

Synthesis of H₂S in the Gastrointestinal Tract

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ABSTRACT

Hydrogen sulfide (H₂S) is the third gaseous signal molecule with interest which is endogenously generated in gastrointestinal tract. H₂S play important physiological and pathophysiological roles in regulation of gastrointestinal functions, for example, gastrointestinal motility, secretion and nociception. In the present review we have mainly reviewed the recent findings about the study of H₂S in regulation of gastrointestinal motility and its mechanism. The effect of H₂S on gastrointestinal smooth muscle shows difference in different concentration of H₂S and different regions. In gastric smooth muscle, H₂S exhibits dual effects, that is, excitatory (low concentration) and inhibitory (high concentration) effects via suppressing voltage-dependent potassium channels and activating ATP-sensitive potassium channels (K_{ATP}), respectively, in guinea-pig and mouse. In intestinal smooth muscle, NaHS induced a biphasic effect, that is, a transient excitatory effect followed a long-lasting inhibitory effect in rat. The excitatory effect is mediated by TRPV1 and NK1 receptors and the inhibitory effect is mediated by K_{ATP}. In colonic smooth muscle, H₂S induces inhibitory effect mediated by both K_{ATP} and SK_{Ca} channels in human, rat and mouse. This review gives a synopsis of the H₂S function in regulation of gastrointestinal motility and its mechanism.

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Key words: Gastrointestinal motility; Hydrogen sulfide (H₂S); Voltage-dependent potassium channel; ATP sensitive potassium channel

INTRODUCTION

Hydrogen sulfide (H₂S) has been regarded as “toxic gas” with an odor of “rotten eggs” for hundreds of years. In 1996, Abe and Kimura^[1] found that endogenous H₂S maybe functioned as a neuromodulator during induction of long-term potentiation in the hippocampus, which brought about a new perception of H₂S. Since then more and more studies have emerged to explore the possible physiological and pathophysiological roles of endogenous H₂S. And so far H₂S has been proved to be involved in many physiological and pathophysiological processes such as vasodilation^[2], angiogenesis^[3,4], pro-^[5,6] or anti-inflammation^[7,8] and cytoprotection^[9,10], which makes it to be regarded as the third gasotransmitter along with nitric oxide (NO) and carbon monoxide (CO)^[11], although others consider “gaseous signaling molecule” to be more accurate^[12]. The roles of H₂S and the mechanisms have been well summarized in some excellent reviews^[11,13-16], in which a little is mentioned in the gastrointestinal tract. Since H₂S has been proved to be endogenously generated in the gastrointestinal tract (see below), the physiological function of H₂S in the gastrointestinal tract is intriguing and also several reviews have covered the roles of H₂S in the gastrointestinal tract such as modulating the gastrointestinal motility and secretion, pro- or anti-inflammation, pro- or anti-nociception^[12,17-22]. As the basis of the gastrointestinal function, gastrointestinal motility can triturate the food into pieces and mix the food with digestive juice sufficiently, which facilitate food digestion and absorption. Consequently, to make sure the effect of endogenous H₂S on the gastrointestinal motility is quite significant, as has been focused on in the present review.

SYNTHESIS OF H₂S IN THE GASTROINTESTINAL TRACT

H₂S can be produced by enzymatic or non-enzymatic pathways in mammalian tissues, of which the enzymatic pathway was mostly

focused on (see reference 14, 16 for review). Three enzymes responsible for H₂S synthesis have been found in the mammals which are cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). CBS and CSE are pyridoxal phosphate-dependent and expressed exclusively in the cytosol. While 3-MST, which catalyzes the generation of H₂S in combination with another enzyme, cysteine aminotransferase, is zinc dependent and is both mitochondrial and cytosolic. All the enzymes use L-cysteine as the common substrate. In the gastrointestinal tract, CBS and CSE are the main enzymes catalyzing the generation of endogenous H₂S.

In the stomach, CBS and CSE have been proved to be expressed in the cultured antral smooth muscle cells of mouse in our previous studies^[23,24]. Western blotting results also demonstrated that both CBS and CSE were expressed in the rat and mouse stomach, and H₂S synthesis in the rat stomach was about 75 nmol/g/h^[25]. CBS and CSE were also identified in the small intestine^[25-30]. In the rat duodenum and jejunum, both enzymes were expressed in the neurons of myenteric plexus, but not the smooth muscle cells or the interstitial cells of Cajal (ICCs)^[27,29]. The expressions of CBS and CSE were also found in the ileum of rat and guinea pig where H₂S production were about 6.6 \pm 1.3 nM/min/g tissue and 20.3 \pm 3.2 nM/min/g protein, respectively. And the inhibitor of CBS or CSE could inhibit the H₂S generation^[26,28]. CSE was found to be mainly expressed in the submucosal and myenteric neurons of the colon^[31-34], but also found in the circular and longitudinal smooth muscle layers in the rat colon^[34]. Myenteric interstitial cells of Cajal were CSE-immunoreactive in the guinea-pig and human colon^[33]. However, CBS was mainly expressed in the neurons of enteric neuron system and in the mucosa of the colon^[31,33,34]. In the presence of L-cysteine, homogenate of rat colonic tissue could generate about 2 nM/min/g protein H₂S^[35].

It is noteworthy that aside from endogenous production, H₂S also can be produced exogenously in colonic tract by colonic microbiota, in which sulfate-reducing bacteria (SRB) is a main source^[21]. Under physiological conditions, H₂S level in the colon is still not so high because of the marked capacity of colonic epithelial cells to metabolize H₂S^[36]. Nevertheless, if the epithelium barrier is impaired, high concentration of H₂S maybe results in some colonic disease because of its toxic effect.

H₂S AND THE GASTROINTESTINAL MOTILITY

Since H₂S can be generated endogenously, it is implied that H₂S play an important role in regulation of gastrointestinal functions under physiological and pathophysiological conditions. Many results about the effect of H₂S on gastrointestinal motility from different researchers exhibit diversity in different regions and different dose of H₂S. In vascular smooth muscle H₂S also reveals the complexity in regulation of smooth muscle tension, for example, H₂S relaxed the vascular smooth muscles in most cases^[2,37,38], however, in the aorta, the inhibitory effect was induced by high concentrations of H₂S, while at low concentrations, H₂S increased the contraction of pre-contracted smooth muscles^[37,38]. Consequently, it is important to clarify the effect of H₂S on the smooth muscle motility for better understanding the physiological significance of this gaseous signal molecule.

In the stomach

Our previous studies demonstrated that exogenous H₂S showed dual effects on the spontaneous contraction of antral smooth muscles, i.e.,

low concentrations of H₂S increased the basal tension, however, at high concentrations, H₂S inhibited the spontaneous contraction^[23,24,39]. In guinea-pig, sodium hydrogen sulfide (NaHS), an H₂S donor, had a dual effect on the spontaneous contraction of gastric antrum muscle strips. At high concentrations (300 μ M~1000 μ M), NaHS suppressed the amplitude of spontaneous contraction. At low concentrations (100 μ M~300 μ M), NaHS enhanced the resting tension of muscle strips while slightly reduced the contractile amplitude^[39]. Similar effect of NaHS was also observed in murine stomach, for example, NaHS inhibited the amplitude and frequency of spontaneous contraction at high concentrations (>200 μ M), however, at low concentrations (<200 μ M) enhanced the basal tension and increased the contractile amplitude of muscle strips. In addition, NaHS at low concentrations (<200 μ M) produced a depolarization of the membrane potential, whereas AOA, an inhibitor of CBS, hyperpolarized the membrane potential and decreased the amplitude of slow waves^[23].

It has been demonstrated that the contractile effect of H₂S on the vascular smooth muscle is associated with NO. As we all know, NO is an endothelium-derived relaxing factor which relaxes vascular smooth muscles. Consequently, H₂S is thought to induce contractile effect by inhibiting endothelial nitric oxide synthase (eNOS) which is a kind of enzyme catalyzing the generation of NO or combining with NO to form a novel nitrosothiol to reduce NO release^[37,38,40]. But in our studies, the excitatory effect was not via these pathways. We found that the excitatory effect was inhibited by 4-AP, an inhibitor of voltage-dependent potassium channel, and exogenous H₂S directly inhibited voltage-dependent potassium current. AOA, an inhibitor of CBS, was shown to increase voltage-dependent potassium current. In another aspect, the inhibitory effect of H₂S was reversed by glibenclamide, an inhibitor of K_{ATP} channel, and exogenous H₂S directly increased K_{ATP} current, as reported in some studies of vascular smooth muscle^[2,38]. All these results indicate that the excitatory effect of H₂S at low concentrations was mediated via inhibition of voltage-dependent potassium channel, while the inhibitory effect was mediated via activation of K_{ATP} channel^[23,24,39] (Figure 1). It has been reported that physiological levels of endogenous H₂S may be from nanomolar to micromolar range in the mammalian tissues with the development of measurement methods^[14]. In our studies, we

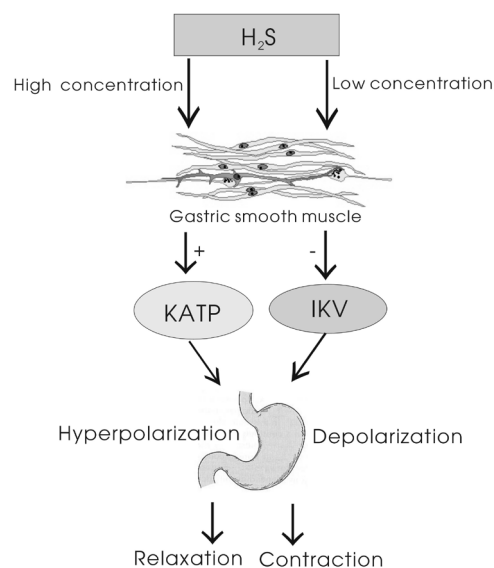


Figure 1 The schematic diagram of the effect of H₂S on gastric motility and its mechanism.

used NaHS as H₂S donor (60 μM–200 μM), one third of which was reported to exist as the undissociated H₂S^[26]. That is, physiological level of endogenous H₂S may be as an excitatory modulator in regulation of gastric motility, and our result that AOAA inhibited the spontaneous contraction of antral smooth muscle partly confirmed the speculation^[23,24].

H₂S donor was also displayed to accelerate gastric emptying of liquid in awake mice in a dose-dependent manner, which maybe mainly resulted from the relaxation effect of H₂S on the pyloric sphincter muscles^[41]. Glibenclamide and a transient receptor potential vanilloid type 1 (TRPV1) receptors antagonist capsazepine abolished the inhibitory effect of H₂S, which indicate the involvement of K_{ATP} channel and TRPV1 receptors located on afferent nerves in this effect^[41].

In the mouse gastric fundus, NaHS inhibited PGF_{2α}-contracted muscle strips in a dose-dependent manner. The exact mechanism of the relaxant effect was unclear because it was not associated with the activation of potassium channels including K_{ATP} channels, Ca²⁺-activated K⁺ channel, voltage dependent K⁺ channel and inward rectifier K⁺ channel, the release of NO, and not influenced by TTX, a nerve blockers. However, the inhibitory effect of NaHS on PGF_{2α}-induced contraction was suppressed by calyculin-A, an inhibitor of myosin light chain phosphatase (MLCP), the result suggests H₂S may be directly participated in MLCP phosphorylation^[42].

Generally speaking, the effect of H₂S on the gastric motility seems to be adapted to the function of the stomach. As the main movement of gastric fundus, receptive relaxation is important for fundus function which makes stomach accommodate more food with less increase of lumen pressure. Since H₂S relax the fundus smooth muscle, it maybe facilitates the receptive relaxation. Meanwhile, the excitatory effect of H₂S on the antrum together with the inhibitory effect on the pyloric sphincter may facilitate the gastric emptying. Therefore, H₂S may be an important modulator in the stomach.

In the small intestine

Effects of H₂S on the intestinal motility were different because of different species and different regions of the intestine. In the duodenum of Wistar rats, NaHS induced a biphasic effect on the spontaneous contraction of the smooth muscle, that is, a transient excitatory effect followed a long-lasting inhibitory effect^[27]. The excitatory effect was attenuated by both TRPV1 antagonist and NK1 receptor antagonist, which is consistent with the H₂S-induced effect on the rat urinary bladder^[43], indicating that NaHS might activate TRPV1 channels in the afferent nerve fibers resulting in the release of substance P and subsequent activation of NK1 receptor on the smooth muscle cells to increase the smooth muscle contraction. That is, the excitatory effect of NaHS resulted from the direct influence on the enteric neurons but not the smooth muscle cells. But the inhibitory effect of NaHS resulted from the open of K_{ATP} channels on the smooth muscle cells. Interestingly, both L-cysteine and SAM, an activator of CBS, increased the duodenal motility, indicating an excitatory effect of endogenous H₂S^[27]. Although not describing in details, the authors of the study also found that NaHS had similar biphasic effects on the spontaneous contraction of the longitudinal muscle in the jejunum, ileum and colon of the Wistar rats^[27], which is different with others studies (see below).

Different from the Wistar rats, in the Lewis rats and mouse jejunum, NaHS induced an inhibitory effect on the smooth muscle motility^[29,44,45]. Gallego *et al.*^[45] demonstrated that NaHS inhibited the spontaneous contraction of the jejunal smooth muscle in mouse, which may be a direct effect on the smooth muscle cells because

TTX had no significant effect on the NaHS-induced inhibition and in the TRPV1^{-/-} animals the inhibitory effect still existed. Similarly, Sarr team^[29,44] also found that NaHS inhibited both spontaneous and bethanechol stimulated contraction of the jejunal smooth muscle in Lewis rats. Although the exact mechanism of NaHS-induced inhibition is unclear, there is a little difference between the circular muscle and the longitudinal muscle. Neither the effect on the circular muscle nor on the longitudinal muscle was via neural pathway, which means a direct effect on the smooth muscle. Interestingly, K_{ATP} channel may be in part involved in the NaHS-induced inhibition of the spontaneous contraction in the circular muscle of the jejunum, but not involved in the effect on the longitudinal muscle, indicating a complex function of H₂S in modulating jejunum motility.

In the ileum, most studies manifested that H₂S inhibited the smooth muscle motility^[26,46-48]. The first study about H₂S modulating the gastrointestinal motility was in the guinea-pig ileum which found that NaHS relaxed acetylcholine pre-contracted smooth muscle^[26]. The following study reported by Teague *et al.*^[46] confirmed the result, which also found that NaHS relaxed both the spontaneous contraction of rabbit ileum and electrical field stimulation (EFS)-induced contraction of guinea-pig ileum. Moreover, the inhibitory effect of NaHS on the guinea-pig ileum to electrical stimulation was not via the open of K_{ATP} channel^[46]. Similarly, the study in the rat ileum also found NaHS inhibited the spontaneous contraction which was not mediated by K_{ATP} channel^[48]. Differently, Teague suspected that endogenous H₂S may be generated by intramural nerves to modulate the smooth muscle motility because PAG, an inhibitor of CSE, increased EFS-induced contraction^[46], whereas, H₂S was supposed not to be produced enough by enteric neurons to play such a role in the rat ileum^[48]. Recent study in mouse ileum also found that AOAA increased the contraction of smooth muscle to repeated electrical stimulations (ES) which was suspected to activate CBS, and substrate for generation of H₂S L-cysteine decreased the EFS-induced contraction, indicating an inhibitory effect of H₂S endogenously produced by enteric neurons^[47]. The results that glibenclamide increased EFS-induced contraction but reduced NaHS-induced inhibition of EFS-induced contraction suggest K_{ATP} channel in the smooth muscle and enteric neurons involved in the inhibitory effect of H₂S. Meanwhile, NaHS induced-decrease of basal tone in the mouse ileum was insensitive to TTX, but blocked by apamin, a blocker of small conductance Ca²⁺-activated K⁺ (SK_{Ca}) channel, which suggest that SK_{Ca} channels in the smooth muscle are responsible for the effect^[47].

Aside from enteric neurons and smooth muscle cells, as the pacemaker cells, the interstitial cell of Cajal (ICC) may be also a target of H₂S to regulate the gastrointestinal motility. Albeit no expression of CBS or CSE in ICC, exogenous H₂S was shown to inhibit the pacemaker activity of ICC^[30] which may be partially responsible for the H₂S-induced inhibition of small intestine motility.

In the colon

In the mouse colon, it has demonstrated that NaHS inhibited the spontaneous and pre-contracted smooth muscle contraction in a dose-dependent manner, which was not via neuronal pathway because both TTX and TRPV1 blocker capsazepine had no significant effects on the inhibition^[45,49]. Since glibenclamide and L-NAME, a nitric oxide synthase, did not affect the NaHS-induced inhibition, so that K_{ATP} channel and NO were not involved in the effect^[49]. In the rat and human colon, NaHS also inhibited the colonic motility^[45,50,51]. Interestingly, H₂S exhibited different effects on different colonic motility patterns, for example, NaHS inhibited rhythmic propulsive

motor complexes (RPMCs) which is associated with outflow, but enhanced the amplitude of ripples which may be associated with promoting mixing. The inhibitory effect of NaHS on the colonic motility was reduced by K_{ATP} channel blocker and small conductance calcium-activated potassium channel (SK_{Ca}) blocker, which suggest that both K_{ATP} and SK_{Ca} channels are involved in the effect^[45,50,51]. Furthermore, the inhibitor of CBS or CSE increased the colonic motility and hyperpolarized the smooth muscle cell, suggesting that the endogenous H₂S induced an inhibitory effect on colon^[34]. However, different from the small intestine, NaHS did not alter the frequency of slow waves, which indicates that ICC may be not the main target of H₂S in the colon^[50].

MECHANISMS OF H₂S IN REGULATING THE GASTROINTESTINAL MOTILITY

From all the studies above, the effect of H₂S on gastrointestinal motility is very complicated (Table 1). For example, the effect is different in different regions of gastrointestinal tract and different species, and the targets of the effect are also dissimilarity. We can summarize the mechanism of H₂S in regulation of gastrointestinal motility as below: (1) H₂S can affect enteric neurons. H₂S can activate TRPV1 channel in the primary afferent neurons to release a neurotransmitter^[27,41], or H₂S can affect the cholinergic neuromuscular transmission^[46,50] to regulate the smooth muscle motility; (2) H₂S can directly influence the channels in the smooth muscle cells, such as K_{ATP} channel^[27,39,41,45,47,50,51], voltage-dependent K⁺ channel^[23,39] and SK_{Ca} channel^[45,47,50]. However, it is not clear how H₂S affect these channels. Recently, it has been well-established that H₂S directly regulates the physiological functions by sulfhydrating a large number of cellular proteins^[16], for example, NaHS sulfhydrating sulfonylurea receptor 2B (SUR2B) subunit of K_{ATP} channel might partially interpret the effect of H₂S on these channels; (3) H₂S also can affect the pacemaker activity of ICC to modulate the smooth muscle motility^[30]; (4) As two of the important gaseous signal molecules, H₂S and NO have interaction in regulating the gastrointestinal smooth muscle motility. They can cooperate with each other to relax the smooth muscle^[46], alternatively, they counteract each other to regulate the motility^[24]. Although the exact mechanism is still needed to investigate, a finding that endogenous H₂S inhibited the production of NO provides a clue^[32]. Albeit these possible pathways, the mechanism of H₂S in regulating the gastrointestinal motility is still ongoing.

Table 1 The effect of H₂S on gastrointestinal motility and its mechanism.

	Region	Effect of H ₂ S on the motility	Mechanism
stomach	Fundus	Inhibitory effect	Unclear
	Antrum	Excitatory effect Inhibitory effect	K _V on smooth muscle(-) K _{ATP} on smooth muscle(+)
Small intestine	Duodenum	Excitatory effect	TRPV1 on enteric nerve(+)
	Jejunum	Inhibitory effect	K _{ATP} on smooth muscle(+) Unclear
	Ileum	Inhibitory effect	K _{ATP} on the smooth muscle and enteric nerve (+) Or Sk _{Ca} on the smooth muscle(+)
	Colon	Inhibitory effect	K _{ATP} (+) Or Sk _{Ca} (+)

(+) means activation, (-) means inhibition.

PROBLEMS IN STUDIES

Since the studies on the physiological function of H₂S are ongoing, there are still some problems to be resolved. The most important problem is that the present used inhibitors of CBS and CSE are considered nonspecific, especially AOAA which was proved to inhibit other pyridoxal phosphate-dependent enzymes^[52,53]. Although it has been the exclusive inhibitor of CBS so far and also proved to reduce the generation of H₂S^[25,34], the possible side effects of AOAA make it suspect to speculate the function of endogenous H₂S. Using the animals targeted deleted CBS and CSE may be an effective method to study the function of endogenous H₂S in the gastrointestinal tract. Secondly, the exact concentration of H₂S *in vivo* is ambiguous. The H₂S levels are from nanomolar to low micromolar ranges by using different measurement methods under different conditions (see reference 14 for review). Consequently, whether the H₂S donor used in the studies is close to the physiological level of H₂S is questionable. Does the effect of H₂S result from its toxicity? H₂S has been reported to inhibit cytochrome C oxidase resulting in decrease of cellular adenosine triphosphate (ATP) which directly activate K_{ATP} channel responsible for many biological functions of H₂S^[54]. If H₂S regulating the gastrointestinal motility via activation of K_{ATP} channel is a toxic effect? Thirdly, as the two important gaseous signal molecules in the gastrointestinal tract, the cross talk between H₂S and NO in regulating the gastrointestinal motility is intriguing. Although there are a few studies on this topic, the exact mechanism is still unclear. In the future studies it is needed to clarify how H₂S changes channel function and why the effect of H₂S on gastrointestinal smooth muscle motility is different in different regions and what the physiological meanings is. In addition, it is also significant to definite whether there is cross talk between H₂S and NO in regulating the gastrointestinal motility and its mechanism.

CONCLUSION

H₂S can be endogenously generated by gastrointestinal tract and significantly affect the gastrointestinal motility. The effect of H₂S on gastrointestinal smooth muscle shows difference in different concentration of H₂S and different regions. In gastric smooth muscle, H₂S exhibits excitatory (low concentration) and inhibitory (high concentration) effects via suppressing voltage-dependent potassium channels and activating ATP-sensitive potassium channels (K_{ATP}), respectively. In intestinal smooth muscle, NaHS induced a transient excitatory effect followed a long-lasting inhibitory effect in rat. The excitatory effect is mediated by TRPV1 and NK1 receptors and the inhibitory effect is mediated by K_{ATP}. In colonic smooth muscle, H₂S induces inhibitory effect mediated by both K_{ATP} and SK_{Ca} channels in human, rat and mouse. Although the exact mechanism of H₂S on the gastrointestinal motility is still unclear and there are still some unresolved problems in the studies, we believe studying the effect of H₂S will contribute to clinical medicine to resolve the disorders of gastrointestinal motility in the future.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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