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FORUM REVIEW ARTICLE

Role of Cystathionine γ-Lyase/Hydrogen Sulfide Pathway in Cardiovascular Disease: A Novel Therapeutic Strategy?

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Abstract

Significance: Hydrogen sulfide (H_2S) has traditionally been considered a toxic environmental pollutant. In the late 1990s, the presumed solely harmful role of H_2S has been challenged because H_2S may also be involved in the maintenance and preservation of cardiovascular homeostasis. ***Recent Advances:*** The production of endogenous H_2S has been attributed to three key enzymes, cystathionine γ-lyase (CSE), cystathionine β-synthase, and 3-mercaptopyruvate sulfurtransferase. The recognition of H_2S as the third gaseous signaling molecule has stimulated research on a multitude of pathophysiologic events in the cardiovascular system. In particular, important roles in cardiovascular disorder processes are ascribed to the CSE/H_2S pathway, such as atherosclerosis, myocardial infarction, hypertension, and shock. ***Critical Issues:*** Many biological activities and molecular mechanisms of H_2S in the cardiovascular system have been demonstrated in studies using different tools, such as the genetic overexpression of CSE, the direct administration of H_2S donors, or the use of H_2S-releasing prodrugs. Unfortunately, the role of the CSE/H_2S pathway in cardiovascular disease remains controversial in numerous areas, and many questions regarding the gaseous molecule still remain unanswered. ***Future Directions:*** Advances in basic research indicate that the CSE/H_2S pathway may provide potential therapeutic targets for treating cardiovascular disorders. But the molecular targets of H_2S still need to be identified. *Antioxid. Redox Signal.* 17, 106–118.

Introduction

Hydrogen sulfide (H_2S), a colorless gas with a characteristic rotten-egg odor, has traditionally been considered to be a toxic environmental pollutant. However, in the late 1990s, H_2S was reclassified as the third physiologically relevant, gaseous signaling molecule with a diverse physiologic profile, alongside nitric oxide (NO) and carbon monoxide (CO) (1, 60). A multitude of works beginning in the mid-1990s have demonstrated that H_2S relaxes blood vessels (77), decreases inflammation, decreases infarct size and mortality after myocardial infarction (79), and protects neurons from oxidative stress (30). The pioneering studies generated from a group led by the neuroscientist Hideo Kimura determined that the physiologic concentration of H_2S enhances N-methyl-D-aspartate receptor–mediated response and facilitates the induction of hippocampal long-term potentiation, and this has opened up a new perspective on H_2S as a gasotransmitter (1). H_2S has become increasingly identified as an important molecule in the physiologic regulation of multiple systems and in the restoration of homeostasis in pathophysiologic states. In the past two decades, H_2S has undergone an impressive transformation from a noxious gas to an important gaseous signaling molecule.

Endogenous H_2S production has been attributed to three key enzymes, cystathionine γ-lyase (CSE) (77), cystathionine β-synthetase (CBS), and the newly discovered 3-mercaptopyruvate sulfurtransferase (3-MST) (53). CSE is abundant in heart, liver, kidney, and vascular smooth muscle and is the most relevant H_2S-producing enzyme in the cardiovascular system (61, 77). Recent research suggests that CSE is also expressed in the vascular endothelial cells and is capable of producing H_2S (67, 71). A number of studies have demonstrated that the CSE/H_2S pathway may be involved in a multitude of pathophysiologic processes of cardiovascular disease, such as myocardial ischemia, atherosclerosis, hypertension, and so on.

In this review, we discuss the molecular mechanisms mediating the protective effects of H_2S and how these might be used therapeutically to overcome some of the major causes of morbidity and mortality worldwide.

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Endogenous H_2S biosynthesis

Bacteria can produce H_2S gas, and H_2S is essential for their survival and proliferation (62). However, a series of excellent studies have shown that vertebrate tissues also synthesize H_2S and this endogenous gas is, in fact, a signaling molecule, thereby joining NO and CO as the third "gasotransmitter" (1, 60).

The biosynthesis of endogenous H_2S has been reviewed in depth elsewhere (18, 29, 31, 34, 41, 56, 61); however, a short description of the principal H_2S-generating enzymes of the processes involved may be useful. H_2S can be produced in mammalian tissues by endogenous enzymes and by nonenzymatic pathways. The nonenzymatic pathways, although less important, proceed via a nonenzymatic reduction of elemental sulfur or organic polysulfide to H_2S via glucose-supported and thiol-dependent cellular reactions as well as glutathione -dependent acellular reactions (3, 41). With regard to the enzymatic route, CBS and CSE are pyroxidal-5′-phosphate-dependent enzymes, which use L-cysteine as their principal substrate for the enzymatic production of H_2S. The expression of H_2S-producing enzymes is tissue specific. In some tissues CSE is the main H_2S-generating enzyme, while CBS and 3-MST are the primary enzymes in others. For example, CBS is most abundantly expressed in the brain, whereas the activity of CSE is highest in liver, kidney, and blood vessels (24). Large amounts of CBS occur in the central nervous system, whereas CSE appears to play a major role in catalyzing the production of H_2S in cardiovascular tissues (77). Unlike CBS and CSE, 3-MST uses 3-mercaptopyruvate, which is a metabolite of cysteine and keto acids (e.g., α-ketoglutarate) catalyzed by cysteine aminotransferase, as a substrate to form H_2S (53). 3-MST also produces H_2S more efficiently than does CBS, which was previously believed to be the sole H_2S-producing enzyme in the brain (29). CBS and CSE are cytosolic enzymes, but 3-MST is both a mitochondrial and cytosolic enzyme with approximately two thirds of 3-MST existing in the mitochondria (31) (Fig. 1).

Pathophysiology and Clinical Role of H_2S

Levels of H_2S in cardiovascular disease

Under physiologic conditions, the H_2S concentrations vary in different organs and tissues. Warenycia *et al.*, (67a) reported that the level of free H_2S in normal brain tissue of rats is about 54 μM, and subsequent studies showed the physiologic circulating level and tissue level of H_2S to be 10–160 μM (56, 61). However, there is some question whether these concentrations are physiologic. Free H_2S at these concentrations in plasma or in tissue should be malodorous and detectable by the human nose. However, we did not smell the characteristic odor of H_2S in baseline blood samples, and baseline H_2S levels in blood and tissues are probably lower than the levels usually mentioned in the literature. Recent studies employing different analytical techniques, such as head-space gas analysis (58), spectrophotometric determination (44), and a silver sulfide or polarographic sensor (11, 22), also identified the level of H_2S in blood or tissue as being much lower than previously reported. For example, analyses of the gas space over rapidly homogenized mouse brain and liver indicated that *in situ* tissue H_2S concentrations were only about 15 nM (13). Human alveolar air measurements indicated the free H_2S concentration in blood is very low (13). In addition, Ishigami *et al.* (22) reported that using silver particles to measure free H_2S shows that free H_2S is maintained at a low level in basal conditions in the brain (<9.2 μM). The discrepancies mentioned above for tissue concentrations of H_2S and H_2S production rates associated with normal or pathophysiologic processes are in part due to the technical difficulties associated with handling H_2S or different detection assays. Therefore, accepted and effective methods of determining and quantifying the exact free and bioavailable concentrations of H_2S in blood and tissues need to be established.

The concentration of H_2S in plasma or in tissue is regulated at the level of its generation and its consumption, and the levels are maintained within a certain range. Significant

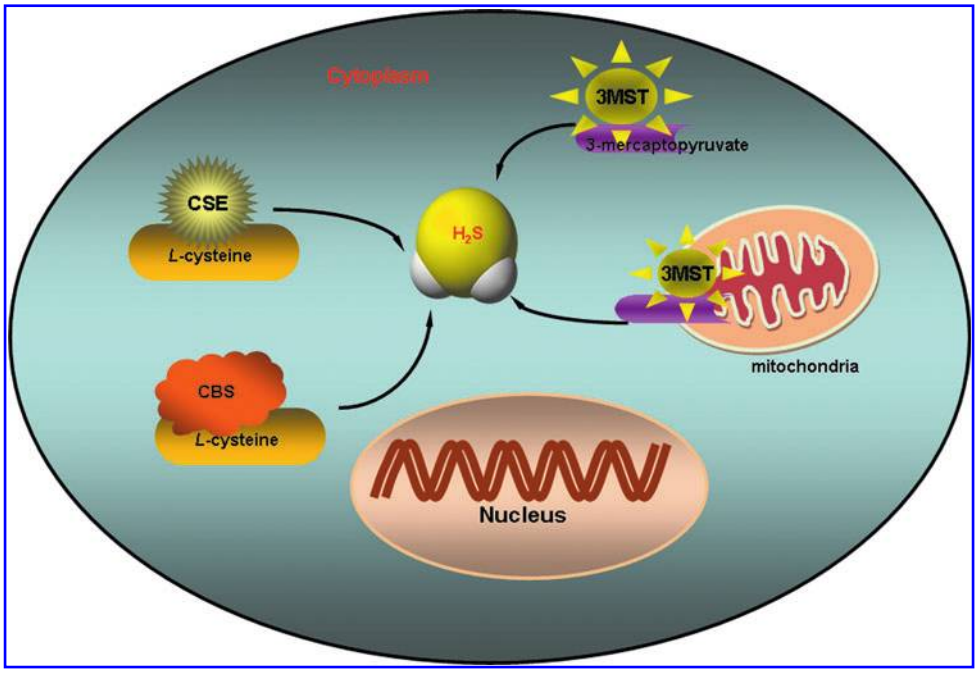

FIG. 1. Schematic diagrams of the H_2S-producing enzymes and their intracellular distributions. Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) use L-cysteine as their principal substrate for the enzymatic production of H_2S and are distributed in the cytoplasm. However, 3-mercaptopyruvate sulfurtransferase (3-MST) uses 3-mercaptopyruvate as a substrate to form H_2S and is both a mitochondrial and cytosolic enzyme. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

changes in the levels contribute to various diseases. Similarly, significant changes of an H_2S-producing enzyme or its activity may have beneficial or detrimental effects in various disease states. For example, in rats with myocardial infarction, beneficial effects of H_2S were seen as decreased infarct size and mortality rate in rats treated with sodium hydrosulfide (NaHS, a H_2S donor), whereas reduced levels of H_2S due to CSE inhibition with propargylglycine (PAG, a CSE inhibitor) resulted in a larger infarct area and higher mortality rate (79). These results were consistent with the report that the plasma H_2S level decreased by 50% in patients with coronary heart disease (56). Additionally, treatment with exogenous H_2S or genetic overexpression of CSE resulted in increased endogenous H_2S production, which was associated with profound protection against ischemia-induced heart failure and decreased mortality in mice with myocardial ischemia–reperfusion injury (6). Similarly, spontaneously hypertensive rats have substantially lower plasma levels of H_2S and H_2S production in aortic tissue and lower H_2S-synthesizing expression and activity than normotensive rats (69). In animal studies, exogenous H_2S significantly decreased blood pressure and prevented the hypertrophy of intramyocardial arterioles and ventricular fibrosis, as well as decreased myocardial reactive oxygen species (ROS) and conjugated diene levels (52, 69). $ApoE^{-/-}$ atherosclerotic mice were also shown to have substantially lower H_2S level in plasma ($ApoE^{-/-}$ mice = 44.6 μM; control mice = 57.6 μM) and aortic production of H_2S ($ApoE^{-/-}$ mice = 1.98 nM/[min·mg protein]; control mice = 4.36 nM/[min·mg protein]) (67). Thus, increases in circulating H_2S concentration or H_2S-producing enzyme expression in tissue have a positive effect in the mentioned diseases. However, in many others diseases, including burn injury and shock, an increase in the circulating level of H_2S can have detrimental effects. Mice subjected to burn injury had significantly enhanced plasma H_2S levels and liver H_2S-synthesizing enzyme activity (76). Additionally, in the plasma of endotoxin-treated mice, there were ~44%–91% increases in H_2S production along with increased liver and kidney H_2S-synthesizing CSE activity and CSE mRNA expression (32).

H_2S and hypoxia/ischemia in the heart

The heart is particularly susceptible to hypoxia since only limited reserves of high-energy phosphates are maintained. The myocardium may be exposed to hypoxia due to major coronary artery occlusion, high altitude, or anemia. A potential function of H_2S in cardiomyocytes was suggested by H_2S being involved in the reduction of cellular damage inflicted on cells exposed to hypoxia or hypoxia/reoxygenation (57, 79). Intermittent hypoxia appears to decrease endothelial CSE expression and reduce endogenous H_2S production, depolarize vascular smooth muscle, and enhance myogenic tone, suggesting that the hypoxic response is mediated through depletion of H_2S biosynthesis (25). Chuah and coworkers (10) first demonstrated that inhibition of CSE resulted in increased myocardial injury and mortality in a rat model of myocardial infarction by showing the pivotal role of endogenous H_2S in the attenuation of myocyte injury. Moreover, exogenous addition of *S*-allylcysteine (SAC), an active compound of garlic and a substrate for CSE, increased the endogenous H_2S production and protein expression of CSE and rendered the heart resistant to ischemic heart disease (10). *S*-propargyl-cysteine (SPRC), an SAC structural analogue, represents a new pharmacological agent that can be used to modulate endogenous H_2S levels (64, 65). In the heart, CBS does not play any significant role in generating H_2S under normal conditions, but CSE appears to be involved in the endogenous generation of H_2S (79). We found that CSE expression was remarkably suppressed after hypoxia treatment, but SPRC significantly increased CSE expression and endogenous H_2S production in primary neonatal rat cardiomyocytes (64). Conversely, the production of H_2S and expression and activity of CSE were reduced after CSE inhibition with PAG (64). It has also been demonstrated that exogenous H_2S may be useful to reduce hypoxic/ischemic injuries in the heart. Our group found that H_2S supplementation displayed a clear protection against left ventricular structural impairment in ischemia-induced heart failure (66) (Fig. 2).

Furthermore, the cardioprotective role of H_2S could also be due to its angiogenic action on the ischemic area in the heart. Angiogenesis plays a pivotal role in the early stage of wound healing. *In vitro* studies demonstrate that exogenously administered H_2S at physiologically relevant concentrations, but not high concentrations, induces angiogenesis in chicken chorioallantoid membranes and stimulates endothelial cell proliferation and migration and tube formation in a Matrigel model (5, 48). It is noteworthy that exogenous H_2S failed to induce vascular endothelial growth factor (VEGF) release in endothelial cells, which suggests that VEGF may not contribute to the angiogenic actions of H_2S (5). However, Papapetropoulos and colleagues (48) demonstrated that endogenously produced H_2S is a key mediator of VEGF-induced angiogenesis. Although the data from these studies are inconsistent, they raise the possibility that exposure of

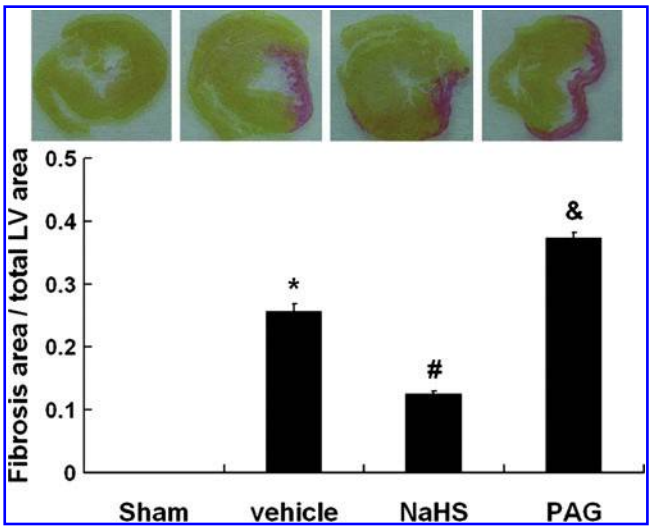

FIG. 2. The effects of H_2S on the area of myocardial fibrosis after heart failure. H_2S treatment significantly decreased the area of fibrosis. However, propargylglycine (PAG) treatment increased the area of fibrosis in the heart failure animals, $^*p < 0.05$ compared with Sham group, $^*p < 0.001$ compared with Vehicle group. Adopted with permission from Wang *et al.* (65). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

endothelial cells to VEGF may lead to H_2S release that in turn contributes to VEGF-stimulated angiogenesis-related properties of endothelial cells. H_2S has also been shown to promote the healing of burn wounds, further confirming the role of H_2S in neovascularization and in wound healing (48). Along these lines, H_2S at lower doses significantly increased collateral vessel growth, capillary density, and regional tissue blood flow in ischemic hind limb muscles (59). The effects of H_2S have been attributed to an increase in VEGF expression in the skeletal muscles and VEGFR2 phosphorylation in the neighboring vascular endothelial cells (59). Consistent with the effects of H_2S in hindlimb ischemia, a recent study (16) reported on the angiogenic effect of H_2S treatment in myocardial hypertrophy. After H_2S treatment in an aortic banding-induced pressure-overload mouse model, functional and histological evaluations performed 3 and 8 weeks later revealed that H_2S supplementation improved cardiac function and the response was associated with a significant increase in capillary density, which mitigated transition from compensatory hypertrophy to heart failure (16). Finally, H_2S is, of course, a potent vasodilator and it is conceivable that, in addition to its direct effect on angiogenesis, this gas also induces angiogenesis indirectly by increasing local blood flow (71). Therefore, pharmacological supplementation or stimulation with H_2S may be of therapeutic relevance for myocardial ischemic therapy.

The data collectively indicate that the activity and expression of CSE are compromised or even abolished during hypoxia, suggesting that increasing the activity and expression of CSE and production of H_2S may protect cardiac cells against hypoxic damage. There is strong evidence that ROS production and oxidative stress are major contributing factors to cardiomyocyte injury after hypoxia, and the indirect or direct antioxidant effects of H_2S may have protective effects under hypoxic conditions (65). In addition, and importantly, preservation of mitochondrial function may also contribute to the protective effects of H_2S (66) (Fig. 3). Multiple signaling mechanisms involved in the angiogenic action of H_2S have been studied. Zhu and colleagues (5, 59) have proposed Akt signaling activation as one of the possible mechanisms of angiogenesis by H_2S. Moreover, H_2S also up-regulated VEGF expression and VEGFR2 phosphorylation in ischemic hind limb muscles (59). However, as observed by Papapetropoulos *et al.* (48), although H_2S activates the Akt pathway, it is the MAPK (ERK1/2 and p38) and ATP-sensitive potassium (K_{ATP}) channel opening pathways, not the Akt pathway, that are involved in H_2S-stimulated angiogenesis. Consistently, however, H_2S is reported to mediate angiogenesis through regulation of anti-angiogenic/angiogenic factors production by regulating the matrix metalloproteinase/tissue inhibitor of metalloproteinase axis (16) (Fig. 4).

H_2S in myocardial ischemia–reperfusion injury

Myocardial ischemia is characterized as a state of insufficient oxygen supply resulting in a decrease of free energy. This leads to irreversible tissue damage within 20 to 30 minutes of sustained ischemia. The most common complication is the occurrence of left ventricular dysfunction and heart failure. The discovery of CSE in the rat heart and identification of H_2S as an important modulator was a breakthrough in the investigation of the role of H_2S in heart function (79). Delivery of H_2S at the time of reperfusion decreases infarct size and preserves left ventricular function in an *in vivo* model of myocardial ischemia–reperfusion, and this effect is probably mediated via preservation of mitochondrial function (12). Transgenic mice expressing cardiospecific CSE protein were generated and showed increased myocardial H_2S production, and it was reported that the heart showed improved recovery in contractile performance and a limited extent of injury following myocardial ischemia–reperfusion (12). This finding

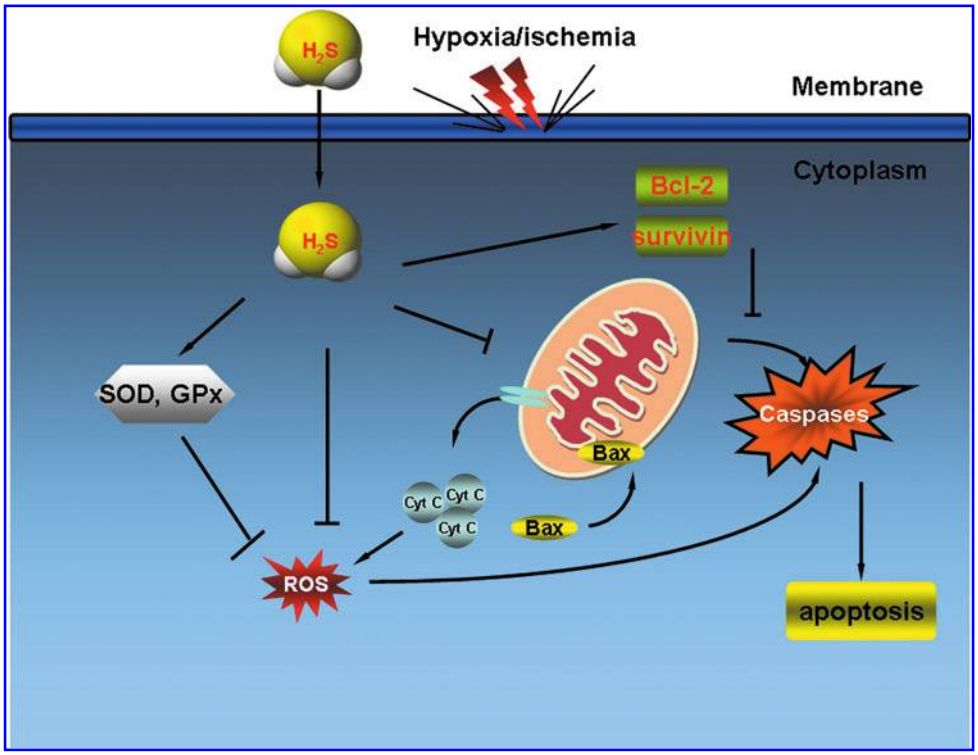

FIG. 3. Schematic diagrams of the cytoprotective effects of H_2S against hypoxia/ischemia-induced injury. H_2S can protect cells from hypoxia/ischemia via different mechanisms. Up-regulation of H_2S by pharmacological agents leads to an increase in the Bcl-2/Bax ratio and preservation of mitochondrial function, resulting in increased cell survival. Simultaneously, H_2S may also directly enhance antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which act as reactive oxygen species (ROS) scavengers to neutralize its pro-oxidant activity. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

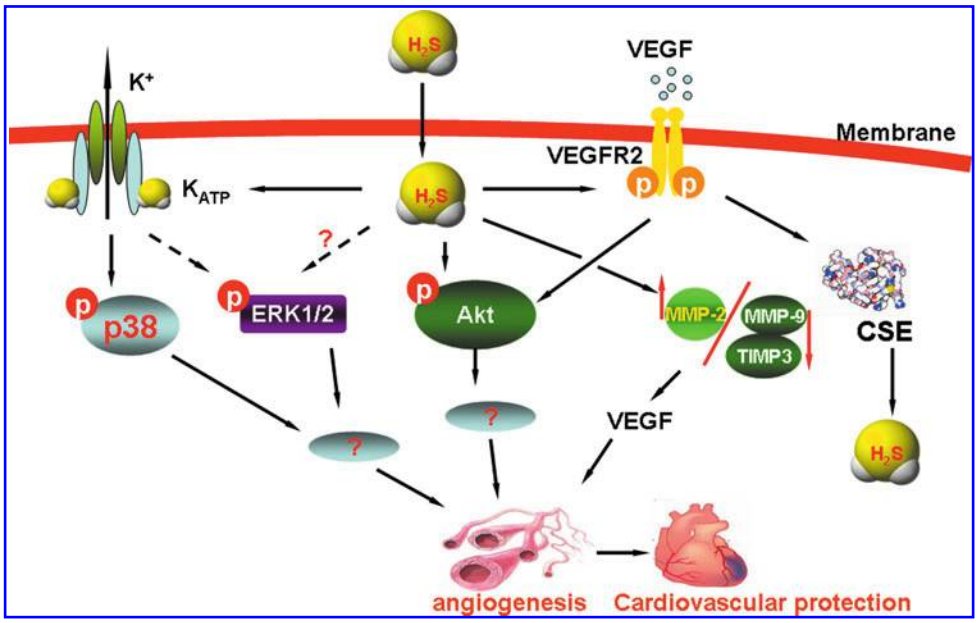

FIG. 4. Proposed signaling pathways of H_2S during angiogenesis. H_2S activates Akt signaling pathways and simultaneously enhances vascular endothelial growth factor (VEGF) production and VEGFR2 phosphorylation, leading to downstream Akt activation. H_2S also exerts angiogenic effects through activation of ATP-sensitive potassium channels (K_{ATP} channels), which in turn facilitate activation of MAPK (ERK1/2 and p38) pathways, leading to new blood vessel formation. VEGF may activate CSE activity and H_2S release that contributes to VEGF-stimulated angiogenesis-related properties of endothelial cells. H_2S promotes VEGF synthesis and angiogenesis by inducing matrix metalloproteinase (MMP)-2, while it suppresses MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-3 levels, thus inhibiting anti-angiogenic factors. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

that modulation of endogenous H_2S is cardioprotective is supported by recent work by our group (80), which showed that pharmacologic inhibition of CSE resulted in an increase in infarct size in a rat model of myocardial ischemia–reperfusion. Conversely, H_2S replacement displayed myocardial protection (80). Similarly, H_2S-releasing derivatives of diclofenac protected isolated rabbit hearts from myocardial ischemia–reperfusion injury (50). In addition, H_2S also played a protective role in myocardial ischemia–reperfusion injury in streptozocin-induced diabetic rats by inhibiting neutrophil accumulation, reducing the production of lipid peroxidation, inhibiting caspase-3 activation, and down-regulating tumor necrosis factor (TNF)-α, Fas, and Fas L expression (15). Since patients with coronary disease have reduced endogenous H_2S levels, these findings indicate that genetic and pharmacologic H_2S therapy may be an effective approach in infarct repair.

To explore the therapeutic potential of CSE gene transfer in long-term myocardial protection, Calvert and co-workers (6) took advantage of transgenic mice with cardiac-restricted overexpression of CSE, which resulted in increased endogenous H_2S production and a profound protection against ischemia-induced heart failure and decreased mortality due to increased levels of endogenous antioxidants, attenuation of apoptosis, and increased mitochondrial biogenesis. On the other hand, we also showed that NaHS decreased the leakage of cytochrome c protein from the mitochondria to the cytoplasm, improved mitochondrial derangements, and increased CSE mRNA and protein levels in heart failure rats (66). In addition, pharmacologic H_2S therapy during heart failure serves to mitigate pathological left ventricular remodeling and to reduce myocardial hypertrophy, oxidative stress, and apoptosis (43).

Myocardial ischemia–reperfusion injury can be due to multiple mechanisms, such as free radical accumulation, reduced availability of NO, Ca^{2+} overload, and mitochondrial permeability transition pore opening, that lead to myocardial cell death and exacerbate tissue injury. Numerous mechanisms have been proposed to account for the cardioprotective effects of H_2S. The mechanism of this protection may be, at least in part, related to the ability of H_2S to activate myocardial K_{ATP} channels. Johansen and colleagues (28) reported that the cardioprotection against myocardial ischemia–reperfusion injury of H_2S was mediated by K_{ATP} channel opening. Moreover, the cardioprotective effect of H_2S is diminished by pretreatment with K_{ATP} channel inhibitor glibenclamide or sodium 5-hydroxydecanoate (27, 28). This finding was further supported by pharmacological inhibitors of K_{ATP} channels increasing myocardial infarct size in normal animals (56). Additional effects of H_2S, whereby it may beneficially affect the outcome of ischemia–reperfusion, are mediated in large part by activation of transcription factors nuclear-factor-E2-related factor-2 (Nrf-2) and nuclear respiratory factor 1 as well as a PKCε-signal transducer and activator of the transcription 3 (STAT-3) signaling cascade, which subsequently modulate antioxidant and anti-apoptotic signaling (6, 7). Furthermore, H_2S-mediated cytoprotection is also associated with an inhibition of myocardial inflammation (12, 54) and preservation of both mitochondrial structure and function after ischemia–reperfusion injury (54). Up-regulating phosphorylation of nitric oxide synthase 3 (42), increasing the phosphorylation of Akt (6), or inducing phosphorylation of glycogen synthase kinase-3beta (Ser9) and subsequent inhibition of mitochondrial permeability transition pore opening

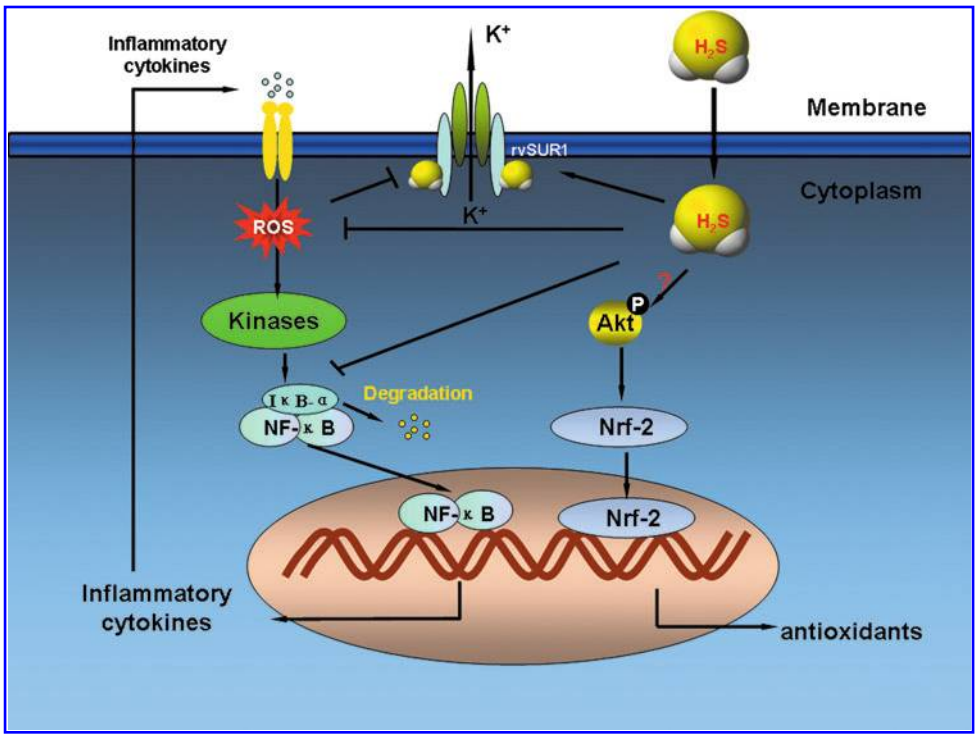

FIG. 5. Potential signaling pathways activated by H_2S leading to tissue protection during ischemia–reperfusion injury. Possible signaling cascades affected by H_2S that are involved in the tissue-protective effects of H_2S. H_2S can activate K_{ATP} channels via direct interaction with the extracellular N terminal of the rvSUR1 subunit. H_2S can activate the Akt pathway, further inducing nuclear-factor-E2–related factor 2 (Nrf-2) nuclear translocation, resulting in increased cell protection. H_2S may cause an inhibition of inflammatory cytokine production through nuclear factor-κB (NF-κB)–dependent pathways, leading to tissue protection. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

(73) may also be involved in H_2S-mediated cardiac protective mechanisms (Fig. 5).

H_2S in atherosclerosis

Atherosclerosis, a chronic, complex, and progressive pathological process in large- and medium-sized arteries, is an important pathological manifestation of cardiovascular disease, the leading cause of death in developed countries. The exact mechanism of this process is still unclear. Vascular inflammation and abnormal immune response, proliferation of smooth muscle cells, endothelial damage, and foam cell accumulation contribute to atherosclerotic plaque formation (62). In fact, the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease.

Considerable evidence suggests that the CSE/H_2S pathway plays important physiologic and pathophysiologic roles and exerts regulatory effects on the pathological process of various cardiovascular diseases, including atherosclerosis (39, 67). Vascular endothelial cells are considered to be a passive monolayer covering the inner part of vascular walls, and these cells are regarded as forming a mechanical barrier between circulating blood and vascular structures. Endothelial dysfunction elicited by inflammatory cytokines is involved in lesion formation by promoting both early and late mechanisms of atherosclerosis. H_2S has been shown to mediate proinflammatory effects by potentiating sulfite production in endotoxic shock (37) and mediating leucocyte activation (74). However, numerous studies characterize H_2S as being an anti-inflammatory molecule (18, 34, 47). These contradictory observations may be a result of the dose of H_2S donor as well as the different models used. However, although the data are conflicting, they raise the possibility that H_2S may be a double-edged sword. Synthesized in appropriate amounts or under physiologic conditions, H_2S may act as an anti-inflammatory mediator to inhibit the leukocyte–endothelium interaction, regulate blood pressure, and attenuate atherosclerosis. Conversely, overproduction of H_2S may contribute to several inflammatory diseases such as septic shock and some forms of chronic inflammation. Combined with previous reports, we investigated the effects of exogenous H_2S on inflammatory signaling and dysfunction induced by TNF-α in human umbilical vein endothelial cells (46). We found that NaHS suppressed, in a concentration-dependent manner, TNF-α–induced mRNA, expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and mRNA expression of P-selectin and E-selectin, as well as monocyte adhesion to human umbilical vein endothelial cells, indicating that direct H_2S administration or the modulation of endogenous H_2S production in vascular cells may attenuate the development of atherosclerosis (46). Our results were consistent with another study's finding that disturbance of the vascular CSE/H_2S pathway may play a vital role in the pathogenesis of atherosclerosis (67). The researchers found a decrease in H_2S level in plasma as well as CSE activity and expression in apoE$^{-/-}$ mice with advanced atherosclerosis (67). Treatment of apoE$^{-/-}$ mice with NaHS resulted in reduced atherosclerotic plaque, whereas inhibition of CSE activity in apoE$^{-/-}$ mice with PAG enlarged plaque size. In agreement with the anti-inflammatory effects of endogenous H_2S, a deficiency contributes to hyperglycemia in endothelial cells and in rats with streptozotocin-induced diabetes. H_2S supplementation is able to improve the endothelial metabolic state and maintain normal endothelial function in hyperglycemic endothelial cells and streptozotocin-induced diabetic rats (55). These results also suggest that an abnormal CSE/H_2S pathway may be another causative factor for endothelial dysfunction, and the administration of exogenous H_2S or the modulation of the CSE/H_2S pathway may be

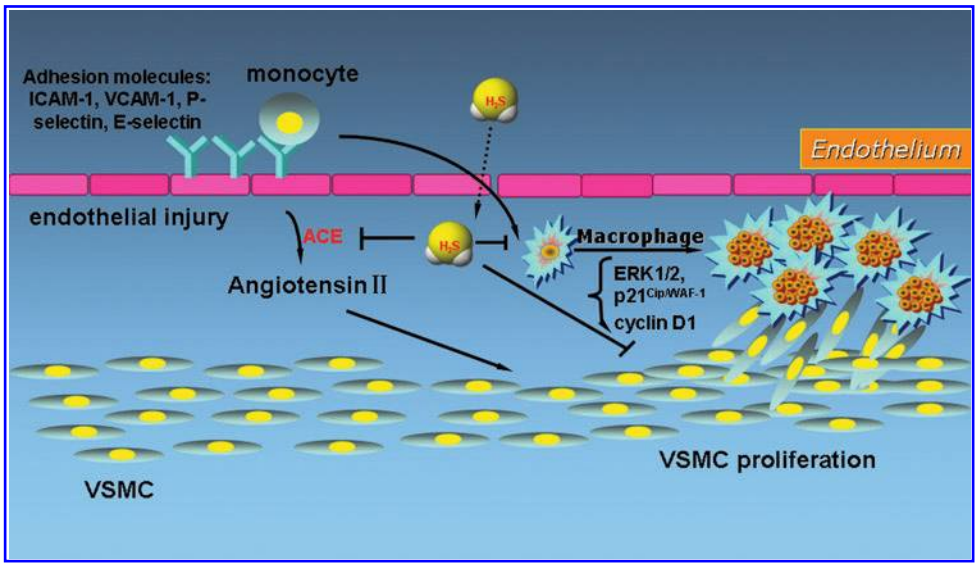

FIG. 6. Schematic representation of the role of H_2S in atherosclerosis. Atherosclerosis is manifested by vascular inflammation, proliferation of smooth muscle cells, and endothelial damage. An increase in H_2S inhibits proliferation of smooth muscle cells via up-regulation of ERK1/2 and $p21^{Cip/WAF-1}$ phosphorylation and down-regulation of cyclin D1. H_2S can attenuate inflammatory cytokine–induced endothelial dysfunction. Meanwhile, H_2S also suppresses foam cell formation. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars). VSMC, vascular smooth muscle cells; ACE, angiotensin-converting enzyme.

of therapeutic benefit in the treatment of atherosclerosis (46, 67) (Fig. 6).

Vascular smooth muscle cell proliferation is an essential step for the development of atherosclerosis. H_2S can be generated in blood vessels in a reaction catalyzed mainly by CSE. Abnormal metabolism and functions of the CSE/H_2S pathway also plays a role in vascular smooth muscle cell proliferation and apoptosis (63, 72). Given that lack of H_2S exacerbates atherosclerosis (67), the next question is whether disturbance of the vascular CSE/H_2S pathway would induce vascular smooth muscle cell proliferation. Using a recombinant defective adenovirus containing the CSE gene, Yang and colleagues (72) found that overexpression of CSE in human aorta smooth muscle cells resulted in a significant increase in the expression of CSE protein and H_2S production, which further stimulated smooth muscle cell apoptosis. They also found that inhibiting endogenous H_2S production with PAG, then adding exogenous H_2S at physiologically relevant concentrations induced much more significant apoptosis. In contrast, Yang (70) further found that smooth muscle cells derived from $CSE^{-/-}$ mice displayed a proliferative phenotype but had an enhanced susceptibility to apoptosis induced by exogenous H_2S at physiologically relevant concentrations. These results indicate that exogenous H_2S induces apoptosis of smooth muscle cells, which can also be significantly affected by the endogenous H_2S level. Therefore, the pro-apoptotic effect of H_2S on vascular smooth muscle cells may have important implications in the vascular remodeling process and the development of atherosclerosis (Fig. 6).

What is the molecular mechanism underlying the effect of H_2S on atherosclerosis? H_2S probably acts via multiple mechanisms under differing conditions. Nuclear factor-κB (NF-κB), a transcription factor, is involved in regulation of a number of proinflammatory genes, including those that encode for inflammatory cytokines/chemokines, adhesion molecules, and so on. Suppression of NF-κB activation may be an anti-atherosclerotic mechanisms. Exogenous H_2S was originally shown to inhibit lipopolysaccharide (LPS)-induced NF-κB activation in cultured RAW 264.7 macrophages (45). We and other researchers also reported a similar phenomenon in other cultured cells. In cultured human umbilical vein endothelial cells, exogenous H_2S suppressed TNF-α–induced IκBα degradation and p65 phosphorylation and nuclear translocation (34, 46, 67). Interestingly, garlic compounds such as diallyl sulfide, a possible H_2S donor, can also down-regulate NF-κB activation (3). Indeed, such a mechanism may contribute to the anti-atherosclerotic effect of garlic compounds (38). In addition, the endogenous H_2S-modulated agent SPRC attenuated NF-κB activation through regulation of H_2S level in beta-amyloid–induced cognitive deficits and pro-inflammatory response in rats (19), LPS-induced spatial learning and memory impairment in rats (17, 45) and LPS-induced inflammatory response in H9c2 cells (47). Apart from limiting activation of NF-κB, the other protective molecular mechanisms of action of H_2S may also be involved in the process. In addition, H_2S is a reductant that can react with ROS and induces increased production of anti-oxidants. Oxidative modification of low-density lipoprotein plays a crucial role in early atherogenesis. Jeney and co-workers (26) found that H_2S inhibited hemin-induced oxidative modification of LDL through a mechanism involving a reduction of lipid hydroperoxide content. As such, the direct or indirect modulation of intracellular ROS level by H_2S may have important implications in the development of atherosclerosis. This notion receives further support from recent studies showing that H_2S exerts a marked antiatherogenic effect in rats in association with elevated plasma glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities (38, 39). Inhibition of proliferation of smooth muscle cells via induction of ERK1/2 phosphorylation (70), opening of K_{ATP}

channels (63), and down-regulation of cyclin D1 and up-regulation of $p21^{Cip/WAF-1}$ (70, 72) may also contribute to the antiatherosclerotic effects of H_2S (Fig. 6).

H_2S and hypertension

It is well established that hypertension is a pathophysiologic state that manifests as high blood pressure and is a major risk factor for multiple cardiovascular disorders. To date, the mechanisms of hypertension have not been fully understood. NO and CO are established physiologic messenger molecules, and NO and CO have important roles as regulators of blood pressure. Recent various studies also showed that H_2S has important physiologic functions, including relaxing rat aortic artery *in vitro* and inhibiting cultured vascular smooth muscle cell proliferation, that indicate a role of H_2S in hypertension (20, 70–72, 77). H_2S has long been known to relax blood vessels, such as the rat thoracic aorta and portal vein as well as peripheral resistance vessels (9, 20, 77). Supporting a function of H_2S in hypertension, the plasma level of H_2S as well as the gene expression and activity of CSE have been shown to decrease in spontaneously hypertensive rats (69). In addition, exogenous administration of H_2S attenuated the elevation of pressure and lessened the aorta structural remodeling during the development of hypertension (69), while the administration of CSE inhibitor PAG reduced plasma H_2S concentration and elevated blood pressure in WKY rats but not in spontaneously hypertensive rats. These results suggest that the CSE/H_2S pathway is involved in the regulation of vascular tone under baseline conditions and that H_2S production is reduced in hypertension. Direct evidence bearing upon potential antihypertensive activity for H_2S was gathered through investigations with $CSE^{-/-}$ mice. Yang and colleagues (71) produced mice lacking CSE, which had markedly reduced H_2S levels in various tissues, including aorta, smaller arteries, liver, and kidney. These $CSE^{-/-}$ mice had pronounced age-dependent hypertension and diminished endothelium-dependent vasorelaxation, and the authors concluded that the reduction of endogenous H_2S in peripheral arteries led to hypertension. Administration of exogenous H_2S acutely reduced blood pressure in normal mice, and this blood pressure lowering response was much exaggerated in $CSE^{-/-}$ mice (71); however, Ishii *et al.* (23) reported that $CSE^{-/-}$ mice appear to be normotensive. This discrepancy may be related to a difference in the genetic backgrounds of the mice. The role of H_2S in exerting antihypertensive activity is further supported by H_2S donor studies. ACS14, a new H_2S-releasing aspirin, reduced blood pressure and levels of thromboxane B2, 8-isoprostane, and insulin in rats with buthionine sulfoximine–induced hypertension (49). In rats with N^G-nitro-L-arginine methyl ester–evoked hypertension and in spontaneously hypertensive rats, a novel, water-soluble, slow-releasing H_2S compound [morpholin-4-ium 4 methoxyphenyl (morpholino) phosphinodithioate (GYY4137)] caused a slow relaxation of rat aortic rings and dilated the perfused rat renal vasculature. The antihypertensive effect might be due to opening vascular smooth muscle K_{ATP} channels (32). These preliminary findings have already shown that the CSE/H_2S pathway participates in hypertension and that exogenous H_2S effectively prevents the development of hypertension. As already mentioned, impaired H_2S biosynthesis was also observed with hypertension related to experimental models of diabetes mellitus (41).

Homocysteine, a metabolite of methionine, could be converted to H_2S through the transsulfuration pathway (34, 41). However, the elevation of homocysteine levels in plasma, known as hyperhomocysteinemia, is an independent and graded risk factor for cardiovascular diseases, including hypertension. In the hyperhomocysteinemia rat model, plasma H_2S levels and CSE activity in the myocardium were significantly decreased (8). Moreover, in $CSE^{-/-}$ mice, plasma levels of homocysteine were 18 times the level seen in wild-type mice, whereas the levels in heterozygous $CSE^{-/+}$ mice were 2 times those in wild-type mice (71). These results were consistent with a recent study that $CSE^{-/-}$ mice suffer from hyperhomocysteinemia and severe pathological conditions, including acute myopathy and vulnerability to oxidative injury (23). One of the pathophysiologic mechanisms of hyperhomocysteinemia-associated hypertension is smooth muscle cell overproliferation (51). This suggests that H_2S production and CSE activity diminish with hyperhomocysteinemia, which may contribute to, or even create, hyperhomocysteinemia-associated hypertension. This is further supported by additional findings that deficiency of CSE promotes cell proliferation primarily through decreased H_2S production (70). Together, these studies suggest that endogenous and exogenous H_2S play a vital role in regulating hyperhomocysteinemia-associated pathological processes.

Therefore, various molecular mechanisms may be responsible for the antihypertensive effect of H_2S. H_2S was reported to open K_{ATP} channels via direct interaction with the extracellular N-terminal of the rvSUR1 subunit in the smooth muscle, thereby contributing to its protective effect (36, 77). Activation of adenyl cyclase and cAMP-mediated vasodilation may be another possible mechanism of vasodilation by H_2S (68). In addition, H_2S causes vasorelaxation by acting as a nonselective endogenous phosphodiesterase inhibitor that boosts cyclic nucleotide levels in tissues (4). Furthermore, inhibition of the elevation in plasma renin activity and angiotensin II levels (40) and attenuation of angiotensin-converting enzyme activity by H_2S have the potential for retarding the development of hypertension. However, the mechanisms by which H_2S exerts its vasodilator activity have not been completely established.

H_2S and circulatory shock

Over the past few years, a number of potential physiologic and pathophysiologic roles for H_2S have been proposed. One of the most controversial areas of H_2S biology at present is its role in circulatory shock, with both pro- and anti-inflammatory effects reported. The role of H_2S in the pathophysiology of shock depends on the disease state. In a cecal ligation and puncture model of septic shock in rat, Hui and co-workers (21) reported that tissue H_2S formation was significantly elevated and negatively correlated with blood pressure, cardiac function, and the degree of hypoglycemia in rats. Further studies showed that pretreatment with PAG not only reduced tissue H_2S formation but also induced marked anti-inflammatory effects; however, administration of exogenous H_2S provoked inflammatory responses coupled with elevated levels of inflammatory cytokines (2, 32). The available data therefore suggest in these animal models, endotoxic, septic, and hemorrhagic shock were associated with elevated plasma H_2S levels alongside augmented tissue H_2S-synthesizing activity.

A similar proinflammatory effect of H_2S has also been reported in other animal models of circulatory shock. In hemorrhagic shock, the CSE inhibitors PAG or β-cyanoalanine (BCA) led to decreased levels of plasma H_2S, accelerated the recovery of blood pressure, and decreased heart rate (44); inhibition of H_2S biosynthesis also reduced hemorrhagic shock–induced inflammatory responses and organ injury. Interestingly, NaHS can improve hemodynamics in resuscitated hemorrhagic shock and attenuate oxidative and nitrosative stresses (14). Coincidentally, burn injury up-regulated CSE expression and activity in the liver and consequently raised plasma H_2S levels. Therefore, administration of PAG significantly alleviated burn injury–associated inflammation and multiple organ damage (76). In contrast to H_2S gas or NaHS, H_2S-releasing molecules such as GYY4137 and *S*-diclofenac exhibited more pronounced anti-inflammatory activity in circulatory shock coupled with decreased inflammatory cytokines and tissue damage (33, 35). The results were further supported by studies showing that CSE overexpression/SPRC decreased LPS-induced inflammatory response in macrophages/H9c2 cells (47, 78). H_2S exerts complex and, at times, opposing effects on inflammation in animals with shock. One possible explanation for these discrepant data may be the choice of H_2S donor used. The available H_2S donors release H_2S at different rates and therefore give rise to different concentrations of the gas at the inflamed site and thus may exert different effects (35). This raises the possibility that H_2S may act as an anti-inflammatory molecule to inhibit inflammatory responses at a physiologic concentration but may contribute to inflammation when production is excessive and uncontrolled. Nevertheless, the role of H_2S during systemic circulatory shock is still a matter of debate.

There are numerous conflicting data regarding the effects of H_2S on circulatory shock. The mechanisms of action of H_2S on circulatory shock may depend on the animal model and the H_2S donor. H_2S donor NaHS significantly enhanced the activation of ERK1/2 in lung and liver, therefore leading to a further rise in tissue NF-κB activity; increased the transcription of NF-κB-dependent genes; and aggravated systemic inflammation in circulatory shock (75). In addition, activation of activating transient receptor potential vanilloid 1 also

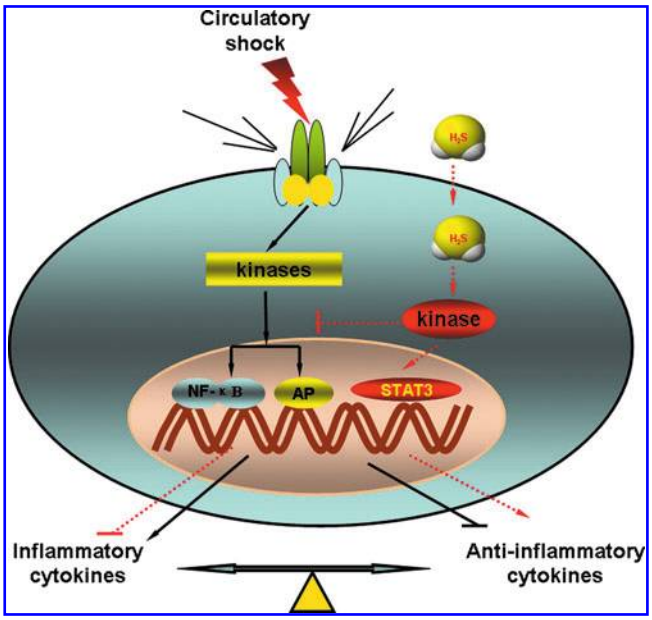

FIG. 7. Schematic diagram illustrates the anti-inflammatory effects of H_2S in circulatory shock. H_2S might control the balance of pro- and anti-inflammatory cytokines in circulatory shock, and this balance plays a major role in inflammatory reactions and tissue injury. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

contributed significantly to the overall pathogenesis of circulatory shock (2). However, slow-releasing H_2S donors, such as GYY4137 and *S*-diclofenac, inhibited NF-κB activation in circulatory shock (33, 35), which is consistent with previous reports in the literature suggesting an inhibitory effect of H_2S on transcription via NF-κB (45, 47). Meanwhile, GYY4137 also interfered with activation of cellular transduction factor STAT-3 and thereby reduced the expression of proinflammatory molecules and/or up-regulated the expression of anti-inflammatory molecules (33). In addition, *S*-diclofenac–derived H_2S reduced expression of inflammatory mediators

Table 1. Selected H_2S-Releasing Compounds and Potential Applications in Cardiovascular Disorders

Compound	*Sources*	*Potential applications*	*References*
GYY4137	A derivative of Lawesson's compound	Circulatory shock; hypertension	29, 32
S-diclofenac	A derivative of diclofenac	Ischemia–reperfusion injury; atherosclerosis vascular obstructive and restenosis; arterial thrombosis or plaque stabilization	31, 44
S-propargyl-cysteine	An analogue of *S*-allylcysteine, a compound of garlic	Circulatory shock; myocardial ischemia	75
ACS 6	Sildenafil	Ischemia–reperfusion injury; vascular obstructive and restenosis	37
Diallyl disulfide	An active compound of garlic	Atherosclerosis; myocardial ischemia; hypertension	3
ASA	A derivative of aspirin	Anti-inflammatory and antithrombotic applications; myocardial ischemia–reperfusion injury; hypertension	50
EXP 3174	An active metabolite of losartan	Vasorelaxing effect, antiplatelet activity, and cardioprotective effect	37
ACS67	A derivative of latanoprost acid	Ischemic diseases	37

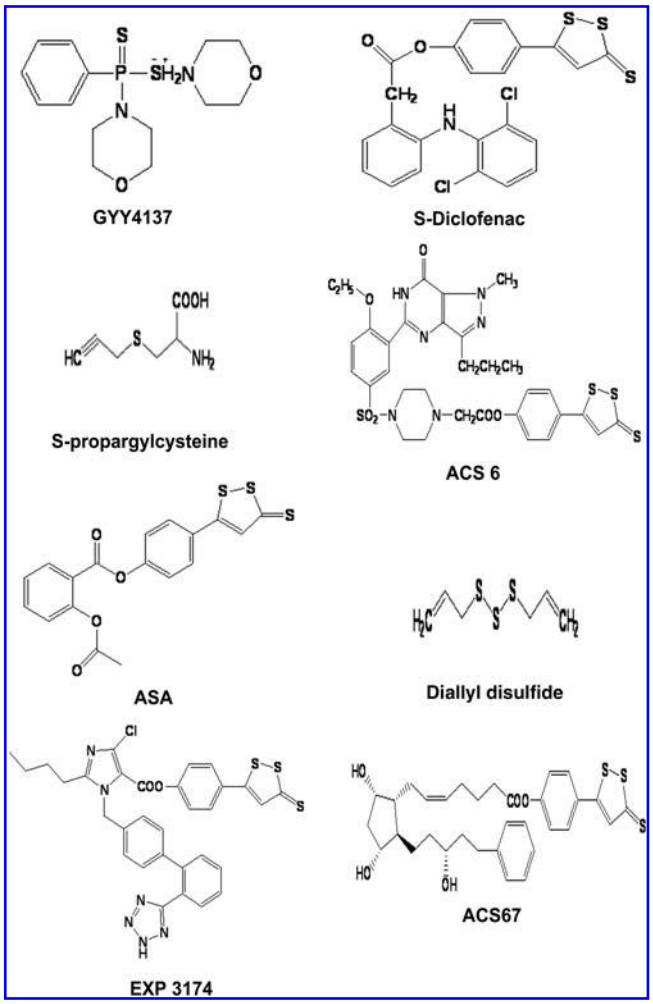

FIG. 8. Chemical structure of some H_2S-releasing drugs that have potential to treat cardiovascular disease.

by inhibiting intracellular transduction via the activator protein 1 pathways (35) (Fig. 7).

Therapeutic potential of H_2S in cardiovascular disease

The protective function of H_2S offers a great genetic and pharmacologic therapeutic potential for cardiovascular diseases. The delivery of cardiac-restricted H_2S-producing enzyme CSE gene to mice has proved successful for achieving increased endogenous H_2S production that decreased myocardial infarct size and attenuated left ventricular dysfunction after myocardial ischemia–reperfusion injury (12). Overexpression of CSE has been shown to attenuate the severity of ischemia-induced heart failure in mice by increasing the levels of endogenous antioxidants, attenuating apoptosis, and increasing mitochondrial biogenesis (6). In contrast, the underexpression of CSE is associated with pronounced hypertension and diminished endothelium-dependent vasorelaxation, substantiating a significant role for H_2S as part of the cellular antioxidant defense system against oxidant-mediated damage in vascular endothelium and as a physiologic vasodilator and regulator of blood pressure (71). With particular regard to the cardiovascular system, the pharmacological modulation of H_2S is becoming a challenging field of research in drug discovery. NaHS behaving as a source of H_2S is viewed as a powerful tool for basic studies for a variety of cardiovascular diseases (41). The release of endogenous H_2S from cells is likely to occur in lesser amounts and at a much slower rate than that from sulfide salts, and therefore NaHS may not mimic the biological effects of naturally produced H_2S (36). Thus ideal H_2S-releasing drug for therapeutic purposes should generate H_2S with slow release rates and mimic the biological effects of endogenous H_2S (41).

Organic polysulfide derivatives of garlic are natural sources of H_2S donors and release H_2S with a relatively slow mechanism. Indeed, the pharmacological properties of H_2S explain the cardiovascular positive effects of garlic well (3). Therefore, organic polysulfide derivatives of garlic are viewed as useful templates for drug discovery and innovative pharmacotherapeutic agents for a variety of cardiovascular diseases. Interestingly, our group found that SPRC exerted cardiovascular protective effects in *in vivo* and *in vitro* studies with an accompanying increase in CSE activity and plasma H_2S concentration (64, 65). A significant amount of effort is currently being channeled into developing novel therapeutics based on delivering H_2S. Aside from the organic polysulfides of natural origin, some synthetic H_2S-releasing donors for the potential treatment of cardiovascular disease have come to the fore (Table 1 and Fig. 8). These include H_2S-releasing derivatives of nonsteroidal anti-inflammatory drugs (ACS14, *S*-diclofenac), an H_2S-releasing moiety that include Lawesson's compound (GYY4137), an H_2S-releasing derivative of latanoprost, H_2S-releasing sartans, H_2S-sildenafil, and so on (41).

Therefore, administration of exogenous H_2S or the modulation of endogenous H_2S production may hold promise as a therapeutic intervention for the treatment of cardiovascular diseases.

Conclusion

H_2S is the third gasotransmitter, after NO and CO, and it is not surprising that it plays an important role in regulating cell functions, cardiovascular responses, and inflammatory/immune functions. We have summarized the current knowledge on the effects of H_2S in cardiovascular disease and discussed the possible molecular mechanisms it mediates in cardiovascular protection as well as its therapeutic potential for cardiovascular disorders. Although the molecular mechanisms underlying H_2S action in cardiovascular diseases have not been clearly elucidated, accumulating evidence from the past two decades has demonstrated that H_2S is an important player in protecting against cardiovascular disease. Therefore, insight into the molecular mechanisms underlying H_2S action in the cardiovascular system may promote the understanding of pathophysiology of these diseases. A better understanding of the biochemical function of the H_2S-producing enzyme CSE as well as its roles in cardiovascular disease may lead to new therapeutic targets based on modulation of H_2S production.

Author Disclosure Statement

No conflicts to disclose.

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Abbreviations Used

3-MST = 3-mercaptopyruvate sulfurtransferase
ACE = angiotensin-converting enzyme
BCA = β-cyanoalanine
CBS = cystathionine β-synthase
CO = carbon monoxide
CSE = cystathionine γ-lyase
GSH-Px = glutathione peroxidase
GYY4137 = morpholin-4-ium 4 methoxyphenyl (morpholino) phosphinodithioate
H_2S = hydrogen sulfide
ICAM-1 = intercellular adhesion molecule-1
K_{ATP} = ATP-sensitive potassium channel
LPS = lipopolysaccharide
NaHS = sodium hydrosulfide
NF-κB = nuclear factor-kappaB
NO = nitric oxide
Nrf-2 = nuclear-factor-E2-related factor-2
PAG = propargylglycine
ROS = reactive oxygen species
SAC = *S*-allylcysteine
SOD = superoxide dismutase
SPRC = *S*-propargyl-cysteine
TNF-α = tumor necrosis factor-alpha
VCAM-1 = vascular cell adhesion molecule-1
VEGF = vascular endothelial growth factor
VSMC = vascular smooth muscle cells