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Invited review

Hydrogen sulfide: third gaseous transmitter, but with great pharmacological potential

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Abstract

Hydrogen sulfide (H_2S), which is well known traditionally as a toxic gas, has been proven to be produced endogenously by 3 enzymes in mammalian tissues and plays important roles in physiological and pathophysiological conditions. In the central nervous system, H_2S functions as not only a neuromodulator, but also a neuroprotectant against oxidative stress. In the cardiovascular system, H_2S relaxes vascular smooth muscles by the activation of K_{ATP} channels and inhibits smooth muscle cell proliferation via the mitogen-activated protein kinase signaling pathway. These effects are important for maintaining blood pressure and preventing vessel structural remodeling, and identifies H_2S as an important factor in the development of some vascular diseases, such as hypertension. H_2S also shows cardioprotective effects in ischemic myocardium and septic and endotoxin shock. Recent studies have demonstrated a new mechanism to explain the motor effect of H_2S on the rat detrusor muscle, which is through the activation of the capsaicin-sensitive primary neuron. This review focuses on the recent research achievements on H_2S and discloses the great potential of H_2S as the third gaseous transmitter in cardiac protection.

Introduction

For hundreds of years, hydrogen sulfide (H_2S) has been known solely as a toxic gas with the smell of rotten eggs. Indeed, H_2S is gradually considered to be a broad spectrum toxicant; its major toxic effects are the toxicity of the central nervous system (CNS) and the inhibition of the respiratory system[1,2]. Recent studies have shown that H_2S is not only a chemical hazard in certain industrial manufacturing, but it can also be produced endogenously in mammalian tissues from *L*-cysteine mainly by 3 enzymes: cystathionine β-synthetase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptosulfurtransferase[3–6]. The expressions of CBS and CSE have been detected in various tissues. H_2S research has been recently focused on its physiological effects and significance under disease conditions. Like nitric oxide (NO) and carbon monoxide (CO), which are considered 2 gaseous transmitters, H_2S has been shown to be the third gaseous transmitter and plays important roles, both in normal physiology conditions and in the process/progress of several diseases.

H_2S is a small molecule and can permeate membranes freely. The endogenous H_2S level is 0–46 μmol/L[7] in rat serum and 50–160 μmol/L in the brain[4]. One-third of H_2S remains undissociated in an aqueous solution and its solubility in lipophilic solvents is 5-fold greater than in water[8]. H_2S is mostly metabolized to sulfate and thiosulfate via the oxidation metabolism in mitochondria, and glutathione triggers the reaction. Very little of H_2S can be converted into lower toxic compounds of methylmercaptan and dimethyl sulfate via the methylation metabolism in cytosol. The metabolic product can exhaust from the kidney and intestinal tract and lungs within 24 h, so the endogenously-generated H_2S under physiological condition is hardly accumulated or toxic to cells due to the balanced cellular metabolism of the gas[9].

Although there are several excellent reviews on the pathophysiological effects of H_2S, such as those by Wang[8] in 2002, Tang *et al*[6] and Du *et al*[10] in 2006, the present study will summarize the latest progress on H_2S studies concerning the CNS, cardiovascular system, a possible novel mecha-

nism for its motor effect, as well as the role of H_2S in ischemia.

Role of H_2S in the CNS

Endogenous H_2S is generated mainly by CBS in the brain[11]. The transcriptional expression of CBS, but not CSE, in the rat brain (hippocampus, cerebellum, cerebral, and brainstem) was confirmed using the Northern blot assay; a similar conclusion was confirmed in CBS knockout mice. The production of H_2S by CBS in the brain is regulated by Ca^{2+} and calmodulin[12]. It is greatly enhanced by the activation of glutamate receptors and electrical stimulation which could cause influx of Ca^{2+}. There are 2 mechanisms through which CBS could produce H_2S. First, CBS could catalyze the production of H_2S from cysteine by a β-elimination or a α,β-elimination reaction; second, CBS can efficiently catalyze the formation of H_2S via the condensation of homocysteine with cysteine, and the latter is not affected by Ca^{2+} and calmodulin[13]. The regulation of CBS activity can affect brain H_2S formation. Eto *et al* found that sodium nitroprusside (SNP), well known as a NO donor, could enhance CBS activity by modifying 4 of 13 cysteine residues of CBS, but this effect is independent of NO production[14]. A human CBS complete genomic sequence has been determined[15] and the transcriptional start sites of 5 CBS mRNA isoforms, designated CBS-1a–1e, have been mapped[16]. The 5 CBS transcripts begin with a different exon. CBS gene transcription might be regulated by more than 1 promoter. Isoforms -1a and -1b form the vast majority of transcripts. Regulation on the transcriptional level is likely to be the mechanism of the tissue-specific manner of CBS expression, and in some senses, the expression of CBS in the brain could be modulated at the gene level under physiological and pathophysiological conditions.

The endogenous H_2S by CBS in the brain indicates it has physiological functions in the CNS. *N*-methyl-*D*-aspartate (NMDA) receptors may be one of its targets. The activation of NMDA receptors is required for the induction of hippocampal long-term potentiation (LTP)[17], a synaptic model of learning and memory. Because of the relatively high concentration of endogenous H_2S in the brain (50–160 μmol/L), the physiological concentration of H_2S facilitates the induction of LTP by enhancing NMDA receptor-induced currents[11]. This activation could be blocked by an adenylyl cyclase-specific inhibitor, indicating that the modulation of NMDA receptors by H_2S is induced by the enhancement of cAMP production[18]. This function ranked H_2S as a neuromodulator in the brain. Another study showed that H_2S could increase intracellular Ca^{2+} and induce Ca^{2+} waves in neighboring astrocytes[19]. Therefore, H_2S may mediate signals between neurons and glia and regulate synaptic activity by modulating the activity of both neurons and glia.

In addition to its role in signal transduction, H_2S can protect neuron cells from oxidative stress, not only by increasing the levels of antioxidant glutathione[20], but also by activating the K^+_{ATP} and Cl^- channels[21]. In human cultured neuron cells, H_2S could inhibit peroxynitrite ($ONOO^-$), which is an important mediator of human neurodegenerative disease, inducing tyrosine nitration, a1-antiproteinase inactivation, cell toxicity, intracellular protein oxidation, and protein nitration. This antioxidant action of H_2S suggests it functions as an endogenous $ONOO^-$ scavenger[22]. Oxidative stress is responsible for neuronal damage and degenerates in brain disorders. These observations suggest that H_2S may act as a neuroprotectant against oxidative stress.

The concentration of H_2S in the brain changes with CNS diseases. The levels of H_2S decreased by 55% in the brains of Alzheimer's disease (AD) patients and CBS activity was also dramatically decreased[23], but the level of AdoMet, a CBS activator, is low in AD brains. The CBS activity was reduced in another disease, homocystinuria[24]. Febrile seizure (FS) frequently occurs in children. Both gamma-aminobutyric acid (GABA) B receptor ($GABA_BR$) subunits and the H_2S/CBS system were involved in FS. H_2S functioned as a protective factor in the development of FS through regulating $GABA_BR$[25]. Intriguingly, the levels of CBS in Down's syndrome brains were approximately 3 times greater than those in normal individuals[26]. The role of H_2S in CNS diseases is not clear and needs to be explored in future. The progress of this field can provide novel therapy in clinical trials.

Role of H_2S in the cardiovascular system

Hosoki *et al* found that H_2S could be generated in the homogenates of the portal vein and thoracic aorta[4]. They also identified that CSE was the major enzyme to generate H_2S in these tissues by detecting the transcription of the mRNA of CSE with the Northern blot assay. The expression levels of CSE mRNA varied in different types of vascular tissues and was ranked as artery>aorta>tail artery>mesenteric artery[27]. A recent study has shown that in the heart, there is very few CBS, but plentiful CSE[28]. It seems that CBS does not play a major role in the cardiovascular system under physiological conditions. These observations suggested the potential physiological functions of H_2S/the CSE system in the cardiovascular system. The biosynthetic underlying mechanism of action of H_2S is summarized in Figure 1[3].

H_2S can be produced enzymatically in vascular tissues

and relaxes vascular smooth muscles both *in vivo* and *in vitro*[4,29]. This vasorelaxant effect is most probably caused by opening vascular smooth muscle cells (VSMC) K_{ATP} channels which leads to membrane hyperpolarization[27]. Therefore, H_2S may reduce extracellular Ca^{2+} entry and relax vascular tissues. The vasorelaxation induced by H_2S can be attenuated by the removal of the endothelium, since H_2S may facilitate the release of vasorelaxant factors from the endothelium, including NO and the endothelium-derived relaxing factor. As opposed to NO and CO, H_2S-induced vasorelaxation is not mediated by the cGMP signaling pathway. This indicates that H_2S is a novel endogenous gaseous modulator of vascular contractility. At the same time, similar to NO and CO, H_2S could inhibit VSMC proliferation and induce apoptosis *in vitro*[30,31]. Using cultured VSMC, exogenous H_2S could dose-dependently suppress the proliferation of VSMC through the mitogen-activated protein kinase (MAPK) signaling pathway. Studies using molecular means to overexpress CSE in cultured VSMC found that endogenous H_2S could also attenuate the rate of cell proliferation and increase the rate of cell apoptosis. The effect is via the activation of MAPK and caspase-3. The possible signaling pathway is shown in Figure 2. So H_2S is not only a vasorelaxant, but also an important regulator of cell growth and may thereby attenuate the structural remodeling of vessel tissues. This can help us understand the mechanism of some vascular diseases and provide links to new therapeutic methods.

The pathophysiological role of H_2S in some cardiovascular diseases has been explored. The endogenous H_2S/CSE pathway participated in the pathophysiological process in vascular diseases, such as spontaneous hypertension[32], hypoxia-induced pulmonary hypertension (HPH)[33–35], and high pulmonary blood flow-induced pulmonary hypertension[36,37]. Hypertension is one of the most common cardiovascular diseases and its mechanism is not fully understood

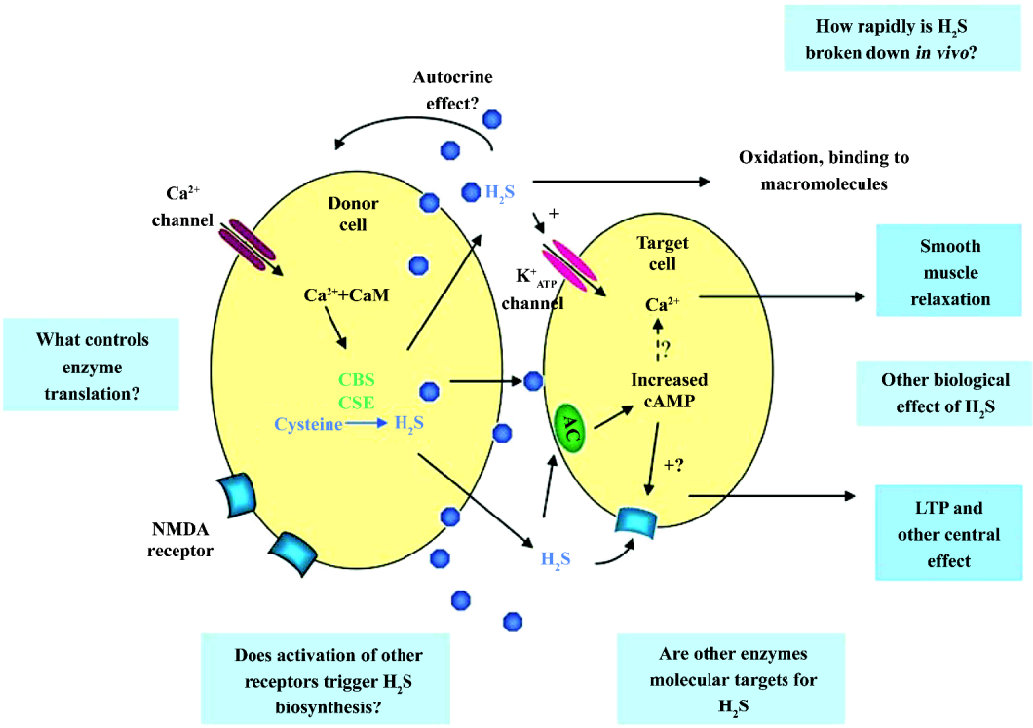

Figure 1. Biosynthetic mechanism of action and principal biological effects of H_2S. Cystathionine γ-lyase (CSE) or cystathionine β-synthetase (CBS) catalyze the production of H_2S from cysteine in the donor cell. An influx of Ca^{2+}, perhaps triggered by the activation of NMDA receptors by glutamate or via separate channels, binds to calmodulin (CaM), thereby activating CBS. Factors that activate CSE have yet to be determined. H_2S is released, presumably by free diffusion, to act on the target cell to increase adenylyl cyclase (AC) activity, thereby raising the intracellular concentration of cAMP, and/or activating K_{ATP} channels directly to cause hyperpolarization (neurons) or smooth muscle relaxation. It should be noted that whether H_2S acts on a separate target cell or on the cell from which it is released (ie an autocrine effect) is not yet clear. H_2S is broken down either chemically or by sequestration with macromolecules, such as hemoglobin or glutathione. Major biological effects of released H_2S include smooth muscle relaxation and the promotion of neuronal long-term potentiation (LTP). Challenging questions that remain to be answered are indicated in green boxes[1].

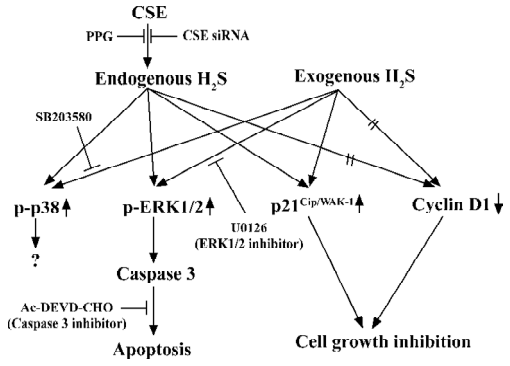

Figure 2. Schematic signal transduction pathways underlying the H_2S-induced cell growth changes. "p" indicates the phosphorylated form.

yet. Vasoconstriction and structural remodeling by VSMC proliferation are essential processes in hypertension development. In the spontaneous hypertension rat model, the plasma level of H_2S is low and the activity of CSE is suppressed. The exogenous administration of H_2S not only attenuated the elevation of blood pressure and vessel remodeling, but also recovered the H_2S level and CSE activity[32]. Guang *et al*'s study found that there was dysfunction of the H_2S/CSE system in *L*-NAME-induced hypertension rats and that exogenous H_2S could effectively prevent the development of hypertension[38]. The exogenous H_2S-induced positive feedback on CSE activity is different from its negative feedback in physiological conditions[39]. A recent study reported that the exogenous administration of H_2S downregulated osteopontin gene expression and ameliorated vascular calcification (which is a common finding in many diseases, such as hypertension, atherosclerosis, diabetes, chronic renal failure, aging, and arterial stenosis)[40]. As it is known, the baroreflex is the major method of blood pressure modulation. Exogenous H_2S could facilitate carotid sinus baroreflex (CSB) by opening K_{ATP} channels and further closing the calcium channels in vascular smooth muscle which suggests that endogenous H_2S might activate the activity of the CSB *in vivo*[41]. In HPH pathophysiological processes, the similar dysfunction of H_2S/CSE was found, and exogenous H_2S could inhibit the proliferative cell nuclear antigen (PCNA) and U-II expressions in the pulmonary wall to depress the proliferation of pulmonary artery smooth muscle cells and reduce the expression of collagen I and III, elastin, and TGF-β3 to decrease the hypoxic pulmonary vascular structural remodeling[34,35]. Olson *et al*'s recent study on the mechanical and electrical responses of select blood vessels to hypoxia and H_2S suggested that H_2S served as an O_2 sensor/transducer in the vascular responses to hypoxia. The inhibition of H_2S synthesis inhibited the hypoxic response of vertebrate blood vessels and the concentration of H_2S in the vessel was regulated by the balance between endogenous H_2S production and its oxidation by available O_2[42]. The exogenous supply of H_2S could alleviate the elevation of pulmonary arterial pressure. At the same time, exogenous supply of propargylglycine (PPG, inhibitor of CSE), plasma CO level, and the expressions of the HO-1 protein and mRNA in pulmonary arteries decreased. The results showed that H_2S could play a regulatory role in the pathogenesis of HPH through the upregulation of the CO/HO pathway[43]. However, in aortic smooth muscle cells, Jin *et al*'s study proved that endogenous CO/HO and the H_2S/CSE pathways downregulated each other under physiological conditions[44]. Exogenous H_2S also ameliorated pulmonary vascular structural remodeling induced by high pulmonary blood flow, downregulated PCNA expression and the ERK/MAPK signal pathway, inhibited the NO/NO synthase pathway, and enhanced the CO/HO pathway in rats with high pulmonary blood flow[36,37]. These studies suggested that endogenous H_2S was one of the key factors in hypertension development and that the deficit of the H_2S/CSE system was one of the major causes of hypertension.

Some studies have proven that the concentration of arterial endogenous H_2S was significantly increased in both septic and endotoxic shock rats, which suggested that endogenous H_2S was still involved in physiological and pathophysiological processes during shock[45].

Human cystathioninuria, which is characterized by high plasma homocysteine and cystathionine, is concerned with a wide range of disease associations, such as cardiovascular injury. Its genomic basis has been shown to be 2 nonsense mutations and 2 sense mutations in CSE[46].

It has been reported that H_2S also activates the K_{ATP} channel in mitochondria and sarcolemmal K_{ATP} channels in cardiac myocytes and has potent cardioprotective effects[47–49]. The cardioprotection of H_2S was also demonstrated in rat isolated ventricular myocytes[50]. NaHS could concentration-dependently increase the cell viability and the percentage of rod-shaped cells, which were exposed to severe metabolic inhibition solution. NaHS-induced cardioprotection following metabolic inhibition preconditioning could be blocked by PPG and HMR-1098 (a sarcolemmal K_{ATP} blocker). Pretreatment with *L*-NAME to block endogenous NO production could also attenuate the cardioprotective effect of NaHS. These results indicate that H_2S may protect the heart most probably by activating sarcolemmal K_{ATP}, which is different from its vasorelaxant effect. NO also plays an important role in cardioprotection. Further, the cardioprotective

effect has similar characteristics to the time-course of ischemic preconditioning; it suggests the possible protective role of H_2S in ischemic myocardium. This has been proven by a recent study[51]. In a rat model of myocardial infarction (MI), NaHS treatment could decrease the mortality rate of MI rats and diminish infarct size as shown in Figure 3. The vessel dilating/relaxing effects of NaHS may dilate coronary arteries and increase coronary blood flow in ischemic diseases, thus reducing cellular damage from ischemia. This heart protective effect could be abolished by the administration of PPG. The results were further confirmed by our *in vitro* hypoxic model (Figure 4). It has been suggested that endogenous H_2S might provide a novel approach to the treatment of MI. Further work needs to be performed to explore whether or not the mechanism of this effect concerns the K_{ATP} channel. H_2S also showed a cardioprotective effect in another isoproterenol injection-induced myocardial ischemic injury model in which the plasma H_2S concentration and CSE activity decreased. The administration of NaHS could effectively protect myocytes and contractile activity[52].

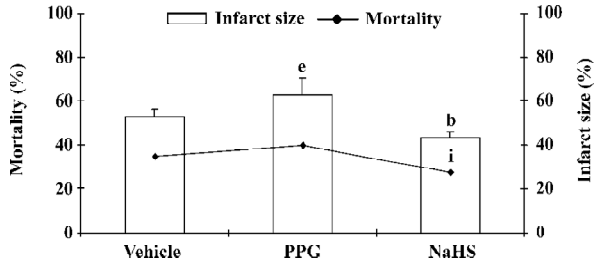

Figure 3. Mortality and myocardial infarct size changes in vehicle, PPG, and NaHS pretreatment groups. $^bP<0.05$ infarct size in NaHS group *vs* vehicle and PPG-treated groups; $^eP<0.05$ infarct size in PPG group *vs* vehicle and NaHS-treated groups. $^iP<0.05$ mortality in NaHS group *vs* vehicle and PPG-treated groups. Figure modified with permission from Zhu *et al*[51].

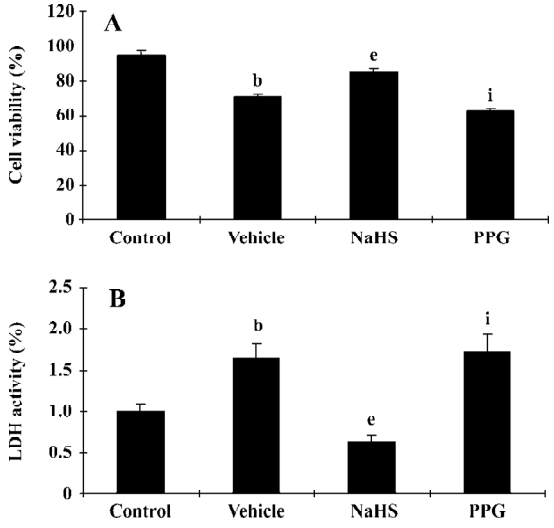

Figure 4. (A) VSMC viability measurement in control (non-hypoxia) and hypoxic vehicle, and NaHS and PPG pretreatment groups. (B) relative LDH liberation in control (non-hypoxia) and hypoxic vehicle, and NaHS, and PPG pretreatment groups. ($^bP<0.05$ hypoxic vehicle *vs* control; $^eP<0.05$ NaHS-treated *vs* vehicle; $^iP<0.05$ PPG *vs* NaHS-treated). Figure modified with permission from Zhu *et al*[51].

Excitatory motor effect of H_2S

In contrast to the vasorelaxant effect, NaHS produced concentration-dependent contractile responses in the detrusor muscle of the rat urinary bladder[53]. This response exhibited rapid and persistent tachyphylaxis similar to the responses of capsaicin[54,55]. The response was abolished by high-capsaicin pretreatment which could desensitize capsaicin-sensitive primary afferent neurons or the pretreatment of tissues with a combination of tachykinin natural killer $(NK)_1$ and NK_2 receptor-selective antagonists. At the same time, the response to NaHS is mostly resistant to tetrodotoxin, as is the effect of capsaicin in this organ[56]. These results show pharmacological evidence that H_2S stimulates capsaicin-sensitive primary afferent nerve terminals with the consequent release of tachykinins, which in turn produces contractile responses of the detrusor muscle. In further studies, the same researchers demonstrated that the transient receptor potential vanilloid receptor 1 (TRPV1, also called the capsaicin receptor) selective antagonist capsazepine and SB366791 could not affect the H_2S contractile activity[53]. However, the unselective cation channel blocker, ruthenium red, almost abolished the contraction similar to its effect on capsaicin, which provided 2 hypotheses: first, H_2S stimulates the TRPV1 receptor by a different way from those known activators; second, H_2S might stimulate other receptors present on the terminals of capsaicin-sensitive sensory neurons.

TRPV1 was cloned from rat sensory neurons[57] in 1997. TRPV1 is non-selective cation channel with high permeability of Ca^{2+} and could be activated by chemical and physical stimuli, such as capsaicin, low pH[58], noxious heat, anandamide[59], 12-hydroperoxyeicosatetraenoic acid[60], and *N*-arachidonoyl-dopamine[61]. The influx of Na^+ causes primary sensory neuron depolarization and the initiation of action potentials. In particular, the influx of Ca^{2+} resulted in the local release of neuropeptides, including the calcitonin gene-related peptide and the tachykinins, substance P, and neurokinin A. TRPV1-positive neurons are not only afferent

neurons which are involved in the perception of somatic and visceral pain, but also have a sensory effector function. These neuropeptides act on different effector cells and cause different responses, including neurogenic inflammation, thermal hyperalgesia, airway constriction, and vasodilatation[62]. TRPV1 is highly expressed in primary sensory neurons of the trigeminal, vagal, and dorsal root ganglion with C- and A-δ fibers, which are called nociceptive neuron. Studies also show that TRPV1 is expressed in non-neuronal cells, including epithelial cells of the urothelium[63], keratinocytes[64], and skeletal muscles[65]. These features of TRPV1 indicate its broad physiological and pathophysiological functions.

In addition to the detrusor muscle, NaHS increases sensory neuropeptide release in the guinea pig airways and causes *in vivo* bronchoconstriction and microvascular leakage in a capsazepine-sensitive manner[66]. This novel mechanism may contribute to the irritant action of H_2S in the respiratory system, possibly through TRPV1 activation. Further research is still required in order to prove whether or not H_2S acts as a endogenous ligand of TRPV1. It will be interesting to detect the odorous activator of TRPV1 and its undergoing mechanism. Therefore, previous studies on the physiological and pathophysiological roles of H_2S need re-evaluation based on this mechanism.

Concluding remarks

H_2S has been shown to be an important biological molecule in the last 2 decades. In addition to its neuromodulator and cardiovascular protection effects, studies also show that H_2S has various effects in mammalian tissues, such as the relaxation effect of the ileum, which indicates that endogenous H_2S could regulate alimentary contractile functions[67]. H_2S has been described as an endogenous mediator with diverse biological effects in a study, including playing an important role in endotoxin-induced inflammation[68]. The studies of the physiological functions of H_2S and its underlying mechanism, the regulation of H_2S concentration and activity, and/or the expression of CBS and/or CSE and its interaction with some diseases may have a significant impact in our understanding of the pathogenesis of these diseases, as well as having far-reaching clinical and therapeutic implications. Further research needs to be undertaken to find new therapeutic methods and ruling out possible side-effects.

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