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Forum Review

Physiological Roles of Hydrogen Sulfide: Synaptic Modulation, Neuroprotection, and Smooth Muscle Relaxation

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ABSTRACT

Nearly 300 years have passed since the first description of the toxicity of hydrogen sulfide (H_2S) in 1713. Although many studies have been devoted to its toxicity, very little attention has been paid to understanding its normal physiological function. Relatively high concentrations of endogenous H_2S, however, have recently been discovered in animal tissues, and its possible function as a biological messenger has been proposed. H_2S enhances the activity of *N*-methyl-D-aspartate receptors and facilitates the induction of hippocampal long-term potentiation, a synaptic model for memory. H_2S also increases intracellular concentrations of Ca^{2+} in glia and induces Ca^{2+} waves, which mediate glial signal transmission. Based on accumulating evidence for the reciprocal interactions between glia and neurons, it has been suggested that glia modulate synaptic transmission. Therefore, H_2S may regulate synaptic activity by modulating the activity of both neurons and glia. In addition to a role in the signal transduction, H_2S protects neurons from oxidative stress and in smooth muscle it may function as a relaxant. H_2S, the toxic gas, may therefore be used as a multifunctional signaling mechanism under normal physiological conditions. *Antioxid. Redox Signal.* 7, 795–803.

INTRODUCTION

HYDROGEN SULFIDE (H_2S) is generally thought of in terms of a poisonous gas. However, relatively high endogenous levels of H_2S have recently been measured in the brains of rats, humans, and bovine (30, 60, 79). As H_2S is chemically a very active gas, endogenous H_2S may have a physiological function. Recently, it has been shown that physiological concentrations of H_2S specifically potentiate the activity of *N*-methyl-D-aspartate (NMDA) receptor and alter the induction of long-term potentiation (LTP) in the hippocampus, a synaptic model of learning and memory (1). H_2S can also regulate the release of corticotropin-releasing hormone from the hypothalamus (58). Two other gases, nitric oxide (NO) and carbon monoxide (CO), are endogenously produced by enzymes localized in the brain (28, 77). Both NO and CO have also been proposed as retrograde messengers in hippocampal LTP (10, 34, 52, 61, 64, 83). These observations suggest the neuromodulatory role of H_2S in the brain (1, 62).

Glial cells have been considered to be the nonexcitable supportive elements in the nervous system, but they are now regarded as elements that respond to neuronal activity, as well as modulate synaptic activity (36). One class of glia, astrocytes, makes neurotransmitters and expresses hormone receptors. A number of conditions, including neurotransmitters and a mechanical stimulation, evoke increases in intracellular Ca^{2+} in astrocytes that propagate into neighboring astrocytes as intercellular Ca^{2+} waves (11, 17, 40, 50). Ca^{2+} waves have been well characterized in cultured astrocytes, as well as acutely isolated hippocampal slices (20, 25, 40, 50). Neurons interact with glia, and the two communicate with each other (20, 51, 55). Neuronal activity evokes glial Ca^{2+} waves (20), and conversely glial Ca^{2+} waves drive neuronal activity (51, 55). Glial cells are therefore integral modulatory elements in synaptic transmission (4), and H_2S may be involved in the glial signal transduction.

There are two forms of glutamate toxicity: receptor-initiated excitotoxicity (14) and non–receptor-mediated oxidative glutamate toxicity (47). Oxidative glutamate toxicity, recently renamed oxytosis (70), is a well-studied programmed cell-death pathway that is independent of ionotropic glutamate receptors (44, 47, 70). It has been observed in primary cultures

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of neuronal cells (48), neuronal cell lines (21, 46, 47), and brain slices (78). Oxidative stress is responsible for neuronal damage and degeneration in brain disorders, including stroke, epilepsy, and Alzheimer's disease (18, 56). Glutamate shares an amino acid transporter with cystine, and it competes with cystine for transport into cells (7). Therefore, elevated extracellular glutamate inhibits the transport of cystine that is the primary source of intracellular cysteine necessary for glutathione synthesis. H_2S increases the activity of γ-glutamylcysteine synthase (γ-GCS) and causes the recovery of cystine transport suppressed by glutamate, resulting in an increase in the levels of glutathione in neurons (42). Thus, H_2S may function as a neuroprotectant against oxidative stress.

When acetylcholine is applied to the thoracic aorta, the endothelial cells release endothelial-derived relaxing factor (EDRF) (26). EDRF relaxes smooth muscle and hyperpolarizes smooth muscle cells (24). NO is a relaxing factor identified as EDRF (53, 54). However, in some blood vessels, a lack of correlation has been noted between the effect of NO and that of EDRF to hyperpolarize the vascular smooth muscle cells (24). Another unidentified factor or component of EDRF, which hyperpolarizes smooth muscle, is thought to be released from endothelial cells and is designated endothelial-derived hyperpolarizing factor (EDHF). In addition to these factors released from endothelial cells, non–endothelium-derived relaxing factors have been proposed (29). Low-molecular-weight *S*-nitrosothiol intermediates may also contribute to the relaxation of coronary smooth muscle, vascular smooth muscle, carotid arteries, and the cerebral artery (12, 24, 29, 49). Cystathionine γ-lyase (CSE), which can produce H_2S, has been identified in smooth muscle. The following paragraphs outline in more detail the possible role of H_2S as a synaptic modulator in the central nervous system and as a relaxant in smooth muscle.

CHEMICAL PROPERTIES OF H_2S

H_2S is a colorless 34 molecular weight gas that is heavier than air. One gram of H_2S dissolves in 242 ml of water, 94.3 ml of ethanol, or 48.5 ml of diethyl ether (57). H_2S easily penetrates biological membranes. In physiological saline, approximately one-third of the H_2S exists as the undissociated form (H_2S), and the remaining two-thirds exists as HS^- at equilibrium with H_2S (57). NaHS has been widely used for studies of H_2S instead of H_2S gas for the following reasons (9, 43, 79). NaHS dissociates to Na^+ and HS^- in solution, then HS^- associates with H^+ and produces H_2S. It does not matter whether the H_2S solution is prepared by bubbling H_2S gas or by dissolving NaHS. The use of NaHS enables us to define the concentrations of H_2S in solution more accurately and reproducibly than bubbling H_2S gas. The influence of <1 m*M* Na^+ on electrophysiological experiments is negligible, because basic salt solution contains 150 m*M* Na^+. NaHS at concentrations of <1 m*M* does not change the pH of basic salt solution.

Like H_2S, both NO and CO are colorless gases and easily penetrate biological membrane. The molecular weight of NO and CO is 30 and 28, respectively. NO is a little heavier than air, whereas CO is lighter. At 20°C, 4.6 ml of NO and 2.3 ml of CO are dissolved in 100 ml of water. NO is a free radical and produces the extremely toxic hydroxyl radical when it combines with superoxide (63). CO is a reducing agent like H_2S. With those differences and similarities, H_2S, NO, and CO elicit several effects; some of them are opposite, whereas others are quite similar.

H_2S PRODUCTION

Endogenous H_2S can be produced from cysteine by pyridoxal 5′-phosphate-dependent enzymes, including cystathionine β-synthetase (CBS) and CSE. CBS mRNA is expressed in the brain, expecially in hippocampus and cerebellum, whereas CSE mRNA is not detectable (1). The production of H_2S from brain homogenates is suppressed by CBS-specific inhibitors, aminooxyacetate and hydroxylamine, whereas it is not suppressed by CSE-specific inhibitors, DL-propargylglycine and β-cyano-L-alanine (1). The H_2S production is enhanced by a CBS activator, *S*-adenosyl-L-methionine. These observations suggest that CBS is a candidate enzyme for the production of H_2S in the brain.

CSE is expressed in the ileum, portal vein, and thoracic aorta. The homogenates of these tissues produce H_2S in the presence of cysteine, and this production is blocked by CSE-specific inhibitors (38, 82). The production of H_2S from homogenized vascular tissues is up-regulated by sodium nitroprusside (SNP) in a concentration-dependent manner, and *S*-nitroso-*N*-acetylpenicillamine (SNAP), another NO donor, increases the transcriptional level of CSE (81).

Other enzymes involved in the transaminative pathway of methionine catabolism have also been proposed to produce H_2S in mammals, and their regulation may also be involved in the normal physiological function of H_2S (74).

REGULATION OF NEURONAL ACTIVITY

Because H_2S is produced in the brain, H_2S may play a role in synaptic transmission. We recently found that physiological concentrations of H_2S modify the induction of LTP in a dose-dependent manner. Although NaHS at concentrations of <130 μ*M* or a weak tetanic stimulation alone does not induce LTP, simultaneous application of both stimulations induces LTP (1). The timing of application of H_2S with a weak tetanic stimulation is an important factor to facilitate the induction of LTP. When NaHS is applied 10 min before or after a weak tetanic stimulation, facilitation of LTP induction does not occur.

NO and CO increase intracellular cyclic GMP, whereas H_2S does not (1, 63). The observation that NO and CO induce LTP even when NMDA receptors are blocked (83) supports the idea that NO and CO act as retrograde messengers at synapses (52, 61, 64). In contrast, H_2S with a weak tetanic stimulation does not induce LTP in the presence of 2-amino-5-phosphonovalerate, a specific blocker for the NMDA receptor (1), suggesting that the induction of LTP by H_2S requires the activation of NMDA receptors.

Hippocampal LTP induced by a tetanic stimulation requires the activation of NMDA receptors (35). H_2S alone does not

induce any apparent currents, but significantly increases the NMDA-induced inward current (1). The enhancing effect of H_2S on the NMDA response is concentration-dependent in the same range as its LTP-facilitating effect and is specific to NMDA receptors. Therefore, H_2S may enhance the induction of LTP by activating NMDA receptors.

Disulfide bonds play a role in modulating the function of many proteins, including NMDA receptors (3, 71). It is therefore possible that H_2S interacts with disulfide bonds or free thiols in NMDA receptors. The irreversible thiol-protecting agent dithiothreitol (DTT) with a weak tetanic stimulation significantly facilitates the induction of LTP. H_2S with a weak tetanic stimulation, however, still induces LTP even after treatment with DTT, demonstrating that DTT does not occlude the effect of H_2S (1). It is therefore unlikely that the thiol redox sites in the NMDA receptor contribute little, if at all, to the potentiating effect of H_2S on the induction of LTP.

H_2S INCREASES INTRACELLULAR Ca^{2+} AND INDUCES Ca^{2+} WAVES IN ASTROCYTES

The observation that H_2S enhances the induction of hippocampal LTP suggests that H_2S may modulate some aspects of synaptic activity. Although H_2S enhances the NMDA receptor-mediated responses to glutamate in neurons, the effects of H_2S on brain cells in the absence of glutamate are not well understood. We recently found that H_2S alone induces Ca^{2+} waves in astrocytes (50) using a Ca^{2+} imaging system with Calcium Green-1 as a Ca^{2+}-sensitive fluorescent dye. Focal application of H_2S increased intracellular concentrations of Ca^{2+} in glial fibrillary acidic protein (GFAP)-positive astrocytes (50). Although H_2S enhances the responses of neurons to NMDA, significant Ca^{2+} responses to H_2S applied directly to neurons were not observed. H_2S may therefore modulate synaptic activity by directly enhancing the responses to glutamate in neurons and indirectly by inducing Ca^{2+} waves in astrocytes (Fig. 1).

There is a difference in the time course of the increase in intracellular Ca^{2+} between the astrocytes exposed directly to H_2S and those activated by the propagated Ca^{2+} waves. The intracellular Ca^{2+} in the astrocytes exposed to H_2S sharply increases and gradually decays, whereas the propagated Ca^{2+} waves show oscillations with a faster decay (50). The initial increase in the intracellular Ca^{2+} induced by H_2S may therefore be regulated by a different mechanism than the propagated Ca^{2+} waves.

Glial cells in primary cultures are GFAP-negative during the first 10 days (72), and then cells become GFAP-positive and A2B5-negative astrocytes. Cells start responding to H_2S at 6 days, and the responses reach a maximum level at ~30 days (50). The responses to H_2S observed in cultures of astrocytes also occur in hippocampal slices (50). It has been difficult to identify neurons and glia in brain slices during electrophysiological recording or imaging, for viable slices cannot be stained for specific cell markers. Recently, it has been found that increases in the intracellular concentrations of Ca^{2+} are specifically induced in astrocytes by low external concentrations of K^+ (19). By using this characteristic of astrocytes, we can define the cells that respond to H_2S in hippocampal slices as astrocytes. Because astrocytes in acute brain slices respond to H_2S, the possibility that responses to H_2S may be the artifact caused by cell culture can be excluded. The requirement of several days in culture before astrocytes respond to H_2S may be due to the expression of proteins that are necessary for responding to H_2S or the formation of cell–cell junctions.

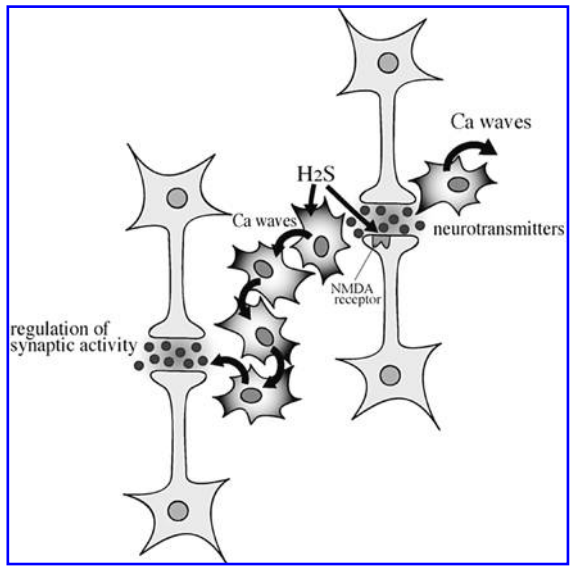

FIG. 1. H_2S induces Ca^{2+} waves in astrocytes. H_2S is released from neurons or glia surrounding synapses and increases the intracellular concentrations of Ca^{2+}. Elevated intracellular Ca^{2+} triggers the induction of Ca^{2+} waves that propagate to the neighboring astrocytes and may reach and regulate the next synapse.

RESPONSES TO H_2S REQUIRE BOTH EXTRACELLULAR Ca^{2+} AND INTRACELLULAR Ca^{2+} STORES

Glutamate and ATP are known to induce Ca^{2+} waves in astrocytes (17, 33). The increase in intracellular Ca^{2+} induced by glutamate is dependent on extracellular Ca^{2+}, whereas that induced by ATP is only dependent on intracellular Ca^{2+} stores (17, 23, 41). The increase in intracellular Ca^{2+} induced by NaHS is greatly suppressed in the Ca^{2+}-free medium, whereas the response to ATP is intact. H_2S increases the influx of Ca^{2+} similarly to that caused by ionomycin, a Ca^{2+} ionophore (50). Responses to H_2S are also suppressed by thapsigargin, a compound that depletes intracellular Ca^{2+} stores, but the suppression of responses to H_2S is much less than that of responses to ATP or glutamate. H_2S increases intracellular concentrations of Ca^{2+}, largely by inducing Ca^{2+} influx, and to a lesser extent through the release from intracellular Ca^{2+} stores.

As H_2S increases intracellular Ca^{2+}, it is possible that H_2S may activate a channel or a receptor associated with a channel that is permeable to Ca^{2+}. Trivalent cations, La^{3+} and Gd^{3+}, well-known blockers of Ca^{2+} channels, potently suppress re-

sponses to H_2S (50). Ruthenium red, which is a blocker of ryanodine receptors and inhibits voltage-gated Ca^{2+} channels (15), also suppresses responses to H_2S. Three additional voltage-dependent Ca^{2+}-channel blockers, flunarizine, nifedipine, and ω-conotoxin GVIA, all potently suppress responses to H_2S.

Although Ca^{2+} channels are clearly activated, the type of Ca^{2+} channels is difficult to determine because a T-type blocker, flunarizine, an L-type, nifedipine, and an N-type, ω-conotoxin, block the effect of H_2S less potently than the nonspecific voltage-dependent Ca^{2+} channel blockers, La^{3+} and Gd^{3+}. Alternatively, La^{3+}, Gd^{3+}, and ruthenium red are also potent inhibitors of the transient receptor potential (TRP) family of channels that are permeable to Ca^{2+} (16, 65, 73), and H_2S may activate these channels. Mg^{2+} and MDL-12,330A block TRP channels (64, 76), and both substances suppress responses to H_2S. Therefore, further work is necessary to identify the specific type of Ca^{2+} channel that is activated by H_2S.

INVOLVEMENT OF H_2S IN Ca^{2+} WAVES IN ASTROCYTES INDUCED BY NEURONAL EXCITATION

Interactions between neurons and glia may modulate synaptic transmission, for neuronal activity can evoke glial Ca^{2+} waves (20), and propagated Ca^{2+} waves in glial cells may modulate neuronal activity (51, 55). After neurons were excited by NMDA, Ca^{2+} waves occurred in neighboring astrocytes (50). The Ca^{2+} waves induced by NMDA were completely suppressed by 10 μ*M* La^{3+} or 10 μ*M* Gd^{3+}. H_2S released in response to neuronal excitation may increase intracellular Ca^{2+} and induce Ca^{2+} waves in neighboring astrocytes. Alternatively, as La^{3+} and Gd^{3+} block Ca^{2+} waves and also inhibit Ca^{2+} channels, La^{3+} and Gd^{3+} may inhibit the exocytosis of glutamate or some other factor from nerve terminals when neurons are stimulated by NMDA.

H_2S AS A NEUROPROTECTANT

The toxic effect of H_2S on the nervous system is well known (57), but the protective effect of H_2S on neuronal cells has not even been imagined. NO may be involved in glutamate neurotoxicity (63). In contrast, sulfur-containing substances, dimethylsulfoniopropionate (DMSP) and its enzymatic cleavage product dimethyl sulfide (DMS), have recently been identified as endogenous scavengers for hydroxyl radicals and other reactive oxygen species in marine algae (69). Because H_2S, an endogenous reducing agent, is produced by oxidative stress (44), it is possible that H_2S functions as an antioxidant. To investigate this possibility, the effect of H_2S on oxytosis was examined using primary cultures of neurons. Primary cultures of cortical immature neurons, which lack ionotropic glutamate receptors during their first few days in culture (48), were prepared from 17-day-old embryonic rat brains and cultured for 1 day. Most of the neurons died within 24 h after the application of 1 m*M* glutamate, because glutamate inhibits cystine uptake causing oxidative stress-induced cell death, a process called oxytosis (70). H_2S protects cells from glutamate toxicity in a dose-dependent manner (42). H_2S alone caused a significant increase in survival following plating, protecting cells from the spontaneous cell death that occurs in primary cultures (2).

Glutamate reduces intracellular glutathione (42), and glutathione is the major endogenous antioxidant (31). H_2S alone increases the levels of the reduced form of glutathione (GSH) and the oxidized form of glutathione (GSSG) for several hours. H_2S also reinstates intracellular glutathione lowered by glutamate (42). GSH can protect cells from oxidative stress, and H_2S increases the GSH levels both in untreated cells and in cells where GSH is normally depleted by glutamate. It is likely that H_2S increases glutathione levels instead of functioning directly as an antioxidant. The endogenous levels of glutathione (1–8 m*M*) (32) are much greater than those of H_2S (50–160 μ*M*) (57). Therefore, H_2S does not itself rescue cells from oxidative stress, but H_2S induces the production of a potent antioxidant, glutathione.

Cells can be rescued from oxidative stress by mechanisms that are either dependent on or independent of glutathione metabolism. For example, antioxidants such as vitamin E protect neuronal cells from oxytosis by acting directly as antioxidants even when the intracellular glutathione levels are decreased (47, 62). In contrast, dihydroxyphenylglycine, an agonist of group I metabotropic glutamate receptors, protects neurons by up-regulating glutathione (59). Because H_2S rescues neurons by increasing the accumulation of glutathione, the protection from oxytosis by H_2S belongs to the latter class of mechanisms. The requirement of glutathione for cell survival induced by H_2S is also supported by another observation. A specific inhibitor of γ-GCS, buthionine sulfoximine (31), dose-dependently suppressed both the levels of glutathione and cell survival induced by H_2S (42).

It is possible that H_2S enhances the activity of γ-GCS to increase the production of γ-glutamylcysteine (γ-GC). We found that H_2S does indeed increase the levels of γ-GC, leading to the increase in the levels of glutathione. In the presence of H_2S, the levels of γ-GC in cells are increased more than twofold of those in cells in the absence of H_2S. Even in the presence of glutamate, H_2S increases the levels of γ-GC in cells approximately twofold (42). The increase in γ-GC induced by H_2S is not caused by the transcriptional regulation of γ-GCS, but either by the direct activation of the enzyme or through a translational mechanism.

Glutathione is synthesized from cysteine that is produced from cystine transported into cells from the outside (7). Oxytosis is caused by the blockade of the cystine/glutamate antiporter that couples the import of cystine and the export of glutamate (6, 47). The transport of cystine into primary neurons is significantly increased by H_2S, and even in the presence of glutamate H_2S significantly reversed the inhibition of cystine transport by glutamate (42). The H_2S-induced recovery of glutamate-suppressed cystine transport may therefore be involved in the increased production of glutathione and neuroprotection.

The cystine uptake by the cystine/glutamate antiporter x_c^- mediates oxytosis (13). The specific inhibitor for x_c^-, glutamate, significantly suppresses the cystine uptake, and this in-

hibition is significantly reduced by H_2S, suggesting that antiporter x_c^- may be involved in the cystine transport recovered by H_2S (42). This is also supported by the following observations: (a) Endogenous levels of cysteine are increased in the presence of H_2S. (b) At each concentration of extracellular cystine tested, the glutathione levels are increased by greater than twofold in the presence of H_2S relative to those in the absence of H_2S. (c) When the extracellular concentrations of cystine are decreased, glutathione levels are decreased in both the presence and absence of H_2S, indicating that the enhancing effect of H_2S on the glutathione levels is dependent on the extracellular concentrations of cystine (42).

Because H_2S is a reducing agent, it is possible that H_2S reduces cystine to cysteine and enhances the transport of cysteine that is transported by the ASC (alanine, serine, and cysteine) transporter. The inhibitors for the ASC transporter, alanine and serine, do not significantly inhibit the cysteine uptake, nor do they significantly inhibit the cysteine uptake in the presence of H_2S, excluding the possibility that H_2S increases the transport of cysteine by enhancing the activity of the ASC transporter. As cells synthesize little cysteine themselves (8), H_2S must function by enhancing cystine transport, leading to the increase in the levels of γ-GC and glutathione (Fig. 2).

H_2S is an active molecule and has a strong effect on several targets. For example, H_2S potentiates the induction of LTP by enhancing the activity of NMDA receptors in neurons, and it activates Ca^{2+} channels to induce Ca^{2+} waves in astrocytes (1, 50). H_2S relaxes smooth muscle by activating ATP-dependent K^+ channels (38, 81). In neurons, H_2S enhances the activity of γ-GCS and cystine/glutamate antiporter x_c^-. The combined enhancement of the activity of these different targets may cause an integrated effect that results in the increase in the levels of glutathione. Although the function is not well understood, the uptake of atmospheric H_2S by leaves also increases the levels of glutathione in plants (37), suggesting that H_2S activates a common pathway in plants and animals to accumulate glutathione.

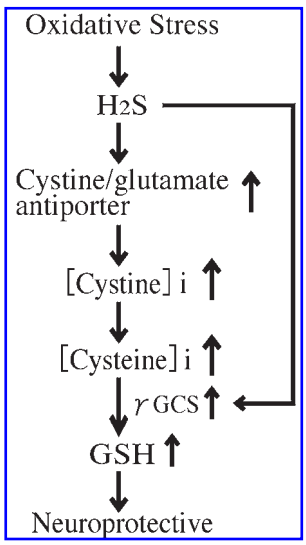

FIG. 2. H_2S protects neurons from oxidative stress. H_2S increases cysteine levels by enhancing glutamate/cystine antiporter and γ-GCS activity, which produces glutathione. H_2S protects neurons from oxidative stress by increasing levels of glutathione, a major intracellular antioxidant.

H_2S AS A RELAXANT FOR SMOOTH MUSCLE

NO has been discovered as an EDRF (26, 54), and CO has been found to be another gaseous messenger in smooth muscle (75). CO relaxes smooth muscle of hepatic microcirculation, whereas it constricts the resistant artery (67, 68). Substances other than NO, including low-molecular-weight *S*-nitrosothiol intermediates, may contribute to the relaxation of smooth muscle (12, 24, 29, 49). A candidate enzyme for the production of H_2S, CSE, is expressed in the ileum, portal vein, and thoracic aorta (38). The homogenates of these tissues produce H_2S, and this production is blocked by CSE inhibitors (38). Exogenously applied H_2S alone relaxes these smooth muscles and modifies the relaxation of smooth muscle induced by NO. Low concentrations of H_2S enhance smooth muscle relaxation induced by NO in the helical tissue strips of the thoracic aorta (38). Although a similar enhancing effect of H_2S on relaxation of vas deferens induced by NO was observed (73), the enhancing effect of H_2S is controversial. In aortic ring preparations, H_2S inhibits the vasorelaxant effect of the NO-producing agent, SNP (80). H_2S production can also be regulated by NO. H_2S production by CSE is increased by SNP, and the expression of CSE is up-regulated by another NO-producing agent, SNAP (81).

Based on the observation that H_2S significantly relaxes thoracic aorta even after the removal of endothelial cells (38, 80), the ability of H_2S to relax thoracic aorta must be a direct effect on smooth muscle cells. However, in the presence of apamin and charybdotoxin, which are used to block the effect of EDHF (22), the ability of H_2S to relax thoracic aorta is attenuated, suggesting that the endothelial cells may release factors such as EDHF in response to H_2S (80).

NO and CO relax smooth muscle by activating guanylyl cyclase to increase the production of cyclic GMP (39, 63). In contrast, H_2S does not have an effect on the production of cyclic GMP (1), suggesting a different mechanism for the effect of H_2S. The hyperpolarization induced by H_2S is similar to that induced by NO in small mesenteric arteries in that the hyperpolarization is suppressed by glibenclamide, a blocker for K_{ATP} channels (27, 81). Although the concentration of glibenclamide is greater than needed to specifically suppress K_{ATP} channels (5, 66), relaxation of the aorta induced by H_2S is also suppressed by glibenclamide (81). Based on these observations, it has been proposed that the relaxation and hyperpolarization of smooth muscle induced by H_2S is mediated by K_{ATP} channels (81). However, in the ileum, glibenclamide does not have any effect on the relaxation induced by H_2S (73). H_2S inhibits contractions of the aorta induced by 20 m*M* KCl (81), whereas H_2S fails to affect the contractile response to KCl in the ileum (73). Therefore, a different mechanism may be involved in the relaxation effect of H_2S on the ileum. Al-

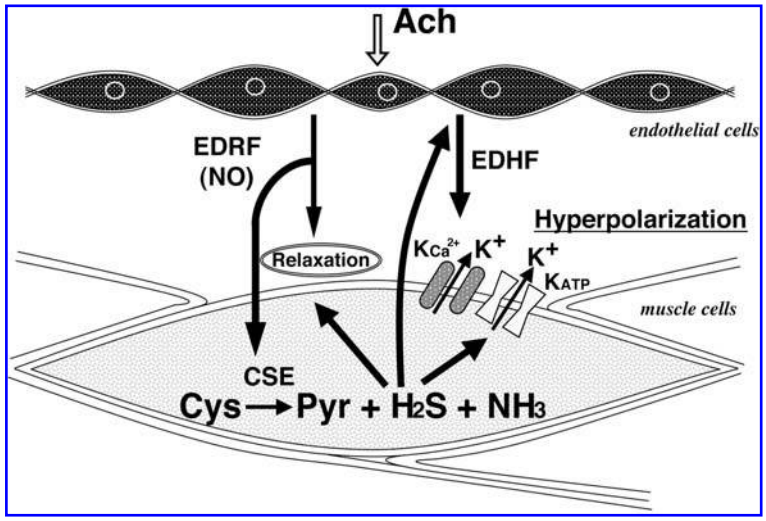

FIG. 3. H_2S relaxes smooth muscle. H_2S relaxes smooth muscle by activating K_{ATP} channels. H_2S released from smooth muscle may enhance the release of EDHF from endothelial cells or directly activate K_{ATP} channels. NO-producing agents enhance the activity of CSE and upregulate transcription of the CSE gene. Ach, acetylcholine.

though further studies are required to elucidate the possibility of an interaction between H_2S and EDHF, accumulating evidence suggests that H_2S is a strong candidate for a smooth muscle relaxant (Fig. 3).

CONCLUSIONS

H_2S is an active molecule and has a strong effect on several targets. H_2S enhances the activity of NMDA receptors in neurons (1) and elicits Ca^{2+} waves in astrocytes by activating Ca^{2+} channels (50). H_2S enhances the induction of LTP, but the mechanism is not well understood. As H_2S targets both neurons and glia, H_2S may be involved in the modulation of tripartite synapse in which the activity of glial cells cross-talk with neurons (1, 4, 50). Another effect on neurons is that H_2S protects neurons by increasing levels of a major and potent antioxidant glutathione by enhancing the activity of γ-GC and cystine/glutamate transporter instead of functioning directly as an antioxidant.

Although K_{ATP} has been proposed to be involved in the vasorelaxation induced by H_2S (81), K_{ATP} may not be the major component of ileum relaxation (73), suggesting additional targets for H_2S in its relaxation effect on smooth muscle. The combined enhancement of the activity of these different targets may cause an integrated effect that results in smooth muscle relaxation.

After H_2S stimulates its targets, it has to be cleared from its site of action. The mechanism of clearance is not understood. The study of H_2S as a physiologically active molecule is just beginning, but understanding the mechanisms underlying its physiological function may provide a new insight into the neurotransmission, protection, and smooth muscle relaxation.

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ABBREVIATIONS

ASC, alanine, serine, and cysteine; CBS, cystathionine β-synthase; CO, carbon monoxide; CSE, cystathionine γ-lyase; DTT, dithiothreitol; EDHF, endothelial-derived hyperpolarizing factor; EDRF, endothelial-derived relaxing factor; γ-GC, γ-glutamylcysteine; γ-GCS, γ-glutamylcysteine synthase; GFAP, glial fibrillary acidic protein; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; H_2S, hydrogen sulfide; K_{ATP} channel, ATP-dependent K^+ channel; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; SNAP, *S*-nitroso-*N*-acetylpenicillamine; SNP, sodium nitroprusside; TRP, transient receptor potential.

REFERENCES

1. Abe K and Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* 16: 1066–1071, 1996.
2. Abe K, Takayanagi M, and Saito H. Effects of recombinant human basic FGF and its modified protein CS23 on survival of primary cultured neurons from various regions of fetal rat brain. *Jpn J Pharmacol* 53: 221–227, 1990.
3. Aizenman E, Lipton DA, and Loring RH. Selective modulation of NMDA responses by reduction and oxidation. *Neuron* 2: 1257–1263, 1989.
4. Araque A, Parpura V, Sanzgiri RP, and Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci* 22: 208–215, 1999.
5. Ashcroft SJH and Ashcroft FM. Properties and functions of ATP-sensitive K-channels. *Cell Signal* 2: 197–214, 1990.
6. Bannai S. Exchange of cystine and glutamate across plasma membrane of human fibroblasts. *J Biol Chem* 261: 2256–2263, 1986.

7. Bannai S and Kitamura E. Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. *J Biol Chem* 255: 2372–2376, 1980.
8. Bannai S, Ishii T, Takada A, and Noriko T. Regulation of glutathione level by amino acid transport. In: *Glutathione Centennial*, edited by Taniguchi N, Higashi T, Sakamoto Y, and Meister A. San Diego, CA: Academic Press, 1989, pp. 407–421.
9. Beauchamp RO Jr, Bus JS, Popp JA, Boreiko CJ, and Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. *Crit Rev Toxicol* 13: 25–97, 1984.
10. Bliss TVP and Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31–39, 1993.
11. Charles AC, Merrill JE, Dirksen ER, and Sanderson MJ. Intercellular signaling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron* 6: 983–992, 1991.
12. Chen G, Yamamoto Y, Miwa K, and Suzuki H. Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. *Am J Physiol* 260: H1888–H1892, 1991.
13. Cho Y and Bannai S. Uptake of glutamate and cystine in C-6 glioma cells and in cultured astrocytes. *J Neurochem* 55: 2091–2097, 1990.
14. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1: 623–634, 1988.
15. Cibulsky SM and Sather WA. Block by ruthenium red of cloned neuronal voltage-gated calcium channels. *J Pharmacol Exp Ther* 289: 1447–1453, 1999.
16. Clapham DE, Runnels LW, and Strubing C. The TRP ion channel family. *Nat Rev Neurosci* 2: 387–396, 2001.
17. Cornell-Bell AH, Finkbeiner SM, Cooper MS, and Smith SJ. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247: 470–473, 1990.
18. Coyle JT and Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262: 689–695, 1993.
19. Dallwig R and Deitmer JW. Cell-type specific calcium responses in acute rat hippocampal slices. *J Neurosci Methods* 116: 77–87, 2002.
20. Dani JW, Chernjavsky A, and Smith SJ. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* 8: 429–440, 1992.
21. Davis JB and Maher P. Protein kinase C activation inhibits glutamate-induced cytotoxicity in a neuronal cell line. *Brain Res* 652: 169–173, 1994.
22. Doughty JM, Plane F, and Langton PD. Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. *Am J Physiol* 276: H1107–H1112, 1999.
23. Fam SR, Gallagher CJ, and Salter MW. P2Y(1) purinoceptor-mediated Ca^{2+} signaling and Ca^{2+} wave propagation in dorsal spinal cord astrocytes. *J Neurosci* 20: 2800–2808, 2000.
24. Feletou M and Vanhoute PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 93: 515–524, 1988.
25. Finkbeiner S. Calcium waves in astrocytes–filling in the gaps. *Neuron* 8: 1101–1108, 1992.
26. Furchgott RF and Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373–376, 1980.
27. Garland CJ and McPherson GA. Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. *Br J Pharmacol* 105: 429–435, 1992.
28. Garthwaite J, Charles SL, and Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 336: 385–388, 1988.
29. Gaw AJ and Bevan JA. Flow-induced relaxation of the rabbit middle cerebral artery is composed of both endothelium-dependent and -independent components. *Stroke* 24: 105–110, 1993.
30. Goodwin LR, Francom D, Dieken FP, Taylor JD, Warenycia MW, Reiffenstein RJ, and Dowling G. Determination of sulfide in brain tissue by gas dialysis/ion chromatography: postmortem studies and two case reports. *J Anal Toxicol* 13: 105–109, 1989.
31. Griffith OW. Mechanism of action, metabolism, and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. *J Biol Chem* 257: 13704–13712, 1982.
32. Griffith OW and Meister A. Glutathione: interorgan translocation, turnover, and metabolism. *Proc Natl Acad Sci U S A* 76: 5606–5610, 1979.
33. Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, and Kater SB. ATP released from astrocytes mediates glial calcium waves. *J Neurosci* 19: 520–528, 1999.
34. Haley JE, Wilcox GL, and Chapman PF. The role of nitric oxide in hippocampal long-term potentiation. *Neuron* 8: 211–216, 1992.
35. Harris EW, Ganong AH, and Cotman CW. Long-term potentiation in the hippocampus involves activation of *N*-methyl-D-aspartate receptors. *Brain Res* 323: 132–137, 1984.
36. Haydon, PG. GLIA: listening and talking to the synapse. *Nat Rev Neurosci* 2: 185–193, 2001.
37. Herschbach C, van Der Zalm E, Schneider A, Jouanin L, De Kok LJ, and Rennenberg H. Regulation of sulfur nutrition in wild-type and transgenic poplar over-expressing γ-glutamylcysteine synthetase in the cytosol as affected by atmospheric H_2S. *Plant Physiol* 124: 461–473, 2000.
38. Hosoki R, Matsuki N, and Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 237: 527–531, 1997.
39. Kajimura M, Shimoyama M, Tsuyama S, Suzuki T, Kozaki S, Takenaka S, Tsubota K, Oguchi Y, and Suematsu M. Visualization of gaseous monoxide reception by soluble guanylate cyclase in the rat retina. *FASEB J* 17: 506–508, 2003.
40. Kang J, Jiang L, Goldman SA, and Nedergaard M. Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat Neurosci* 1: 683–692, 1998.
41. Kim WT, Rioult MG, and Cornell-Bell AH. Glutamate-induced calcium signaling in astrocytes. *Glia* 11: 173–184, 1994.

42. Kimura Y and Kimura H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J* 18: 1165–1167, 2004.
43. Kombian SB, Reiffenstein RJ, and Colmers WF. The actions of hydrogen sulfide on dorsal raphe serotonergic neurons in vitro. *J Neurophysiol* 70: 81–96, 1993.
44. Kwak WJ, Kwon GS, Jin I, Kuriyama H, and Sohn HY. Involvement of oxidative stress in the regulation of H_2S production during ultradian metabolic oscillation of *Saccharomyces cerevisiae. FEMS Microbiol Lett* 219: 99–104, 2003.
45. Maher P and Davis J. The role of monoamine metabolism in oxidative glutamate toxicity. *J Neurosci* 16: 6394–6401, 1996.
46. Miyamoto M, Murphy TH, Schnaar RL, and Coyle JT. Antioxidants protect against glutamate-induced cytotoxicity in a neuronal cell line. *J Pharmacol Exp Ther* 250: 1132–1140, 1989.
47. Murphy TH, Miyamoto M, Sastre A, Schnaar RL, and Coyle JT. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron* 2: 1547–1558, 1989.
48. Murphy TH, Schnaar RL, and Coyle JT. Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. *FASEB J* 4: 1624–1633, 1990.
49. Myers PR, Minor RL Jr, Guerra R Jr, Bates JN, and Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble *S*-nitrosocysteine than nitric oxide. *Nature* 345: 161–163, 1990.
50. Nagai Y, Tsugane M, Oka J-I, and Kimura H. Hydrogen sulfide induces calcium waves in astrocytes. *FASEB J* 18: 557–559, 2004.
51. Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 263: 1768–1771, 1994.
52. O'Dell TJ, Hawkins RD, Kandel ER, and Arancio O. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. *Proc Natl Acad Sci U S A* 88: 11285–11289, 1991.
53. Palmer RMJ, Ferrige AG, and Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327: 524–526, 1987.
54. Palmer RMJ, Ashton DS, and Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333: 664–666, 1988.
55. Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, and Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature* 369: 744–747, 1994.
56. Perry G, Cash AD, and Smith MA. Alzheimer disease and oxidative stress. *J Biomed Biotechnol* 2: 120–123, 2002.
57. Reiffenstein RJ, Hulbert WC, and Roth SH. Toxicology of hydrogen sulfide. *Annu Rev Pharmacol Toxicol* 32: 109–134, 1992.
58. Russo CD, Tringali G, Ragazzoni E, Maggiano N, Menini E, Vairano M, Preziosi P, and Navarra P. Evidence that hydrogen sulphide can modulate hypothalamo–pituitary–adrenal axis function: *in vitro* and *in vivo* studies in the rat. *J Neuroendocrinol* 12: 225–233, 2000.
59. Sagara Y and Schubert D. The activation of metabotropic glutamate receptors protects nerve cells from oxidative stress. *J Neurosci* 18: 6662–6671, 1998.
60. Savage JC and Gould DH. Determination of sulfide in brain tissue and rumen fluid by ion-interaction reversed-phase high-performance liquid chromatography. *J Chromatogr* 526: 540–545, 1990.
61. Schuman EM and Madison DV. A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science* 254: 1503–1506, 1991.
62. Sen CK, Khanna S, Roy S, and Packer L. Molecular basis of vitamin E action. Tocotrienol potently inhibits glutamate-induced $pp60^{c\text{-}Src}$ kinase activation and death of HT4 neuronal cells. *J Biol Chem* 275: 13049–13055, 2000.
63. Snyder SH and Ferris CD. Novel neurotransmitters and their neuropsychiatric relevance. *Am J Psychiatry* 157: 1738–1751, 2000.
64. Stevens CF and Wang Y. Reversal of long-term potentiation by inhibitors of haem oxygenase. *Nature* 364: 147–149, 1993.
65. Strubing C, Krapivinsky G, Krapivinsky L, and Clapham DE. TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron* 29: 645–655, 2001.
66. Sturgess NC, Kozlowski RZ, Carrington CA, Hales CN, and Ashford MLJ. Effects of sulphonylureas and diazoxide on insulin secretion and nucleotide-sensitive channels in an insulin-secreting cell line. *Br J Pharmacol* 95: 83–94, 1988.
67. Suematsu M, Goda N, Sano T, Kashiwagi S, Egawa T, Shinoda Y, and Ishimura Y. Carbon monoxide: an endogenous modulator of sinusoidal tone in the perfused rat liver. *J Clin Invest* 96: 2431–2437, 1995.
68. Suematsu M, Suganuma K, and Kashiwagi S. Mechanistic probing of gaseous signal transduction in microcirculation. *Antioxid Redox Signal* 5: 485–492, 2003.
69. Sunda W, Kieber DJ, Kiene RP, and Huntsman S. An antioxidant function for DMSP and DMS in marine algae. *Nature* 418: 317–320, 2002.
70. Tan S, Schubert D, and Maher P. Oxytosis: a novel form of programmed cell death. *Curr Top Med Chem* 1: 497–506, 2001.
71. Tang L-H and Aizenman E. The modulation of *N*-methyl-D-aspartate receptors by redox and alkylating reagents in rat cortical neurons in vitro. *J Physiol (Lond)* 465: 303–323, 1993.
72. Tardy M, Fages C, Riol H, LePrince G, Rataboul P, Charriere-Bertrand C, and Nunez J. Developmental expression of the glial fibrillary acidic protein mRNA in the central nervous system and in cultured astrocytes. *J Neurochem* 52: 162–167, 1989.
73. Teague B, Asiedu S, and Moore PK. The smooth muscle relaxant effect of hydrogen sulphide in vitro: evidence for a physiological role to control intestinal contractility. *Br J Pharmacol* 137: 139–145, 2002.
74. Toohey JI. Sulphane sulphur in biological systems: a possible regulatory role. *Biochem J* 264: 625–632, 1989.
75. Utz J and Ullrich V. Carbon monoxide relaxes ileal smooth muscle through activation of guanylate cyclase. *Biochem Pharmacol* 41: 1195–1201, 1991.
76. van Rossum DB, Patterson RL, Ma HT, and Gill DL. Ca^{2+} entry mediated by store depletion, *S*-nitrosylation, and TRP3 channels. Comparison of coupling and function. *J Biol Chem* 275: 28562–28568, 2000.

77. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, and Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 259: 381–384, 1993.
78. Vornov JJ and Coyle JT. Glutamate neurotoxicity and the inhibition of protein synthesis in the hippocampal slice. *J Neurochem* 56: 996–1006, 1991.
79. Warenycia MW, Goodwin LR, Benishin CG, Reiffenstein RJ, Francom DM, Taylor JD, and Dieken FP. Acute hydrogen sulfide poisoning: demonstration of selective uptake of sulfide by the brainstem by measurement of brain sulfide levels. *Biochem Pharmacol* 38: 973–981, 1989.
80. Zhao W and Wang R. H_2S-induced vasorelaxation and underlying cellular and molecular mechanisms. *Am J Physiol Heart Circ Physiol* 283: H474–H480, 2002.
81. Zhao W, Zhang J, Lu Y, and Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. *EMBO J* 20: 6008–6016, 2001.
82. Zhao W, Ndisang JF, and Wang R. Modulation of endogenous production of H_2S in rat tissues. *Can J Physiol Pharmacol* 81: 848–853, 2003.
83. Zhuo M, Small SA, Kandel ER, and Hawkins RD. Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus. *Science* 260: 1946–1950, 1993.

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3. Jun Zhou, Peng-Fei Wu, Fang Wang, Jian-Guo Chen. 2012. Targeting gaseous molecules to protect against cerebral ischaemic injury: Mechanisms and prospects. *Clinical and Experimental Pharmacology and Physiology* **39**:6, 566-576. [CrossRef]
4. Ya Fatou Njie-Mbye, Madhura Kulkarni, Catherine A. Opere, Sunny E. Ohia. 2012. Mechanism of action of hydrogen sulfide on cyclic AMP formation in rat retinal pigment epithelial cells. *Experimental Eye Research* **98**, 16-22. [CrossRef]
5. M. Scott Vandiver, Solomon H. Snyder. 2012. Hydrogen sulfide: a gasotransmitter of clinical relevance. *Journal of Molecular Medicine* . [CrossRef]
6. Schwefelwasserstoff [MAK Value Documentation in German language, 2007] . [CrossRef]
7. Xinxing Xie, Aijun Sun, Wenqing Zhu, Zheyong Huang, Xinying Hu, Jianguo Jia, Yunzeng Zou, Junbo Ge. 2012. Transplantation of Mesenchymal Stem Cells Preconditioned with Hydrogen Sulfide Enhances Repair of Myocardial Infarction in Rats. *The Tohoku Journal of Experimental Medicine* **226**:1, 29-36. [CrossRef]
8. Prem Kumar, Nanduri R. PrabhakarPeripheral Chemoreceptors: Function and Plasticity of the Carotid Body . [CrossRef]
9. Fabiao Yu, Peng Li, Ping Song, Bingshuai Wang, Jianzhang Zhao, Keli Han. 2012. An ICT-based strategy to a colorimetric and ratiometric fluorescence probe for hydrogen sulfide in living cells. *Chemical Communications* . [CrossRef]
10. Neville N. Osborne, Dan Ji, Aman Shah Abdul Majid, Piero Del Sodato, Anna Sparatore. 2012. Glutamate oxidative injury to RGC-5 cells in culture is necrostatin sensitive and blunted by a hydrogen sulfide (H2S)-releasing derivative of aspirin (ACS14). *Neurochemistry International* . [CrossRef]
11. Jason A. Bennett, Marc A. Neiswonger, Christopher D. Wheeler, James E. Pander, Stephanie E. McKinney. 2012. Cyanide-Coordinated Fe(III) Meso-Tetra(4-carboxyphenyl) Porphyrin as a Possible Electrocatalytic Material for Selective H2S Oxidation. *Journal of The Electrochemical Society* **159**:5, F119. [CrossRef]
12. M. Kwiatkoski, R.N. Soriano, H.D.C. Francescato, M.E. Batalhao, T.M. Coimbra, E.C. Carnio, L.G.S. Branco. 2011. Hydrogen sulfide as a cryogenic mediator of hypoxia-induced anapyrexia. *Neuroscience* . [CrossRef]
13. Doris Abele, José Pablo Vázquez-Medina, Tania Zenteno-SavínIntroduction to Oxidative Stress in Aquatic Ecosystems 1-5. [CrossRef]
14. Xianfeng Gu, Chunhua Liu, Yi-Chun Zhu, Yi-Zhun Zhu. 2011. Development of a boron-dipyrromethene-Cu2+ ensemble based colorimetric probe toward hydrogen sulfide in aqueous media. *Tetrahedron Letters* . [CrossRef]
15. Claudio Bucolo, Filippo Drago. 2011. Carbon monoxide and the eye: Implications for glaucoma therapy. *Pharmacology & Therapeutics* **130**:2, 191-201. [CrossRef]
16. Zhong-Shi Xu, Xin-Yu Wang, De-Ming Xiao, Li-Fang Hu, Ming Lu, Zhi-Yuan Wu, Jin-Song Bian. 2011. Hydrogen sulfide protects MC3T3-E1 osteoblastic cells against H2O2-induced oxidative damage—implications for the treatment of osteoporosis. *Free Radical Biology and Medicine* **50**:10, 1314-1323. [CrossRef]
17. Jason A. Bennett, James E. Pander III, Marc A. Neiswonger. 2011. Investigating the viability of electrodeposited vanadium pentoxide as a suitable electrode material for in vivo amperometric hydrogen sulfide detection. *Journal of Electroanalytical Chemistry* **654**:1-2, 1-7. [CrossRef]
18. Xianfeng Gu, Yi Zhun Zhu. 2011. Therapeutic applications of organosulfur compounds as novel hydrogen sulfide donors and/or mediators. *Expert Review of Clinical Pharmacology* **4**:1, 123-133. [CrossRef]
19. Benjamin L. Predmore, Maikel J. Alendy, Khadija I. Ahmed, Christiaan Leeuwenburgh, David Julian. 2010. The hydrogen sulfide signaling system: changes during aging and the benefits of caloric restriction. *AGE* **32**:4, 467-481. [CrossRef]
20. Nilkantha Sen, Solomon H. Snyder. 2010. Protein modifications involved in neurotransmitter and gasotransmitter signaling. *Trends in Neurosciences* **33**:11, 493-502. [CrossRef]
21. Chi Xin Tiong, Ming Lu, Jin-Song Bian. 2010. Protective effect of hydrogen sulphide against 6-OHDA-induced cell injury in SH-SY5Y cells involves PKC/PI3K/Akt pathway. *British Journal of Pharmacology* **161**:2, 467-480. [CrossRef]
22. Qi-Hai Gong, Qian Wang, Li-Long Pan, Xin-Hua Liu, Hui Huang, Yi-Zhun Zhu. 2010. Hydrogen sulfide attenuates lipopolysaccharide-induced cognitive impairment: A pro-inflammatory pathway in rats. *Pharmacology Biochemistry and Behavior* **96**:1, 52-58. [CrossRef]

23. Rong Hu, Jianqiang Lu, Xingji You, Xiaoyan Zhu, Ning Hui, Xin Ni. 2010. Hydrogen sulfide inhibits the spontaneous and oxytocin-induced contractility of human pregnant myometrium. *Gynecological Endocrinology* 1-5. [CrossRef]
24. Y.-J. Peng, J. Nanduri, G. Raghuraman, D. Souvannakitti, M. M. Gadalla, G. K. Kumar, S. H. Snyder, N. R. Prabhakar. 2010. H2S mediates O2 sensing in the carotid body. *Proceedings of the National Academy of Sciences* **107**:23, 10719-10724. [CrossRef]
25. Nilce Mitiko Matsuda, Steven M. Miller. 2010. Non-adrenergic non-cholinergic inhibition of gastrointestinal smooth muscle and its intracellular mechanism(s). *Fundamental & Clinical Pharmacology* **24**:3, 261-268. [CrossRef]
26. Moataz M. Gadalla, Solomon H. Snyder. 2010. Hydrogen sulfide as a gasotransmitter. *Journal of Neurochemistry* **113**:1, 14-26. [CrossRef]
27. E Ekundi-Valentim, KT Santos, EA Camargo, A Denadai-Souza, SA Teixeira, CI Zanoni, AD Grant, JL Wallace, MN Muscará, SK Costa. 2010. Differing effects of exogenous and endogenous hydrogen sulphide in carrageenan-induced knee joint synovitis in the rat. *British Journal of Pharmacology* **159**:7, 1463-1474. [CrossRef]
28. Ya Fatou Njie-Mbye, Odelia Y. N. Bongmba, Chinwe C. Onyema, Abhishek Chitnis, Madhura Kulkarni, Catherine A. Opere, Angela M. LeDay, Sunny E. Ohia. 2010. Effect of Hydrogen Sulfide on Cyclic AMP Production in Isolated Bovine and Porcine Neural Retinae. *Neurochemical Research* **35**:3, 487-494. [CrossRef]
29. Yuka Kimura , Yu-Ichi Goto , Hideo Kimura . 2010. Hydrogen Sulfide Increases Glutathione Production and Suppresses Oxidative Stress in Mitochondria. *Antioxidants & Redox Signaling* **12**:1, 1-13. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
30. Alma Martelli, Lara Testai, Maria Cristina Breschi, Corrado Blandizzi, Agostino Virdis, Stefano Taddei, Vincenzo Calderone. 2010. Hydrogen sulphide: novel opportunity for drug discovery. *Medicinal Research Reviews* n/a-n/a. [CrossRef]
31. Catherine A. Opere, Emmanuel M. Monjok, Kaustubh H. Kulkarni, Ya Fatou Njie, Sunny E. Ohia. 2009. Regulation of [3H] d-Aspartate Release from Mammalian Isolated Retinae by Hydrogen Sulfide. *Neurochemical Research* **34**:11, 1962-1968. [CrossRef]
32. Alejandro K. Samhan-Arias, Miguel A. Garcia-Bereguiain, Carlos Gutierrez-Merino. 2009. Hydrogen sulfide is a reversible inhibitor of the NADH oxidase activity of synaptic plasma membranes. *Biochemical and Biophysical Research Communications* **388**:4, 718-722. [CrossRef]
33. Wang Hua, Jianbin Jiang, Xing Rong, Rongzhou Wu, Huixian Qiu, Yuanhai Zhang, Qi Chen. 2009. The dual role of the cystathionine #-lyase/hydrogen sulfide pathway in CVB3-induced myocarditis in mice. *Biochemical and Biophysical Research Communications* **388**:3, 595-600. [CrossRef]
34. John T. Pinto, Tetyana Khomenko, Sandor Szabo, Gordon D. McLaren, Travis T. Denton, Boris F. Krasnikov, Thomas M. Jeitner, Arthur J.L. Cooper. 2009. Measurement of sulfur-containing compounds involved in the metabolism and transport of cysteamine and cystamine. Regional differences in cerebral metabolism#. *Journal of Chromatography B* **877**:28, 3434-3441. [CrossRef]
35. Peng Zhao, Xu Huang, Zuo-yu Wang, Zhang-xun Qiu, Yan-fei Han, Hong-li Lu, Young-chul Kim, Wen-xie Xu. 2009. Dual effect of exogenous hydrogen sulfide on the spontaneous contraction of gastric smooth muscle in guinea-pig. *European Journal of Pharmacology* **616**:1-3, 223-228. [CrossRef]
36. Winnie W. Pong, William D. Eldred. 2009. Interactions of the gaseous neuromodulators nitric oxide, carbon monoxide, and hydrogen sulfide in the salamander retina. *Journal of Neuroscience Research* **87**:10, 2356-2364. [CrossRef]
37. Xilong Li, Fuller W. Bazer, Haijun Gao, Wenjuan Jobgen, Gregory A. Johnson, Peng Li, Jason R. McKnight, M. Carey Satterfield, Thomas E. Spencer, Guoyao Wu. 2009. Amino acids and gaseous signaling. *Amino Acids* **37**:1, 65-78. [CrossRef]
38. Elena Perrino, Caterina Uliva, Cecilia Lanzi, Piero Del Soldato, Emanuela Masini, Anna Sparatore. 2009. New prostaglandin derivative for glaucoma treatment. *Bioorganic & Medicinal Chemistry Letters* **19**:6, 1639-1642. [CrossRef]
39. Kaustubh H. Kulkarni, Emmanuel M. Monjok, Robert Zeyssig, Ghislaine Kouamou, Odelia N. Bongmba, Catherine A. Opere, Ya Fatou Njie, Sunny E. Ohia. 2009. Effect of Hydrogen Sulfide on Sympathetic Neurotransmission and Catecholamine Levels in Isolated Porcine Iris-Ciliary Body. *Neurochemical Research* **34**:3, 400-406. [CrossRef]
40. Anna Sparatore, Elena Perrino, Valerio Tazzari, Daniela Giustarini, Ranieri Rossi, Giuseppe Rossoni, Kati Erdman, Henning Schröder, Piero Del Soldato. 2009. Pharmacological profile of a novel H2S-releasing aspirin. *Free Radical Biology and Medicine* **46**:5, 586-592. [CrossRef]
41. Suzanne Durocher, Asad Rezaee, Caroline Hamm, Chitra Rangan, Silvia Mittler, Bulent Mutus. 2009. Disulfide-Linked, Gold Nanoparticle Based Reagent for Detecting Small Molecular Weight Thiols. *Journal of the American Chemical Society* **131**:7, 2475-2477. [CrossRef]

42. Myung Gil Choi, Sunyoung Cha, Haekyung Lee, Hye Lim Jeon, Suk-Kyu Chang. 2009. Sulfide-selective chemosignaling by a Cu2+ complex of dipicolylamine appended fluorescein. *Chemical Communications* :47, 7390. [CrossRef]
43. E MONJOK, K KULKARNI, G KOUAMOU, M MCKOY, C OPERE, O BONGMBA, Y NJIE, S OHIA. 2008. Inhibitory action of hydrogen sulfide on muscarinic receptor-induced contraction of isolated porcine irides. *Experimental Eye Research* **87**:6, 612-616. [CrossRef]
44. d. gallego, p. clavé, j. donovan, r. rahmati, d. grundy, m. jiménez, m. j. beyak. 2008. The gaseous mediator, hydrogen sulphide, inhibits in vitro motor patterns in the human, rat and mouse colon and jejunum. *Neurogastroenterology & Motility* **20**:12, 1306-1316. [CrossRef]
45. Mostafa Saadat, Zahra Zendeh-Boodi. 2008. Association between genetic polymorphism of GSTT1 and depression score in individuals chronically exposed to natural sour gas. *Neuroscience Letters* **435**:1, 65-68. [CrossRef]
46. Miguel Angel García-Bereguiaín , Alejandro Khalil Samhan-Arias , Francisco Javier Martín-Romero , Carlos Gutiérrez-Merino . 2008. Hydrogen Sulfide Raises Cytosolic Calcium in Neurons Through Activation of L-Type Ca2+ Channels. *Antioxidants & Redox Signaling* **10**:1, 31-42. [Abstract] [Full Text PDF] [Full Text PDF with Links]
47. David W. Kraus, Jeannette E. Doeller, Xueji ZhangElectrochemical sensors for the determination of hydrogen sulfide production in biological samples 213-235. [CrossRef]
48. D TRUONG, A MIHAJLOVIC, P GUNNESS, W HINDMARSH, P OBRIEN. 2007. Prevention of hydrogen sulfide (H2S)-induced mouse lethality and cytotoxicity by hydroxocobalamin (vitamin B12a). *Toxicology* **242**:1-3, 16-22. [CrossRef]
49. Ken Umemura , Hideo Kimura . 2007. Hydrogen Sulfide Enhances Reducing Activity in Neurons: Neurotrophic Role of H2S in the Brain?. *Antioxidants & Redox Signaling* **9**:11, 2035-2042. [Abstract] [Full Text PDF] [Full Text PDF with Links]
50. Chang-qing CHEN, Hong XIN, Yi-zhun ZHU. 2007. Hydrogen sulfide: third gaseous transmitter, but with great pharmacological potential. *Acta Pharmacologica Sinica* **28**:11, 1709-1716. [CrossRef]
51. Eric Blackstone, Mark B. Roth. 2007. SUSPENDED ANIMATION-LIKE STATE PROTECTS MICE FROM LETHAL HYPOXIA. *Shock* **27**:4, 370-372. [CrossRef]
52. Mamiko Tsugane , Yasuo Nagai , Yuka Kimura , Jun-Ichiro Oka , Dr. Hideo Kimura . 2007. Differentiated Astrocytes Acquire Sensitivity to Hydrogen Sulfide That Is Diminished by the Transformation into Reactive Astrocytes. *Antioxidants & Redox Signaling* **9**:2, 257-269. [Abstract] [Full Text PDF] [Full Text PDF with Links]
53. Li-Fang Hu, Peter T.-H. Wong, Philip K. Moore, Jin-Song Bian. 2007. Hydrogen sulfide attenuates lipopolysaccharide-induced inflammation by inhibition of p38 mitogen-activated protein kinase in microglia. *Journal of Neurochemistry* **100**:4, 1121-1128. [CrossRef]
54. Hong-fang JIN, Jun-bao DU, Xiao-hui LI, Yan-fei WANG, Yin-fang LIANG, Chao-shu TANG. 2006. Interaction between hydrogen sulfide/cystathionine ?-lyase and carbon monoxide/heme oxygenase pathways in aortic smooth muscle cells. *Acta Pharmacologica Sinica* **27**:12, 1561-1566. [CrossRef]
55. Mamiko Tsugane, Yasuo Nagai, Yuka Kimura, Jun-Ichiro Oka, Hideo Kimura. 2006. Differentiated Astrocytes Acquire Sensitivity to Hydrogen Sulfide That Is Diminished by the Transformation into Reactive Astrocytes. *Antioxidants & Redox Signaling*, ahead of print061121054212007. [CrossRef]
56. David Lloyd. 2006. Hydrogen sulfide: clandestine microbial messenger?. *Trends in Microbiology* **14**:10, 456-462. [CrossRef]
57. Hideo Kimura . 2005. Hydrogen Sulfide as a Biological Mediator. *Antioxidants & Redox Signaling* **7**:5-6, 778-780. [Citation] [Full Text PDF] [Full Text PDF with Links]
58. Li Xiaohui, Du Junbao, Shi Lin, Li Jian, Tang Xiuying, Qi Jianguang, Wei Bing, Jin Hongfang, Tang Chaoshu. 2005. Down-Regulation of Endogenous Hydrogen Sulfide Pathway in Pulmonary Hypertension and Pulmonary Vascular Structural Remodeling Induced by High Pulmonary Blood Flow in Rats. *Circulation Journal* **69**:11, 1418-1424. [CrossRef]