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Review

Hydrogen sulfide (H_2S) – the third gas of interest for pharmacologists

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Abstract:
Nitric oxide (NO) and carbon monoxide (CO) synthesized from L-arginine by NO synthase and from heme by heme oxygenase, respectively, are the well-known neurotransmitters and are also involved in the regulation of vascular tone. Recent studies suggest that 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), both using pyridoxal 5'-phosphate (vitamin B_6) as a cofactor. H_2S stimulates ATP-sensitive potassium channels (K_{ATP}) in the vascular smooth muscle cells, neurons, cardiomyocytes and pancreatic β-cells. In addition, H_2S may react with reactive oxygen and/or nitrogen species limiting their toxic effects but also, attenuating their physiological functions, like nitric oxide does. In contrast to NO and CO, H_2S does not stimulate soluble guanylate cyclase. H_2S is involved in the regulation of vascular tone, myocardial contractility, neurotransmission, and insulin secretion. H_2S deficiency was observed in various animal models of arterial and pulmonary hypertension, Alzheimer's disease, gastric mucosal injury and liver cirrhosis. Exogenous H_2S ameliorates myocardial dysfunction associated with the ischemia/reperfusion injury and reduces the damage of gastric mucosa 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 states.

Key words:
hydrogen sulfide, arterial hypertension, atherosclerosis, homocysteine, septic shock, inflammation, diabetes mellitus

Abbreviations: cAMP – cyclic adenosine monophosphate, CBS – cystathionine β-synthase, CDO – cysteine dioxygenase, cGMP – cyclic guanosine monophosphate, CO – carbon monoxide, CSE – cystathionine γ-lyase, fMLP – N-formyl-methionyl-leucyl-phenylalanine, GSH – reduced glutathione, Hcy – homocysteine, HO – heme oxygenase, K_{ATP} – ATP-sensitive potassium channels, LPS – lipopolysaccharide, MPO – myeloperoxidase, MTHFR – methylenetetrahydrofolate reductase, NaHS – sodium hydrosulfide, NMDA – N-methyl D-aspartate, NO – nitric oxide, NOS – nitric oxide synthase, PAG – propargylglycine, SAH – S-adenosylhomocysteine, SAM – S-adenosylmethionine, SHR – spontaneously hypertensive rats, SUR – sulfonylurea receptor, TRPV – transient receptor potential vanilloid receptor

Introduction

It was a great surprise for scientific community when the endothelium-derived relaxing factor (EDRF) was identified as nitric oxide (NO), a simple inorganic molecule, because all hormones, mediators and neurotransmitters known before were organic compounds. Now there is no doubt that NO plays important regulatory roles in almost all tissues [104]. Soon thereafter, the second inorganic gaseous compound, carbon monoxide (CO), was recognized as an endoge-

nously produced mediator and neurotransmitter. CO is synthesized during the catabolism of heme to biliverdin by heme oxygenase (HO). Interestingly, NO and CO share at least one common mechanism of action, i.e. they stimulate soluble guanylate cyclase and increase intracellular cGMP concentration, although CO is a much weaker activator than NO [6, 47]. Recent studies indicate that another "toxic gas", hydrogen sulfide (H_2S), is also produced in substantial amounts by mammalian tissues and exerts many physiological effects suggesting its potential role as a regulatory mediator. H_2S, the colourless gas with a strong odour of rotten eggs, was known for decades only as a toxic environmental pollutant. The main mechanism of its toxicity is a potent inhibition of mitochondrial cytochrome c oxidase. In fact, H_2S is a more potent inhibitor of mitochondrial respiration than cyanide [97]. Although endogenous hydrogen sulfide was found in the brain at the end of 1980s [114], it was initially suggested to be an artifact since sulfide concentration rapidly increases postmortem in mammalian tissues [84], and may be easily released from so called "sulfane sulfur" (compounds containing sulfur atoms bound only to other sulfur atoms) during tissue preparation [52]. That H_2S may operate as an endogenous neurotransmitter was first suggested a decade ago 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 [1]. Now H_2S is increasingly recognized as a member of a growing family of "gasotransmitters", together with its two counterparts, NO and CO. However, much less is known about the physiological role of H_2S than about either NO or CO. Several review articles about H_2S have been published [7, 11, 28, 36, 58, 61, 62, 68, 71, 86, 112, 113], however, they do not cover many important findings obtained during the last 2–3 years. In this paper, we briefly characterize biochemistry of H_2S, its physiological effects, changes in H_2S in pathology and possible pharmacological implications.

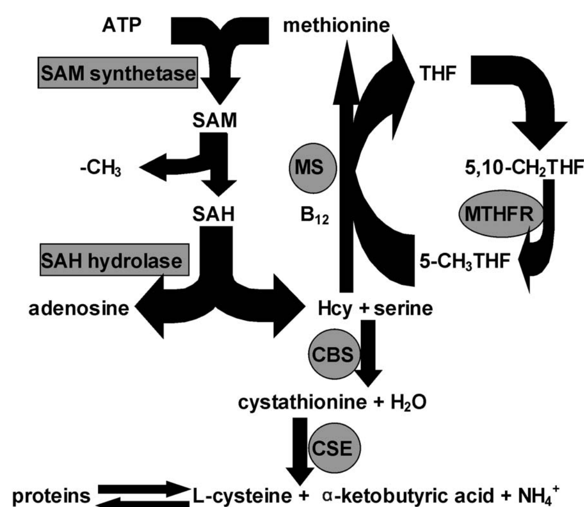


Fig. 1. Metabolism of homocysteine and synthesis of cysteine. Homocysteine is derived from methionine which is first transformed to S-adenosylmethionine (SAM), a donor of methyl groups for various methylation reactions. During these reactions SAM is transformed to S-adenosylhomocysteine (SAH) subsequently decomposed to Hcy by SAH hydrolase. Hcy may be remethylated to methionine by methionine synthase (MS) which uses vitamin B_{12} as a cofactor and 5-methyltetrahydrofolate (5-CH_3THF) as a donor of a methyl group. 5-CH_3THF is formed from 5,10-methylenetetrahydrofolate (5,10-CH_2 THF) by methylenetetrahydrofolate reductase (MTHFR). Apart from this "remethylation pathway", Hcy is metabolized to cysteine in the transsulfuration pathway by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE)

Biochemistry of H_2S

Chemical properties

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), which subsequently may decompose to H^+ and sulfide ion (S^{2-}). However, the latter reaction occurs only at high pH, thus S^{2-} does not occur *in vivo* at substantial amounts. Sodium hydrosulfide (NaHS) is commonly used as an H_2S donor since it dissociates to Na^+ and HS^-; the latter then partially binds H^+ to form undissociated H_2S. Similarly to NO and CO, H_2S is lipophilic and freely permeates plasma membranes, although due to partial dissociation membranes are relatively less permeable to H_2S than to both other gases. H_2S is detectable in serum and most tissues at a concentration of about 50 μM. Its physiological level in the brain is up to three-fold higher than in serum and is in fact close to toxic concentration.

Synthesis of H_2S

H_2S is produced at significant amounts in most tissues. The highest rate of production was noted in the brain, cardiovascular system, liver and kidney [25].

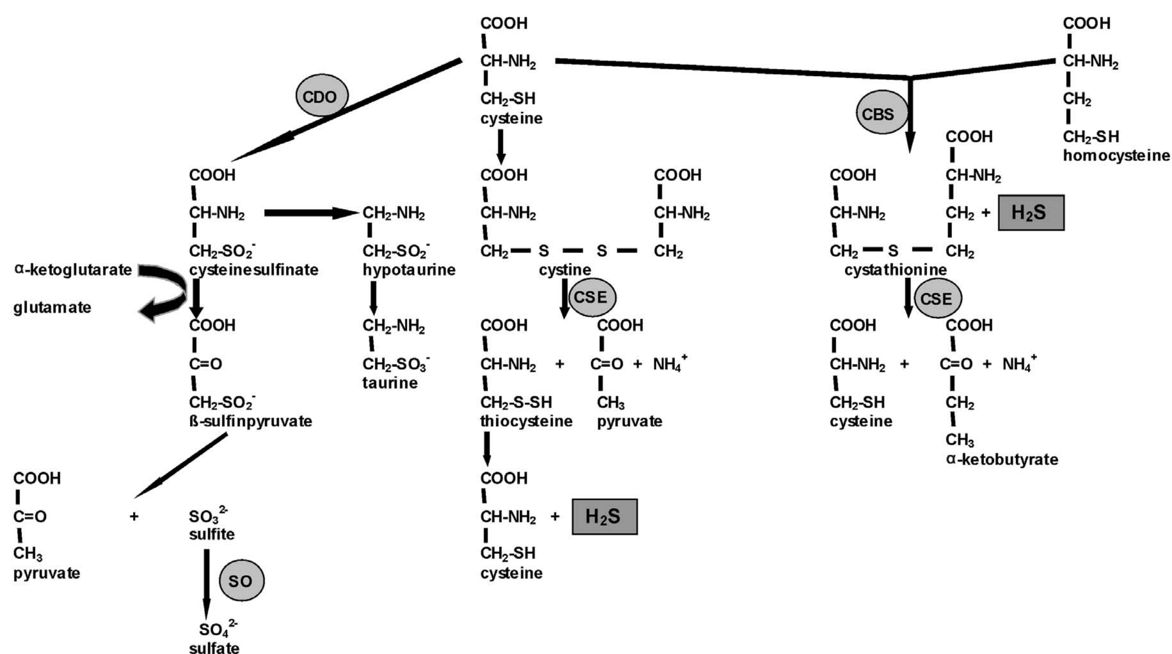


Fig. 2. Cysteine metabolism. CBS – cystathionine β-synthase, CDO – cysteine dioxygenase, CSE – cystathionine γ-lyase, SO – sulfite oxidase

The only substrate for the generation of endogenous H_2S is L-cysteine, a sulfur-containing amino acid derived from alimentary sources, synthesized from L-methionine through the so-called "transsulfuration pathway" with homocysteine (Hcy) as an intermediate, or liberated from endogenous proteins (Fig. 1). There are two major pathways of cysteine catabolism [52, 106] (Fig. 2). One of them is oxidation of –SH group by cysteine dioxygenase (CDO) to cysteine sulfinate, which may be decarboxylated to hypotaurine or converted to pyruvate and sulfite, subsequently oxidized to sulfate by sulfite oxidase. The second pathway, referred to as "desulfhydration", is associated with the removal of cysteine sulfur atom without its oxidation and results in H_2S production. This process may be catalyzed by either of the two enzymes of the Hcy transsulfuration pathway: cystathionine β-synthase (CBS, EC 4.2.1.22) and cystathionine γ-lyase (CSE, EC 4.4.1.1). Both are pyridoxal 5'-phosphate (vitamin B_6)-dependent but differ in the specific mechanism of H_2S formation (Fig. 2). CSE catalyzes the conversion of cystine (a cysteine disulfide) to thiocysteine, pyruvate and ammonia; thiocysteine then nonenzymatically decomposes to cysteine and H_2S. The major mechanism of H_2S production by CBS involves probably the condensation of homocysteine with cysteine to yield cystathionine; H_2S is liberated during this reaction [14]. It should be noted that this reaction is closely related to the canonical reaction catalyzed by CBS in the transsulfuration pathway (Fig. 1), with the exception that cysteine instead of serine condenses with homocysteine, and H_2S instead of H_2O is released. CBS and CSE are widely distributed in tissues, however, CBS is a predominant source of H_2S in the central nervous system whereas CSE is a major H_2S-producing enzyme in the cardiovascular system. In some tissues such as the liver and kidney, both enzymes contribute to H_2S generation. In general, it is assumed that quantitatively predominant route of cysteine catabolism is *via* dioxygenase, however, some *in vitro* studies suggest that desulfhydration with subsequent H_2S production accounts for up to 50% of cysteine metabolism in some tissues such as renal tubular cells [105].

Regulation of H_2S-producing enzymes

Little is known about the regulation of H_2S-producing enzymes. In the brain, electrical stimulation and excitatory neurotransmitter, glutamate, rapidly increase CBS activity in Ca^{2+}/calmodulin-dependent manner [33]. Both N-methyl-D-aspartate (NMDA) and

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptors are involved in this effect. S-adenosyl-methionine (SAM), an intermediate product of methionine metabolism and a major donor of methyl groups (Fig. 1), is an allosteric activator of CBS [32]. Sex hormones seem to regulate brain H_2S since CBS activity and H_2S level are higher in male than in female mice and castration of male mice decreases H_2S formation [32]. A nitric oxide donor, sodium nitroprusside, increases the activity of brain CBS *in vitro*, however, this effect is NO-independent and results from chemical modification of the enzyme's cysteine groups [31]. In contrast, NO itself may bind to and inactivate the CBS. Interestingly, CO is a much more potent CBS inhibitor than NO and it is suggested that CBS may be one of the molecular targets for CO in the brain [93, 108]. In homogenates of the rat aorta, NO donors acutely increase CSE-dependent H_2S generation in a cGMP-dependent manner [134]. Moreover, prolonged incubation of cultured vascular smooth muscle cells in the presence of NO donors increases CSE mRNA and protein levels [136]. The physiological significance of NO in the regulation of H_2S production is also supported by the observation that circulating H_2S level as well as CSE gene expression and enzymatic activity in the cardiovascular system are reduced in rats chronically treated with NOS inhibitor. Thus, NO is probably a physiological regulator of H_2S production in the cardiovascular system.

Catabolism of H_2S

Catabolism of H_2S is less recognized and most data were obtained by using exogenous H_2S; thus these studies have important toxicological but not necessarily physiological implications. H_2S is rapidly oxidized, mainly in mitochondria, initially to thiosulfate which is further converted to sulfite and sulfate (Fig. 3). Oxidation of H_2S to thiosulfate is probably a nonenzymatic process associated with mitochondrial respiratory electron transport, although superoxide dismutase may also catalyze this reaction [101]. Conversion of thiosulfate to sulfite is catalyzed by thiosulfate:cyanide sulfurtransferase (TST, rhodanese, EC 2.8.1.1) which transfers sulfur from thiosulfate to cyanide or other acceptors [92]. Sulfite, which originates in this reaction, is rapidly oxidized to sulfate by sulfite oxidase. Thus, under physiological conditions sulfate is a major end-product of H_2S metabolism, although most of urinary sulfate originates from cysteine oxidation by CDO rather than from H_2S-derived thiosulfate (Fig. 2). Although thiosulfate is also excreted in urine, its concentration is less then 1% of sulfate [58]. However, urinary thiosulfate is considered to be a specific marker of whole-body H_2S production [5].

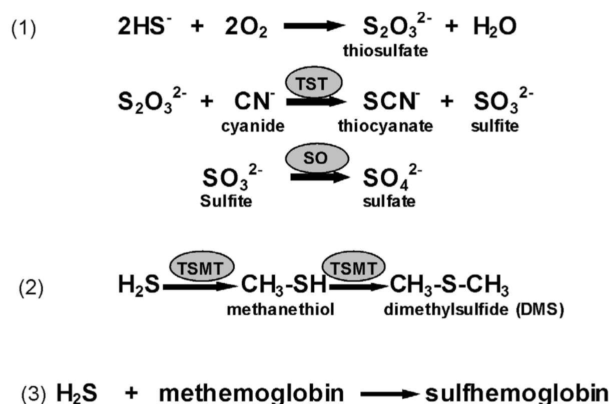


Fig. 3. Catabolism of H_2S. (1) – mitochondrial oxidation, (2) – cytosolic methylation, (3) – binding to hemoglobin. SO – sulfite oxidase, TSMT – thiol S-methyltransferase, TST – thiosulfate:cyanide sulfurtransferase (rhodanese)

The second pathway of H_2S metabolism is the methylation by thiol S-methyltransferase (TSMT) to methanethiol and dimethylsulfide [38] (Fig. 3). This reaction occurs mainly in the cytosol. Some studies question the significance of this pathway, at least in the gastrointestinal tract [70, 92]. 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.

Signal transduction mechanisms

Signaling mechanisms triggered by H_2S recognized so far are listed in Table 1. H_2S is a strong reducing agent. Therefore, it has been suggested that some of its effects may be mediated by protection of protein thiol groups from oxidation. However, all studies performed to date indicate that effects of H_2S applied at physiological concentration are not reproduced or only partially mimicked by thiol-protecting agents, suggesting that this is not a major mechanism of H_2S action [1]. In many systems, the effect of H_2S is mediated by ATP-sensitive potassium channels (K_{ATP}). This conclusion is mostly based on the observation

that many effects of H_2S are mimicked by K_{ATP} openers such as pinacidil or diazoxide and abolished by their inhibitors (sulfonylurea derivatives) such as glibenclamide. Only in few studies the stimulatory effect of H_2S on K_{ATP} channel was demonstrated directly by measuring K_{ATP} channel current by the patch-clamp method [16, 107]. The precise mechanism through which H_2S stimulates K_{ATP} channels is not clear.

H_2S is a highly reactive molecule and may easily react with other compounds, especially with reactive oxygen and nitrogen species (ROS and RNS). It has been demonstrated that H_2S reacts with at least four different ROS, superoxide radical anion [80], hydrogen peroxide [41], peroxynitrite [115] and hypochlorite [116]. All of them are physiologically relevant ROS or RNS. Superoxide anion (O_2^-) is produced by NADPH oxidase present in phagocytes as well as by the related non-phagocytic NADPH oxidases expressed in many cell types, in particular in the cardiovascular system and the kidney. H_2O_2 is produced from O_2^- in the reaction catalyzed by superoxide dismutase. Peroxynitrite ($ONOO^-$) is the product of spontaneous reaction between superoxide and NO, whereas hypochlorite (ClO^-) is produced from H_2O_2 by neutrophil myeloperoxidase (MPO). All these compounds are highly reactive and their interaction with H_2S results in the protection of proteins and lipids from ROS/RNS-mediated damage [115, 116]. Significance of H_2S reaction with O_2^- is ambiguous since the product, sulfite, may have both toxic [19] and antioxidant [77] properties, most likely depending on its concentration. H_2S also reacts with NO to form a nitrosothiol compound with yet undefined chemical structure [117]. Interestingly, in contrast to other nitrosothiols (R-S-NO) which are considered to be a reservoir of NO and often mimic its activity, the nitrosothiol originating from H_2S and NO is inactive. It has been suggested that H_2S may scavenge the excess of NO produced in the inflammatory state [117], but may also limit the availability of NO continuously produced at physiological concentrations [2]. Additional mechanism through which H_2S may exert antioxidant effect involves stimulation of cysteine transport to the cells and enhancement of glutathione synthesis [65]. Moreover, H_2S has been demonstrated to stimulate heme oxygenase expression and CO production, and to have bidirectional effects on the extracellular signal-regulated kinases (ERK) and inducible NO synthase (Tab. 1). It is unclear if these effects are primary or result from the stimulation of other targets such as K_{ATP} channels.

Tab. 1. Intracellular signaling mechanisms triggered by H_2S

Signaling mechanism	Ref.
Stimulation of K_{ATP} channels in:	
blood vessels	[16, 107]
myocardium	[42
pancreatic β-cells	[129]
neurons	[64]
carotid sinus	[122]
smooth muscle of the colon	[23]
Maintaining protein –SH groups in the reduced state	[1]
Stimulation of adenylate cyclase	[63, 67]
Reaction with ROS and RNS	
O_2^-	[80]
H_2O_2	[41]
$ONOO^-$	[115]
ClO^-	[116]
NO	[2, 117]
Stimulation of cysteine transport to the cell and GSH synthesis	[65]
Stimulation of ERK	[85]
Inhibition of ERK	[29]
Stimulation of HO–CO pathway	[85]
Stimulation of iNOS	[55]
Inhibition of iNOS	[85]
Increase in intracellular Ca^{2+}	[83]
Stimulation of TRPV1 channel	[91]

H_2S in the nervous system

In 1996 Abe and Kimura first demonstrated high expression of CBS in the rat hippocampus and cerebellum and H_2S production by brain homogenates *in vitro* [1]. H_2S generation in the brain was blocked by CBS but not CSE inhibitors and was markedly reduced in CBS-deficient mice [31]. In addition, physiological concentrations of NaHS facilitated hippocampal long-term potentiation, a synaptic model of learning and memory. NaHS *per se* had no effect on postsynaptic potential but concentration-dependently enhanced the NMDA-induced currents. Subsequently, the same research group has shown that H_2S increases

the sensitivity of NMDA receptors to glutamate in a cAMP-dependent manner [63].

H_2S may regulate not only neurocytic but also astrocytic function. Both H_2S and NaHS increase intracellular Ca^{2+} in primary cultures of rat brain astrocytes, which is achieved largely by Ca^{2+} influx from the extracellular space and to a lesser extent by its release from the intracellular stores [83]. Unlike in neurons, H_2S production in astrocytes is driven by the CSE and its effect on Ca^{2+} influx is mediated by cAMP and protein kinase A [67].

H_2S may also have some effects in the peripheral nervous system. In particular, accumulating body of evidence suggests that H_2S stimulates the capsaicin-sensitive sensory nerves and evokes the release of tachykinins such as substance P (SP) and neurokinin-A. H_2S induces a concentration-dependent contraction of the rat urinary bladder detrusor muscle [90]. However, this is not a direct effect on the muscle because it was abolished by the combination of neurokinin NK_1 and NK_2 receptor antagonists as well as by desensitizing afferent sensory nerves by high-dose capsaicin. The specific mechanism through which H_2S elicits this response is unclear, however, it is abolished by ruthenium red – a nonspecific blocker of transient receptor potential vanilloid receptor-1 (TRPV-1) calcium channel [91]. TRPV-1 is a nonselective cation channel which serves as a nonspecific receptor of sensory terminals for various noxious physical and chemical stimuli. These data suggest that H_2S may stimulate TRPV-1 or a related ion channel in the sensory nerve endings.

Dello Russo et al. [21] have demonstrated that H_2S donor, NaHS, as well as H_2S precursor and CBS activator, S-adenosylmethionine, decreased potassium-stimulated release of corticotropin releasing hormone (CRH) by the rat hypothalamic slices. In addition, SAM attenuated stress-induced increase in plasma glucocorticoids suggesting that H_2S may be a negative regulator of hypothalamo-pituitary-adrenal axis.

Implication of H_2S in central nervous system diseases

The CBS gene in humans is located on the chromosome 21 and, therefore, H_2S should be expected to be overproduced in the brain of patients with Down syndrome. In 2001 Belardinelli et al. demonstrated that urinary excretion of thiosulfate was increased two-fold in patients with Down syndrome in comparison to healthy individuals [5]. In contrast, excretion of sulfate, cysteine and taurine did not differ between groups. Assuming that thiosulfate is a specific end product of H_2S, these results indirectly suggest the overproduction of H_2S, and are consistent with lower plasma Hcy concentration [13] and sulfhemoglobin level [57] in these patients. It has been 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 [59].

Qu et al. [95] have observed that administration of NaHS or L-cysteine aggravated, whereas CBS or CSE inhibitors reduced the volume of brain infarct induced by unilateral occlusion of the middle cerebral artery. In addition, H_2S concentration in the cerebral cortex increased in that model of stroke. These data suggest a detrimental effect of H_2S in experimental stroke. This conclusion may explain the observation that high concentration of cysteine, presumably associated with high H_2S, inversely correlates with clinical outcome in patients with ischemic stroke [118].

However, there are also some data suggesting that H_2S may be protective for neurons and thus its deficiency in the brain may be detrimental. In particular, H_2S protects neurons against neurotoxicity of glutamate independently of the stimulation of excitatory amino acid receptors. Overproduction of glutamate is observed in certain pathological conditions, such as seizures, brain ischemia, trauma, etc. Neurotoxic effect of this excitatory amino acid is generally attributed to the overstimulation of its receptors. However, glutamate may exert a neurotoxic effect also through the receptor-independent manner referred to as "oxytosis". This mechanism of injury is associated with the inhibition of cystine transport to neurons by the x_c^- system (cystine/glutamate antiporter) which drives the import of cystine coupled to export of glutamate. Extracellular glutamate inhibits this exchange, leading to intracellular cysteine deficiency and to the impairment of glutathione (GSH) synthesis, which renders the cell more sensitive to oxidative stress. NaHS increases intracellular GSH concentration in cultured rat cortical neurons both under baseline conditions and when the cells are challenged with glutamate. In addition, NaHS protects cells against glutamate-

induced death. NaHS increases intracellular concentration of cysteine and γ-glutamylcysteine (γ-GC), an immediate GSH precursor, and its protective effect is abolished by the γ-GC synthase inhibitor, buthionine sulfoximine [65]. The mechanism through which H_2S stimulates the x_c^- transporter is unclear but is not associated with the reductive properties of this gas. Recently, Kimura et al. have demonstrated that the protective effect of H_2S against ischemia- or glutamate induced neurotoxicity is mimicked by K_{ATP} openers and abolished by K_{ATP} blockers in a mouse neuronal cell line [64].

The other mechanism through which H_2S might protect neurons is scavenging of reactive oxygen and/or nitrogen species. Whiteman et al. have demonstrated that H_2S reduces peroxynitrite-induced tyrosine nitration and attenuates its cytotoxicity in cultured human neuroblastoma SH-SY5Y cells [115]. Similarly, H_2S potently limited the toxic effect of hypochlorous acid (HClO) on these cells [116]. Thus, H_2S may play a beneficial role in nervous system pathologies associated with the increased generation of reactive oxygen and nitrogen species. One of such diseases is Alzheimer disease (AD). Interestingly, the concentration of H_2S in the brain of AD patients was demonstrated to be severely depressed in comparison to controls, which is probably associated with the deficiency of CBS activator, SAM [30]. It is suggested that H_2S deficiency may lead to the increased concentration of peroxynitrite and neuronal injury in AD patients. In addition, HClO is formed from H_2O_2 and Cl^- in the brain by myeloperoxidase. MPO activity and brain level of 3-chlorotyrosine, a marker of HClO-induced neuronal injury, are increased in AD patients [43]. Since H_2S scavenges HClO, its deficiency may also aggravate hypochlorite-induced cytotoxicity. Finally, considering the role of H_2S in long-term potentiation [1], its deficiency may directly contribute to cognitive impairment by compromising synaptic transmission.

Han et al. [46] have demonstrated that a CBS inhibitor, hydroxylamine, aggravated neuronal damage, whereas NaHS reduced damage induced in the rat by recurrent febrile seizures. Neither of these compounds had any effect on seizure development in response to raising body temperature [46]. In addition, febrile seizures resulted in the increase in CBS mRNA level in the hippocampus and H_2S concentration in plasma. Taken together, these results suggest that CBS-H_2S pathway may be up-regulated by seizures in order to protect neurons from seizure-induced damage. Further studies revealed that NaHS increases whereas hydroxylamine reduces the expression of γ-aminobutyric acid (GABA) receptor in rats subjected to seizures. Since seizures *per se* reduced the expression of GABA receptors, protective effect of H_2S against seizure-induced injury could be partially attributed to the improvement of inhibitory GABA neurotransmission [44]. It has also been suggested that CBS-H_2S and HO-1/CO pathways are synergistically up-regulated by seizures. Hippocampal HO-1 mRNA and plasma CO concentration increase after seizures, and administration of NaHS further increases their level whereas hydroxylamine has the opposite effect. On the other hand, the increase in hippocampal CBS mRNA and plasma H_2S concentration induced by seizures were attenuated by zinc protoporphyrin IX, a HO-1 inhibitor, and augmented by hemin, a HO-1 substrate [45].

H_2S in the cardiovascular system

Synthesis

While CBS is a major H_2S-generating enzyme in the brain, H_2S in the cardiovascular system is mainly produced by CSE. Immunohistochemical studies and reverse transcription-polymerase chain reaction revealed that CSE is expressed in vascular smooth muscle but not in endothelial cells [136]. One study [111] reported CBS expression in human umbilical vein endothelial cells, however, these cells were cultured for 14 days in the presence of high concentration of homocysteine, which could up-regulate this enzyme. Nevertheless, this study suggests that CBS may be induced in cardiovascular tissue under certain conditions.

Effects on blood pressure and vascular tone

Intravenous bolus injection of H_2S induces a transient dose-dependent decrease in mean arterial pressure in anesthetized rats [136]. *In vitro*, H_2S and NaHS relaxed rat thoracic aorta and portal vein preconstricted with norepinephrine. Importantly, H_2S relaxed also rat mesenteric arteries which are peripheral resistance vessels more significant for the regulation of vascular resistance and blood pressure than large conduit arteries [16]. In addition, H_2S exerted this effect at concentrations lower than required to relax the aorta. The effect of H_2S was mimicked by L-cysteine, and relax-

ing effect of L-cysteine was abolished by the CSE inhibitor, propargylglycine (PAG), suggesting that cysteine acted through its conversion to H_2S.

Initial studies demonstrated that neither endothelial denudation nor vascular denervation affected H_2S-induced vasorelaxation, suggesting the direct effect on smooth muscle cells [136]. However, subsequent more detailed study revealed that NOS inhibitors or endothelial removal shifted the dose-response curve of H_2S-induced relaxation to the right without affecting the maximal response, indicating that a small portion of the effect is endothelium-dependent [135]. Neither cyclooxygenase nor protein kinase A inhibitors had any effect on vasodilatory properties of H_2S, indicating that its effect was not mediated by prostacyclin or cyclic AMP. Also, soluble guanylyl cyclase inhibitor did not attenuate but rather augmented the vasodilatory effect of H_2S, demonstrating that cyclic GMP was not involved, in contrast to its well-known role in vascular action of NO and CO [135]. In the mesenteric artery, H_2S-induced relaxation at higher gas concentrations was weakened by endothelial removal or by the mixture of apamin and charybdotoxin which block potassium channels and are commonly used to inhibit the portion of endothelium-dependent vasorelaxation sensitive to endothelium-derived hyperpolarizing factor (EDHF) [16].

The currently available data indicate that H_2S relaxes blood vessels mostly, if not exclusively, by opening ATP-regulated potassium channels in the vascular smooth muscle cells. First, glibenclamide, a K_{ATP} channel antagonist, attenuated the hypotensive effect of H_2S *in vivo* and vasodilatory effect *in vitro* [136]. Second, vasodilatory effect of H_2S was attenuated when vessels were incubated in a high-K^+ medium. Third, patch-clamp studies have demonstrated that H_2S increases K_{ATP}-dependent current and induces hyperpolarization in isolated vascular smooth muscle cells [16, 136]. In smooth muscle cells isolated from the rat mesenteric artery, H_2S increased the open-probability of K_{ATP} channels without altering their conductance [107]. Interestingly, CSE inhibitors reduced K_{ATP} channel current indicating that endogenous H_2S continuously stimulated the channel under baseline conditions. Unlike direct effect on smooth muscle cells, the endothelium-dependent component of H_2S-induced vasorelaxation is independent of K_{ATP} channels [16]. It should be noted that NO and CO may directly activate smooth muscle cell Ca^{2+}-activated K^+ channel [119]. Although NO also activates K_{ATP} channels, this effect is indirect and is mediated by cGMP [82]. Thus, H_2S seems to have a unique mechanism of action among vasodilatory gases. Interestingly, H_2S is synthesized and has vasorelaxing properties in all vertebrates studied so far, i.e. fish, amphibians, reptiles, birds and mammals, and thus seems to be phylogenetically more ancient than NO which originated in amphibians during the evolution [26, 27].

The recent study [2] suggests that low doses of H_2S may induce vasoconstriction by scavenging endothelial NO. Mixing NaHS with NO donors inhibited the vasorelaxant effect of the latter *in vitro* and hypotensive *in vivo*. In addition, low concentrations of NaHS or H_2S gas reversed the relaxant effects of NO-dependent vasodilators such as acetylcholine and histamine but not that of NO-independent ones such as isoprenaline. *In vivo*, low doses of NaHS increase the mean arterial pressure in anesthetized rats [2]. Thus, H_2S may negatively modulate NO availability in the cardiovascular system. It should be noted that CO may also attenuate the NO-dependent vasorelaxation but not through the chemical interaction with NO but by competing with it for a common pool of soluble guanylate cyclase [68].

Myocardial contractility

NaHS decreases myocardial contractility both *in vitro* and *in vivo* [42]. This effect is attenuated, but not completely abolished, by pretreatment with glibenclamide, suggesting only partial involvement of K_{ATP} channels.

Central regulation of hemodynamics

Recently, Xiao et al. [122] have demonstrated that perfusion of the rat carotid sinus with the NaHS solution dose-dependently enhanced the reflex decrease in blood pressure induced by elevated intrasinus pressure. These data indicate that H_2S may regulate the hemodynamics also through its central effect on the baroreceptor reflex.

Role of H_2S in cardiovascular pathology

Arterial hypertension

As H_2S induces vasorelaxation, one may ask whether its deficiency contributes to the pathogenesis of arterial hypertension. Several data suggest that this may

indeed be the case, at least in some animal models. First, plasma H_2S concentration as well as aortic CSE mRNA expression and enzymatic activity 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 [124]. Administration of CSE inhibitor, PAG, decreases plasma H_2S concentration and aortic H_2S production and elevates blood pressure in normotensive rats but not in SHR, indicating that vascular H_2S is involved in the regulation of vascular tone under baseline conditions, and that H_2S-generating system is suppressed in the hypertensive strain. H_2S deficiency, decreased CSE activity and gene expression, and hypotensive effect of an exogenous H_2S donor have also been demonstrated in experimental hypertension induced by chronic inhibition of nitric oxide synthase [137]. Future studies have to address the question whether vascular H_2S is involved in human hypertension.

Atherosclerosis

Both NO and CO produced in the arterial wall inhibit atherogenesis through their anti-inflammatory, anti-platelet, and antiproliferative activities. Therefore, the question arises if H_2S is also involved in atherogenesis. *In vivo*, administration of NaHS attenuates vascular remodeling in spontaneously hypertensive rats, hypoxia-induced pulmonary hypertension, and in hypertension induced by chronic NOS blockade [124, 137]. These data suggest that H_2S may have some direct effects on the vascular wall. Indeed, H_2S suppresses endothelin-induced proliferation of rat aortic smooth muscle cells by down-regulating mitogen-activated protein kinases [29]. In addition, H_2S induces apoptosis of human aortic smooth muscle cells [127, 128]. Thus, H_2S might reduce the growth of atherosclerotic lesions. The other effect of H_2S relevant to atherogenesis is its influence on vascular inflammatory reaction, which plays an important role in plaque destabilization and rupture. This effect is, however, controversial. For example, H_2S has an anti-inflammatory effect on macrophages [85] but proinflammatory on vascular smooth muscle cells [55].

Vascular calcifications often develop in patients with hypertension and/or atherosclerosis. Calcifications reduce arterial compliance (increase arterial stiffness) as well as promote thrombosis and plaque rupture. Calcification development is associated with the transformation of smooth muscle cells into the osteoblast-like phenotype, the process accompanied by the expression of alkaline phosphatase (ALP), bone morphogenetic proteins (BMP), osteopontin, osteocalcin and osteonectin. Experimental vascular calcification induced in the rat by vitamin D and nicotine is associated with reduced plasma H_2S level and decreased expression and activity of CSE in the aortic wall. In addition, exogenous NaHS ameliorated calcification process as evidenced by the reduction of vascular Ca content, ALP activity and osteopontin expression [121].

If potential role of H_2S in atherogenesis is considered, one should first focus on patients with hyperhomocysteinemia. As may be concluded from the metabolic pathways of Hcy and H_2S (Fig. 1 and 2), deficiency of the latter may be associated with at least some forms of hyperhomocysteinemia. Hyperhomocysteinemia may result from homo- or heterozygous CBS deficiency (homocystinuria), reduced activity of methylenetetrahydrofolate reductase (MTHFR), or deficiency of vitamins B_6, B_{12} or folate. Whereas abnormalities of the remethylation pathway will shift homocysteine toward transsulfuration and thus will increase cysteine availability and presumably H_2S production, impaired transsulfuration due to CBS or vitamin B_6 deficiency will be expected to reduce H_2S synthesis (Fig. 1). Unfortunately, plasma H_2S has not been measured in various animal models of hyperhomocysteinemia or in humans with hyperhomocysteinemia of different etiology. In addition, the effect of exogenous H_2S on atherosclerosis progression in animal models unrelated to homocysteine should be investigated. It seems that three facts support, although very indirectly, the hypothesis that H_2S deficiency may contribute to atherogenesis. First, some studies indicate that the risk of acute cardiovascular events is not increased in humans with reduced activity of MTHFR despite elevated plasma homocysteine [75]. As stated previously, MTHFR deficiency should increase H_2S generation which may partially attenuate the proatherogenic effect of homocysteine. Second, the risk of cardiovascular events in patients with homocystinuria, especially those treated with vitamin B_6, is increased but is not as high as in patients with mild hyperhomocysteinemia, although plasma Hcy is much greater in the former group [54]. This dissociation between Hcy level and atherogenesis could be explained if we assume that vitamin B_6 supplementation increases cardiovascular CSE-dependent H_2S

generation in these subjects, despite only small reduction in total plasma homocysteine. Finally, progression of atherosclerosis is significantly slower in patients with Down syndrome, a state of H_2S overproduction [57]. Whether this is in fact a consequence of H_2S excess, remains to be established because hypohomocysteinemia could also play a protective role.

Myocardial injury

Myocardial cells contain large density of K_{ATP} channels consisting of inwardly rectifying K^+ channel Kir6.2 and a sulfonylurea receptor, SUR2A. Multiple studies have documented a protective effect of K_{ATP} channel activators in myocardial ischemia-reperfusion injury (for review, see [87]). Thus, it is of interest whether H_2S activates myocardial K_{ATP} channel and, if so, what is its effect on myocardial ischemic injury. Geng et al. [41] investigated the role of H_2S in the "infarct-like" myocardial necrosis induced in the rat by isoproterenol. H_2S concentrations in myocardium and plasma were by 60% lower in isoproterenol-treated rats, which was associated with reduced myocardial CSE activity despite up-regulation of CSE gene expression. Exogenous NaHS decreased the mortality rate of isoproterenol-treated rats as well as significantly attenuated the isoproterenol-induced decrease in myocardial contractility and ameliorated myocyte necrosis.

In the isolated perfused rat heart preparation, NaHS limited the size of infarction induced by left coronary artery ligation and this protective effect was abolished by K_{ATP} channel blockers [56]. These findings have been recently confirmed in the intact rat [138]. In addition, it has been demonstrated that CSE is expressed in the infarct area which is accompanied by the increase in plasma H_2S concentration. NaHS protected also cultured myocardial cells against hypoxia-induced death [138].

Myocardium subjected to episodes of sublethal ischemia becomes less sensitive to the subsequent more severe ischemic insult; the phenomenon referred to as "ischemic preconditioning". Perfusion of the isolated rat heart with H_2S before ischemia attenuated arrhythmias induced by the subsequent ischemia/reperfusion episode, and protected isolated cardiac myocytes against death induced by subsequent hypoxia [12]. Thus, exogenous H_2S applied before the ischemic insult may confer myocardial protection which was referred to as "sulfide preconditioning". In addition, blockade of endogenous H_2S production by PAG reduced the protective effect of ischemic preconditioning [12]. Moreover, H_2S production by cultured myocytes was markedly reduced by severe hypoxia but was stimulated by previous moderate hypoxia [12]. These data suggest that endogenous H_2S may be involved in the phenomenon of ischemic preconditioning. Apart from ischemia/hypoxia, myocardial protection may be conferred *in vitro* by the "metabolic inhibition", i.e. handling of myocardium in the absence of glucose and in the presence of the glycolysis inhibitor, 2-deoxy-D-glucose. Pan et al. [89] have demonstrated that preexposure to NaHS confers protection against myocardial necrosis induced by subsequent severe metabolic inhibition. In addition, CSE inhibitors attenuated cardioprotection induced by mild metabolic inhibition, suggesting the involvement of endogenous H_2S in this phenomenon. The mechanism through which H_2S confers myocardial protection is incompletely understood. Although in the above-mentioned studies it was abolished or at least attenuated by the K_{ATP} antagonists, other mechanisms such as ROS scavenging may also be important.

Pulmonary hypertension

In experimental pulmonary hypertension induced in the rat by a 3-week hypobaric hypoxia (hypoxic pulmonary hypertension, HPH), plasma H_2S concentration was reduced by about one third, which was accompanied by a twofold decrease in H_2S generation by lung homogenates, as well as by the reduced pulmonary CSE gene expression and enzymatic activity. Administration of NaHS decreased pulmonary arterial pressure, prevented right ventricular hypertrophy, and attenuated remodeling of pulmonary artery in this model [17]. In particular, NaHS reduced the accumulation of extracellular matrix in the vascular wall, inhibited the proliferation of smooth muscle cells, and reduced the expression of urotensin-II, a strong vasoconstricting and proliferatory peptide which is up-regulated in HPH [48]. Interestingly, plasma concentration of CO and expression of heme oxygenase-1 in pulmonary vasculature are increased in the HPH model and NaHS supplementation causes further stimulation of the HO-1/CO system [94]. These data suggest that H_2S may relieve pulmonary hypertension at least in part by stimulating the vasodilatory CO signaling. Plasma H_2S concentration, H_2S production in lung homogenates and CSE expression were also

reduced in pulmonary hypertension induced by high pulmonary blood flow produced by aortocaval shunt [123], and NaHS alleviated pulmonary artery remodeling, reduced pulmonary blood pressure and prevented right ventricular hypertrophy in this model [73].

H_2S in inflammation

Sepsis and septic shock

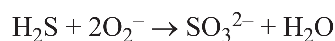
Septic shock, which often accompanies sepsis induced by infection with Gram-negative bacteria, is characterized by generalized vasodilation and hypotension. Overproduction of NO and CO by cytokine-inuced enzymes, inducible NO synthase and heme oxygenase-1, respectively, contributes to this vasodilation [130]. H_2S is also overproduced in vascular tissue of rats with experimental septic shock induced by coecal ligation and puncture, as well as in endotoxemic shock induced by lipopolysaccharide (LPS) administration [50]. In addition, H_2S level negatively correlates with blood pressure and myocardial contractility, suggesting its pathogenic role in the hemodynamic collapse. Notably, at least one study has demonstrated that LPS-induced hypotension is attenuated by glibenclamide suggesting the involvement of abnormal activation of K_{ATP} channels [40]. In addition, Mok et al. [81] have demonstrated that plasma H_2S is increased in rats with hemorrhagic shock and that CSE inhibitors and glibenclamide increase mean arterial pressure in these animals, which indicates that deleterious hemodynamic effect of H_2S excess is not confined to the septic shock.

Several subsequent studies confirmed that plasma H_2S concentration as well as CSE expression and activity in the liver and kidney are increased in LPS-treated mice [72] and in cecal ligation and puncture model of sepsis in the mouse [133]. In addition, these studies strongly suggest that H_2S not only contributes to hypotension but also augments the inflammatory response and end-organ damage associated with sepsis. Thus, PAG treatment attenuates the inflammatory response as evidenced by the reduction of myeloperoxidase activity (a marker of neutrophil infiltration) in the lung and liver in a mouse model of sepsis [133]. In addition, PAG reduced mortality after cecal ligation and puncture [133]. In contrast, NaHS aggravated histological lesions in the lung and liver as well as increased the tissue MPO activity and plasma tumor necrosis factor-α (TNF-α) concentration [133]. The similar results were obtained in LPS-injected rat [18] and mice [72]. Interestingly, in LPS-injected rats PAG reduced liver and skeletal muscle damage without altering blood pressure, which suggests that its protective effect was independent of the improvement of hemodynamics [18].

Pro- and antiinflammatory effects of H_2S

The above-mentioned *in vivo* studies strongly suggest that H_2S is a proinflammatory mediator. However, the mechanism through which H_2S aggravates inflammation is not clear. In addition, the results of *in vitro* experiments are not so unambiguous and demonstrate either pro- or antiinflammatory potential of H_2S. For example, Na_2S inhibited fMLP-induced chemotaxis and degranulation of polymorphonuclear leukocytes [76]. In addition, H_2S donors inhibited aspirin-induced leukocyte adhesion to the endothelium of rat mesenteric venules, whereas inhibitors of H_2S synthesis elicited leukocyte adhesion [132]. On the other hand, NaHS inhibited apoptosis of isolated human neutrophils while having no effect on their bactericidal properties [99]. Interestingly, in the same study NaHS had no effect on viability of eosinophils and enhanced apoptosis of lymphocytes. In cultured murine RAW264.7 macrophages, H_2S solution suppressed the LPS-induced expression of inducible NO synthase (iNOS). This effect was mediated by H_2S-induced activation of ERK, increased expression of heme oxygenase-1 and carbon monoxide production, and CO-mediated inhibition of proinflammatory transcription factor, nuclear factor-κB [85]. Interestingly, LPS as well as proinflammatory cytokines upregulated the CSE gene expression, which may explain other observations of increased H_2S production in various inflammatory states [85].

Recently, Mitsuhashi et al. [80] have demonstrated that fMLP-activated neutrophils nonenzymatically convert H_2S to sulfite (SO_3^{2-}) in NADPH oxidase and ROS-dependent manner, most likely through the following reaction:



$$H_2S + 2O_2^- \rightarrow SO_3^{2-} + H_2O$$

These observations were confirmed *in vivo* by demonstrating that LPS increased serum sulfite level [80], and are consistent with previous findings that

LPS induced sulfite production by isolated leukocytes [79]. Serum sulfite is increased in patients with pneumonia [78], as well as in LPS-treated rats [79]. Sulfite may be an important bactericidal compound produced by inflammatory cells, but may also contribute to tissue injury during inflammation. Normal level of sulfite in tissues and body fluids is very low due to its rapid conversion to sulfate by sulfite oxidase. However, increased concentration of sulfite is highly toxic as observed in sulfur dioxide poisoning or in patients with inherited sulfite oxidase deficiency [88]. Indeed, sulfite itself dose-dependently stimulates the oxidative burst of neutrophils [66] and their adhesion to the endothelium [102]. Sulfite may also react with peroxynitrite to form toxic sulfite radicals [98]. These observations suggest that overproduction of H_2S during the inflammation, although primarily aimed to enhance nonspecific host defense, may perpetuate the inflammatory reaction and cause tissue damage.

H_2S in local inflammatory reactions

Several studies indicate that H_2S is overproduced not only in sepsis but also in more localized forms of inflammation. For example, experimental acute pancreatitis induced in the mouse by repeated caerulein administration is associated with the increase in CSE mRNA expression level and enzymatic activity in the pancreas. Moreover, PAG administered before or after caerulein reduced pancreatic acinar cell injury, decreased MPO activity and inflammatory cell infiltration in the pancreatic tissue, and partially normalized plasma amylase activity. In addition, PAG ameliorated pancreatitis-associated lung inflammation [9].

Hindpaw edema induced by intraplanar injection of carrageenan in the rat is a commonly used model of local inflammation and a good example of ambiguous results concerning the role of H_2S in inflammatory reaction. Bhatia et al. [8] have observed that carrageenan increases local H_2S formation and that PAG reduces edema formation as well as leukocyte infiltration in this model. On the other hand, Zanardo et al. have observed that carrageenan-induced paw edema was suppressed by NaHS and Na_2S and enhanced by inhibitors of H_2S synthesis [132]. Suppression of edema formation by H_2S donors was mimicked by K_{ATP} channel agonists and reversed by their antagonists. The reason for the discrepancies between these two studies [8, 132] is not clear.

H_2S in neurogenic inflammation

Stimulation of afferent sensory nerves may contribute to the inflammatory response through the so-called "neurogenic inflammation", associated with the release of substance P (SP), neurokinin-A and calcitonin gene-related peptide (CGRP). These mediators induce a series of inflammatory responses, especially in the airways, which include vasodilation, extravasation of plasma proteins leading to edema, bronchoconstriction, mucus secretion and recruitment of inflammatory and immune cells. Trevisani et al. [110] have demonstrated that NaHS, similarly to capsaicin, induced CGRP and SP release from the sensory nerves in the guinea-pig airways. NaHS induced a dose-dependent contraction of isolated bronchial and tracheal rings and its effect was abolished by the desensitization of sensory nerves with high concentration of capsaicin, by TRPV1 antagonists, capsazepine and ruthenium red, as well as by a mixture of neurokinin NK_1 and NK_2 receptor antagonists. Interestingly, intraperitoneal injection of NaHS to healthy mice induced substantial inflammatory reaction in the lung as evidenced by increased concentration of substance P, proinflammatory cytokines, TNF-α and interleukin-1β (IL-1β), and lung MPO activity [10]. These effects were abolished by a specific NK_1 receptor (a substance P receptor) antagonist, CP-96,345, but not by NK_2 or CGRP receptor antagonists. In addition, the inflammatory effect of H_2S was abolished by capsazepine and was not observed in mice lacking substance P and neurokinin-A due to the knockout of their common precursor gene, preprotachykinin-A [10]. These data indicate that H_2S *per se* may induce neurogenic inflammation even in the absence of other noxious insults.

Is H_2S a mediator of inflammation in humans?

Interestingly, before the "H_2S research era" Lyons et al. demonstrated that plasma sulfhemoglobin was higher in children with sepsis than in healthy controls, which indirectly suggested the overproduction of H_2S [74]. In addition, plasma H_2S concentration is increased by almost 50% in patients with stable chronic obstructive bronchopulmonary disease (COPD) in comparison to the control group [15]. Interestingly, in patients with exacerbated COPD, H_2S level was lower than in those with stable disease and was inversely correlated with pulmonary artery systolic pressure, which suggests that pulmonary hypertension *per se* has a deleterious effect on H_2S production in humans.

Effect of hydrogen sulfide on 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. In contrast to vascular K_{ATP} channels which consist of Kir6.2 and sulfonylurea receptor SUR2B, pancreatic K_{ATP} channel contains Kir6.2 and SUR1. Pancreatic K_{ATP} channels play a major role in the regulation of insulin secretion. Indeed, increased concentration of glucose leads to the accumulation of ATP in the cell, blockade of K_{ATP} channels, depolarization of plasma membrane, Ca^{2+} influx and insulin secretion. Transfection of cultured rat insulinoma cell line, INS-1E, with the adenovirus containing the CSE gene as well as exogenous H_2S inhibit glucose-induced insulin release, which is associated with the increase in open probability of K_{ATP} channel. In contrast, lowering of endogenous H_2S by PAG or CSE-targeted short interfering mRNA has the opposite effect. These data suggest that endogenous H_2S inhibits insulin secretion. In addition, glucose reduces islet H_2S production, suggesting that down-regulation of H_2S may contribute to glucose-induced insulin secretion [129]. The similar results were obtained in isolated mouse islets [60], but the authors of that study suggested that H_2S acted by inhibiting glucose metabolism and ATP production rather than by a direct effect on K_{ATP} channels. Since plasma concentration of L-cysteine as well as the expression of CBS and CSE in various tissues are increased in patients with diabetes [53], overproduction of H_2S may contribute to the impairment of insulin secretion. Indeed, Yusuf et al. [131] have recently demonstrated that although plasma H_2S is unchanged in rats with streptozotocin-induced diabetes, its production by pancreatic and liver homogenates is increased. CBS expression in the pancreas was also higher in diabetic animals and all these abnormalities were corrected by insulin therapy [131].

H_2S in the gastrointestinal system

Both CSE and CBS are expressed in the gastric mucosa and endogenous H_2S seems to be a protective factor against mucosal injury. Both acetylsalicylic acid (ASA) and nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the expression of CSE gene and H_2S production in the gastric mucosa. NaHS prevents the reduction of mucosal blood flow induced in rats by ASA and NSAIDs [34]. In addition, NaHS reduces NSAIDs-induced adherence of leukocytes to vascular endothelium and mucosal leukocyte infiltration assessed as MPO activity, normalizes increased expression of TNF-α and intracellular adhesion molecule-1 (ICAM-1), and improves prostaglandin E_2 synthesis impaired by these agents [34]. Moreover, NaHS attenuates histological lesions of the gastric mucosa.

Several studies have demonstrated that H_2S reduces spontaneous or acetylcholine-induced contractility of the ileum in various animal species [49, 109]. In addition, NaHS administered intraperitoneally relaxes the rat colon *in vivo* [23]. In contrast to the relaxing effect on rabbit or guinea-pig ileum observed *in vitro* [49, 109], the effect on colon contractility was abolished by glibenclamide [23].

CBS and CSE were immunohistochemically detected in guinea pig and human colonic submucosal and myenteric nerve plexuses. Serosal application of NaHS or L-cysteine stimulated luminal chloride secretion by guinea pig and human colonic tissues [100]. This effect was blocked by tetrodotoxin, desensitization of afferent nerves with capsaicin, or TRPV1 antagonist, capsazepine. In addition, secretory effect of NaHS was not observed in human colonic epithelial cell line, T84. Taken together, these data indicate that H_2S is generated in the enteric nervous system and indirectly stimulates mucosal secretion by acting on TRPV1-containing sensory nerve endings which then send collaterals to the mucosa or to submucosal secretomotor neurons [100]. Thus, H_2S is involved in the regulation of gut motility and secretory function.

Recently, Distrutti et al. [23] have demonstrated that NaHS dose-dependently ameliorates visceral nociception evoked in the rat by colorectal distension (CRD). This effect could not be attributed to colonic relaxation since the latter was observed only at the highest NaHS dose. Rather, antinociceptive effect might be associated with the direct impact on neurotransmission since NaHS attenuated the CRD-induced increase in c-Fos gene expression in the spinal cord. As expected, in animals with experimentally-induced colitis, the behavioral response to CRD was much greater than in control rats. NaHS almost completely abolished the allodynic response (perception of non-painful stimuli as painful) and markedly reduced hyperalgesia (perception of painful stimuli as more painful) in animals with colitis [23]. Experimental colitis induced by trinitrobenzene sulfonic acid was associ-

ated with the increase in CBS and CSE expression in the colonic mucosa and slight increase in CSE expression in the spinal cord [24].

Fiorucci et al. [35] have recently demonstrated that H_2S attenuates the norepinephrine-induced vasoconstriction in the liver of healthy rat as well as in animals with experimental liver cirrhosis induced by bile duct ligation. It was shown that CSE was expressed in hepatocytes and hepatic stellate cells but not in hepatic endothelial cells, and that H_2S relaxed the isolated stellate cells thus contributing to the relaxation of hepatic microvessels. Experimental cirrhosis induced by either bile duct ligation or carbon tetrachloride administration is associated with the reduced expression of CSE, decreased production of H_2S by liver homogenates, and the decrease in plasma H_2S concentration [35]. Relaxing effects of L-cysteine on hepatic stellate cells as well as on hepatic microvessels are impaired in cirrhotic animals, whereas relaxing effects of NaHS are intact suggesting reduced formation of H_2S from cysteine and normal sensitivity to this gas. These findings are complementary to the increase in hepatic vascular resistance in cirrhotic animals and humans and suggest that H_2S deficiency may contribute to the development of portal hypertension. Although H_2S level in human cirrhosis has not been studied, it is well known that cirrhosis is associated with the reduced flux of homocysteine through the transsulfuration pathway [39]. Endogenous H_2S may also be involved in the regulation of bile secretion. Indeed, PAG stimulates choleresis and biliary bicarbonate excretion and this effect is reversed by NaHS [37].

It should be noted that colonic mucosa is continuously exposed to high amounts of H_2S generated from alimentary sulfate by commensal sulfate-reducing bacteria. It has been suggested that H_2S of bacterial origin may contribute to various bowel diseases including ulcerative colitis and colorectal cancer [51]. It has been demonstrated that H_2S stimulates proliferation of cultured rat intestinal epithelial cells, IEC-18 [22]. Interestingly, H_2S up-regulated the expression of a potent angiogenic factor, vascular endothelial growth factor (VEGF), which is involved in tumor vascularization. Colonic mucosa metabolizes H_2S very efficiently due to high expression of rhodanese at the luminal surface of enterocytes [96], and is able to adapt metabolically to sulfide excess [69]. Indeed, colonic cells are much less sensitive to genotoxic effect of H_2S than other cell lines [4]. Intestinal expression of rhodanese is stimulated by H_2S and increases during epithelial cell differentiation and, interestingly, is lower in patients with ulcerative colitis or colorectal cancer than in healthy controls [96].

Tab. 2. Diseases associated with changes in H_2S generation

	Ref.
Increased H_2S formation:	
Down syndrome	[5*[a], 57*[b]]
septic shock	[74]*[b]
NSAID-induced gastric mucosal injury	[34]
colitis	[24]
caerulein-induced pancreatitis	[9]
diabetes mellitus	[131]
myocardial ischemia/reperfusion	[138]
ischemic preconditioning	[12, 89]
COPD	[15]*
ischemic stroke	[95]
febrile seizures	[46]
Decreased H_2S formation:	
spontaneously hypertensive rats	[124]
arterial hypertension induced by NOS blockade	[137]
hypoxia-induced pulmonary hypertension	[15]*
isoproterenol-induced myocardial injury	[41]
myocardial ischemia/reperfusion injury	[12]
NSAID-induced gastric mucosal injury	[34]
liver cirrhosis	[35, 25*[c]]
Alzheimer's disease	[30]*

* demonstrated in humans, [a] indirect evidence (increased urinary thiosulfate), [b] indirect evidence (increased plasma sulfhemoglobin), [c] indirect evidence (reduced Hcy flux through the transsulfuration pathway)

H_2S in pharmacotherapy

As may be concluded from the data presented above, production of endogenous H_2S is altered in many diseases, at least in experimental studies (Tab. 2). In addition, both exogenous and endogenous H_2S has been demonstrated to exert either protective or deleterious effect in many pathologies (Tab. 3). Thus, the question arises if pharmacological modulation of H_2S level could be of a potential therapeutic value.

In theory, the level of H_2S may be modulated by any of four approaches: (1) administration of H_2S itself or of currently available H_2S donors, (2) administration of specific CBS or CSE inhibitors, (3) generating new H_2S-releasing compounds, (4) targeting H_2S production by currently used drugs. With regard to the first possibility, a therapeutic potential of H_2S gas seems to be limited due to difficulties in obtaining precisely controlled concentrations and possible toxic impact of H_2S excess, although in view of attempts to use inhaled NO or CO [120] it cannot be definitely excluded. 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. Also 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, PAG is a lead-containing compound and thus is expected to be toxic after long-term administration. Finally, inhibitors other than PAG have a very limited ability to permeate plasma membranes. More suitable H_2S donors as well as inhibitors of H_2S producing enzymes are currently under development. However, even completely specific CBS or CSE inhibitors will not only affect H_2S level but also Hcy metabolism, which may be unbeneficial in certain circumstances.

Tab. 3. Diseases in which H_2S has been demonstrated to be protective or deleterious

	Ref.
Protective	
arterial hypertension	[124, 137]
pulmonary hypertension	[17, 73]
myocardial ischemia/reperfusion injury	[12, 138]
erectile dysfunction	[103]
gastric mucosal injury	[34]
colitis, irritable bowel syndrome	[24]
neuronal damage induced by febrile seizures	[46]
Deleterious	
septic shock	[18, 72]
caerulein-induced pancreatitis	[9]
ischemic stroke	[95]

Only those diseases are listed in which the effect of H_2S donors or inhibitors of H_2S-producing enzymes on the disease course was examined in *in vivo* models

H_2S may also be affected by currently used drugs or their derivatives. For example, L-arginine supplementation, primarily expected to augment NO formation [20], also corrects the impaired H_2S production in high blood flow-induced pulmonary hypertension [125]. Recently, Anuar et al. [3] have demonstrated that an NO-releasing derivative of anti-inflammatory drug flurbiprofen, nitroflurbiprofen, dose-dependently inhibits hepatic H_2S production and CSE mRNA in LPS-treated rats. In addition, nitroflurbiprofen but not its parent drug decreases plasma concentration of pro-inflammatory cytokines, TNF-α and IL-1β, as well as MPO activity [3]. These data suggest that H_2S may be targeted by drugs which affect nitric oxide. Distrutti et al. [24] have synthesized a mesalamine derivative, ATB-429, and examined its therapeutic potential in rats with experimentally induced colitis. Mesalamine is widely used to maintain remission in inflammatory bowel diseases such as ulcerative colitis, and ATB-429 is a derivative of mesalamine which contains also an H_2S-releasing moiety. Intraperitoneally administered ATB-429 increased plasma H_2S concentration and markedly reduced the nociceptive response to colorectal distension in colitic rats, whereas mesalamine had no antinociceptive effect [24]. In addition, long-term treatment with ATB-429 reduced the expression of inflammatory markers, cyclooxygenase-2 and IL-1β, in the colon [24]. Acetylsalicylic acid and nonsteroidal anti-inflammatory drugs have an inhibitory effect on the CSE-H_2S pathway in gastrointestinal mucosa [34]. This effect may contribute to gastric mucosal injury induced by these drugs but, on the other hand, may be involved in their antineoplastic effect in the gastrointestinal tract, since H_2S facilitates the neoplastic growth of intestinal epithelial cells [22]. Interestingly, NSAIDs reduce CSE expression also in cultured human renal epithelial cell line, HEK293, and NaHS exerts a protective effect against NSAIDs-induced injury in these cells [34]. 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. Thus, unbeneficial modulation of H_2S signaling may also contribute to side effects of certain drugs.

It should be noted that H_2S overproduction in a given disease does not necessarily indicate that it is a deleterious agent (Tab. 2 and 3). If its deleterious role has been established by protective effect of CBS/CSE inhibitors, it may be concluded that H_2S is indeed a link in the pathogenetic chain. In such cases (e.g. sepsis) blocking H_2S production is a potential therapeutic strategy. However, H_2S overproduction may also be associated with its protective role (e.g. febrile seizures). In such cases, H_2S production may be considered as an adaptive response, although probably insufficient to block disease development, and additional supplementation of H_2S may be therapeutically useful. Deficiency of H_2S is observed in diseases in which it plays a protective role, e.g. arterial hypertension, gastric mucosal injury, etc., and H_2S donors may provide some therapeutic benefit. In theory, reduced production of H_2S could also be a compensatory response to limit its unbeneficial effects, however, such examples are not known to date. Until now, H_2S generation was not specifically targeted in humans. Clearly, the effect of currently used therapies on H_2S level in human diseases should be explored before any attempts to use specific H_2S modulators are made.

Taking into account the potential role of H_2S deficiency in arterial hypertension and atherosclerosis, the effect of drugs widely used in pharmacotherapy of cardiovascular diseases such as diuretics, angiotensin-converting enzyme inhibitors, β-adrenergic antagonists, statins, fibrates, thiazolidinediones, nitrovasodilators and ASA administered at low "cardiologic" doses on H_2S metabolism is of great interest, but was not explored yet, even in animals. Finally, because K_{ATP} channels mediate many effects of H_2S (Tab. 1), sulfonylurea derivatives widely used in the treatment of type 2 diabetes may interfere with the effects of endogenous H_2S.

Conclusions and perspectives for the future

Taken together, the data presented above strongly suggest that apart from NO and CO, H_2S is another inorganic gaseous mediator in the cardiovascular and nervous system. However, our current knowledge about its role in physiology and pathology is still fragmentary. Many effects of H_2S are controversial. For example, H_2S has been demonstrated to either stimulate or inhibit certain intracellular transduction pathways (Tab. 1), to stimulate [22] or inhibit [29, 126] cell proliferation, to activate [127, 128] or block [99] apoptosis, to be overproduced [138] or deficient [12, 41] in myocardial ischemia, to be pro- [8] or antiinflammatory [132] in the model of hindpaw edema, etc. Only few studies demonstrated alterations in H_2S level in human diseases (Tab. 2), and in most cases it was done indirectly by measuring H_2S-related compounds such as thiosulfate [5] or sulfhemoglobin [74] rather than H_2S itself. Given a potential role of H_2S in cardiovascular pathology, its level should be examined in patients with various risk factors of atherosclerosis such as arterial hypertension, hyperlipidemia, diabetes mellitus, etc., and the relationship between H_2S and the progression of atherosclerosis must be addressed in the progressive studies. Until now only one cardiovascular risk factor, i.e. smoking, was demonstrated to reduce H_2S level in humans [15]. Novel exciting aspects of the H_2S research continuously emerge. For example, given that H_2S is quenched by ROS [115], and considering the important role of oxidative stress in many diseases such as atherosclerosis, arterial hypertension, Alzheimer's disease, etc., one may wonder if excessive ROS production may cause H_2S deficiency. Whereas the mechanisms regulating CSE and CBS expression and activity are known only fragmentarily, virtually nothing is known about the regulation of H_2S-degrading enzymes such as rhodanese. Fortunately, some of the controversial issues are being explained. For example, the effect of H_2S on K_{ATP} channels in myocardium and insulin-secreting cells, outlined among future directions in the conclusions of a previous review in this field published 3 years ago [7], is now to large extent elucidated [6, 12]. This allows us to hope that some of the controversial aspects outlined above may also be clarified in the near future.

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