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HYDROGEN SULFIDE (H_2S): THE NEW MEMBER OF GASOTRANSMITTER FAMILY

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Recent studies indicate that apart from nitric oxide (NO) and carbon monoxide (CO), hydrogen sulfide (H_2S) is the third gaseous mediator in mammals. H_2S is synthesized from L-cysteine by either cystathionine β-synthase (CBS) or cystathionine γ-lyase (CSE), vitamin B_6-dependent enzymes also involved in homocysteine metabolism. H_2S stimulates ATP-sensitive potassium channels (K_{ATP}) in vascular smooth muscle cells, neurons, cardiomyocytes and pancreatic β-cells. H_2S is involved in neurotransmission, regulation of vascular tone and blood pressure, regulates gastrointestinal motility and secretory function and inhibits insulin secretion. Deficiency of endogenous H_2S was observed in various models of arterial and pulmonary hypertension, gastric mucosal injury and liver cirrhosis. Exogenous H_2S or its donors decrease blood pressure and reduce vascular hypertrophy in spontaneously hypertensive rats, inhibit neointima formation induced by arterial injury, ameliorate myocardial dysfunction associated with ischemia/reperfusion, and reduce gastric mucosal damage induced by anti-inflammatory drugs. On the other hand, excessive production of H_2S may contribute to the pathogenesis of inflammatory diseases, septic shock, cerebral stroke and mental retardation in patients with Down syndrome, and reduction of its production may be of potential therapeutic value in these diseases. ***Biomed Rev 2007; 18: 75-83.***

Key words: hydrogen sulfide, vascular tone, neurotransmission, arterial hypertension, atherosclerosis, inflammation

INTRODUCTION

The long-lasting paradigm in biomedical sciences stated that all hormones, mediators and neurotransmitters are specialized organic molecules synthesized by specific enzyme systems. Therefore, it was a great surprise when endothelium-derived relaxing factor was identified as nitric oxide (NO) – a simple inorganic molecule. Soon thereafter, the second inorganic gaseous compound, carbon monoxide (CO), was recognized as an endogenously produced mediator and neurotransmitter. CO, together with biliverdin, is a product of heme catabolism by heme oxygenase (HO). Recent studies indicate that another gas, hydrogen sulfide (H_2S), is also a physiological mediator

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in mammalian tissues. H_2S is a water-soluble, colorless and flammable gas with a strong odor of rotten eggs, known for decades only as a toxic environmental pollutant. The main mechanism of its toxicity is a potent inhibition of mitochondrial cytochrome c oxidase (1). That H_2S may operate as an endogenous neurotransmitter was first suggested in 1996 by Abe and Kimura who described the enzymatic mechanism of H_2S production in the brain, its biological effects at physiological concentrations, and its specific cellular targets (2). Now, H_2S is increasingly recognized as a member of a growing family of "gasotransmitters", together with its two "older brothers", NO and CO.

Several comprehensive reviews about H_2S have been published recently (3–6). Herein, I will briefly highlight only mainstream aspects of H_2S research including new important findings published during the recent 12 months.

CHEMICAL PROPERTIES OF H_2S, ITS SYNTHESIS AND METABOLISM

Under physiologically relevant conditions, i.e. in aqueous solutions and at pH 7.4, one third of H_2S is undissociated and two thirds dissociate into H^+ and HS^- (hydrosulfide ion). Sodium hydrosulfide (NaHS) is commonly used experimentally as a H_2S donor since it dissociates into Na^+ and HS^-; the latter than partially combines with H^+ to form H_2S. H_2S is lipophilic and freely permeates plasma membranes. H_2S is detectable in serum and most tissues at a relatively high concentration (about 50 μM), and even three-fold higher concentrations are found in the brain.

H_2S is synthesized from L-cysteine by either cystathionine β-synthase (CBS, EC 4.2.1.22) or cystathionine γ-lyase (CSE, EC 4.4.1.1), both being vitamin B_6-dependent enzymes. CBS and CSE also act sequentially to form cysteine from homocysteine; the so-called transsulfuration pathway of homocysteine metabolism (7). CBS is the major H_2S-generating enzyme in the nervous system, whereas CSE catalyzes H_2S production in the cardiovascular system. In some tissues such as the liver and kidney, both enzymes are expressed in comparable amounts. In general, H_2S production from cysteine by either CBS or CSE constitutes only a minor fraction of cysteine metabolism. Most cysteine is catabolized by cysteine oxidase with its thiol group being directly oxidized to sulfite (SO_3^{2-}), which is rapidly oxidized to sulfate (SO_4^{2-}) by sulfite oxidase (SO, EC 1.8.3.1). However, in some tissues such as renal tubules desulfhydration of cysteine to H_2S accounts for up to 50% of cysteine metabolism (8).

Both CBS and CSE are regulated by various factors at both transcriptional and posttranslational levels (4). Interestingly, NO and CO are potent CBS inhibitors and it is suggested that CBS may be a major target for CO in the brain (9). In contrast, NO donors acutely stimulate CSE-dependent H_2S generation in a cGMP-dependent manner in the rat aorta (10), and increases CSE mRNA and protein expression in the long run (11).

H_2S is rapidly oxidized to thiosulfate (SSO_3^{2-}) in mitochondria, but the mechanism of this reaction is unclear. Thiosulfate is further converted to sulfite by thiosulfate sulfurtransferase (TST, rhodanese, EC 2.8.1.1), and sulfite is then oxidized to sulfate by sulfite oxidase. The second pathway of H_2S metabolism is its methylation by thiol S-methyltransferase (TSMT, EC 2.1.1.9) to methanethiol (CH_3SH) and dimethylsulfide (CH_3SCH_3) (4). Finally, H_2S may bind to methemoglobin to form sulfhemoglobin. Because hemoglobin may also bind NO and CO, it is a common "sink" for all three gasotransmitters.

Colonic mucosa is continuously exposed to high concentrations of H_2S produced by enteric bacteria, and is equipped with extraordinarily high amounts of TST and TSMT. Genotoxicity of H_2S (12) and reduced expression of H_2S-metabolizing enzymes in patients with colorectal cancer (13), suggesting that impaired H_2S metabolism may contribute to the pathogenesis of this disease. In addition, colonic epithelial cells are able to use H_2S as an electron donor for the mitochondrial respiratory chain (14). This is a first described example of mitochondrial oxidation of inorganic substrate in mammalian cells since previously only bacteria, worms and fish adapted to high H_2S concentration were known to utilize sulfide as an electron donor.

SIGNAL TRANSDUCTION MECHANISMS

The best characterized molecular targets stimulated by H_2S are ATP-sensitive potassium channels (K_{ATP}) composed of inwardly rectifying potassium channel Kir6.2 and tissue-specific isoforms of sulfonylurea receptor (SUR). K_{ATP} are cellular energy sensors activated under conditions of ATP depletion such as hypoxia to drive potassium efflux from the cell. H_2S also reacts with other molecular targets. As a strong reductant, H_2S may protect protein sulfhydryl groups from oxidation, although until now little data support it as a mechanism of physiological effects of this gas. Moreover, H_2S reacts with reactive oxygen and nitrogen species such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and hypochlorite (ClO^-), and thus protects cells from being damaged by these noxious molecules (15–18).

Activated neutrophils generate O_2^- which reacts with H_2S to form sulfite – an important bactericidal compound which may also contribute to tissue damage in inflammatory states. Indeed, increased sulfite concentration is observed in patients with septic shock (19). H_2S has also been demonstrated to stimulate heme oxygenase, to stimulate or inhibit inducible NO synthase, and to activate or suppress extracellular signal-regulated kinases (ERK), however, it is unclear if these effects are primary or result from activation of other signaling pathways such as K_{ATP} channels (4).

H_2S IN THE NERVOUS SYSTEM

Several effects of H_2S in the nervous system have been described, suggesting that it may function as a neurotransmitter/neuromodulator. H_2S facilitates hippocampal long-term potentiation, a synaptic model of learning and memory (2), at least in part by increasing the sensitivity of N-methyl D-aspartate (NMDA) receptors to their ligand, glutamate (20). In addition, H_2S stimulates Ca^{2+} influx to astrocytes (21) and neurons (22) from the extracellular space. Accumulating body of evidence suggests that H_2S stimulates capsaicin-sensitive sensory nerves and evokes the release of tachykinins such as substance P and neurokinin-A. This effect is mediated by transient receptor potential vanilloid receptor-1 (TRPV-1) calcium channel (23), a nonselective cation channel which serves as a nonspecific receptor of sensory terminals for various noxious physical and chemical stimuli.

Abnormalities of H_2S production have been implicated in certain nervous system diseases. Human CBS-encoding gene is located on chromosome 21 and, therefore, H_2S is overproduced in the brain of patients with Down syndrome. Indeed, urinary excretion of thiosulfate is increased two-fold in patients with Down syndrome in comparison to healthy individuals (24). It is hypothesized that excess of H_2S exerts a toxic effect on neurons through the inhibition of cytochrome c oxidase and/or overstimulation of NMDA receptors, and thus contributes to a progressive mental retardation in patients with 21 trisomy (25). Qu et al. (26) have observed that administration of NaHS or L-cysteine aggravates, whereas CBS or CSE inhibitors reduce the volume of brain infarct induced by cerebral artery occlusion. In addition, H_2S concentration in the cerebral cortex increased in this model of stroke. These data suggest a detrimental effect of H_2S in experimental stroke. Indeed, H_2S may induce neuronal cell death by overactivating NMDA receptors (27).

However, H_2S may also be protective for neurons under certain conditions, and thus its deficiency in the brain may be detrimental. In particular, H_2S protects neurons against neurotoxicity of glutamate independent of the stimulation of excitatory amino acid receptors. Overproduction of glutamate is observed in certain pathological conditions, such as seizures, brain ischemia, trauma, etc., and may damage neurons not only by activating specific membrane receptors but also in the receptor-independent manner referred to as "oxytosis". Cystine is transported to the cell by the x_c^- system (cystine/glutamate antiporter), which drives import of cystine coupled to export of glutamate. Extracellular glutamate inhibits this exchange, leading to intracellular cysteine deficiency. Since cysteine is a rate-limiting substrate for glutathione (GSH) synthesis, excess glutamate induces GSH depletion which renders the cell more sensitive to oxidative stress. NaHS increases intracellular GSH concentration in rat cortical neurons and protects these cells against ischemia or glutamate-induced death (28, 29). H_2S may also protect neurons by scavenging reactive oxygen and/or nitrogen species (17, 18). Interestingly, H_2S concentration in the brain of patients with Alzheimer disease is severely depressed in comparison to control individuals, which may contribute to cognitive impairment and progressive neuronal injury (30).

METABOLIC EFFECTS OF H_2S

Recently, it has been demonstrated that H_2S inhaled at low concentrations induces a hibernation-like phenotype in mice, characterized by reduced metabolic rate, decrease in body temperature, heart and respiratory rates, most likely by inhibiting mitochondrial respiration (31). These data suggest that protective effect of H_2S in some experimental models may result, at least partially, from reduced oxygen demand. Indeed, pretreatment with H_2S improved survival of mice subsequently exposed to low oxygen tension (32). The similar protective effect was observed in a rat model of rapid lethal hemorrhage (3). In addition, H_2S added to the incubation medium reduces injury of the donor rat heart used for transplantation (33). This metabolic effects may also contribute to increased life span of *Caenorhabditis elegant* exposed to low H_2S concentrations (34). However, it is unclear if the analogous effect will be observed in large animals such as primates, who spend much less energy for thermoregulation (35).

H_2S IN THE CARDIOVASCULAR SYSTEM

Initial studies have unequivocally indicated that H_2S has a hypotensive/vasodilating activity. Intravenously injected H_2S induces a transient dose-dependent decrease in mean arterial pressure (11). *In vitro*, H_2S and NaHS relax isolated rat arter-

ies and veins (36). In contrast to NO and CO, H_2S does not stimulate soluble guanylate cyclase but activates K_{ATP} channels in vascular smooth muscle cells, which results in hyperpolarization of plasma membrane, reduction of Ca^{2+} influx, and attenuation of the contractile response. Vasodilatory effect of H_2S is abolished by K_{ATP} channel antagonist, glibenclamide (11). Moreover, patch-clamp studies have demonstrated that H_2S increases K_{ATP}-dependent current by increasing channel's open probability, but has no effect on its conductance (37). Interestingly, CSE inhibitors reduce K_{ATP} channel-dependent current indicating that endogenous H_2S continuously stimulates the channel under baseline conditions. It seems that H_2S activates K_{ATP} channels directly and no intermediate signaling mechanism is involved, however, the specific molecular background of channel activation is not known.

Recent studies suggest that H_2S has a more complex effect on vascular tone. First, O_2-dependent effect on rat thoracic aorta was observed, i.e. H_2S constricted it at high but relaxed at low O_2 concentration (38). Second, both *in vivo* (39) and *in vitro* (40) studies indicate that low H_2S concentrations may induce vasoconstriction and blood pressure elevation by inactivating endothelial NO which combines with H_2S to form inactive nitrosothiol (41). Finally, H_2S directly inhibits endothelial NO synthase (42). Other effects of H_2S in the cardiovascular system include augmentation of baroreceptor reflex (43), decrease in myocardial contractility (44), and stimulation of angiogenesis (45). H_2S also inhibits angiotensin-converting enzyme (ACE) – a zinc-containing protein – by binding Zn^{2+} cations (46).

Plasma H_2S concentration as well as aortic CSE expression are lower in spontaneously hypertensive rats (SHR) than in control Wistar-Kyoto rats. In addition, chronic administration of NaHS lowers blood pressure in SHR but not in normotensive rats (47). The similar results were obtained in rats with experimental hypertension induced by chronic NO synthase blockade (48). Plasma H_2S level is lower in patients with essential hypertension than in normotensive controls (49). Taken together, these data suggest that deficiency of H_2S may contribute to the pathogenesis of arterial hypertension.

Both NO and CO produced in the arterial wall inhibit atherogenesis through their anti-inflammatory, antiplatelet, and antiproliferative activities. Therefore, the question arises if H_2S is also involved in atherogenesis. Indeed, H_2S exerts some effects potentially associated with suppressing atherogenesis. H_2S inhibits proliferation (50) and induces apoptosis (51) of vascular smooth muscle cells, suppresses oxidative modification of low-density lipoproteins (52), inhibits platelet aggregation (53), attenuates prooxidant effect of homocysteine (54), and may inhibit vascular inflammatory reactions, although the latter is controversial (see below). *In vivo*, NaHS attenuates vascular remodeling and inhibits neointima formation after balloon-induced injury of the rat carotid artery (55), and ameliorates experimentally-induced vascular calcifications (56). Further studies are required to elucidate if H_2S production is impaired in human atherosclerosis. It should be noted that low CBS/CSE activity will promote not only H_2S deficiency but also homocysteine excess, so low H_2S may accompany at least some forms of hyperhomocystinemia. Dissecting the role of homocysteine vs. H_2S deficiency in atherogenesis will be an important and demanding aspect of future research in this field.

Myocardial cells contain large amounts of K_{ATP} channels consisting of Kir6.2 and a sulfonylurea receptor, SUR2A. Activators of K_{ATP} channels have documented a protective effect in myocardial ischemia-reperfusion injury (57). Several studies have demonstrated that H_2S donors reduce myocardial damage and improve hemodynamic function in various models of ischemia-reoxygenation in isolated cardiomyocytes, isolated perfused heart, and in intact animals (58,59). Perfusion of isolated rat heart with H_2S before ischemia prevented arrhythmias induced by the subsequent ischemia/reperfusion episode, and protected isolated cardiac myocytes against death induced by subsequent hypoxia (60). In addition, blockade of endogenous H_2S production reduced the protective effect of modest ischemia against deleterious effect of subsequent severe ischemia, suggesting that endogenous H_2S is involved in the phenomenon of "ischemic preconditioning".

Finally, H_2S deficiency is observed in experimental pulmonary hypertension induced by hypobaric hypoxia (61) or by high pulmonary blood flow (62), and sodium hydrosulfide attenuates vascular remodeling and right ventricular hypertrophy as well as reduces pulmonary arterial pressure in these models (63,64).

H_2S AND INFLAMMATION

H_2S seems to play a very complex role in the inflammatory reactions. Both pro- and antiinflammatory effects of H_2S have been described both *in vitro* and *in vivo,* depending on the experimental model and dose/concentration of this gasotransmitter (reviewed in 3,4,65). For example, H_2S is overproduced in experimental models of septic shock and may contribute to hypotension, impaired myocardial contractility, and lung and

liver damage (66–68). Suppressing H_2S production reduces inflammatory reaction and improves survival in these models. In addition, increased CSE expression/activity and H_2S production are observed in experimental caerulein-induced pancreatitis (69). H_2S may also induce "neurogenic inflammation", especially in the airways, by activating TRPV1 in sensory nerve endings and increasing local release of substance P, neurokinin-A and calcitonin gene-related peptide (CGRP). These mediators induce a series of inflammatory responses including vasodilation, extravasation of plasma proteins, edema, bronchoconstriction, mucus secretion and recruitment of inflammatory and immune cells (70,71). Plasma H_2S concentration is increased by almost 50% in patients with stable chronic obstructive bronchopulmonary disease (COPD) in comparison to the control group (72). Interestingly, in patients with exacerbated COPD, H_2S level is lower than in those with stable disease and is inversely correlated with pulmonary artery systolic pressure, suggesting that pulmonary hypertension *per se* has a deleterious effect on H_2S production in humans. Finally, H_2S concentration in exhaled breath is increased in patients with chronic pancreatitis (73).

INSULIN SECRETION AND DIABETES MELLITUS

Apart from vascular smooth muscle cells and cardiomyocytes, K_{ATP} channels are abundantly expressed in insulin-secreting pancreatic β cells. Pancreatic K_{ATP} channels, consisting of Kir6.2 and SUR1, play an important role in the regulation of glucose-induced insulin secretion. Glucose increases intracellular ATP concentration leading to K_{ATP} channel blockade, plasma membrane depolarization, Ca^{2+} influx through the voltage-sensitive Ca^{2+} channels, and insulin release. Several studies have demonstrated that both exo- and endogenous H_2S inhibits insulin release by activating K_{ATP} channels in β-cells (74, 75). Moreover CBS expression and H_2S production are increased in pancreas of rats with streptozotocin-induced diabetes, suggesting that excess of H_2S may contribute to abnormal insulin secretion (76). H_2S was not measured in diabetic humans, but plasma L-cysteine as well as the expression of CBS and CSE in various tissues are increased in patients with diabetes (77).

H_2S IN THE GASTROINTESTINAL SYSTEM

Both acetylsalicylic acid (ASA) and nonsteroidal anti-inflammatory drugs (NSAIDs) reduce CSE expression and H_2S production in the gastric mucosa. NaHS attenuates gastric lesions induced in the rat by ASA or NSAIDs (78).

Serosal application of NaHS or L-cysteine stimulates luminal chloride secretion by guinea pig and human colonic tissues. This effect results from TRPV1-mediated stimulation of the enteric nervous system (79). Recently, Distrutti *et al* (80) have demonstrated that NaHS ameliorates visceral nociception evoked in the rat by colorectal distension. Fiorucci *et al* (81) have shown that H_2S attenuates norepinephrine-induced vasoconstriction in the liver of healthy rat as well as in animals with experimental liver cirrhosis induced by bile duct ligation. Experimental cirrhosis is associated with reduced CSE expression, decreased H_2S production by liver homogenates, and decrease in plasma H_2S concentration (81). Deficiency of H_2S may thus contribute to enhanced hepatic vascular resistance and to the development of portal hypertension.

THERAPEUTIC IMPLICATIONS

According to the experimental studies mentioned above, elevating H_2S may be beneficial in diseases such as arterial and pulmonary hypertension, atherosclerosis, myocardial infarction, gastrointestinal ulcer and some inflammatory diseases, whereas suppressing H_2S production may be indicated in conditions such as septic shock, pancreatitis, cerebral stroke or diabetes. Although there are attempts to use gaseous NO and CO in some diseases such as pulmonary hypertension, H_2S application via this route may be more difficult due to problems with precise control of concentration, low therapeutic index, manufacturing and formulation difficulties and, last but not least, a very unpleasant smell even at low concentrations. Such therapy may be considered only in acute states such as myocardial infarction. Most of problems with gaseous H_2S will also emerge while trying to administer H_2S solutions parenterally. NaHS, although widely used as a research tool, releases H_2S quickly and is thus a short-lasting donor. In addition, rapid release of H_2S may cause acute changes in blood pressure. Ideal H_2S donors, from therapeutic point of view, should release H_2S slowly in moderate amounts. Such compounds are, unfortunately, still not available. Recently, two H_2S-releasing derivatives of currently used drugs were synthesized: mesalamine derivative, ATB-429, and S-diclofenac. They differ from their parent compounds in that they contain a H_2S-releasing moiety, and initial results suggest that this augments their antiinflammatory properties while reducing deleterious effects on the gastrointestinal system (82). The other possible approach is pharmaco- or gene therapy aimed to increase CBS/CSE expression. Interestingly, biologically active garlic component, S-allylcysteine, is a H_2S precursor *in*

vivo, and H_2S seems to drive its beneficial effects on vascular tone on myocardial ischemic insults (83,84).

Therapeutic approach based on suppressing H_2S-generating enzymes is a matter of future. Currently used CSE and CBS inhibitors are unsuitable for pharmacotherapy and non ideal even for research. First, they are not completely specific and inhibit also other vitamin B_6-dependent enzymes. Second, the most commonly used CSE inhibitor, propargylglycine, is a lead-containing compound and may be toxic after long-term administration. Finally, most inhibitors have a very limited ability to permeate plasma membranes. However, even completely specific CBS or CSE inhibitors will not only reduce H_2S level but also impair homocysteine metabolism, which may be unbeneficial in certain circumstances. Thus, their administration may be considered only for a short time.

ASA and NSAIDs have an inhibitory effect on the CSE-H_2S pathway in gastrointestinal mucosa (78), which may contribute to mucosal injury induced by these drugs but, on the other hand, may be involved in their antineoplastic effect of these medications in the gastrointestinal tract. NSAIDs reduce CSE expression also in renal epithelial cells, and NaHS exerts a protective effect against NSAIDs-induced injury in these cells (78). These data are intriguing in view of high expression of H_2S-producing enzymes in the kidney and the well-known nephrotoxic effect of NSAIDs. Because K_{ATP} channels mediate many effects of H_2S, sulfonylurea derivatives widely used in the treatment of type 2 diabetes may interfere with the effects of this gasotransmitter by blocking K_{ATP} channels outside the pancreas. In this context, it should be noted that some sulfonylurea derivatives have adverse effects in experimental myocardial ischemia and increase the amount of cardiovascular events in clinical trials (85). Thus, unbeneficial modulation of H_2S signaling may also contribute to adverse effects of certain drugs. Finally, taking into account the potential role of H_2S deficiency in arterial hypertension and atherosclerosis, the effect of drugs currently used in pharmacotherapy of cardiovascular diseases on H_2S metabolism is of great interest.

CONCLUSIONS AND PERSPECTIVES

H_2S emerges as an important regulator of many physiological functions, and is also involved in the pathogenesis of certain diseases. However, our knowledge about its role in physiology and pathology is far from being complete. Some aspects of its activity are controversial and the results are often contradictory depending on the experimental conditions. Only few studies demonstrated alterations in H_2S level in human diseases, and in most cases it was done indirectly by measuring H_2S-related compounds such as thiosulfate, sulfite or sulfhemoglobin rather than H_2S itself. The most important issues for future research are: (i) improvement of methods used for measurement of H_2S concentration in biological samples, (*ii*) more detailed characterization of specific molecular targets for this transmitter, (*iii*) unraveling the mechanisms which regulate H_2S-producing and H_2S-degrading enzymes, (*iv*) more systematic analysis of H_2S system in human diseases, including prospective studies focused on its role as a cardiovascular risk factor, and (*v*) synthesis of H_2S donors and CBS/CSE inhibitors more suitable for research and potentially for therapy than currently used compounds. Great progress made in this field in the last five years allows to hope that we will be able to answer many unresolved questions in the near future.

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