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REVIEW ARTICLE

Hydrogen sulfide: its production, release and functions

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Abstract Hydrogen sulfide (H_2S), which is a well-known toxic gas, has been recognized as a signal molecule as well as a cytoprotectant. It is produced by three enzymes, cystathionine β-synthase, cystathionine γ-lyase and 3-mercaptopyruvate sulfurtransferase along with cysteine aminotransferase. In addition to an immediate release of H_2S from producing enzymes, it can be stored as bound sulfane sulfur, which may release H_2S in response to physiological stimuli. As a signal molecule, it modulates neuronal transmission, relaxes smooth muscle, regulates release of insulin and is involved in inflammation. Because of its reputation as a toxic gas, the function as a cytoprotectant has been overlooked: the nervous system and cardiovascular system are protected from oxidative stress. In this review, enzymatic production, release mechanism and functions of H_2S are focused on.

Keywords H_2S · Cystathionine β-synthase · Cystathionine γ-lyase · 3-Mercaptopyruvate sulfurtransferase · Bound sulfane sulfur · Neuromodulator · Neuroprotectant · Cardioprotectant · Smooth muscle relaxant · EDRF

Introduction

The first toxic gas identified as a signal molecule is nitric oxide (NO), which is produced from arginine by NO synthase (Furchgott and Zawadzki 1980). Another toxic gas, carbon monoxide (CO), is produced from biliverdin by heme oxygenase (Verma et al. 1993). Both NO and CO were identified as smooth muscle relaxants, and recognized later as neurotransmitters (O'Dell et al. 1991; Stevens and Wang 1993). Hydrogen sulfide (H_2S), which is produced from cysteine or homocysteine, was initially found to be a neuromodulator (Abe and Kimura 1996). It facilitates the induction of hippocampal long-term potentiation (LTP) by enhancing the activity of NMDA receptors in neurons and increases the influx of Ca^{2+} into astrocytes. Like NO and CO, H_2S relaxes the thoracic aorta, portal vein and the ileum, which mainly express cystathionine γ-lyase (CSE) (Hosoki et al. 1997). Hypertension is observed in CSE knockout mice, confirming that H_2S is a smooth muscle relaxant and suggesting that it may regulate blood pressure (Yang et al. 2008).

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Enzymatic regulation of H_2S production

H_2S can be produced from cysteine by pyridoxal-5′-phosphate (PLP)-dependent enzymes, including cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Abe and Kimura 1996; Hosoki et al. 1997; Stipanuk and Beck 1982; Zhao et al. 2001). CBS and CSE are expressed in many tissues including the liver and the kidney. Although the expression of CSE in the brain is controversial, both CBS and CSE were reported to be expressed in the brain and produce H_2S from cysteine (Abe and Kimura 1996; Diwakar and Ravindranath 2007; Ishii et al. 2004; Vitvitsky et al. 2006). CBS and CSE also catalyze the condensation reaction of homocysteine with cysteine to produce H_2S (Chen et al. 2004; Chiku et al. 2009). H_2S production by CBS changes depending on the extent of allosteric activation of *S*-adenosylmethionine, and the contribution of

CBS is decreased under hyperhomocysteinemic conditions (Singh et al. 2009).

Cystathionine β-synthase (CBS) is expressed in the liver, kidney, brain, ileum, uterus, placenta and pancreatic islets. In the brain, CBS is mainly localized to the cerebellar Bergmann glia and astrocytes (Enokido et al. 2005; Ichinohe et al. 2005). CBS is localized to neuroepithelial cells in the ventricular zone at the early developmental stages, but its localization changes to radial glial cells and astrocytes during the late embryonic and neonatal periods. The expression of CBS is enhanced by epidermal growth factor (EGF), transforming growth factor-α (TGF-α), cyclic adenosine monophosphate (cAMP) and dexamethasone in astrocytes (Enokido et al. 2005). In addition to the transcriptional up-regulation of CBS, a CBS activator, *S*-adenosyl methionine enhances the enzymatic production of H_2S (Abe and Kimura 1996).

Cystathionine γ-lyase (CSE) produces H_2S from cysteine or homocysteine. Under normal conditions, approximately 70% of H_2S is produced from cysteine and the remaining 30% is from homocysteine. However, under conditions with higher concentrations of homocysteine such as homocysteinemia, homocysteine rather than cysteine becomes the preferred source for H_2S production (Chiku et al. 2009).

Cystathionine γ-lyase (CSE) is expressed in the liver, kidney, thoracic aorta, ileum, portal vein, uterus, brain as well as pancreatic islets and the placenta (Diwakar and Ravindranath 2007; Hosoki et al. 1997; Kaneko et al. 2006; Patel et al. 2009; Vitvitsky et al. 2006). The expression of CSE is increased by the NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP), and the enzymatic activity of CSE is enhanced by another NO donor, sodium nitroprusside (SNP) (Zhao et al. 2001), although these effects of NO on CSE are controversial (Chiku et al. 2009). Although the requirement for Ca^{2+} concentrations was high (1 mM), a recent study showed that CSE activity was regulated by calcium calmodulin (Yang et al. 2008). Since the interaction between CSE and calmodulin is abolished by EGTA and the calmodulin inhibitor W7, the enhanced activity of CSE may be caused by its direct binding to calmodulin.

We recently found that H_2S is produced from cysteine with brain homogenates of CBS knockout mice and that the production depends on the presence of α-ketoglutarate (Shibuya et al. 2009b). These observations suggest that there is another H_2S-producing enzyme, which is neither CBS nor CSE, in the brain. 3-Mercaptopyruvate sulfurtransferase (3MST) along with cysteine aminotransferase (CAT), which is identical with aspartate aminotransferase (AAT), can produce H_2S from cysteine in the presence of α-ketoglutarate (Cooper 1983; Frendo and Wrobel 1997; Kuo et al. 1983). The production of H_2S by 3MST along with CAT in the presence of cysteine and α-ketoglutarate supports the existence of 3MP, which has not been identified. The existence of 3MP has been implicated based solely on the release of mercaptolactate-cysteine disulfide, a metabolite of 3MP, to urine. Our observation that the pathway produces H_2S even without the addition of 3MP indicates the existence of 3MP as an intermediate (Shibuya et al. 2009b).

3MST is localized to the liver, kidney, heart, lung, thymus, testis, thoracic aorta and the brain (Nagahara et al. 1998; Shibuya et al. 2009a, b). 3MST with CAT efficiently produces H_2S from cysteine and α-ketoglutarate, and this production is suppressed by a competition with aspartate, a preferred substrate for CAT or AAT.

Mechanism of H_2S release

There are at least two mechanisms for the release of H_2S. H_2S is immediately released after its production by enzymes. Another possible mechanism is that the produced H_2S is stored and released in response to a physiologic signal. Two forms of sulfur stores in cells have been identified (Ogasawara et al. 1993; Toohey 1989). Acidic conditions release H_2S from acid-labile sulfur. Another form of storage is called bound sulfane sulfur, which releases H_2S under the reducing conditions (Ogasawara et al. 1994). Acid-labile sulfur is mainly sulfur atoms in the iron–sulfur complexes, which play a critical role in a wide range of redox reactions in enzymes of the respiratory chain in mitochondria. The critical pH below which H_2S is released from acid-labile sulfur is 5.4 (Ishigami et al. 2009). Because mitochondrial pH is between 7 and 8, which is higher than the critical pH, acid-labile sulfur may not release H_2S under physiologic conditions.

The sulfur of H_2S can be incorporated into proteins as bound sulfane sulfur, which is divalent sulfur bound only to the other sulfur, such as outer sulfur atoms of the persulfides and inner chain atoms of polysulfides. Cysteine is metabolized to produce sulfide that is incorporated into a pool of bound sulfane sulfur (Daniels and Stipanuk 1982). Exogenously applied free H_2S is immediately absorbed in a sulfur store as bound sulfane sulfur, suggesting that enzymatically produced H_2S may also be stored as bound sulfane sulfur (Ishigami et al. 2009). Cells expressing 3MST and CAT increase the levels of bound sulfane sulfur, while cells expressing a defective mutant of 3MST, which does not produce H_2S, maintain the levels of bound sulfane sulfur at the control levels (Nagahara and Nishino 1996; Shibuya et al. 2009b). The intracellular levels of bound sulfane sulfur are dependent on the H_2S producing activity of 3MST, and that H_2S produced by 3MST is stored as bound sulfane sulfur in the cells (Fig. 1).

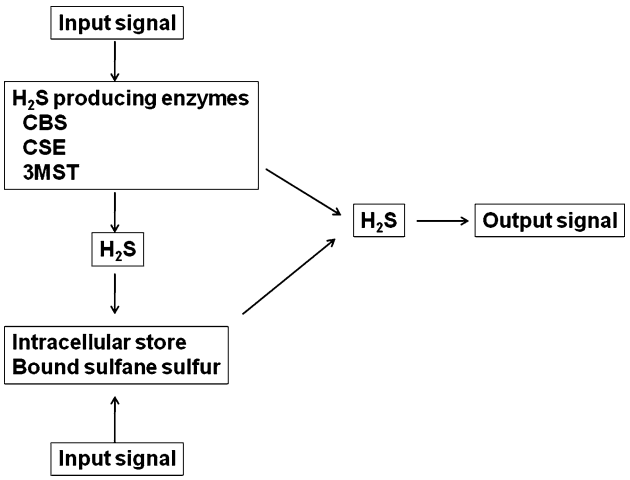

Fig. 1 Bound sulfane sulfur is a store of H_2S. H_2S produced by enzymes functions as a signal molecule. It can also be stored as bound sulfane sulfur, which in turn may release H_2S when cells receive certain physiological signals

In the presence of major cellular reducing substances, glutathione and cysteine at their physiologic concentrations, H_2S is released from lysates of cultured neurons and astrocytes at pH 8.4 (Ishigami et al. 2009). Because the reducing activity of thiols is greater in alkaline conditions than at a neutral pH, H_2S release is observed at pH higher than 8.4. Although the systemic pH approximately changes up to 0.2 in either alkalosis or acidosis, it is possible that pH can locally be changed more greatly. When neurons are excited, sodium ions enter and potassium ions exit from cells, resulting in high potassium concentrations in the extracellular environment. It depolarizes the membrane of surrounding astrocytes and activates their Na^+/HCO_3^- cotransporters. Entrance of HCO_3^- causes the alkalinization of the cells (Brookes and Turner 1994). Although H_2S has not been detected, approximately 10% of the primary cultures of astrocytes responded well to 10 mM K^+ and shifted their intracellular pH to 8.4 (Ishigami et al. 2009).

Neuromodulator, the signal function in the nervous cells

Since H_2S is enzymatically produced from cysteine, H_2S was predicted to have a physiological function in the brain (Abe and Kimura 1996). The studies of H_2S toxicity showed that H_2S suppressed synaptic potentials and modified K^+ channels (Kombian et al. 1993). Although H_2S does not affect the excitatory postsynaptic potentials (EPSPs) at low concentrations, it induces hippocampal LTP when simultaneously applied with a weak titanic stimulation, which alone does not induce LTP (Abe and Kimura 1996). LTP induced in the presence of H_2S shares common mechanisms with LTP induced by a regular electrical stimulation. H_2S activates LTP at active, but not quiescent, synapses, and may be involved in associative learning as defined by Hebb in which the synergistic effect is observed between two inputs simultaneously entered into the same neuron (Hebb 1949). Although H_2S, like other gaseous messengers, NO and CO, facilitates the induction of LTP (O'Dell et al. 1991; Stevens and Wang 1993), the mechanism of action of H_2S is different from those of NO and CO. NO and CO activate soluble guanylyl cyclase and increase intracellular cGMP (Verma et al. 1993), while H_2S does not (Abe and Kimura 1996). It is well known that the activation of NMDA receptors is required for the induction of LTP. NO and CO, which function as retrograde neurotransmitters, do not require NMDA receptor activation (Zhuo et al. 1993), while H_2S does not facilitate the LTP induction under the blockade of NMDA receptors (Abe and Kimura 1996). H_2S facilitates the induction of LTP by enhancing the activity of NMDA receptors.

Disulfide bonds play a role in modulating the function of many proteins, including NMDA receptors (Aizenman et al. 1989). It is possible that H_2S reduces disulfide bonds or makes bound sulfane sulfur in NMDA receptors. A high concentration of dithiothreitol (DTT) facilitates the induction of LTP, while even a low concentration of H_2S further facilitates the induction of the greater amplitudes of LTP. Since H_2S further facilitates the induction of LTP enhanced by DTT, mere reducing activity of H_2S may not contribute to its facilitating effect on the induction of LTP. The formation of bound sulfane sulfur at thiols of NMDA receptors may be a possible mechanism.

Glial mediator for the interaction with neurons

Although cells with no electrical changes have been thought to be glia, recent studies have shown that glia are electrically active cells, which have neurotransmitter receptors and respond to transmitters (Cornell-Bell et al. 1990). There are reciprocal interactions between neurons and glia. Neural activity evokes glial calcium waves, and glial calcium waves drive neuronal activity (Dani et al. 1992). The glia communicate with each other by propagating their signals as Ca^{2+} waves (Parri et al. 2001). The Ca^{2+} waves often appear to be initiated at sites of contact with neurons, suggesting that the glial Ca^{2+} waves are initiated by neuronal excitation (Charles 1994).

Astrocytes, a type of glia, respond to a neurotransmitter released from neurons. In neurons, H_2S shows modulatory function by enhancing the activity of NMDA receptors activated by a neurotransmitter, glutamate (Abe and Kimura 1996). In astrocytes, H_2S elicits direct activation of Ca^{2+} influx (Nagai et al. 2004). Because astrocytes elicit intercellular Ca^{2+} waves by electrical stimulation of nearby

neurons as well as by the activation of neurons by NMDA, it has been suggested that astrocytes respond directly to a neurotransmitter released from neurons (Dani et al. 1992; Haydon 2001). NMDA induces Ca^{2+} waves in astrocytes only in the presence of neurons, and TTX suppresses the induction, suggesting that neuronal excitation is required to release H_2S (Nagai et al. 2004). Neuronal excitation induces Ca^{2+} waves in astrocytes, which are propagated to neighboring astrocytes and reach nearby synapse and may modulate its activity.

Although the sensitive molecule or receptors for H_2S in astrocytes has not been identified, considering the facts that responses to H_2S were suppressed by La^{3+}, Gd^{3+} and ruthenium red, which are known as blockers of voltage-dependent Ca^{2+} channels or TRP channels, TRP channels may mediate the responses to H_2S (Nagai et al. 2004). Contraction of the detrusor muscle is regulated by capsaicin-sensitive primary afferent neurons in the urinary bladder. Contraction induced by H_2S is suppressed by unselective TRP channel blocker, ruthenium red, suggesting that TRP channels may be involved in the responses to H_2S (Patacchini et al. 2005). The release of a neuropeptide and contraction induced by H_2S in the airways are significantly attenuated by a TRPV1 channel blocker, capsazepine. TRPV1 channels may mediate contraction induced by H_2S. The other example that supports TRP channels as a group of H_2S -sensitive molecules or receptors is that CHO cells expressing TRPA1 channels respond to NaHS (Streng et al. 2008).

Smooth muscle relaxant

The production of H_2S by the thoracic aorta, portal vein and the ileum was suppressed by CSE inhibitors, and H_2S relaxes these tissues (Hosoki et al. 1997). Relaxation occurs mostly by opening ATP-dependent K^+ channels in a non-ATP-associated manner (Zhao et al. 2001). Deficiency of CSE causes hypertension in mice, suggesting that H_2S is a physiologic vascular smooth muscle relaxant and may regulate blood pressure (Yang et al. 2008). Smooth muscle of the other tissue that was recently demonstrated to be regulated by H_2S is the corpus cavernosum smooth muscle (di Villa Bianca et al. 2009). Both CBS and CSE are expressed in human penile tissue, and homogenates of the tissue produce H_2S. Exogenously applied H_2S and cysteine cause a concentration-dependent relaxation of strips of human corpus cavernosum. Cysteine-induced relaxation is suppressed by a CBS inhibitor, aminooxyacetate. In rats, H_2S and cysteine promote penile erection, and the response to cysteine is blocked by a CSE inhibitor, propargylglycine. A functional cysteine/H_2S pathway may be involved in mediating penile erection in humans and other mammals.

Although the relaxation effect of H_2S alone is weak, there is a synergy between NO and H_2S on vascular smooth muscle relaxation (Hosoki et al. 1997). A similar synergistic effect between H_2S and NO was observed in the inhibition of the twitch responses of the ileum to electrical stimulation (Teague et al. 2002). In addition to the synergistic effect of H_2S with NO, CSE activity is also enhanced by NO. CSE contains 12 cysteine residues that are potential targets for *S*-nitrosation, which may enhance the activity of CSE (Koenitzer et al. 2007). H_2S-induced vasorelaxation was partially attenuated either by removal of the endothelium, by blockade of NO synthase or by the Ca^{2+}-dependent K^+ channel blockers, suggesting that H_2S might stimulate endothelial cells to release certain factors that facilitate the relaxation of smooth muscle (Zhao and Wang 2002). This observation also supports the finding that H_2S enhances relaxation activity of NO.

Oxygen modulates the sensitivity of vessels to H_2S-induced relaxation. H_2S relaxes vessels under physiological O_2 conditions (Koenitzer et al. 2007) and causes contraction of aortic smooth muscle at high O_2 levels, while H_2S induces rapid relaxation at lower physiological O_2 levels. Peripheral vessels with a smaller diameter and wall thickness have greater proportion of smooth muscle cells than collagen compared with the aorta and higher rate of O_2 consumption. However, the levels of O_2 in periphery are lower. Peripheral arteries normally operate at lower O_2 concentrations than the aorta, and H_2S efficiently relaxes arteries under low O_2 conditions. *S*-nitrosothiols in vascular tissue caused by liberated NO may also contribute to vessel tone (Koenitzer et al. 2007). H_2S catalyzes the release of NO from *S*-nitrosoglutathione in an O_2-dependent manner (Koenitzer et al. 2007). This observation further supports the interaction between H_2S and NO.

In contrast to these observations, H_2S has been reported to suppress the activity of NO to relax smooth muscle. For example, H_2S may induce vascular constriction by scavenging endothelial NO and increase the mean arterial pressure in rats (Ali et al. 2006). Pretreatment with H_2S inhibits the relaxation of aortic tissues induced by SNP (Zhao and Wang 2002).

A candidate of EDRFs

Since the relaxation effect of H_2S on vascular smooth muscle was not affected or slightly enhanced in the presence of endothelium, it was thought that exogenously applied H_2S might stimulate the endothelium to release EDRF or EDHF and interact with them (Hosoki et al. 1997; Zhao et al. 2001). Because H_2S still significantly relaxes vascular tissues after endothelium removal, the contribution of endothelium to the relaxation effect of exogenously

applied H_2S must be low (Hosoki et al. 1997; Zhao and Wang 2002).

The possibility that EDRF is not identical to NO has been raised based on the following observations. NO relaxed nonvascular smooth muscles, but EDRF released from cultured endothelial cells did not (Shikano et al. 1988). The activity of EDRF is lost on passage over anionic exchange resins, but that of NO is not. In addition, EDRF hyperpolarizes smooth muscle, but NO does not have such an effect. These observations suggest that EDRF consists of more than one component. One of components, which could cause hyperpolarizing of the membrane of the smooth muscle, is designated as EDHF (Chen et al. 1988). Although H_2S has properties similar to those of EDRF (its major producing enzyme CSE is localized to the smooth muscle), it has not been thought to be a component of EDRF (Hosoki et al. 1997; Zhao et al. 2001). Although species were different, it was recently reported that CSE was found in endothelial cells of mice, bovine and humans (Yang et al. 2008). Western blot analysis and immunohistochemistry in rats show that 3MST and CAT, which we recently demonstrated as H_2S producing enzymes in the brain, are localized to the vascular endothelium (Shibuya et al. 2009a, b). Lysates of endothelial cells produce H_2S from 3MP as a substrate and also from cysteine in the presence of α-ketoglutarate. Since H_2S production in endothelium is highly dependent on α-ketoglutarate, 3MST along with CAT rather than CSE may be a major enzyme that produces H_2S in the endothelium at least in rats. H_2S is a candidate for a component of EDRFs (Shibuya et al. 2009a) (Fig. 2).

Neuroprotectant, the cytoprotective function in the nervous cells

Cells can be rescued from oxidative stress by mechanisms that are either dependent on or independent of glutathione metabolism. Vitamine E protects cells from oxidative glutamate toxicity by acting directly as an antioxidant even when the intracellular glutathione levels are decreased (Murphy et al. 1989). In contrast, dihydroxyphenylglycine, an agonist of group 1 metabotropic glutamate receptors, protects neurons by up-regulating glutathione (Sagara and Schubert 1998). Extracellular H_2S protects cells from oxidative stress by enhancing the production of glutathione, and H_2S produced by 3MST and CAT may suppress oxidative stress in mitochondria (Kimura et al. 2010; Kimura and Kimura 2004).

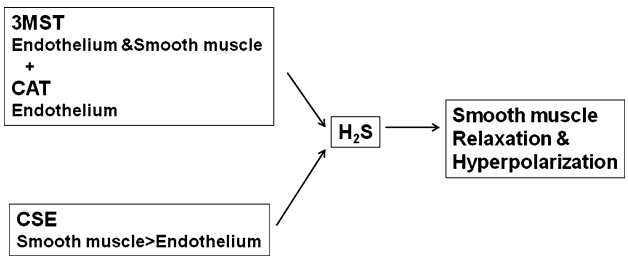

Fig. 2 H_2S is a candidate of EDRFs. It can be produced by 3-mercaptopyruvate sulfurtransferase (3MST) along with cysteine aminotransferase (CAT) or cystathionine γ-lyase (CSE) localized to the vascular endothelium. H_2S relaxes smooth muscle and hyperpolarizes its membrane

There are two forms of glutamate toxicity: ionotropic receptor-initiated excitotoxicity and non-receptor mediated oxidative glutamate toxicity (Choi 1988; Murphy et al. 1989). Oxidative glutamate toxicity is a well-studied programmed cell death pathway that is independent of ionotropic glutamate receptors (Murphy et al. 1989). It has been observed in primary cultures of neuronal cells (Kimura and Kimura 2004), neuronal cell lines (Kimura et al. 2006; Murphy et al. 1989) and brain slices (Vornov and Coyle 1991).

Sulfur-containing substances, dimethylsulphoniopropionate (DMSP) and its enzymatic cleavage product dimethylsulphide (DMS), have recently been identified as endogenous scavengers for hydroxyl radicals and other reactive oxygen species in marine algae (Sunda et al. 2002). H_2S, a reducing agent that readily reacts with hydrogen peroxide, may suppress reactive oxygen species (Devai and Delaune 2002). H_2S-producing enzymes 3MST and CAT are mainly localized to the mitochondria, which are a major source of oxidative stress and play a key role in cell death pathways (Shibuya et al. 2009b). Most of cell death signals may converge on the mitochondria (Murphy and Steenbergen 2007). Perturbation of the mitochondrial function causes the release of apoptogenic factors such as cytochrome *C* into the cytosol and ultimately results in cell death. Mitochondrial dysfunction caused by oxidative stress leads to numerous neurodegenerative diseases (Lin and Beal 2006). Neuronal cells expressing 3MST and CAT show significant resistance against oxidative glutamate toxicity (Kimura et al. 2010), suggesting that H_2S produced by 3MST along with CAT can scavenge reactive oxygen species in mitochondria and protect cells from oxidative stress.

H_2S increases the levels of glutathione, which is decreased under oxidative stress caused by glutamate (Kimura and Kimura 2004). The gradient of glutamate drives cystine import by cystine/glutamate antiporter that can be impaired under conditions where extracellular glutamate concentrations are increased (Bannai and Kitamura 1980). Elevated extracellular glutamate inhibits the import of cystine that is a substrate for the production of glutathione. H_2S enhances the activity of cystine/glutamate antiporter to increase the levels of cysteine, resulting in the

increased production of glutathione (Kimura and Kimura 2004).

In addition to the cystine transport, cysteine is also efficiently transported into cells by H_2S. It has been thought that cysteine normally exists as its oxidized form, cystine, in the extracellular space. However, significant amounts of cysteine (approximately 20 μM) were measured in plasma and blood (Richie and Lang 1987). H_2S produced in the cells can be released into the extracellular space and reduces cystine to cysteine that can readily be imported into cells by cysteine transporters and used for glutathione production (Kimura et al. 2010; Mathai et al. 2009).

Glutathione is produced through sequential metabolism by two enzymes, γ-glutamyl cysteine synthetase (γ-GCS) and glutathione synthetase (GS). H_2S enhances the activity of γ-GCS and increases the levels of γ-glutamyl cysteine, but it has no effect on GS (Kimura and Kimura 2004) (Fig. 3). The levels of γ-GCS mRNA or protein are not changed in cells exposed to H_2S, and the activity of γ-GCS in brain homogenates is not affected in the presence of H_2S (Kimura et al. 2010). These observations indicate that the enhancement of γ-GCS activity by H_2S is caused by neither transcriptional regulation nor the direct contact of H_2S with γ-GCS. Since the activity of γ-GCS is enhanced only when cells are exposed to H_2S, it may activate some receptors on the cell surface to initiate an intracellular signal that may enhance the activity of the enzyme.

In addition to the protection from oxidative glutamate toxicity, H_2S protects cells from broader oxidative stress including that caused by H_2O_2 (Kimura et al. 2010). Another example of protection from cell injury is that H_2S suppresses cell death induced by rotenone, a commonly used toxin to establish Parkinson's disease models by

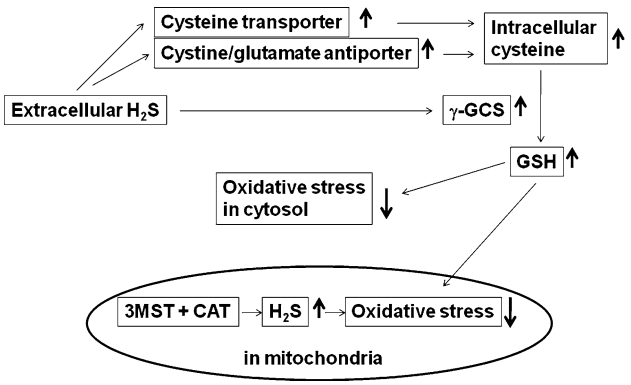

Fig. 3 H_2S is a neurotrotectant. It increases the levels of glutathione (GSH) by enhancing the activity of cystine/glutamate antiporter, cysteine transporter and γ-glutamyl cysteine synthetase (γ-GCS). H_2S produced by 3MST along with CAT suppresses oxidative stress in mitochondria

stabilizing mitochondrial membrane potential (Hu et al. 2009).

H_2S protects fetal brains by reinstating GSH levels decreased by oxidative stress caused by ischemia–reperfusion of maternal–fetal blood supply (Kimura et al. 2010). All the fetal brains 24 h after reperfusion from the ischemia were macerated, and the GSH levels in the fetal brains were severely decreased to 24% of the control. In contrast, brains of only one-fourth of fetuses were macerated by a treatment of NaHS prior to the ischemia–reperfusion. Although the GSH levels of macerated brains even with an NaHS application before ischemia–reperfusion were decreased to 36% of that of the control, those of non-macerated brains were maintained at approximately 90% of that of the control. These in vivo data support the in vitro results that H_2S protects brains by reinstating the GSH levels decreased by oxidative stress (Kimura et al. 2010).

In addition to mechanisms of suppressing oxidative stress in mitochondria and increasing glutathione levels, H_2S also protects cells by stabilizing membrane potentials (Kimura et al. 2006). K_{ATP} channels are involved in protection against ischemia and excitotoxicity (Heurteaux et al. 1993). A K_{ATP} channel opener, pinacidil, suppresses glutamate toxicity, and the protection is increased by the simultaneous application of H_2S (Kimura et al. 2006). Because a blocker selective to mitochondrial K_{ATP} channels, 5-hydroxydecanate, does not modulate protection by H_2S, K_{ATP} channels localized to plasma membrane, but not those to mitochondria, may mediate H_2S-induced neuroprotection.

CFTR Cl^- channels and a component of K_{ATP} channels belong to the ATP-binding cassette superfamily, and both channels share significant sequence homologies (Higgins 1995). The Cl^- channel blockers suppress protection by H_2S, and an opener of Cl^- channels efficiently suppresses glutamate toxicity (Kimura et al. 2006). CFTR Cl^- channels are also involved in protection by H_2S against oxidative stress with the same mechanism as with K_{ATP} channels.

Cardioprotectant, the cytoprotective function in the cardiovascular cells

As H_2S protects neurons from oxidative stress (Kimura and Kimura 2004), it protects cardiac muscle from ischemic injury. Exogenously applied H_2S decreases the myocardial necrosis induced by isoproterenol and the rate of mortality (Geng et al. 2004). It also attenuated the isoproterenol-induced decrease in myocardial contractility. NaHS decreased the size of infarction induced by left coronary artery ligation and the protection was abolished by K_{ATP} channel blockers (Johansen et al. 2006). The activation of

K_{ATP} as well as CFTR Cl channels protects neurons from oxidative stress by stabilizing membrane potential (Kimura et al. 2006), and the same mechanism may be also involved in the protection of the myocardium from ischemic injury. Episodes of sublethal ischemia make myocardium less sensitive to the subsequent more severe ischemic insult. Blockade of H_2S production by propargylglycine, a CSE inhibitor, reduced the protective effect of ischemic preconditioning (Bian et al. 2006).

Application of H_2S at the time of reperfusion limits infarct size and preserves left ventricular function (Elrod et al. 2007). Cardiac-specific transgenic mice with overexpression of CSE increased myocardial levels of H_2S and have a reduction in infarct size after ischemia reperfusion injury. Histological analysis shows that the administration of H_2S substantially decreased hemorrhage and necrosis as well as the number of leukocytes within the ischemic zone. H_2S also decreased myocardial levels of interleukin-1β and leukocyte-–endothelial cell interactions in vivo. Inhibition of leukocyte transmigration is one possible mechanism by which H_2S restrains the extent of inflammation and limits the extent of myocardial infarction (Elrod et al. 2007). Another important function of H_2S is the preservation of mitochondrial function. H_2S reversibly decreased mitochondrial oxygen consumption in a dose-dependent manner. Mitochondria isolated from mice applied H_2S at the time of reperfusion displayed preserved mitochondrial function, and mitochondrial swelling was decreased and matrix density increased in mice receiving H_2S (Elrod et al. 2007).

Conclusion

H_2S is produced by three enzymes, CBS, CSE and 3MST. Although CSE has been reported to be regulated by calcium/calmodulin, the regulation of the activity of CBS and 3MST has not been understood well. H_2S may function as a signal molecule immediately after release from the enzyme; it is also stored as bound sulfane sulfur, which may in turn release H_2S when cells receive a certain physiological signals. Although the levels of free H_2S were thought to be relatively high, it was recently clarified that free H_2S is maintained at low levels in the basal conditions that may be necessary for cells to properly respond to H_2S (Furne et al. 2008; Ishigami et al. 2009). The long exposure to relatively high concentrations of H_2S may cause desensitization of sensitive molecules or exert toxic effects.

H_2S was initially found as a neuromodulator, which facilitated the induction of hippocampal LTP by enhancing NMDA-induced currents. The direct effect of H_2S was found on astrocytes. H_2S induces the influx of Ca^{2+}, which propagates as calcium waves to the surrounding astrocytes. There are reciprocal interactions between glial calcium waves and neuronal activity, and glial cells are integral modulatory elements in synaptic transmission. H_2S may mediate the interaction and modulate synaptic activity.

H_2S also functions as signal molecule in smooth muscle relaxation. It relaxes tissues consisting of smooth muscle, including the thoracic aorta. Since CSE was the only enzyme known to be expressed in smooth muscle, but not in endothelium of the thoracic aorta in rats, H_2S has not been thought to be a component of EDRFs. However, recently 3MST and CAT were found to be localized to the endothelium and even CSE was reported to be in the endothelium of mice, humans and bovine. Based on these observations, it has been proposed that H_2S is a candidate of EDRFs.

In addition to the function as a signal molecule, H_2S also functions as a cytoprotectant in neurons and cardiac muscle. It increases the levels of glutathione and redistributes it to the mitochondria. H_2S produced by 3MST and CAT, which localize to the mitochondria, also suppresses oxidative stress in mitochondria and preserves the function of this organelle. The application of H_2S and the regulation of endogenous production may be of clinical benefit.

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