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Invited Review

The physiological role of hydrogen sulfide and beyond

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ABSTRACT

Hydrogen sulfide (H_2S) has been considered to be a physiological mediator since the identification of endogenous sulfides in the mammalian brain. H_2S is produced from L-cysteine by enzymes such as cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), 3-mercaptopyruvate sulfurtransferase (3MST), and cysteine aminotransferase (CAT). CSE and CAT are regulated by Ca^{2+}. At steady-state low intracellular concentrations of Ca^{2+}, CSE and the 3MST/CAT pathway produce H_2S. However, after intracellular concentrations of Ca^{2+} increase in stimulated cells, the production of H_2S by these enzymes decreases. We recently identified a fourth pathway, by which H_2S is produced from D-cysteine by the enzymes D-amino acid oxidase (DAO) and 3MST. This pathway is mainly localized in the cerebellum and the kidney. The production of H_2S from D-cysteine is 80 times more efficient than that from L-cysteine in the kidney, and the administration of D-cysteine to mice ameliorates renal ischemia-reperfusion injury more effectively than L-cysteine. These results suggest that D-cysteine might be used to treat renal diseases or even increase the success of kidney transplantation. We found that H_2S-derived polysulfides exist in the brain and activate transient receptor potential ankyrin-1 (TRPA1) channels 300 times more potently than H_2S. Although TRPA1 channels mediate sensory transduction and respond to a variety of stimuli, including cold temperature, pungent compounds and environmental irritants, their endogenous ligand(s) has not been identified. The sulfane sulfur of polysulfides is a reactive electrophile that is readily transferred to a nucleophilic protein thiolate to generate the protein persulfide or bound sulfane sulfur by sulfhydration (as referred to as sulfuration). The bound sulfane sulfur-producing activity of polysulfides is much greater than that of H_2S. This review focuses on the physiological roles of H_2S and H_2S-derived polysulfides as signaling molecules.

Contents

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Introduction

The discovery of endogenous sulfides in the mammalian brain first suggested that hydrogen sulfide (H_2S) might have a physiological role(s) [1–3]. We found that H_2S is produced by cystathionine β-synthase (CBS) and that H_2S facilitates the induction of hippocampal LTP by enhancing the activity of N-methyl-D-aspartate (NMDA) receptors [4]. Subsequently, we found that H_2S is produced by cystathionine γ-lyase (CSE) in the thoracic aorta, portal vein, and ileum, and that H_2S relaxes these smooth muscles in synergy with NO [5–7]. Recently, the minimal nitrosothiol HSNO was found to be produced in human umbilical vein endothelial cells (HUVEC) [8]. Moreover, NOSH compounds, which release both H_2S and NO, were found to have potent anti-cancer activity as well as anti-inflammatory effects [9]. The synergistic interaction between H_2S and other molecules, including NO, implies the therapeutic potential of these compounds.

Given that CSE was not found in the brain, CBS was thought to be a unique H_2S-producing enzyme in the brain. However, we detected H_2S-producing activity in the brain of CBS-knockout mice. This led to the identification of a pathway, which is regulated by 3-mercaptopyruvate sulfurtransferase (3MST) along with cysteine aminotransferase (CAT) [10]. Based on our observation that brain homogenates produce H_2S from D-cysteine (a negative control of L-cysteine) we identified a novel pathway that comprises D-amino acid oxidase (DAO) and 3MST [11].

Given that H_2S was initially considered to be a toxic gas, its cytoprotective effects were initially overlooked. Later, we and others found that H_2S protects the neurons from oxidative stress by facilitating the production of glutathione, a major intracellular antioxidant, and by scavenging reactive oxygen species (ROS) in mitochondria [12–15]. In the retina, H_2S protects photoreceptor cells from light-induced degeneration by suppressing the excessive elevation of intracellular Ca^{2+} concentrations [16]. H_2S also regulates endoplasmic reticulum (ER) stress and facilitates the translocation of Nrf2 and NF-κB into the nucleus to up-regulate the transcription of antioxidant and antiapoptotic genes, respectively [17–19].

The recent re-evaluation of the endogenous levels of H_2S revealed them to be much lower than previously estimated [20–22]. Nevertheless, it confirmed the existence of H_2S in tissues. The K_m values of H_2S-producing enzymes are greater than the endogenous concentrations of their substrate cysteine. Therefore, the quantity and rate of production of H_2S are not adequate to induce physiological responses. To compensate for the inadequate production, H_2S is stored as bound sulfane sulfur, which may release H_2S when cells are stimulated [21]. On the other hand, H_2S is oxidized to polysulfides (H_2S_n) (n = 2–7), which are more stable than H_2S. We recently found that polysulfides are derived from H_2S in the brain, and that the activation of TRPA1 channels by polysulfides is approximately 300 times more potent than parental H_2S [23]. Although TRPA1 channels are activated by various stimuli, including other pungent substances, their endogenous ligand has not been identified. Polysulfides are potential ligands for TRPA1 channels.

H_2S production

H_2S-producing enzymes

During the 1950s–1970s, CBS, CSE, 3MST, and CAT were shown to produce H_2S in vitro [24–26]. However, rather than being recognized as a physiologically active molecule in itself, H_2S was initially thought of simply as a byproduct of metabolic pathways or as a marker for the evaluation of enzyme activity.

CBS is expressed in several organs and tissues, such as liver, kidney, brain, ileum, uterus, placenta and pancreatic islets [5,27–29]. CBS produces H_2S from cysteine via a β-elimination reaction, and more efficiently via a β-replacement reaction in which cysteine is condensed with homocysteine [30]. The production of H_2S by CBS is enhanced by S-adenoxylmethionine, an allosteric activator of CBS, and suppressed by NO and CO [4,31,32].

CSE is expressed in the liver, kidney, thoracic aorta, ileum, portal vain, uterus and placenta, but is weakly detected in the brain [5,27–29,33,34]. Under normal conditions, α,β-elimination of cysteine generates H_2S by CSE. The high concentrations of homocysteine caused by homocysteinemia result in the α,γ-elimination and γ-replacement reactions of homocysteine becoming the dominant source of H_2S production [35].

Although CBS and CSE were thought to be the major H_2S-producing enzymes, we found that brain homogenates prepared from CBS knockout mice still produce H_2S. This observation suggested that other H_2S-producting enzymes are present. These enzymes were identified as 3MST and CAT [10,36–38]. 3MST produces H_2S from 3-mercaptopyruvate (3MP), which is, in turn, produced from cysteine and α-ketoglutarate by CAT. Whereas CBS and CSE are localized to the cytoplasm, 3MST is mainly localized to the mitochondrial matrix, with an optimal pH of approximately 8 [39]. Unlike the cytosol, mitochondria contain sufficiently high concentrations of cysteine (approximately 1 mM) to enable the 3MST/CAT pathway to produce H_2S [40,41].

Cofactors for 3MST

Three pathways produce H_2S from L-cysteine. These are defined by the enzymes CBS, CSE, and 3MST, which acts together with cysteine amino transferase (CAT), which is identical to aspartate amino transferase (AAT) [10,42]. Whereas 3MST requires a reducing substance such as dithiothreitol (DTT; the endogenous counterpart of which has not been identified) to produce H_2S, CBS and CSE do not require reductant. For this reason 3MST has not been recognized as an H_2S-producing enzyme [43]. The observation that 3MST can interact with thioredoxin suggested that thioredoxin might be an endogenous reducing substance that enables 3MST to produce H_2S [44]. The presence of thioredoxin, which exists at a concentration of approximately 20 μM in cells, increased the production of H_2S by 3MST by 10-fold [45,46]. Another endogenous reducing substance, dihydrolipoic acid (DHLA), which exists approximately 40 μM in the brain [47,48], increased the production of H_2S by 3MST by 3-fold [46]. The other endogenous reducing substances, such as cysteine, GSH, NADPH, NADH and CoA, have little effect on H_2S production by 3MST. Thioredoxin, DHLA and DTT have negative redox potentials in the range from −0.22 to −0.33 V, but there is no correlation between these potentials and the production of H_2S [46,49–52]. Among these substances, DTT and DHLA are dithiols and thioredoxin has two cysteine residues at its active site. Dithiols may be critically required for these substances to associate with 3MST to produce H_2S. A possible mechanism for the production of H_2S is that 3MST receives sulfur from 3MP to produce 3MST-persulfide, which is transferred to a thiol of thioredoxin or DHLT to produce thioredoxin- or DHLA-persulfide. The remaining thiol of thioredoxin or DHLA reduces the persulfide to release H_2S [46]. Similar results for thioredoxin as an endogenous reducing substance were shown by Banerjee and colleagues [53], and for DHLA for the production of H_2S [54].

3MST is localized in both mitochondria and the cytosol, with two forms of CAT known as mitochondrial CAT and cytosolic CAT. Therefore, this pathway can produce H_2S both in the mitochondria and the cytoplasm. Considering the fact that the concentrations of cysteine in the cytoplasm are 0.15–0.25 mM and those in mitochondria are 0.7–0.99 mM, it seems likely that most of