MOLECULE PAGE

Hydrogen Sulfide

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1. General Information

Background

Hydrogen sulfide (H\textsubscript{2}S; CAS No. 7783-06-4) is a colorless, flammable gas with a characteristic odor of rotten eggs. It is produced naturally and as a result of human activity. Natural sources account for about 90% of the total H\textsubscript{2}S in the atmosphere (56). Concentrations of H\textsubscript{2}S in ambient air as a result of natural sources have been estimated to be between 0.14 and 0.4 µg/m\textsuperscript{3} (56). The odor threshold of H\textsubscript{2}S is 0.011 mg/m\textsuperscript{3}. Human exposure at ranges between 5 and 700 mg/m\textsuperscript{3} have reported to result in ocular, respiratory and neurological complications. At concentrations \( \geq 700\text{mg/m}^3 \), incidences of death have been reported (5).

Endogenous H\textsubscript{2}S synthesis and metabolism

Apart from environmental and bacterial sources, mammals too are capable of synthesizing H\textsubscript{2}S. Endogenous levels of H\textsubscript{2}S have been measured in the circulatory system with rat serum being reported to contain \( \sim 46\text{µM} \) H\textsubscript{2}S (67). H\textsubscript{2}S synthesizing activity has also been shown in rat tissue extracts of liver, kidney, heart, brain, small intestine, skeletal muscle and pancreas (22). H\textsubscript{2}S can be hydrolyzed to hydrosulfide and sulfide ions. In an aqueous solution, about one third of H\textsubscript{2}S remains undissociated at pH 7.4. H\textsubscript{2}S is permeable to plasma membranes as its solubility in lipophilic solvents is \( \sim \) fivefold greater than in water (53).

Endogenous H\textsubscript{2}S can be synthesized via the desulfuration of cystine/cysteine by three enzymes; Cystathionine beta synthase (CBS; EC 4.2.1.22), Cystathionine gamma Lyase (CSE; EC 4.4.1.1) and mercaptopyruvate sulfurtransferase (MST; EC 2.8.1.2) MST is found both in the mitochondria and cytosol while CBS and CSE are mainly produced in the cytosol. (22). Among the 3 enzymes, MST contributes the least towards endogenous H\textsubscript{2}S production (36, 42) while CBS seems to be the main H\textsubscript{2}S-forming enzyme in the CNS and CSE is the main H\textsubscript{2}S-forming enzyme in the cardiovascular system (34). In mice, CSE expression has been detected mainly in the liver and kidney, and in lower abundance in adipose tissue, stomach, small intestine, brain, heart and lung (20). CBS expression has been found in all
parts of the brain, liver and pancreas (4, 38). In mouse pancreas, CBS is ubiquitously distributed but CSE was found mostly in the exocrine and in very small amounts in the freshly prepared islets. However, high glucose increased the CSE expression in the beta-cells (23).

Both CSE and CBS are pyridoxal 5'-phosphate dependant enzymes. CBS is able to synthesize H$_2$S directly by substituting the thiol group L-cysteine with a variety of thiol compounds to form H$_2$S and the corresponding thioether. CSE on the other hand catalyzes the desulfhydration of cystine which results in pyruvate, NH$_4^+$, and thiocysteine. Thiocysteine then reacts with cysteine or other thiols to form H$_2$S (42) (Figure 1) The metabolism of H$_2$S can be divided to 3 distinct pathways: oxidation to sulfate, methylation, and reaction with metallo or disulfide containing proteins (5). Oxidation of sulfide to sulfate and subsequent excretion by the kidney is thought to represent the major metabolic and secretory pathway.

### Physiological and pathological functions:

Rapid development in the H$_2$S field has revealed numerous possible physiological and pathological roles for H$_2$S. Below is a brief introduction into the current areas in H$_2$S have been discovered to play a role.

#### Neuromodulation

Physiological concentrations of H$_2$S selectively enhance NMDA receptor-mediated responses and facilitate the induction of hippocampal long term potentiation (1) as well as regulate the release of the corticotrophin-releasing hormone from the hypothalamus (14). An antinociceptive role of H$_2$S has been reported in colorectal distensions in rats mediated by K$_{ATP}$ channels and NO (15). Subsequent studies have however, reported a pronociceptive role for H$_2$S through the sensitization/ activation of T-type Ca$^{2+}$ channels (31, 16).

*Figure 1. Endogenous H$_2$S synthesis and metabolism.*
**Cardiovascular function**

$\text{H}_2\text{S}$ has been demonstrated to be a vasoactive factor that relaxes rat thoracic aorta and portal vein, and guinea pig ileum in a concentration dependent manner (18). This action has been shown to be mediated by activation of $K_{\text{ATP}}$ channels resulting in a hyperpolarized membrane leading to smooth muscle relaxation (67). A growing body of evidence suggests that $\text{H}_2\text{S}$ preconditioning protects against myocardial injury (9, 21, 40, 41).

**Interaction with reactive- oxygen and nitrogen species**

$\text{H}_2\text{S}$ has been shown to possess scavenging properties towards peroxynitrite (54) and hypochlorous acid (27, 55). An indirect antioxidant effect was observed in primary neuron cultures where $\text{H}_2\text{S}$ was found to increase glutathione levels by enhancing the activity of gamma-glutamylcysteine synthetase and up-regulating cystine transport (26). $\text{H}_2\text{S}$ has also been shown to interact with reactive oxygen species from activated neutrophils resulting in its oxidation to sulfite which is toxic at high levels (32).

**Inflammation**

The role of $\text{H}_2\text{S}$ in inflammation is still a matter of debate. In most of the studies, $\text{H}_2\text{S}$ has been shown to be pro-inflammatory. This was observed in various animal models of inflammation such as LPS induced endotoxemia (28), pancreatitis (8), cecal-ligation and puncture induced sepsis (65), hemorrhagic shock (33), burns injury (66) and cisplatin induced renal injury (13). In these studies, pre-treatment with inhibitor of endogenous $\text{H}_2\text{S}$ synthesis, PAG afforded protection while administration of $\text{H}_2\text{S}$ in the form of NaHS resulted in exacerbation.

On the other hand, studies have reported an anti-inflammatory effect on gastritis and colitis using Lawesson reagent as a sulfide donor as well as NaHS at a low dose (51, 52). A slow releasing $\text{H}_2\text{S}$ molecule, GYY4137 has also been shown to protect mice against LPS induced endotoxemia (30) by the same author that previously showed pro-inflammatory effect using NaHS (28). $\text{H}_2\text{S}$ releasing drugs have also been shown to have anti-inflammatory effect attributed to the sulfide moiety and not the parent drug, diclofenac (6, 29).

Conflicting observations have also been reported on the effect of $\text{H}_2\text{S}$ on leukocyte rolling and adhesion. Studies have shown that $\text{H}_2\text{S}$ both positively and negatively regulate leukocyte activation and migration. (3, 12, 64, 65, 68). It is becoming apparent that $\text{H}_2\text{S}$ has a multifaceted role in inflammation and current studies vary in opinions largely based on the dose, rate of release, and type of $\text{H}_2\text{S}$ donor used.

**2. Pancreatic Information**

1. $\text{H}_2\text{S}$ and Insulin secretion

Mobilization and secretion of insulin-containing vesicles into the extracellular space is regulated by cellular Ca movements via the regulation of the voltage gated Ca channel which is activated based on membrane polarization. $\text{H}_2\text{S}$ which is actively synthesized in the pancreas has been shown to activate $K_{\text{ATP}}$ channels in smooth muscle cells which regulate membrane polarization. Therefore it has become of interest to study the possible role of $\text{H}_2\text{S}$ in insulin mobilization.

Freshly prepared pancreatic islet and related cell lines are reported to express $\text{H}_2\text{S}$ synthesizing enzymes, CSE and CBS (24, 60, 63). $\text{H}_2\text{S}$ synthesizing rates were reported in INS-1E cells at 12 nmole/g/min in 5mM glucose (60) and fresh rat pancreas extract at 38 nmole/g/30min (63) and 8 nmole/g/min (57). Increased glucose concentration (20mM) in INS-1E cells resulted in a decrease in $\text{H}_2\text{S}$ synthesis to 6 nmole/g/min (60). Altered $\text{H}_2\text{S}$ synthesis was also observed in fresh pancreatic extracts of Streptozotocin-diabetic and Zucker diabetic fatty rats which increased to 65 nmole/g/30min (63) and 12 nmole/g/min (57) respectively. However the
identified enzyme that was responsible for this observed increase in H\textsubscript{2}S synthesis differed between these two models of diabetes. In the Streptozocin-diabetic rats, the increased H\textsubscript{2}S synthesis was attributed due to an increased CBS expression of up to 2-fold (63), while in Zucker diabetic fatty rats it was due to increased CSE expression (57).

These changes in H\textsubscript{2}S levels affect the amount of insulin secreted, the general consensus being H\textsubscript{2}S functions as an inhibitor to insulin secretion. It has been reported that exposure of beta islet cells to exogenous or increased endogenous H\textsubscript{2}S significantly reduced glucose-induced insulin secretion (2, 24, 57, 63) The observed suppression of insulin secretion by H\textsubscript{2}S has been associated with the opening of K\textsubscript{ATP} channels. The reported effective dose of NaHS (exogenous source of H\textsubscript{2}S) that resulted in activation of K\textsubscript{ATP} channels and reduced insulin secretion in INS-1E and HIT-T15 cells was 100uM (60, 2). This was abolished following pre-treatment with a K\textsubscript{ATP} channel blocker, glibenclamide (2). Similar findings were reported in freshly prepared islet cells from rats (57). Further molecular mechanism studies revealed that exogenous H\textsubscript{2}S prevents Ca\textsuperscript{2+} influx (2) and oscillation (24) which are essential to insulin secretion (37).

2. H\textsubscript{2}S and pancreatic cell death

H\textsubscript{2}S has been reported to induce apoptosis in both exocrine and endocrine cells of the pancreas. Pancreatic acinar cells treated with 10 uM NaHS for 3 hours showed positive Annexin V staining (10). This was accompanied with the activation of effector caspase -3 and initiator caspase- 8 and 9 as well as loss of mitochondrial membrane integrity and release of cytochrome C. Additionally pro-apoptotic proteins Bax was upregulated while anti-apoptotic protein FLIP was downregulated. INS-1E cells stimulated with 100 uM H\textsubscript{2}S (pure H\textsubscript{2}S gas) and CSE over-expressing INS-1E cells were found to be apoptotic at 12 hours and 48 hours respectively (59). The authors reported an inhibition of ERK 1/2 and activation of p38 MAPK as well as upregulation of ER stress regulators; BiP and CHOP following H\textsubscript{2}S stimulation. Inhibition of p38 MAPK inhibited expression of BiP and CHOP leading to a decrease in H\textsubscript{2}S mediated apoptosis in INS-1E cells.

Interestingly, H\textsubscript{2}S has also been shown to have anti-apoptotic effect on pancreatic endocrine cells. 100 uM NaHS and 3 mM L-Cysteine suppressed freshly prepared mouse islet cell apoptosis induced with high glucose (20 mM) for 18 hours (23). H\textsubscript{2}S has also been shown to protect freshly prepared mouse islet cells against apoptosis induced by palmitate, H\textsubscript{2}O\textsubscript{2} and cytokine mixture (TNF-α, IL-1β and INF-δ) but did not protect against thapsigargin and tunicamycin (49). Similar findings were found with MIN6 cells (23). Administration of NaHS was found to reduce oxidative stress by increasing glutathione content (23) and suppression of ROS production (49) in MIN6 cells following insult.

3. H\textsubscript{2}S modulates pancreatic nociception

NaHS was shown to be pronociceptive when administered into the pancreatic duct of rats at 500 nmole /rat (35, 16). Nociception was determined by expression of Fos proteins as well as phosphorylation of ERK in the spinal dorsal horns of the animals. The upregulation of Fos proteins as well as phosphorylation of ERK was abolished following pre-treatment with a T-type Ca\textsuperscript{2+} channel blocker, glibenclamid (2). In the caerulein induced pancreatitis model, the prevention of pancreatitis associated alldynia/hyperalgesia has been reported by pre-treatment with a CSE blocker (PAG) and treatment with a T-type Ca\textsuperscript{2+} channel blocker (Mibefadril) (35).

4. H\textsubscript{2}S regulates the severity of inflammatory response in acute pancreatitis and associated lung injury

Caerulein induced acute pancreatitis upregulates CSE expression and H\textsubscript{2}S production whereas
inhibition of endogenous H₂S formation with DL-propargylglycine (PAG), a CSE inhibitor, reduces the severity of caerulein-induced acute pancreatitis and affords protection against the associated lung injury in mice (8). H₂S donor, NaHS provoked inflammation in both mouse pancreatic acinar cells (46) and primary human monocyte cells (68). Therefore, observations using H₂S donor in murine cells could still be extrapolated to human cells, and may provide useful guidance on the potential therapeutic benefits of using H₂S in inflammatory conditions.

Studies have suggested that H₂S has both pro and anti-inflammatory properties, depending largely on the experimental conditions and the cell type under scrutiny. The mechanism through which H₂S is able to promote and delay inflammatory response still remains to be fully elucidated. However, it has been proposed that low concentrations (NaHS- 5 and 10 µM) (48) of H₂S, have a protective effect. On the other hand higher concentrations (100 µM) (46) induce inflammation in the acini. Another H₂S donor ACS15, is a H₂S releasing derivative of Diclofenac. ACS15 reduced lung inflammation without having any effect on pancreatic injury (6). In addition Nishimura et al showed that pancreatic NaHS/ H₂S most probably targets T-type Ca(2+) channels, leading to nociception, and that endogenous H₂S produced by CSE and possibly T-type Ca(2+) channels are involved in pancreatitis-related pain (35).

Altering endogenous or exogenous H₂S may have unanticipated effects because H₂S has a wide array of actions for example H₂S regulates chemokines, cytokines and adhesion molecule expression and exerts explicit biphasic effect in acute pancreatitis and associated lung injury (Fig 2) (39, 45). At present, there are several pathways that appear to be involved in the potential effects of H₂S on acute pancreatitis. Although the mechanism of the pro-inflammatory effect of H₂S has not yet been fully investigated, it is likely that H₂S stimulates neutrophil adhesion to caerulein treated pancreatic acinar cells and subsequent intracellular adhesion molecule (ICAM)-1 upregulation through Src family kinases (44).

One of the major messengers in the upregulation of ICAM-1, and neutrophil adhesion, is nuclear transcription factor-κB (NF-κB). NF-κB is a transcription factor that translocates to the cell nucleus on activation by H₂S, whereupon it acts as a signal for increased transcription of ICAM-1. This increase in ICAM-1 mRNA was consistent with Western blot analysis of increased NF-κB in caerulein treated pancreatic acinar cells. The mechanism of this enhanced ICAM-1 mRNA appears to relate to IκB-α, since H₂S donor inhibits IκB-α phosphorylation. IκB-α is the inhibitory subunit of NF-κB and thus inhibition of IκB-α activates the expression of NF-κB, effectively upregulating ICAM-1 expression on pancreatic acinar cells. In caerulein treated pancreatic acini, PAG reduces ICAM-1 expression which led to decrease in neutrophil adhesion to acini via Src family kinases and NF-κB (44).

Another potential mechanism of action of H₂S is its interaction with the neuropeptide Substance P in both an in vivo (7) and an in vitro model of acute pancreatitis (46). As a consequence of this interaction Substance P is upregulated via activation of TLR4 and NF-κB pathway (7). Blockade of H₂S biosynthesis by PAG and PPTA deficiency attenuates H₂S induced TLR4 and NF-κB pathway possibly by stimulation of IκB-α phosphorylation (Fig 2) (47).

Recently, we analyzed the mechanism by which H₂S reduces caerulein induced inflammation in pancreatic acini. We found that H₂S activation of the PI3K/AKT pathway negatively regulates the intracellular signaling pathway, ERK, transcription factor NF-κB and the cytokines in caerulein treated pancreatic acinar cells (Fig 2) (48). Activation of PI3K/AKT signaling mediates the anti-inflammatory effects in inflammation (62).
Differences may exist in the mechanisms by which H₂S induces inflammation. It is also important to realize that the concentration of H₂S donor used may not necessarily reflect the concentration of H₂S to which the cells are exposed. Equivalent concentrations of different H₂S donors may liberate H₂S to different extents or at different rates. Therefore, the concentration of free H₂S in the vicinity of the cells at any given time may vary, and the H₂S concentration in the system needs to be measured in order to directly compare different H₂S donors. The vast majority of work on this subject has been carried out using in vitro and in vivo systems, utilizing animals. How the results obtained in these systems relate to the in vivo situation during inflammation in humans still largely remains to be determined, but recent studies in mice show that H₂S is a promising candidate for treatment or prevention of inflammatory conditions. Further studies are required to elucidate completely the mechanism of action of H₂S on inflammation, in order to identify potential targets for the treatment of human inflammatory conditions and to evaluate the sources of H₂S that provide greatest therapeutic potential.

**Summary**

H₂S is produced by three enzymes, CSE, CBS and MST which are present in most mammals including humans. Both CBS and CSE are expressed in the pancreas. H₂S has been found to have both physiological and pathological roles in the pancreas. Currently there have been multiple conflicting reports on the effect of H₂S which could be largely attributed to the source and dose of H₂S studied.

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**Figure 2.** Based on our findings, this overview shows that H₂S (5µM) induced (PI3K/AKT) anti-inflammatory signals down-regulate proinflammatory gene induction by abrogating caerulein-induced IκBα degradation and thus NF-κB nuclear translocation. On the contrary, H₂S at 100µM concentration stimulates SFK, TLR4 phosphorylation, IκBα degradation and thus nuclear translocation of NF-κB to up-regulate proinflammatory gene induction.
3. Tools for Study of H$_2$S

1. Knockout mice and transgenic cell lines
   a. PPTA knockout mice of BALB/c background (11)
   b. CSE knockout mice of C57BL/6J background (58)
   c. CSE knockout mice of C57BL/6J background (19)
   d. CSE overexpressed HEK-293 cells (47)

2. CSE inhibitors
   a. D,L-propargylglycine, Compound ID: 95575 (1)
   b. β-cyano-L-alanine, Compound ID: 439742 (1)

3. CBS inhibitors
   a. Hydroxylamine, Compound ID: 787 (1)
   b. Aminooxyacetate, Compound ID 286 (1)

4. CBS activator
   a. S-adenosyl-L-methionine(AdoMet), Compound ID: 34756 (25)

5. H$_2$S donor compounds
   a. NaHS (Compound ID: 28015)
   b. Na$_2$S (Compound ID: 237873)
   d. GYY4137 (30)

6. PCR primers (24)
   a. Mouse Cystathionine-γ-lyase
      Forward 5’ – ATG GAT GAA GTG TAT GGA GAG GT- 3’
   b. Mouse Cystathionine-β-synthase
      Forward 5’ – ACT ACG ATG ACA CCG CCG AG – 3’
      Reverse 5’ – AGT CCT TCC TGT GCG ATG AG – 3’

7. Antibodies
   a. Rabbit anti mouse CSE and CBS antibody for immunofluorescence staining in pancreatic tissue and western blotting of pancreatic islet cell protein extract (23).

8. Method of measuring H$_2$S
   To date, several methods of measuring sulfides in biological samples have been developed (50). Among the more commonly used methods are:
   a) Spectrophotometric method (Methylene Blue Assay)
      • A more high-throughput method
      • Sensitivity in the micromolar range
   b) Chromatography (Ion, Liquid and Gas)
      • More specific as sulfide is separated before measurement
      • Sensitivity depends on the method of sulfide binding and detection employed.
   c) Sulfide Specific Electrode
      • Currently the most specific and sensitive method
      • Allows for real-time measurement
      • Very slow turn-over rate

4. References


53. Wang R. Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter?. *FASEB J* 16: 1792-1798, 2002. PMID: 12409322


