Hydrogen Sulfide and Cell Signaling

Ling Li,[1] Peter Rose,[2] and Philip K. Moore[2,*]

[1]Section of Inflammation and Signal Transduction, Division of Immunology and Inflammation, Imperial College London, London W12 0NN, United Kingdom

[2]Pharmaceutical Science Division, King's College London, London SE1 9NH, United Kingdom; email: phillip.moore@kcl.ac.uk

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

Annu. Rev. Pharmacol. Toxicol. 2011. 51:169–87

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

This article's doi:
10.1146/annurev-pharmtox-010510-100505

0362-1642/11/0210-0169$20.00

Keywords

cysteine, transcription factors, kinases, sulfhydration, antioxidant

Abstract

Hydrogen sulfide (H_2S) is a gaseous mediator synthesized from cysteine by cystathionine γ lyase (CSE) and other naturally occurring enzymes. Pharmacological experiments using H_2S 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 H_2S 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. H_2S activates K_{ATP} and transient receptor potential (TRP) channels but usually inhibits big conductance Ca^{2+}-sensitive K^+ (BK_{Ca}) channels, T-type calcium channels, and M-type calcium channels. H_2S 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 H_2S and/or its ability to promote sulfhydration of protein cysteine moieties within the cell.

INTRODUCTION

H_2S: hydrogen sulfide

NO: nitric oxide

CO: carbon monoxide

CSE: cystathionine γ lyase

Hydrogen sulfide (H_2S), 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 O_2 and CO_2, play equally, if not more, important and fundamental roles in human biology, yet H_2S, NO, and CO have attracted attention because they exert fine, modulatory control over cellular functions by influencing an array of intracellular signaling processes.

H_2S 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 H_2S acts physiologically and, if so, what advantages it confers over NO and CO? Indeed, recent work points to an important cell signaling role for H_2S 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 H_2S on cell signaling processes that are particularly relevant to two of the most important areas of H_2S research: the cardiovascular system and inflammation. Although other possible roles for endogenous H_2S 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

H_2S 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 H_2S, 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 H_2S interacts with cysteine thiols to form stable persulfides, which, under reducing conditions, can release stored H_2S. 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 H_2S 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 H_2S biosynthesis (8, 13, 15). Interestingly, these enzymes are evolutionarily conserved and occur in many lower species as well as in mammals. H_2S 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 H_2S and L-serine; (*b*) cystathionine γ lyase (CSE, EC 4.4.1.1) forms thiocysteine from cystine, which then rearranges to form H_2S; (*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 H_2S; and (*d*) cysteine lyase (CL, EC 4.4.1.10) converts L-cysteine and sulfite to L-cysteate and H_2S. 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 H_2S is synthesized in the body, less is known about its metabolism (see **Figure 1**). Multiple chemical and biochemical catabolic

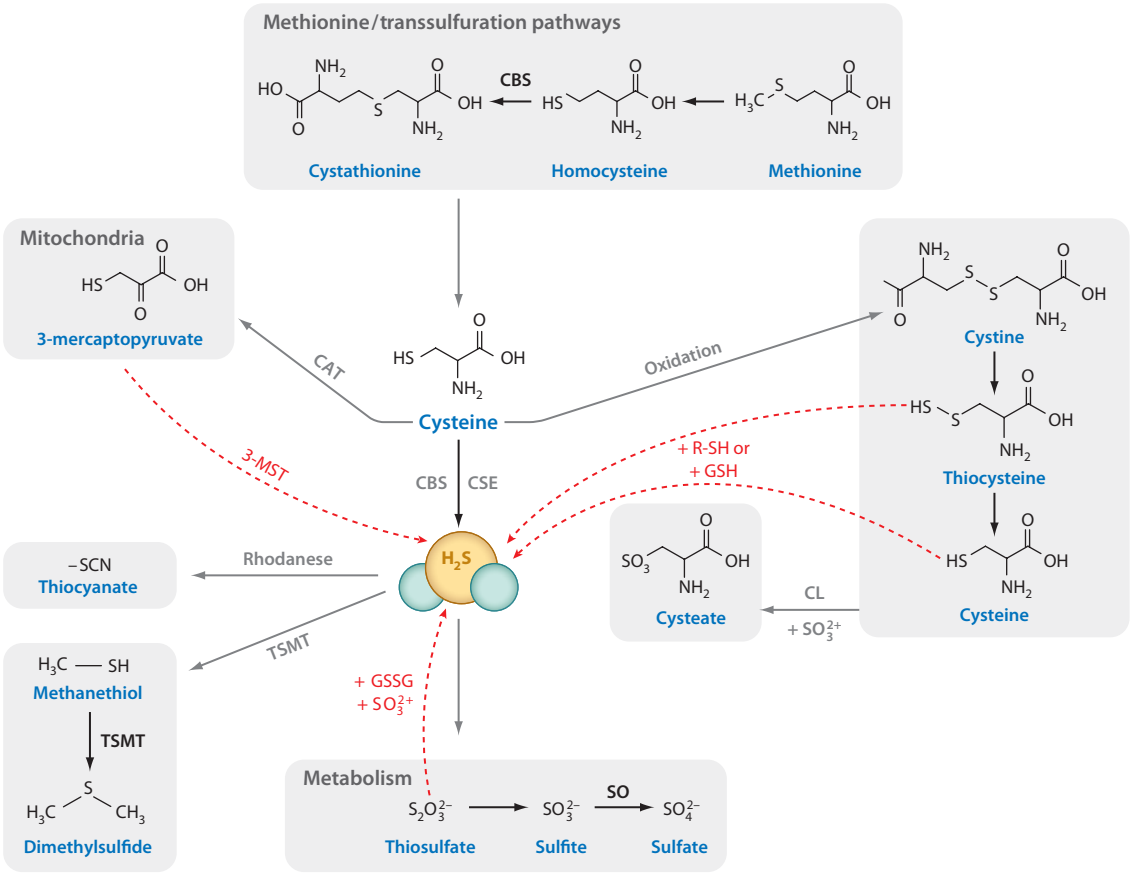

Figure 1

The biosynthesis and degradation of hydrogen sulfide (H_2S) in mammalian cells. H_2S is synthesized inside the cell from cysteine via cystathionine γ lyase (CSE), cystathionine β synthetase (CBS), or 3-mercaptopyruvate sulfurtransferase (3-MST) as an extension of the transsulfuration pathway. Interconversion of sulfur-containing amino acids and metabolites is achieved via cysteine aminotransferase (CAT), cysteine dioxygenase (CDO), and cysteine lyase (CL). GSSG is oxidized glutathione, GSH is reduced glutathione, R-SH represents a thiol-bearing intermediate, SO represents sulfite oxidase, and TSMT is thiol S-methyltransferase.

fates await newly synthesized H_2S, and many more are probably still to be discovered. For example, H_2S reacts readily with methemoglobin to form sulfhemoglobin, which might act as a metabolic sink for H_2S. Furthermore, H_2S is rapidly oxidized to thiosulfate ($S_2O_3^{2-}$) by mitochondria and is subsequently converted to sulfite (SO_3^{2-}) and sulfate (SO_4^{2-}). H_2S can also undergo methylation by thiol-*S*-methyltransferase (EC 2.1.1.9) to yield methanethiol (CH_3SH) and dimethylsulfide (CH_3SCH_3), 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 SO_4^{2-}. Notwithstanding these biochemical means for H_2S catabolism, H_2S 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 H_2S 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 H_2S 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.

NaHS: sodium hydrogen sulfide

Cardioprotection: the process by which H_2S (and other mediators) protects the heart against damage due to ischemia/reperfusion

Ischemia/reperfusion injury: damage to heart, kidney, and other organs following temporary loss of blood flow

THE PRINCIPAL BIOLOGICAL EFFECTS OF HYDROGEN SULFIDE

To date, much research into H_2S 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 H_2S 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 H_2S research most likely started with the finding that H_2S (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 H_2S. Thus H_2S 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. H_2S 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 H_2S 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 H_2S donor better mimics the biological effects of endogenous H_2S and potentially which might be taken forward as candidates for therapeutic entities. The number of H_2S donors is growing rapidly, and the chemical structures of some of those described to date are shown in **Figure 2**.

Given that H_2S 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 H_2S 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 H_2S) 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 H_2S 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 H_2S 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 H_2S is involved in blood pressure regulation, angiogenesis, and cardioprotection.

Over the past few years, numerous other cardiovascular effects of H_2S 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)

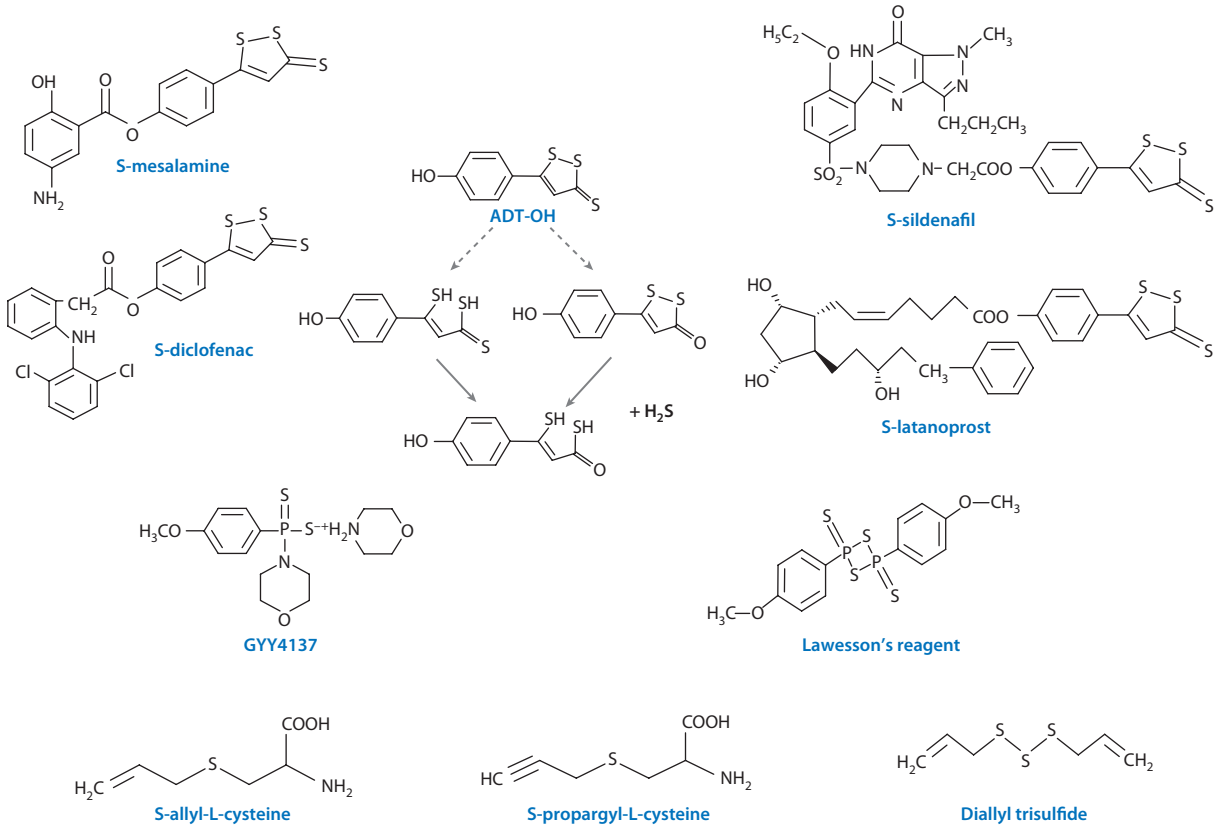

Figure 2

Chemical structures of some hydrogen sulfide (H_2S)-releasing drugs. Slow-releasing H_2S adduct drugs include S-diclofenac, S-sildenafil, S-latanoprost, and S-mesalamine. These drugs are prodrugs and are split at the ester linkage to yield the parent compound and 5-(4-hydroxyphenyl)-3*H*-1,2-dithiol-3-thione (ADT-OH), which in turn breaks down to H_2S. The following have also been used experimentally as H_2S donors: the allium derivatives S-allyl-L-cysteine and diallyl trisulfide; the cysteine derivative S-propargyl-L-cysteine; Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,2,3,4-dithiadiphosphetane 2,4-disulfide]; and GYY4137 [morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate].

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, H_2S 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 H_2S in inflammation. Injection of NaHS causes tissue inflammation, as evidenced by increased organ myeloperoxidase (MPO) activity resulting from tissue neutrophil infiltration (49). Moreover, plasma H_2S concentration, tissue H_2S 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 H_2S. H_2S-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 H_2S donor, GYY4137, is also anti-inflammatory in a mouse model of endotoxic shock (53), and H_2S-releasing mesalamine reduces colitis-associated leukocyte infiltration and

expression of several proinflammatory cytokines (54). H_2S 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_2S 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_2S? 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_2S 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_2S 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_2S in biological tissues and fluids. The most common assay relies on trapping H_2S 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_2S and that human and rat serum contain 50–100 μM H_2S. 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_2S but also other species such as hydrosulfide anion (HS^-) and sulfide (S^{2-}). Other techniques have also been used to measure plasma H_2S 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_2S 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_2S in the submicromolar range (56) and suggest that basal H_2S 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_2S 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_2S in human plasma (58). For further information regarding the issues associated with the measurement of H_2S, 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_2S detectable in plasma and tissues are also indicative of biological significance?

Where Should Hydrogen Sulfide Be Measured?

H_2S 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_2S 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_2S are inside the cell. Indeed, measuring H_2S 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_2S. Such advances in technology are eagerly awaited, as they are probably the only answer to the conundrum of how to link free H_2S levels with biological response.

HYDROGEN SULFIDE—A MEDIATOR OF CELL SIGNALING?

H_2S 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_2S is an environmental toxin and poison has been known for centuries; this rationale results from the ability of H_2S to inhibit purified cytochrome *c* oxidase at low micromolar concentrations in vitro and with a potency similar to cyanide (CN^-) (61). Thus cells exposed to H_2S exhibit reduced cell oxidative phosphorylation and ATP biosynthesis (62). Indeed, some reports suggest that ATP starvation may contribute to the vasodilator effect of H_2S (63). The possibility that H_2S is formed naturally and exerts fine control over cellular metabolic processes is a more modern concept introduced only in the past decade. Quite legitimately, this possibility has been met with healthy skepticism from the scientific community. Some of the most important effects of H_2S on cell signaling are summarized in **Figure 3**.

Effect of Hydrogen Sulfide on Ion Channels

With the exception of cytochrome *c* oxidase, the first reported molecular target for H_2S was the K_{ATP} channel (21). H_2S-induced dilation of blood vessels relies, in part at least, on its ability to open vascular smooth muscle K_{ATP} channels (21, 22, 25, 32, 64), as evidenced by sensitivity to a range of antagonists for this channel including glibenclamide, pinacidil, and PNU37883A. H_2S also opens K_{ATP} channels in rat atrial and ventricular myocytes (65). Direct confirmation of the effect of this gas on the K_{ATP} channel has come from electrophysiological studies in which NaHS increased the K_{ATP} current in rat aortic and mesenteric smooth muscle cells (21, 25). The opening of the K_{ATP} channel also underpins the relaxant effect of H_2S in colonic (66) and eye (67) but not bronchial (68) smooth muscle. Recently, researchers have raised the possibility that some smooth muscles (e.g., mouse gastric fundus) relax in response to NaHS, not via the opening of K_{ATP} channels but by the activation of myosin-light-chain phosphatase (69).

The precise manner by which H_2S activates K_{ATP} channels is not clear, but the finding that H_2S activated cloned rvKir6.1/rvSUR$_1$ channels by interacting with extracellular cysteine residues may be important (70). Recently, the K_{ATP} channel has been reported to be sulfhydrated by H_2S (71). Whether sulfhydration affects the function of these channels is not known and is discussed in more detail later.

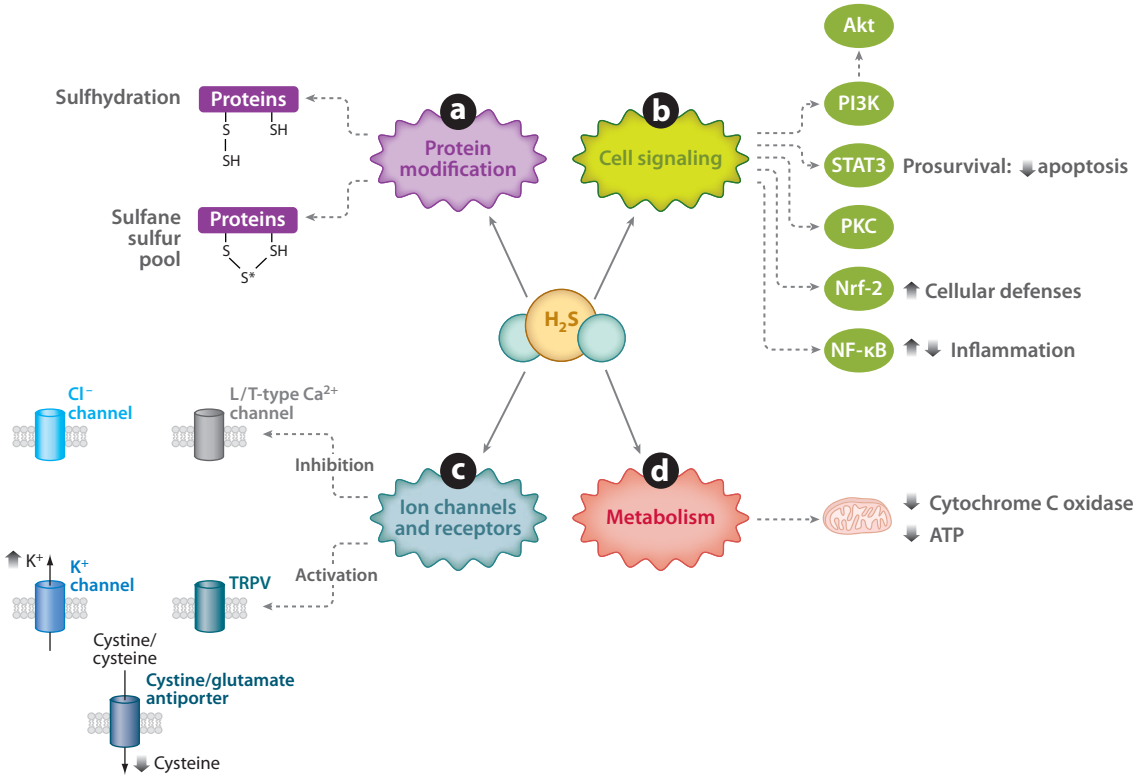

Figure 3

Known molecular targets for hydrogen sulfide (H_2S). 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_2S in blood vessels, heart, and nerves; and (*d*) metabolism (effects on mitochondrial ATP production). Abbreviations: ATP, adenosine triphosphate; NF-κB, nuclear factor κB; 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_2S 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_2S (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_2S is also known to act on a number of other ion channels. H_2S 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_2S 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_2S 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.

NF-κB: nuclear factor κB

IκB-α: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α

Effect of Hydrogen Sulfide on Transcription Factors

The interaction of H_2S 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 κB (NF-κB) 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 IκB-α (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α) degradation and NF-κB translocation in tumor necrosis factor α (TNFα)-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 IκB-α degradation and thereby decreasing translocation of NF-κB to the nucleus, the precise molecular mechanism of action of H_2S remains unknown. It would be interesting, for example, to determine whether H_2S affected the activity of the IκB kinase, which usually serves to phosphorylate and hence activate the proteolysis of IκB-α.

NaHS is not the only H_2S donor that has been studied for effects on NF-κB activation. The slow-releasing H_2S donor drug, GYY4137, also reduces LPS-induced NF-κB activation in mouse macrophages and decreases the LPS-induced signaling via NF-κB 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-κB activation (31). Interestingly, garlic compounds such as diallyl sulfide (DAS), a possible H_2S donor (89), can also downregulate NF-κB 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) IκB-α degradation and thence increase (not reduce) NF-κB activation. This occurs, for example, in interferon-γ (IFN-γ)-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_2S is also found to upregulate proinflammatory gene expression in synoviocytes by a mechanism independent of the NF-κB pathway (91). Clearly, there is considerable variation in the way that the NF-κB system responds to H_2S, perhaps due to differences in cell type and/or culture conditions used in many of these studies.

The functional consequence of reduced NF-κB 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-κB in inflammation is changing. Although NF-κB 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 β (TGF-β). Thus H_2S may either inhibit or activate NF-κB 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_2S. 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

cell survival (e.g., survivin), proliferation (e.g., c-fos), and angiogenesis (e.g., vascular endothelial growth factor) (93). NaHS also induces the nuclear localization of the transcription factor Nrf-2 (NF-E2-related factor 2) in rat hearts during experimental myocardial ischemia (94). Nrf-2 controls the gene expression of a number of cellular protective enzymes, including heme oxygenase-1 (HO-1) and thioredoxin-1 (Trx-1), and increased expression of these proteins is believed to limit cardiac damage. Finally, H_2S increases hypoxia-inducible factor-1 (Hif-1) activity in the nematode, *Caenorhabditis elegans* (95). Whether H_2S exerts a similar effect on Hif—which affects the way cells adapt to changes in ambient oxygen tension—in mammalian cells remains to be seen.

MAPK: mitogen-activated protein kinase

ERK: extracellular signal-regulated kinase

Oxidative stress: imbalance of the formation and removal of reactive oxygen species

GSH: glutathione

Effect of Hydrogen Sulfide on Kinases

Much of the data regarding the effect of H_2S 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. H_2S 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 H_2S 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 H_2S 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 H_2S. H_2S 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). H_2S 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 preconditioning, 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 H_2S 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 H_2S biology, it is of paramount importance to determine the conditions under which H_2S can either promote or block the effects of these important enzymes.

Effect of Hydrogen Sulfide on Oxidative Stress

H_2S 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, H_2S was reported to open K_{ATP} and Cl^- channels in these cells, thereby contributing to its protective effect (110). Recently, H_2S 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 H_2S via 3-MST is particularly interesting.

The powerful reducing properties of H_2S 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_2S 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_2O_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).

S-sulfhydration: the process whereby cysteine thiols react with H_2S to form hydropersulfides

The antioxidant effect of H_2S has recently been examined in more detail through the use of H_2S-releasing derivatives. ACS67 (a H_2S-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_2S-releasing derivative of sildenafil) inhibits O_2^- formation and NADH oxidation in endothelial cells.

Whether H_2S 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_2S 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_2S 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_2S may be analogous to that of protein *S*-nitrosylation by NO. In this process, a sulfur (derived from H_2S) 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_2S biology, perhaps results from *S*-sulfhydration. Interestingly, *S*-nitrosylation of GAPDH reduces the activity of this enzyme, suggesting that NO and H_2S 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_2S.

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_2S 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 H_2S? 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 H_2S 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 H_2S 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 H_2S 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 H_2S. Indeed, H_2S 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 H_2S exerts its biological effects and to measure its concentration at that active site.

There is clearly dispute regarding the biological effects of H_2S 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 H_2S 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 H_2S may, of course, depend on the local concentration of the gas achieved under differing conditions, but H_2S is arguably unique in that it not only acts directly on cells (e.g., to open the K_{ATP} channel or to influence NF-κB translocation) but it also triggers indirect effects. More specifically, cells exposed to H_2S may "respond" to the toxic effect of this gas by altering their own metabolic pathways. In this case, the effect of H_2S 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 H_2S experienced by the cell and with the natural well-being of cells when they encounter H_2S. 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 H_2S may, after all, be inextricably linked with its toxicity.

SUMMARY POINTS LIST

1. H_2S is synthesized from cysteine in mammals by four enzymes, of which CSE is likely to be the most physiologically relevant. Once formed, H_2S is broken down rapidly by a combination of chemical and enzyme reactions. Plasma concentration of free H_2S is probably low (<1 μM), although sulfide concentration is much higher.
2. H_2S 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 H_2S is a physiologically relevant vasodilator. H_2S 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_2S reduces nuclear translocation of NF-κB; 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_2S 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_2S are eagerly sought. The ability to measure free H_2S 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_2S and exogenous H_2S provided by slow- and fast-releasing donor drugs.
3. A wider range of drugs that release H_2S 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_2S using genetically modified animals is needed.
5. Greater insights into the mechanism of the cell signaling activity of H_2S 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

1. Bannenberg GL, Vieira HL. 2009. Therapeutic applications of the gaseous mediators carbon monoxide and hydrogen sulfide. *Expert Opin. Ther. Pat.* 19:663–82
2. Elsey DJ, Fowkes RC, Baxter GF. 2010. Regulation of cardiovascular cell function by hydrogen sulfide (H_2S). *Cell Biochem. Funct.* 28:95–106
3. Wang R. 2009. Hydrogen sulfide: a new EDRF. *Kidney Int.* 76:700–4
4. Li L, Moore PK. 2007. An overview of the biological significance of endogenous gases: new roles for old molecules. *Biochem. Soc. Trans.* 35:1138–41
5. Hughes MN, Centelles MN, Moore KP. 2009. Making and working with hydrogen sulfide: The chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: a review. *Free Radic. Biol. Med.* 47:1346–53
6. Kapoor A, Thiemermann C. 2010. Hydrogen sulfide, neurogenic inflammation, and cardioprotection: a tale of rotten eggs and vanilloid receptors. *Crit. Care Med.* 38:728–30
7. Kimura H. 2010. Hydrogen sulfide: its production, release and functions. *Amino Acids.* In press, doi:10.1007/s00726-010-0510-x
8. Leffler CW, Parfenova H, Jaggar JH, Wang R. 2006. Carbon monoxide and hydrogen sulfide: gaseous messengers in cerebrovascular circulation. *J. Appl. Physiol.* 100:1065–76
9. Li L, Hsu A, Moore PK. 2009. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation—a tale of three gases! *Pharmacol. Ther.* 123:386–400
10. Li L, Moore PK. 2008. Putative biological roles of hydrogen sulfide in health and disease: a breath of not so fresh air? *Trends Pharmacol. Sci.* 29:84–90
11. Linden DR, Levitt MD, Farrugia G, Szurszewski JH. 2010. Endogenous production of H_2S in the gastrointestinal tract: still in search of a physiologic function. *Antioxid. Redox Signal.* 12(9):1135–46
12. Olson KR. 2008. Hydrogen sulfide and oxygen sensing: implications in cardiorespiratory control. *J. Exp. Biol.* 211:2727–34
13. Qu K, Lee SW, Bian JS, Low CM, Wong PTH. 2008. Hydrogen sulfide: neurochemistry and neurobiology. *Neurochem. Int.* 52:155–65
14. Szabo C. 2007. Hydrogen sulphide and its therapeutic potential. *Nat. Rev. Drug Discov.* 6:917–35
15. Whiteman M, Moore PK. 2009. Hydrogen sulfide and the vasculature: a novel vasculoprotective entity and regulator of nitric oxide bioavailability? *J. Cell. Mol. Med.* 13:488–507
16. Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, Kimura H. 2009. A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid. Redox Signal.* 11:205–14
17. Stipanuk MH, Ueki I. 2010. Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. *J. Inherit. Metab. Dis.* In press, doi:10.1007/s10545-009-9006-9
18. Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, et al. 2004. The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger'? *J. Neurochem.* 90:765–68
19. Chang L, Geng B, Yu F, Zhao J, Jiang H, et al. 2008. Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats. *Amino Acids* 34:573–85
20. Geng B, Yang J, Qi Y, Zhao J, Pang Y, et al. 2004. H_2S generated by heart in rat and its effects on cardiac function. *Biochem. Biophys. Res. Commun.* 313:362–68
21. Zhao W, Zhang J, Lu Y, Wang R. 2001. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. *EMBO J.* 20:6008–16
22. Hosoki R, Matsuki N, Kimura H. 1997. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem. Biophys. Res. Commun.* 237:527–31
23. Ali MY, Ping CY, Mok YYP, Ling L, Whiteman M, et al. 2006. Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide? *Br. J. Pharmacol.* 149:625–34
24. Kubo S, Kajiwara M, Kawabata A. 2008. Dual modulation of the tension of isolated gastric artery and gastric mucosal circulation by hydrogen sulfide in rats. *Inflammopharmacology* 15:288–92
25. Cheng Y, Ndisang JF, Tang G, Cao K, Wang R. 2004. Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. *Am. J. Physiol. Heart Circ. Physiol.* 287:H2316–23

26. Srilatha B, Adaikan PG, Moore PK. 2006. Possible role for the novel gasotransmitter hydrogen sulphide in erectile dysfunction—a pilot study. *Eur. J. Pharmacol.* 535:280–82
27. d'Emmanuele di Villa Bianca R, Sorrentino R, Maffia P, Mirone V, Imbimbo C, et al. 2009. Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation. *Proc. Natl. Acad. Sci. USA* 106:4513–18
28. Siebert N, Cantré D, Eipel C, Vollmar B. 2008. H_2S contributes to the hepatic arterial buffer response and mediates vasorelaxation of the hepatic artery via activation of K_{ATP} channels. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295:G1266–73
29. Webb GD, Lim LH, Oh VMS, Yeo SB, Cheong YP, et al. 2008. Contractile and vasorelaxant effects of hydrogen sulfide and its biosynthesis in the human internal mammary artery. *J. Pharmacol. Exp. Ther.* 324:876–82
30. Cai WJ, Wang MJ, Moore PK, Jin HM, Yao T, Zhu YC. 2007. The novel proangiogenic effect of hydrogen sulfide is dependent on Akt phosphorylation. *Cardiovasc. Res.* 76:29–34
31. Li L, Rossoni G, Sparatore A, Lee LC, Del Soldato P, Moore PK. 2007. Anti-inflammatory and gastrointestinal effects of a novel diclofenac derivative. *Free Radic. Biol. Med.* 42:706–19
32. Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, et al. 2008. Characterization of a novel, water-soluble hydrogen sulfide–releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. *Circulation* 117:2351–60
33. Chunyu Z, Du J, Dingfang B, Hui Y, Xiuying T, Tang C. 2003. The regulatory effect of hydrogen sulfide on hypoxic pulmonary hypertension in rats. *Biochem. Biophys. Res. Commun.* 302:810–16
34. Yanfei W, Lin S, Du J, Tang C. 2006. Impact of L-arginine on hydrogen sulfide/cystathionine-γ-lyase pathway in rats with high blood flow–induced pulmonary hypertension. *Biochem. Biophys. Res. Commun.* 345:851–57
35. Zhong G, Chen F, Cheng Y, Tang C, Du J. 2003. The role of hydrogen sulfide generation in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase. *J. Hypertens.* 21:1879–85
36. Yan H, Du J, Tang C. 2004. The possible role of hydrogen sulfide on the pathogenesis of spontaneous hypertension in rats. *Biochem. Biophys. Res. Commun.* 313:22–27
37. Yang G, Wu L, Jiang B, Yang W, Qi J, et al. 2008. H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ-lyase. *Science* 322:587–90
38. Papapetropoulos A, Pyriochou A, Altaany Z, Yang G, Marazioti A, et al. 2009. Hydrogen sulfide is an endogenous stimulator of angiogenesis. *Proc. Natl. Acad. Sci. USA* 106:21972–77
39. Salloum FN, Chau VQ, Hoke NN, Abbate A, Varma A, et al. 2009. Phosphodiesterase-5 inhibitor, tadalafil, protects against myocardial ischemia/reperfusion through protein-kinase G–dependent generation of hydrogen sulfide. *Circulation* 120:S31–36
40. Shi YX, Chen Y, Zhu YZ, Huang GY, Moore PK, et al. 2007. Chronic sodium hydrosulfide treatment decreases medial thickening of intramyocardial coronary arteries, interstitial fibrosis, and ROS production in spontaneously hypertensive rats. *Am. J. Physiol. Heart Circ. Physiol.* 293:H2093–100
41. Zhao X, Zhang LK, Zhang CY, Zeng XJ, Yan H, et al. 2008. Regulatory effect of hydrogen sulfide on vascular collagen content in spontaneously hypertensive rats. *Hypertens. Res.* 31:1619–30
42. Wang Y, Zhao X, Jin H, Wei H, Li W, et al. 2009. Role of hydrogen sulfide in the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Arterioscler. Thromb. Vasc. Biol.* 29:173–79
43. Zagli G, Patacchini R, Trevisani M, Abbate R, Cinotti S, et al. 2007. Hydrogen sulfide inhibits human platelet aggregation. *Eur. J. Pharmacol.* 559:65–68
44. Zanardo RCO, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL. 2006. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEB J.* 20:2118–20
45. Zhang H, Zhi L, Moore PK, Bhatia M. 2006. The role of hydrogen sulfide in cecal ligation and puncture-induced sepsis in the mouse. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 290:L1193–201
46. Zhu YZ, Wang ZJ, Ho P, Loke YY, Zhu YC, et al. 2007. Hydrogen sulfide and its possible roles in myocardial ischemia in experimental rats. *J. Appl. Physiol.* 102:261–68
47. Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, et al. 2007. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc. Natl. Acad. Sci. USA* 104:15560–65

48. Sivarajah A, McDonald MC, Thiemermann C. 2006. The production of hydrogen sulfide limits myocardial ischemia and reperfusion injury and contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia in the rat. *Shock* 26:154–61
49. Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, et al. 2005. Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *FASEB J.* 19:1196–98
50. Bhatia M, Wong FL, Fu D, Lau HY, Moochhala SM, Moore PK. 2005. Role of hydrogen sulfide in acute pancreatitis and associated lung injury. *FASEB J.* 19:623–25
51. Mok YYP, Atan MS, Ping CY, Jing WZ, Bhatia M, et al. 2004. Role of hydrogen sulphide in haemorrhagic shock in the rat: protective effect of inhibitors of hydrogen sulphide biosynthesis. *Br. J. Pharmacol.* 143:881–89
52. Wallace JL, Caliendo G, Santagada V, Cirino G, Fiorucci S. 2007. Gastrointestinal safety and anti-inflammatory effects of a hydrogen sulfide–releasing diclofenac derivative in the rat. *Gastroenterology* 132:261–71
53. Whiteman M, Li L, Rose P, Tan CH, Parkinson DB, Moore PK. 2010. The effect of hydrogen sulfide donors on lipopolysaccharide-induced formation of inflammatory mediators in macrophages. *Antioxid. Redox Signal.* 12(10):1147–54
54. Fiorucci S, Orlandi S, Mencarelli A, Caliendo G, Santagada V, et al. 2007. Enhanced activity of a hydrogen sulphide–releasing derivative of mesalamine (ATB-429) in a mouse model of colitis. *Br. J. Pharmacol.* 150:996–1002
55. Wallace JL, Dicay M, McKnight W, Martin GR. 2007. Hydrogen sulfide enhances ulcer healing in rats. *FASEB J.* 21:4070–76
56. Olson KR, Dombkowski RA, Russell MJ, Doellman MM, Head SK, et al. 2006. Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J. Exp. Biol.* 209:4011–23
57. Furne J, Saeed A, Levitt MD. 2008. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295:R1479–85
58. Whitfield NL, Kreimier EL, Verdial FC, Skovgaard N, Olson KR. 2008. Reappraisal of H_2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294:R1930–37
59. Tangerman A. 2009. Measurement and biological significance of the volatile sulfur compounds hydrogen sulfide, methanethiol and dimethyl sulfide in various biological matrices. *J. Chromatogr. B* 877:3366–77
60. Hall CN, Garthwaite J. 2009. What is the real physiological NO concentration in vivo? *Nitric Oxide* 21:92–103
61. Glass DC. 1990. A review of the health effects of hydrogen sulphide exposure. *Ann. Occup. Hyg.* 34:323–27
62. Cooper CE, Brown GC. 2008. The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *J. Bioenerg. Biomembr.* 40:533–39
63. Kiss L, Deitch EA, Szabo C. 2008. Hydrogen sulfide decreases adenosine triphosphate levels in aortic rings and leads to vasorelaxation via metabolic inhibition. *Life Sci.* 24:589–94
64. Fiorucci S, Antonelli E, Mencarelli A, Orlandi S, Renga B, et al. 2005. The third gas: H_2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. *Hepatology* 42:539–48
65. Zhong GZ, Li YB, Liu XL, Guo LS, Chen ML, Yang XC. 2009. Hydrogen sulfide opens the K_{ATP} channel on rat atrial and ventricular myocytes. *Cardiology* 115:120–26
66. Distrutti E, Sediari L, Mencarelli A, Renga B, Orlandi S, et al. 2006. Evidence that hydrogen sulfide exerts antinociceptive effects in the gastrointestinal tract by activating K_{ATP} channels. *J. Pharmacol. Exp. Ther.* 316:325–35
67. Monjok EM, Kulkarni KH, Kouamou G, McKoy M, Opere CA, et al. 2008. Inhibitory action of hydrogen sulfide on muscarinic receptor-induced contraction of isolated porcine irides. *Exp. Eye Res.* 87:612–16
68. Kubo S, Doe I, Kurokawa Y, Kawabata A. 2007. Hydrogen sulfide causes relaxation in mouse bronchial smooth muscle. *J. Pharmacol. Sci.* 104:392–96
69. Dhaese I, Van Colen I, Lefebvre RA. 2010. Mechanisms of action of hydrogen sulfide in relaxation of mouse distal colonic smooth muscle. *Eur. J. Pharmacol.* 628:179–86

70. Jiang B, Tang G, Cao K, Wu L, Wang R. 2010. Molecular mechanism for H_2S-induced activation of K_{ATP} channels. *Antioxid. Redox Signal.* 12:1167–78
71. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, et al. 2009. H_2S signals through protein S-sulfhydration. *Sci. Signal.* 2:ra72
72. Zhang Z, Huang H, Liu P, Tang C, Wang J. 2007. Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening K_{ATP} channels. *Can J. Physiol. Pharmacol.* 85:1248–53
73. Ji Y, Pang QF, Xu G, Wang L, Wang JK, Zeng YM. 2008. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. *Eur. J. Pharmacol.* 587:1–7
74. Johansen D, Ytrehus K, Baxter GF. 2006. Exogenous hydrogen sulfide (H_2S) protects against regional myocardial ischemia-reperfusion injury—evidence for a role of K_{ATP} channels. *Basic Res. Cardiol.* 101:53–60
75. Fiorucci S, Antonelli E, Distrutti E, Rizzo G, Mencarelli A, et al. 2005. Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. *Gastroenterology* 129:1210–24
76. Dawe GS, Han SP, Bian JS, Moore PK. 2008. Hydrogen sulphide in the hypothalamus causes an ATP-sensitive K^+ channel-dependent decrease in blood pressure in freely moving rats. *Neuroscience* 152:169–77
77. Telezhkin V, Brazier SP, Cayzac S, Müller CT, Riccardi D, Kemp PJ. 2009. Hydrogen sulfide inhibits human BK_{Ca} channels. *Adv. Exp. Med. Biol.* 648:65–72
78. Sitdikova GF, Weiger TM, Hermann A. 2010. Hydrogen sulfide increases calcium-activated potassium (BK) channel activity of rat pituitary tumor cells. *Pflüg. Arch.* 459:389–97
79. Sun YG, Cao YX, Wang WW, Ma SF, Yao T, Zhu YC. 2008. Hydrogen sulphide is an inhibitor of L-type calcium channels and mechanical contraction in rat cardiomyocytes. *Cardiovasc. Res.* 79:632–41
80. Matsunami M, Tarui T, Mitani K, Nagasawa K, Fukushima O, et al. 2009. Luminal hydrogen sulfide plays a pronociceptive role in mouse colon. *Gut* 58:751–61
81. Malekova L, Krizanova O, Ondrias K. 2009. H_2S and HS^- donor NaHS inhibits intracellular chloride channels. *Gen. Physiol. Biophys.* 28:190–94
82. Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, et al. 2008. Distribution and function of the hydrogen sulfide–sensitive TRPA1 ion channel in rat urinary bladder. *Eur. Urol.* 53:391–99
83. Trevisani M, Patacchini R, Nicoletti P, Gatti R, Gazzieri D, et al. 2005. Hydrogen sulfide causes vanilloid receptor 1-mediated neurogenic inflammation in the airways. *Br. J. Pharmacol.* 145:1123–31
84. Oh GS, Pae HO, Lee BS, Kim BN, Kim JM, et al. 2006. Hydrogen sulfide inhibits nitric oxide production and nuclear factor-κB via heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide. *Free Radic. Biol. Med.* 41:106–19
85. Lee M, Schwab C, Yu S, McGeer E, McGeer PL. 2009. Astrocytes produce the antiinflammatory and neuroprotective agent hydrogen sulfide. *Neurobiol. Aging* 30:1523–34
86. Tamizhselvi R, Moore PK, Bhatia M. 2008. Inhibition of hydrogen sulfide synthesis attenuates chemokine production and protects mice against acute pancreatitis and associated lung injury. *Pancreas* 36:e24–31
87. Tripatara P, Patel NS, Collino M, Gallicchio M, Kieswich J, et al. 2008. Generation of endogenous hydrogen sulfide by cystathionine γ-lyase limits renal ischemia/reperfusion injury and dysfunction. *Lab. Investig.* 88:1038–48
88. Jin HF, Sun Y, Liang JM, Tang CS, Du JB. 2008. Hypotensive effects of hydrogen sulfide via attenuating vascular inflammation in spontaneously hypertensive rats. *Zhonghua Xin Xue Guan Bing Za Zhi* 36:541–45 (In Chinese)
89. Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, et al. 2007. Hydrogen sulfide mediates the vasoactivity of garlic. *Proc. Natl. Acad. Sci. USA* 104:17977–82
90. Zhi L, Ang AD, Zhang H, Moore PK, Bhatia M. 2007. Hydrogen sulfide induces the synthesis of proinflammatory cytokines in human monocyte cell line U937 via the ERK-NF-κB pathway. *J. Leukoc. Biol.* 81:1322–32
91. Stuhlmeier KM, Bröll J, Iliev B. 2009. NF-κB independent activation of a series of proinflammatory genes by hydrogen sulfide. *Exp. Biol. Med.* 234:1327–38
92. Li L, Salto-Tellez M, Tan C-H, Whiteman M, Moore PK. 2009. GYY4137, a novel hydrogen sulfide–releasing molecule, protects against endotoxic shock in the rat. *Free Radic. Biol. Med.* 47:103–13

93. Aggarwal BB, Kunnumakkara AB, Harikumar KB, Gupta SR, Tharakan ST, et al. 2009. Signal transducer and activator of transcription-3, inflammation, and cancer: How intimate is the relationship? *Ann. NY Acad. Sci.* 1171:59–76
94. Calvert JW, Jha S, Gundewar S, Elrod JW, Ramachandran A, et al. 2009. Hydrogen sulfide mediates cardioprotection through Nrf2 signaling. *Circ. Res.* 105:365–74
95. Budde MW, Roth MB. 2010. Hydrogen sulfide increases hypoxia-inducible factor-1 activity independently of von Hippel–Lindau tumor suppressor-1 in *C. elegans*. *Mol. Biol. Cell* 21:212–17
96. Du J, Hui Y, Cheung Y, Bin G, Jiang H, et al. 2004. The possible role of hydrogen sulfide as a smooth muscle cell proliferation inhibitor in rat cultured cells. *Heart Vessels* 19:75–80
97. Osipov RM, Robich MP, Feng J, Chan V, Clements RT, et al. 2010. Effect of hydrogen sulfide on myocardial protection in the setting of cardioplegia and cardiopulmonary bypass. *Interact. Cardiovasc. Thorac. Surg.* 10:506–12
98. Rinaldi L, Gobbi G, Pambianco M, Micheloni C, Mirandola P, Vitale M. 2006. Hydrogen sulfide prevents apoptosis of human PMN via inhibition of p38 and caspase 3. *Lab. Investig.* 86:391–97
99. Hu LF, Wong PT, Moore PK, Bian JS. 2007. Hydrogen sulfide attenuates lipopolysaccharide-induced inflammation by inhibition of p38 mitogen-activated protein kinase in microglia. *J. Neurochem.* 100:1121–28
100. Gobbi G, Ricci F, Malinverno C, Carubbi C, Pambianco M, et al. 2009. Hydrogen sulfide impairs keratinocyte cell growth and adhesion inhibiting mitogen-activated protein kinase signaling. *Lab. Investig.* 89:994–1006
101. Yang G, Sun X, Wang R. 2004. Hydrogen sulfide-induced apoptosis of human aorta smooth muscle cells via the activation of mitogen-activated protein kinases and caspase-3. *FASEB J.* 18:782–84
102. Yang G, Wu L, Wang R. 2006. Pro-apoptotic effect of endogenous H_2S on human aorta smooth muscle cells. *FASEB J.* 20:553–55
103. Yonezawa D, Sekiguchi F, Miyamoto M, Taniguchi E, Honjo M, et al. 2009. A protective role of hydrogen sulfide against oxidative stress in rat gastric mucosal epithelium. *Toxicology* 241:11–18
104. Yusof M, Kamada K, Kalogeris T, Gaskin FS, Korthuis RJ. 2009. Hydrogen sulfide triggers late-phase preconditioning in postischemic small intestine by an NO- and p38 MAPK-dependent mechanism. *Am. J. Physiol. Heart Circ. Physiol.* 296:H868–76
105. Jeong SO, Pae HO, Oh GS, Jeong GS, Lee BS, et al. 2006. Hydrogen sulfide potentiates interleukin-1β-induced nitric oxide production via enhancement of extracellular signal-regulated kinase activation in rat vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 345:938–44
106. Cai WJ, Wang MJ, Ju LH, Wang C, Zhu YC. 2010. Hydrogen sulfide induces human colon cancer cell proliferation: role of Akt, ERK and p21. *Cell Biol. Int.* 34(6):565–72
107. Wang MJ, Cai WJ, Li N, Ding YJ, Chen Y, Zhu YC. 2010. The hydrogen sulfide donor NaHS promotes angiogenesis in a rat model of hind limb ischemia. *Antioxid. Redox Signal.* 12(9):1065–77
108. Yong QC, Lee SW, Foo CS, Neo KL, Chen X, Bian JS. 2008. Endogenous hydrogen sulphide mediates the cardioprotection induced by ischemic postconditioning. *Am. J. Physiol. Heart Circ. Physiol.* 295:H1330–40
109. Kimura Y, Kimura H. 2004. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J.* 18:1165–67
110. Kimura Y, Dargusch R, Schubert D, Kimura H. 2006. Hydrogen sulfide protects HT22 neuronal cells from oxidative stress. *Antioxid. Redox Signal.* 8:661–70
111. Kimura Y, Goto Y, Kimura H. 2010. Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxid. Redox Signal.* 12:1–13
112. Rossoni G, Sparatore A, Tazzari V, Manfredi B, Del Soldato P, Berti F. 2008. The hydrogen sulphide–releasing derivative of diclofenac protects against ischaemia–reperfusion injury in the isolated rabbit heart. *Br. J. Pharmacol.* 153:100–9
113. Jha S, Calvert JW, Duranski MR, Ramachandran A, Lefer DJ. 2008. Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling. *Am. J. Physiol. Heart Circ. Physiol.* 295:H801–6
114. Liu H, Bai XB, Shi S, Cao YX. 2009. Hydrogen sulfide protects from intestinal ischaemia-reperfusion injury in rats. *J. Pharm. Pharmacol.* 61:207–12

115. Sen U, Basu P, Abe OA, Givvimani S, Tyagi N, et al. 2009. Hydrogen sulfide ameliorates hyperhomocysteinemia-associated chronic renal failure. *Am. J. Physiol. Renal. Physiol.* 297:F410–19
116. Tyagi N, Moshal KS, Sen U, Vacek TP, Kumar M, et al. 2009. H_2S protects against methionine-induced oxidative stress in brain endothelial cells. *Antioxid. Redox Signal.* 11:25–33
117. Yan SK, Chang T, Wang H, Wu L, Wang R, Meng QH. 2006. Effects of hydrogen sulfide on homocysteine-induced oxidative stress in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 351:485–91
118. Jeney V, Komódi E, Nagy E, Zarjou A, Vercellotti GM, et al. 2009. Suppression of hemin-mediated oxidation of low-density lipoprotein and subsequent endothelial reactions by hydrogen sulfide (H_2S). *Free Radic. Biol. Med.* 46:616–23
119. Perrino E, Uliva C, Lanzi C, Del Soldato P, Masini E, Sparatore A. 2009. New prostaglandin derivative for glaucoma treatment. *Bioorg. Med. Chem. Lett.* 19:1639–42
120. Osborne NN, Ji D, Abdul Majid AS, Fawcett RJ, Sparatore A, Del Soldato P. 2010. ACS67, a hydrogen sulfide–releasing derivative of latanoprost acid, attenuates retinal ischemia and oxidative stress to RGC-5 cells in culture. *Investig. Ophthalmol. Vis. Sci.* 51:284–94
121. Gadalla MM, Snyder SH. 2010. Hydrogen sulfide as a gasotransmitter. *J. Neurochem.* 113(1):14–26

Annual Review of Pharmacology and Toxicology

Volume 51, 2011

Contents

Indexes

Errata

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