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

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Hydrogen sulfide (H_2S) promotes gastric acid secretion in rats. The present study aimed to test the hypothesis that H_2S regulates this response *via* activating TRPV1 channel and through activation of the nuclear factor-κB (NF-κB) pathway. Male Wistar rats were randomly divided into the sodium hydrosulfide (NaHS, 100 μmol/kg b.w.) group, pyrrolidine dithiocarbamate (PDTC, 100 μmol/kg b.w.) group, PDTC (100 μmol/kg b.w.) + NaHS (100 μmol /kg b.w.) group, capsazepine (0.1 mM) + NaHS (100 μmol /kg b.w.) group and L703606 (0.1 mM) + NaHS (100 μmol /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_2S 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 capsazepine, a transient receptor potential vanilloid 1 (TRPV1) antagonist; L703606, a neurokinin 1 (NK_1) receptor antagonist; and PDTC, a NF-κB 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-κB -dependent manner.

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

INTRODUCTION

Recent studies suggest that hydrogen sulfide (H_2S) 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_2S is generated in mammalian cells mainly by cystathionine β-synthase (CBS) and cystathionine γ-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_2S modulates a range of physiological and pathological processes involving K(ATP) channels, studies on its effects on other ion channels have just begun. H_2S 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), the donor of H_2S, caused secretion in the guinea-pig and human colon by activating TRPV1 receptors located in afferent nerves, which resulted in the local release of substance P (SP) (16). NaHS exerts an excitatory effect on intestinal motility in rats through activating TRPV1 channels in sensory nerve terminals with the consequent release of substance P (20). Because NK1 receptors are expressed in a cell-specific manner in the digestive system, such as smooth muscle cells and interstitial cells of Cajal (ICC) (21), it is possible that H_2S-induced release of SP influences both the amplitude and frequency of the muscle contraction. Previous research found that the expression of CSE appears to be induced in parietal cells (22), and NaHS promoted gastric acid secretion (23). However, whether a similar mechanism is involved in the regulation of gastric acid secretion induced by H_2S is still unclear.

Hydrogen sulfide protected gastric epithelial cell from ischemia/reperfusion injury by Keap1 s-sulfhydration, MAPK-dependent anti-apoptosis, and NF-κB dependent anti-inflammation pathway (24). NaHS, the donor of H_2S, plays a protective role against RWIS injury in rats, possibly through modulation of K^+(ATP) channel opening and an NF-κB-dependent pathway (25). Ang *et al*. found that H_2S regulated TRPV1-mediated neurogenic inflammation in polymicrobial sepsis through enhancement of SP production and activation of the ERK-NF-κB pathway (26).

Therefore, in this study, we evaluated whether the excitatory effect of H_2S gastric acid secretion in rats occurred through activation of TRPV1 channels in sensory nerve terminals with the consequent release of substance P, in an NF-κB-dependent manner.

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

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 H_2S 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 NK_1 receptor antagonist. The pH of gastric juice changed from 5.45 ± 0.56 (before

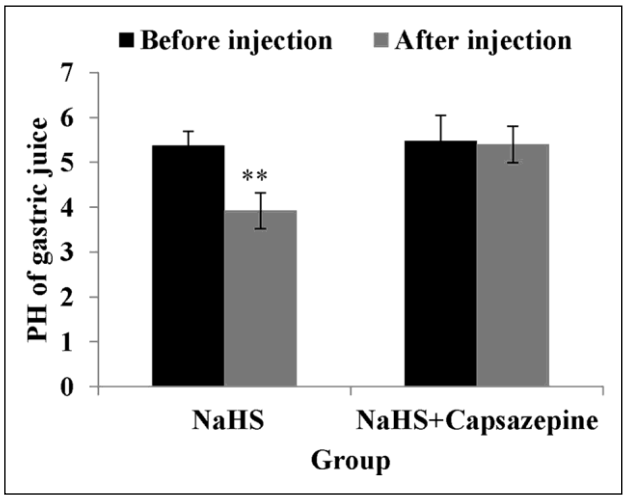

Fig. 1. The pH of gastric juice in the NaHS (100 μmol/kg b.w.) group and Capsazepine + NaHS (100 μmol/kg b.w.) group. **P < 0.01, versus before injection.

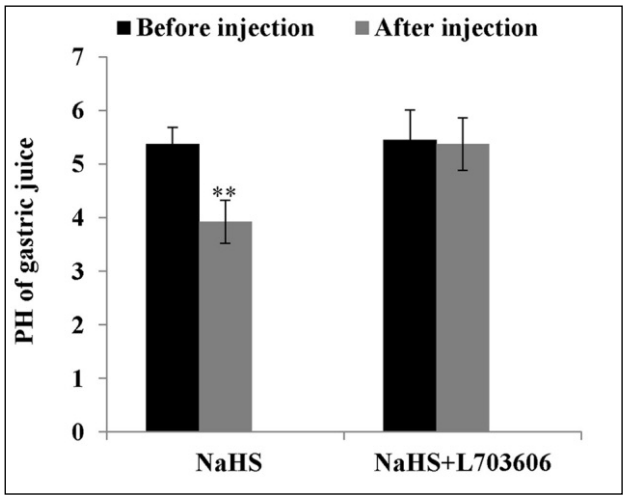

Fig. 2. The pH of gastric juice in the NaHS (100 μmol/kg body weight) group and L703606 + NaHS (100 μmol/kg body weight) group. **P < 0.01, versus before injection.

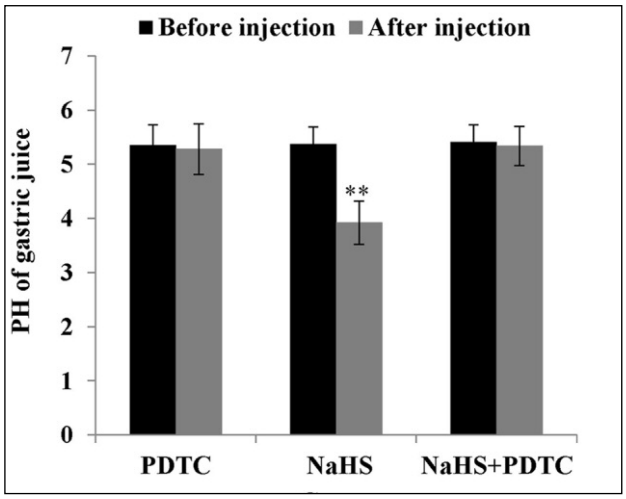

Fig. 3. The pH of gastric juice in the NaHS (100 μmol/kg b.w.) group and PDTC + NaHS (100 μmol/kg b.w.) group. **P < 0.01, versus before injection; PDTC, pyrrolidine dithiocarbamate.

injection) to 5.37 ± 0.49 (after injection, P > 0.05). There are no obvious difference after L703606 + NaHS was injected into the enterocoelia (*Fig. 2*). These results suggest that NaHS was involved 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. 3*), 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 enterocoelia (*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 NK_1 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, such as the skin, gut, airway and conjunctiva (28-29), and which is abundantly expressed in primary afferent nerve endings (30). Capsaicin, the agonist of TRPV1 receptors, excites intestinal motility by activating the local efferent function of sensory nerves, this effect might be mediated by tachykinins (most likely SP) released from afferent nerves (31). Wen Lu *et al*. found that capsazepine, a TRPV1 channel antagonist, and L703606, a NK1 receptor antagonist, in smooth muscle cells and interstitial cells of Cajal (ICC), significantly attenuated the excitatory responses evoked by NaHS, indicating that NaHS might activate TRPV1 channels in the afferent nerve fibres with the consequent release of SP (20). Schicho *et al*. reported that H_2S activates TRPV1 receptors on extrinsic primary afferent terminals, 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 H_2S donors did not change the volume of gastric juice, pH and total acidity, ascompared 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 umol/kg), while the Medeiros *et al*. and Wallace *et al*. were NaHS (50 umol/kg) and NaHS (30 umol/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, H_2S 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 H_2S, 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 H_2S 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 H_2S 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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Conflict of interests: None declared.

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