INTRODUCTION

Recent studies suggest that hydrogen sulfide (H\textsubscript{2}S) is a biologically relevant signaling molecule in mammals, which modulates a range of physiological and pathological processes in the nervous system, cardiovascular system, respiratory system, and digestive system, as well as regulating metabolism and immunity (1-8). H\textsubscript{2}S is generated in mammalian cells mainly by cystathionine \textit{b}-synthase (CBS) and cystathionine \textit{g}-lyase (CSE), using L-cysteine as the main substrate (9). CBS and CSE have been found throughout the gastrointestinal tract, including the enteric nervous system (ENS) (10-17).

Although it is well recognized that H\textsubscript{2}S modulates a range of physiological and pathological processes involving K\textsubscript{ATP} channels, studies on its effects on other ion channels have just begun. H\textsubscript{2}S stimulates transient receptor potentials of vanilloid 1 (TRPV1) channels in rat urinary bladder and guinea-pig airway, causing bladder constriction and airway contraction through a neurogenic inflammation mechanism (18-19). Moreover, Schicho et al. demonstrated that sodium hydrosulfide (NaHS), an exogenous H\textsubscript{2}S donor, significantly reduced the pH of gastric juice when injected into the enterocoelia. Further, the promotional effect of NaHS on gastric acid secretion could be attenuated by capsaepine, a transient receptor potential vanilloid 1 (TRPV1) antagonist; L703606, a neurokinin 1 (NK\textsubscript{1}) receptor antagonist; and PDTC, a NF-\textkappaB inhibitor. The data from these experiments suggest that NaHS exerts an excitatory effect on gastric acid secretion possibly mediated by TRPV1 channel activation in sensory nerve terminals with the consequent release of substance P and in a NF-\textkappaB-dependent manner.

Key words: gastric acid secretion, hydrogen sulfide, nuclear factor-\textkappaB, substance P, transient receptor potential vanilloid 1, neurokinin 1 receptor antagonist

MATERIALS AND METHODS

Animals

Wistar male rats (220 – 280 g) were provided by the Experimental Animal Center of Shandong University. Animals were fed in a temperature-controlled environment on a 12-h

HYDROGEN SULFIDE MODULATES GASTRIC ACID SECRETION IN RATS VIA INVOLVEMENT OF SUBSTANCE P AND NUCLEAR FACTOR-\textkappaB SIGNALING

College of Life Science, Qilu Normal University, Zhangqiu, Jinan, P.R. China

Hydrogen sulfide (H\textsubscript{2}S) promotes gastric acid secretion in rats. The present study aimed to test the hypothesis that H\textsubscript{2}S regulates this response via activating TRPV1 channel and through activation of the nuclear factor-\textkappaB (NF-\textkappaB) pathway. Male Wistar rats were randomly divided into the sodium hydrosulfide (NaHS, 100 \mu mol/kg b.w.) group, pyrrolidine dithiocarbamate (PDTC, 100 \mu mol/kg b.w.) group, PDTC (100 \mu mol/kg b.w.) + NaHS (100 \mu mol/kg b.w.) group, capsaepine (0.1 mM) + NaHS (100 \mu mol/kg b.w.) group and L703606 (0.1 mM) + NaHS (100 \mu mol/kg b.w.) group. The acidity of gastric juice before injection and after injection were determined by a pH meter. The results showed that sodium hydrosulfide (NaHS), an exogenous H\textsubscript{2}S donor, significantly reduced the pH of gastric juice when injected into the enterocoelia. Further, the promotional effect of NaHS on gastric acid secretion could be attenuated by capsaepine, a transient receptor potential vanilloid 1 (TRPV1) antagonist; L703606, a neurokinin 1 (NK\textsubscript{1}) receptor antagonist; and PDTC, a NF-\textkappaB inhibitor. The data from these experiments suggest that NaHS exerts an excitatory effect on gastric acid secretion possibly mediated by TRPV1 channel activation in sensory nerve terminals with the consequent release of substance P and in a NF-\textkappaB-dependent manner.

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light/dark cycle. Prior to the experiments, the animals were deprived of food for 24 h, but allowed free access to water. All the procedures described were approved by the Ethics Committee for Research on Animals, Qi Lu Normal University. All studies involving animals were performed according to the guidelines of the International Association for the Study of Pain (27).

**Chemicals**

NaHS (100 µmol/kg b.w.), PDTC (100 µmol/kg b.w.), capsazepine (0.1 mM), and L703606 (0.1 mM) were bought from Sigma (Saint Louis, MO, USA). NaHS were dissolved in 0.9% saline, but other chemicals were dissolved in dimethyl sulfoxide (DMSO). All chemicals are available now and by an intraperitoneal injections.

**Experimental group**

The rats were randomly divided into five groups, with 10 rats per group:

1. Effect of NaHS (100 µmol/kg b.w., 1 mL/100 g b.w.) on gastric acid secretion;
2. Effect of PDTC (100 µmol/kg b.w., 1 mL/100 g b.w.) on gastric acid secretion;
3. PDTC + NaHS group, gastric acid secretion was observed after pretreatment with i.p. injection of PDTC and NaHS (100 µmol/kg b.w.);
4. Capsazepine + NaHS group, gastric acid secretion was observed after pretreatment with i.p. injection of capsazepine (0.1 mM) and NaHS (100 µmol/kg b.w.);
5. L703606 + NaHS group, gastric acid secretion was observed after pretreatment with i.p. injection of L703606 (0.1 mM) and NaHS (100 µmol/kg b.w.).

**Collecting gastric juice and determining pH**

Esophageal perfusion was used to collect gastric secretions. Animals were anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg b.w., i.p.). Body temperature was maintained at 37 ± 1°C with a radiant heat lamp. A 2.5-mm cannula was inserted into the trachea. A polyethylene tube (2 mm in diameter) was inserted into the esophagus to perfuse warm (37°C) normal saline at 2.0 mL/min. A 3-mm polyethylene tube was inserted into the stomach at the joint between the pylorus and duodenum to collect the gastric secretions. Basal secretion values were determined from three consecutive 20-min values before injection. Three consecutive 20-min values were also taken from the commencement of injecting chemicals into enterocoelia to assess the change in secretion. The acidity of gastric juice was determined by a pH meter (PHS-3B, Shanghai Optical Instrument Factory, Shanghai, China).

**Data analysis**

All values were analyzed using the SPSS22.0 software (SPSS Inc. Chicago, Ill., USA) and presented as mean ± SD was performed by the Student’s t-test. Significance was accepted at the level of P < 0.05.

**RESULTS**

**Effects of capsazepine on gastric acid secretion**

NaHS, an exogenous H2S donor, by an intraperitoneal injections, significantly reduced the pH of gastric juice, from 5.37 ± 0.32 (before injection) to 3.92 ± 0.40 (after injection, P < 0.01; Fig. 1). However, the same volume of physiological saline (PS, 1 mL/100 g b.w.) administered similarly did not change pH of gastric juice (23).

The promotional effect of NaHS on gastric acid secretion could be weakened by capsazepine, a TRPV1 antagonist. The pH of gastric juice changed from 5.47 ± 0.58 (before injection) to 5.40 ± 0.41 (after injection, P > 0.05). There were no obvious differences after capsazepine + NaHS was injected into the enterocoelia (Fig. 1). These results suggest that NaHS was involved in the control of gastric acid secretion by TRPV1 channels.

**Effects of L703606 on gastric acid secretion**

The promotional effect of NaHS on gastric acid secretion could also be reduced by L703606, a NK1 receptor antagonist. The pH of gastric juice changed from 5.45 ± 0.56 (before injection) to 5.30 ± 0.36 (after injection, P < 0.01; Fig. 2). The pH of gastric juice in the NaHS (100 µmol/kg body weight) group and Capsazepine + NaHS (100 µmol/kg b.w.) group. **P < 0.01, versus before injection.

**Fig. 1.** The pH of gastric juice in the NaHS (100 µmol/kg b.w.) group and Capsazepine + NaHS (100 µmol/kg b.w.) group. **P < 0.01, versus before injection.

**Fig. 2.** The pH of gastric juice in the NaHS (100 µmol/kg body weight) group and L703606 + NaHS (100 µmol/kg body weight) group. **P < 0.01, versus before injection.
H2S activates TRPV1 receptors on extrinsic primary afferent the consequent release of SP (20). Schicho et al. might activate TRPV1 channels in the afferent nerve fibres with excitatory responses evoked by NaHS, indicating that NaHS interstitial cells of Cajal (ICC), significantly attenuated the (most likely SP) released from afferent nerves (31). Wen Lu et al. sensory nerves, this effect might be mediated by tachykinins involvement in the control of gastric acid secretion through enhancement of SP production.

Effects of pyrrolidine dithiocarbamate on gastric acid secretion

After PDTC, an NF-κB inhibitor, was injected, the pH of gastric juice did not change significantly (from 5.35 ± 0.38 to 5.28 ± 0.47, P > 0.05; Fig. 2), but the promotional effect of NaHS on gastric acid secretion could be prevented by PDTC. The pH of gastric juice changed from 5.41 ± 0.32 (before injection) to 5.34 ± 0.36 (after injection, P > 0.05). There are no significant difference after PDTC + NaHS was injected into the enterocelia (Fig. 3). These results indicate that NaHS was involved in the control of gastric acid secretion by activating NF-κB pathway.

DISCUSSION

In this study, we found that the promotional effect of NaHS on gastric acid secretion could be attenuated by capsazepine (a TRPV1 antagonist) and L703606 (a NK1 receptor antagonist). These results suggest that the excitatory effect of NaHS on gastric acid secretion might be mediated by activation of TRPV1 channels in sensory nerve terminals, with the consequent release of substance P. TRPV1 is broadly expressed in all ‘port of entry’ tissues, which in turn activate enteric neurons resulting in mucosal Cl– secretion (16). All of these reports provide support to our hypothesis that the excitatory effect of NaHS on gastric acid secretion might be regulated by activation of capsaicin sensitive primary afferent nerves with the consequent release of substance P.

Medeiros et al. and Wallace et al. reported that L-cysteine or H2S donors did not change the volume of gastric juice, pH and total acidity, as compared with the saline group (32-33). This result is different with our result in this article. We assume that the difference is perhaps due to three reasons. One was that we injected with different concentrations of NaHS (100 µmol/kg), while the Medeiros et al. and Wallace et al. were NaHS (50 µmol/kg) and NaHS (30 µmol/kg), respectively. We use perfusion to collect gastric juice, and they use pyloric ligation. Thirdly, the collection time of gastric juice is different. We collected changes in gastric juice within an hour before and after the injection, and they collected changes in gastric juice within 3 – 4 hours after the injection. Our result is from scientific experiments and is indeed credible.

As a small gas molecule, H2S can affect multiple signaling pathways, such as NF-κB signaling, mitogen-activated protein kinase (MAPK) signaling pathways, and phosphoinositide 3-kinase (PI3K) and its downstream molecules, such as serine/threonine protein kinase AKT (PI3K/AKT) (34-35). Hydrogen sulfide protected gastric epithelial cells from ischemia-reperfusion injury by activation of Keap1 s-sulfhydration, MAPK-dependent anti-apoptosis, and the NF-κB-dependent anti-inflammation pathway (24). NaHS, the donor of H2S, plays a protective role against RWIS injury in rats, possibly through modulation of K+ (ATP) channel opening and an NF-κB-dependent pathway (25). There are some studies showing that H2S is actually downregulation of NF-κB (36-37). However, in this study, we found that NaHS promoted gastric acid secretion in rats, possibly through an NF-κB-dependent mechanism. These differences between our present study and previous reports might be attributed to the different species and tissues used.

In conclusion, the present study suggests that exogenous H2S promoted gastric acid secretion, which may occur via the activation of TRPV1 channels in sensory nerve terminals with the consequent release of substance P in a NF-κB-dependent manner.

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REFERENCES


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Author’s address: Dr. Hong-Zhao Sun, College of Life Science, Qi Lu Normal University, No. 2, Wenbo Road, Zhangqiu 250200, Jinan, PR. China.
E-mail: sunhongzhao18@126.com