypertrophy of intramyocardial arterioles and aortic thickening of spontaneously hypertensive rats (SHR) (Du et al. 2003; Zhao et al. 2008). Injection of a single dose of GYY4137 (a H_2S donor) alleviated L-NAME-induced hypertension in rats, and chronic treatment with GYY4137 successfully reduced blood pressure of SHR (Li et al. 2008a). ACS14, another H_2S-releasing donor, reduced blood pressure in buthionine sulfoximine-induced hypertensive rats (Rossoni et al. 2010). Inhibition of H_2S production by PPG worsened hypoxic pulmonary hypertension in rats. Direct evidence also demonstrated that complete deficiency of CSE in mice markedly reduced endogenous H_2S levels in the vascular system and led to age-dependent development of hypertension (Yang et al. 2008). Preeclampsia is a disorder of pregnancy characterized by high blood pressure and contributes to maternal and fetal morbidity and mortality worldwide. The cause and pathogenesis of preeclampsia has yet to be definitively uncovered. Wang et al. recently showed that plasma H_2S and CSE expression in the placenta were reduced in pregnancies complicated by preeclampsia in comparison with gestational age-matched controls (Wang et al. 2013a). Inhibition of CSE activity induces hypertension and causes placental abnormalities in pregnant mice owing to the inhibition of H_2S production. These discoveries suggest that a dysfunctional CSE/H_2S pathway may contribute to the pathogenesis of preeclampsia and targeting at the CSE/H_2S system would be an effective therapy for preeclampsia (Wang et al. 2013a, b).

7.2 H_2S and Atherosclerosis

Atherosclerosis is a chronic progressive pathological process in large- and medium-sized arteries, caused by the buildup of fatty/cholesterol plaques on the ECs of arteries, endothelial inflammation, and SMC proliferation. H_2S plays an anti-atherosclerotic role, and its deficiency leads to the development and progression of atherosclerosis (Zhang et al. 2012a, b; Mani et al. 2013, 2014; Xu et al. 2014;

Wang et al. 2009). Plasma H_2S level and aortic CSE activity was decreased in apolipoprotein E (ApoE) knockout mice with advanced atherosclerosis. Treatment of ApoE knockout mice with NaHS resulted in reduced atherosclerosis plaque, while inhibition of CSE activity enlarged plaque size (Wang et al. 2009). GYY4137, a H_2S donor, was also shown to decrease aortic atherosclerotic plaque formation and partially restored aortic endothelium-dependent relaxation in ApoE knockout mice (Li et al. 2008a; Liu et al. 2013; Qiao et al. 2010). Another study showed that onion extracts boosted endogenous production of H_2S and lessened atherosclerotic lesions in rats (Zhang et al. 2012a, b). In a high-fat and high-vitamin D1 diet-induced atherosclerotic rat model, H_2S was shown to slow down the development of atherosclerosis by improving the damage of vessels and inhibiting the expression of VEGF (Zhang et al. 2012a, b). Treatment with NaHS significantly inhibited arterial restenosis following balloon angioplasty in rabbits by reducing the intimal area and the intima/media ratio, while PPG treatment had a tendency to worsen the severe restenosis (Ma et al. 2012). Whether CSE deficiency impacts on the development of atherosclerosis has been directly addressed by knocking out the CSE gene in high-fat-fed mice. Decreased endogenous H_2S production in CSE knockout mice leads to the vascular remodeling and early development of atherosclerosis (Mani et al. 2013). The atherosclerotic plaque development is rescued by H_2S donor NaHS via reducing vessel intimal proliferation and inhibiting adhesion molecule expression. In contrast, antihypertensive (hydralazine), antioxidant (N-acetylcysteine), or lipid-lowering (ezetimibe) agents have no effect on high-fat-diet-induced plaque formation in CSE knockout mice, implying that hypertension, higher oxidative stress, and abnormal lipid profile do not play major roles in atherosclerosis development in CSE knockout mice. In addition, the knockout of CSE from ApoE knockout mice accelerated plaque formation even under normal diet, indicative of a potential therapeutic implication of endogenous H_2S (generated by CSE) in atherosclerosis. Interestingly, estrogen attenuates atherosclerosis development by stimulating H_2S production in female ovariectomized ApoE knockout mice fed with a high-fat diet, suggesting that H_2S mediates estrogen-induced vascular protection (Zhou et al. 2013; Fu et al. 2013).

7.3 H_2S and Aortic Aneurysms

Aortic aneurysms, including thoracic and abdominal aortic aneurysms, are the most life-threatening cardiovascular complication in Marfan syndrome, leading to aortic expansion, dissection, rupture, and sudden death. Enzymatic degradation of extracellular matrix (ECM) protein by matrix metalloproteinases (MMPs) leads to dilation of the aortic wall and constitutes the most prominent characters of aortic aneurysms. The regulatory role of H_2S in vascular remodeling during aortic aneurysms has not been explored yet. Early studies showed that H_2S is involved in vascular remolding during the development of hypertension and neointimal formation. Rats with high pulmonary blood flow for 11 weeks showed a significant pulmonary hypertension and pulmonary artery collagen remodeling in association

with a decrease in lung tissue H_2S content (Li et al. 2008b). Supplement of exogenous H_2S lowered pulmonary artery collagen I and collagen III protein levels and normalized pulmonary hypertension, suggesting that the downregulation of H_2S is involved in the development of pulmonary artery collagen remodeling induced by high pulmonary blood flow. Vascular H_2S production was lower in SHR compared with Wistar Kyoto (WKY) rats, and the inhibitory role of H_2S on collagen generation was stronger in the SHR than in the WKY rats (Zhao et al. 2008). In a carotid artery-injured mouse model, H_2S was shown to mitigate vascular remodeling from endothelial damage by decreasing the expressions of TIMP-3 and MMP-9 (Vacek et al. 2010). In addition, we found that H_2S inhibited SMC migration and neointima formation by suppressing α5β1-integrin-dependent MMP-2 expression. The inhibitory roles of H_2S on the MMP/TIMP system suggest that H_2S can block excess degradation of ECM and maintain the normal structure of the aorta (Yang et al. 2012a, b). Future studies need to determine the role of H_2S in vascular degeneration and aortic aneurysm formation. The molecular mechanisms involved in the pathogenesis of aortic aneurysm formation as well as the mechanisms underlying CSE/H_2S system-regulated MMP activation are also waiting to be explored. All these endeavors will provide a new therapeutic avenue for the prevention and treatment of aortic aneurysms.

8 Prospective

Undoubtedly, the research scope and depth on H_2S signaling in the cardiovascular system, especially in blood vessel-related disorders, will continue to expand and deepen. The identification of new cellular targets and development of novel agents to enhance endogenous H_2S generation are highly expected. A better understanding of the roles of H_2S in vascular remodeling and the regulatory mechanisms for endogenous production of H_2S in blood vessel can provide insight into potential therapeutic interventions against blood vessel-associated disorders. The interaction of H_2S with numerous biological molecules, such as NO and CO, needs to be better characterized. At the end of the day, all these fundamental and mechanistic studies will become meaningless if the related discoveries cannot be translated from bench side to bedside so that human health and welfare will be improved. That day will not be too far away.

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