Regulation of Aqueous Humor Dynamics by Hydrogen Sulfide: Potential Role in Glaucoma Pharmacotherapy

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Abstract

Hydrogen sulfide (H₂S) is a gaseous transmitter with well-known biological actions in a wide variety of tissues and organs. The potential involvement of this gas in physiological and pathological processes in the eye has led to several *in vitro*, *ex vivo*, and *in vivo* studies to understand its pharmacological role in some mammalian species. Evidence from literature demonstrates that 4 enzymes responsible for the biosynthesis of this gas (cystathionine β -synthase, CBS; cystathionine γ -lyase, CSE; 3-mercaptopyruvate sulfurtransferase, 3MST; and D-amino acid oxidase) are present in the cornea, iris, ciliary body, lens, and retina. Studies of the pharmacological actions of H₂S (using several compounds as fast- and slow-releasing gas donors) on anterior uveal tissues reveal an effect on sympathetic neurotransmission and the ability of the gas to relax precontracted iris and ocular vascular smooth muscles, responses that were blocked by inhibitors of CSE, CBS, and K_{ATP} channels. In the retina, there is evidence that H₂S can inhibit excitatory amino acid neurotransmission and can also protect this tissue from a wide variety of insults. Furthermore, exogenous application of H₂S-releasing compounds was reported to increase aqueous humor outflow facility in an *ex vivo* model of the porcine ocular anterior segment and lowered intraocular pressure (IOP) in both normotensive and glaucomatous rabbits. Taken together, the finding that H₂S-releasing compounds can lower IOP and can serve a neuroprotective role in the retina suggests that H₂S prodrugs could be used as tools or therapeutic agents in diseases such as glaucoma.

Keywords: hydrogen sulfide, neurotransmitters, neuroprotection, aqueous humor dynamics

Introduction

FOR CENTURIES HYDROGEN SULFIDE (H₂S), was known as a toxic gas with deleterious consequences for the environment and animals. In animal models, acute exposure to high concentrations of H₂S was reported to alter neuronal activity through an action on calcium channels.^{1,2} In humans, victims of H₂S poisoning have problems with coordination, memory loss, and other psychiatric disorders.² Warenycia et al.³ found that exposure of rats to H₂S by inhalation or through intraperitoneal injections of the H₂S releasing compound, sodium hydrosulfide (NaHS), caused a dose-dependent increase in the concentration of this gas in the brain. Interestingly, these workers measured basal levels of H₂S in both rat and postmortem human brains suggesting an endogenous source of this gas in mammals.^{3,4} Although enzymes involved in the metabolism of H₂S in mammalian

tissues were characterized earlier,^{5,6} it was assumed that this gas was a by-product of metabolic pathways or a marker of activities of some enzymes. The possible role of H₂S as an endogenous neuromodulator was first described by Abe and Kimura.⁷ These investigators found that H₂S can facilitate long-term potentiation by enhancing the activity of *N*-methyl-D-aspartate (NMDA) receptors and that cystathionine β -synthase (CBS) can synthesize this gas in the brain.⁷ It was subsequently reported by Snyder and colleagues⁸ that a H₂S produced its biological action by adding sulfur to cysteine residues of target proteins, a process known as sulphydration.^{8,9}

The discovery of enzymes responsible for the biosynthesis of this gas from cysteine and homocysteine and the presence of transsulfuration pathways in mammals supports a biological role for H₂S as a multifunctional signaling molecule with important regulatory roles in processes such

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as learning and memory, modulation of synaptic activities, inflammation, and in the maintenance of vascular tone.¹⁰ Furthermore, H₂S has been shown to protect various organs and tissues such as heart, kidney, brain, and retina from ischemic, hypoxic, oxidative, and hyperglycemic insults.^{11–15} Recently, H₂S was hypothesized to play a major role in aging and aging-related pathologies^{16,17} and was reported to play a role in erectile responses to NaHS and Na₂S.¹⁸ There is evidence that H₂S can utilize different signal transduction pathways in eliciting its physiological and pharmacological actions. For example, H₂S-releasing compounds have been reported to relax vascular smooth muscle by stimulating ATP-sensitive K⁺ channels (K_{ATP}); they can promote angiogenesis and vascular remodeling through phosphatidylinositol 3-kinase/Akt/surviving axis in endothelial cells; they can stimulate long-term synaptic potentiation by enhancing the activity of NMDA receptors through the cyclic AMP/protein kinase A pathway; and they can downregulate pro-inflammatory genes involved in cardiac ischemic/reperfusion injury by preventing the translocation of nuclear factor-κB.^{19–21} In addition to K_{ATP} channels, H₂S-releasing compounds have been reported to interact with calcium channels, chloride channels, sodium channels, transient receptor ankyrin 1 (TRPA1), and transient receptor vanilloid channels.^{22–24}

Apart from H₂S, hydrogen polysulfide (H₂S_n) compounds that contain a higher concentration of sulfur atoms than H₂S have been reported to act as potential signaling molecules with pharmacological activity at TRPA1 channels,^{25,26} regulation of the activity of a tumor suppressor, phosphatase, and tensin homolog,²⁷ facilitation of the translocation of Nrf2 to the nucleus for upregulation of transcription of antioxidant genes,²⁸ and in the relaxation of vascular smooth muscle by an action on protein kinase G1α.²⁹ It appears that the ability of H₂S_n to modify these processes is based on their possession of 2 sensitive cysteine residues at active sites (either of which can be sulfureted by H₂S_n) to generate a cysteine disulfide bond by reacting with each other.³⁰ It is pertinent to note that the potential pharmacological actions of H₂S_n in ocular tissues are yet to be determined.

Biosynthesis of Hydrogen Sulfide

The biosynthesis of H₂S occurs through 4 major pathways involving amino acids: 3 from L-cysteine and 1 from D-cysteine.³⁰ The two pyridoxal-5'-phosphate-dependent enzymes found in the cytosol, cystathionine β-synthase (CBS), and cystathionine γ-lyase (CSE) convert L-cysteine, along with L-homocysteine into H₂S.^{6,7,31,32} A third enzyme cyste-

ine aminotransferase converts L-cysteine, along with α-ketoglutarate to 3-mercaptopyruvate, which is then further metabolized by 3-mercaptopyruvate sulfur transferase (3MST) to H₂S in the presence of thioredoxin.^{6,33–38} A fourth enzyme, D-amino acid oxidase (DAO) converts D-cysteine to achiral 3-mercaptopyruvate, which is then metabolized to H₂S by 3MST.^{39,40} 3MST is found in the mitochondria, whereas DAO is present in peroxisomes.⁴¹ The regulation of H₂S concentrations in mammalian tissues appears to depend upon the balance between its production and clearance.^{42–44}

In the eye, the 4 enzymes responsible for the biosynthesis of H₂S (CBS, CSE, 3MST, and DAO) have been localized in tissues such as the cornea, iris, ciliary body, lens, and retina.^{11,45–53} The reported expression of CBS and CSE enzymes in ocular tissues^{46,47} correlated with the endogenous production of H₂S in these tissues.⁵⁴ Kulkarni et al.⁵⁴ found that inhibitors of CBS (propargylglycine) and CSE (aminooxyacetic acid) attenuated, while the activator of CBS (S-adenosyl-L-methionine) enhanced endogenous production of H₂S in the retina. In summary, the presence of the 4 enzymes of the transsulfuration pathway in the eyes suggests that H₂S may play a vital role in the pathophysiology of ocular diseases.

Pharmacological Actions of Hydrogen Sulfide in Ocular Tissues

Due to the inherent difficulties of working with H₂S as a gas, the pharmacological actions of H₂S are studied using donor molecules or gas-releasing compounds, which include inorganic sulfide salts, synthetic organic slow-releasing H₂S donors, H₂S-releasing nonsteroidal anti-inflammatory drugs, cysteine analogs, nucleoside phosphorothioates, and plant-derived polysulfide contained in garlic.^{55,56}

Anterior uveal tissues

Experimental studies on the pharmacological actions of H₂S (using several compounds as fast- and slow-releasing gas donors) on the anterior uvea (Table 1) have focused on the regulation of sympathetic neurotransmission and their direct effects on iris muscle tone. Furthermore, the pharmacological effects of H₂S-releasing compounds have also been studied on aqueous humor (AH) outflow facility in a porcine anterior segment model, *ex vivo*. Since the autonomic nervous system plays an important role in the regulation of AH dynamics,⁵⁷ Kulkarni et al.⁵⁸ studied the pharmacological actions of H₂S (using sodium hydrosulfide,

TABLE 1. ANTERIOR UVEA

Tissue	Pharmacological effect	Mechanism of action	References
Iris-ciliary body	Inhibition of sympathetic neurotransmission in bovine and porcine iris-ciliary bodies (NaHS, Na ₂ S, ACS67, GYY4137)	Endogenous biosynthesis of H ₂ S; biosynthesis of prostaglandins; activation of K _{ATP} channels.	58,59
	Decrease in norepinephrine and epinephrine content in porcine iris-ciliary bodies (Na ₂ S, NaHS)	Endogenous biosynthesis of H ₂ S	58
Iris	Relaxation of induced tone in porcine irides (NaHS, Na ₂ S, L-cysteine)	Endogenous biosynthesis of H ₂ S; biosynthesis of prostaglandins; activation of K _{ATP} channels.	60,61

A summary of the pharmacological effects and potential mechanism/s of action of hydrogen sulfide-releasing compounds in the anterior uvea.

NaHS, and Na₂S as donors) on sympathetic neurotransmission in the anterior uvea.^{58,59} In porcine isolated iris-ciliary bodies, release of radiolabeled norepinephrine ([³H]NE) triggered by electrical field stimulation was reduced by both NaHS and Na₂S in a concentration-dependent manner.⁵⁸ The inhibitors of CSE (propargylglycine) and CBS (aminooxyacetic acid) antagonized the inhibitory action of the H₂S donors on NE release. Furthermore, both H₂S donors elicited a concentration-dependent decrease in NE and epinephrine content in the iris-ciliary bodies. Kulkarni et al.⁵⁸ concluded that H₂S donors can block sympathetic neurotransmission in the porcine anterior uvea, an action that was dependent, at least in part, on the intramural biosynthesis of this gas and a direct action of H₂S on neurotransmitter pools.⁵⁸ Recently, Salvi et al.⁵⁹ investigated the pharmacological actions of different sources of H₂S on sympathetic neurotransmission in the bovine isolated iris-ciliary bodies: ACS67, a hybrid of latanoprost and a H₂S donating moiety; L-cysteine, a substrate for endogenous production of H₂S; and GYY4137, a slow donor of H₂S. All 3 donors caused a concentration-dependent attenuation of [³H]NE release evoked by electrical field stimulation, a response that was antagonized by an inhibitor of CBS, aminooxyacetic acid, and glibenclamide (a K_{ATP} channel blocker). Interestingly, flurbiprofen (a cyclooxygenase inhibitor) enhanced the inhibitory effect of ACS67 and L-cysteine on stimulated NE release. Taken together, Salvi et al.⁵⁹ concluded that the ability of H₂S donors to reduce sympathetic neurotransmission in bovine anterior uvea was partially dependent upon the intramural production of this gas and prostanoids and was mediated by an action on K_{ATP} channels. It is pertinent to note that reduced sympathetic neurotransmission in the anterior uvea could have an indirect action on vascular tone and blood flow to ciliary body. Indeed, there is evidence of a relationship between the activity level of sympathetic nerves, choroidal blood flow, and intraocular pressure (IOP) in experimental animals.^{60–63} Furthermore, autonomic regulation of blood vessels of the ciliary body and ciliary epithelium has been reported to play a major role in the formation of AH, while such regulatory process in the trabecular meshwork and episcleral blood vessels is associated with AH outflow.⁵⁷ Clearly, the finding that H₂S-releasing compounds can alter sympathetic neurotransmission indicates that this gas is capable of eliciting an indirect action on AH dynamics.

A possible direct action of H₂S donors on isolated porcine iris smooth muscle was studied by Monjok et al.⁶⁴ and Ohia et al.⁶⁵ In the presence of tone induced by muscarinic receptor stimulation, both NaHS and Na₂S elicited a concentration-dependent relaxation porcine isolated irides, a response that was blocked by inhibitors of CSE (propargylglycine and β-cyanoalanine) and CBS (aminooxyacetic acid and hydroxylamine) and a blocker to K_{ATP} channels (glibenclamide) but enhanced by S-adenosylmethionine, an activator of CBS.⁶⁴ These workers also found that while endogenously generated prostanoids were involved in the inhibitory action of H₂S donors, nitric oxide (NO) was not a mediator of this response. Monjok et al.⁶⁴ concluded that relaxations induced by H₂S donors in porcine irides were partially due to intramural generation of the gas and by an effect on K_{ATP} channels. In a related study, the pharmacological actions of L-cysteine, a substrate for the biosynthesis of H₂S was examined under the presence of tone induced by muscarinic receptor activation in porcine irides.⁶⁵ L-Cysteine produced a concentration-related

relaxation of tone that was inhibited by aminooxyacetic acid, propargylglycine, and glibenclamide but enhanced by flurbiprofen, a cyclooxygenase inhibitor, indicating that the response induced by the H₂S substrate was dependent upon the endogenous biosynthesis of this gas and prostanoids. Furthermore, the relaxation elicited by L-cysteine was mediated by K_{ATP} channels.⁶⁵ While the observed direct pharmacological actions of H₂S-releasing compounds on iris muscle tone are interesting, its relevance in regulation of AH dynamics is unclear and merits further investigation.

A study to investigate the possible direct pharmacological effects of H₂S-releasing compounds on pathways involved in AH outflow was reported in an *ex vivo* model. The pharmacological actions of H₂S-releasing compounds on AH dynamics in whole animals are discussed in the appropriate section below (Pharmacological Actions of Hydrogen Sulfide on AH Dynamics). To determine whether H₂S donors can alter AH dynamics, Robinson et al.⁶⁶ studied the pharmacological actions of NaHS and the H₂S substrate, L-cysteine, on AH outflow facility on porcine ocular anterior segment model, *ex vivo*. Both compounds elicited a concentration-dependent increase in AH outflow facility, an effect that was antagonized by aminooxyacetic acid, glibenclamide, and SQ22536 (an inhibitor of adenylyl cyclase). Morphological studies of the effect of L-cysteine on the architecture of the trabecular meshwork did not reveal any significant anatomical changes compared to controls. These findings led Robinson et al.⁶⁶ to conclude that the stimulatory effects of these compounds on AH outflow facility are mediated, at least in part, by endogenously produced H₂S, K_{ATP} channels, and adenylyl cyclase. Interestingly, the observation that increases in AH outflow facility induced by NaHS and L-cysteine can be blocked by an inhibitor of adenylyl cyclase is noteworthy based on the fact that this nucleotide has been implicated in the overall regulation of AH dynamics.⁶⁷ The perfused ocular anterior segment model preserves the architecture of the outflow pathway and has been used extensively in *ex vivo* studies of the pharmacological actions of drugs on AH dynamics.^{68–70} Clearly, the increase in outflow facility induced by H₂S-releasing compounds suggests that an effect on conventional outflow channels could underlie, at least in part, a mechanism of IOP-lowering action of this gas in mammals.

Ocular vasculature

In the eye, the pharmacological actions of H₂S (using several compounds as fast- and slow-releasing gas donors) have been reported in the posterior ciliary artery, the ophthalmic artery, and the retinal artery (Table 2). Ocular blood flow has been shown to be regulated directly through autonomic influences on the vasculature of the optic nerve head, choroid, ciliary body, and iris, as well as through indirect autonomic influences on retinal blood flow.⁵⁷ The first biological activity ascribed to H₂S was its relaxant action on the vasculature.⁷¹ After publication of the report by Hosoki et al.,⁷¹ other workers have provided evidence for the involvement of this gas in the pathophysiology of vascular smooth muscles and the endothelium.^{72–74} In the eye, Chitnis et al.⁷⁵ were the first investigators to describe the pharmacological actions of H₂S donors on vascular tone in the bovine isolated long posterior ciliary artery.⁷⁵ Both NaHS (a fast-releasing H₂S donor) and GYY4137 (a slow-releasing H₂S donor) caused a concentration-dependent

TABLE 2. OCULAR VASCULATURE

<i>Tissue</i>	<i>Pharmacological effect</i>	<i>Mechanism of action</i>	<i>References</i>
Posterior ciliary artery	Relaxation of induced tone in bovine posterior ciliary arteries (NaHS, GYY4137, AP67, AP72)	Endogenous biosynthesis of H ₂ S; biosynthesis of prostaglandins; activation of K _{ATP} channels; NO synthesis (AP67 and AP72)	68,70
	Relaxation of induced tone in porcine retinal arteries (NaHS and GYY4137)	Endogenous biosynthesis of H ₂ S	72
Ophthalmic artery	Reversal of NO mediated relaxation of induced tone in rabbit ophthalmic artery (GYY4137)		69
Retinal artery	Relaxation of induced tone in bovine retinal arteries (NaHS)	Activation of voltage-dependent potassium channels (K _v); activation of inwardly rectifying potassium channels (K _{ir})	71

A summary of the pharmacological effects and potential mechanism/s of action of hydrogen sulfide-releasing compounds on the ocular vasculature.

NO, nitric oxide.

relaxation of phenylephrine-induced tone in the long posterior ciliary artery. Relaxations elicited by GYY4137 were blocked by aminooxyacetic acid, propargylglycine, and glibenclamide but enhanced by flurbiprofen suggesting that endogenously produced H₂S, prostanoids, and K_{ATP} channels were involved in the observed response. Chitnis et al.⁷⁵ also reported that *N*(ω)-nitro-L-arginine methyl ester (L-NAME) had no effect on relaxations evoked by GYY4137 indicating that NO did not mediate responses of the bovine long posterior ciliary artery to the slow-releasing H₂S donor. In the rabbit isolated ophthalmic artery, Salomone et al.⁷⁶ found that carbon monoxide and NO can synergize to induce relaxations of phenylephrine-induced tone and that H₂S released by GYY4137 counteracted the effects produced by endogenous NO. It is pertinent to note that the concentrations of GYY4137 utilized by Salomone et al.⁷⁶ in their studies with the rabbit ophthalmic artery were higher than those reported to elicit relaxations of the bovine long posterior ciliary artery.⁷⁵ In a subsequent study, Kulkarni-Chitnis et al.⁷⁷ examined the pharmacological actions of 2 novel slow-releasing H₂S donors, (4-methoxyphenyl)pyrrolidin-1-ylphosphinodithioc acid (AP67) and (4-methoxyphenyl)piperidin-1-ylphosphinodithioc acid (AP72), on bovine isolated long posterior ciliary arteries.⁷⁷ Both AP67 and AP72 caused a concentration-related relaxation of phenylephrine-induced tone, a response that was blocked by aminooxyacetic acid, propargylglycine, L-NAME, glibenclamide, and flurbiprofen (AP67 only). Kulkarni-Chitnis et al.⁷⁷ concluded that the inhibitory action of novel slow-releasing H₂S donors on isolated bovine long posterior ciliary arteries was dependent upon the endogenous production of H₂S and that it could involve products of the NO pathways and activity of K_{ATP} channels. It is of interest to note that in comparison with AP67 and AP72, GYY4137 did not interact with the NO pathway indicating that differences exist in the mechanism of action of these compounds on vascular tone in the bovine long posterior ciliary arteries. In bovine retinal arteries, Takir et al.⁷⁸ found that relaxations of PGF_{2α}- and potassium-induced tone elicited by NaHS were blocked by inhibitors of voltage-dependent potassium channels and inwardly rectifying potassium channels suggesting that H₂S could play a major role in the regulation of retinal arterial tone through an action on these channels. In endothelin precontracted porcine retinal arterioles, Winther et al.⁷⁹ demonstrated that both GYY4137 and NaHS evoked

concentration-dependent relaxations that were consistent with reports from other investigators.^{75,78} Interestingly, inhibitors of H₂S biosynthetic enzymes, aminooxyacetic acid and propargylglycine, enhanced the endothelin contractions, a response that was more pronounced in hypoxic (1% O₂) conditions. These findings led Winther et al.⁷⁹ to conclude that the presence of perivascular retina and hypoxia reduces arteriolar vasoconstriction and that endogenously produced H₂S plays an important role in the regulation of arteriolar tone.⁷⁹ Apart from ocular vasculature, the ability of H₂S donors to relax other vascular beds has been well documented.^{74,80,81} The observed ability of H₂S-releasing compounds in altering vascular tone in posterior ciliary, ophthalmic, and retinal arteries is of pharmacological importance because of the role of these blood vessels in the regulation of AH dynamics and other visual processes in the eye.⁵⁷

Retina

The pharmacological effects of H₂S (using several compounds as fast- and slow-releasing gas donors) in the retina have been studied with emphasis on its potential neuroprotective role in this tissue (Table 3). In published reports, effects of H₂S-releasing compounds were examined on excitatory amino acid neurotransmission and under conditions of ischemia, oxidative stress, and glutamate excitotoxicity. In 2009, Opere et al.⁸² studied the pharmacological actions of H₂S donors, NaHS and Na₂S, on excitatory amino acid transmission [using [³H]D-aspartate as a marker for glutamate] in isolated, superfused bovine and porcine neural retinae. Both H₂S donors caused a concentration-dependent inhibition of evoked [³H]D-aspartate release from bovine and porcine tissues, a response that was blocked by propargylglycine indicating a role for endogenously produced H₂S in the regulation of amino acid transmission in neural retina from both species.⁸² Since an increase in retinal glutamate concentrations has been linked to excitotoxicity,^{83,84} the ability of H₂S to reduce glutamate release suggests a potential neuroprotective action of this gas in the retina.²³ In both isolated bovine and porcine neural retinae⁸⁵ and rat retinal pigment epithelial cells (RPE-J),⁸⁶ NaHS, Na₂S, and L-cysteine enhanced cyclic AMP production through mechanisms that were dependent upon the intramural biosynthesis of H₂S and an action on K_{ATP} channels. In a rat

TABLE 3. RETINA

<i>Pharmacological effect</i>	<i>Mechanism of action</i>	<i>References</i>
Inhibition of excitatory amino acid transmission in bovine and porcine neural retina (NaHS, Na ₂ S)	Endogenous biosynthesis of H ₂ S	75
Neuroprotection against ischemia, ocular hypertension, oxidative stress, and glutamate excitotoxicity in retinal tissues (ACS, ADTOH, NaHS)	Decrease in caspase-3 activity; increase Hsp 90 expression; regulation of calcium homeostasis; ROS scavenging; increase in glutathione production	11,78,81, 82,84,91
Increase cyclic AMP production in isolated bovine and porcine neural retinae and rat retinal pigment epithelial cells (NaHS, Na ₂ S, L-cysteine)	Endogenous biosynthesis of H ₂ S; activation of K _{ATP} channels	79,80

A summary of the pharmacological effects and potential mechanism/s of action of hydrogen sulfide-releasing compounds in the retina.

model of ischemia/reperfusion injury, Biermann et al.⁸⁷ showed that preconditioning of animals with inhalational H₂S decreased the death of retinal ganglion cells and reduced caspase-3 activity while elevating HSP-90 expression indicating that the gas mediates antiapoptotic/neuroprotective actions. H₂S donors have also been reported to protect the mouse retina from light-induced degeneration through an action on calcium homeostasis.^{11,88} In rats, Sakamoto et al.,⁸⁹ showed that NaHS inhibited NMDA induced oxidative damage in the retina affirming a neuroprotective role for H₂S in this model. In summary, it appears that H₂S can protect the retina from a wide variety of insults, an action that may play an important role in the pathophysiology of ocular diseases such as diabetic retinopathy⁹⁰ and glaucoma.⁹¹ A potential neuroprotective action of H₂S has also been described in some diseases of the central nervous system such as Stroke, Alzheimer's disease, and Parkinson's Disease.^{92,93}

Pharmacological Actions of Hydrogen Sulfide on AH Dynamics

Despite the evidence presented above that H₂S-releasing compounds can elicit pharmacological effects on various ocular tissues, there are few reports in literature about the ability of H₂S-releasing compounds to alter AH dynamics in whole animals (Table 4). Latanoprost, an ester prodrug of PGF_{2α}, has been used for the treatment of glaucoma for more than a decade.^{94,95} In 2009, Perrino et al.⁹⁶ reported that ACS67, a H₂S-releasing derivative of latanoprost, caused a time-dependent decrease in IOP in glaucomatous

TABLE 4. AQUEOUS HUMOR DYNAMICS

<i>Pharmacological effect</i>	<i>Mechanism of action</i>	<i>References</i>
Time-dependent decrease in IOP in glaucomatous rabbits (ACS67)	?	91
Time- and dose-dependent decrease in IOP in normotensive rabbits (NaHS, L-cysteine, ACS67, and GYY4137)	?	93
Dose-dependent increase in aqueous humor outflow facility (NaHS and L-cysteine)	Endogenous biosynthesis of H ₂ S; activation of K _{ATP} channels; activation of adenylyl cyclase.	62

A summary of the pharmacological effects and potential mechanism/s of action of hydrogen sulfide-releasing compounds in the retina. IOP, intraocular pressure.

pigmented rabbits that was significantly higher than those elicited by latanoprost. Interestingly, these investigators observed that while latanoprost failed to lower IOP in glaucomatous albino rabbits, ACS was effective in reducing IOP by the fourth hour after treatment.⁹⁶ The increased IOP lowering action of ACS67 was accompanied by a significant increase in glutathione levels in the AH of ACS67-treated rabbits compared to their latanoprost counterparts. Perrino et al.⁹⁶ then concluded that addition of the H₂S releasing moiety enhanced the therapeutic potential of ACS67 and that its ability to affect glutathione levels suggests a potential neuroprotective action of this compound in their animal glaucoma model. In a preliminary report, NaHS was found to lower IOP in normotensive albino rabbits indicating that on its own, H₂S can alter AH dynamics.⁹⁷ In a recent study, Salvi et al.⁹⁸ compared the IOP lowering activity of GYY4137 with those produced by L-cysteine and ACS67 in normotensive male albino rabbits. All 3 H₂S-producing compounds lowered IOP and also caused significant contralateral effects in vehicle-treated control eyes. Salvi et al.⁹⁸ found that GYY4137 displayed a profile of response that showed a slow onset and a prolonged duration of action compared with L-cysteine and ACS67, a fact that is consistent with its chemical nature as a slow-releasing gas donor. In a new model of chronic ocular hypertension in rats that simulates glaucoma, Huang et al.⁹⁹ studied the involvement of H₂S in both the IOP response and retinal ganglion cell (RGC) survival. These workers showed that retinal H₂S levels were markedly decreased when retinal protein expressions of CBS, CSE, and 3MST were down-regulated.⁹⁹ Furthermore, the decrease in retinal H₂S concentrations and loss of RGCs were both reversed by prior intraperitoneal injection of animals with NaHS 3 days before the induction of chronic ocular hypertension. Huang et al.⁹⁹ then concluded that H₂S had a protective action against RGC death in a chronic ocular hypertension model, suggesting a role for this gas in retinal neuroprotection. In summary, H₂S-releasing compounds are effective in

lowering IOP and exerting a neuroprotective action in animal models of glaucoma. Further studies are, therefore, needed to determine if these compounds can elicit the ocular hypotensive action response in primates or humans.

Hydrogen Sulfide-Releasing Compounds and Glaucoma Pharmacotherapy

Evidence from the literature discussed above demonstrates that H₂S-releasing compounds exert pharmacological action on ocular tissues involved in the regulation of AH dynamics and IOP in experimental animals. Furthermore, these compounds have been reported to have neuroprotective actions in both nonocular and ocular tissues, an additional attribute that may prove useful in the pharmacotherapy of glaucoma. As gaseous molecules, H₂S and NO share common traits such as free diffusion across cell membranes and an endogenous enzymatic biosynthetic pathway that can be regulated at several levels. Furthermore, both gases can modulate many physiological processes in the central and peripheral nervous systems.¹⁰⁰ In both nonocular and ocular blood vessels, there are reports of interaction between H₂S and NO in the regulation of vascular tone.^{76,101,102} In preclinical and clinical studies, the NO-donating latanoprost has been shown to lower IOP more effectively than latanoprost alone.^{103,104} The observation that a H₂S-releasing derivative of latanoprost can lower IOP in rabbits⁹¹ strongly suggests that such a formulation may also find utility in the treatment of glaucoma in humans. Consequently, it is feasible to speculate that a new generation of compounds could be developed whose mechanism of action is associated with the H₂S metabolic pathway. Indeed, there is a new focus on the development of H₂S prodrugs that can be classified into: plant-derived natural products; hydrolysis-based H₂S compounds, and controlled-release compounds.¹⁰⁵ It is possible that these H₂S prodrugs could serve as tools or therapeutic agents for the therapy of some cardiovascular and neurological diseases of which glaucoma can be added to the list.

Author Disclosure Statement

No competing financial interests exist.

References

1. Kombian, S.B., Warenycia, M.W., Mele, F.G., and Reiffenstein, R.J. Effects of acute intoxication with hydrogen sulfide on central amino acid transmitter systems. *Neurotoxicology*. 9:587–595, 1988.
2. Reiffenstein, R.J., Hulbert, W.C., and Roth, S.H. Toxicology of hydrogen sulfide. *Annu. Rev. Pharmacol. Toxicol.* 32:109–134, 1992.
3. Warenycia, M.W., Goodwin, L.R., Benishin, C.G., et al. 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.
4. Goodwin, L.R., Francom, D., Dieken, F.P., et al. Determination of sulfide in brain tissue by gas dialysis/ion chromatography: postmortem studies and two case reports. *J. Anal. Toxicol.* 13:105–109, 1989.
5. Braunstein, A.E., Goryachenkova, E.V., Tolosa, E.A., Willhardt, I.H., and Yefremova, L.L. Specificity and some other properties of liver serine sulphhydrylase: evidence for its identity with cystathionine-synthase. *Biochim. Biophys. Acta*. 242:247–260, 1971.

6. Stipanuk, M.H., and Beck, P.W. Characterization of the enzymic capacity for cysteine desulphhydration in liver and kidney of the rat. *Biochem. J.* 206:267–277, 1982.
7. Abe, K., and Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* 16:1066–1071, 1996.
8. Paul, B.D., and Snyder, S.H. H₂S: A novel gaso-transmitter that signals by sulfhydration. *Trends Biochem. Sci.* 40:687–700, 2015.
9. Mustafa, A.K., Gadalla, M.M., Sen, N., et al. H₂S signals through protein S-sulfhydration. *Sci. Signal.* 2:ra72, 2009.
10. Wang, R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol. Rev.* 92:791–896, 2012.
11. Mikami, Y., Shibuya, N., Kimura, Y., Nagahara, N., Yamada, M., and Kimura, H. Hydrogen sulfide protects the retina from light-induced degeneration by the modulation of Ca²⁺ influx. *J. Biol. Chem.* 286:39379–39386, 2011.
12. Chatzianastasiou, A., Bibli, S.I., Andreadou, I., et al. Cardioprotection by H₂S donors: nitric oxide-dependent and independent mechanisms. *J. Pharmacol. Exp. Ther.* 358:431–440, 2016.
13. Shi, H.Q., Zhang, Y., Cheng, M.H., et al. Sodium sulfide, a hydrogen sulfide-releasing molecule, attenuates acute cerebral ischemia in rats. *CNS Neurosci. Ther.* 22:625–632, 2016.
14. Cao, X., and Bian, J.S. The role of hydrogen sulfide in renal system. *Front Pharmacol.* 7:385, 2016.
15. Gero, D., Torregrossa, R., Perry, A., et al. The novel mitochondria-targeted hydrogen sulfide (H₂S) donors AP123 and AP39 protect against hyperglycemic injury in microvascular endothelial cells in vitro. *Pharmacol. Res.* 113:186–198, 2016.
16. Perridon, B.W., Leuvenink, H.G., Hillebrands, J.L., van, G.H., and Bos, E.M. The role of hydrogen sulfide in aging and age-related pathologies. *Aging (Albany NY)*. 8:2264–2289, 2016.
17. Tabibzadeh, S. Nature creates, adapts, protects and sustains life using hydrogen sulfide. *Front. Biosci. (Landmark Ed)*. 21:528–560, 2016.
18. Jupiter, R.C., Yoo, D., Pankey, E.A., et al. Analysis of erectile responses to H₂S donors in the anesthetized rat. *Am. J. Physiol. Heart Circ. Physiol.* 309:H835–H843, 2015.
19. Li, L., Rose, P., and Moore, P.K. Hydrogen sulfide and cell signaling. *Annu. Rev. Pharmacol. Toxicol.* 51:169–187, 2011.
20. Mancardi, D., Penna, C., Merlino, A., Del, S.P., Wink, D.A., and Pagliaro, P. Physiological and pharmacological features of the novel gasotransmitter: hydrogen sulfide. *Biochim. Biophys. Acta*. 1787:864–872, 2009.
21. Tan, B.H., Wong, P.T., and Bian, J.S. Hydrogen sulfide: a novel signaling molecule in the central nervous system. *Neurochem. Int.* 56:3–10, 2010.
22. Munaron, L., Avanzato, D., Moccia, F., and Mancardi, D. Hydrogen sulfide as a regulator of calcium channels. *Cell Calcium*. 53:77–84, 2013.
23. Njie-Mbye, Y.F., Opere, C.A., Chitnis, M., and Ohia, S.E. Hydrogen sulfide: role in ion channel and transporter modulation in the eye. *Front. Physiol.* 3:295, 2012.
24. Tang, G., Wu, L., and Wang, R. Interaction of hydrogen sulfide with ion channels. *Clin. Exp. Pharmacol. Physiol.* 37:753–763, 2010.
25. Kimura, Y., Mikami, Y., Osumi, K., Tsugane, M., Oka, J., and Kimura, H. Polysulfides are possible H₂S-derived signaling molecules in rat brain. *FASEB J.* 27:2451–2457, 2013.

26. Hatakeyama, Y., Takahashi, K., Tominaga, M., Kimura, H., and Ohta, T. Polysulfide evokes acute pain through the activation of nociceptive TRPA1 in mouse sensory neurons. *Mol. Pain*. 11:24, 2015.
27. Greiner, R., Palinkas, Z., Basell, K., et al. Polysulfides link H₂S to protein thiol oxidation. *Antioxid. Redox Signal*. 19: 1749–1765, 2013.
28. Koike, S., Ogasawara, Y., Shibuya, N., Kimura, H., and Ishii, K. Polysulfide exerts a protective effect against cytotoxicity caused by t-buthylhydroperoxide through Nrf2 signaling in neuroblastoma cells. *FEBS Lett*. 587:3548–3555, 2013.
29. Stubbett, D., Pryszazhna, O., Rudyk, O., Scotcher, J., Burgoyne, J.R., and Eaton, P. Protein kinase G α 1a oxidation paradoxically underlies blood pressure lowering by the reductant hydrogen sulfide. *Hypertension*. 64: 1344–1351, 2014.
30. Kimura, H. Hydrogen polysulfide (H₂S)_n signaling along with hydrogen sulfide (H₂S) and nitric oxide (NO). *J. Neural Transm. (Vienna)*. 123:1235–1245, 2016.
31. Cavallini, D., Mondovi, B., De Marco, C., and Scioscia-Santoro, A. The mechanism of desulphhydration of cysteine. *Enzymologia*. 24:253–266, 1962.
32. Singh, S., Padovani, D., Leslie, R.A., Chiku, T., and Banerjee, R. Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H₂S biogenesis via alternative trans-sulfuration reactions. *J. Biol. Chem*. 284: 22457–22466, 2009.
33. Meister, A., Fraser, P.E., and Tice, S.V. Enzymatic desulfuration of beta-mercaptopyruvate to pyruvate. *J. Biol. Chem*. 206:561–575, 1954.
34. Kuo, S.M., Lea, T.C., and Stipanuk, M.H. Developmental pattern, tissue distribution, and subcellular distribution of cysteine: alpha-ketoglutarate aminotransferase and 3-mercaptopyruvate sulfurtransferase activities in the rat. *Biol. Neonate*. 43:23–32, 1983.
35. Nagahara, N., Okazaki, T., and Nishino, T. Cytosolic mercaptopyruvate sulfurtransferase is evolutionarily related to mitochondrial rhodanese. Striking similarity in active site amino acid sequence and the increase in the mercaptopyruvate sulfurtransferase activity of rhodanese by site-directed mutagenesis. *J. Biol. Chem*. 270:16230–16235, 1995.
36. Shibuya, N., Mikami, Y., Kimura, Y., Nagahara, N., and Kimura, H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J. Biochem*. 146:623–626, 2009.
37. Shibuya, N., Tanaka, M., Yoshida, M., et al. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid. Redox Signal*. 11:703–714, 2009.
38. Yadav, P.K., Yamada, K., Chiku, T., Koutmos, M., and Banerjee, R. Structure and kinetic analysis of H₂S production by human mercaptopyruvate sulfurtransferase. *J. Biol. Chem*. 288:20002–20013, 2013.
39. Cooper, A.J. Biochemistry of sulfur-containing amino acids. *Annu. Rev. Biochem*. 52:187–222, 1983.
40. Shibuya, N., Koike, S., Tanaka, M., et al. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. *Nat. Commun*. 4:1366, 2013.
41. Kimura, H. Signaling molecules: hydrogen sulfide and polysulfide. *Antioxid. Redox Signal*. 22:362–376, 2015.
42. Hildebrandt, T.M., and Grieshaber, M.K. Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *FEBS J*. 275: 3352–3361, 2008.
43. Kimura, H. Metabolic turnover of hydrogen sulfide. *Front. Physiol*. 3:101, 2012.
44. Vitvitsky, V., Kabil, O., and Banerjee, R. High turnover rates for hydrogen sulfide allow for rapid regulation of its tissue concentrations. *Antioxid. Redox Signal*. 17:22–31, 2012.
45. De, L.G., Ruggeri, P., and Macaione, S. Cystathionase activity in rat tissues during development. *Ital. J. Biochem*. 23:371–379, 1974.
46. Persa, C., Osmotherly, K., Chao-Wei, C.K., Moon, S., and Lou, M.F. The distribution of cystathionine beta-synthase (CBS) in the eye: implication of the presence of a trans-sulfuration pathway for oxidative stress defense. *Exp. Eye Res*. 83:817–823, 2006.
47. Pong, W.W., Stouracova, R., Frank, N., Kraus, J.P., and Eldred, W.D. Comparative localization of cystathionine beta-synthase and cystathionine gamma-lyase in retina: differences between amphibians and mammals. *J. Comp. Neurol*. 505:158–165, 2007.
48. Ganapathy, P.S., Roon, P., Moister, T.K., Mysona, B., and Smith, S.B. Diabetes accelerates retinal neuronal cell death in a mouse model of endogenous hyperhomocysteinemia. *Ophthalmol. Eye Dis*. 1:3–11, 2009.
49. Ganapathy, P.S., Perry, R.L., Tawfik, A., et al. Homocysteine-mediated modulation of mitochondrial dynamics in retinal ganglion cells. *Invest. Ophthalmol. Vis. Sci*. 52:5551–5558, 2011.
50. Yu, M., Sturgill-Short, G., Ganapathy, P., Tawfik, A., Peachey, N.S., and Smith, S.B. Age-related changes in visual function in cystathionine-beta-synthase mutant mice, a model of hyperhomocysteinemia. *Exp. Eye Res*. 96:124–131, 2012.
51. Markand, S., Tawfik, A., Ha, Y., et al. Cystathionine beta synthase expression in mouse retina. *Curr. Eye Res*. 38: 597–604, 2013.
52. Gustafson, E.C., Morgans, C.W., Tekmen, M., et al. Retinal NMDA receptor function and expression are altered in a mouse lacking D-amino acid oxidase. *J. Neurophysiol*. 110:2718–2726, 2013.
53. Romero, G.E., Lockridge, A.D., Morgans, C.W., Bandyopadhyay, D., and Miller, R.F. The postnatal development of D-serine in the retinas of two mouse strains, including a mutant mouse with a deficiency in D-amino acid oxidase and a serine racemase knockout mouse. *ACS Chem. Neurosci*. 5:848–854, 2014.
54. Kulkarni, M., Njie-Mbye, Y.F., Okpobiri, I., Zhao, M., Opere, C.A., and Ohia, S.E. Endogenous production of hydrogen sulfide in isolated bovine eye. *Neurochem. Res*. 36:1540–1545, 2011.
55. Guo, W., Cheng, Z.Y., and Zhu, Y.Z. Hydrogen sulfide and translational medicine. *Acta Pharmacol. Sin*. 34:1284–1291, 2013.
56. Beltowski, J. Hydrogen sulfide in pharmacology and medicine—an update. *Pharmacol. Rep*. 67:647–658, 2015.
57. McDougal, D.H., and Gamlin, P.D. Autonomic control of the eye. *Compr. Physiol*. 5:439–473, 2015.
58. Kulkarni, K.H., Monjok, E.M., Zeyssig, R., et al. Effect of hydrogen sulfide on sympathetic neurotransmission and catecholamine levels in isolated porcine iris-ciliary body. *Neurochem. Res*. 34:400–406, 2009.
59. Salvi, A., Bankhele, P., Jamil, J.M., et al. Pharmacological actions of hydrogen sulfide donors on sympathetic neurotransmission in the bovine anterior uvea, in vitro. *Neurochem. Res*. 41:1020–1028, 2016.

60. Gallar, J., and Liu, J.H. Stimulation of the cervical sympathetic nerves increases intraocular pressure. *Invest. Ophthalmol. Vis. Sci.* 34:596–605, 1993.
61. Hubbard, W.C., Robinson, J.C., Schmidt, K., Rohen, J.W., Tamm, E.R., and Kaufman, P.L. Superior cervical ganglionectomy in monkeys: effects on refraction and intraocular pressure. *Exp. Eye Res.* 68:637–639, 1999.
62. Chou, P., Lu, D.W., and Chen, J.T. Bilateral superior cervical ganglionectomy increases choroidal blood flow in the rabbit. *Ophthalmologica.* 214:421–425, 2000.
63. Zhan, G.L., Lee, P.Y., Ball, D.C., et al. Time dependent effects of sympathetic denervation on aqueous humor dynamics and choroidal blood flow in rabbits. *Curr. Eye Res.* 25:99–105, 2002.
64. Monjok, E.M., Kulkarni, K.H., Kouamou, G., et al. Inhibitory action of hydrogen sulfide on muscarinic receptor-induced contraction of isolated porcine irides. *Exp. Eye Res.* 87:612–616, 2008.
65. Ohia, S.E., Opere, C.A., Monjok, E.M., Kouamou, G., Leday, A.M., and Njie-Mbye, Y.F. Role of hydrogen sulfide production in inhibitory action of L-cysteine on isolated porcine irides. *Curr. Eye Res.* 35:402–407, 2010.
66. Robinson, J., Okoro, E., Ezuedu, C., et al. Effects of hydrogen sulfide-releasing compounds on aqueous humor outflow facility in porcine ocular anterior segments, ex vivo. *J. Ocul. Pharmacol. Ther.* 33:91–97, 2017.
67. Lee, Y.S., and Marmorstein, A.D. Control of outflow resistance by soluble adenylyl cyclase. *J. Ocul. Pharmacol. Ther.* 30:138–142, 2014.
68. Johnson, D.H. Human trabecular meshwork cell survival is dependent on perfusion rate. *Invest. Ophthalmol. Vis. Sci.* 37:1204–1208, 1996.
69. Pang, I.H., McCartney, M.D., Steely, H.T., and Clark, A.F. Human ocular perfusion organ culture: a versatile ex vivo model for glaucoma research. *J. Glaucoma.* 9:468–479, 2000.
70. Njie, Y.F., Kumar, A., Qiao, Z., Zhong, L., and Song, Z.H. Naladin ether acts on trabecular meshwork cannabinoid (CB1) receptors to enhance aqueous humor outflow facility. *Invest. Ophthalmol. Vis. Sci.* 47:1999–2005, 2006.
71. 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.
72. Beltowski, J., and Jamroz-Wisniewska, A. Hydrogen sulfide and endothelium-dependent vasorelaxation. *Molecules.* 19:21183–21199, 2014.
73. Wang, R., Szabo, C., Ichinose, F., Ahmed, A., Whiteman, M., and Papapetropoulos, A. The role of H₂S bioavailability in endothelial dysfunction. *Trends Pharmacol. Sci.* 36:568–578, 2015.
74. Yang, G., and Wang, R. H₂S and blood vessels: an overview. *Handb. Exp. Pharmacol.* 230:85–110, 2015.
75. Chitnis, M.K., Njie-Mbye, Y.F., Opere, C.A., Wood, M.E., Whiteman, M., and Ohia, S.E. Pharmacological actions of the slow release hydrogen sulfide donor GYY4137 on phenylephrine-induced tone in isolated bovine ciliary artery. *Exp. Eye Res.* 116:350–354, 2013.
76. Salomone, S., Foresti, R., Villari, A., Giordanella, G., Drago, F., and Bucolo, C. Regulation of vascular tone in rabbit ophthalmic artery: cross talk of endogenous and exogenous gas mediators. *Biochem. Pharmacol.* 92:661–668, 2014.
77. Kulkarni-Chitnis, M., Njie-Mbye, Y.F., Mitchell, L., et al. Inhibitory action of novel hydrogen sulfide donors on bovine isolated posterior ciliary arteries. *Exp. Eye Res.* 134:73–79, 2015.
78. Takir, S., Ortakoylu, G.Z., Toprak, A., and Uydes-Dogan, B.S. NaHS induces relaxation response in prostaglandin F(2alpha) precontracted bovine retinal arteries partially via K(v) and K(ir) channels. *Exp. Eye Res.* 132:190–197, 2015.
79. Winther, A.K., Dalsgaard, T., Hedegaard, E.R., and Simonsen, U. Involvement of hydrogen sulfide in perivascular and hypoxia-induced inhibition of endothelin contraction in porcine retinal arterioles. *Nitric Oxide.* 50:1–9, 2015.
80. Sun, Y., Tang, C.S., Du, J.B., and Jin, H.F. Hydrogen sulfide and vascular relaxation. *Chin. Med. J. (Engl).* 124:3816–3819, 2011.
81. Holwerda, K.M., Karumanchi, S.A., and Lely, A.T. Hydrogen sulfide: role in vascular physiology and pathology. *Curr. Opin. Nephrol. Hypertens.* 24:170–176, 2015.
82. Opere, C.A., Monjok, E.M., Kulkarni, K.H., Njie, Y.F., and Ohia, S.E. Regulation of [3H] D-aspartate release from mammalian isolated retinae by hydrogen sulfide. *Neurochem. Res.* 34:1962–1968, 2009.
83. Seki, M., and Lipton, S.A. Targeting excitotoxic/free radical signaling pathways for therapeutic intervention in glaucoma. *Prog. Brain Res.* 173:495–510, 2008.
84. Ishikawa, M. Abnormalities in glutamate metabolism and excitotoxicity in the retinal diseases. *Scientifica (Cairo).* 2013:528940, 2013.
85. Njie-Mbye, Y.F., Bongmba, O.Y., Onyema, C.C., et al. Effect of hydrogen sulfide on cyclic AMP production in isolated bovine and porcine neural retinae. *Neurochem. Res.* 35:487–494, 2010.
86. Njie-Mbye, Y.F., Kulkarni, M., Opere, C.A., and Ohia, S.E. Mechanism of action of hydrogen sulfide on cyclic AMP formation in rat retinal pigment epithelial cells. *Exp. Eye Res.* 98:16–22, 2012.
87. Biermann, J., Lagreze, W.A., Schallner, N., Schwer, C.I., and Goebel, U. Inhalative preconditioning with hydrogen sulfide attenuated apoptosis after retinal ischemia/reperfusion injury. *Mol. Vis.* 17:1275–1286, 2011.
88. Mikami, Y., and Kimura, H. A mechanism of retinal protection from light-induced degeneration by hydrogen sulfide. *Commun. Integr. Biol.* 5:169–171, 2012.
89. Sakamoto, K., Suzuki, Y., Kurauchi, Y., Mori, A., Nakahara, T., and Ishii, K. Hydrogen sulfide attenuates NMDA-induced neuronal injury via its anti-oxidative activity in the rat retina. *Exp. Eye Res.* 120:90–96, 2014.
90. Si, Y.F., Wang, J., Guan, J., Zhou, L., Sheng, Y., and Zhao, J. Treatment with hydrogen sulfide alleviates streptozotocin-induced diabetic retinopathy in rats. *Br. J. Pharmacol.* 169:619–631, 2013.
91. Sun, M.H., Pang, J.H., Chen, S.L., et al. Retinal protection from acute glaucoma-induced ischemia-reperfusion injury through pharmacologic induction of heme oxygenase-1. *Invest. Ophthalmol. Vis. Sci.* 51:4798–4808, 2010.
92. Zhou, C.F., and Tang, X.Q. Hydrogen sulfide and nervous system regulation. *Chin. Med. J. (Engl).* 124:3576–3582, 2011.
93. Dou, Y., Wang, Z., and Chen, G. The role of hydrogen sulfide in stroke. *Med. Gas Res.* 6:79–84, 2016.
94. Alm, A. Latanoprost in the treatment of glaucoma. *Clin. Ophthalmol.* 8:1967–1985, 2014.
95. Doucette, L.P., and Walter, M.A. Prostaglandins in the eye: function, expression, and roles in glaucoma. *Ophthalmic Genet.* 38:108–116, 2017.

96. Perrino, E., Uliva, C., Lanzi, C., Soldato, P.D., Masini, E., and Sparatore, A. New prostaglandin derivative for glaucoma treatment. *Bioorg. Med. Chem. Lett.* 19:1639–1642, 2009.
97. Sunny Edet Ohia CAOGZEMKKGEK. U.S. Patent 8092838: Use of Hydrogen Sulfide in the Treatment of Eye Diseases. 1-10-2012.
98. Salvi, A., Bankhele, P., Jamil, J., et al. Effect of hydrogen sulfide donors on intraocular pressure in rabbits. *J. Ocul. Pharmacol. Ther.* 32:371–375, 2016.
99. Huang, S., Huang, P., Liu, X., et al. Relevant variations and neuroprotective effect of hydrogen sulfide in a rat glaucoma model. *Neuroscience*. 341:27–41, 2017.
100. Wang, R. Two's company, three's a crowd: can H₂S be the third endogenous gaseous transmitter? *FASEB J.* 16: 1792–1798, 2002.
101. Ali, M.Y., Ping, C.Y., Mok, Y.Y., et al. Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide? *Br. J. Pharmacol.* 149: 625–634, 2006.
102. Nagpure, B.V., and Bian, J.S. Interaction of hydrogen sulfide with nitric oxide in the cardiovascular system. *Oxid. Med. Cell Longev.* 2016:6904327, 2016.
103. Krauss, A.H., Impagnatiello, F., Toris, C.B., et al. Ocular hypotensive activity of BOL-303259-X, a nitric oxide donating prostaglandin F₂α agonist, in preclinical models. *Exp. Eye Res.* 93:250–255, 2011.
104. Kaufman, P.L. Latanoprostene bunod ophthalmic solution 0.024% for IOP lowering in glaucoma and ocular hypertension. *Expert Opin. Pharmacother.* 18:433–444, 2017.
105. Zheng, Y., Ji, X., Ji, K., and Wang, B. Hydrogen sulfide prodrugs-a review. *Acta Pharm. Sin B.* 5:367–377, 2015.

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