Significance of Hydrogen Sulfide Production in the Pancreatic β-Cell

Shigeki Taniguchi1 and Ichiro Niki1,∗

1Department of Pharmacology, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama, Oita 879-5593, Japan

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Abstract. Hydrogen sulfide (H2S) is an important signaling molecule in various mammalian cells and tissues. H2S is synthesized from L-cysteine and regulates several cellular and physiological phenomena (vasorelaxation, hormone secretion, and apoptosis) and multicellular events (neuromodulation and inflammatory responses). H2S can be produced in pancreatic β-cells by cystathionine β-synthase (CBS) or cystathionine γ-lyase (CSE). H2S inhibits insulin release and regulates β-cell survival. We found that glucose stimulation increased CSE expression at transcript and protein levels in mouse pancreatic islets. We also found that H2S protects β-cells that were chronically exposed to high glucose from apoptotic cell death. Loss of β-cell mass and failures of β-cell function are important in the pathogenesis and/or progression of diabetes mellitus; therefore, molecular analyses of the mechanisms of H2S production and its protective effects on β-cells may lead to new insights into diabetes mellitus.

Keywords: hydrogen sulfide, gasotransmitter, pancreatic β-cell, cytoprotection, diabetes mellitus

1. Introduction

Hydrogen sulfide (H2S) is a toxic gas with the distinct smell of rotten eggs. However, H2S is also produced in mammals. In 1989, Warenycia et al. measured endogenous levels of H2S in brain tissue of anesthetized rats (1). Their initial idea was that general anesthesia might affect brain sulfide levels because signs of H2S poisoning resemble an anesthetized state. Their findings did not demonstrate that anesthesia affects brain sulfide levels, but they did find a high level of sulfide in untreated brain tissue. The same group also made postmortem measurements of endogenous levels of H2S in human brains and found high levels of acid-labile sulfur (2). Subsequent research identified metabolic pathways that produce H2S and its physiological and pathological roles (3–5).

2. Biosynthesis of H2S

In mammalian tissues, H2S is synthesized from L-cysteine, a sulfur-containing amino acid, by a few enzymes, including cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (3–6). These two enzymes are distributed in distinct patterns among various tissues (3–6); CBS is the predominant H2S-producing enzyme in the brain, and CSE is a major source of H2S in the heart and vasculature system. A recent report suggests that an enzymatic pathway that includes cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3-MST) may also produce H2S from L-cysteine in the brain (7). The existence of the alternative synthetic pathway was indicated by the finding that H2S can be generated in mouse brains that lack CBS (7). 3-MST is also distributed in the liver, kidney, heart, and lung (6, 8).

3. H2S as a gasotransmitter

Application of H2S or the H2S donor NaHS is known to cause various cellular responses, including smooth muscle tone, neurotransmission, hormonal secretion, and cell death/survival (5, 9). In early studies, H2S was found to facilitate the induction of hippocampal long-term potentiation by enhancing the activity of NMDA receptors in neurons (10). It also enhances NO-induced relaxation of vascular and intestinal smooth muscles (11) and inhibits high K+-induced corticotropin secretion from hypothalamic slices (12). Furthermore, H2S affects cell sur-
H₂S exhibits its protective effects via multiple mechanisms, including the opening of the ATP-sensitive K⁺ (K<sub>ATP</sub>) channels, induction of anti-oxidative molecules (e.g., thioredoxin), reduction of lipid peroxide formation, upregulation of the anti-apoptotic molecule Bcl-2, and activation of the anti-apoptotic signaling by Akt and MAP kinases (5, 9, 14). In contrast, exposure to H₂S is reported to facilitate apoptotic cell death in aortic smooth muscle cells, lung fibroblasts, and pancreatic acinar cells (13). Clearly, H₂S can exhibit opposite effects on cellular survival/death among different tissue types, but the reasons for these different effects are not fully understood.

We still have very limited knowledge about the relevant molecular mechanisms that mediate the physiological and pathological actions of H₂S. It is likely that there are multiple direct target molecules that mediate the actions of H₂S (4). A recent study indicates that H₂S influences its target molecules via S-sulfhydration of a cysteine residue; specifically, H₂S activates glyceraldehyde-3-phosphate dehydrogenase via S-sulfhydration at the Cys<sup>150</sup> residue (15). This finding may enable us to search for other targets of H₂S, although this chemical reaction seems to be rather common. Intracellular signaling via H₂S-mediated S-sulfhydration may be similar to the nitric oxide (NO)-mediated S-nitrosylation that may transduce various intracellular signals by NO.

NO and carbon monoxide (CO) are recognized as gaseous transmitters. Endogenous NO is produced from l-arginine in a reaction catalyzed by NO synthase (NOS), and CO is synthesized from heme by heme oxygenase (HO). Like NO and CO, H₂S can freely penetrate cell membranes without using specific transporters and participate in intra- and inter-cellular signal transduction. Accordingly, H₂S is now recognized as a third gasotransmitter.

### 4. Production of H₂S in pancreatic β-cells

Pancreatic β-cells, distributed in the islets of Langerhans, secrete insulin, a glucose-lowering hormone, which is crucial for homeostasis of the blood glucose levels in vivo. Some studies report that H₂S is produced in pancreatic β-cell lines (16 – 18). H₂S production in homogenates of rat (INS-1E) and hamster (HIT-T15) β-cell lines was measured in the presence of an excess amount of exogenous l-cysteine (16, 17). We measured H₂S content as acid-labile sulfur in the mouse insulin-secreting cell line MIN6 (18), and we found that H₂S levels in this β-cell line were comparable with those in brain homogenates (5, 9 – 11). We also found that CSE and CBS mRNAs are expressed in mouse pancreatic islets (18, 19). Further analyses revealed that expression of CSE, but not CBS, dramatically increases in the islets following glucose stimulation (19). This finding was the first to indicate that expression of H₂S-producing enzymes can be induced by a physiological stimulus. The finding also indicates that H₂S production can be modulated by inducible enzymes, as NO and CO are induced by NOS and HO-1, respectively (20, 21). We also found that a stimulatory concentration of glucose increased the levels of endogenous H₂S (Fig. 1).

In contrast, glucose stimulation has been reported to decrease the H₂S-producing activity in the homogenates of INS-1E cells (16). The contradictory effects of glucose on H₂S production may be due to species-specific differences in the conditions for gene induction of CSE. Indeed, CSE expression levels in intact mouse islets were different from those in rat islets (Fig. 2). Notably, tumor cell lines possess some features that are distinct from their original cells, and these differences may affect their H₂S production. Although H₂S-producing enzymes and its potential functions in β-cells have been studied in several papers, the pathophysiological roles of CBS is apparently less pronounced (17 – 19, 22).

### 5. Roles of H₂S in β-cell functions

Insulin release from pancreatic β-cells is controlled by various substances, including nutrients, neurotransmitters, and hormones. However, the molecular mechanisms underlying the regulation of insulin release are not fully understood. For example, we still have limited understanding of the inhibitory messengers that suppress insulin release, although it is clear that many substances inhibit insulin secretion. Inhibition of insulin release by H₂S has been reported in a few studies (16 – 18). Evi...
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dently, H2S inhibits insulin release via multiple mechanisms. Studies with insulin-secreting cell lines suggest that H2S may inhibit insulin secretion via opening of KATP (16, 17). However, we also found that H2S may decrease insulin release via mechanisms independent of the opening of KATP. For example, NaHS decreases insulin secretion from permeabilized mouse islets, in which all ionic events across the plasma membrane are abolished (18). NaHS also decreases intracellular ATP concentrations in MIN6 cells (18). Moreover, application of NaHS to intact mouse β-cells abolishes glucose-induced oscillation of intracellular Ca2+ concentration ([Ca2+]i) without changing the mean [Ca2+]i.

Reportedly, H2S affects β-cell death/survival. Yang et al. reported that H2S application or CSE overexpression induced the expression of ER-stress–related molecules and apoptosis in rat insulinoma INS-1E cells via p38 MAPK activation (23). In contrast, we have recently reported that H2S may act as a cytoprotectant for intact mouse islets and MIN6 cells. NaHS or l-cysteine reduced apoptotic cell death of mouse islets or MIN6 cells exposed to high glucose, fatty acids, or a mixture of cytotoxic cytokines (19, 24). Moreover, concomitant incubation of mouse islets with l-cysteine restored the secretory activity of islets subjected to chronic exposure to high glucose (19). Because the l-cysteine effect was inhibited by the CSE inhibitor, DL-propargylglycine (PPG), H2S production by CSE seems to be important to β-cell protection. Notably, these effects of H2S on MIN6 cells were found to ameliorate cellular damage by oxidative stress, but not the damage caused by ER stress (24). Therefore and again, these findings on the effects of H2S on mouse islets and MIN6 cells contradict findings with INS-1E cells (23), possibly because of species-specific differences in the sensitivity of INS-1E cells to H2S and/or the experimental conditions used to induce cell death.

We studied the molecular mechanisms underlying the H2S-mediated protection of β-cells. NaHS or l-cysteine increases the content of glutathione and reduces the production of reactive oxygen species (19, 24). PPG treatment partially inhibited l-cysteine–induced increases in glutathione levels, suggesting the involvement of CSE (19). Apart from these anti-oxidative actions, NaHS application to MIN6 cells also activates Akt phosphorylation, an important molecule for β-cell survival (24). Inhibitory effects of H2S on [Ca2+]i oscillation may also be involved because persistent elevation of [Ca2+]i causes β-cell death (25). Based on our findings mainly using native mouse islets, H2S may prevent glucotoxicity and other insults that occur under diabetic conditions. We hypothesize that high-glucose–induced CSE expression increases H2S production to protect β-cells, which thereby inhibit insulin release and protect themselves (Fig. 3).

We suggest that H2S may function as an ‘intrinsic brake’ in pancreatic β-cells.

6. H2S and diabetes mellitus

There are only a few reports on the content of H2S and the activities of H2S-producing enzymes in diabetic animals. Previous studies demonstrated the induction of H2S-producing enzymes in animals treated with the β-cell
toxin streptozotocin (26, 27). The mRNA and protein expression levels of CSE and CBS are higher in the liver of streptozotocin-treated rats relative to control animals; however, the expression of CBS, but not of CSE, is increased in the diabetic pancreas (mostly composed of pancreatic exocrine cells) (27). The H$_2$S-producing activity increases in the liver and pancreas of streptozotocin-treated diabetic rats (27, 28), whereas the plasma H$_2$S levels are unchanged (27). Increases in CBS and CSE expression and H$_2$S production in these diabetic tissues are reversed by insulin treatment, suggesting that this may be a secondary result from hyperglycemia or hypoinsulinemia. In Zucker diabetic fatty (ZDF) rats, which are a diabetic model with obesity and hyperinsulinemia, CSE expression is elevated in the islets (22). Intraperitoneal administration of PPG, but not of aminooxyacetate, a putative selective inhibitor of CBS, improves glycemic control in vivo in ZDF rats (22). However, non-obese-diabetic (NOD) mice, another model of type 1 diabetes that results from an autoimmune disorder, show a progressive reduction of plasma H$_2$S levels (29). A recent study showed that plasma H$_2$S levels are reduced in human diabetic subjects (30, 31) and streptozotocin-treated rats (31). These findings do not support the idea that an excess of H$_2$S production exacerbates diabetes. These conflicting findings might occur because changes in H$_2$S synthesis and breakdown may depend on the stage of diabetes.

H$_2$S seems to contribute to the pathogenesis of several diseases, including Alzheimer’s disease, hypertension, and cardiac infarction (4, 9, 32). Therefore, modulation of its production may be a potential therapeutic strategy for these diseases. This possibility led us to investigate H$_2$S-related substances as treatments for diabetes (33). The cytoprotective effects of H$_2$S are particularly interesting because exhaustion of $\beta$-cells is an important process in the pathogenesis of type 2 diabetes mellitus, and the prevention of $\beta$-cell exhaustion may be a new strategy for the treatment of this disease (34). However, the available data on the roles of H$_2$S in diabetes are limited and apparently contradictory in some instances.

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