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Synthesis and Biological Effects of Hydrogen Sulfide (H_2S): Development of H_2S-Releasing Drugs as Pharmaceuticals

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Introduction

Gaseous transmitters are a growing family of regulatory molecules involved in multilevel regulation of physiological and pathological functions in mammalian tissues.[1] Hydrogen sulfide (H_2S[a]) is best known for its characteristic smell of rotten eggs. It is now widely recognized that H_2S, along with nitric oxide (NO) and carbon monoxide (CO), is involved in a multitude of physiological functions.[2] The generation of H_2S by mammalian tissues is likely to occur in a slow and constant rate, and it appears to be involved in several processes including neuromodulation,[3] hypertension,[4] inflammation,[5,6] edema,[7] hemorrhagic shock,[8] pain perception,[9] gastric mucosal integrity,[10] and vascular tone.

This Perspective has been designed in order to give to the reader an updated overview on the physiology and biochemistry of H_2S. In particular we have summarized the effects of H_2S inhibitors and H_2S donors in animal models of disease ordered for apparatus. Finally there is a section that addresses the potential for therapeutic exploitation of H_2S and provides an update on the patents so far filed.

Hydrogen Sulfide (H_2S): Chemistry and Metabolic Pathways

H_2S is a gas with a structure is very similar to that of water, but this is where the similarity ends (Figure 1). The sulfur atom is not nearly as electronegative as oxygen so that hydrogen sulfide is much less polar than water. Because of this, comparatively weak intermolecular forces exist for H_2S and the melting and boiling points are much lower than they are in water. The boiling temperatures of hydrogen sulfide and water are −60.7 and +100.0 °C, respectively.

Hydrogen sulfide is weakly acidic, dissociating in aqueous solution into hydrogen cation (H^+) and hydrosulfide anion (HS^-), which subsequently may decompose to H^+ and sulfide ion (S^{2-}) ($K_{a1} = 1.3 \times 10^{-7}$ M, $K_{a2} = 1 \times 10^{-19}$ M (Scheme 1).[11−13] Under physiological conditions, i.e., at pH 7.4, one-third of hydrogen sulfide is undissociated and present in biological fluids as H_2S. Conversely, the chemical form S^{2-} is not present in appreciable amounts, since the dissociation of HS^- occurs only at high pH values.

Hydrogen sulfide is rapidly oxidized, mainly in mitochondria, initially to thiosulfate and subsequently to sulfite and sulfate. This oxidation is not enzymatically driven, while thiosulfate conversion to sulfate and/or sulfite is catalyzed by thiosulfate cyanide sulfurtransferase (TST).

Also, sulfite originating through this reaction is quickly oxidized to sulfate, as sulfate is the major end-product of H_2S metabolism under physiological conditions; however, urinary thiosulfate is considered to be a nonspecific marker of whole-body H_2S production.[14]

Another catabolic pathway is represented by methylation by thiol-*S*-methyltransferase (TSMT) to methanethiol and dimethyl sulfide. This reaction occurs mainly in cytosol, and some studies have questioned the significance of this pathway in the gastrointestinal tract.[15,16] Methemoglobin, considered a "common sink" for endogenous gases including CO and NO, also binds H_2S as sulfhemoglobin (Scheme 2).[17]

Toxic effects of hydrogen sulfide in humans (at concentrations of < 100 ppm) include eye irritation, sore throat, dizziness, nausea, shortness of breath, and chest tightness.[18,19] Exposure to hydrogen sulfide at > 1000 ppm may cause severe adverse effects, especially for the central nervous system (CNS) and respiratory depression, ranging from loss of consciousness to death.[20] The primary cause of death from H_2S poisoning has been attributed to respiratory paralysis.[18] In addition, pulmonary edema has consistently been reported as the single most notable lesion in autopsies of individuals killed by H_2S poisoning.[21] At present, although the mechanism of action for these toxic effects is not clear, it is widely believed that H_2S targets mitochondria at low micromolar concentrations via reversible inhibition of cytochrome *c* oxidase.[19] Measurement of the concentration of thiosulfate in blood and urine is useful for determining hydrogen sulfide poisoning.[22]

In mammals, H_2S is endogenously produced by enzymatic reactions, even if some nonenzymatic pathways are involved in the biochemistry of hydrogen sulfide. H_2S is present in micromolar concentrations in blood[23] and can be synthesized from L-cysteine primarily via two enzymes: cystathionine-γ-lyase (CSE) and cystathionine-β-synthetase (CBS).[24] L-Cysteine is a sulfur-containing amino acid derived from alimentary

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[a] Abbreviations: AdoMet, *S*-adenosyl-L-methionine; BCA, β-cyanoalanine; CBS, cystathionine-β-synthetase; CD, Crohn's disease; CLP, cecal ligation puncture; CNS, central nervous system; CO, carbon monoxide; CRD, colorectal distension; CSE, cystathionine-γ-lyase; CTH, cystathionase; Hcy, homocysteine; H_2S, hydrogen sulfide; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PAG, propargylglycine; PDE5, phosphodiesterase-5; PKA, protein kinase A; PLP, pyridoxal 5′-phosphate; SP, substance P; TNF-α, tumor necrosis factor α; TSMT, thiol-*S*-methyltransferase; TST, thiosulfate cyanide sulfurtransferase; UC, ulcerative cholitis; ZDF, Zucker diabetic fatty.

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sources, synthesized from L-methionine through the so-called "trans-sulfuration pathway" with homocysteine (Hcy) as an intermediate or liberated from endogenous protein (Scheme 3).[25,26]

Another pathway that leads to the release of H_2S is represented by a "desulfhydration" reaction, consisting of the removal of the cysteine sulfur atom without its oxidation. This process may be catalyzed by both trans-sulfuration pathway enzymes, CBS and CSE. On the other hand, the H_2S catabolic pathway is not well-defined yet, and most of the data have been obtained by using exogenous H_2S.

Figure 1. Similarity in molecular structure between water (H_2O) and hydrogen sulfide (H_2S).

Scheme 1. Hydrogen Sulfide Dissociation

$$H_2S \underset{}{\overset{Ka_1}{\rightleftharpoons}} H^+ + HS^- \overset{Ka_2}{\rightleftharpoons} H^+ + S^{2-}$$

Scheme 2. Catabolism of H_2S[a]

a) $2\,HS^- + 2\,O_2 \longrightarrow \underset{\text{thiosulfate}}{S_2O_3^{2-}} + H_2O$

$$S_2O_3^{2-} + CN^- \xrightarrow{TST} \underset{\text{thiocyanate}}{SCN^-} + \underset{\text{sulfite}}{SO_3^{2-}}$$

b) $H_2S \xrightarrow{TSMT} \underset{\text{methanethiol}}{CH_3SH} \xrightarrow{TSMT} \underset{\text{dimethylsulfide (DMS)}}{CH_3\text{-}S\text{-}CH_3}$

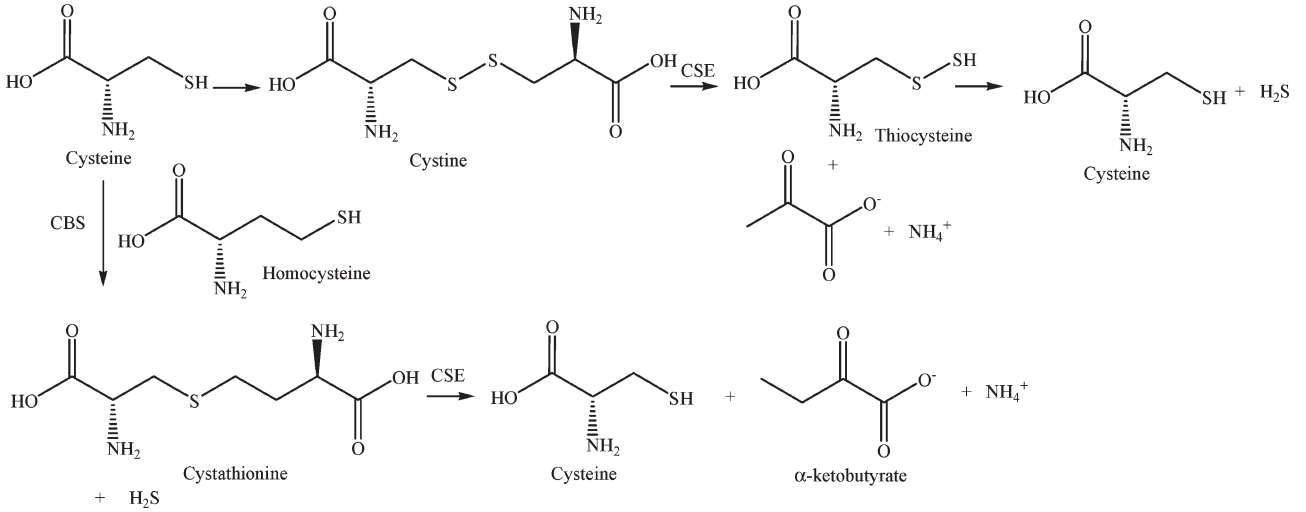
c) H_2S + methemoglobin $\longrightarrow$ sulfhemoglobin

[a] (a) mitochondrial oxidation; (b) cytosolic methylation; (c) binding to hemoglobin. SO: sulfite oxidase. TSMT: thiol-*S*-thiomethyl transferase. TST: thiosulfate cyanide sulfur transferase (rhodanese).

CBS and CSE are responsible for the majority of the endogenous production of H_2S in mammalian tissues and have been detected in human and other mammalian cells.[27] Their expression is thought to be tissue-specific.[23] Indeed, CBS is expressed in hippocampus, cerebellum, cerebral cortex, and brainstem, and its activity is 30-fold greater than CSE.[28] On the other hand, CSE expression and activity have been shown to be higher than CBS in aorta, mesenteric artery, portal vein, and other vascular tissue.[23,29] In some tissues, CSE and CBS are both required for H_2S synthesis, whereas in others only one of these enzymes is necessary.[1] CSE appears to be the predominant enzymatic source of H_2S in the vasculature and heart,[1] while in the central nervous system (CNS) CBS predominates.[1,30] As reported above, H_2S formation is related to the activity of both CBS and CSE, which are pyridoxal 5′-phosphate (PLP) dependent enzymes that differ in their specific mechanism (Scheme 3).

CSE, also known as cystathionase (CTH), is mainly able to convert cystathionine to cysteine by catalyzing the elimination reactions of L-homoserine to form H_2O, NH_3, and 2-oxobutanoate, of L-cystine to produce thiocysteine, pyruvate, and NH_3, and of L-cysteine to produce pyruvate, NH_3, and H_2S.[31] CSE is a protein of 405aa and is a tetramer formed by two homodimers, both contributing to the active site pocket. On the other hand, CBS is a cystathionine-forming enzyme even if, in general, it catalyzes β-replacement reactions between L-serine, L-cysteine, cysteine thioethers, or some other β-substituted α-L-amino acids, and a variety of mercaptans.[32−34] In cases in which the condensation reaction involves cysteine and homocysteine, the final products will be H_2S and cystathionine. Recently Qingxiang et al. reported the crystal structures of human CSE (hCSE), in apo form and in complex with PLP and PLP-DL-propargylglycine (PAG).[35] Structural characterization and biophysical and biochemical studies provide new insights into the inhibition mechanism of hCSE-mediated production of H_2S. Transition from the open form of apo-hCSE to the closed PLP-bound form reveals large conformational changes hitherto not reported. In addition, PAG binds hCSE via a unique binding mode not previously observed in PAG−enzyme complexes. The interaction of PAG-hCSE was not predicted based on existing information from known PAG complexes. The structure of hCSE.PLP-PAG complex

Scheme 3. Endogenous Hydrogen Sulfide Synthesizing Pathways[a]

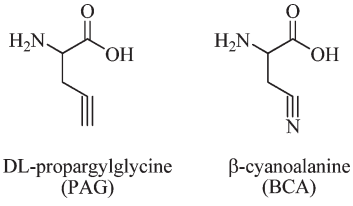


[a] Homocysteine is the physiological substrate for CBS that in the presence of cysteine releases both H_2S and cystathionine. Cystine and cystathionine are both substrates for CSE, which is able to produce both cysteine and thiocysteine. The latter can release H_2S in a nonenzymatic manner.

highlights the particular importance of Tyr_{114} in hCSE and the mechanism of PAG-dependent inhibition of hCSE. These results provided significant insights, which will facilitate the structure-based design of novel inhibitors of hCSE to aid in the development of therapies for diseases involving disorders of sulfur metabolism.

CBS (cystathionine β-synthase) domains are small protein motifs, usually associated in tandem, that are involved in binding to adenosyl groups.[36,37] Human CBS is a homotetramer consisting of 63 kDa subunits which bind two cofactors, pyridoxal 5′-phosphate (PLP) and heme. Each CBS subunit of 551 amino acid residues binds two substrates (homocysteine and serine) and is further regulated by *S*-adenosyl-L-methionine (AdoMet).[37] While the role of heme in CBS activity is unknown, catalysis by CBS can be explained solely by participation of PLP in the reaction mechanism.[38] In humans, several genetic diseases have been associated with mutations in CBS domains, and they can be considered as promising targets for the rational design of new drugs. Recently, Lucas et al. reported the purification, crystallization, and preliminary X-ray diffraction analysis of the CBS-domain pair from the *Methanococcus jannaschii* protein MJ0100.[39] CBS domains consist of a three-stranded β-sheet and two α-helices that are present in proteins of all kingdoms of life and in proteins with completely different functions. The C-terminal domain of the *Methanococcus jannaschii* protein MJ0100 includes a CBS-domain pair and has been overexpressed, purified, and crystallized. Preliminary analysis of the X-ray data indicated that there were eight molecules per asymmetric unit in both cases. Successively Martinez-Cruz et al.[40] explored in this study the oligomerization state, the stability, and conformational properties of the CBS domain protein MJ0729 from *Methanocaldococcus jannaschii* by using a combination of hydrodynamic (namely, ultracentrifugation, DLS, DOSY-NMR, and gel filtration) and spectroscopic techniques (fluorescence, CD, NMR, and FTIR). The results indicated that the protein had a pH-dependent oligomerization equilibrium; at pH 7, the dominant species is a dimer, where each monomer is a two-CBS domain protein, and at pH 4.5−4.8, the dominant species is a tetramer with an oblong shape, as shown by X-ray. Deconvolution of the FTIR spectra indicated that the monomer at physiology pH has 26% α-helical structure and 17% β-sheet, with most of the structure disordered.

Recently it has been proposed that H_2S can signal through protein S-sulfhydration. S-Sulfhydration affects cysteines in target proteins, yielding an −SSH moiety that has enhanced chemical reactivity.[41]



DL-propargylglycine (PAG) β-cyanoalanine (BCA)

Figure 2. Structures of PAG and BCA.

Biological Activity

There are several pathological and physiological conditions in which H_2S appears to be involved. All the papers published thus far have dealt with this research topic by either using an exogenous source of H_2S or using inhibitors of H_2S synthesis acting either on CBS or on CSE. Pharmacological inhibitors of H_2S biosynthesis include DL-propargylglycine (PAG) and β-cyanoalanine (BCA) (Figure 2).

Although characterized by low potency, poor selectivity, and limited cell-membrane permeability, these compounds have been used in several studies in order to verify the effects of inhibition of H_2S production. They are nonspecific and are likely to interact with other pyridoxal phosphate dependent enzymes. Another important issue to be pointed out is that while deletion of the CBS gene leads to mice with retarded growth that die within 3 weeks, knockout of CSE generates viable mice that display a reduced vascular response.

For brevity, in the following paragraphs summarize the effect of H_2S in different systems and literature relevant data are reported in Tables 1−5.

H_2S and Cardiovascular System

Hydrogen sulfide activity in the cardiovascular system has been investigated in several species. The role of H_2S has been investigated by using mainly two approaches: (i) by inhibiting endogenous H_2S; (ii) by administering exogenous H_2S mainly using NaHS as donor. On the basis of these studies, a role for H_2S in modulating vascular tone has emerged. For brevity, Table 1 summarizes the main effects displayed by H_2S in the cardiovascular system.

Table 1. Effects and Role of H_2S in the Cardiovascular System

species	tissues	effect in vitro	effect in vivo	effect of inhibitor	ref
rat	mesenteric artery, aorta portal vein	relaxation	hypotensive	K_{ATP} channel blocker inhibits, PAG and BCA block relaxation	23, 42−45
hypertensive rat	aorta, plasma, thoracic aorta	relaxation, regulation of excess vascular collage	reduces blood pressure	the effect was reduced by PAG	4, 46, 47
rat	liver, ileum		increases systolic blood pressure (PAG)	amino oxyacetate, PAG inhibit H_2S production	48
human	internal thoracic artery	relaxation, contraction (low dose)		K_{ATP} channel blocker inhibits	49
mouse lacking CSE	serum, heart, aorta	reduced H_2S levels, reduced vasorelaxation	hypertension	not tested	50
rat	plasma myocardium	reduced lipid peroxidation, increased antioxidation, inhibition oxidative stress injury	improves cardiac function	not tested	51
rat	pulmonary artery	effect on pulmonary collagen remodeling		PAG in vivo in a model of pulmonary high flow	52

Table 2. Pro- and Anti-Inflammatory Role for H_2S

species	tissues/cells	effect in vitro	effect in vivo	effect of inhibitor/mediator involvement	ref
			Proinflammatory Effects for H_2S		
mouse	lung liver		proinflammatory in septic shock cecal ligation and puncture (CLP)induced sepsis	PAG reduced lung and liver myeloperoxidase activity. PAG reduced leukocyte rolling and adherence significantly in mesenteric venules coupled with decreased mRNA and protein levels of adhesion molecules in lung and liver.	56, 57
guinea pig	airways	tachykinin-mediated neurogenic inflammatory responses in guinea pig airways	intratracheal instillation of NaHS increased the total lung resistance and airway plasma protein extravasation	Capsazepine and SR140333 or SR48968 inhibit NaHS effect.	58
mouse	lung		H_2S involvement in the generation of substance P (SP) via NK-1 receptor	PAG pretreatment or post-treatment significantly decreased the production of SP in lung.	59
mouse	pancreas		H_2S mediates pancreatitis induced by caerulein hyperstimulation	PAG treatment reduced pancreatic and lung edema, diminished acinar-cell injury/necrosis.	6
rat, mouse			H_2S proinflammatory: paw edema, hemorrhagic shock	Pretreatment with PAG significantly reduces proinflammatory effects.	7, 8, 60−62
human	IFNγ-primed human U937	H_2S is proinflammatory		proinflammatory cytokines, Involvement ERK-NF-κB pathway	63
rat			nociceptive in formalin assay	PAG attenuated both nociceptive flinching and hind paw edema.	64
			Anti-Inflammatory Effects of H_2S		
rat	mesenteric venules		leukocyte adherence in mesenteric venules, leucocyte infiltration in the air pouch carrageenan-induced hindpaw edema	activation of ATP-sensitive K^+ (K_{ATP}) channels	65
mouse	caerulein-treated acinar cells	H_2S inhibits the production of proinflammatory cytokines by activation of the (PI3K)/AKT pathway		PI3K inhibitor in LY294002 abolished the H_2S-mediated activation of AKT and increases TNF-α and IL-1β levels	66
mouse	lung		H_2S exerts protective effects in acute lung injury	activation of anti-inflammatory and antioxidant pathways	67
rat	stomach		H_2S promotes GI healing in the rat.		65, 68
mouse	LPS-stimulated microglia and astrocytes		H_2S produced an antiinflammatory effect.	inhibition of inducible nitric oxide synthase and p38 MAPK signaling pathways	69
rat	lung		H_2S attenuates the development of pulmonary hypertension.	elevating $I_\kappa B_\alpha$ expression, down-regulating NF-κB p65 expression	70
rat	myocardium		cardioprotective effect of NaHS	antiapoptotic and anti-inflammatory effects	71
macrophages	RAW2647	LPS-stimulated	anti-inflammatory	H_2S can inhibit NO production and NF-κB activation. BCA enhanced NO production.	72
Yorkshire swine	myocardium		protection in response to ischemia−reperfusion injury	Therapeutic sulfide reduces tissue levels of IL-6, IL-8, TNF-α, and MPO activity.	73
rat	colon		Inhibition of H_2S synthesis in rats resulted in inflammation and mucosal injury.	Intracolonic administration of H_2S donors reduced the severity of colitis and reduced colonic expression of mRNA for TNF-α	74

Table 3. Effects of H_2S in the Nervous System Central in Processes of Animal Models of Disease

species and tissues	effect in vitro or in vivo and effect of inhibitor	ref
rat, brain	NaHS down-regulated the expression of c-fos and increased the expression of γ-aminobutyric acid B receptor subunits 1 and 2 ($GABA_{(B)}R2$) in recurrent febrile seizures. Hydroxylamine (an inhibitor of CBS) up-regulates c-fos and down-regulates GABA(B)R2.	80
rat, primary hepatocyte	H_2S protects neurons from oxidative stress rat primary hepatocytes.	81
human, cultured human SH-SY5Y cells	H_2S inhibits HOCl-mediated inactivation of α(1)-antiproteinase and protein oxidation, HOCl-induced cytotoxicity, intracellular protein oxidation, and lipid peroxidation.	82
mouse, hippocampal cell line	H_2S protects from oxidative glutamate toxicity through ATP-dependent K^+ and Cl^- channels and increases glutathione levels.	83
mouse, brain endothelial cells (bEnd3)	H_2S protects from oxidative stress induced by hyperhomocysteinemia.	84
glia cells	H_2S has a protective effect on oxidative stress on brain endothelial cells.	85−87
rat, primary cultures of astrocytes and hippocampal slices	H_2S increases the influx of Ca^{2+} and causes the release of stored intracellular Ca^{2+}, La^{3+}, and Gd^{3+}. Inhibitors of Ca^{2+} channels block Ca^{2+} waves induced by neuronal excitation or from exogenously applied H_2S.	88
rat, primary cultured microglial cells	Hydrogen sulfide regulates calcium homeostasis in microglial cells. PAG and BCA significantly decrease $[Ca^{2+}]_i$.	89
mouse, primary cultured microglia and immortalized murine BV-2 microglial cells	Hydrogen sulfide attenuates lipopolysaccharide-induced inflammation by inhibition of p38 mitogen-activated protein kinase (MAPK) in microglia.	69

Table 4. Effects of H_2S in the Endocrine System in Processes of Animal Models of Disease

species and tissues	effect in vitro or in vivo and effect of inhibitor	ref
	H_2S Role on Insulin Metabolism	
Zucker diabetic fatty (ZDF) rats, pancreatic β-cells	H_2S significantly increases K_{ATP} channel activity rat pancreatic β-cells. Insulin release is impaired in ZDF by a high pancreatic production of H_2S. PAG increased serum insulin level, lowered hyperglycemia, and reduced hemoglobin A1c level.	90
mouse, mouse pancreas	High glucose increased CSE expression in β-cells. L-Cys or NaHS suppressed islet cell apoptosis with high glucose and increased glutathione content in MIN6 β-cells. The CSE inhibitor PAG antagonized L-cysteine effects.	91
rat, INS1-E cells derived from a rat insulinoma	H_2S at physiologically relevant concentrations induced apoptosis of insulin-secreting β cells by enhancing ER stress via p38 MAPK activation.	92
	H_2S Role on Catecolamine System	
rainbow trout; chromaffin cells (posterior cardinal vein and anterior kidney)	H_2S elicits catecholamine secretion via membrane depolarization followed by Ca^{2+}-mediated exocytosis. H_2S-induced catecholamine secretion is unaltered by the nicotinic receptor blocker hexamethonium.	93
	H_2S Role on Hypothalamo−Pituitary	
rat, hippocampus	NaHS caused a concentration-dependent decrease in KCl stimulated corticotropin-releasing hormone release. *S*-Adenosylmethionine mimics the effects of NaHS but did not affect hypothalamo−pituitary−adrenal function under resting conditions while inhibiting stress-related glucocorticoid increase.	94

H_2S and Inflammation

Recently, many studies and reviews[53−55] were published on the involvement of hydrogen sulfide in inflammatory events. Evidence has been shown for a pro- or anti-inflammatory role of H_2S. In Table 2 is reported a summary of the evidence for the role of H_2S in inflammation.

H_2S and Central Nervous System

The possible role of hydrogen sulfide as an endogenous neuromodulator and an intracellular messenger was reported by Abe et al.[75] In the mammalian CNS, H_2S is present at relatively high levels in the brain, and CBS, which is highly expressed in the hippocampus, is involved in the production of brain H_2S. As CBS is a calcium- and calmodulin-dependent enzyme, the biosynthesis of H_2S should be acutely controlled by the intracellular concentration of calcium. In addition, it is also regulated by *S*-adenosylmethionine which acts as an allosteric activator of CBS.

Physiological concentrations of H_2S selectively enhance NMDA receptor-mediated currents and facilitate the induction of hippocampal long-term potentiation (LTP). The NMDA receptor subunits are directly phosphorylated at specific sites by protein kinase A (PKA), resulting in the activation of NMDA-receptor-mediated excitatory postsynaptic currents. PKA activation is also observed in the induction of LTP. Kimura showed that the physiological concentrations of H_2S increase the production of cAMP in primary cultures of brain cells, neuronal and glial cell lines, and *Xenopus* oocytes.[76] NMDA receptors expressed on *Xenopus* oocyte membrane are modulated by H_2S. This modulation by H_2S is specifically inhibited by adenylyl cyclase-specific inhibitor MDL-12, 330A.[76] H_2S is also involved in CNS pathologies such as stroke[77] and Alzheimer's[78] disease. In stroke, H_2S appears to act as a mediator of ischemic injuries, and thus, inhibition of its production has been suggested to be a potential treatment approach in stroke therapy.

On the basis of the known actions of H_2S described above, the potential physiological functions of H_2S in the brain may

Table 5. Effects of H_2S in the Gastrointestinal System in Processes of Animal Models of Disease

species and tissues	effect in vitro or in vivo and effect of inhibitor	ref
mouse, human, rat; Jejunum, colon, muscular strips	H_2S inhibits in vitro intestinal and colonic motor patterns. This effect is dependent on SK channels and glybenclamide-sensitive $K_{(ATP)}$ channels.	97
mouse, small intestine	H_2S prevents in ischemia−reperfusion, leukocyte rolling, and leukocyte adhesion through an eNOS- and p38 MAPK-dependent mechanism.	98
mouse, intestine	Intraperitoneal injections of lysine acetyl salicylate increased concentration of endogenous hydrogen sulfide	99
rat, liver	In normal hyperhomocysteinemic and cirrhotic rat livers, endothelial dysfunction caused by homocysteine was reversed by perfusion of the livers with sodium sulfide.	100
mouse, liver	H_2S protects the murine liver against ischemia−reperfusion injury through an up-regulation of intracellular antioxidant and antiapoptotic signaling pathways.	101
mouse, cecal ligation and puncture (CLP) induced sepsis	Inhibition of H_2S formation by PAG significantly reduced the phosphorylation of ERK1/2 in lung and liver 4 h after CLP, coupled with decreased degradation of $I_\kappa B_\alpha$ and activation of NF-κB.	102
rat, hepatic arteria	CSE-derived H_2S contributes to hepatic arterial buffer response and partly mediates vasorelaxation of the hepatic artery via activation of $K_{(ATP)}$ channels. PAG significantly reduced the buffer capacity.	103
human, erythrocytes and colonic mucosa	No evidence of defective enzymic detoxication of sulfide by *Rhodanese* or thiol methyltransferase was found in patients with ulcerative colitis or Crohn's disease.	104
mouse, colon	H_2S-releasing mesalamine derivative, ATB-429, reduces colitis-associated leukocyte infiltration and expression of several proinflammatory cytokines.	105
mouse, pancreatic acini	The proinflammatory effect of H_2S may be mediated by substance P neurokinin-1 receptor (SP-NK-1R) related pathway in mouse pancreatic acinar cells.	106
mouse, cecal ligation and puncture induced sepsis	H_2S acts as an important endogenous regulator of leukocyte activation and trafficking during an inflammatory response (in cecal ligation and puncture-induced sepsis).	57
rat, gastric mucosal epithelial cells	NaHS/H_2S protects gastric mucosal epithelial cells against oxidative stress through stimulation of MAP kinase pathways.	107
rat, colorectal distension (CRD)	ATB-429, a novel H_2S-releasing derivative of mesalamine, inhibits hypersensitivity induced by CRD in both healthy and postcolitic allodynic rats by a $K_{(ATP)}$ channel-mediated mechanism.	108
rat and mouse, pancreas	NaHS/H_2S targets T-type Ca^{2+} channels, leading to nociception. Endogenous H_2S produced by CSE and possibly T-type Ca^{2+} channels are involved in pancreatitis-related pain.	109
rat and mouse, gastrointestinal tract	The capacity for H_2S synthesis varies throughout the rodent gastrointestinal tract, as does the distribution and contribution of the two key enzymes (CSB and CSE).	110
guinea pig, gastric antrum muscle strips	H_2S has multiple actions during the regulation of gastric motility in the guinea pig. An excitatory effect is mediated via inhibition of the voltage-gated K^+ channel, and an inhibitory effect is mediated via activation of the $K_{(ATP)}$ channel.	111
rat	H_2S synthesis is markedly up-regulated during experimental colitis. Inhibition of H_2S synthesis interfered with resolution/healing of the colitis, while administration of H_2S donors accelerated resolution/healing.	74

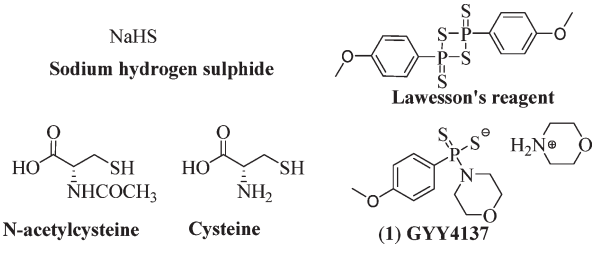


Figure 3. Structures of H_2S donors used for basic research.

include calcium homeostasis, potentiation of LTP, suppression of oxidative stress, and modulation of neurotransmission.[79] In Table 3 is summarized the main evidence for the role of H_2S in CNS.

H_2S and the Endocrine System

The potential roles of H_2S in regulating endocrine function have also been recently investigated. Table 4 is a summary of the evidence for the role of H_2S in insulin metabolism and in catecolamine and hypothalamo−pituitary endocrine system.

H_2S and the Gastrointestinal System[95b,c,96]

Studies of H_2S-induced chloride secretion in guinea pig and human colon imply that the presence of H_2S in the mucosa/submucosa of the colon stimulates primary afferent nerve fibers, which leads to increased chloride secretion. With this reasoning, it might also be possible that H_2S affects contractile function not only by direct effects on the smooth muscle itself but by an effect on neurons of the enteric nervous system that might, in turn, affect smooth muscle function. Another potential source of H_2S, besides the neuronal endogenous production, might be H_2S produced by bacteria within the bowel lumen; however, a physiologic role of H_2S originating from this source has not yet been described. The pathways that appear to modulate the contractile function of the gastrointestinal (GI) tract by H_2S have not yet been studied and serve as fertile territory for future experiments.

In addition to the effect of H_2S as a gasotransmitter affecting GI contractile activity, H_2S may play a role as a proinflammatory mediator in abdominal sepsis, endotoxemia, and pancreatitis in contrast to its anti-inflammatory effects in animal models of gastritis and colitis. Furthermore, H_2S has an antinociceptive effect on visceral pain perception, but H_2S has also been demonstrated to activate primary sensory neurons. Some of the major findings on the role of H_2S in the GI tract are reported in Table 5.

H_2S-Related Compounds in Development

In Figure 3 are reported the structures of H_2S donors used for basic research, and in Figure 4 are reported drug candidates in

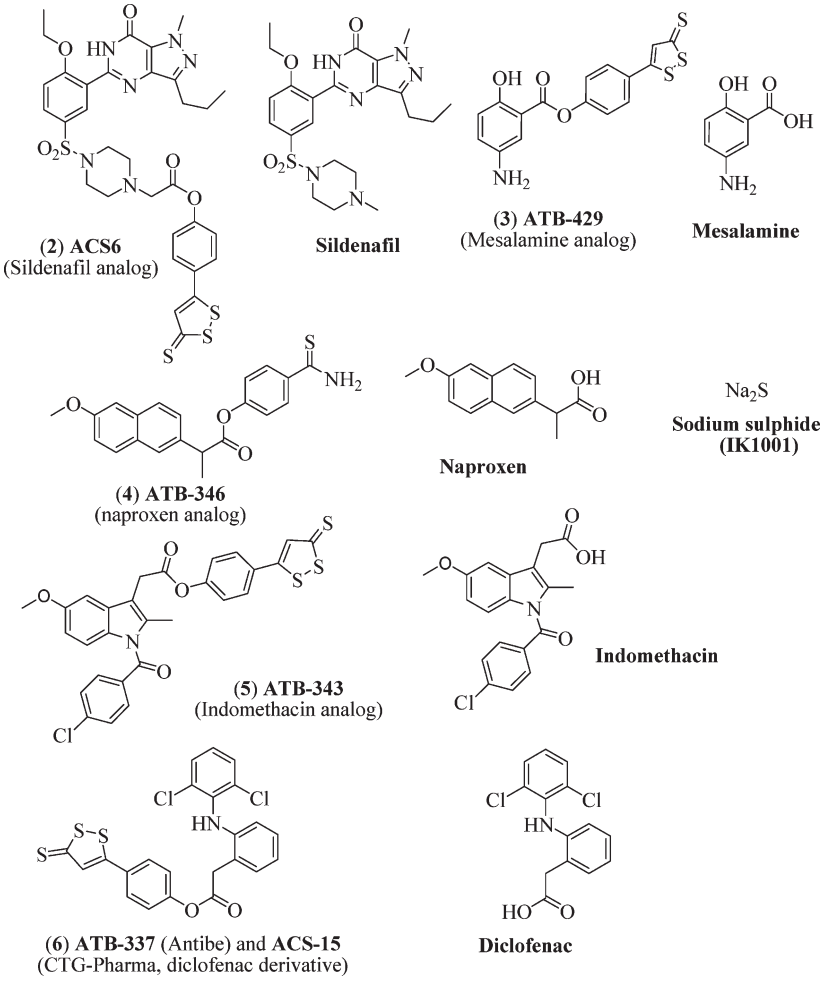
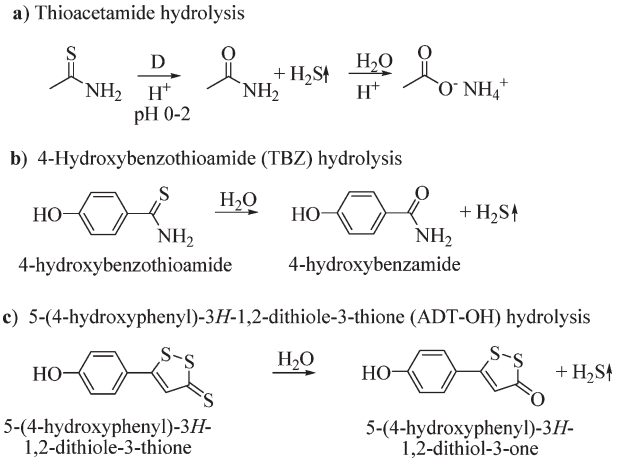


Figure 4. Structures of H_2S-releasing agents and their parent drugs (sildenafil, mesalamine, naproxen, indomethacin, diclofenac) reported in the literature as drug candidates in development.

Scheme 4. Hypothesis of Chemical Transformation of the H_2S Releasing Moieties



development. Among them, there are agents that either directly release H_2S when in solution (NaHS, Na_2S, *Lawesson's* reagent, GYY4137[112]) or function as a precursor for endogenous H_2S synthesis (*N*-acetylcysteine,[65] L-cysteine).

Pharmacologically useful donors should be soluble in aqueous media, should not be not toxic, should not metabolize quickly, and should release H_2S in vivo slowly over a period of time. The most widely used tool to generate H_2S is NaHS[2,53−55,68,113] Sodium hydrogen sulfide (NaHS) is a known donor, but it releases H_2S too quickly and is not useful for many studies. A large release of H_2S causes the blood pressure to fall rapidly and may have detrimental effects on the patient or animal. The effects from the release of H_2S also will be fairly short-lived. Some papers have shown that Lawesson's reagent is a H_2S releasing compound. In a smaller number of papers, Na_2S has been used, which is also currently under development by Ikaria (see below). More recently, a water-soluble compound GYY4137 has been synthesized that is a slow-releasing H_2S compound with vasodilatator and antihypertensive activity.[112]

One of the major issues in this field is the rate of hydrolysis of H_2S donors. In other words it is critical for research in this field to have tools that are clearly defined. In Scheme 4 we have reported hypothetic hydrolysis mechanisms of some H_2S donors obtained by comparison with some analogue structures reported in the literature.[114−116]

Hydrolysis mechanism of TBZ could be similar to that of thioacetamide.[114] Moreover, TBZ is more prone to undergo this conversion because of the presence of an aromatic ring that makes the formed intermediates more stable than that of thioacetamide. Concerning ADT-OH, it has been reported that trithiones can be converted to the corresponding dithiolone by means of oxidant reagents such as $Hg(OAc)_2$ and $KMnO_4$.[115,116] In addition we could also consider the oxidative desulfurization of thiobarbiturates performed by CYP450.[117] However,

H_2S-related pharmacological research is a rapidly emerging field, which is likely to yield a number of therapeutic possibilities, and early stage drug candidates are now in development.

Ikaria is investigating the impact of the sulfide ion, administered as sodium sulfide (IK-1001),[118,119] in a number of disease models. Preclinical data demonstrate the therapeutic potential of Na_2S for injection, a parenteral injectable formulation of hydrogen sulfide, in a variety of disease models including myocardial infarction, cardiopulmonary bypass surgery, thoracoabdominal aortic aneurysm surgery, liver ischemia and reperfusion, organ storage and transplantation, and acute lung injury.[118,119]

Another main line of research deals with the synthesis of known chemical entities bearing in their structure a moiety that releases hydrogen sulfide. A H_2S donating derivative of sildenafil, namely, ACS6 (CTG-Pharma, **2**, Figure 4), relaxed cavernosal smooth muscle equipotently to sildenafil citrate. The formation of superoxide and expression of p47 and PDE5 were reduced by **2**, sildenafil citrate, and NaHS. Compound **2** was more active than sildenafil.[120] Recently it has been shown that the H_2S pathway is involved in the erectile function in humans.[121]

Another line of research that is producing new molecules and exploiting the H_2S concept is aimed at reducing the toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs). H_2S donors were shown to reduce the severity of NSAID-induced damage in the rat stomach.[121] Moreover, NSAIDs decreased endogenous H_2S synthesis.[122] Antibe Therapeutics has produced an interesting chemical entity, ATB 337 (**6**, Figure 4), a derivative of diclofenac, that exhibits enhanced anti-inflammatory effects to the parent drug.[54] The same compound, ACS15 (**6**, Figure 4), has been been synthesized and tested by CTG Pharma.[123] While diclofenac dose-dependently damages the stomach, **6** displays significantly reduced GI toxicity and has no effect on hematocrit.[95a,124] Diclofenac, but not **6**, elevates gastric granulocyte infiltration and expression of TNF-α, lymphocyte function-associated antigen 1, and intercellular adhesion molecule 1. Compound **6** inhibited COX-1 and COX-2 activity as effectively as diclofenac. Compound **6** did not induce leukocyte adherence, whereas diclofenac did and was more potent at reducing paw edema.

ATB-429[105,108] (**3**, Figure 4) is a derivative of mesalamine, a drug commonly used in the treatment of inflammatory bowel disease (IBD). IBD is divided into two subtypes: Crohn's disease (CD) and ulcerative colitis (UC). Compound **3** has been widely characterized in animal models of CD and UC, and it has been shown to be significantly more effective than mesalamine. Moreover, there is also considerable preclinical evidence that **3** is significantly more effective in treating the visceral pain associated with IBD.[105,108]

ATB-346[125] (**4**, Figure 4) is a derivative of naproxen, the latter being a drug that is widely used as pain relief in osteoarthritis, the most common form of arthritis. However, like other NSAIDS, naproxen carries a significant risk of serious gastrointestinal bleeding and cardiovascular effects (e.g., heart attacks, elevated blood pressure). In preclinical studies, **4** exhibits increased effectiveness over its parent drug and a remarkable reduction in the gastrointestinal and cardiovascular toxicity. The drug at the present stage is in preclinical effectiveness and toxicology studies.[125]

Conclusions and Future Directions

In this review we have made an effort to give an overview of the recent contributions to this research field. It is clear that the H_2S research field is growing rapidly, and considerable evidence is accumulating regarding the role of this mediator in different diseases. The data produced in the field can be divided into two main groups: (i) data obtained using an exogenous sources of H_2S and (ii) data obtained by modulating endogenous H_2S synthesis by using the substrate L-cysteine or inhibitors or CBS and/or CSE. In the majority of the studies published so far NaHS has been used as an exogenous source of H_2S and Na_2S to a lesser extent. In both cases the donors have been used in different micromolar ranges and in some cases very high concentrations (millimolar) have been used. In addition, at the present stage we do not know how much of the exogenous H_2S generated by NaHS or Na_2S actually reaches the cells, so it is not clear if the studies are examining physiological or pathological effects of this mediator. Does it matter if Na_2S is used instead of NaHS? Both salts generate a limited amount of H_2S, particularly when they are dissolved in buffers at a pH of $\sim$7.4. Recently, in order to overcome this problem, medicinal chemists have started to synthesize organic donors aiming to produce drugs with a controlled H_2S release. Thus, in our view, the first important issue to be addressed by medicinal chemists is the synthesis and characterization of H_2S donors that release the gas with controlled kinetics at a defined pH. Indeed, synthesis of organic water-soluble H_2S donors, hopefully with controlled release, will contribute dramatically to the development of this field.

With respect to inhibitors of CBS and CSE, the available drugs lack selectivity, are not well characterized, and can be poorly soluble. These issues make the data presently available complex to interpret. Indeed, we do not know the relative selectivity of these inhibitors toward CSE or CBS in terms of IC_{50} (i.e., studies performed using isolated enzyme systems). In addition, at least some of these inhibitors interact with other related enzymes. All these considerations indicate without a doubt that this research field opens an important opportunity for medicinal chemists to design and characterize selective inhibitors for CBS or CSE. The development of such agents, particularly if water-soluble, will allow researchers to modulate the endogenous physiological or pathological levels of H_2S. With these tools it will also be possible to evaluate the cross-talk of H_2S pathways with other relevant pathways (e.g., NO, COX, etc.). Therefore, the design, synthesis, and characterization of new H_2S donors and new selective inhibitors will enormously boost this research field. This field, even if relatively young, has already produced significant output in terms of numbers of patents filed. In conclusion, we believe that for researchers in medicinal chemistry this represents a challenging opportunity to contribute to the development of this exciting and promising new research field.

Biographies

Giuseppe Caliendo received his B.Sc. in Pharmacy from University of Naples Federico II, Italy, in 1981. Currently, he is Full Professor of Medicinal Chemistry at the Faculty of Pharmacy of Naples. He has published over 120 peer-reviewed papers and some scientific books in the field of peptidomimetics and synthesis by microwave of biologically active compounds. His research is focused on identification and characterization of pharmacologically active heterocyclic derivatives. In the recent years, he has been interested in the development of hybrid structures containing H_2S-releasing moiety in order to produce novel pharmacological tools able to highlight the therapeutic potential of modulation of the H_2S pathway.

Giuseppe Cirino received his degree in Pharmacy at the University of Naples Federico II, Italy, in 1980 and his Ph.D.

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Vincenzo Santagada received his B.Sc. in Pharmacy from University of Naples Federico II, Italy, in 1982 and his Ph.D. in Medicinal Chemistry in 1987. Currently, he is Full Professor of Medicinal Chemistry at the Faculty of Pharmacy of Naples. He has published over 120 peer-reviewed papers and some scientific books in the field of peptidomimetics and synthesis by microwave. His scientific interests cover design and synthesis of pharmacologically active peptides and peptidomimetics that have been evaluated for their antitumoral, anti-inflammatory, antiviral, or analgesic properties. Recent years have seen a deep interest in the application of microwave technology to medicinal chemistry and in the development of H_2S-releasing molecules.

John L. Wallace received his B.Sc. and M.Sc. degrees in Biology from Queen's University, Canada, his Ph.D. from the University of Toronto, Canada, and an MBA from the University of Birmingham, U.K. He is presently the Director of the Farncombe Family Digestive Health Research Institute at McMaster University in Hamilton, Ontario, Canada. He has published over 350 peer-reviewed articles and over 80 book chapters. His major research interests are inflammation and ulceration in the gastrointestinal tract.

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