Hydrogen Sulfide and Cell Signaling

Ling Li,1 Peter Rose,2 and Philip K. Moore2,*

1Section of Inflammation and Signal Transduction, Division of Immunology and Inflammation, Imperial College London, London W12 0NN, United Kingdom
2Pharmaceutical Science Division, King’s College London, London SE1 9NH, United Kingdom; email: philip.moore@kcl.ac.uk

*Current address: Departments of Pharmacology and Pharmacy, National University of Singapore, Singapore 117597; email: phcmpk@nus.edu.sg

Abstract

Hydrogen sulfide (H2S) is a gaseous mediator synthesized from cysteine by cystathionine γ lyase (CSE) and other naturally occurring enzymes. Pharmacological experiments using H2S donors and genetic experiments using CSE knockout mice suggest important roles for this vasodilator gas in the regulation of blood vessel caliber, cardiac response to ischemia/reperfusion injury, and inflammation. That H2S inhibits cytochrome c oxidase and reduces cell energy production has been known for many decades, but more recently, a number of additional pharmacological targets for this gas have been identified. H2S activates KATP and transient receptor potential (TRP) channels but usually inhibits big conductance Ca2+-sensitive K+ (BKCa) channels, T-type calcium channels, and M-type calcium channels. H2S may inhibit or activate NF-κB nuclear translocation while affecting the activity of numerous kinases including p38 mitogen-activated protein kinase (p38 MAPK), extracellular signal-regulated kinase (ERK), and Akt. These disparate effects may be secondary to the well-known reducing activity of H2S and/or its ability to promote sulfhydration of protein cysteine moieties within the cell.
INTRODUCTION

Hydrogen sulfide (H₂S), along with nitric oxide (NO) and carbon monoxide (CO), forms part of a group of biologically active gases that are termed gasotransmitters or gasomediators. Many other gases, including O₂ and CO₂, play equally, if not more, important and fundamental roles in human biology, yet H₂S, NO, and CO have attracted attention because they exert fine, modulatory control over cellular functions by influencing an array of intracellular signaling processes.

H₂S is the most recently discovered gasotransmitter and, perhaps not surprisingly, has attracted a great deal of controversy over the past few years. To date, few scientists comprehend how such a poisonous molecule might be formed naturally at levels sufficient to modify cell function without causing cell death. Others query whether H₂S acts physiologically and, if so, what advantages it confers over NO and CO? Indeed, recent work points to an important cell signaling role for H₂S that may be of fundamental importance for cellular functions.

This review describes recent advances in our understanding of the biosynthesis, catabolism, and cell biology of this intriguing gas. Specifically, we focus on the effect of H₂S on cell signaling processes that are particularly relevant to two of the most important areas of H₂S research: the cardiovascular system and inflammation. Although other possible roles for endogenous H₂S have been postulated, these fields lie beyond the scope of this review, and interested readers are referred elsewhere (1–15).

BIOSYNTHESIS, CATABOLISM, AND OCCURRENCE OF HYDROGEN SULFIDE

H₂S is synthesized in mammalian tissues via endogenous enzymes and by nonenzymatic pathways (e.g., reduction of thiols and thiol-containing molecules). Whereas most emphasis has been placed on the enzymatic formation of H₂S, the generation of this gas from bound sulfane sulfur (intracellular sulfur stores) may be important in certain cells and under certain conditions (16).

Bound sulfide most likely occurs when H₂S interacts with cysteine thiols to form stable persulfides, which, under reducing conditions, can release stored H₂S. This mechanism seems to have a pH optimum of 8.4 (at which thiol-reducing activity is highest), and because of this, its physiological relevance is not yet clear (see Reference 6 for a review).

The enzymes and the pathways that catalyze H₂S production have been known for many years and are not described in detail here. Rather, interested readers are referred to Figure 1, which summarizes these pathways, and also to several excellent reviews dealing with both general aspects of cysteine sulfur metabolism (17) and specific accounts of H₂S biosynthesis (8, 13, 15). Interestingly, these enzymes are evolutionarily conserved and occur in many lower species as well as in mammals. H₂S is produced from L-cysteine by at least four separate pathways. Thus, (a) cystathionine β synthetase (CBS, EC 4.2.1.22) acts on L-cysteine to produce H₂S and L-serine; (b) cystathionine γ lyase (CSE, EC 4.4.1.1) forms thiocysteine from cystine, which then rearranges to form H₂S; (c) cysteine aminotransferase (CAT, EC 2.6.1.3) catalyzes the reaction of L-cysteine with keto acids (e.g., α-ketoglutarate) to form 3-mercaptopyruvate, which is then desulfurated by 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2) to form H₂S; and (d) cysteine lyase (CL, EC 4.4.1.10) converts L-cysteine and sulfite to L-cysteate and H₂S. Pyridoxal 5’-phosphate (PPP) is required as a necessary cofactor by CBS, CAT, CSE, and CL, whereas 3-MST is zinc dependent. CAT and 3-MST are both mitochondrial and cytosolic, whereas CBS and CSE appear to be exclusively cytosolic.

Although much has been discovered about the way in which H₂S is synthesized in the body, less is known about its metabolism (see Figure 1). Multiple chemical and biochemical catabolic
fates await newly synthesized H₂S, and many more are probably still to be discovered. For example, H₂S reacts readily with methemoglobin to form sulhemoglobin, which might act as a metabolic sink for H₂S. Furthermore, H₂S is rapidly oxidized to thiosulfate (S₂O₃²⁻) by mitochondria and is subsequently converted to sulfite (SO₃²⁻) and sulfate (SO₄²⁻). H₂S can also undergo methylation by thiol-S-methyltransferase (EC 2.1.1.9) to yield methanethiol (CH₃SH) and dimethylsulfide (CH₃SCH₃), and it is also a substrate (especially in the colon) for rhodanese (thiosulfate:cyanide sulfurtransferase; EC 2.8.1.1), leading to the formation of thiocyanate (SCN⁻) and SO₄²⁻. Notwithstanding these biochemical means for H₂S catabolism, H₂S is a powerful reducing agent and is likely to be consumed by endogenous oxidant species in the vasculature, such as peroxynitrite (18), superoxide (19), and hydrogen peroxide (20).

These degradative pathways exist to ensure that H₂S is removed quickly from the cellular environment, and this seems entirely appropriate given the physiological functionality of this molecule. Sadly, there are no single biomarkers of H₂S identified in the same way that nitrite
and nitrate, for example, have been widely used as biomarkers for NO. This certainly adds to the difficulty of identifying physiological and pathophysiological roles for this gas.

THE PRINCIPAL BIOLOGICAL EFFECTS OF HYDROGEN SULFIDE

To date, much research into H₂S has centered upon its effects on individual body systems. Although many such systems have come under the spotlight, the effect of this gas on the cardiovascular system and in inflammation has attracted the most attention. These areas are reviewed here. Many other possible roles for endogenous H₂S have been postulated in, for example, the peripheral and central nervous systems, pain appreciation and neurodegeneration, control of gastrointestinal and urogenital function, and endocrinology. Unfortunately, these areas lie outside the immediate scope of this review, and interested readers are referred elsewhere for further information (7–10, 12, 13).

The modern era of H₂S research most likely started with the finding that H₂S (generated from sodium hydrogen sulfide, or NaHS) dilates rat blood vessels both in vitro and in vivo by a mechanism that involves the opening of vascular smooth muscle K⁺ATP channels (21, 22). Since these seminal reports, other researchers have confirmed a vasodilator effect of H₂S. Thus H₂S dilates many isolated mammalian blood vessels including rat aorta, gastric artery, and portal vein (21–24), along with the mesenteric (25), cavernosal (26, 27), and hepatic (28) vascular beds. H₂S also relaxes the human internal mammary artery (29). In whole animals, acute (21, 22) and chronic administration of NaHS (30) reduces blood pressure, as does injection of other H₂S donors such as ADT-OH, S-diclofenac (31), and GYY4137 (32). In the various studies referenced, the concentration of NaHS required to dilate blood vessels in vitro is high. In general, >50–100 μM NaHS is needed to relax blood vessels in the organ bath, whereas 10–50 μmol kg⁻¹ are needed to bring about significant falls in blood pressure. This requirement fuels the debate as to which H₂S donor better mimics the biological effects of endogenous H₂S and potentially which might be taken forward as candidates for therapeutic entities. The number of H₂S donors is growing rapidly, and the chemical structures of some of those described to date are shown in Figure 2.

Given that H₂S dilates most mammalian blood vessels, the possibility that a deficiency in the biosynthesis of this gas contributes to or predisposes to cardiovascular diseases is appealing. Reduced CSE expression and tissue H₂S biosynthesis are features of experimentally induced hypoxic pulmonary hypertension (33), high blood flow–induced pulmonary hypertension (34), and high blood pressure induced by NG-nitro-L-arginine methyl ester (L-NAME) (35); they are also features of blood vessels from spontaneously hypertensive rats (36). Collectively, these data suggest that a deficiency of CSE (and hence H₂S) may predispose to hypertension. The cellular whereabouts of the CSE involved in this phenomenon has been debated for several years. This enzyme occurs in cultured vascular smooth muscle cells (37), but the idea that this could be the source of the H₂S generated naturally to control blood vessel caliber seems unlikely. It was originally thought, based on measurements of CSE mRNA (21), that endothelial cells were devoid of this enzyme. However, that CSE-mediated H₂S biosynthesis occurs not only in vascular smooth muscle but also in vascular endothelial cells is now known (37). Indeed, CSE knockout mice exhibit pronounced hypertension (37), reduced wound healing most likely resulting from the inhibition of angiogenesis (38), and decreased cardioprotection evoked by tadalafil, a long-acting inhibitor of phosphodiesterase-5 (39). These studies provide further experimental support that endogenous H₂S is involved in blood pressure regulation, angiogenesis, and cardioprotection.

Over the past few years, numerous other cardiovascular effects of H₂S have been noted. These include proangiogenesis in vitro and in vivo (30, 38), a remodeling effect (i.e., reduced vascular hyperplasia) in blood vessels of hypertensive rats (40, 41), antiatherosclerotic activity (42), platelet antiaggregatory activity at high concentrations (43), and either reduced (44) or enhanced (45)
binding of neutrophils to the vascular endothelial layer during inflammation—an effect likely due to altered expression of adhesion molecules including intercellular adhesion molecule 1 (ICAM-1). Furthermore, H₂S is cardioprotective in isolated rat hearts following coronary artery ligation (46), ischemia (47), or lipopolysaccharide (LPS) injection (48).

Another area attracting considerable attention is the part played by H₂S in inflammation. Injection of NaHS causes tissue inflammation, as evidenced by increased organ myeloperoxidase (MPO) activity resulting from tissue neutrophil infiltration (49). Moreover, plasma H₂S concentration, tissue H₂S synthesizing activity, and CSE expression are increased in animal models of inflammation (e.g., endotoxic, septic and hemorrhagic shock, pancreatitis, carrageenan-evoked hind-paw edema) (49–51). However, plentiful evidence also supports an anti-inflammatory effect of H₂S. H₂S-releasing derivatives of diclofenac (cf diclofenac alone) exhibit enhanced anti-inflammatory activity in endotoxic shock and against carrageenan-induced hind-paw swelling (31, 52). The slow-releasing H₂S donor, GYY4137, is also anti-inflammatory in a mouse model of endotoxic shock (53), and H₂S-releasing mesalamine reduces colitis-associated leukocyte infiltration and
expression of several proinflammatory cytokines (54). H$_2$S also promotes ulcer healing in the rat (55) and reduces both leukocyte infiltration in an air pouch model and decreases carrageenan-induced hind-paw edema (44). There now seems little debate that H$_2$S can be both pro- and anti-inflammatory, although the responsible mechanisms remain largely unknown.

Researchers have thrown themselves enthusiastically into the task of probing the roles of this “new” biologically active gas but possibly at the expense of more fundamental issues such as these: What is the effective concentration range for endogenous H$_2$S? To what extent is this mimicked by NaHS and other donors? Exactly how does this gas work at the cellular level? This review seeks to provide insights for each of these important questions.

HYDROGEN SULFIDE BIOSYNTHESIS AND HYDROGEN SULFIDE CONCENTRATIONS IN THE BODY

Any discussion of the biological effects of a newly discovered mediator such as H$_2$S needs to be tempered by the consideration of the mediator’s achieved concentration at its site of action. As such, it is important to have in place (a) robust assays for H$_2$S measurement and (b) a clear idea of where this gas acts at the tissue and cellular levels.

What Is the Concentration of Endogenous Hydrogen Sulfide?

Many researchers have sought to measure H$_2$S in biological tissues and fluids. The most common assay relies on trapping H$_2$S with a metal (usually zinc) followed by acidification and reaction with a dye, N,N-dimethyl-p-phenylenediamine (DMPD), to form methylene blue, which is then measured spectrophotometrically (for further details, see Reference 15). This method revealed several decades ago that brain tissue contains approximately 50–160 μM H$_2$S and that human and rat serum contain 50–100 μM H$_2$S. The majority of authors using this method are, or should be, aware of its limitations and have clarified that this technique monitors not only free H$_2$S but also other species such as hydrosulfide anion (HS$^-$) and sulfide (S$_2^-$). Other techniques have also been used to measure plasma H$_2$S concentrations in the rat: an amperometric (i.e., sulfide-selective probe) technique produced reports of approximately 50 μM, and gas chromatography–mass spectrometry showed approximately 80 μM. Bearing in mind the potent effect of H$_2$S on mitochondrial cytochrome c oxidase activity, these values are extremely high and, as has been pointed out, probably not consistent with life (9, 14). More recent estimates have put the plasma concentration of free H$_2$S in the submicromolar range (56) and suggest that basal H$_2$S in mouse brain and liver homogenates is on the order of ~15 nM (57). As assay techniques are refined and more is learned about the catabolic fate of H$_2$S in different experimental conditions, we can speculate that the true plasma concentration of this gas is likely to be lower. Indeed, one report failed to find any detectable free H$_2$S in human plasma (58). For further information regarding the issues associated with the measurement of H$_2$S, we refer the reader to the excellent review by Tangerman (59).

It is of interest that 10–15 years ago, the plasma concentration of NO was widely believed to be in the low micromolar range. As existing assays were refined and as new, sensitive biosensor techniques were developed, the actual plasma levels of NO were found to be in the range of 1–5 nM, which is several orders of magnitude lower than first believed (reviewed in Reference 60). That plasma levels of NO are this low is testament to the physiological importance of NO and the need to maintain circulating levels of this highly biologically active molecule within tightly controlled limits. Indeed, the inability of researchers to find anything other than a mere trace of
NO in plasma likely adds to its biological relevance rather than detracts from it. Perhaps the low levels of H$_2$S detectable in plasma and tissues are also indicative of biological significance?

**Where Should Hydrogen Sulfide Be Measured?**

H$_2$S is synthesized naturally by enzymes located either in the cytosol (CSE, CBS) or in mitochondria (3-MST). Because it is a highly diffusible gas, upon formation it is likely to be either sequestered or catabolized rapidly. The molecular targets for H$_2$S are largely unknown but are likely to include intracellular proteins, enzymes, and transcription factors, as well as an array of membrane ion channels (see next section). It seems reasonable, therefore, that the active sites for many of the biological effects of H$_2$S are inside the cell. Indeed, measuring H$_2$S either in plasma or in homogenized tissue is not reflective of its cellular site of action. Unfortunately, there are no techniques with the sensitivity, selectivity, and real-time capability to measure intracellular H$_2$S. Such advances in technology are eagerly awaited, as they are probably the only answer to the conundrum of how to link free H$_2$S levels with biological response.

**HYDROGEN SULFIDE—A MEDIATOR OF CELL SIGNALING?**

H$_2$S is going through a reclassification from environmental toxin/poison to mammalian intracellular cell signaling molecule. This is a well-trodden route that both NO and CO have taken in the past decade. That H$_2$S is an environmental toxin and poison has been known for centuries; this rationale results from the ability of H$_2$S to inhibit purified cytochrome c oxidase at low micromolar concentrations in vitro and with a potency similar to cyanide (CN$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^
Figure 3
Known molecular targets for hydrogen sulfide (H$_2$S). These can be grouped broadly into effects on (a) protein (cysteine thiol) modifications, the biological significance of which is yet to be elucidated; (b) intracellular signaling proteins and transcription factors, which likely account for effects in inflammation and cell protection; (c) ion channels, which underpin many of the systemic responses to H$_2$S in blood vessels, heart, and nerves; and (d) metabolism (effects on mitochondrial ATP production). Abbreviations: ATP, adenosine triphosphate; NF-$\kappa$B, nuclear factor $\kappa$B; Nrf-2, NF-E2-related factor 2; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; -SH, thiol; -SSH, hydropersulfide; STAT3, signal transducer and activator of transcription 3; TRPV, transient receptor potential vanilloid.

In the heart, the negative inotropic effect of H$_2$S is antagonized by glibenclamide (20), as is the cardioprotection associated with ischemia/reperfusion injury in isolated hearts (72–74), aspirin-mediated leukocyte adherence in mesenteric venules (75), suppression of carrageenan-induced hind-paw edema (44), blood pressure reduction following intrahypothalamic injection of H$_2$S (76), and pain due to colorectal distension (66). Overall, the evidence suggests that many of the reported biological effects of NaHS are, either in part or in whole, mediated by the opening of K$_{ATP}$ channels.

H$_2$S is also known to act on a number of other ion channels. H$_2$S inhibits BK$_{Ca}$ (big conductance Ca$^{2+}$-sensitive K$^+$) channels in stably transfected HEK-293 cells (77), whereas the opposite effect (i.e., stimulation) was noted in rat pituitary tumor cells (78). Furthermore, H$_2$S inhibits L-type Ca$^{2+}$ channels in cardiomyocytes (79), T-type Ca$^{2+}$ channels mediating visceral pain in the mouse (80), and intracellular chloride channels in rat heart lysosomal vesicles (81); it activates transient receptor potential vanilloid (TRPV) channels in both urinary tract (82) and airway smooth muscle (83).
The biological significance of these various effects of H\(_2\)S on ion channels (other than those on the K\(_{ATP}\) channel) has yet to be properly addressed, but clearly this gas is more promiscuous in its effects on ion channels than was first envisaged.

**Effect of Hydrogen Sulfide on Transcription Factors**

The interaction of H\(_2\)S with intracellular transcription factors has received much attention, particularly with respect to its role in inflammation and tissue ischemia/reperfusion injury. NaHS was originally shown to inhibit LPS-induced nuclear factor \(\kappa\)B (NF-\(\kappa\)B) activation in cultured RAW 264.7 macrophages by Oh and colleagues (84). Since then, other researchers using cultured cells and whole animals have reported a similar phenomenon. Thus NaHS inhibits I\(\kappa\)B-\(\alpha\) (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, \(\alpha\)) degradation and NF-\(\kappa\)B translocation in tumor necrosis factor \(\alpha\) (TNF\(\alpha\))-challenged human umbilical vein endothelial cells (44), LPS-challenged astrocytes (85), and caerulein-provoked rat pancreatic acinar cells (86); it also inhibits these processes in kidney (87) and heart (48) after ischemia/reperfusion injury and in aorta from spontaneously hypertensive rats injected with NaHS (88). Apart from limiting I\(\kappa\)B-\(\alpha\) degradation and thereby decreasing translocation of NF-\(\kappa\)B to the nucleus, the precise molecular mechanism of action of H\(_2\)S remains unknown. It would be interesting, for example, to determine whether H\(_2\)S affected the activity of the I\(\kappa\)B kinase, which usually serves to phosphorylate and hence activate the proteolysis of I\(\kappa\)B-\(\alpha\).

NaHS is not the only H\(_2\)S donor that has been studied for effects on NF-\(\kappa\)B activation. The slow-releasing H\(_2\)S donor drug, GYY4137, also reduces LPS-induced NF-\(\kappa\)B activation in mouse macrophages and decreases the LPS-induced signaling via NF-\(\kappa\)B in livers of treated rats (32, 53). In addition, S-diclofenac reduced inflammation in rats following LPS injection; this is associated with the inhibition of liver NF-\(\kappa\)B activation (31). Interestingly, garlic compounds such as diallyl sulfide (DAS), a possible H\(_2\)S donor (89), can also downregulate NF-\(\kappa\)B activation. Indeed, such a mechanism may contribute to the anti-inflammatory effect of garlic compounds.

However, under some experimental circumstances, NaHS can be proinflammatory and can augment (not inhibit) I\(\kappa\)B-\(\alpha\) degradation and thence increase (not reduce) NF-\(\kappa\)B activation. This occurs, for example, in interferon-\(\gamma\) (IFN-\(\gamma\))-primed human monocytic cells (U937) (90) and in mice subjected to cecal ligation puncture (to mimic a state of septic shock) followed by NaHS injection (to promote the ongoing inflammation) (45). H\(_2\)S is also found to upregulate proinflammatory gene expression in synoviocytes by a mechanism independent of the NF-\(\kappa\)B pathway (91). Clearly, there is considerable variation in the way that the NF-\(\kappa\)B system responds to H\(_2\)S, perhaps due to differences in cell type and/or culture conditions used in many of these studies.

The functional consequence of reduced NF-\(\kappa\)B activation in inflammatory cells is downregulation of a number of proinflammatory genes, including those that encode for inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as well as downregulation of inflammatory cytokines/chemokines and adhesion molecules. However, our understanding of the role of NF-\(\kappa\)B in inflammation is changing. Although NF-\(\kappa\)B activation in the early stages of inflammation is indeed proinflammatory, its activation in the resolution phase is coupled to expression of anti-inflammatory factors such as transforming growth factor \(\beta\) (TGF-\(\beta\)). Thus H\(_2\)S may either inhibit or activate NF-\(\kappa\)B and, depending on the precise stage of the inflammatory response, may bring about an anti-inflammatory effect in both cases.

Additional transcription factors are also targets for H\(_2\)S. For example, administration of GYY4137 to LPS-injected rats resulted in the activation of signal transducer and activator of transcription 3 (STAT3) (92). STAT3 is known to regulate the expression of many genes that mediate...
Effect of Hydrogen Sulfide on Kinases

Much of the data regarding the effect of H₂S on mitogen-activated protein kinase (MAPK) signaling in the literature are conflictive and likely result from cell type effects and concentrations used. To date, NaHS has been found to inhibit endothelin-provoked rises in p38 MAPK activity in cultured rat aortic vascular smooth muscle cells (96), yet it upregulates p38 MAPK activity in cultured human umbilical vein endothelial cells (38); this effect was associated with the stimulation of angiogenesis. H₂S also promotes the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 in pigs subjected to coronary artery occlusion and reperfusion (97). The anti-apoptotic effects of H₂S in human polymorphonuclear leukocytes appear to be associated with the inhibition of p38 MAPK (98) and with the attenuation of LPS-induced p38 MAPK phosphorylation in BV-2 microglial cells (99) and in keratinocytes (100). The following effects of H₂S are reportedly secondary to p38 MAPK activation: (a) apoptosis in human aorta smooth muscle cells (101, 102), (b) protection of gastric mucosal epithelial cells against oxidative stress (103), and (c) reduced intestinal leukocyte attachment and rolling in mouse postcapillary venules subjected to ischemia/reperfusion injury (104).

Additional kinases are also targets of H₂S. H₂S generated as a result of overexpression of CSE causes a sustained activation/phosphorylation of ERK in HEK-293 cells (101), and a similar effect has also been observed in rat vascular smooth muscle cells (105) and human colon cancer cells (106). H₂S also activates/phosphorylates Akt in cultured endothelial cells (30), colon cancer cells (105), and ischemic hind-limb muscles in the rat (107). In isolated hearts subjected to ischemic pre-conditioning, a brief infusion of NaHS was found to stimulate both cardiac Akt and protein kinase C (PKC) activity; this effect has been associated with improved cardiac mechanical performance and reduced injury (108).

Overall, the principal effect of H₂S on a broad range of intracellular cell signaling kinases would appear to be activation. However, the literature contains reports of the opposite effect, i.e., inhibition of kinases. For a fuller understanding of H₂S biology, it is of paramount importance to determine the conditions under which H₂S can either promote or block the effects of these important enzymes.

Effect of Hydrogen Sulfide on Oxidative Stress

H₂S protects primary cultures of neurons from cell death by increasing reduced glutathione (GSH) levels (109). This effect was originally ascribed to increased activity of γ-glutamylcysteine synthetase and upregulation of cystine transport. Subsequently, H₂S was reported to open KᵥATP and Cl⁻ channels in these cells, thereby contributing to its protective effect (110). Recently, H₂S has been found to increase intracellular reduced GSH concentrations and to suppress oxidative stress in mitochondria (111). Thus the finding from the same group that mitochondria are able to synthesize H₂S via 3-MST is particularly interesting.
The powerful reducing properties of H$_2$S may also be highly relevant in terms of its anti-inflammatory effect. Inflammation is associated with the generation of both NO and reactive oxygen species that can lead to tissue and cellular damage. The antioxidant activity of H$_2$S may explain a number of the reported biological effects of this gas, including protection against (a) heart (112), liver (113), and intestinal (114) damage following ischemia/reperfusion injury; (b) H$_2$O$_2$-induced damage in rat gastric epithelial cells (103); (c) myocardial (19) and renal (115) injury due to hyperhomocysteinemia in rats; (d) methionine-induced (116) and homocysteine-induced (117) oxidative stress; and (e) hemin-mediated oxidation of low-density lipoprotein (118).

The antioxidant effect of H$_2$S has recently been examined in more detail through the use of H$_2$S-releasing derivatives. ACS67 (a H$_2$S-releasing derivative of latanoprost) reduced intraocular pressure in the rat (119) and attenuated retinal damage following experimental elevation of intraocular pressure (120). ACS67 was more potent than latanoprost in both cases. In addition, ACS-6 (a H$_2$S-releasing derivative of sildenafil) inhibits O$_2^-$ formation and NADH oxidation in endothelial cells.

Whether H$_2$S is antioxidant by virtue of its undoubted reducing activity or by its activation of endogenous defense systems—or both—needs to be examined further.

**HYDROGEN SULFIDE IN CELL SIGNALING—THE ROLE OF S-SULFHYDRATION**

H$_2$S causes S-sulfhydration of a large number of cellular proteins (71); this process is potentially significant because it provides a possible mechanism by which H$_2$S alters the function of a wide range of cellular proteins and enzymes. This subject has recently been reviewed (121).

The process of S-sulfhydration of proteins by H$_2$S may be analogous to that of protein S-nitrosylation by NO. In this process, a sulfur (derived from H$_2$S) is added to the thiol groups of cysteine residues to yield a hydropersulfide (-SSH) moiety. Protein cysteines equipped with an -SSH group in this way appear to exhibit increased chemical reactivity when compared with base cysteines with sulfur in the thiol state. Importantly, the enhanced chemical reactivity of these modified cysteines may also be reflected in enhanced biological activity. Certainly, S-sulfhydrated GAPDH exhibits greater enzyme activity than the nonsulfhydrated enzyme. Whether S-sulfhydration of other enzymes in this way also enhances their activity awaits further work, but it is certainly intriguing to postulate that kinase activation, which seems to play such an important part in H$_2$S biology, perhaps results from S-sulfhydration. Interestingly, S-nitrosylation of GAPDH reduces the activity of this enzyme, suggesting that NO and H$_2$S may—by virtue of S-nitrosylation and S-sulfhydration, respectively—act together to regulate cell signaling. This possibility therefore sheds new light on the complex cross-talk capability of these two gases and reinforces the point that any consideration of the biological roles of NO must, by necessity, also take into account the possible controlling influence of H$_2$S.

S-sulfhydration seems to be a common posttranslational modification event in the cell, with 10–25% of all mouse liver proteins reported to exist in this state (71). The possibility that this process represents an entirely novel form of cell signaling is obviously an intriguing one. However, many questions remain to be answered. For example, does sulfhydration occur in tissue other than liver and in species other than rat (i.e., in man)? To date, a considerable body of work in animals has identified numerous experimental disease states in which cell and tissue H$_2$S biosyntheses are either upregulated (e.g., inflammation) or downregulated (e.g., hypertension)—is there a parallel change in S-sulfhydration status of the intracellular proteins in these cells and tissues? If so, does this change occur in proteins across the board or only in selected proteins that contribute to the pathology? Is S-sulfhydration exclusively synonymous with cell signaling? Or may it also
reflect a defensive adaptation of cells, perhaps evolved over the millennia, to regulate and control intracellular concentrations of H₂S? Finally, is S-sulfhydration a reversible process, as might be imagined for a cell signaling mechanism? One thing is clear: More research is needed to evaluate fully the physiological and pathophysiological implications of this fascinating posttranslational protein modification.

CONCLUSIONS

Much has been learned about the pharmacology of H₂S in the past few years. Accumulated evidence points to physiological roles of this gas in, for example, control of vascular reactivity, and to pathophysiological roles in hypertension, heart disease, and inflammation. References to new H₂S drugs are appearing in the literature on a more or less monthly basis, and a CSE knockout mouse has been developed.

Despite these advances, there are still many uncertainties. The original estimates of plasma/serum/tissue H₂S were too high, and this gas does not occur naturally at concentrations of tens (or even hundreds) of micromolars. However, plasma/serum/tissue levels of this gas are most likely poor markers for the formation or activity of H₂S. Indeed, H₂S in plasma may simply be the excess spillover from its cellular sites of synthesis. It will be crucial to identify exactly where in the cell/tissue H₂S exerts its biological effects and to measure its concentration at that active site.

There is clearly dispute regarding the biological effects of H₂S in the cardiovascular system, in inflammation, and in its actions on cellular proteins (ion channels and enzymes) and transcription factors. A recurring theme in the literature, as highlighted in this article, is that H₂S can, under differing experimental conditions, exert diametrically opposite biological and molecular effects both at the cell signaling level and at the functional level. The overall effect of H₂S may, of course, depend on the local concentration of the gas achieved under differing conditions, but H₂S is arguably unique in that it not only acts directly on cells (e.g., to open the Kᵥ ATP channel or to influence NF-κB translocation) but it also triggers indirect effects. More specifically, cells exposed to H₂S may “respond” to the toxic effect of this gas by altering their own metabolic pathways. In this case, the effect of H₂S in any particular cell, tissue, or animal will depend on the balance between these opposing influences, and this effect will undoubtedly alter with the concentration of H₂S experienced by the cell and with the natural well-being of cells when they encounter H₂S. This, in turn, is a reflection of the rate of biosynthesis versus the rate of breakdown/storage. It might be argued that NO and CO act similarly. After years of study, it is fascinating that the biological significance of H₂S may, after all, be inextricably linked with its toxicity.

SUMMARY POINTS LIST

1. H₂S is synthesized from cysteine in mammals by four enzymes, of which CSE is likely to be the most physiologically relevant. Once formed, H₂S is broken down rapidly by a combination of chemical and enzyme reactions. Plasma concentration of free H₂S is probably low (<1 μM), although sulfide concentration is much higher.

2. H₂S exhibits vasodilator, proangiogenic, and antiatherosclerotic activity and also protects the heart, kidney, and other organs from damage following ischemia/reperfusion injury. CSE knockout mice exhibit pronounced hypertension, suggesting that endothelial H₂S is a physiologically relevant vasodilator. H₂S causes both pro- and anti-inflammatory activity depending on experimental conditions and is both vasculoprotective and cardioprotective.
3. The bulk of the available evidence suggests that H\textsubscript{2}S reduces nuclear translocation of NF-\kappa B; enhances the nuclear accumulation of Nrf-2 and STAT3; enhances p38 MAPK, ERK1/2, and Akt activity; and interacts with NO and its biosynthesis in a complex manner.

4. H\textsubscript{2}S is an important signaling molecule in the cell. The most likely molecular mechanisms of action are alteration of the function of redox-sensitive enzymes/transcription and S-sulfhydration—a newly discovered process in which cysteine thiols (-SH) in susceptible cell proteins are converted to hydropersulfides (-SSH). This process is associated with activation of selected enzymes such as GAPDH.

FUTURE ISSUES

1. New assays that are able to detect free H\textsubscript{2}S are eagerly sought. The ability to measure free H\textsubscript{2}S generated by/within cells at low concentrations in real time would be a significant advance.

2. It is important to get a clearer idea of the plasma/serum/tissue concentration and its relevance in terms of predicting the biological effects of endogenous H\textsubscript{2}S and exogenous H\textsubscript{2}S provided by slow- and fast-releasing donor drugs.

3. A wider range of drugs that release H\textsubscript{2}S at different rates and a better understanding of their precise cellular and intracellular sites of action/breakdown will go some way toward establishing whether this mediator can be targeted with drugs for therapeutic benefit.

4. A thorough evaluation of both the physiological and pathophysiological roles of H\textsubscript{2}S using genetically modified animals is needed.

5. Greater insights into the mechanism of the cell signaling activity of H\textsubscript{2}S are needed. It will be important to determine the relative contributions of redox mechanisms and intracellular protein S-sulfhydration and their interrelationship with the known effects of this gas on ion channels, transcription factors, and kinases, which seem to be the major molecular targets.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We wish to thank all colleagues, collaborators, and fellow researchers for their critical input in generating the ideas and the data that support the various hypotheses put forward in this review. We would also like to take the opportunity to apologize to all the scientists worldwide who have added to this fascinating story in the past few years but whose contribution has not been reported herein. Unfortunately, space limitations are such that some interesting research has had to be abbreviated, and other areas have been omitted completely.
LITERATURE CITED


Contents

A 40-Year Journey in Search of Selective Antiviral Chemotherapy
   Erik De Clercq ................................................................. 1

microRNAs: Master Regulators as Potential Therapeutics in Cancer
   Michela Garofalo and Carlo M. Croce ........................................... 25

Physiologically-Based Pharmacokinetics in Drug Development
   and Regulatory Science
   Malcolm Rowland, Carl Peck, and Geoffrey Tucker ............................ 45

Transcriptional and Epigenetic Regulation of Opioid Receptor Genes:
   Present and Future
   Li-Na Wei and Horace H. Lab ..................................................... 75

Membrane Receptor for Thyroid Hormone: Physiologic and
   Pharmacologic Implications
   Paul J. Davis, Faith B. Davis, Shaker A. Mousa, Mary K. Luidens,
   and Hung-Yun Lin .................................................................... 99

Strategies to Discover Unexpected Targets for Drugs Active at
   G Protein–Coupled Receptors
   John A. Allen and Bryan L. Roth .................................................. 117

Bioactivation of Drugs: Risk and Drug Design
   John S. Walsh and Gerald T. Miwa ................................................ 145

Hydrogen Sulfide and Cell Signaling
   Ling Li, Peter Rose, and Philip K. Moore ...................................... 169

Schizophrenia: Treatment Targets Beyond Monoamine Systems
   Hisham M. Ibrahim and Carol A. Tamminga .................................... 189

Modulation of Monoamine Receptors by Adaptor Proteins and Lipid
   Rafts: Role in Some Effects of Centrally Acting Drugs and
   Therapeutic Agents
   Karl Björk and Per Svenningsson ................................................... 211

Orexin Receptors: Pharmacology and Therapeutic Opportunities
   Thomas E. Scammell and Christopher J. Winrow .............................. 243
Macrophages and Tissue Injury: Agents of Defense or Destruction?  
*Debra L. Laskin, Vasanthi R. Sunil, Carol R. Gardner, and Jeffrey D. Laskin* …… 267

Drugging the Cancer Stem Cell Compartment: Lessons Learned from the Hedgehog and Wnt Signal Transduction Pathways  
*Michael E. Dodge and Lawrence Lum* ………………………………………… 289

Mechanisms of the Anti-Cancer and Anti-Inflammatory Actions of Vitamin D  
*Aruna V. Krishnan and David Feldman* ………………………………………… 311

Spatiotemporal Regulation of Small GTPases as Revealed by Probes Based on the Principle of Förster Resonance Energy Transfer (FRET): Implications for Signaling and Pharmacology  
*Etsuko Kiyokawa, Kazuhiro Aoki, Takeshi Nakamura, and Michiyuki Matsuda* …… 337

Mechanisms of Monoclonal Antibody–Drug Interactions  
*Hongbui Zhou and Mary Ann Mascelli* ………………………………………… 359

Molecular Mechanisms and Treatment Options for Muscle Wasting Diseases  
*Markus A. Rüegg and David J. Glass* ………………………………………… 373

Curing HIV: Pharmacologic Approaches to Target HIV-1 Latency  
*Shailesh K. Choudhury and David M. Margolis* ………………………………………… 397

**Indexes**

Contributing Authors, Volumes 47–51 ………………………………………… 419

Chapter Titles, Volumes 47–51 ………………………………………… 422

**Errata**

An online log of corrections to *Annual Review of Pharmacology and Toxicology* articles may be found at http://pharmtox.annualreviews.org/errata.shtml