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Forum Review

The Gasotransmitter Role of Hydrogen Sulfide

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ABSTRACT

A novel concept of "gasotransmitter" arrived recently. Gasotransmitters are small molecules of endogenous gases with important physiological functions. Their production and metabolism are enzymatically regulated, and their effects are not dependent on specific membrane receptors. Following the identification of nitric oxide and carbon monoxide as gasotransmitters, hydrogen sulfide (H_2S) may be qualified as the third gasotransmitter. Recent studies have shown that H_2S is generated from vascular smooth muscle cells (SMCs), catalyzed by specific H_2S-generating enzyme. At physiologically relevant concentrations, H_2S relaxes vascular tissues, an effect mediated by the activation of ATP-sensitive K^+ (K_{ATP}) channels in vascular SMCs. H_2S directly alters the activity of K_{ATP} channels without the involvement of second messengers. Furthermore, the endogenous production of H_2S in the cardiovascular system is likely regulated by nitric oxide, whereas the vasorelaxant effect of nitric oxide is inhibited by H_2S. It is anticipated that future studies will better reveal the molecular mechanisms underlying the effect of H_2S on K_{ATP} channel proteins, the interaction of H_2S and other gasotransmitters in cardiovascular system, the endogenous stimulators and inhibitors of H_2S metabolism, the role of H_2S in the regulation of heart function, and the abnormal H_2S production and action under various pathophysiological conditions. *Antioxid. Redox Signal.* 5, 493–501.

INTRODUCTION

ABOUT 15 YEARS AGO, NITRIC OXIDE (NO) was known only as a toxic gas related to air pollution. Both NO and its oxidative derivative, nitrogen dioxide (NO_2), present a serious health hazard especially for people with circulatory and respiratory diseases. Similarly, carbon monoxide (CO) is among the most abundant of air pollutants in North America. As it is colorless and odorless, intoxication by CO is hard to detect, which earns CO a reputation of a "silent killer." The presence of hydrogen sulfide (H_2S) in our environment is easily recognizable for its peculiar rotten-egg smell (44, 57). The intoxication induced by H_2S is also very well known. Acute intoxication by H_2S leads to the loss of central respiratory drive (11, 12, 44, 57). It appears that all these gases are vicious and exert only detrimental influence on human health. This conventional thinking has gradually lost its ground. First to arrive is the evidence that NO is actually endogenously generated and has profound biological and physiological effects. Endogenous production of CO, on the other hand, has been known for a long time. But the reevaluation and realization of the physiological importance of CO to the homeostatic control of the human body has been achieved only in the last 10 years or so (52). Like NO and CO, H_2S at physiologically relevant levels affects the structures and functions of the human body at molecular, cellular, tissue, and system levels. The latest development in the identification of H_2S as an important *gasotransmitter* (53) will be the focus of this article. Among the topics to be covered are the following: the measurement of detectable levels of endogenous H_2S in the circulation and in specific tissues; the determination of genes that code for specific enzymes responsible for endogenous H_2S production; the elucidation of H_2S effects both *in vivo* and *in vitro* at physiologically relevant concentrations at different levels; and the identification of specific cellular and membrane targets of H_2S. Although the metabolism and function of H_2S in the cardiovascular system are emphasized, the basic action mechanisms of H_2S as well as progress and experimen-

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tal approaches described in this article will also help readers to understand better the biological and physiological roles of H_2S in other systems, such as the neuronal and endocrine systems.

GASOTRANSMITTER—THE CONCEPT

Vehicles for intercellular communication are either electrical signals via gap junction or chemicals. The conventional doctrine about chemical-mediated communication claims that the binding of neurotransmitters or humoral factors to receptors located on the plasma membrane is the essential triggering event. The ligand–receptor interaction generates intracellular second messengers that relay and direct the extracellular signals to different intracellular destinations, resulting in modulated cellular activity.

The Nobel Prize-winning discovery of NO in the 1990s sets the stage for recognizing a membrane receptor-independent signaling mechanism, emphasizing the necessity to modify the conventional doctrine about cellular signal transduction. The resurgence of CO and the discovery of H_2S as important endogenous signaling gases strengthen and endorse the physiological importance of this group of gas molecules. To distinguish NO, CO, and H_2S from classical neurotransmitters and humoral factors while acknowledging their common natures, these endogenous gaseous transmitters have been defined as "gasotransmitters," gauged by the following criteria (53): (i) They are small molecules of gas. (ii) They are freely permeable to membranes. As such, their effects do not rely on the cognate membrane receptors, and they can have endocrine, paracrine, and autocrine effects. (iii) They are endogenously and enzymatically generated and regulated. (iv) They have well defined and specific functions at physiologically relevant concentrations. (v) Their cellular effects may or may not be mediated by second messengers, but should have specific cellular and molecular targets. The gasotransmitter family may consist of many yet unknown endogenous gaseous molecules, such as ammonia and acetaldehyde.

THE ENDOGENOUS PRODUCTION OF H_2S IN CARDIOVASCULAR TISSUES

H_2S is endogenously generated via both nonenzymatic and enzymatic pathways (53). The enzymatic production of H_2S in mammalian tissues from L-cysteine is catalyzed by one of two pyridoxal-5′-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (5, 10, 15, 47, 53) (Fig. 1). Ammonium and pyruvate are the other two coproducts of this L-cysteine metabolism, but their involvements in physiological modulation of cellular functions are not clear yet. For more detailed discussions on H_2S metabolism, the readers are referred to another recent review article (53).

CSE is also named cysteine desulfhydrase (42). The CBS locus is mapped to chromosome 21 (21q22.3) (43). Several specific blockers for CBS and CSE are currently available. D,L-Propargylglycine and β-cyano-L-alanine selectively inhibit CSE (15, 45, 50, 63). L-Cysteine metabolites, including ammonia, H_2S, and pyruvate, cannot inhibit CSE activity (21). CBS activity can be specifically inhibited by aminooxyacetate and hydroxylamine (Fig. 1).

Determination of the circulating level and tissue level of H_2S under physiological conditions is essential for assigning this gasotransmitter a physiological role. It has been reported that the endogenous concentration of H_2S is 50–160 μ*M* in rat, human, and bovine brains (13, 40, 55). Mason *et al.* (26) reported that the normal blood level of H_2S in Wistar rats is ~10 μ*M*. We recently determined that the plasma level of H_2S in Sprague–Dawley rats is ~46 μ*M*. In human, 10–100 μ*M* H_2S in blood was reported (38). The tissue level of H_2S is known to be higher than its circulating level. The physiological concentration of H_2S in brain tissue is as high as 160 μ*M* (1). Significant amounts of H_2S are generated from vascular tissues, and this production varies among different types of vascular tissues. For instance, the homogenates of thoracic aortae yielded more H_2S than that of portal vein of rats (63). When the specific inhibitor of CSE, D,L-propargylglycine, was added to the reaction medium, H_2S production was completely abolished in all tested arteries (63). This observation indicates that the generation of H_2S from vascular tissues is due mainly to the specific catalytic activity of CSE.

The roles of gasotransmitters as physiological factors or toxic insults have been constantly debated. In this regard, H_2S is no exception. The first report of H_2S intoxication can be traced back ~300 years ago. The major lethal consequence of H_2S intoxication is the loss of central respiratory drive due to biochemical lesions of the respiratory centers of the brainstem (12). Many enzymes are inhibited by toxicological level of H_2S, including cytochrome oxidase (31, 36), carbonic anhydrase (25), and monoamine oxidase (54). Nevertheless, the recently proposed physiological effects of H_2S can be distinguished from the toxicological profile of this gas. The balanced cellular metabolism prevents intoxication of cells from the accumulation of the endogenously generated H_2S under physiological conditions. It has been shown that the oxidative phosphorylation was not disturbed by HS^- at concentrations higher than 30 μ*M*. This could result from rapid oxidation of H_2S in mitochondria (3, 31). On the other hand, the reported toxic level of H_2S is less than twofold greater than its endogenous level in rat brain tissues (55). In the case of obvious intoxication of mice with NaHS, sulfide concentrations in brain, liver, and kidney were increased by only 57%, 18%, and 64%, respectively (27). The proximity of physiological and toxicological levels of H_2S suggests two things. First, the dose-response relationship of H_2S at the physiological concentration range must be very steep before the physiological effect of H_2S sharply transforms to a highly toxic effect (63). Second, a delicate mechanism must exist in the human body to maintain the H_2S level within the physiological range.

IDENTIFICATION, DIFFERENTIAL EXPRESSION, AND MOLECULAR BASIS OF H_2S-GENERATING ENZYMES IN CARDIOVASCULAR TISSUES

The enzymatic activity of CBS has been demonstrated in rat liver, pancreas, and kidney; human brain and liver (2, 29);

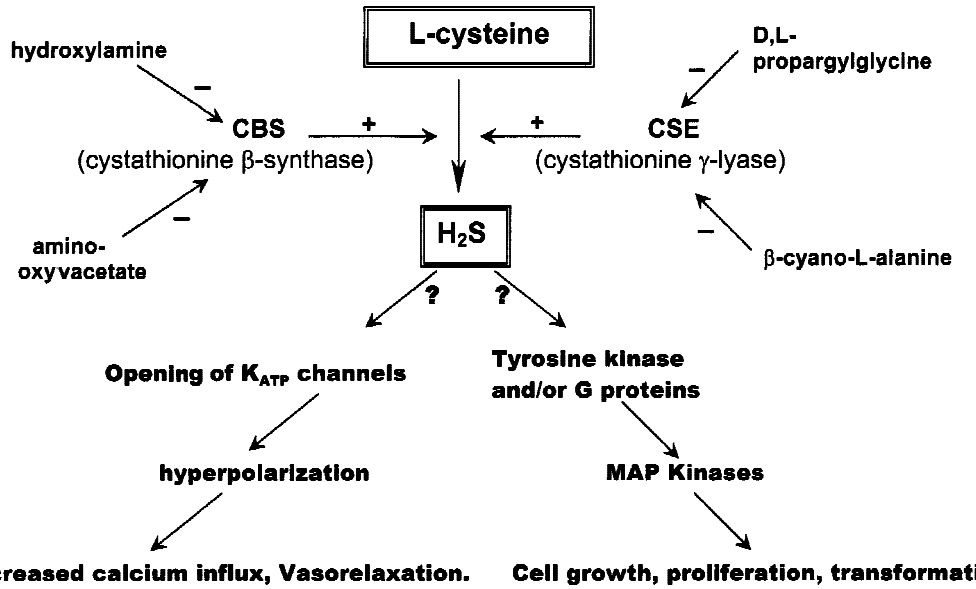

FIG. 1. The enzymatic production and putative physiological actions of endogenous H_2S. MAP, mitogen-activated protein.

and mouse pancreas, liver, kidney, and brain (2). No expression of CBS in human atrium and ventricle tissues could be detected using an enzyme assay and western blot analysis (7). Limited studies on human vascular tissues, including internal mammary arteries, saphenous veins, coronary arteries, and aortic arteries, also reported the absence of the activity and/or expression of CBS (2, 7). Wang *et al.* (51) reported the activity of CBS as reflected by the production of cystathionine in cultured human umbilical venous endothelial cells. These cells had been cultured for 14 days in Dulbecco's modified Eagle medium with the addition of 100 μ*M* L-homocysteine. Unfortunately, this study did not actually measure the protein or mRNA levels of CBS. Whether the detected CBS activity was induced by homocysteine in the culture medium is also not clear. Therefore, it was premature to conclude that CBS is expressed in these endothelial cells. The absence of CBS in rat vascular tissues has been recently reported (63). Taken together, the generation of H_2S from cardiovascular tissues seem to involve an enzymatic system excluding CBS.

A partial sequence of a CSE clone was originally obtained from a rat cDNA library (10). Northern blot hybridization revealed a higher abundance of mRNA of this clone in liver than in brain. Nishi *et al.* (32) later on reported the full sequence of CSE in rat tissues. CSE has a unique tissue distribution and is not detectable in brain and lungs (1, 42). The human CSE gene has been cloned, and its expression in *E. coli* yields high γ-lyase activity (46). Adult human liver tissues exhibit CSE activity, which is, however, absent in fetal, premature, and full-term neonatal liver tissue. This developmental stage-dependent CSE activity seems to be related to the age-dependent posttranscriptional regulation of this enzyme (22). Whether a similar age-dependent expression of CSE is present in cardiovascular tissues has not yet been determined. Animal experiments have identified the expression of CSE in portal vein, thoracic aorta (15), mesenteric artery, tail artery, and pulmonary arteries of rats (63). The transcriptional expression level of CSE has been quantitated, using RNase protection assay, among different vascular tissues (63). The intensity rank of expression levels of CSE mRNA is pulmonary artery > aorta > tail artery > mesenteric artery (63). Further studies demonstrated that CSE mRNA is solely distributed to the vascular smooth muscle layer, not the endothelial layer, of rat aortic wall, as detected by *in situ* hybridization study. This selective topological location of CSE mRNA was confirmed by RT-PCR study on purified cultured vascular smooth muscle cells (SMCs) and endothelial cells. The SMC localization of H_2S-generating enzyme is also quite different from that of NO- and CO-generating enzymes, which are located in both endothelial cells and SMCs. These studies, however, have not been able to correlate the mRNA levels of CSE to H_2S levels in different vascular tissues because the protein expression level of CSE has not been determined. This gap definitely needs to be filled up before a tissue-specific production of H_2S can be fully understood.

Zhao *et al.* (63) for the first time cloned CSE from rat mesenteric artery. They used a pair of primers covering the translation initiation and termination codons of CSE to obtain a cDNA clone of CSE. This clone contained an opening reading frame of 1,197 bp, encoding a 398-amino acid peptide. The full sequence of this clone (GenBank no. AB052882) was identical to the CSE clone derived from rat liver from the same laboratory (GenBank no. AY032875).

THE HYPOTENSIVE EFFECTS OF H_2S *IN VIVO*

H_2S affects cardiovascular function of the whole animal. Intravenous injection of H_2S provoked a transient, but significant, decrease in mean arterial blood pressure of anesthetized rats. It might be argued that this effect could result from the

inhibition of the respiratory system by H_2S, which led to a reflective change of heart beat. Interestingly, H_2S injection in these rats did not alter heart rate. Pretreatment of rats for 20 min with glibenclamide to specifically block ATP-sensitive K^+ (K_{ATP}) channels significantly antagonized the hypotensive effect of H_2S. On the other hand, pinacidil application mimicked the H_2S-induced transient decrease in blood pressure. These *in vivo* results indicated that the hypotensive effect of H_2S was likely provoked by the relaxation of resistance blood vessels through the opening of K_{ATP} channels. The short duration of the hypotensive effect of H_2S could be attributed to the scavenging of H_2S by metalloproteins, disulfide-containing proteins, thio-*S*-methyltransferase, and heme compounds. The administration of H_2S as a bolus injection also partially explains the transient effect (63).

THE MUSCULAR RELAXANT EFFECTS OF H_2S *IN VITRO*

The biological effects of H_2S have been studied using either H_2S gas-saturated solution or NaHS, a donor of H_2S. Teague *et al.* (49) reported the relaxant effects of NaHS on various muscular tissues. NaHS not only reduced the acetylcholine-precontracted or electrical field-stimulated guinea-pig ileum tissues, but also relaxed the spontaneously contracted rabbit ileum (EC_{50}, 76 μ*M*) and the electrical field-stimulated rat vas deferens (EC_{50}, 65 μ*M*) (49). Hayden *et al.* (14) showed that H_2S inhibited the oxytocin-induced contraction of rat uterine tissues. The spontaneous contraction of isolated pregnant rat uterine strips was also inhibited by NaHS (41).

H_2S induced a concentration-dependent relaxation of the phenylephrine-precontracted rat aortic tissues (15, 62). The vasorelaxation induced by H_2S was even greater when the vascular tissues were precontracted by low concentration of KCl (20 m*M*). For instance, the threshold concentrations of H_2S to initiate relaxation were 18 μ*M* and 60 μ*M* for 20 m*M* KCl- and phenylephrine-precontracted tissues, respectively. Controversial results have been obtained on the endothelium dependence of the vasorelaxant effects of H_2S. Hosoki *et al.* (15) noticed that the relaxation of rat aortic tissues induced by H_2S was not altered by the removal of endothelium. However, that report did not specify the concentration of H_2S used to study the endothelium-dependent vasorelaxation. Two studies from Wang's team claimed that the vasorelaxant effects of H_2S were endothelium-dependent, but this endothelium dependence was closely related to the concentrations. The removal of endothelium attenuated the relaxation of rat aortic tissues induced by H_2S at a single dose (180 μ*M*) (63), but the maximum relaxation induced by H_2S at concentrations equal to or greater than 1 m*M* was irrelevant to the presence of endothelium (63). The absence of an intact endothelium shifted the H_2S concentration-response curve to the right with IC_{50} changed from 136 μ*M* to 273 μ*M*. Taken together, the H_2S-induced vasorelaxation is partially facilitated by an endothelium-mediated mechanism, and the major target cell types in vascular wall of H_2S are vascular SMCs.

The endothelium-dependent effect of H_2S can be explained by several mechanisms. Absorbance and retention of H_2S by endothelium may prolong the vascular effect of H_2S by maintaining the local concentration of H_2S in the vascular wall. Release of endothelium-dependent vasoactive factors is another means by which H_2S exerts its influence on vascular tone. Zhao and Wang (62) showed that pretreatment of the endothelium-intact tissues with L-NAME (N^G-nitro-L-arginine methyl ester; 100 μ*M*) to block endogenous NO production from endothelium attenuated the concentration-dependent vasorelaxant effect of H_2S. Moreover, coapplication of charybdotoxin and apamin to the endothelium-intact tissues reduced the H_2S-induced vasorelaxation, suggesting the inhibited release or effect of endothelium-derived hyperpolarizing factor.

The involvement of various signal transduction pathways in the vascular effect of H_2S has been studied. Treatment of vascular tissues with indomethacin, or staurosporine, or SQ22536 did not change the effect of H_2S. Thus, the vasorelaxant effects of H_2S were unlikely mediated by prostaglandin, protein kinase C, or cyclic AMP pathways, respectively. The generation of superoxide anion or hydrogen peroxide was also unlikely responsible for the H_2S-induced vasorelaxation because the inclusion of superoxide dismutase and catalase failed to alter the effect of H_2S (62, 63).

Finally, the cardiac effect of H_2S has not been fully investigated. An early study showed that a low concentration of H_2S (0.1 mg/L air or 72 ppm) exposure changed electrocardiogram features of rabbit heart with flattened and inverted T waves (18).

H_2S IS AN ENDOGENOUS OPENER OF K_{ATP} CHANNELS IN VASCULAR SMCS

K_{ATP} channels are a heterooctamer assembly of four pore-forming subunits (Kir6.x) and four regulatory SUR subunits $(Kir6.x/SUR)_4$ (24). These channels are inhibited by intracellular ATP and extracellular sulfonylureas, but stimulated by K_{ATP} channel openers (KCOs) (35). Originally discovered in cardiac muscle (34), K_{ATP} channels were later identified in many other tissues, including pancreatic β-cells, skeletal muscle cells, and many types of vascular SMCs (8, 9, 30, 33, 39, 59, 61). The activation of K_{ATP} channels leads to membrane hyperpolarization and a relaxation of vascular SMCs. Kir6.1 and Kir6.2 belong to a class of inwardly rectifying K^+ channels with two membrane-spanning regions. SURs belong to the ATP-binding cassette superfamily. The binding sites for sulfonylureas and KCOs are on SURs. Different combinations of Kir6.x and SURs yield tissue-specific K_{ATP} channels with different electrophysiological and pharmacological features. The transcripts of Kir6.1, Kir6.2, SUR2B, and SUR1 have been detected in rat mesenteric artery SMCs; the corresponding genes have been cloned and their full sequences are available (6). It is possible that vascular SMCs possess multiple types of K_{ATP} channels constructed by Kir6.1 with either SUR1 or SUR2B being the regulatory subunit. This is because Kir6.1 confers the relative ATP insensitivity (not inhibited by ATP at concentrations lower than 1 m*M*), which is one of the fingerprints of K_{ATP} channels in vascular SMCs.

Among the known endogenous K_{ATP} channel modulators is endothelin, which inhibits K_{ATP} channels of vascular SMCs (28). NO hyperpolarizes SMCs from rabbit mesenteric arteries by activating K_{ATP} channels with cyclic GMP (cGMP) as the intermediate factor (30). However, another study found

that sodium nitroprusside (SNP), a NO donor, had no effect on K_{ATP} channel currents in porcine coronary artery SMCs (56). Atrial natriuretic factor also activated K_{ATP} channels in rat aortic SMCs via the stimulation of particulate guanylate cyclase (20). Calcitonin gene-related peptide activated K_{ATP} channels in vascular SMCs mediated by a cyclic AMP pathway (37, 48, 56). The effect of these endogenous substances appears to be mediated by either membrane receptor- or cellular metabolism-coupled mechanisms.

Studies from Wang's laboratory provided several lines of evidence, from vascular tissues and isolated single vascular SMCs, to substantiate the notion that H_2S may be the first identified endogenous substance that directly interacts with K_{ATP} channels.

High concentration of K^+ in extracellular solution alleviates the H_2S-induced vasorelaxation

The maximum vascular relaxation induced by H_2S was 91% or 19% when the tissues were precontracted with 20 or 100 m*M* KCl, respectively. This study indicates that the vasorelaxant effect of H_2S is mainly mediated by the increased K^+ conductance, which was largely nullified by a significantly reduced driving force for K^+ outflow with 100 m*M* in the bath solution (63). The inability of NaHS to relax the guinea-pig ileum tissues precontracted with 60 m*M* KCl also supports the importance of K^+ conductance in the relaxant effect of H_2S (49). Interestingly, NaHS did not alter the contractility of the guinea-pig ileum tissues in the presence of 10 m*M* KCl in the bath solution (49). Whether the 10 m*M* KCl-induced contraction of these ileum tissues could be reduced by another known vasorelaxant such as SNP was not further investigated. It is also worth noting that these experiments were carried out by preincubating tissues with NaHS before the KCl stimulation was given. Comparison of the effects of NaHS given before and during KCl stimulation may be intriguing. At any rate, Teague *et al.* (49) also observed that glibenclamide failed to alter the effect of NaHS, disproving the involvement of K_{ATP} channels in the relaxant effect of NaHS on the guinea-pig ileum tissues. Therefore, it is likely that H_2S may act on different cellular targets in different types of cells. Whether the direct application of H_2S gas to nonvascular smooth muscle tissues can generate the same effects through the same mechanisms as NaHS needs to be further investigated.

Selective K^+ channel blockers abolished the H_2S-induced vasorelaxation

Also using the isolated aortic ring preparations, Zhao *et al.* (63) demonstrated that 10 m*M* tetraethylammonium, but not charybdotoxin or iberiotoxin (two specific K_{Ca} channel inhibitors), abolished the H_2S-induced vasorelaxation. Furthermore, 4-aminopyridine did not alter the vascular effect of H_2S. These results show that K^+ channels are responsible for the vascular effects of H_2S, but the involved K^+ channels are not big-conductance K_{Ca} channels or 4-aminopyridine-sensitive Kv channels. Pharmacological studies revealed that the H_2S-induced vasorelaxation was concentration-dependently inhibited by glibenclamide. The H_2S effect was also mimicked by pinacidil, which relaxed the phenylephrine-precontracted vascular tissues in a concentration-dependent manner (63). An interaction between H_2S and K_{ATP} channels is thus indicated at the tissue level.

H_2S increased K_{ATP} channel currents and hyperpolarized membrane potential in single vascular SMCs

Direct evidence on the stimulation of K_{ATP} channels by H_2S was derived from the patch-clamp studies on single SMCs. The whole-cell K_{ATP} channel currents in rat aortic SMCs were significantly increased by H_2S. This effect of H_2S was significantly reduced by glibenclamide.

The opening of K_{ATP} channels would lead to membrane hyperpolarization, which in turn can close voltage-gated calcium channels. The first scenario has been unmasked. After SMCs were exposed to H_2S, cell membrane was hyperpolarized from a resting level of -36 to -53 mV. The hyperpolarization developed within 3 min of the application of H_2S, which was antagonized by the subsequently applied glibenclamide (63). Membrane hyperpolarization induced by H_2S had also been shown in dorsal raphe serotonergic neurons (17). The second scenario is also confirmed by a recent study (62). The application of nifedipine, a classic blocker of voltage-gated calcium channels, reduced the H_2S-induced relaxation of rat aortic tissues. Similarly, when a calcium-free bath solution was used, the H_2S-induced relaxation was reduced by 50%. It should be noted, however, that a direct inhibitory effect of H_2S on voltage-gated calcium channels in vascular SMCs as opposed to an indirect effect via the membrane hyperpolarization cannot be ruled out or ruled in yet.

It may be argued that the increase in K_{ATP} channel currents in the presence of H_2S resulted from the alteration of ATP metabolism by H_2S. However, the reversibility of the H_2S effect was not in favor of this argument. Moreover, in the aforementioned experiments, the cells were dialyzed with the pipette solution that contained a predetermined ATP concentration, *i.e.,* 0.5 m*M*. In another set of experiments, the concentrations of ATP in the pipette solution were intentionally altered. When ATP concentrations of the pipette solution were changed to 0.2 or 1 m*M*, H_2S still significantly increased K_{ATP} channel currents. Interestingly, the increases in K_{ATP} channel currents with different concentrations of ATP in the pipette solution did not show significant difference ($p > 0.05$). These results indicate that the activation of K_{ATP} channels by H_2S in rat aortic SMCs was not dependent on the ATP concentrations of the intracellular milieu. The actual mechanisms for the H_2S-enhanced K_{ATP} channel activity are still under investigation. Potentially, H_2S may induce the reduction of disulfide bonds of the K_{ATP} channel protein (54).

THE ACUTE AND CHRONIC INTERACTION OF H_2S AND NO

H_2S may provide a tonic influence on vascular tone under different conditions. It may also be acutely released upon the arrival of a specific stimulus. In either case, the production of H_2S needs to be regulated should this gasotransmitter fulfill important physiological functions. We for the first time showed that NO is an endogenous regulator for the production of H_2S in vascular tissues (63). The NO-stimulated H_2S

production takes place following two schemes. Acutely, NO increases CSE activity in vascular tissues. Incubating the homogenized rat vascular tissues with different concentrations of SNP, a NO donor, increased the accumulated H_2S production during a 90-min period. Putatively, NO increases the activity of cGMP-dependent protein kinases, which in turn stimulates CSE. This mechanism is supported by the finding that the blockade of cGMP-dependent protein kinase abolished the NO-induced increase in H_2S level in vascular tissues (unpublished observation). It is also possible that NO acts directly on CSE protein. Rat mesenteric artery CSE protein contains 12 cysteines that are the potential substrate of *S*-nitrosylation. Currently, the three-dimensional structure of CSE is unknown, and which cysteine contains a free –SH group cannot be assured yet. However, the nitrosylation of a certain free –SH group of CSE in the presence of NO does represent a possibility (45).

Chronically, NO up-regulates the expression of CSE (63). NO has been shown to regulate protein expression and synthesis, including growth factors, leukocyte adhesive proteins, and extracellular matrix proteins (16, 19, 60). In our study, incubating the cultured vascular SMCs with SNAP, another NO donor, for 6 h significantly increased the transcriptional level of CSE. The mechanism by which NO increased CSE transcription is not clear yet.

Although H_2S or NO alone relaxed vascular tissues, the integrated vascular effects of the two gasotransmitters are more complicated than a simple algebraic summation of individual actions. Hosoki *et al.* (15) observed that the vasorelaxant effect of SNP was enhanced by incubating rat aortic tissues with 30 μ*M* NaHS. In contrast, pretreating aortic tissues in another study with 60 μ*M* H_2S inhibited the vasorelaxant effect of SNP (62). This discrepancy may be partially explained by the experimental conditions of these studies. Hosoki *et al.* (15) used norepinephrine (1 μ*M*)-precontracted helical tissue strips of aorta from Wistar rats, and Zhao and Wang (62) used phenylephrine (0.3 μ*M*)-precontracted aortic rings from Sprague–Dawley rats. The tissue damage of helical strips is certainly greater than that of ring preparations. Moreover, the maximal contraction can be induced by 1 μ*M* norepinephrine, whereas 0.3 μ*M* phenylephrine (a submaximal concentration) only induced ~90% of the maximal contraction of rat aortic tissues. The advantage of using a submaximal concentration of phenylephrine is that the tissue can react with the relaxant agent in a more sensitive way. Should sulfide interact with a number of enzymes and other macromolecules, H_2S may inhibit cGMP accumulation. The inhibition of the cGMP pathway by H_2S *per se* may not suffice to alter the vascular tone, *i.e.,* to evoke contraction. It might exert an inhibitory influence, however, on the NO-induced vasodilation as the latter is mainly mediated by the cGMP pathway. An earlier study showed that the NO-induced relaxation of rabbit aorta and the increase in cGMP level were inhibited by L-cysteine and L-homocysteine (23). As both L-cysteine and L-homocysteine are endogenous precursors of H_2S and L-cysteine shares a relaxant effect similar to H_2S (41), this study also lends support to a possible inhibitory effect of H_2S on the vascular effect of NO. Another recent study reported that coapplication of NaHS and SNP produced a relaxation of the guinea-pig ileum much stronger than the summation of the relaxant effects of NaHS and SNP alone (49). The underlying mechanisms for this superadditive effect are not clear yet.

CONCLUSIONS

In summary, recent studies have provided convincing evidence that H_2S is a gasotransmitter. It is a small molecule of gas. It is freely permeable to membranes. It is endogenously generated by specific enzymes and its production is regulated. It has important physiological function in general and vasorelaxant effect in particular. K_{ATP} channels on plasma membrane may be the specific targets of H_2S in vascular SMCs (63) and, likely, in neurons (17).

H_2S is also a unique endogenous vasorelaxant factor as compared with other gasotransmitters. The vasorelaxation induced by H_2S comprises a minor endothelium-dependent effect and a major direct effect on smooth muscles, which differs from the effects of NO and CO, which act only on smooth muscles. While the activation of the cGMP pathway is an important mechanism for the NO- and CO-induced vasorelaxation, the H_2S-induced vasorelaxation is mediated mainly by the opening of K_{ATP} channels in vascular SMCs and partially through a K^+ conductance in endothelial cells. H_2S directly increases the activity of K_{ATP} channels without the involvement of second messengers. Similar direct interactions between NO and CO and K_{Ca} channels in vascular SMCs have been reported (58). Thus, different gasotransmitters use different mechanisms to regulate vascular tone.

PERSPECTIVES

Our understanding of the vascular effects of H_2S is relatively limited to conduit blood vessels. The cardiac effects of H_2S have not received due attention, especially in the context of ischemia/reperfusion heart injury, a process in which K_{ATP} channels play important roles. As reviewed in this article, the stimulation of K_{ATP} channels in vascular SMCs has been documented. We do not know yet whether H_2S will modulate the activity of K_{ATP} channels in other cell types, such as pancreatic β cells, skeletal muscle cells, and cardiomyocytes. The K_{ATP} channel complex is composed of different Kir6.x and SURx subunits. The interaction of H_2S with different K_{ATP} channel subunits and with the specific amino acid residues of a given K_{ATP} channel subunit is also intriguing. Future studies are also expected to reveal the vasorelaxant effect of H_2S on peripheral resistant arteries, such as mesenteric artery and kidney interlobal arteries, at physiologically relevant concentrations. Moreover, the chronic effects of this gasotransmitter on vascular tissue remodeling and/or apoptosis have not been explored. Potential interactions between mitogen-activated protein kinases and H_2S may lead to altered cell growth, proliferation, and transformations in a cell type-specific manner (Fig. 1), which merits intensive study in the future.

Abnormal metabolism of H_2S may have significant impact on the cardiovascular functions. It is known that, for example, in subjects with inherited abnormalities of the methionine metabolism, the homocysteine level is elevated, and coincidently, a reduced H_2S production is suspected. Therefore, arteriosclerotic vascular complications of these subjects at their child-

hood should not be simply explained by the homocysteine-induced endothelial cell injury and cell detachment (4). Lower H_2S may also affect the structure and function of vascular SMCs, thus joining homocysteine as compounding pathogenetic factors for arteriosclerotic cerebrovascular disease.

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ABBREVIATIONS

CBS, cystathionine β-synthase; cGMP, cyclic GMP; CO, carbon monoxide; CSE, cystathionine γ-lyase; H_2S, hydrogen sulfide; K_{ATP} channel, ATP-sensitive K^+ channel; KCO, K_{ATP} channel opener; NO, nitric oxide; SMC, smooth muscle cell; SNP, sodium nitroprusside.

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