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Hydrogen Sulfide as a Neuromodulator

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Abstract

Hydrogen sulfide (H_2S) is a well-known toxic gas with the smell of rotten eggs. Since the first description of the toxicity of H_2S in 1713, most studies about H_2S have been devoted to its toxic effects. Recently, H_2S has been proposed as a physiologically active messenger. Three groups discovered that the brain contains relatively high concentrations of endogenous H_2S. This discovery accelerated the identification of an H_2S-producing enzyme, cystathionine β-synthase (CBS) in the brain. In addition to the well-known regulators for CBS, *S*-adenosyl-L-methionine (SAM) and pyridoxal-5′-phosphate, it was recently found that Ca^{2+}/calmodulin-mediated pathways are involved in the regulation of CBS activity. H_2S is produced in response to neuronal excitation, and alters hippocampal long-term potentiation (LTP), a synaptic model for memory. can also regulate the release of corticotropin-releasing hormone (CRH) from hypothalamus. Another H_2S producing enzyme, cystathionine γ-lyase (CSE), has been identified in smooth muscle, and H_2S relaxes smooth muscle in synergy with nitric oxide (NO). Recent progress in the study of H_2S as a novel neuromodulator/transmitter in the brain is briefly reviewed.

Index Entries: Hydrogen sulfide; neuromodulator; neurotransmitter; smooth muscle relaxant; cystathionine β-synthase; cystathionine γ-flyase; calmodulin.

Introduction

Since the first description of H_2S toxicity in 1713 *(1)*, most studies about H_2S have been devoted to its toxic effects with little attention paid to its physiological function *(2)*. Warenycia et al. found that the rat brain contains endogenous H_2S *(3)*, and endogenous concentrations of H_2S have also been measured in human and bovine brain *(4,5)*. The relatively high concentrations of H_2S in the brain (50–160 μ*M*) suggest that it has a physiological function.

Endogenous H_2S in the brain is formed from L-cysteine by a pyridoxal-5′-phosphate-dependent enzyme, cystathionine β-synthase (CBS) *(6–11)*. CBS inhibitors, hydroxylamine

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Table 1
Characteristics of H_2S-producing Enzymes

	Cystathionine β-synthase (CBS)	Cysthathionine γ-lyase (CSE)	References
Tissue localization	Brain, liver, kidney, ileum.	Thoracic aorta, ileum, portal vein, liver, kidney.	*(10,11,44,49)*
Activators	Pyridoxal 5′-phosphate *S*-adenosyl-L-methionine Ca^{2+}/calmodulin	Pyridoxal 5′-phosphate	*(6–11,26)*
Inhibitors	Hydroxylamine Amino-oxyacetate	D,L-propargylglycine β-cyano-L-alanine	*(6,10,44)*
Functional roles	H_2S production in the brain	H_2S production in smooth muscle	*(10,11,44,49)*

and amino-oxyacetate, suppress H_2S production, while a CBS activator, *S*-adenosyl-L-methionine (SAM), enhances it. Observations with CBS knockout mice clearly show that CBS is the only enzyme that produces H_2S in the brain *(11).*

Two other gases, nitric oxide (NO) and carbon monoxide (CO), are endogenously produced by enzymes localized to the brain. NO is synthesized by NO synthase via the metabolism of arginine to citrulline *(12,13),* and CO is produced by heme oxygenase via the metabolism of heme to biliverdin *(14,15).* Both NO and CO enhance the induction of hippocampal long-term potentiation (LTP), a synaptic model of learning and memory *(16–22).* The activities of NO synthase are regulated by Ca^{2+}/calmodulin, and NO is released when N-methyl-D-aspartate (NMDA) receptors are activated by L-glutamate *(23,24).* In contrast, the regulation of CO production by neuronal excitation is not understood *(22).*

H_2S production in the brain is enhanced in response to neuronal excitation via the Ca^{2+} and calmodulin-mediated pathways *(11).* In addition, physiological concentrations of H_2S specifically potentiate the activity of NMDA receptors, and hippocampal LTP is altered in CBS knockout mice *(10,11).* H_2S can also regulate the release of corticotropin-releasing hormone from the hypothalamus *(25).* Based on these observations, it has been proposed that H_2S may function as a neuromodulator or transmitter in the brain *(10,11).* The following paragraphs outline the identification of an H_2S-producing enzyme, CBS, in the brain and its regulation in response to neuronal excitation. The possible involvement of H_2S in disease is also discussed.

H_2S-Producing Enzyme

The discovery of endogenous H_2S in the brain prompted us to identify the enzyme that produces H_2S. H_2S can be formed from cysteine by pyridoxal-5′-phosphate-dependent enzymes, including CBS and CSE *(6–9).* Both CBS and CSE have been intensively studied in the liver and kidney, but little was known about them in the brain. CBS has recently been identified as a major H_2S producing enzyme in the brain by the following observations: 1) CBS mRNA is highly expressed in the brain, especially in the hippocampus, while CSE mRNA is not detectable *(10);* 2) the production of H_2S from brain homogenates is suppressed by CBS specific inhibitors hydroxylamine and aminooxyacetate, while it is not suppressed by CSE specific inhibitors D,L-propargylglycine and β-cyano-L-alanine; 3) a CBS activator, *S*-adenocyl-L-methionine (SAM), enhances the production of brain H_2S *(10);* 4) endogenous H_2S is under detectable levels in the brains of CBS knockout mice *(11).*

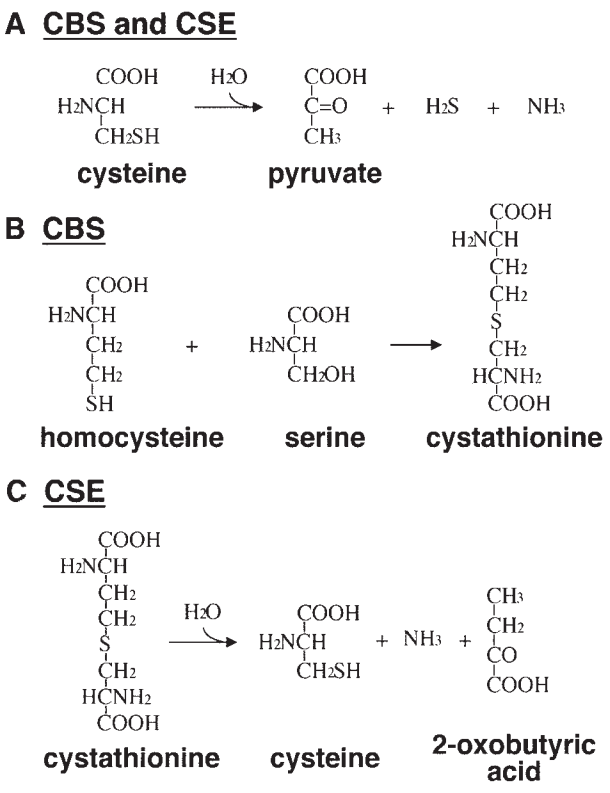

Fig. 1. Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) catalyze two metabolic reactions. **(A)** is a common reaction for CBS and CSE to produce H_2S. **(B)** is the specific reaction for CBS and C for CSE (6–9).

Regulation of H_2S Production

CBS is dependent on pyridoxal 5′-phosphate and heme, and its activity is enhanced by SAM *(26,27).* No other regulators for this enzyme had been found. We have, however, recently shown that CBS activity is mediated by Ca^{2+} and calmodulin *(11).* CBS activity is suppressed by calmodulin-specific inhibitors, W13 and trifluoroperazine, and CBS and calmodulin coimmunoprecipitate. The calmodulin-binding consensus sequence has also been identified in CBS *(11,28).* The enzymatic activity of CBS has two metabolic outcomes *(6,29;* Fig. 1). Most studies have been devoted to a pathway in which CBS catalyzes the reaction with substrate homocysteine to produce cystathionine *(29),* but little attention has been paid to another pathway in which CBS produces H_2S from L-cysteine as a substrate (*6,10,11;* Fig. 1A). SAM regulates CBS activity in both metabolic pathways *(10,11,26),* and a model for CBS regulation by SAM has been proposed *(30).* A similar mechanism may also function in the regulation of CBS by Ca^{2+}/calmodulin (*11;* Fig. 2). In the absence of Ca^{2+}/calmodulin the carboxy-terminal domain may cover the catalytic domain, and CBS activity remains at a basal level. When Ca^{2+}/calmodulin binds to the 19 amino acid calmodulin binding consensus sequence, the catalytic domain is exposed by opening of the carboxy-terminal domain and CBS becomes active (Fig. 2). This model is supported by the observation that the CBS mutant (1–396), which is deficient in the 19 amino acid Ca^{2+}/calmodulin binding sequence, is constantly active even in the absence of Ca^{2+}/calmodulin (*11;* Fig. 2).

When neuronal cells are excited by an excitatory neurotransmitter glutamate, as well as electrical stimulation, H_2S production is increased (*11;* Fig. 3). H_2S production is not simply increased linearly in response to neuronal excitation, for there is a regulatory mechanism to maintain the production of H_2S levels within nontoxic levels. For example, longer stimulation by glutamate or electrical activity, and greater concentrations of the Ca^{2+} ionophore A23187, do not effectively increase H_2S production *(11).*

Regulation of Synaptic Activity by H_2S

What is the function of H_2S in the brain? Physiological concentrations of H_2S modify LTP, and LTP is altered in the brains of CBS knockout mice *(10,11).* In contrast, concentrations of H_2S greater than the physiological basal level specifically suppress excitatory postsynaptic potentials (EPSPs) *(10).* This suppression was initially thought to be due to the toxic effect of H_2S. However, H_2S production can be locally and transiently increased in response to neuronal excitation, and the suppression of EPSPs still occurs *(10,11).*

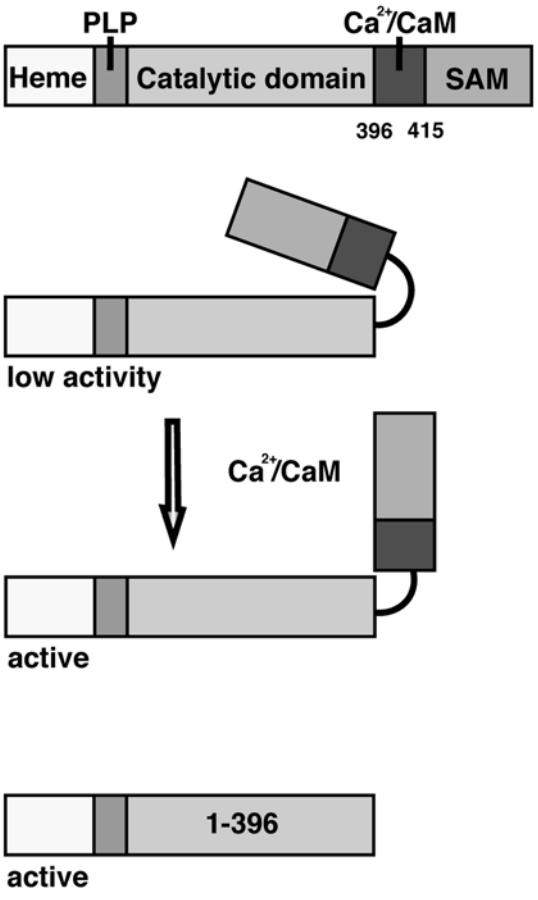

Fig. 2. The regulation of CBS activity by Ca^{2+} and calmodulin. CBS consists of domains for heme binding (Heme), pyridoxal-5′-phosphate binding (PLP), catalytic, Ca^{2+} and calmodulin binding (Ca^{2+}/CaM) and *S*-adenosyl-L-methionine binding (SAM). A model for CBS regulation by SAM has been proposed *(30).* A similar mechanism may also function in the regulation of CBS by Ca^{2+} calmodulin. In the absence of Ca^{2+}/calmodulin the carboxyl-tenninal domain may cover the catalytic domain, and CBS activity remains at a basal level. When Ca^{2+}/calmodulin binds to the 19 amino acid sequence (amino acids 396–415), the catalytic domain is exposed by opening of the carboxy-teiminal domain and CBS becomes active. This model is supported by our observation that the CBS mutant (1–396), which is deficient in the 19 amino acid Ca^{2+}/calmodulin binding sequence, is constantly active even in the absence of Ca^{2+}/calmodulin *(11).*

Because H_2S regulates the release of corticotropin-releasing hormone (CRH) from hypothalamus *(25,31),* it is possible that H_2S may modify the release of neurotransmitters. Therefore the effect of H_2S on glutamate receptor activation was examined, and it was shown that physiological concentrations of H_2S specifically enhance NMDA receptor-mediated responses *(10).* This modification of NMDA receptor activity by H_2S may not be the direct effect of H_2S, but may partly be due to the activation of cAMP pathways by H_2S *(32).* The NMDA receptor subunits have specific sites directly phosphorylated by protein kinase A, and H_2S may activate this pathway *(33,34).* It is not clear at present whether or not there is an effect of H_2S on glutamate release.

Involvement of H_2S in Diseases of the Nervous System

There is a good amount of data that suggests that defects in H_2S metabolism may be involved in CNS disease. The CBS gene is encoded on chromosome 21q22.3 *(35,36),* a region associated with Down syndrome *(37,38),* and it has been proposed that H_2S may be involved in the cognitive dysfunction associated with Down syndrome *(39).* Loss of CBS activity causes homocystinurea, an autosomal recessive disease characterized, in part, by mental retardation *(29).* CBS interacts with Huntingtin, mutants of which cause Huntington's disease *(40).* Finally polymorphisms of CBS gene is significantly underrepresented in children with high IQ compared with those with average IQ, suggesting that CBS activity may be involved in the cognitive function *(41).* These observations in conjunction with the findings described earlier suggest that CBS and its product H_2S may regulate some aspects of synaptic activity and modify cognitive function.

Recent studies have shown that abnormalities in the cerebral microvasculature are relevant to the cause of dementia, including Alzheimer's disease *(42,43).* Although CBS is the major, if not exclusive, enzyme-producing

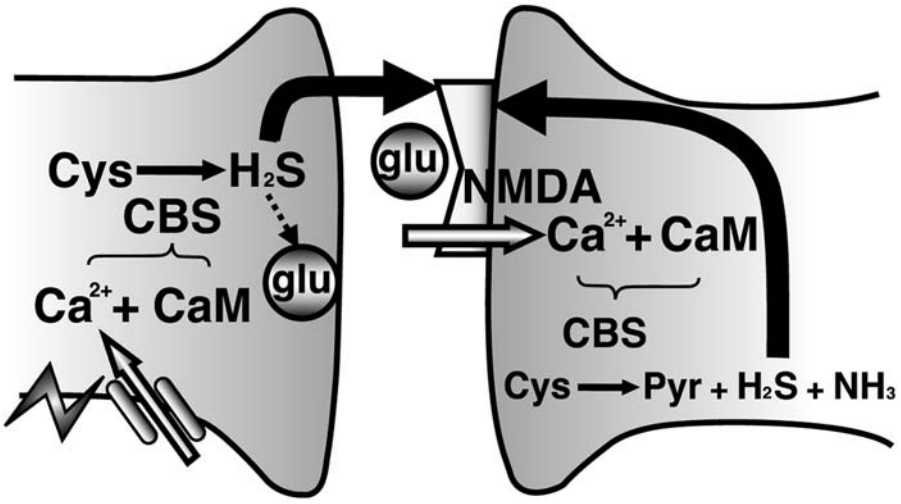

Fig. 3. Production and function of H_2S in the central nervous system. When the electrical signals descend to the axon terminal, Ca^{2+} enters into the nerve terminal and interacts with calmodulin. The Ca^{2+}/calmodulin activates CBS to produce H_2S. H_2S can pass through the membrane and reach the postsynaptic membrane to modify the activity of the NMDA receptor, allowing greater Ca^{2+} influx. H_2S also can modulate the release of transmitters and hormones *(10,25,31).* When the NMDA receptor is activated, Ca^{2+} enters through NMDA receptors and Ca^{2+}/calmodulin activates CBS to produce H_2S. H_2S can regulate NMDA receptor activity and modulate the induction of LTP *(10,11).*

H_2S in the brain, another H_2S-producing enzyme, CSE, was identified as the major H_2S-producing enzyme in the smooth muscle *(44).* Although exogenously applied H_2S alone relaxes smooth muscle, much lower concentrations of H_2S greatly enhance the smooth muscle relaxation induced by NO *(44).* H_2S also hyperpolarizes smooth muscle by activating K_{ATP} channels. *(45).* Based on these observations, it is likely that H_2S may also regulate cerebral blood flow.

Conclusions

The physiological relevance of two H_2S-producing enzymes, CBS and CSE has been identified, and the regulation of their activities has also been determined. A novel finding that H_2S production by CBS is regulated by Ca^{2+} and calmodulin lead to the observation that H_2S is produced in response to neuronal excitation. Exogenously applied H_2S modifies LTP, and LTP is altered in the brain of CBS knockout mice. H_2S can regulate some aspects of synaptic activity and modify cognitive function. In smooth muscle, H_2S enhances NO induced relaxation and can regulate the activity of CSE. Both gaseous smooth muscle relaxants strongly interact with each other.

A few candidates of molecular targets for H_2S have been identified. These are NMDA receptors in the brain and the K_{ATP} channel in smooth muscle *(10,32,45).* The mechanism of the activation of these targets has not been solved, and it is not known if it is a direct or indirect effect. Because H_2S is a very active molecule, more targets are expected to be found. After H_2S stimulates its targets, it has to be cleared from its site of action. The mechanism of clearance is not understood. The study of H_2S as a physiologically active molecule has just beginning, but understanding the mechanisms underlying its physiological function may provide a new insights into neurotransmission.

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