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Review

Hydrogen sulfide and translational medicine

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Hydrogen sulfide (H₂S) along with carbon monoxide and nitric oxide is an important signaling molecule that has undergone large numbers of fundamental investigations. H₂S is involved in various physiological activities associated with the regulation of homeostasis, vascular contractility, pro- and anti-inflammatory activities, as well as pro- and anti-apoptotic activities etc. However, the actions of H₂S are influenced by its concentration, reaction time, and cell/disease types. Therefore, H₂S is a signaling molecule without definite effect. The use of existing H₂S donors is limited because of the instant release and short lifetime of H₂S. Thus, translational medicine involving the sustained and controlled release of H₂S is of great value for both scientific and clinical uses. H₂S donation can be manipulated by different ways, including where H₂S is given, how H₂S is donated, or the specific structures of H₂S donors. This review briefly summarizes recent progress in research on the physiological and pathological functions of H₂S and H₂S-releasing drugs, and suggests hope for future investigations.

Keywords: hydrogen sulfide; K_ATP channel; calcium channel; TRPA1; NF-κB; cytochrome oxidase; antioxidant; H₂S donor; H₂S-releasing drug; translational medicine

Introduction

Hydrogen sulfide (H₂S) is a colorless, gaseous molecule at room temperature. It is widely known and disliked for its pungent, rotten egg-like smell. H₂S is very water-soluble and lipophilic, resulting in quick and convenient transport between cells and tissues[1]. It is also strongly toxic, especially to the central nervous system. In fact, H₂S acts as a more potent inhibitor of mitochondrial respiration than cyanide[2]. The maximum concentration in the air is approximately 10 mg/m³, and high concentrations of H₂S may cause an instant loss of olfactory sensation or fainting. However, H₂S, resembling CO and NO, is converted from a noxious molecule to a signaling molecule that may have promise in the medical and pharmaceutical fields.

Endogenous H₂S is produced from L-cysteine by both CSE and CBS, with the cofactor cysteine amino transferase, through a so-called “trans-sulfuration pathway”[3-5]. Both CSE and CBS enzymes are pyridoxal-5′-phosphate-dependent with different concentrations in various tissues. It was previously reported that CBS is predominantly located in the central nervous system (hippocampus, cerebellum, cerebral cortex, and brain stem)[6], while CSE emerges more in the cardiovascular system (aorta, mesenteric artery, portal vein, and other vascular tissue)[7-8]. An amount of CBS or CSE is tissue-specific, and coincidence and sole incidence are both allowed. A recent investigation also suggests the presence of another enzyme besides CBS and CSE that can generate endogenous H₂S: 3-MST, which generates H₂S from cysteine with the help of α-ketoglutarate in the brain[9]. 3-MST is located in the liver, kidney, heart, lung, thymus, testis, thoracic aorta, and brain. Three pathways of H₂S degradation exist: (1) desulfurization to thiosulfate by mitochondrial oxidation, then to sulfite or sulfate; (2) cytosolic methylation to dimethylsulfide; and (3) sulfhemoglobin formation by the binding to hemoglobin[9, 10]. A previous investigation shows that after H₂S is synthesized, it is quickly absorbed or stored as a form of bound or acid-labile sulfur, while free H₂S is maintained at a baseline level[11]. Bound sulfur is stored intracellularly in neurons and astrocytes of rats and releases H₂S in the presence of physiologic concentrations of the endogenous reducing substances glutathione and cysteine. Acid-labile sulfur is another form of stored H₂S. It localizes in the mitochondria and the release of H₂S occurs at a pH below 5.4.

Methods to study H₂S conventionally included three aspects. First, changes in substances involved in the down-regulation of the H₂S concentration, such as CBS and CSE antagonists and inhibitors of potent targets are observed. Second, symptoms are compared between a range of H₂S concentrations.
by enhancing the dosage of H$_2$S, mainly through exogenous H$_2$S donors, such as NaHS or other H$_2$S-releasing compounds. Third, the variance in H$_2$S concentration in certain diseases is measured, and the function of this variability is determined. The effort put into studying the pathological and physiological functions of H$_2$S has allowed for a general understanding of its behavior in the body, including its synthesis, metabolism, potent targets, and effects. The large amount of evidence gathered through various experiments and observations has shown that the role of H$_2$S as a vital endogenous gasotransmitter is ubiquitous throughout the human body. The use of H$_2$S in the central nervous system, cardiovascular system, and gastrointestinal system is promising, and the investigation of its role is important because cardiovascular and cerebrovascular diseases as well as gastrointestinal cancers are among the top causes of human mortality. Hence, a breakthrough in dealing with diseases that severely affect our health in hope of longevity to occur is necessary. Furthermore, H$_2$S-associated drugs with effectiveness similar to that of nitroglycerin in cases of angina pectoris may be developed in the future.

Recent evidence for the therapeutic application of H$_2$S
Since 1996, when Ade and Kimura began their investigation of H$_2$S, the role of H$_2$S has been gradually revealed by various contributions from scientists worldwide[3]. Recent discoveries regarding H$_2$S demonstrate its critical importance, much like NO and CO, to the medical field. This small, gaseous molecule appears to be both powerful and ubiquitous.

In the central nervous system, H$_2$S ameliorates ischemic injuries but leads to the aggravation of stroke. Enhancement of the H$_2$S concentration in the brain induces brain infarct, while CBS or CSE inhibitors can reverse this effect. The role of H$_2$S in neuron protection has been shown in glutamate-induced death with increasing concentrations of cysteine and γ-glutamylcysteine, which causes enhanced GSH concentrations[11–14]. In patients with AD, localized increases in H$_2$S resulted in a delay of aggravation and exacerbation of symptoms[15, 16]. In addition, patients with Down syndrome overproduce H$_2$S because their urinary excretion of thiosulfate, a specific H$_2$S end product, was increased, suggesting a positive relationship between H$_2$S concentration and the aggravation of Down syndrome[17–18].

In the cardiovascular system, H$_2$S is related to hypertension, atherosclerosis, and myocardial injury. Its utility for treating hypertension may correspond to its vasodilatory effect. H$_2$S has the ability to relax the rat thoracic aorta, portal vein, and mesenteric artery, which suggests a more fundamental role in the regulation of contractility and blood pressure[19–23]. Conversely, further study has suggested that H$_2$S is a vasoconstrictor at low concentrations with a possible mechanism for suppressing NO, which is also involved in contractility. As plaque is destabilized and aortic smooth muscle cell proliferation is possibly reduced by H$_2$S, atherosclerosis is also palliated owing to an enhanced H$_2$S concentration[24, 25]. Similarly, H$_2$S is able to help patients recover from myocardial injury, particularly ischemia-reperfusion injury[26–31]. In summary, many cardiovascular diseases demonstrated a relationship with H$_2$S modulation, prognosticating a comprehensive utility of H$_2$S in heart diseases.

In the gastrointestinal system, H$_2$S remains important in the regulation of local homeostasis. H$_2$S-releasing bacteria in the intestine are a major source of endogenous H$_2$S. Donation of H$_2$S contributes to chloride secretion, which aggravates certain types of gastritis[30]. Gastrointestinal contractility shows sensitivity to H$_2$S, which affects the gastrointestinal smooth muscle cells as well as the neurons in the enteric nervous system[15, 35, 36]. The concentration of H$_2$S is enhanced when abdominal sepsis or endotoxemia occurs, and administration of H$_2$S leads to exacerbation of these conditions, mainly because of its pro-inflammatory effect[36, 37]. Apart from these negative effects, H$_2$S has been shown to have a protective anti-inflammatory effect on the gastrointestinal system in some types of gastritis and colitis[38–40]. An obvious contradiction, thus, emerges, leading to puzzling study results in terms of the actual pro- or anti-inflammatory effects of H$_2$S. This conflict implies the presence of fundamental unknown mechanisms in addition to those that have been observed. The observed results may be derived from a balance of multiple mechanisms because a balanced result always changes with circumstances.

Furthermore, insulin secretion and diabetes mellitus can be affected by local H$_2$S concentrations because the pancreas is among the targets of H$_2$S[41–44]. In the pancreases, CSE is the main enzyme converting cysteine to H$_2$S. It has also been reported that the concentration of H$_2$S rose in response to the presence of pancreatitis, which is ascribed to its pro-inflammatory effect. Therefore, it is clear that H$_2$S-releasing drugs may make a difference in pancreatitis or diabetes. However, the functions of H$_2$S remain ambiguous and demand further investigation to elucidate its anfractuous signaling network. Current studies suggest that the effects of H$_2$S are often influenced by its concentration and reaction time as well as the cell and disease type it is acting upon. Hence, the application of H$_2$S in certain dysfunctions is bound to be difficult considering its potentially low specificity for certain organs and tissues (see Figure 1). On the other hand, various uses of H$_2$S may be possible because of its power.

New therapeutic targets associated with H$_2$S
The mechanisms and pathways of H$_2$S signaling are not yet completely understood. The regulation of H$_2$S is so complicated that new mechanisms are continuously discovered before the former mechanisms are fully understood. Fortunately, several fundamental mechanisms have been determined and have drawn much attention, and we have gradually begun to fully understand them (shown in Figure 2).
Activation of K\textsubscript{ATP} channels

A hallmark role of H\textsubscript{2}S is the regulation of contractility, which is the most fundamental and valuable use of H\textsubscript{2}S. The mechanism directing this effect is a major research topic. Vasodilation has been shown to be caused by the activation of the K\textsubscript{ATP} channel\textsuperscript{[8, 26, 45]}. Evidence for this hypothesis comes from the observation that the vasodilatory effect of H\textsubscript{2}S is attenuated by K\textsubscript{ATP} channel antagonists, such as glibenclamide, while the K\textsubscript{ATP}-dependent current is increased by H\textsubscript{2}S, as shown by patch-clamp technology. In another study, the non-selective K\textsubscript{ATP} channel antagonist glibenclamide, the selective K\textsubscript{ATP} channel antagonist HMR-1098, and the selective mitochondrial K\textsubscript{ATP} channel antagonist 5-HD were compared to show the effect of H\textsubscript{2}S on the K\textsubscript{ATP} channel\textsuperscript{[46–48]}. The results of this study suggest that H\textsubscript{2}S selectively activates the plasma-membrane K\textsubscript{ATP} channel (only 5-HD treatment lacked mediation of neuroprotection during hypoxia). In addition to having a vasodilatory effect, the activation of the K\textsubscript{ATP} channel preconditions the body against ischemia-reperfusion injury and promotes myocardial protection, which together provide a theoretical and experimental basis for the application of H\textsubscript{2}S in heart disease\textsuperscript{[26, 27, 45]}.

Activation of T-type calcium channels

Research has revealed that H\textsubscript{2}S is involved in visceral pain-like nociception and somatic hyperalgesia in mice. This observation led to further investigation of the role of indirect activation of T-type calcium channels in facilitation of visceral nociception\textsuperscript{[49–52]}. Because Zn\textsuperscript{2+} acts as an inhibitor of T-type calcium channels, especially the Cav\textsubscript{3.2} isoform, H\textsubscript{2}S, which chelates Zn\textsuperscript{2+}, is reasonably regarded to indirectly activate T-type calcium channels by inhibiting Zn\textsuperscript{2+}\textsuperscript{[53]}. This hypothesis is further proven by the observation that Zn\textsuperscript{2+} chelates similarly to dipicolinic acid, showing the aggravation of colonic pain as well as hyperalgesia.

Activation of TRPA1

The TRP superfamily is comprised of nonselective cation channels that are divided into six main subfamilies: TRPC, TRPV, TRPM, TRPP, TRPML, and TRPA\textsuperscript{[54]}. Among them, both TRPV and TRPA are important targets of pathological research. It is
well known that TRPV1 in the bladder wall produces detrusor overactivity. TRPA1 is present on unmyelinated sensory nerve fibers within the urothelium, suburothelial space, and muscle layer as well as around blood vessels throughout the bladder. Because TRPA1 coexists with TRPV1 in major loci, such as sensory neurons in rodent dorsal root and trigeminal ganglia, and because intracellular TRPA1 activators including allyl isothiocyanate and cinnamaldehyde also initiate detrusor overactivity resembling that caused by TRPV1, it is believed that TRPA1 is involved in sensory transduction\(^{[59]}\). A recent investigation demonstrated that H\(_2\)S activated TRPA1 in the presence of inflammation by the induction of calcium responses in TRPA1-expressing CHO cells. Moreover, this result is suppressed by ruthenium red, which is a non-selective TRP antagonist, and HC-030031, which is a selective TRPA1 antagonist. This reaction is not observed with the selective TRPV1 antagonist idoxaresiniferatoxin. These findings reveal the presence of H\(_2\)S-specific activation of TRPA1 channels\(^{[56]}\). Further investigation of H\(_2\)S-specific TRPA1 channel activation may provide new solutions to various bladder disorders caused by hyperreflexia, and H\(_2\)S may affect nociception, which results from complicated functions of TRPA1, upon activation via the elicitation of pain, protective reflexes, and local release of neurotransmitters in the periphery.

**The NF-κB signaling pathway**

H\(_2\)S-induced inflammation has a relationship with the NF-κB signaling pathway. This pathway is involved in cecal ligation and puncture-induced sepsis, in which H\(_2\)S regulates the expression of cytokines, chemokines, and adhesion molecules\(^{[57]}\). There are data indicating that the inhibition of SFKs causes the down-regulation of H\(_2\)S-induced NF-κB activity and ICAM-1 expression, suggesting that H\(_2\)S is involved in regulating the activity of NF-κB by activating the phosphorylation of SFKs, eventually resulting in mediation of ICAM-1 expression\(^{[58]}\). This pathway may also be an answer to H\(_2\)S-induced anti-apoptosis. Mouse embryonic fibroblasts lacking p65, which is a subunit of NF-κB, are sensitive to TNF-α-induced cell apoptosis, indicating that NF-κB is a target molecule involved in apoptosis. Further research has elucidated a possible mechanism of enhanced transcription of CSE, which generates H\(_2\)S, during TNF-α-induced apoptosis\(^{[6]}\). H\(_2\)S or sulfhydrates then generates the p65 subunit of NF-κB at cysteine-38 and binds to the coactivator ribosomal protein, S3. Finally, the promotion of anti-apoptotic genes occurs, including the cellular inhibitors of apoptosis, caspase-8-c-FLIP, A1, TRAF1, and TRAF2\(^{[59]}\).

**Cytochrome oxidase**

H\(_2\)S has long been regarded as a strong toxin that inhibits mitochondrial respiration by combining the cytochrome c oxidase copper and/or heme iron site, resembling hydrogen cyanide, a well-known lethal toxin\(^{[2]}\). Studies suggest that H\(_2\)S also binds and reduces ferric heme in microsomal cytochrome P450, generating a state of oxidative stress\(^{[60]}\). On the other hand, an interesting phenomenon called “suspended animation,” a form of hypometabolism, results from the above mechanism. This phenomenon was first found in rodents that inhaled H\(_2\)S gas, and subsequent findings suggest that it is size-dependent or species-related\(^{[61–63]}\). Animals in suspended animation resembled those in hibernation, with reductions in cardiac output, ventilation frequency, and core temperature, finally leading to a decreased demand for energy. Hypometabolism can make great difference, if occurring in the human body, as an emergent protection against ischemia-associated tissue damage and infarction or providing a time delay in patients with stroke.

**A scavenger of reactive oxygen and nitrogen species**

H\(_2\)S is highly predisposed to react with cytochrome oxidase; therefore, the hypothesis that H\(_2\)S is also a potent antioxidant has been theorized. Recent studies have suggested that H\(_2\)S has the ability to react with ROS and RNS, including the superoxide radical anion, hydrogen peroxide, peroxynitrite, and hypochlorite\(^{[5, 64, 65]}\). Thus, a protective effect against organ damage caused by ROS and/or RNS may be proven. Because the concentration of H\(_2\)S in patients with AD is often severely suppressed and because AD has been proven to have a relationship with the increased generation of ROS and RNS, the role of H\(_2\)S in the cure of AD merits investigation\(^{[6, 66]}\).

H\(_2\)S is also reported to have a specific relationship with NO. They both have a synergistic effect on vasodilation as well as an inhibitory effect on the twitching of the ileum stimulated by electricity. While NO may induce CSE activity and then enhance the production of H\(_2\)S, H\(_2\)S also possess the ability to catalyze release of NO from S-nitrosoglutathione\(^{[67]}\). This type of relationship reveals a deeply unexplored mechanism between H\(_2\)S and NO. At a low concentration, H\(_2\)S displays only a weak relaxation of vessels, mainly due to the scavenging of NO. Thus, the specific concentration of H\(_2\)S play a critical role in determining its usability as a biomarker or its therapeutic application\(^{[68, 69]}\).

**H\(_2\)S donor molecules and H\(_2\)S-releasing drugs**

Ordinarily, H\(_2\)S-related drugs are categorized as either administrators of H\(_2\)S that enhance local or whole-body H\(_2\)S concentrations and cause pathological and physiological changes or as inhibitors of H\(_2\)S production enzymes that reduce local or whole-body H\(_2\)S concentrations and cause pathological and physiological changes. H\(_2\)S donors, including donor molecules and H\(_2\)S-releasing drugs, can be divided into three types: inorganic substances, such as NaHS; organic compounds, represented by GYY4137, which is derived from Lawesson’s reagent; and agonists of H\(_2\)S-synthesized enzymes. Inhibitors of enzymes involved in the synthesis of H\(_2\)S are of great importance, especially in the study of the mechanisms of H\(_2\)S functions. DL-propargylglycine, which easily permeates the cell membrane without obvious damage due to its high lipophilic property, is widely used as a nonspecific inhibitor of CSE and CBS. Disproof is a basic yet practical method for scientific investigation; therefore, the study of the effect of H\(_2\)S on certain targets by diminishing its concentration may be more
effective than directly administering it. In fact, these inhibitors are effective agents in diseases induced by the overproduction of H$_2$S.

Inorganic H$_2$S donors

Simple molecules, such as NaHS and Na$_2$S, are basic tools used for H$_2$S research. Their donation of H$_2$S is rapid upon reaction with water because of high solubility. Gaseous H$_2$S is sometimes used by respiratory passages through direct absorption by pulmonary alveoli due to high bioavailability. However, there is a fundamental shortage of the use of inorganic H$_2$S donors in both research and clinical applications because of the need to attain long-term release at a controlled rate.$^{[50,71]}$ A too rapid release implies increasing the concentration of H$_2$S instantly, resembling a pulse cure, which is associated with a variety of problems, including concentrations that are too high for the local tissue and a lack of a long-term effect. Both problems led to imprecise experimental parameters, including dose and time, random results, and misleading causes. Thus, this data shortage may be a partial explanation for the emergence of divergent results, even when the same function is investigated.

To obtain a better H$_2$S donor for sustained and controlled use, organic compounds containing chemically synthesized molecules and natural plant extracts have become prevalent. Some organic compounds have shown ideal therapeutic effects and a large commercial potential.

Precursors for endogenous H$_2$S synthesis

N-acetylcysteine and L-cysteine are precursors for endogenous H$_2$S synthesis.$^{[70]}$ Enhancing these precursors causes H$_2$S to increase with the catalysis of CSE and CBS. This precursor increase has minimal side effects associated with their production in the body.

Synthetic H$_2$S donors

As in other fields, synthetic drugs are predominant among H$_2$S-releasing drugs. GYY4137, a derivative of Lawesson’s reagent, acts as a synthetic H$_2$S donor. Its sustained release of H$_2$S in aqueous media with a concentration above baseline has been demonstrated.$^{[72]}$ GYY4137 causes the concentration-dependent suppression of cancer cells, such as MCF-7, by the promotion of apoptosis without an obvious impact in normal cells, such as IMR90. That is to say, our demand for the sustained moderate release of H$_2$S is met. In a recent study, a series of new H$_2$S donors based on the N-(benzoylthio)benzamide template were synthesized. The authors utilized the theory that the S-N bond is cysteine-activated under certain conditions, which needs to be demonstrated in future studies.$^{[73]}$ The release rate could be manually adjusted by the purposeful modification of the core structure.

H$_2$S-nonsteroidal anti-inflammatory drug hybrids

NSAIDs, among which aspirin is well known, are widely used for their anti-inflammatory and anti-gout properties. Nevertheless, they are associated with side effects, such as serious gastrointestinal bleeding and heart attack. Aspirin, as a delegate of NSAIDs, is famous for its anti-inflammatory ability as well as its prevention of atherosclerosis by inhibiting COX and platelet aggregation. However, aspirin also stimulates gastric bleeding and, worse, the prevention of platelet aggregation will hinder the cessation of bleeding. H$_2$S-NSAID hybrids have, thus, been devised based on the protective effect of H$_2$S on the gastrointestinal and cardiovascular systems.$^{[74,75]}$ ATB 337, which is derived from diclofenac, shows significantly reduced gastrointestinal toxicity compared with diclofenac and has an enhanced anti-inflammatory effect with no effect on hematocrit or leukocyte adherence. ATB 429, which is derived from mesalamine, also shows an improvement in treating inflammatory bowel disease compared with its parent drug. ATB 346, which is derived from naproxen, remarkably reduces gastrointestinal and cardiovascular toxicity, such as the two aforementioned drugs.$^{[70]}$ The side effects of NSAIDs mainly come from the single inhibition of only COX-1 or COX-2, which generates an imbalance in the expression of COX-1 and COX-2 in different tissues and organs. Therefore, these H$_2$S-NSAID hybrid compounds can release H$_2$S when dissolved by enzymes, which will protect organs, such as the stomach as well as the heart, because of the anti-oxidation or other effects of H$_2$S. It should also be noted that these types of compounds are not a simple addition of both NSAIDs and H$_2$S but because they can affect each other when in the same place at the same time, they make a more magnified effect than a simple two part addition.

Natural plant-derived compounds

Vegetables, represented by garlic and ginger, are ordinary salubrious foods that have anti-inflammatory functions. In recent years, garlic-derived polysulfide compounds have drawn great attention due to their potential anti-inflammatory and anti-cancer effects. DATS is the first garlic-derived molecule discovered to possess vasoactivity along with the ability to specifically suppress cancer cells.$^{[76]}$ Compounds similar to DATS, such as DAS, DADS, and DATTS, have been evaluated for similar functions, but they are complicated. Different effects have been found. DAS reportedly activates the nuclear receptor CAR which induces various hepatic drug-metabolizing phase I and phase II enzymes in the mouse liver and preventive effects on some types of gastric cancer.$^{[77]}$ DADS has demonstrated vasorelaxation effect in aortic ring preparations via glucose- and thiol-dependent cellular reactions. Interestingly, DADS modified hemoglobin’s β-chain at cysteine-93 or cysteine-112 in deoxygenated human red blood cells. This was the first instance of a garlic-derived compound that was shown to be able to modify an intracellular protein.$^{[77]}$ Normally, garlic-derived compounds are simply regarded as precursors of H$_2$S when absorbed and metabolized in blood. Therefore, because DADS itself is able to generate direct protein changes, it is worthy of future study.

S-allylcysteine is believed to have a cardioprotective effect and is a potential source of H$_2$S. However, whether it plays a role as a H$_2$S precursor or a modulator of H$_2$S-related enzymes
is controversial. Another novel garlic-derived compound, S-propargyl-cysteine, is now under investigation. It was recently shown to attenuate lipopolysaccharide-induced spatial learning and memory impairment through TNF signaling and the NF-κB pathway in rats, which contributes to studies about AD and other diseases related to neuronal damage.

Compounds extracted from other plants display similar effects. Sulforaphane, which is derived from isothiocyanates found in cruciferous vegetables, exhibits an anti-cancer property. Sulforaphane suppresses the proliferation of prostate cancer cells and enhances the expression of CBS and CSE. In addition, sulforaphane activates p38 mitogen-activated protein kinase and c-Jun N-terminal kinase, suggesting a potent mechanism. Although most of the significant results have been gained from in vitro studies, the study of H_{2}S-releasing donors in vivo is more complex. Therefore, the identification of a feasible drug for clinical use requires further research.

**Future developments: challenges for translation of the toxic molecule H_{2}S**

Investigation of the role of H_{2}S is still in its infancy. H_{2}S has both scientific and technological value. The latter tends to dominate because of its financial value and direct applicability, which substantiates research efforts. However, the scientific investigation of H_{2}S is also important for the elucidation of its base fundamental roles. Scientific investigation strives to address disease, enhance quality of life, and disclose the secrets of the human body. Certainly problems and challenges will arise and the limitations of experimental materials will at times constrain further H_{2}S research. A sustained and controlled H_{2}S-releasing donor that functions both in vitro and in vivo has not yet been found. An effective and quick method of measuring the concentration of H_{2}S is also required, especially a non-invasive method. With an increased understanding of the various H_{2}S mechanisms in the body, further study of H_{2}S becomes more difficult and complicated. H_{2}S is known as a third gaseous signaling molecule, which means that it plays the role of a messenger. The concentration of H_{2}S has been proven to have relevance in particular diseases; for instance, it is overproduced in sepsis and found at inadequate levels in AD. Therefore, the mechanism controlling the actual concentration of H_{2}S in certain tissues may become the ultimate problem for H_{2}S related research. It should be emphasized that a relevant relationship does not mean a relationship of causation. By regulating the H_{2}S concentration in particular tissues, symptoms of a specific disease can be controlled, which implies that the origin of the disease has not been addressed. That is to say, the regulation of H_{2}S can only provide transient protection from certain diseases, such as hypertension. The challenges of the sustained and controlled release of H_{2}S-releasing drugs were mentioned above. Another difficulty in H_{2}S related research comes from the multiple functions of H_{2}S, which cause a shortage of specific effects. The effects of H_{2}S are dose-, time-, and tissue-dependent. For patients with different diseases, H_{2}S may need to be administered as different drugs. Therefore, a focus on the general effects of H_{2}S, such as on cardiovascular protection, is rational.

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**Abbreviations**

H_{2}S, Hydrogen sulfide; CO, carbon monoxide; NO, nitric oxide; CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; 3-MST, 3-mercaptopyruvate sulfurtransferase; NaHS, sodium hydrosulfide; AD, Alzheimer’s disease; K_{ATP} channels, potassium-dependent ATP-sensitive channels; TRP, transient receptor potential; TRPC, canonical transient receptor potential channel; TRPV, vanilloid transient receptor potential channel; TRPM, melastatin transient receptor potential channel; TRPP, polycystin transient receptor potential channel; TRPML, mucolipin transient receptor potential channel; CHO cells, Chinese hamster ovary cells; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SFKs, SRC family kinases; ICAM-1, intracellular adhesion molecule-1; TRAF1, TNFR-associated factor 1; TRAF2, TNFR-associated factor 2; ROS, reactive oxygen species; RNS, reactive nitrogen species; NSAIDs, nonsteroidal anti-inflammatory drugs; DATS, diallyl trisulfide; DAS, diallyl sulfide; DADS, diallyl disulfide; DATTs, diallyl tetrasulfide; CAR, orphan member of the nuclear steroid hormone receptor superfamily.

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