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Hydrogen Sulfide

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1. General Information

Background

Hydrogen sulfide (H_2S; 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_2S in the atmosphere (56). Concentrations of H_2S in ambient air as a result of natural sources have been estimated to be between 0.14 and 0.4 µg/m3 (56). The odor threshold of H_2S is 0.011 mg/m^3. Human exposure at ranges between 5 and 700 mg/m^3 have reported to result in ocular, respiratory and neurological complications. At concentrations $\geq$ 700mg/m^3, incidences of death have been reported (5).

Endogenous H_2S synthesis and metabolism

Apart from environmental and bacterial sources, mammals too are capable of synthesizing H_2S. Endogenous levels of H_2S have been measured in the circulatory system with rat serum being reported to contain ~46uM H_2S (67). H_2S synthesizing activity has also been shown in rat tissue extracts of liver, kidney, heart, brain, small intestine, skeletal muscle and pancreas (22). H_2S can be hydrolyzed to hydrosulfide and sulfide ions. In an aqueous solution, about one third of H_2S remains undissociated at pH 7.4. H_2S is permeable to plasma membranes as its solubility in lipophilic solvents is ~ fivefold greater than in water (53).

Endogenous H_2S 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_2S production (36, 42) while CBS seems to be the main H_2S-forming enzyme in the CNS and CSE is the main H_2S-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

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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_2S directly by substituting the thiol group L-cysteine with a variety of thiol compounds to form H_2S 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_2S (42) (Figure 1) The metabolism of H_2S 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_2S field has revealed numerous possible physiological and pathological roles for H_2S. Below is a brief introduction into the current areas in H_2S have been discovered to play a role.

Neuromodulation

Physiological concentrations of H_2S 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_2S 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_2S through the sensitization/ activation of T-type Ca^{2+} channels (31, 16).

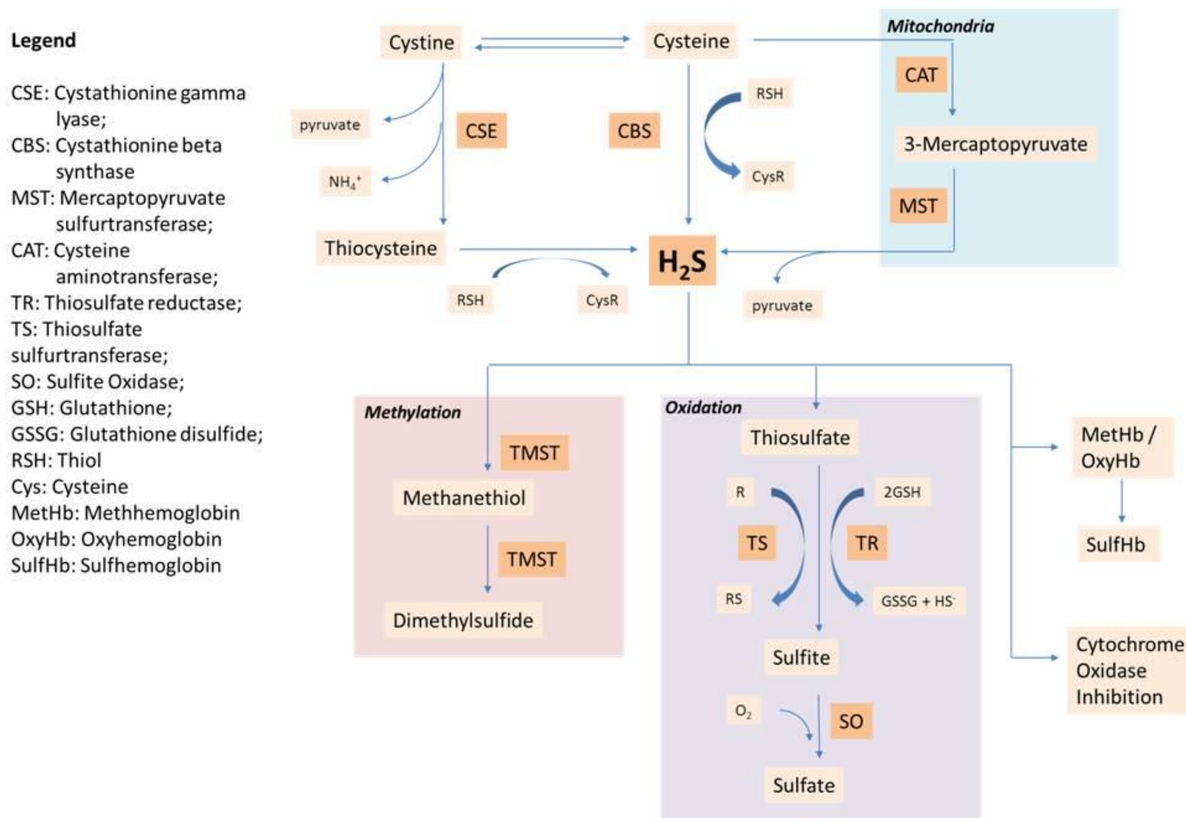

Figure 1. Endogenous H_2S synthesis and metabolism.

Cardiovascular function

H_2S 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_{ATP} channels resulting in a hyperpolarized membrane leading to smooth muscle relaxation (67). A growing body of evidence suggests that H_2S preconditioning protects against myocardial injury (9, 21, 40, 41)

Interaction with reactive- oxygen and nitrogen species

H_2S has been shown to possess scavenging properties towards peroxynitrite (54) and hypochlorous acid (27, 55). An indirect anti-oxidant effect was observed in primary neuron cultures where H_2S was found to increase glutathione levels by enhancing the activity of gamma-glutamylcysteine synthetase and up-regulating cystine transport (26). H_2S 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 H_2S in inflammation is still a matter of debate. In most of the studies, H_2S 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 H_2S synthesis, PAG afforded protection while administration of H_2S 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 H_2S 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). H_2S 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 H_2S on leukocyte rolling and adhesion. Studies have shown that H_2S both positively and negatively regulate leukocyte activation and migration. (3, 12, 64, 65, 68). It is becoming apparent that H_2S has a multifaceted role in inflammation and current studies vary in opinions largely based on the dose, rate of release, and type of H_2S donor used.

2. Pancreatic Information

1. H_2S 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. H_2S which is actively synthesized in the pancreas has been shown to activate K_{ATP} channels in smooth muscle cells which regulate membrane polarization. Therefore it has become of interest to study the possible role of H_2S in insulin mobilization.

Freshly prepared pancreatic islet and related cell lines are reported to express H_2S synthesizing enzymes, CSE and CBS (24, 60, 63). H_2S 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 H_2S synthesis to 6 nmole/g/min (60). Altered H_2S 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_2S synthesis differed between these two models of diabetes. In the Streptozocin-diabetic rats, the increased H_2S 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_2S levels affect the amount of insulin secreted, the general consensus being H_2S functions as an inhibitor to insulin secretion. It has been reported that exposure of beta islet cells to exogenous or increased endogenous H_2S significantly reduced glucose-induced insulin secretion (2, 24, 57, 63) The observed suppression of insulin secretion by H_2S has been associated with the opening of K_{ATP} channels. The reported effective dose of NaHS (exogenous source of H_2S) that resulted in activation of K_{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_{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_2S prevents Ca influx (2) and oscillation (24) which are essential to insulin secretion (37)

2. H_2S and pancreatic cell death

H_2S 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_2S (pure H_2S 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_2S stimulation. Inhibition of p38 MAPK inhibited expression of BiP and CHOP leading to a decrease in H_2S mediated apoptosis in INS-1E cells.

Interestingly, H_2S 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_2S has also been shown to protect freshly prepared mouse islet cells against apoptosis induced by palmitate, H_2O_2 and cytokine mixture (TNF-α, IL-1β and INF-δ) but did not protect against thapsigarin 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_2S 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 and phosphorylation of ERK was abolished following pre-treatment with Mibefadril, a T-type Ca^{2+} channel blocker. In the caerulein induced pancreatitis model, the prevention of pancreatitis associated allodynia/hyperalgesia has been reported by pre-treatment with a CSE blocker (PAG) and treatment with a T-type Ca^{2+} channel blocker (Mibefadril) (35).

4. H_2S regulates the severity of inflammatory response in acute pancreatitis and associated lung injury

Caerulein induced acute pancreatitis upregulates CSE expression and H_2S production whereas

inhibition of endogenous H_2S 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). H_2S donor, NaHS provoked inflammation in both mouse pancreatic acinar cells (46) and primary human monocyte cells (68). Therefore, observations using H_2S donor in murine cells could still be extrapolated to human cells, and may provide useful guidance on the potential therapeutic benefits of using H_2S in inflammatory conditions.

Studies have suggested that H_2S has both pro and anti-inflammatory properties, depending largely on the experimental conditions and the cell type under scrutiny. The mechanism through which H_2S 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 H_2S, have a protective effect. On the other hand higher concentrations (100 μM) (46) induce inflammation in the acini. Another H_2S donor ACS15, is a H_2S 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/ H_2S most probably targets T-type Ca(2+) channels, leading to nociception, and that endogenous H_2S produced by CSE and possibly T-type Ca(2+) channels are involved in pancreatitis-related pain (35).

Altering endogenous or exogenous H_2S may have unanticipated effects because H_2S has a wide array of actions for example H_2S 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 H_2S on acute pancreatitis. Although the mechanism of the pro-inflammatory effect of H_2S has not yet been fully investigated, it is likely that H_2S 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 H_2S, 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 H_2S 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 H_2S 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 H_2S biosynthesis by PAG and PPTA deficiency attenuates H_2S induced TLR4 and NF-κB pathway possibly by stimulation of IκB-α phosphorylation (Fig 2) (47).

Recently, we analyzed the mechanism by which H_2S reduces caerulein induced inflammation in pancreatic acini. We found that H_2S 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 H_2S induces inflammation. It is also important to realize that the concentration of H_2S donor used may not necessarily reflect the concentration of H_2S to which the cells are exposed. Equivalent concentrations of different H_2S donors may liberate H_2S to different extents or at different rates. Therefore, the concentration of free H_2S in the vicinity of the cells at any given time may vary, and the H_2S concentration in the system needs to be measured in order to directly compare different H_2S 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 H_2S is a promising candidate for treatment or prevention of inflammatory conditions. Further studies are required to elucidate completely the mechanism of action of H_2S on inflammation, in order to identify potential targets for the treatment of human inflammatory conditions and to evaluate the sources of H_2S that provide greatest therapeutic potential.

Summary

H_2S 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. H_2S has been found to have both physiological and pathological roles in the pancreas. Currently there have been multiple conflicting reports on the effect of H_2S which could be largely attributed to the source and dose of H_2S studied.

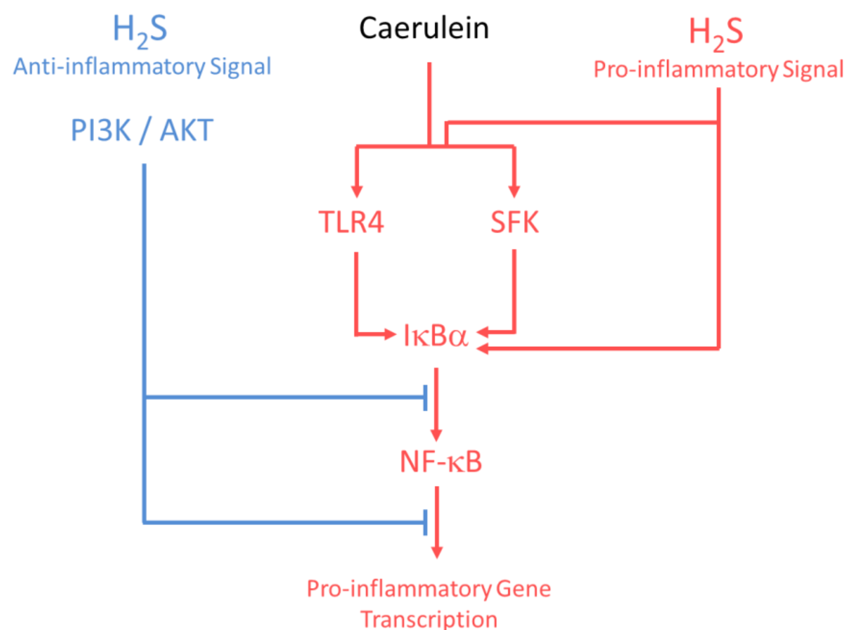

Figure 2. Based on our findings, this overview shows that H_2S (5μM) induced (PI3K/AKT) anti-inflammatory signals down-regulate proinflammatory gene induction by abrogating caerulein-induced IkBα degradation and thus NF-kB nuclear translocation. On the contrary, H_2S at 100μM concentration stimulates SFK, TLR4 phosphorylation, IkBα degradation and thus nuclear translocation of NF-kB to up-regulate proinflammatory gene induction.

3. Tools for Study of H_2S

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_2S donor compounds***
    a. NaHS (Compound ID: 28015)
    b. Na_2S (Compound ID: 237873)
    c. SPRC (US Patent application – US 2009/0036534 A1
    d. GYY4137 (30)
6. ***PCR primers (24)***
    a. Mouse Cystathionine-γ-lyase
    Forward 5' – ATG GAT GAA GTG TAT GGA GG -3'
    Reverse 5′ -ACG AAG CCG ACT ATT 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_2S***
    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

1. **Abe K, Kimura H.** The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* 16: 1066-1071,1996. PMID: 8558235
2. **Ali MY, Whiteman M, Low CM, Moore PK.** Hydrogen sulphide reduces insulin secretion from HIT-T15 cells by a KATP channel-dependent pathway. *J Endocrinol* 195: 105-112, 2007. PMID: 17911402
3. **Andruski B, McCafferty DM, Ignacy T, Millen B, McDougall JJ.** Leukocyte trafficking and pain behavioral responses to a hydrogen sulfide donor in acute monoarthritis. *Am J Physiol Regul Integr Comp Physiol* 295: R814-R820, 2008. PMID: 18667709
4. **Bao L, Vlcek C, Paces V, Kraus JP.** Identification and tissue distribution of human cystathionine beta-synthase mRNA isoforms. *Arch Biochem Biophys* 350: 95-103,1998. PMID: 9466825
5. **Beauchamp RO Jr, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA.** A critical review of the literature on hydrogen sulfide toxicity. *Critical Reviews in Toxicology* 13: 25-97, 1984. PMID: 6378532

6. **Bhatia M, Sidhapuriwala JN, Sparatore A, Moore PK.** Treatment with H_2S-releasing diclofenac protects mice against acute pancreatitis-associated lung injury. *Shock* 29: 84-88, 2008. PMID: 17621252
7. **Bhatia M, Sidhapuriwala JN, Ng SW, Tamizhselvi R, Moochhala SM.** Pro-inflammatory effects of hydrogen sulphide on substance P in caerulein-induced acute pancreatitis. *J Cell Mol Med* 12: 580-590, 2008. PMID: 18419599
8. **Bhatia M, Wong FL, Fu D, Lau HY, Moochhala SM, Moore PK.** Role of hydrogen sulfide in acute pancreatitis and associated lung injury. *FASEB J* 19: 623-625, 2005. PMID: 15671155
9. **Bian JS, Yong QC, Pan TT, Feng ZN, Ali MY, Zhou S, Moore PK.** Role of hydrogen sulfide in the cardioprotection caused by ischemic preconditioning in the rat heart and cardiac myocytes. *J Pharmacol Exp Ther* 316: 670-678, 2005. PMID: 16204473
10. **Cao Y, Adhikari S, Ang AD, Moore PK, Bhatia M.** Mechanism of induction of pancreatic acinar cell apoptosis by hydrogen sulfide. *Am J Physiol Cell Physiol* 291: C503-C510, 2006. PMID:16597918
11. **Cao YQ, Mantyh PW, Carlson EJ, Gillespie AM, Epstein CJ, Basbaum A.** Primary afferent tachykinins are required to experience moderate to intense pain. *Nature* 392: 390-394, 1998. PMID: 9537322
12. **Dal-Secco D, Cunha TM, Freitas A, Alves-Filho JC, Souto FO, Fukada SY, Grespan R, Alencar NM, Neto AF, Rossi MA, Ferreira SH, Hothersall JS, Cunha FQ.** Hydrogen sulfide augments neutrophil migration through enhancement of adhesion molecule expression and prevention of CXCR2 internalization: role of ATP-sensitive potassium channels. *J Immunol* 181: 4287-4298, 2008. PMID: 18768887
13. **Della Coletta Francescato H, Cunha FQ, Costa RS, Barbosa Júnior F, Boim MA, Arnoni CP, da Silva CG, Coimbra TM.** Inhibition of hydrogen sulphide formation reduces cisplatin-induced renal damage. *Nephrol Dial Transplant* 26: 479-488, 2011. PMID: 20656754
14. **Dello Russo C, Tringali G, Ragazzoni E, Maggiano N, Menini E, Vairano M, Preziosi P, Navarra P.** Evidence that hydrogen sulphide can modulate hypothalamo-pituitary-adrenal axis function: in vitro and in vivo studies in the rat. *J Neuroendocrinol* 12: 225-33, 2000. PMID: 10718918
15. **Distrutti E, Sediari L, Mencarelli A, Renga B, Orlandi S, Antonelli E, Roviezzo F, Morelli A, Cirino G, Wallace JL, Fiorucci S.** Evidence that hydrogen sulfide exerts antinociceptive effects in the gastrointestinal tract by activating KATP channels. *J Pharmacol Exp Ther* 316: 325-335, 2006. PMID: 16192316
16. **Fukushima O, Nishimura S, Matsunami M, Aoki Y, Nishikawa H, Ishikura H, Kawabata A.** Phosphorylation of ERK in the spinal dorsal horn following pancreatic pronociceptive stimuli with proteinase-activated receptor-2 agonists and hydrogen sulfide in rats: Evidence for involvement of distinct mechanisms. *J Neurosci Res* 88: 3198-3205, 2010. PMID: 20806405
17. **Goodwin LR, Francom D, Dieken FP, Taylor JD, Warenycia NW, Reiffenstein RJ, Dowling G.** Determination of sulfide in brain tissue by gas dialysis/ion chromatography: post-mortem studies and two case reports. *J Anal Toxicol* 13:105-109, 1989. PMID: 2733387
18. **Hosoki R, Matsuki N, Kimura H.** The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 237: 527-531, 1997. PMID: 9299397
19. **Ishii I, Akahoshi N, Yamada H, Nakano S, Izumi T, Suematsu M.** Cystathionine gamma-Lyase-deficient mice require dietary cysteine to protect against acute lethal myopathy and oxidative injury. *J Biol Chem* 285: 26358-26368, 2010. PMID: 20566639
20. **Ishii I, Akahoshi N, Yu XN, Kobayashi Y, Namekata K, Komaki G, Kimura H.** Murine cystathionine gamma-lyase: complete cDNA and genomic sequences, promoter activity, tissue distribution and developmental expression. *Biochem J* 381: 113-123, 2004. PMID: 15038791
21. **Johansen D, Ytrehus K, Baxter GF.** Exogenous hydrogen sulfide (H_2S) protects against regional myocardial ischemia-reperfusion injury--Evidence for a role of K ATP channels. *Basic Res Cardiol* 101: 53-60, 2006. PMID: 16328106
22. **Kamoun P.** Endogenous production of hydrogen sulphide in mammals. *Amino Acids* 26: 243-254, 2004. PMID: 15221504
23. **Kaneko Y, Kimura T, Taniguchi S, Souma M, Kojima Y, Kimura Y, Kimura H, Niki I.** Glucose-induced production of hydrogen sulfide may protect the pancreatic beta-cells from apoptotic cell death by high glucose. *FEBS Lett* 583: 377-382, 2009. PMID: 19100738
24. **Kaneko Y, Kimura Y, Kimura H, Niki I.** L-cysteine inhibits insulin release from the pancreatic beta-cell: possible involvement of metabolic production of hydrogen sulfide, a novel gasotransmitter. *Diabetes* 55: 1391-1397, 2006. PMID: 16644696
25. **Kery V, Bukovska G, Kraus JP.** Transsulfuration depends on heme in addition to pyridoxal 5'-phosphate. Cystathionine beta-synthase is a heme protein. *J Biol Chem* 269: 25283-25288, 1994. PMID: 7929220

26. **Kimura Y, Kimura H.** Hydrogen sulfide protects neurons from oxidative stress. *FASEB J* 18: 1165-1167, 2004. PMID: 15155563
27. **Laggner H, Muellner MK, Schreier S, Sturm B, Hermann M, Exner M, Gmeiner BM, Kapiotis S.** Hydrogen sulphide: a novel physiological inhibitor of LDL atherogenic modification by HOCl. *Free Radic Res* 41: 741-747, 2007. PMID: 17577734
28. **Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, Wang ZJ, Anuar FB, Whiteman M, Salto-Tellez M, Moore PK.** Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *FASEB J* 19: 1196-1198, 2005. PMID: 15863703
29. **Li L, Rossoni G, Sparatore A, Lee LC, Del Soldato P, Moore PK.** Anti-inflammatory and gastrointestinal effects of a novel diclofenac derivative. *Free Radic Biol Med* 42: 706-719, 2007. PMID: 17291994
30. **Li L, Salto-Tellez M, Tan CH, Whiteman M, Moore PK.** GYY4137, a novel hydrogen sulfide-releasing molecule, protects against endotoxic shock in the rat. *Free Radic Biol Med* 47: 103-113, 2009. PMID: 19375498
31. **Matsunami M, Tarui T, Mitani K, Nagasawa K, Fukushima O, Okubo K, Yoshida S, Takemura M, Kawabata A.** Luminal hydrogen sulfide plays a pronociceptive role in mouse colon. *Gut* 58: 751-761, 2009. PMID: 18852258
32. **Mitsuhashi H, Yamashita S, Ikeuchi H, Kuroiwa T, Kaneko Y, Hiromura K, Ueki K, Nojima Y.** Oxidative stress-dependent conversion of hydrogen sulfide to sulfite by activated neutrophils. *Shock* 24: 529-534, 2005. PMID: 16317383
33. **Mok YY, Moore PK.** Hydrogen sulphide is pro-inflammatory in haemorrhagic shock. *Inflam Res* 57: 512-518, 2008. PMID: 19109743
34. **Moore PK, Bhatia M, Moochhala S.** Hydrogen sulfide: from smell of the past to the mediator of the future. *Trends Pharmacol Sci* 24: 609-611, 2003. PMID: 14654297
35. **Nishimura S, Fukushima O, Ishikura H, Takahashi T, Matsunami M, Tsujiuchi T, Sekiguchi F, Naruse M, Kamanaka Y, Kawabata A.** Hydrogen sulfide as a novel mediator for pancreatic pain in rodents. *Gut* 58: 762-770, 2009. PMID: 19201768
36. **Ogasawara Y, Isoda S, Tanabe S.** Tissue and subcellular distribution of bound and acid labile sulfur, and the enzymic capacity for sulphide production in the rat. *Biol Pharm Bull* 17: 1535-1542, 1994. PMID: 7735193
37. **Prentki M, Matschinsky FM.** Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol Rev* 67: 1185-1248, 1987. PMID: 2825225
38. **Quéré I, Paul V, Rouillac C, Janbon C, London J, Demaille J, Kamoun P, Dufier JL, Abitbol M, Chassé JF.** Spatial and temporal expression of the cystathionine beta-synthase gene during early human development. *Biochem Biophys Res Commun* 254: 127-137, 1999. PMID: 9920745
39. **Sidhapuriwala JN, Ng SW, Bhatia M.** Effects of hydrogen sulfide on inflammation in caerulein-induced acute pancreatitis. *J Inflamm (Lond)* 6: 35, 2009. PMID: 20040116
40. **Sivarajah A, Collino M, Yasin M, Benetti E, Gallicchio M, Mazzon E, Cuzzocrea S, Fantozzi R, Thiemermann C.** Anti-apoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial I/R. *Shock* 31: 267-274, 2009. PMID: 18636044
41. **Sodha NR, Clements RT, Feng J, Liu Y, Bianchi C, Horvath EM, Szabo C, Stahl GL, Sellke FW.** Hydrogen sulfide therapy attenuates the inflammatory response in a porcine model of myocardial ischemia/reperfusion injury. *J Thorac Cardiovasc Surg* 138: 977-984, 2009. PMID: 19660398
42. **Stipanuk MH, Beck PW.** Characterization of the enzymic capacity for cysteine desulphydration in the liver and kidney of the rat. *Biochem J* 206: 267-277, 1982. PMID: 7150244
43. **Takahashi T, Aoki Y, Okubo K, Maeda Y, Sekiguchi F, Mitani K, Nishikawa H, Kawabata A.** Upregulation of Ca(v)3.2 T-type calcium channels targeted by endogenous hydrogen sulfide contributes to maintenance of neuropathic pain. *Pain* 150: 183-191, 2010. PMID: 20546998
44. **Tamizhselvi R, Koh YH, Sun J, Zhang H, Bhatia M.** Hydrogen sulfide induces ICAM-1 expression and neutrophil adhesion to caerulein-treated pancreatic acinar cells through NF-kappaB and Src-family kinases pathway. *Exp Cell Res* 316: 1625-1636, 2010. PMID: 20211170
45. **Tamizhselvi R, Moore PK, Bhatia M.** Inhibition of hydrogen sulfide synthesis attenuates chemokine production and protects mice against acute pancreatitis and associated lung injury. *Pancreas* 36: e24-e31, 2008. PMID: 18437075
46. **Tamizhselvi R, Moore PK, Bhatia M.** Hydrogen sulfide acts as a mediator of inflammation in acute pancreatitis: in vitro studies using isolated mouse pancreatic acinar cells. *J Cell Mol Med* 11: 315-326, 2007. PMID: 17488480

47. **Tamizhselvi R, Shrivastava P, Koh YH, Zhang H, Bhatia M.** Preprotachykinin-a gene deletion regulates hydrogen sulfide-induced toll-like receptor 4 signaling pathway in cerulein-treated pancreatic acinar cells. *Pancreas* 40: 444-452, 2011. PMID: 21289528
48. **Tamizhselvi R, Sun J, Koh YH, Bhatia M.** Effect of hydrogen sulfide on the phosphatidylinositol 3-kinase-protein kinase B pathway and on caerulein-induced cytokine production in isolated mouse pancreatic acinar cells. *J Pharmacol Exp Ther* 329: 1166-1177, 2009. PMID: 19258518
49. **Taniguchi S, Kang L, Kimura T, Niki I.** Hydrogen sulphide protects mouse pancreatic beta-cells from cell death induced by oxidative stress, but not by endoplasmic reticulum stress. *Br J Pharmacol* 162: 1171-1178, 2011. PMID: 21091646
50. **Ubuka T.** Assay methods and biological roles of labile sulfur in animal tissues. *J Chromatogr B Analyt Technol Biomed Life Sci* 781: 227-249, 2002. PMID: 12450661
51. **Wallace JL, Dicay M, McKnight W, Martin GR.** Hydrogen sulfide enhances ulcer healing in rats. *FASEB J* 21: 4070-4076, 2007. PMID: 176343
52. **Wallace JL, Vong L, McKnight W, Dicay M, Martin GR**. Endogenous and exogenous hydrogen sulfide promotes resolution of colitis in rats. *Gastroenterology* 137: 569-578, 2009. PMID: 19375422
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
54. **Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Cheung NS, Halliwell B, Moore PK.** The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger'?. *J Neurochem* 90: 765-768, 2004. PMID: 15255956
55. **Whiteman M, Cheung NS, Zhu YZ, Chu SH, Siau JL, Wong BS, Armstrong JS, Moore PK.** Hydrogen sulphide: a novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain?. *Biochem Biophys Res Commun* 326: 794-798, 2005. PMID: 15607739
56. **World Health Organization.** Hydrogen Sulfide: Human Health Aspects. Concise International Chemical Assessment Document 53. Stuttgart, Germany, 2003.
57. **Wu L, Yang W, Jia X, Yang G, Duridanova D, Cao K, Wang R**. Pancreatic islet overproduction of H_2S and suppressed insulin release in Zucker diabetic rats. *Lab Invest* 89: 59-67, 2009. PMID: 19002107
58. **Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R.** H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 587-590, 2008. PMID: 18948540
59. **Yang G, Yang W, Wu L, Wang R.** H2S, endoplasmic reticulum stress, and apoptosis of insulin-secreting beta cells. *J Biol Chem* 282: 16567-16576, 2007. PMID: 17430888
60. **Yang W, Yang G, Jia X, Wu L, Wang R.** Activation of KATP channels by H_2S in rat insulin-secreting cells and the underlying mechanisms. *J Physiol* 569: 519-531, 2005. PMID: 16179362
61. **Yao LL, Huang XW, Wang YG, Cao YX, Zhang CC, Zhu YC.** Hydrogen sulfide protects cardiomyocytes from hypoxia/reoxygenation-induced apoptosis by preventing GSK-3beta-dependent opening of mPTP. *Am J Physiol Heart Circ Physiol* 298: H1310-H1319, 2010. PMID: 20154265
62. **Yong QC, Lee SW, Foo CS, Neo KL, Chen X, Bian JS.** Endogenous hydrogen sulphide mediates the cardioprotection induced by ischemic postconditioning. *Am J Physiol Heart Circ Physiol* 295: H1330-H1340, 2008. PMID: 18660450
63. **Yusuf M, Kwong Huat BT, Hsu A, Whiteman M, Bhatia M, Moore PK.** Streptozotocin-induced diabetes in the rat is associated with enhanced tissue hydrogen sulfide biosynthesis. *Biochem Biophys Res Commun* 333: 1146-1152, 2005. PMID: 15967410
64. **Zanardo RC, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL.** Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEB J* 20: 2118-2120, 2006. PMID: 16912151
65. **Zhang H, Zhi L, Moochhala S, Moore PK, Bhatia M.** Hydrogen sulfide acts as an inflammatory mediator in cecal ligation and puncture-induced sepsis in mice by upregulating the production of cytokines and chemokines via NF-kappaB. *Am J Physiol Lung Cell Mol Physiol* 292: L960-L971, 2007. PMID: 17209138
66. **Zhang J, Sio SW, Moochhala S, Bhatia M.** Role of hydrogen sulfide in severe burn injury-induced inflammation in mice. *Mol Med* 16: 417-424, 2010. PMID: 20440442
67. **Zhao W, Zhang J, Lu Y Wang R.** The vasorelaxant effect of H_2S as a novel endogenous KATP channel opener. *EMBO J* 20: 6008-6016, 2001. PMID: 11689441
68. **Zhi L, Ang AD, Zhang H, Moore PK, Bhatia M.** Hydrogen sulfide induces the synthesis of proinflammatory cytokines in human monocyte cell line U937 via the ERK-NF-kappaB pathway. *J Leukoc Biol* 81: 1322-1332, 2007. PMID: 17289797