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Hydrogen Sulfide's Involvement in Modulating Nociception

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Hydrogen sulfide (H_2S) is a malodorous gas which functions as an endogenous gasotransmitter in humans. It is becoming appreciated that H_2S may be involved in a wide variety of processes including nociceptive processes. The molecular mechanisms responsible for many of the activities of H_2S remain uncertain, however, H_2S increases cAMP levels in neuronal and glial cell lines and primary neuron cultures with hyperpolarization. H_2S may be involved in multiple signaling pathways and produce various effects on ion channels (e.g. T-type calcium channel currents, ATP-sensitive K+ (K_{ATP}) channels) which may inhibit or promote nociception. It is also conceivable that H_2S may affect the n-methyl-d aspartate (NMDA) receptor complex and/or TRPA1 ion channels which may modulate nociceptive processes. It appears that H_2S may regulate key neuronal functions, including the induction of hippocampal long-term potentiation, a synaptic model of learning and memory thought to involve the NMDA receptor as well as the release of corticotrophin-releasing hormone from the hypothalamus. It seems that the primary role of H_2S in nociceptive processes is the activation of T-type calcium channels leading to facilitation of pronociceptive processes. A secondary contribution to the facilitation of pronociceptive processes may come from H_2S-induced activation. It would appear that much like other gasotransmitters (e.g. nitric oxide), endogenous H_2S may be involved in multiple physiologic processes and its effects remain complex, difficult to predict, and may vary depending on the specific environment/circumstances/location where it is generated.

A greater understanding of the clinically significant human physiology of H_2S and hydrogen sulfide's effects on modulating nociceptive processes may potentially lead to novel targets for improving analgesia.

Key words: Pain, nociception, hydrogen sulfide, calcium channels, analgesia, potassium channels

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Hydrogen sulfide (H_2S), an endogenous gasotransmitter, is generated from L-cysteine by cystathionine-β-synthase (CBS) and/or cystathionine-γ-lyase (CSE) (1,2), and is also produced by sulfate-reducing bacteria (SRB), members of the normal enterobacteria, in the intestinal lumen (3). Several studies demonstrate a relationship between colonic luminal SRB-derived H_2S and the development of the inflammatory bowel disease (IBD) or colorectal cancer (3-5).

NaHS, an H_2S donor, activates neuronal and vascular ATP-sensitive K^+ (K_{ATP}) channels (1,6,7) and also sensitizes NMDA receptors (1). A cross talk between H_2S and NO has also been recently described (8-10). Most recently, the Kawabata Laboratory has provided electrophysiological evidence that NaHS enhances T-type Ca^{2+} currents (T-currents) through redox modulation in NG108-15 cells, and have shown that intraplantar (i.pl.) injection of NaHS produces mechanical hyperalgesia in rat hindpaw through activation of T-type Ca^{2+} channels (11). These findings are consistent with previous evidence for redox modulation of $Ca_v3.2$, a form of T-type Ca^{2+} channels, and its involvement in activation of primary nociceptive neurons (12-15). Several reports have recently shown H_2S-evoked activation of capsaicin-sensitive sensory neurons in the

guinea-pig and human colon (2), rat urinary bladder (16) and guinea-pig airway (17) supporting a pro-nociceptive role for H_2S.

Gasotransmitters

Gasotransmitters are a growing family of regulatory molecules involved in regulation of physiological and pathological functions in mammalian tissues (1,18).

In some tissues, CSE and CBS are both needed for generation of H_2S, whereas in others one enzyme suffices. The expression of CBS and CSE has been identified in several mammalian tissues, including liver, kidney, brain, ileum, and blood lymphocytes. In the cardiovascular system, H_2S, mostly derived from CSE, modulates endothelium-dependent and endothelium-independent vasodilatation (1,19), whereas CBS-derived H_2S is a physiologically relevant neuromodulator in the central nervous system (CNS) (1,18). Consistent with this view, it has been shown that H_2S is present at relatively high levels in the mammalian brain and that, in the CNS, the activity of CBS is >30-fold greater than that of CSE (20). In addition, the reduced H_2S production after inhibition of CBS and the fact that CSE inhibitors do not suppress H_2S production in the CNS further pinpoint CBS to be the major H_2S-producing enzyme in neural tissues (21).

H_2S regulates key neuronal functions, including the induction of hippocampal long-term potentiation, a synaptic model of learning and memory (21,22), and the release of the corticotropin-releasing hormone from the hypothalamus (23). Although the molecular mechanisms involved in these activities are only partially known, it has been shown that H_2S increases cAMP levels in neuronal and glial cell lines and primary neuron cultures and hyperpolarizes dorsal raphe neurons by activating the ATP-sensitive K^+(K_{ATP}) channels. In addition, H_2S causes a cAMP-dependent potentiation of *N*-methyl-D-aspartate receptors (24). Previous studies have shown that, at low concentrations, H_2S enhances the smooth muscle relaxation effect of nitric oxide (NO), suggesting that a crosstalk between the 2 gases may exist (25). The NO donor sodium nitroprusside enhances brain CBS activity in vitro (26).

H_2S generating enzymes CSE and CBS are expressed in the spinal cord and colon and that detectable amounts of H_2S are produced by these tissues in presence of L-cysteine, a CSE/CBS substrate. Furthermore, the antinociceptive and relaxant actions of L-cysteine are inhibited by DL-propargylglycine (PPG), a CSE inhibitor, suggesting that generation of H_2S mediates the effect of L-cysteine (27). Distrutti et al (27) also found that H_2S administration decreased spinal cord expression of c-Fos mRNA. Yang et al (28) have established CSE as the physiologic source of H_2S in multiple tissues, especially the vascular system.

Hydrogen Sulfide's Potential Role in the Modulation of Nociception

H_2S is a malodorous gas, well known as the agent contributing to the olfactory experience of exposure to rotten eggs. It seems clear that H_2S is an irritant (especially to the head, eyes, and airways). H_2S may function as a mammalian endogenous gasotransmitter [along with nitric oxide (NO) and carbon monoxide (CO)] and modulate multiple physiologic processes.

Lee et al (29) showed that formalin caused a concentration-dependent increase in nociceptive flinching which effect peaked at 5% formalin. Hind paw H_2S concentrations was also elevated at the peak of nociceptive behavior following intraplantar injection of 5% formalin but not following injection of 1.25% formalin (a dose which does not give rise to an increase in H_2S). Moreover, hind paw injection of the H_2S donor NaHS, together with 1.25% formalin, increased the nociceptive behavior during the second phase to a level seen with 5% formalin (29). Pretreatment of rats with PPG, at a dose that blocks H_2S synthesis reduced the formalin (5%) induced nociceptive behavior. Consistent with the lack of elevated hind paw H_2S at 1.25%, PPG did not influence the nociceptive behavior observed with that concentration, or with the low concentration of 0.625% formalin tested in the study (29).

Furthermore, PPG pretreatment attenuated the induction of c-Fos in spinal laminae I–II following injection of 5% formalin. In contrast, co-injection of 1.25% formalin with sodium hydrogen sulfide (NaHS; 1 nmol/0.1 mL), a H_2S donor, into the hind paw increased animal nociceptive behavior (29).

H_2S and T-Type Calcium Channels

Jevtovic-Todorovic and Todorovic (30) have reviewed the role of peripheral T-type calcium channels in pain transmission. Jevtovic-Todorovic and Todorovic examined evidence obtained using an array of techniques such as electrophysiological recordings, pharmacological behavior experiments as well as molecular techniques that strongly supports the role of peripheral T-type Ca^{2+} channels in boosting nocicep-

tive transmission in a variety of experimental pain modules (30).

In relatively small neurons (approximately <30 nm in diameter) prepared from mouse lower thoracic and upper lumbar dorsal root ganglion neurons that would respond to colonic stimulation, typical T-currents were observed (31). The T-currents were significantly enhanced by addition of NaHS at 1.5 mM, but not 0.5 or 3 mM, leading to a bell-shaped dose-response curve (31), while high voltage-activated calcium currents were resistant to NaHS. In the presence of mibefradil at 1 μM, NaHS at 1.5 mM failed to facilitate T-currents; i.e. mibefradil significantly ($P < 0.01$) suppressed the T-currents enhanced by NaHS (31).

Among 3 forms of T-type Ca^{2+} channels, $Ca_v3.1$–3.3, only $Ca_v3.2$ is sensitized by distinct reducing agents (14). Therefore, $Ca_v3.2$ that plays a major pro-nociceptive role in sensory neurons (15) is the most probable target molecule for H_2S/NaHS, since the pro-nociceptive effects of i.col. NaHS were abolished by the T-type Ca^{2+} channel inhibitor mibefradil and the oxidant 5, 5′-dithio-bis-(2-nitrobenzoic acid) [DTNB] (31). The most recent study has demonstrated that reducing agents such as dithiothreitol and L-cysteine relieve Zn^{2+}, inhibition of $Ca_v3.2$ by chelating Zn^{2+}, leading to nociceptor sensitization (32). This novel mechanism for sensitization of $Ca_v3.2$ may contribute to the pro-nociceptive roles for H_2S/NaHS in the colonic lumen, because H_2S/NaHS is also a strong Zn^{2+}, chelator (31).

Like capsaicin, NaHS, administered intracolonic (i.col.) at 0.5-5 nmol/mouse, triggered visceral nociceptive behavior accompanied with referred allodynia/hyperalgesia in mice (31). Phosphorylation of extracellular signal-regulated kinase (ERK) in the spinal dorsal horn was detected following i.col. NaHS or capsaicin (31). The behavioral effects of i.col. NaHS were abolished by a T-type channel blocker or an oxidant, but not inhibitors of L-type Ca^{2+} channels or ATP-sensitive K+ (KATP) channels (31). Intraperitoneal NaHS at 60 μmol/kg facilitated i.col. capsaicin-evoked visceral nociception, an effect being abolished by the T-type channel blocker, although it alone produced no behavioral effect. In DRG neurons, T-currents were enhanced by NaHS. Matsunami and colleagues (31) concluded that their findings suggest that colonic luminal H_2S/NaHS plays pro-nociceptive roles, and imply that the underlying mechanisms might involve sensitization/activation of T-type channels probably in the primary afferents, aside from the issue of the selectivity of mibefradil.

L-cysteine (but not D-cysteine), administered intraplantar (i.pl.) at 100 nmol/paw, produced hyperalgesia (similar to NaHS) in rats (11). Intraperitoneal administration of DL-propargylglycine (PPG) at 37.5 mg/kg and β-cyanoalanine (BCA) at 50 mg/kg, inhibitors of CSE, completely blocked the L-cysteine-induced hyperalgesia (11).

A patch-clamp technique was used to examine if H_2S could modulate barium (Ba^{2+}) currents through T-type Ca^{2+} channels in undifferentiated NG108-15 cells where T-type Ca^{2+} channels, but not high-voltage activated Ca^{2+} channels, were functionally detectable (11), as reported elsewhere (33). Like dithiothreitol (DTT), a reducing agent, NaHS facilitated the T-type currents at a test potential of -20 mV, an effect being abolished by DTNB, an oxidizing agent, at a subeffective concentration (11). Suppression of T-type currents by DTNB at a high concentration was reversed by NaHS or DTT at subeffective concentrations (11).

This in vivo study demonstrates the development of hyperalgesia in rat hindpaw following local application of the H_2S donor NaHS into the peripheral tissue (11). The inhibition experiments indicate that the NaHS-evoked hyperalgesia is attributable to redox modulation of T-type Ca^{2+} channels, possibly present in primary afferent neurons. CSE is considered responsible for production of endogenous H_2S in the peripheral tissue, because PPG and/or BCA, inhibitors of CSE, abolished the L-cysteine-induced hyperalgesia and attenuated the LPS-induced hyperalgesia (11). The electrophysiological studies of Kawabata and colleagues (34) in undifferentiated NG108-15 cells demonstrate that NaHS sensitizes T-type Ca^{2+} channels via a reducing effect; suggesting a role for H_2S as a nociceptive gasotransmitter.

The results of Nishimura et al (35) demonstrating a pro-nociceptive role for pancreatic NaHS/H_2S through T-type Ca^{2+} channels are consistent with evidence for NaHS facilitation of T-type Ca^{2+} channel-dependent membrane currents in NG108-15 cells and with evidence for the mibefradil-reversible hyperalgesia following intraplantar administration of NaHS). The finding that NaHS-evoked Fos expression was not clearly dose-dependent is consistent with our previous studies that indicated a bell-shaped dose-response curve for NaHS-evoked somatic and visceral hyperalgesia (34,36). The in vivo effects of NaHS may be characteristic of the action of NaHS on T-type Ca^{2+} channels, because the dose-response curve for NaHS facilitation of T-type Ca^{2+} channel-dependent mem-

brane currents in NG108-15 cells and DRG neurons in vitro is bell-shaped also (34-36). The observation that mibefradil blocked the expression of spinal Fos caused by the ductal application of NaHS, but not capsaicin, indicates distinct mechanisms for nociceptive processing by those 2 agents in terms of the involvement of T-type Ca^{2+} channels (35).

The Kawabata laboratory has also demonstrated that infusion of NaHS into the pancreatic duct (37) [and colonic lumen (36)] triggers behavioral nociception and/or nociceptive signaling in a mibefradil-reversible manner, similar to the pro-nociceptive role played by H_2S for somatic pain (38).

NaHS, administered i.t. at 0.1 nmol/rat, elicited prompt decrease in nociceptive threshold in the i.t. MM-ODN-treated rats, but caused no hyperalgesia in the AS-ODN-Ca_v3.2-treated rats (38). Also, the hyperalgesia induced by i.pl. administration of NaHS at 1 nmol/paw was abolished by i.t. preadministration of AS-ODN-Ca_v3.2. It is noteworthy that i.t. AS-ODNCa_v3.2 itself did not alter nociceptive threshold in rats (38).

The data of Maeda et al demonstrated that i.t. administration of the H_2S donor, NaHS, causes mibefradil-reversible hyperalgesia in rats, as does i.pl. NaHS, implying that H_2S may play a pro-nociceptive role in the spinal cord as well as in the peripheral tissues, the latter evidence being in agreement with the previous reports (34,36,39,40). Inhibition of either i.t. or i.pl. NaHS-induced hyperalgesia by mibefradil, a T-type Ca^{2+} channel blocker, or ZnCl2 is suggestive of the importance of Ca_v3.2 among T-type Ca^{2+} channel isoforms, and the data from the experiments using AS-ODN-Ca_v3.2 supports the involvement of Ca_v3.2 in the pro-nociceptive effects of i.t. and i.pl. NaHS (38).

Molecular mechanisms for sensitization/activation of Ca_v3.2 T-type Ca^{2+} channels by H_2S remain uncertain. A recent study has shown that Ca_v3.2 is tonically inhibited by Zn^{2+} and many of reducing agents are capable of sensitizing/activating Ca_v3.2 T-type Ca^{2+} channels by chelating Zn^{2+} (41). Given that H_2S is a powerful Zn^{2+} chelator, inhibition of H_2S-evoked hyperalgesia by pretreatment with ZnCl2 in the study of Maeda and colleagues (38) suggests the possibility that H_2S might reverse Zn^{2+} inhibition of Ca_v3.2 T-type Ca^{2+} channels, leading to hyperalgesia.

H_2S and $K^+_{(ATP)}$ Channels

Distrutti et al (27,41) have demonstrated that the systemic administration of different H_2S donors inhibits visceral nociception by opening ATP-sensitive potassium channels ($K^+_{(ATP)}$ channels) (27,41,42). Kawabata et al using a different model demonstrated that H_2S exhibits peripheral pronociceptive activity (11,42). This conclusion is largely substantiated by the observation that intraplantar administration of a H_2S donor induces a mechanical hypernociception of fast onset (25 min) and that the inhibition of endogenous H_2S formation reduces lipopolysaccharide (LPS)-induced inflammatory hypernociception (11,42). The mechanisms of H_2S mediation of inflammatory hyperalgesia seem to be dependent on the direct modulation of T-type Ca^{2+} channel activity in nociceptors — independent of $K^+_{(ATP)}$ channels (11,42). Thus, these studies detected discrepant nociceptive roles for the same mediator. Cunha et al (42) used 2 models of mechanical inflammatory hypernociception to provide more evidence that could explain these contradictory findings concerning the nociceptive role of H_2S.

PGE_2 appears to contribute to sensitization by acting directly on receptors present on nociceptor membranes (43). Thus, hypernociception produced by PGE_2 is independent of the production of other inflammatory mediators or cells such as neutrophils (44,45). The role of endogenous H_2S in the recruitment of neutrophils has been demonstrated in many inflammatory models (42,46,47). It seems that increased production of H_2S during inflammation modulates neutrophil rolling and adhesion as well as their locomotion. H_2S may mediate the increase in the expression of intercellular adhesion molecule 1 (ICAM-1) in the endothelial cells of mesenteric vessels induced by LPS challenge in the peritoneal cavity (42). H_2S also promotes more availability of chemokine (C-X-C motif) ligand 2 (CXCR2) receptors on the neutrophil membrane, which may explain the enhancement of macrophage inflammatory protein-2 (MIP-2)-induced neutrophil chemotaxis by H_2S (47). Furthermore, the various effects of H_2S which promote events responsible for neutrophil migration, as described above, was dependent on the activation of $K^+_{(ATP)}$ channel (42).

H_2S production increases during carrageenin-induced rat paw inflammation (46), perhaps from local leukocytes (although this is unknown). Cunha et al has shown that the activation of neutrophils with MIP-2/(CXCL2), a CXCR1/2 ligand, induces H_2S production, suggesting that H_2S could be produced in the inflammatory focus by migrating leukocytes (42).

The activation of $K^+_{(ATP)}$ channels in the peripheral nociceptive system has been shown to be involved in the modulation of nociception (48,49). For instance,

peripheral antinociceptive drugs that directly block ongoing hypernociception induced by PGE_2, such as morphine and dipyrone, exert their effects by opening $K^+_{(ATP)}$ channels stimulated by the L-arginine/nitric oxide synthase (NOS)/NO/cyclic guanosine monophosphate (cGMP) antinociceptive pathway (42,48-51). Concerning the H_2S system, several biological effects produced by this gas have been attributed to the regulation of $K^+_{(ATP)}$ channel activity (19,52,53). The vasodilator action of H_2S is prevented by the inhibition of this K^+ channel with glibenclamide, suggesting that $K^+_{(ATP)}$ channel activation could be playing a role (19). Cunha et al (42) tested the hypothesis that the antinociceptive effect of H_2S on direct hypernociception induced by prostaglandin E_2 (PGE_2) is dependent on $K^+_{(ATP)}$ channels in the periphery. Supporting this hypothesis, glibenclamide prevented the antinociceptive effect of exogenous H_2S in mice and in rats. A possible direct hypernociceptive effect of glibenclamide was excluded, as glibenclamide administration alone in the rat paw did not produce mechanical hypernociception (51). Further supporting these findings, local administration of a $K^+_{(ATP)}$ channel opener also directly blocks hypernociception induced by PGE_2 (42,52). Electrophysiologically, it has been shown that $K^+_{(ATP)}$ channel activation reduces the enhanced excitability of rat nociceptive sensory neurons induced by PGE_2 (42,54).

A key event in inflammation is the recruitment of circulating leukocytes into the damaged tissue. Use of intravital fluorescence microscopy to visualize leukocyte-endothelial cell interactions in vivo has revealed a complex series of stages in which engaged leukocytes undergo rolling, adhesion, and finally emigration through microvascular fenestrations (55,56). A few studies have reported these phenomena in joint tissues (57-59); however, the surgical approaches employed in these experiments were fairly invasive, involving the exposure of the intraarticular environment. Andruski et al (60) used a minimally invasive procedure, where only the overlying skin was removed, thereby leaving the joint microvasculature completely intact. By focusing on a region of the joint capsule with its underlying synovium, leukocytes could clearly be seen to roll and adhere to the endothelium of kaolin/carrageenan-inflamed knees, although cellular extravasation was not readily discernible in this preparation. Saline-injected control joints did not show any of the typical signs of leukocyte activation (60). Thus intravital fluorescence microscopy appears to be a robust and reproducible means of assessing leukocyte behavior in an intact rodent knee joint (60).

Cunha and colleagues (42) have provided data which reveals the importance of H_2S system in the modulation of the nociceptive process mainly during the inflammatory process. Cunha et al (42) suggest that H_2S may play a dual role in inflammatory hypernociception. Endogenously produced H_2S acts predominantly on neutrophil/endothelium adhesion, enhancing this process, and is consequently involved in the cascade of events leading to neutrophil migration and inflammatory hypernociception (42).

Andruski and colleagues (60) utilized intravital fluorescence microscopy to assess leukocyte behavior in an intact rodent knee joint and concluded that local treatment of acutely inflamed knee joints with an H_2S donor reduced leukocyte recruitment and trafficking, as well as decreased synovial blood flow. These anti-inflammatory effects of H_2S were mediated via the K_{ATP} channel, since responses could be blocked by glibenclamide treatment. Intraarticular administration of NaHS had no effect on joint pain sensation nor secondary allodynia in the rat, although this observation needs to be corroborated in other animal species. Thus, it is conceivable that H_2S may function as an endogenous regulator of joint function and whose action is distinctly anti-inflammatory (60). However, exogenously administered H_2S acts on sensitive neurons promoting the opening of $K^+_{(ATP)}$ channels and consequently antinociception (42).

H_2S and TRPA1 Ion Channels

It is conceivable that another mechanism whereby H_2S may be involved in nociceptive processes is via its potential effects on TPA1 ion channels. Macpherson et al (61) noted that many TRPA1-activating compounds are electrophiles able to react with cysteines. For example, the nucleophilic mercapto group of cysteines can attack the a,b-unsaturated bond of cinnamaldehyde (CA) via a Michael addition. In support of this mechanism, a more reactive cinnamaldehyde-like Michael acceptor with a carbonyl substitution adjacent to the enone (62) [a substituted oxindole, here referred to as super cinnamaldehyde, (SC)] is also a more potent activator of TRPA1. Interestingly, chemically inert structural analogues of TRPA1 agonists, such as propionaldehyde, cinnamic alcohol, and SC alcohol, do not activate TRPA1 (63,64). Other activators including isothiocyanates such as mustard oil (MO) could be

conjugated with cysteines via an addition to form dithiocarbamates (65). Macpherson and colleagues (61) predicted that TRPA1 could be activated by covalent binding of electrophiles to cysteines. They tested if structurally unrelated cysteine modifying agents could also activate TRPA1, and found TRPA1 was activated by both the commonly used cysteine-modifying alkylating agent iodoacetamide (IA; a standard reagent in mass spectrometry used to bind covalently with free cysteines to avoid protein aggregation) and a reagent that forms disulphide bonds with cysteines, (2-aminoethyl) methanethiosulphonate (MTSEA) (61,66,67). N-hydroxyl succinimide (NHS), a lysine modifying agent (68), did not activate TRPA1 in calcium imaging experiments at 100 mM (not shown). In vitro, MO, CA, SC, and IA formed adducts with the cysteine-containing tripeptide glutathione (Glu-Cys-Gly). These data indicate that solvent-accessible cysteine residues in TRPA1 might be covalently modified by these reactive compounds (61).

In the rat bladder, TRPA1 is present on unmyelinated sensory nerve fibres that express TRPV1, and the sensory neuropeptides calcitonin gene-related peptide (CGRP) and substance P (69). Previous investigations have found that TRPA1 is present on a subpopulation of TRPV1-expressing neuronal cell bodies in rodent dorsal root and trigeminal ganglia (71-73). Streng et al (69) have demonstrated a complete colocalisation of TRPA1 and TRPV1 in rat bladder afferents.

The inflammatory mediator H_2S has been shown to activate capsaicin-sensitive sensory neurons in rat isolated urinary bladder by a mechanism yet to be defined (73). Streng et al (69) have shown that H_2S, similar to cinnamaldehyde (CA), stimulated the micturition reflex after protamine sulfate pretreatment, which is in agreement with recruitment of capsaicin-sensitive sensory nerve fibres. Streng and colleagues (69) showed that H_2S caused activation of human and mouse TRPA1, providing TRPA1 as a likely molecular target for H_2S in the bladder. Bladder inflammation may be triggered by TRPA1 activation (74,75) and potential pathogens (e.g. Escherichia coli) can produce H_2S (76). H_2S may conceivably contribute to the symptoms, including urgency and pain, during lower urinary tract infection (69).

Overexposure to zinc can cause pain and inflammation through unknown mechanisms. Hu and colleagues (77) show that zinc excites nociceptive somatosensory neurons and causes nociception in mice through TRPA1. Zinc activates TRPA1 through a unique mechanism that requires zinc influx through TRPA1 channels and subsequent activation via specific intracellular cysteine and histidine residues (77). TRPA1 is highly sensitive to intracellular zinc, as low nanomolar concentrations activate TRPA1 and modulate its sensitivity (77). It is conceivable that H_2S may ameliorate nociceptive input from zinc-induced TPA1 activation via zinc chelation; however, this theoretic role would be expected to be minor at best.

A Global View of H_2S Effects on the Modulation of Nociception

The precise actions of H_2S in modulating nociceptive processes in the human body remain incompletely elucidated and H_2S appears to exhibit multiple functions. H_2S may be involved in multiple signaling pathways and produce various effects on ion channels (e.g. T-type calcium channel currents, ATP-sensitive K^+ [K_{ATP}] channels) which may promote or inhibit nociception. It is also possible that H_2S may affect the n-methyl-d aspartate (NMDA) receptor complex (79) and/or TRPA1 ion channels which may modulate nociceptive processes. Additionally, H_2S may be involved in the accumulation of neutrophils which may further fuel nociceptive processes.

Despite the fact that H_2S may exhibit multiple functions in a variety of environments, it appears that the predominant role of H_2S in nociception is the activation T-type calcium channels leading to facilitation of pronociceptive processes. In certain circumstances/locations (e.g. bladder) the activation of TRPA1 may also play a key role in facilitation of pronociceptive processes. In most settings the activation of K_{ATP} channels by H_2S appears to have relatively minor contributions to the modulation of nociceptive processes (Fig. 1). In fact, there is evidence that pretreatment with glibenclamide, an inhibitor of K_{ATP} channels, does not modify i.pl. NaHS-elicited hyperalgesia in rats (34). Furthermore, although H_2S may activate KATP channels, which play a role in suppression of nociception (27), such an action of H_2S, if any, would be expected to be overcome by its pro-nociceptive action through T-type Ca^{2+} channels (35).

Summary

H_2S is a maloderous gas functioning as an endogenous gasotransmitter. Currently, its precise roles are not well defined, and it appears to exhibit multiple functions. It would appear that much like other gasotransmitters (e.g. nitric oxide), endogenous H_2S may

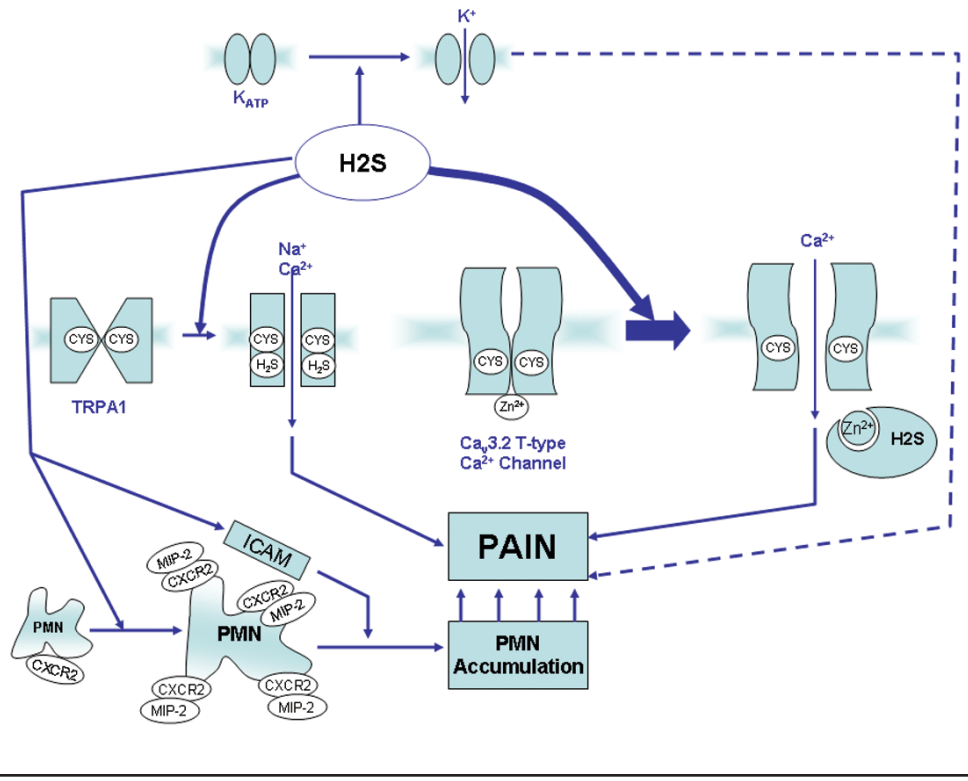

Fig. 1. *Schematic of potential roles of H_2S in the modulation of nociception.*

be involved in multiple physiologic processes and its effects remain complex, difficult to predict, and may vary depending on the specific environment/circumstances/location where it is generated.

Although, talk of hydrogen sulfide-based drugs and H_2S inhibitors may be premature for practioners, interest in the clinical potential of the rotten egg gas in other areas of medicine is growing. It has been described to exhibit cardioprotective roles (79), lower metabolic rate (80), contribute to long-term potentiation (78), and act as a mediator of human corpus cavernosum smooth-muscle relaxation (81).

It appears that the predominant role of H_2S in terms of its effects on nociception is the activation of T-type calcium channels leading to facilitation of pronociceptive processes. A secondary contribution to the facilitation of pronociceptive processes may come from H_2S-induced activation of TRPA1.

Thus, inhibition of endogenous generation of H_2S might serve as a novel therapeutic strategy for the treatment of certain clinical pain states. A better understanding of clinically significant hydrogen sulfide physiology — especially as it pertains to the modulation of nociception may help alleviate patient discomfort in certain circumstances.

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