Hydrogen sulfide signaling in the central nervous system -Comparison with nitric oxide-

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Abstract

Hydrogen sulfide (H2S) together with polysulfides (H2Sn, n>2) are signaling molecules like nitric oxide (NO) with various physiological roles including regulation of neuronal transmission, vascular tone, inflammation, oxygen sensing etc. H2S and H2Sn diffuse to the target proteins to S-sulfurate their cysteine residues to induce the conformational changes to alter the activity. On the other hand, 3-mercaptopyruvate sulfurtransferase transfers sulfur from a substrate 3-mercaptopyruvate to the cysteine residues of acceptor proteins. A similar mechanism has also been identified in S-nitrosylation. S-sulfuration and S-nitrosylation by enzymes proceed only inside the cell, while reactions induced by H2S, H2Sn and NO even extend to the surrounding cells. Disturbance of signaling by these molecules as well as S-sulfuration and S-nitrosylation causes many neuronal diseases. This review focuses on the signaling by H2S and H2Sn with S-sulfuration compared with those of NO and S-nitrosynation, and discuss on their roles in physiology and pathophysiology.

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Abstract

Hydrogen sulfide (H_2S) together with polysulfides (H_2S_n, n>2) are signaling molecules like nitric oxide (NO) with various physiological roles including regulation of neuronal transmission, vascular tone, inflammation, oxygen sensing etc. H_2S and H_2S_n diffuse to the target proteins to S-sulfurate their cysteine residues to induce the conformational changes to alter the activity. On the other hand, 3-mercaptopyruvate sulfurtransferase transfers sulfur from a substrate 3-mercaptopyruvate to the cysteine residues of acceptor proteins. A similar mechanism has also been identified in S-nitrosylation. S-sulfuration and S-nitrosylation by enzymes proceed only inside the cell, while reactions induced by H_2S, H_2S_n and NO even extend to the surrounding cells. Disturbance of signaling by these molecules as well as S-sulfuration and S-nitrosylation causes many neuronal

diseases. This review focuses on the signaling by H_2S and H_2S_n with S-sulfuration compared with those of NO and S-nitrosynation, and discuss on their roles in physiology and pathophysiology.

Abbreviations

Aβ1-42, amyloid β-protein; AD, Alzheimer's disease; ADAM17, disintegrin and metalloproteinase domain-containing protein 17; AGEs, advanced glycation end products; APP, amyloid precursor protein; ATP, adenosine trisphosphate; BACE, β-secretase; CAT, cysteine aminotransferase; CBS, cystathionine β-synthase; CFTR, cystic fibrosys transmembrane receptor; CSE, cystathionine γ-lyase; CysSSH, cysteine persulfide; DAO, D-amino acid oxidase; DS, Down's syndrome; DTT, dithiothreitol; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxation factor; EE, ethylmalonyl encephalopathy; ETHE1, sulfur dioxygenase; $FADH_2$, flavin adenine dinucleotide; γ-GCS, γ-glutamyl cysteine synthetase; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GCL, glutamate cysteine ligase; GSH, glutathione; GSSH, GSH persulfide; HD, Huntington's disease; H_2S, hydrogen sulfide; H_2S_n, hydrogen polysulfides; HNO, nitroxyl; HSNO, thionitrous acid; HSSNO, nitrosopersulfide; Keap1, kelch ECH-associating protein 1; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; 3MP, 3-mercaptopyruvate; MPST, 3-mercaptopyruvate sulfurtransferase; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, NO synthase; Nrf2, nuclear factor erythroid 2-related factor 2; PD, Parkinson's disease; PIP2, phospholipid phosphatidylinositol (4,5)-biphosphate; PK3K, phosphoinositide 3-kinase; PKG1α, protein kinase G1α· PPI, prepulse inhibition; PS, presenilin; PTEN, tumour suppressor phosphatase and tensin homologue; ROS, reactive oxygen species; SOD, super oxide dismutase; SQR, sulfur quinon oxidoreductase; SSNO-, nitrosopersulfide; STAT3, signal transducer and activator of transcription 3; TRPA1, transient receptor potential ankyrin 1

Introduction

A diffusing factor, which is released from vascular endothelium by a stimulation with acetylcholine to relax vascular smooth muscle, was discovered and called endothelium-derived relaxation factor (EDRF) (Furchgott and Zawadzki, 1980). It was later identified as nitric oxide (NO) (Ignarro et al., 1987; Palmer et al., 1987), which activates soluble guanylate cyclase and subsequently protein kinase G through cyclic GMP (Arnold et al., 1977). In the brain an excitatory neurotransmitter glutamate had been recognized to induce cGMP (Mao et al., 1974), and the activation of N-methyl-D-aspartate (NMDA) receptors by glutamate was found to induce a release of a diffusible factor which had similar properties to EDRF in a Ca^{2+} -dependent manner (Garthwaite et al., 1988). NO producing activity was identified as an enzymatic reaction with arginine as a substrate in nicotinamide adenine dinucleotide phosphate (NADPH) and Ca^{2+}/calmoduline-dependent manner (Bredt and Snyder, 1990), and the activity is localized to neurons as well as vascular endothelial cells (Bredt et al., 1991).

Hydrogen sulfide (H_2S), which was identified in the brain (Warenycia et al., 1989; Goodwin et al., 1990; Savage and Gould, 1990), changes the levels of neurotransmitters when administered to animals (Warenycia et al., 1989), facilitates the induction of hippocampal long-term potentiation, a synaptic model of memory formation in the brain (Abe and Kimura, 1996), and relaxes vascular smooth muscle in synergy with NO (Hosoki et al., 1997; Zhao et al., 2001). H_2S is produced by three enzymes cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MPST) along with cysteine aminotransferase (CAT) or D-amino acid oxidase (DAO), from D/L-cysteine as a source of sulfur (Stipanuk and Beck, 1982; Abe and Kimura, 1996; Hosoki et al., 1997; Nagahara, 2007; Shibuya et al., 2009; Chiku et al., 2009; Mikami et al., 2011; Shibuya et al., 2013).

H_2S induces Ca^{2+} influx in astrocytes by activating transient receptor potential ankyrin 1 (TRPA1) channels, and we later found hydrogen polysulfide (H_2S_n, $n > 2$) more effectively activate the channels than H_2S does (Nagai et al., 2004; Nagai et al., 2006; Oosumi et al., 2010; Kimura et al., 2013). H_2S_2 and H_2S_3 were identified in the brain, and MPST produces H_2S_n, and other persulfurated molecules such as cysteine persulfide, glutathione persulfide, and persulfurated proteins (Kimura et al., 2015; Kimura et al., 2017; Koike et al., 2017; Nagahara et al., 2018a; Nagahara et al., 2018b; see also Kimura, 2020).

Two modes of action have been proposed for both H_2S and NO signaling. One is that both molecules diffuse to the heme for redox reaction (Vitvitsky et al., 2015; Ruetz et al., 2017) as well as to cysteine residues of the target proteins to S-sulfurate or S-nitrosylate them (Mustafa et al., 2009; Lancaster, 2017). The other is that sulfur and NO are transferred from respective enzymes to the target proteins for S-sulfuration and S-nitrosylation. An example of the first mode of action of NO is EDRF, which diffuses from endothelium to vascular smooth muscle (Furchgott and Zawadsky, 1980). In the nervous system NO diffuses from postsynapse to presynapse as a retrograde transmitter to induce a release of neurotransmitter glutamate (Garthwaite et al., 1988; Garthwaite, 1991; Zhuo et al., 1993). On the other hand, the enzyme-mediated S-nitrosylation proceeds by clusters of enzymes which generate NO, synthesize S-nitrosylated proteins, and transnitrosylate it (Seth et al., 2018).

H_2S diffuses to heme of target proteins to regulate their activity or to the target cysteine disulfide to reduce it (Aizenman et al., 1989; Abe and Kimura, 1996; Matsui et al., 2018). H_2S and H_2S_n diffuse to the targets to S-sulfurate (S-sulfhydrate) cysteine residues to modify their activity (Mustafa et al., 2009; Kimura et al., 2015). As to enzyme-mediated S-sulfuration, MPST transfers sulfur from 3MP to cysteine residues of target proteins (Nagahara et al., 2012; Kimura et al., 2017).

H_2S exerts regulatory, beneficial and protective effects at physiological concentrations, while it is toxic at higher concentrations. In Down's syndrome (DS) and ethylmalonyl encephalopathy (EE), the levels of H_2S and/or H_2S_n are increased and cause damage to the brain (Tiranti et al., 2009; Panagaki et al., 2019). For DS and EE a decrease in the levels of H_2S has been proposed to have therapeutic potential. In contrast, in Huntington's disease and Alzheimer's disease H_2S levels are not enough to properly function (Paul et al., 2014; Sbodio et al., 2016; Sbodio et al., 2018; Cao et al., 2018; Vandini et al., 2019). For these diseases a supplementation of H_2S may have a benefit. In schizophrenia both beneficial and toxic effects of H_2S and H_2S_n have been reported (Koike et al., 2016; Topcuoglu et al., 2017; Unal et al., 2018; Xiong et al., 2018; Ide et al., 2019). The balance of H_2S, H_2S_n and NO as well as S-sulfuration together with S-nitrosylation plays an important role for the pathogenesis of these neuronal diseases.

S-sulfuration by H_2S and H_2S_n and by MPST

Mustafa et al. (2009) have demonstated that S-sulfuration of cysteine residues of target proteins as a mode of action of H_2S. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is activated through S-sulfuration by H_2S (Mustafa et al., 2009). It was later reported that the activity of GAPDH is suppressed by S-sulfuration by H_2S_n rather than H_2S (Jarosz et al., 2015). S-sulfuration depends on the redox condition of the target cysteine residues; H_2S S-sulfurates oxidized cysteine like Cys-SOH or Cys-SNO, while H_2S_n S-sulfurate cysteine (Cys-SH) (Fig. 1) (Mishanina et al., 2015). It is possible that the former preparation of GAPDH (Mustafa et al., 2009) might be oxidized of which the activity may be suppressed, then H_2S S-sulfurated to activate it. In contrast, the latter preparation (Jarosz et al., 2015) might be under reducing conditions and GAPDH was active, and H_2S_n S-sulfurated it to suppress its activity.

H_2S_n activate TRPA1channels which have two sensitive cysteine residues at the amino terminus, suggesting that the two residues are S-sulfurated or one of them is S-sulfurate to react with the other to make cysteine disulfide bridge (Nagai et al., 2004; Nagai et al., 2006; Streng et al., 2008; Oosumi et al., 2010; Kimura et al., 2013; Hatakeyama et al., 2015; Kimura, 2015a). A similar mechanism was reported for the regulation of PTEN in which one cysteine residue is S-sulfurated by H_2S_n then reacts with another non-S-sulfurated one to produce a cysteine disulfide bond, leading to the conformational change (Greiner et al., 2013). Protein kinase G1α is inactive at its monomer, while it is activated by forming dimer which is generated by S-sulfuration of one cysteine residue of a monomer that reacts with a counterpart cysteine residue of another monomer to produce cysteine disulfide bridge between the two (Stubbert et al., 2014; Kimura, 2020).

MPST produces H_2S and H_2S_n as well as other persulfurated molecules (Fig. 2) (Kimura et al., 2015; Kimura et al., 2017). The endogenous levels of H_2S and H_2S_2 in the brain are 0.030 +0.004 μmol/gram protein (approximately 3.0 μM) and 0.026 + 0.002 μmol/gram protein (2.6 μM), respectively (Koike et al., 2017). Because H_2S_2 and H_2S_3 efficiently S-sulfurate cysteine and glutathione to produce cysteine persulfide

and glutathione persulfide (Kimura et al., 2017), once H_2S_n are produced, cysteine and glutathione exist nearby can immediately be S-sulfurated. Since no enzyme has been identified to mediate the reaction of H_2S with heme containing proteins such as haemoglobin, neuroglobin and catalase as well as SQR and cupper/zinc super oxide dismutase (SOD) (Vitvitsky et al, 2015; Ruetz et al., 2017; Olson et al., 2017; Searcy et al., 1995; Searcy, 1996; Olson et al., 2018), H_2S must reach these targets by diffusion.

MPST transfers sulfur from 3MP to cysteine as observed in the following. 1) MPST can produces H_2S, H_2S_n, cysteine persulfide and glutathione persulfide by transferring sulfur to cysteine (Kimura et al., 2015; Kimura et al., 2017). 2) The levels of persulfurated molecules, which were measured as H_2S released in the presence of a reducing agent dithiothreitol (DTT) called as bound (sulfane) sulfur (Warenycia et al., 1990; Ogasawara et al., 1993; Ogasawara et al., 1994; Ishigami et al., 2009; Kimura, 2015a), were greater in cells expressing MPST compared to a control (Shibuya et al., 2009). 3) Administration of a substrate cysteine to mice increased the amounts of bound sulfur in tissues expressing MPST, while there was no change in those administered saline (Shibuya et al., 2013). 4) The levels of bound sulfur were less than half in the brains of MPST knockout mice than those in the wild type mice (Kimura et al., 2017). S-sulfuration by MPST is specific but restricted inside cells, while that by diffusion extends the signaling to nearby cells.

S-nitrosylation and the interaction between H_2S and NO

For S-nitrosylation two mechanisms have been proposed. NO was initially thought only to diffuse after being produced by NO synthase (NOS) to the target proteins to S-nitrosylate them (Lancaster, 2017). However, it has been demonstrated that S-nitrosylation proceeds by clusters of enzymes which generate NO, synthesize S-nitrosylated proteins, and transnitrosylate it (Seth et al., 2018). Signaling by H_2S, H_2S_n and NO as well as S-sulfuration and S-nitrosylation must have both diffusion mediated- and enzyme oriented- mechanisms for regulating the activity of targets.

Synergistic effect of H_2S with NO on vascular relaxation led to the identification of two mechanisms (Hosoki et al., 1997). One is that the chemical interaction between H_2S and NO produces H_2S_n, nitosothiol, thionitrous acid (HSNO), and nitrosopersulfide (HSSNO) that have the relaxation effect greater than each parental molecule (Nagai et al., 2006; Whiteman et al, 2006; Oosumi et al., 2010; Filipovic et al., 2012; Ebenhardt et al, 2014; Stubbert et al., 2014; Cortese-Krott et al., 2015; Moustafa and Habara, 2016; Miyamoto et al., 2017; See also Kimura, 2020). Another mechanism is that H_2S and NO mutually regulate their synthesizing enzymes (Zhao et al., 2001; Minamishima et al., 2009; Kondo et al., 2013; King et al., 2014; Kimura, 2016). For chemical interaction between H_2S and NO, both molecules should diffuse to interact with each other.

After the identification of EDRF as NO, there appeared a discrepancy. EDRF hyperpolarizes the membrane of vascular smooth muscle, while NO has little hyperpolarizing effect. Based on this observation it has been suggested that EDRF contains an additional factor, endothelium-derived hyperpolarizing factor (EDHF) (Chen et al., 1988). Because H_2S activates K_{ATP} channels and hyperpolarizes the membrane potential, it has been proposed as a potential EDHF (Hosoki et al., 1997; Zhuo et al., 2001; Mustafa et al., 2011). Another possibility is H_2S_n, which are produced by the chemical interaction of H_2S with NO and activate protein kinase G1α (PKG1α) to relax vasculature, are also potent substances to activate K_{ATP} channels by S-sulfurating the active cysteine residue (Nagai et al., 2006; Whiteman et al, 2006; Oosumi et al., 2010; Mustafa et al., 2011; Filipovic et al., 2012; Ebenhardt et al, 2014; Stubbert et al., 2014; Cortese-Krott et al., 2015; Moustafa and Habara, 2016; Miyamoto et al., 2017; See also Kimura, 2020).

Physiological roles of H_2S, H_2S_n and NO

Neuronal Plasticity

When a neurotransmitter glutamate activates NMDA receptors at postsynapse, Ca^{2+} influx is induced and Ca^{2+}/calmodulin-dependent neuronal NOS is subsequently activated to produce NO (Garthwaite et al., 1988). NO produced at postsynapse crosses synaptic cleft to presynapse as a retrograde transmitter, which modifies a release of neurotransmitter glutamate, leading to the facilitation of hippocampal long-term potentiation (LTP) (O'Dell et al., 1991). NO also modifies LTP formation to modifies the activity of

postsynapse (Taqatqeh et al., 2009).

H_2S enhances the activity of NMDA receptors at the active synapses by reducing the cysteine disulfide bond located at the hinge of a ligand binding domain of the receptors to facilitate LTP induction (Abe and Kimura, 1996; Aizenman et al., 1989). On the other hand, H_2S_n activate TRPA1 channels in astrocytes surrounding the synapse to induce Ca^{2+}influx, which triggers a release of a gliotransmitter D-serine to the synaptic cleft to enhance the activity of NMDA receptors (Nagai et al., 2004; Nagai et al., 2006; Oosumi et al., 2010; Kimura et al., 2013; Shigetomi et al., 2013). Genetic knockdown of CBS impairs LTP, while S-sulfuration of serine racemase, which generates D-serine from L-serine, restores LTP (Li et al., 2017).

Neuroprotective role

There are two forms of glutamate toxicity; excitotoxicity is caused by the immoderate excitation of NMDA receptors which transport excessive Ca^{2+} into neurons to death (Choi, 1988). In oxidative glutamate toxicity called oxytosis, which is identical to ferroptosis, high concentrations of glutamate suppress cystine/glutamate antiporter, leading to the decreased transport of cystine into cells that causes decreased production of glutathione, an intracellular major antioxidant, resulting in making cells vulnerable to oxidative stress (Murphy et al., 1989; Tan et al., 2001; Lewerenz et al., 2018). H_2S protects embryonic neurons from oxidative glutamate toxicity (Kimura and Kimura, 2004). H_2S enhances the activity of cystine/glutamate antiporter xCT to transport cystine, which is reduced to cysteine to be used for the production of glutathione. H_2S also augments the activity of glutamate cysteine ligase (GCL), a rate limiting enzyme for glutathione production, also known as γ-glutamyl cysteine synthetase (γ-GCS) (Kimura and Kimura, 2004; Kimura et al., 2010). The activity of ATP-dependent K^+ channels and cystic fibrosys transmembrane receptor (CFTR) Cl^- channels are also activated by H_2S to suppress the excess excitation of the neurons (Kimura et al., 2006). H_2S_n S-sulfurate Kelch-like ECH-associated protein 1 (Keap1) to release Nuclear factor erythroid 2-related factor 2 (Nrf2) from Keap1/Nrf2 complex to nucleus where Nrf2 upregulates antioxidant genes, leading to the protection of neurons from oxidative stress (Koike et al., 2016).

Sulfite, which is produced by further oxidization of H_2S, also protects embryonic neurons from oxidative stress (Kimura et al,. 2018). It reacts with cystine to produce cysteine, which is more efficiently transported into cells than cystine, leading to the effective production of glutathione (Clarke, 1932). A counterpart product of this reaction is S-cysteinesulfonate, agonist of NMDA receptors (Clarke, 1932; Kumar et al., 2018). Because matured neurons express NMDA receptors, of which the activity is enhanced by H_2S and S-cysteinesulfonate (Choi, 1988; Murphy et al., 1989; Tan et al., 2001; Abe and Kimura, 1996; Kumar et al., 2018), the simultaneous application of inhibitors for NMDA receptors must be required for the protection of matured neurons.

Nagahara et al. have proposed that S-sulfuration has a role to protect proteins from oxidative stress. In subsequent oxidation of cysteine-SH to –SOH, -SO_2H, -SO_3H, only –SOH is reversible to –SH but further oxidized forms are irreversible. In contrast, the oxidation of persulfurated cysteine-SSO_2H and -SSO_3H can be converted to cysteine-SH by the reduced form of thioredoxin (Nagahara et al. 2012). These observations were reproduced and confirmed by another group (Doka et al., 2020).

NO protects neurons from excitotoxicity by decreasing the excessive influx of Ca^{2+} by suppressing the activity of NMDA receptors through S-nitrosylation (Jeffrey et al., 2001; Choi et al., 2000). NO also exerts cytoprotective effects by suppressing caspase activity through S-nitrosylation of its active site cysteine (Melino et al., 1997). Because the suppression of soluble guanylate cyclase showed a similar cell death effect to that by the deprivation of NO, cGMP may be involved in the cytoprotective effect of NO (Contestabile et al., 2004).

Mitochondrial energy formation

H_2S is well-known toxic gas at high concentrations, and its toxicity attributed to the inhibition of mitochondrial cytochrome c oxidase by suppressing the binding of oxygen (Hill et al., 1984), though its effect is not so potent compared to that of azide (Umemura and Kimura, 2007). There has been the hypothesis

that mitochondria originated from sulfide-oxidizing symbionts. Yong and Searcy (2001) demonstrated that chicken liver mitochondria consumed oxygen at an accelerated rate when supplied with low concentrations of H_2S, and H_2S oxidation is coupled to adenosine trisphosphate (ATP) generation. ATP synthesis requires less than 5 μM H_2S, and maximum respiration is induced at 10 μM and less efficient up to 60 μM (Yong and Searcy, 2001).

The balance between as the electron donor and the inhibitor of cytochrome c oxidase is likely controlled by H_2S and oxygen availability. Low concentrations of H_2S preserve the respiratory rate, while high concentrations inhibit it (Abou-Hamdan et al., 2016). Because sulfide oxidation requires three times more oxygen than that of nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide ($FADH_2$), sulfide may be a poor energy substrate (Lagoutte et al., 2010). However, Szabo et al. suggested that this low energy yield is balanced by unique properties of H_2S. 1) H_2S freely diffuses across membrane without the need of transporters, and 2) the affinity of H_2S to sulfide oxidation unit including SQR is high and 100% is oxidized (Szabo et al., 2014). In the brain the expression of SQR is very low (Linden et al., 2012), it is predicted that a haemoprotein neuroglobin, which is primarily expressed in neurons, plays a role in H_2S oxidation (Ruetz et al., 2017).

Pathophysiological role of H_2S, H_2S_n and NO

The overproduction of H_2S suppresses cytochrome c oxidase that is likely to be involved in the pathogenesis of Down's syndrome and ethylmalonic encephalopathy (Marechal et al., 2019; Panagaki et al., 2019; Tiranti et al., 2009; Fernandez Cardoso et al., 2017). In contrast, the lack of H_2S production may cause the pathology of Parkinson's, Huntington's, and Alzheimer's diseases (Chung et al., 2004; Vandiver et al., 2013; Xie et al., 2013; Paul et al., 2014; Sbodio et al., 2016; Wright et al., 2016; He et al., 2016; Cao et al., 2018; Sbodio et al., 2018). The detrimental effects caused by the excess production of H_2S and H_2S_n (Ide et al., 2019) as well as those by lacking in the neuroprotective effect of these molecules (Xiong et al., 2018; Topcuoglu et al., 2017; Unal et al., 2018; Koike et al., 2016) have been reported for the pathogenesis of schizophrenia.

1. **Toxicity of H_2S and H_2S_n**
2. Down's syndrome

Down's syndrome (DS) is characterized by impaired brain growth and maturation, which causes mental retardation, has a trisomy of chromosome 21. CBS is encoded on chromosome 21 (21q22.3) and the expression of CBS mRNA is 12 times greater in myeloblasts of DS children than those of normal individuals (Taub et al., 1999). We found that CBS protein levels in DS brains are approximately 3 times greater than those in the normal brains that is twice greater than those expected from the trisomy (Ichinohe et al., 2005). In addition, elderly adults of DS are associated with an Alzheimer's type of dementia where CBS is localized to astrocytes that surround senile plaques in the brain (Ichinohe et al., 2005). The greater levels of thiosulfate, a metabolite of H_2S, were detected in urine of DS patients compared to that of normal individuals (Kamoun et al., 2003). The increased production of H_2S by overexpressed CBS may be the cause of the neurological impairments in DS patients (Fig. 4). In a mouse model of DS, three copies of CBS gene are necessary to cause the DS-related recognition memory deficit (Marechal et al., 2019). Panagaki et al (2019) showed that the levels of CBS and H_2S are markedly elevated in DS fibroblast cells, and the mitochondrial electron transport, oxygen consumption and ATP generation are profoundly suppressed. They suggested the therapeutic potential of CBS inhibitors.

Ethylmalonyl encephalopathy

Ethylmalonyl encephalopathy (EE) is an autosomal recessive early-onset and defective in cytochrome c oxidase in muscle and brain and excrete ethylmalonic acid in urine. In this disease ETHE1, a gene encoding a beta-lactamase-like iron-coordinating metalloprotein or sulfur dioxygenase, is deficient. A great amount of thiosulfate, a metabolite of H_2S, is excreted in urine of ETHE1 knockout mice and patients of this disease (Tiranti et al., 2009). H_2S is mainly metabolized by mitochondrial enzymes, SQR, sulfur dioxygenase, and sulfur transferase. Deficiency of sulfur dioxygenase (ETHE1) increases in the brain and skeletal muscle the basal levels of H_2S which suppress the cytochrome c oxidase that may lead to progressive neurological

failure (Tiranti et al., 2009) (Fig. 4). In addition, acyl-protein thioesterase, which hydrolyses fatty acids bound to cysteine, and glutathione transferases are suppressed in ETHE1 knockout mice probably due to the increased levels of H_2S or persulfides (Hildebrandt et al., 2013). The administration of metronidazole, an antibiotic, or N-acetylcysteine prolonged the lifespan of ETHE1 knockout mice and marked clinical improvement in patients with EE (Viscomi et al., 2010). Metronidazole may be expected to decrease the levels of H_2S incorporated into blood from intestinal bacteria. N-acetylcystein is metabolized in cells to cysteine, which is a precursor of gluthatione being predicted to buffer H_2S, while it is also a substrate to produce H_2S. In this instance the former may be a dominant mechanism of N-acetylcysteine for the improvement of the disease. H_2S decreased the activities of citrate synthase, aconitase and creatine kinase in the brain of mouse model of EE (Fernandez Cardoso et al., 2017). H_2S also suppressed mitochondrial respiration, decreased mitochondrial membrane potential, and induced swelling caused by calcium in brain mitochondria. Changes in mitochondrial membrane potential and the swelling caused by H_2S may be due to opening of mitochondrial permeability transition pore (Fernandez Cardoso et al., 2017). Bioenergetics disturbance, lipid peroxidation, and mitochondrial permeability transition pore opening mediated by H_2S may be involved in the pathophysiology of brain damage observed in this disease (Fernandez Cardoso et al., 2017).

1. **H_2S and $H_2S_n$are beneficial**
2. Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder mainly causing motor dysfunction. Parkin and α-synuclein are associated with pathophysiology of PD. Alpha-synuclein is a major component of Lewy bodies associated with rare cases of PD, while parkin, which is a E3 ubiquitin ligase that ubiquitinates diverse substrates and responsible for the clearance of misfolded proteins including α-synuclein, and its mutations cause autosomal recessive PD (Choi et al., 2001; Jesko et al., 2019). Parkin is S-nitrosylated and suppressed its activity and thereby decreases its protective role in the brains of PD patients (Chung et al., 2004). In contrast, in the brains of normal individuals it is active and S-sulfurated. Cys95, Cys 59 and Cys182 are S-sulfurated in normal individuals, while the same cysteine residues are S-nitrosylated in PD brains (Vandiver et al., 2013) (Fig. 5). H_2S may be involved in the conversion of S-nitrosylated cysteine to S-sulfurated one in parkin to improve its function (Yao et al., 2004; Chung et al., 2004; Vandiver et al., 2013). Systemic administration of sodium salt of H_2S, NaHS, dramatically reversed the progression of movement dysfunction and loss of dopaminergic neurons in the PD model rats (Hu et al., 2010). A similar effect was observed with a H_2S-releasing L-dopa derivative compound (Xie et al., 2013). Overexpression of CBS increases the endogenous levels of H_2S, reversed the behavior induced in PD model rats, decreased apoptotic neuronal loss of the nigral dopaminergic neurons (Xie et al., 2013). The application of H_2S and the regulation of CBS activity have a therapeutic potential (Yin et al., 2017).

Huntington's disease

Huntington's disease (HD) is a devastating neurodegenerative disorder characterized by the progressive development of involuntary movements, neuropsychiatric symptoms and cognitive impairment (Cepeda and Tong, 2018). HD belongs to triplet repeat diseases, in which elongated polyglutamine stretches affect the protein product, and the mutant huntingtin disrupts a number of vital cellular processes, including neuronal transmission, metabolism and gene transcription (Chaganti et al., 2017). Although the levels of CSE are very low in the brain compared to other tissues (Ishii et al., 2004), those are even much lower in mouse models of HD and human HD brain compared to a control mice and normal individuals, respectively (Paul et al., 2014). It is caused by the suppression of the transcription factor specificity protein 1 (SP1), which regulates the transcription of CSE, by mutant huntingtin (Ishii et al., 2004; Paul et al., 2014) (Fig. 5). The expression of CSE is also regulated by transcription factor 4 (ATF4), which is dysfunctional in HD, under ER stress or amino acid deficiency (Sbodio et al., 2016). Striatal cell lines derived from HD model mice have decreased levels of cystine/glutamate antiporter xCT mRNA and protein expression, leading to the lower basal levels of GSH and higher basal levels of reactive oxygen species (ROS) (Wright et al., 2016) (Fig.5). Administration of N-acetylcysteine ameliorates HD pathology in this model.

Alzheimer's disease

Alzheimer's disease (AD) is a chronic neurodegenerative disease that affects cognitive functions and memory formation. The cause of the disease is mostly sporadic with much less familial. In familial cases of AD the mutations in genes encoding amyloid precursor protein (APP), presenilin (PS) 1 and 2, which constitute the catalytic subunits of γ-secretase, have been identified (Selkoe and Hardy, 2016). In the process of metabolism in normal individual, APP is digested by α-secretase following by γ-secretase, leading to the product which is easily eliminated, while in AD brain the metabolism by β-secretase (BACE) and γ-secretase generates amyloid β-protein (Aβ1-42), which aggregates and exerts neurotoxicity. Phosphorylated tau protein also causes deposition as neurofibrillary tangles (Reddy and Oliver, 2019). Neuropolypeptide h3, also known as hippocampal cholinergic neurostimulating peptide, upregulates the levels of choline acetyltransferase whose activity is decreased in patients with AD in cholinergic neurons (Reed et al., 2009; Ojika et al., 1998). Nitration of tyrosine in this peptide decreases its neurotropic activity on cholinergic neurons and may lead to the decline in cognitive function (Reed et al., 2009). The levels of BACE1, PS1 and pp38 mitogen-activated protein kinase (MAPK) are increased while those disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) are decreased in the APP/PS1 transgenic mice (He et al., 2016). The administration of NaHS into the transgenic mice restores the changes in these factors characterized in AD, suggesting that H_2S inhibits the expression of BACE1 and PS1 by activating phosphoinositide 3-kinase (PI3K)/Akt pathway in AD (He et al., 2016). H_2S inhibited exogenous ATP-induced inflammatory responses through reducing pro-inflammatory cytokines, ROS, and activation of NF-kB pathway. H_2S also suppressed the production of Aβ1-42, which was induced by exogenous ATP probably due to the augmented production of amyloid precursor protein and the activation of β- and γ-secretase (Cao et al., 2018) (Fig. 5). As a mechanism for H_2S reducing inflammation and the production of Aβ1-42, they suggested that H_2S suppresses the activities of signal transducer and activator of transcription 3 (STAT3) by inhibiting ATP-induced phosphorylation and decreases the activity of cathepsin S through S-sulfuration of this enzyme (Cao et al., 2018) (Fig. 5).

H_2S and $H_2S_n$are beneficial or toxic

3-1) Schizophrenia

Schizophrenia is a chronic and severe mental disorder that affects a person's thinking, feeling and behaviors. Symptoms, which typically come on gradually and begin in young adulthood, fall into three categories; positive symptoms (hallucinations, delusions, etc.), negative symptoms (flat affect, reduced feeling, etc.) and cognitive symptoms (poor executive function, trouble focusing, etc.) (American Psychiatric Association, 2013). Although the excessive NO production has been shown to be involved in the pathology of this disease (Pitsikas, 2016), the potential beneficial effects have been reported both NO donors and inhibitors on schizophrenia symptoms induced by amphetamine such as prepulse inhibition disruption and hyperlocomotion (Issy et al., 2018), suggesting the deviation of both decrease and increase of NO from the normal levels may be involved in the pathology of this disease. Both excess and deficiency of H_2S and H_2S_n have also been proposed to be involved in the pathogenesis of schizophrenia. Plasma H_2S levels were significantly lower in patients with schizophrenia relative to healthy control subjects, and a positive association was observed between plasma H_2S levels and working memory, visual memory or executive function in patients, suggesting that decreased H_2S is involved in the psychopathology and cognitive deficits of this disease (Xiong et al., 2018) (Fig. 5). Untreated schizophrenia patients had significantly higher (disulfide/total thiol) and (disulfide/free thiol) ratio in blood and a significantly lower (free thiol/total thiol) ratio compared to those of healthy individuals (Fig. 5). Thiol homeostasis is disturbed by a shift to the disulfide bond formation (oxidized) in patients (Topcuoglu et al., 2017). Similar results were also obtained in schizophrenia patients using medication (Unal et al., 2018). Methylglyoxal, a highly reactive dicarbonyl compound, is a major precursor for advanced glycation end products (AGEs), of which the production is associated with various neurological disorders including schizophrenia (Toyoshima et al., 2019; Ohnishi et al., 2019). H_2S_n protect differentiated human neuroblastoma SH-SY5Y cells from methylglyoxal-induced cytotoxicity, suggesting that H_2S_n scavenge methylglyoxal and suppress the accumulation of AGEs and incidents induced by carbonyl stress (Koike et al., 2013; Koike et al., 2016). Excess production of H_2S and H_2S_n has also been proposed

to be involved in the pathogenesis of schizophrenia. Yoshikawa and colleagues found that C57BL/6N (B6) strain mice exhibited greater scores of prepulse inhibition (PPI), which is the normal suppression of a startle response, than C3H/HeN (C3H) mice did (Watanabe et al., 2007). The impaired PPI is regarded as an endophenotype for schizophrenia (Braff et al., 2001). By proteomics analysis the same group found that the expression of MPST is increased in C3H mice compared to B6 (Ide et al., 2019). DNA methylation levels at MPST gene was highly enhanced in C3H mice, and the mean methylation levels of the sites were positively correlated with the expression levels of MPST. MPST levels in schizophrenia were positively correlated with symptom severity scores. In MPST -transgenic mice the expression of genes for energy formation was decreased and mitochondrial energy metabolism was impaired (Ide et al., 2019) (Fig. 4). Maternal immune activation model, which is induced by the injection of polyriboinosinic-polyribocytidilic acid to mother, shows perturbed early neural development via inflammation and oxidative insults (Meyer and Feldon, 2012; Giovanoli et al., 2013; Bundo et al., 2014). In this model the expression of MPST and other inflammatory and oxidative genes were elevated in the brain when pups grow to the adult (Ide et al., 2019). In human brains, catalase gene product was upregulated in schizophrenia samples compared to the controls (Ide et al., 2019). Inflammatory/oxidative insults in early brain development induce upregulation of H_2S/polysulfides production excessively as an antioxidative response that suppresses cytocrhome c oxidase, leading to schizophrenia.**Perspective**Neurotransmitters, cytokines, and hormones diffuse to reach their targets to react with their receptors. An advantage of diffusion is that many targets localized in the area within its reach can be activated. After transmitting the signal, they are properly eliminated. For example, neurotransmitters are released from presynapse and diffuse the synaptic cleft to the receptors at postsynapse to exert their effects, and for cessation of responses the transmitters are recovered by uptake or degraded by enzymes to clear the synaptic cleft. NO barely dissolve in water (5.6 mg/100 ml at 20°C), while H_2S does well (413 mg/100 ml at 20°C) (Kimura, 2015b). H_2S dissociates to H^+ and HS^- ($pK_1 = 7.04$), and further to S^{2-} ($pK_2 = 11.96$). Because H_2S is also lipophilic, it readily passes through plasma membrane. These characteristics of H_2S have an advantage to transmit signals across the membrane by diffusion. HS^- channels or transporters, which have been identified in both bacteria and mammals (Czyzwski and Wang, 2012; Jennings, 2013), enable H_2S to pass through plasma membrane even more efficiently and selectively. H_2S_n, which can pass through the plasma membrane, diffuse to the targets on nearby cells (Nagai et al., 2006; Oosumi et al., 2010; Kimura et al., 2013; Greiner et al., 2013; Kimura et al., 2017). For example, H_2S_nactivate TRPA1 channels by passing through the plasma membrane to S-sulfurate the amino terminus located in the cytoplasm (Nagai et al., 2006; Oosumi et al., 2010; Kimura et al., 2013). Although 23 out of 31 cysteine residues are localized to the amino-terminus (Wang et al., 2012), only Cys422 and Cys634 are responsible for regulating the activity of TRPA1 channels (Hatakeyama et al, 2015; Kimura, 2015a). The remaining 21 cysteine residues were not involved in the activation of channels whether or not they are S-sulfurated. Signaling by diffusion may be less specific to target proteins than that by enzymatic modification. In this case, however, S-sulfuration of the two specific cysteine residues by sulfur transferases, if any, may not have any advantage on specificity to activate the channels compared to the diffusion of H_2S_n. S-sulfuration is compared to phosphorylation, in which kinases incorporate phosphate to residues of serine, threonine and tyrosine (Kimura, 2020). H_2S and H_2S_n S-sulfurate cysteine residues even in the other cells but not specific ones. In contrast, MPST transfers sulfur from 3MP to specific cysteine residues, but the reaction is restricted only inside the cells (Shibuya et al., 2009; Shibuya et al., 2013; Kimura et al., 2017). H_2S and H_2S_n endogenously exist (Kimura et al,, 2015; Koike et al., 2017), and both their diffusion and enzyme mediated mechanisms play a role in S-sulfuration under physiological conditions. Bacteria use H_2S produced by MPST to protect themselves from antibiotics (Shatalin et al., 2011). Because bacteria have channels specific to HS^- (Czyzewski and Wang, 2012), signaling by H_2S with other cells must have emerged in the early history of life on earth. Mammalian have developed HS^-/Cl^- transporter on erythrocytes to rapidly and selectively exchange HS^- with the extracellular milieu (Jennings, 2013). Patients exposed to high concentrations of H_2S are suffered from cognitive disability (Reiffenstain et al., 1992), and the levels of neurotransmitters in the brain were affected in animals exposed to H_2S (Warenycia et al., 1989). The expression of SQR, which is the first step for the metabolism of H_2S, is very low in the brain (Linden et al., 2012). These observations suggest the vulnerability of the brain to excessive H_2S. In DS, EE, and schizophrenia, the

high levels of H_2S or its producing enzymes exert a toxic effect on cytochrome c oxidase, leading to neuronal dysfunction (Taub et al., 1999; Ichinohe et al., 2004; Panagaki et al., 2019; Tiranti et al., 2009; Viscomi et al., 2010; Linden et al., 2012; Ide et al., 2019). In addition, high concentrations of H_2S metabolized by SQR must generate the toxic levels of polysulfides on neurons. The inhibitors of H_2S producing enzymes, which may also decrease the levels of polysluflde, have suggested to have therapeutic potential in these diseases. The accurate comparisons of the levels of H_2S and polysulfides between patients and normal individuals are required to examine the efficiency of these inhibitors for therapeutic uses. S-sulfuration makes parkin active in the brains of normal individuals, while S-nitrosylation inactivates it in those of PD patients (Paul et al., 2014). It is intriguing to know whether or not exogenously applied H_2S or the enhancement of H_2S producing enzymes is able to convert S-nitrosylated cysteine to S-sulfurated one. The difference in the levels of H_2S between patients and normal individuals is not well understood in PD. The concentrations of polysulfides, which must be accompanied by the changes of H_2S levels, should also be clarified. Controlling the levels of these signaling molecules and the activities of enzymes related to S-nitrosylation and S-sulfuration may have a therapeutic benefit for diseases in the central nervous system.

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Conflict of Interest

The author declared no conflicts of interest.

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Figure legends

Fig.1. H_2S_n can transmit signals across cells, while S-sulfuration by MPST is restricted only inside the cells.

1. H_2S and H_2S_n can pass through plasma membrane of the cell in which they are produced and reach the nearby cells as well as react with the targets inside the produced cell. H_2S S-sulfurates the S-nitrosylated cysteine residues, while H_2S_nS-sulfurate the cysteine residues.
2. MPST transfers sulfur from 3MP to targets inside the cell.

Fig. 2. S-sulfuration by H_2S_n diffusion and that by MPST.

1. MPST produces H_2S_n which reach by diffusion to S-sulfurate H_2S, cysteine, glutathione, and cysteine residues of proteins.
2. MPST sulfur transfers from 3MP to H_2S, cysteine, glutathione, and cysteine residues of proteins without being mediated by H_2S_n.

This figure is produced by modifying Kimura et al., 2017.

Fig. 3. H_2S is used for ATP production at physiological concentrations, while it inhibits cytochrome c oxidase at higher concentrations in mitochondria. H_2S is metabolized by SQR, ETHE1, and rhodanese (TST) to thiosulfate through sulfite. Electrons are sent to coenzyme Q to complex IV though III and used for pumping out H^+ from matrix to intermembrane space. ATP synthase produces ATP using the gradient of H^+. In contrast, high concentrations of H_2S suppress cytochrome c oxidase and the energy formation.

Fig. 4 Diseases caused by an excess amount of H_2S and H_2S_n in the central nervous system

A trisomy of choromosome 21, on which CBS is encoded, increases the levels of CBS in Down's syndrome. Sulfur dioxygenase, which is encoded in ETHE1 and one of the enzymes metabolizing H_2S and H_2S_n, is defective in ethylmalonyl encephalopathy. Methylation of MPST gene increases the production of MPST in schizophrenia. Glycolysis is decreased by the suppression of several enzymes such as triosephosphate isomerase, phosphoglycerate kinase, phosphopyruvate hydratase. In these diseases cytochrome c oxidase is suppressed by high concentrations of H_2S, resulting in the decreased production of ATP.

Fig. 5. Diseases caused by a lack of H_2S or H_2Sn in the central nervous system

S-sulfurated parkin in normal individuals is active, while parkin with the same cysteine residues being S-nitrosylated in PD brains is inactive. Mutant huntingtin suppresses the transcription of CSE gene from SP1, resulting in the decrease in the production of H_2S. In HD animal model the activity of cystine/glutamate antiporter xCT is suppressed and glutathione levels are decreased, while those of ROS increased. The animal model of Alzheimer disease shows that H_2S suppresses PI3K/Akt, while enhances the activity of STAT3. Both effects result in the decreased production of Aβ1-42 and suppression of inflammation. In some report the plasma levels of H_2S are lower in patients of schizophrenia than normal individuals.