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Role of KATP channels and TRPV1 receptors in hydrogen sulfide-enhanced gastric emptying of liquid in awake mice ☆

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ABSTRACT

Hydrogen sulphide (H_2S) has shown to relax gastrointestinal muscle. Here in, we evaluated the effects of H_2S donors on gastric emptying and in pyloric sphincter muscle relaxation, and whether these effects involved K_{ATP} channels or TRPV1 receptors. Mice were treated with L-cysteine (alone or with propargylglycine-an inhibitor of H_2S synthesis), NaHS, Lawesson's reagent (H_2S donors) or saline. After 30 min, mice were gavaged with a liquid meal containing a nonabsorbable marker and then killed at 10, 20 or 30 min intervals to assess marker recovery from the stomach and intestine. This experiment was repeated in mice pre-treated with K_{ATP} channel (glibenclamide) or TRPV1 receptor (capsazepine) antagonists. In addition, pyloric sphincter muscles were mounted in an organ bath, incubated with saline, glibenclamide or capsazepine, and NaHS dose–responses were determined. H_2S donors and L-cysteine enhanced gastric emptying in a dose-dependent manner; propargylglycine reversed the effect of L-cysteine. Both glibenclamide and capsazepine abolished L-cysteine and H_2S donors' augmentation of gastric emptying. Dose-dependent inductions of pyloric sphincter relaxation by NaHS were abolished by glibenclamide or capsazepine. These data suggest that H_2S donors-induced acceleration of gastric emptying and relaxation of pyloric sphincter muscle by K_{ATP} channel and TRPV1 receptor activations.

1. Introduction

Hydrogen sulphide (H_2S), in the presence of its substrate L-cysteine, is endogenously produced in several mammalian tissues (Guidotti, 1996; Moore et al., 2003). Importantly, the metabolism of L-cysteine by the two enzymes responsible for the production of H_2S, cystathionine γ-lyase (CSE) and cystathionine β-synthetase (CBS), have both been localized to the gastrointestinal tract (Kawabata et al., 2007). Moreover, there appear to be functional effects. For example, H_2S has recently been shown to be involved in the regulation of smooth muscle tone, which suggests that endogenous H_2S may have modulating effects on gut motor function. This is likely through a neuromodulatory mechanism (Fiorucci et al., 2005; Kawabata et al., 2007; Teague et al., 2002), and at relatively high concentrations, H_2S relaxed vascular smooth muscle in a manner involving ATP-sensitive potassium channels (K_{ATP}) (Kubo et al., 2007; Zhao et al., 2001).

In addition to that, H_2S has also shown to induce concentration-dependent contractile responses on the detrusor muscle of the rat urinary bladder (Patacchini et al., 2005). The persistent tachyphylaxis observed, similarly to that induced by capsaicin (Maggi et al., 1986, 1997), was abolished by desensitization of capsaicin-sensitive primary afferent neurons (Maggi et al., 1997). These authors suggested that an important effect of capsaicin was to activate the sensory nerve fibers by transient receptor potential vanilloid receptors type 1 (TRPV1) (Caterina et al., 1997). However, the role of TRPV1 in modulating the effect of H_2S on gut motor function remains unclear.

Thus, many effects of H_2S on gastrointestinal tract seem to depend of K_{ATP} channels and TRPV1 receptors. Therefore, K_{ATP} channels and TRPV1 receptors play a crucial role in gastrointestinal sensory and motor disorders (Fiorucci et al., 2006; Geppetti and Trevisani, 2004). In addition, our research group suggested that H_2S activates the K_{ATP}

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channels and afferent neurons/TRPV1 receptors, and this effect is important to prevents ethanol-induced gastric damage in mice (Medeiros et al., 2009).

Several authors demonstrated that H_2S donors relax the gastrointestinal smooth muscle in vitro (Gallego et al., 2008; Zhao et al., 2009). However, reports addressing the *in vivo* effects of H_2S on gastrointestinal motility are limited. In this study, we investigated the effect of H_2S donors or L-cysteine on the gastric emptying of liquid meals, and in pyloric sphincter muscle relaxation in mice. In addition, we evaluated the possibility that K_{ATP} channels and/or TRPV1 receptors were involved in these processes.

2. Materials and methods

2.1. Animals

Male Swiss mice (25–30 g) were fasted 18 to 24 h before the experiments. Animals were housed in cages in temperature controlled rooms and received water and food ad libitum. All animal treatments and surgical procedures were performed in accordance with the *Guide for Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD) and were approved by the local ethics committee (protocol 63/07).

2.2. Drugs

L-cysteine, DL-propargylglycine (PAG), capsazepine, and glibenclamide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lawesson's reagent was obtained from Fluka (Mumbai, India). NaHS was synthesized by Prof. Dr. Alberto Federman Neto. Vehicle solutions consisted of saline. Glibenclamide was dissolved in 0.01 N NaOH containing 4% glucose.

2.3. Gastric emptying

Gastric emptying measurement was conducted using a modification of the technique previously described by Reynell and Spray (1978). First, mice were treated intraperitoneally with saline, L-cysteine (50 and 100 mg/kg), NaHS (5, 15 and 50 μmol/kg) or Lawesson's reagent (9, 27 and 81 μmol/kg). The doses were selected according to Medeiros et al. (2009). Another group received DL-propargylglycine (an inhibitor of the CSE, 50 mg/kg, i.p) alone or 30 min before cysteine administration. Thirty min later, the mice were gavage fed (300 μl) with a standard liquid bolus containing a nonabsorbable marker (0.5 mg/ml phenol red in 5% glucose). After 10, 20 or 30 min, animals were sacrificed by cervical dislocation ($N=5$–8 animals/group). After laparotomy, the pylorus, the gastro-esophageal and the gastroduodenal junctions were quickly clamped and the gut removed and stretched along a meter stick on a tabletop and divided into two consecutive segments: stomach and small intestine. Each segment volume was measured by adding 0.1 N NaOH solution (10 ml) to a graduated cylinder and then homogenized for 30 s. One ml of the supernatant was centrifuged for 10 min (2800 rpm). Proteins in 500 μl of homogenate were precipitated with 50 μl of trichloroacetic acid (20% w/v), centrifuged for 20 min (2800 rpm), and 150 μl from the supernatant was added to 200 μl of 0.5 N NaOH solution. The sample absorbance was read at 560 nm wavelength and expressed as optical density. A standard dilution curve was obtained in every experiment relating the phenol red concentration to the optical density of 0.1 N NaOH solution. The linear coefficient (a) of the standard dilution curve was established and used to determine the dye concentration (C) of the solution ($C=O\times D$) and then the amount of phenol red (m) recupered in each segments ($m=C\times$ volume).

The fractional dye retention was expressed in percentage, according to the following equation: gastric dye retention=amount of phenol red recovered in stomach/total amount of phenol red recovered from two segments (stomach and small intestine).

2.4. Sphincter pyloric muscle

Mice (20–25 g) were euthanized, the abdomen was opened and sphincter pyloric was rapidly cut into segments of 1.0–2.0 cm in length. Circular muscle layers were mounted vertically in an organ bath containing Tyrode's solution bubbled with 95% O_2/5% CO_2 and maintained at 37 °C, pH 7.4. The preparation was stabilized under an initial resting tension of 1 g for 1 h before the experimental protocols. Active tension was developed isometrically using a force transducer connected to a computerized data acquisition system (LabChart 6.1; PowerLab, ADInstruments). Experimental protocols were initialized with a contraction control with KCl (60 mM), following tissue washing with Tyrode,s solution. After 1 h of equilibration, it was performed a cumulative curve concentration-response with NaHS (1–1000 μM). Data were expressed as percentages of the maximum relaxation obtain by papaverine (1–1000 μM).

2.5. Role of K_{ATP} in H_2S enhanced gastric emptying of liquid and relaxation in the pyloric sphincter relaxation

To study the role of K_{ATP} in H_2S accelerated gastric emptying of liquid, mice were, initially, pretreated with glibenclamide (10 mg/kg, i.p.). After 1 h, animals received NaHS (50 μmol/kg, i.p) or Lawesson's reagent (81 μmol/kg, i.p). 30 min later, the mice were gavage fed (300 μl) with the test meal (0.5 mg/ml phenol red in 5% glucose). After 20 min, animals were killed for measurement of gastric retention as previously described before.

In order to study the role of K_{ATP} in the NaHS- induced pyloric sphincter relaxation, glibenclamide (10 μM) was added after 1 h of equilibration at 1 g. The drug was allowed to incubate for 30 min before the administration of NaHS (1–1000 μM). Data were expressed as percentage of the contraction control of KCl (60 mM), as described before.

2.6. Role of TRPV1 receptors in H_2S accelerated gastric emptying and in the pyloric sphincter relaxation

To evaluate the involvement of TRPV1 receptors in H_2S accelerated the gastric emptying of liquid, other mice were treated with capsazepine (competitive TRPV1 receptor antagonist, 5 mg/kg, i.p.) 30 min prior to NaHS (50 μmol/kg, p.o.) or Lawesson's reagent (81 μmol/kg, p.o.). Thirty min later, the mice were gavage- fed (300 μl) with test meal (0.5 mg/ml phenol red in 5% glucose). After 20 min, animals were sacrificed for measurement of gastric retention as described before.

In order to study the role of TRPV1 receptors in the NaHS-induced pyloric sphincter relaxation, capsazepine (3 μM) was added after 1 h of equilibration at 1 g. The drug was allowed to incubate for 30 min before the administration of NaHS (1–1000 μM). Data were expressed as percentage of the contraction control of KCl (60 mM), as described before.

2.7. Gastric acid secretion

It was used the technique previously described by Shay et al. (1945). First, pylorus ligature were carefully done in mice under inhalatory anesthesia. After 4 h, saline, L-cysteine (50 mg/kg), NaHS (50 μmol/kg) or Lawesson's reagent (81 μmol/kg) were

injected intraperitoneally. In other control groups, it was tested the gastric acid secretion induced in pylorus-ligated mice treated by ranitidine (5 mg/kg) or histamine (5 mg/kg) trough i.p. injection. After 4 h, the animals were sacrificed by deep inhalatory anesthesia, the stomach was opened and the gastric content was collected. The final volume and pH were directly determined after washing the mucosal side of the stomach with 2 ml of distilled water. Total acidity of the gastric juice was titrated with 0.01 N and using 2% phenolphthalein as an indicator.

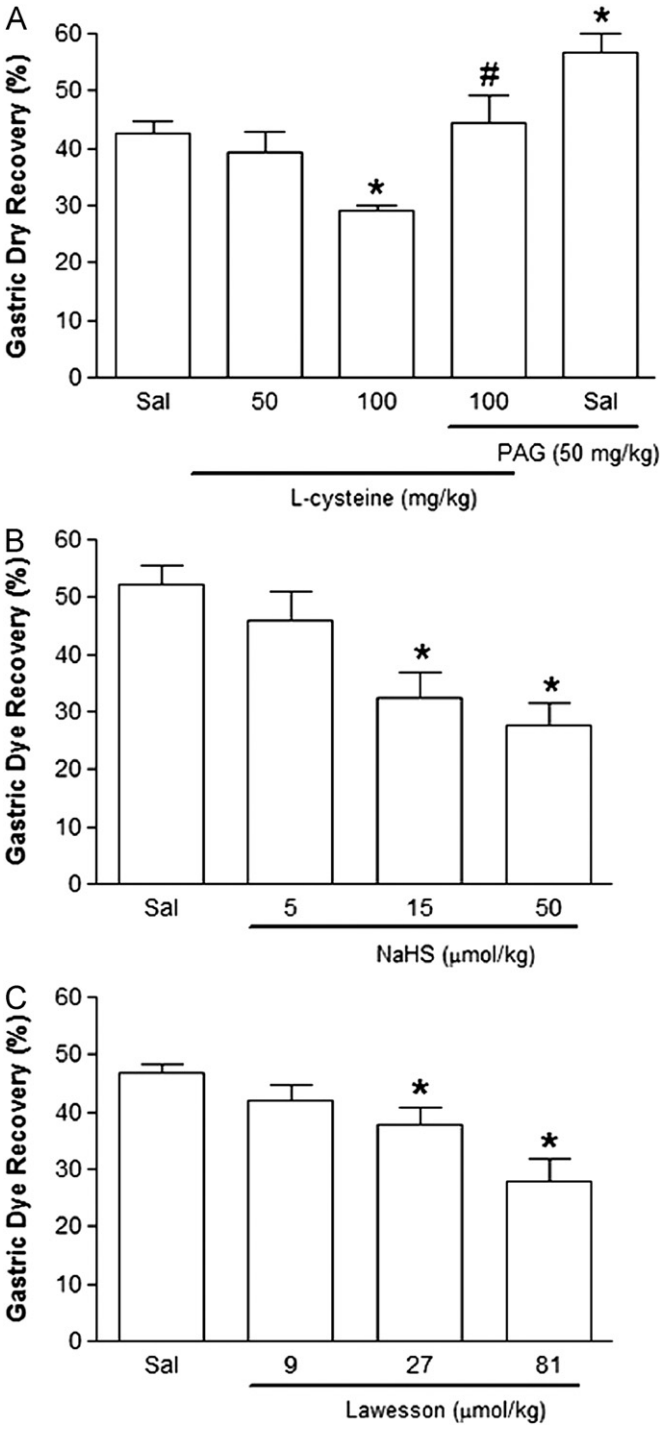

Fig. 1. L-cysteine and H_2S donors (NaHS and Lawesson's reagent) accelerate gastric emptying in mice. Each control and experimental subset consisted of 5–8 animals. Animals were treated intraperitoneally with L-cysteine alone or with propargylglycine (PAG) (panel A), NaHS (panel B) or Lawesson's reagent (panel C). Thirty min later, the mice were gavage-fed with a standard liquid test meal containing a nonabsorbable marker. After 20 min, animals were sacrificed by cervical dislocation and gastric dye recovery assessment was performed by spectrophotometry. Results are expressed as the means ± S.E.M. $^*P < 0.05$, when compared to the saline group; $\#P < 0.05$, when compared the L-cysteine 100 mg/kg group. ANOVA and Newman–Keuls test.

2.8. Statistical analysis

All values are expressed as means ± S.E.M. The analysis of variance (ANOVA Student-Newman–Keuls test) or Student's *t*-test were used to determine the statistical significance of differences between groups. Differences were considered as significant at $P \leq 0.05$.

3. Results

In Fig. 1, shows the effect of L-cysteine (panel A), NaHS (panel B) or Lawesson's reagent (panel C) treatments on the fractional gastric dye retention in awake mice. It can be seen that gastric retention decreased, in a dose-dependent manner, by 36.9% when treated by L-cysteine (100 mg/kg), 47.1% when treated by NaHS (50 μmol/kg) and 39.9% when treated by Lawesson's reagent (81 μmol/kg). The administration of propargylglycine, an inhibitor of CSE, which reduce CSE-synthesized hydrogen sulphide, reversed the drive effect of L-cysteine on gastric emptying (Fig. 1, panel A). In addition, propargylglycine alone increased gastric dye retention when compared with saline group.

Fig. 2 shows that H_2S donors (NaHS and Lawesson's reagent), and H_2S precursor (L-cysteine), also decreased the gastric retention at 10, 20 and 30 min postprandial intervals, as compared to theirs respective values obtained in the control group (saline).

To assess the contribution of K_{ATP} channels to the decrease of gastric retention induced by H_2S donors, mice were pretreated with glibenclamide, which prevented the effects of NaHS or Lawesson's reagent (panel A) on gastric retention (Fig. 3). Likewise, the Fig. 3 also shows that pretreatment with capsazepine, a

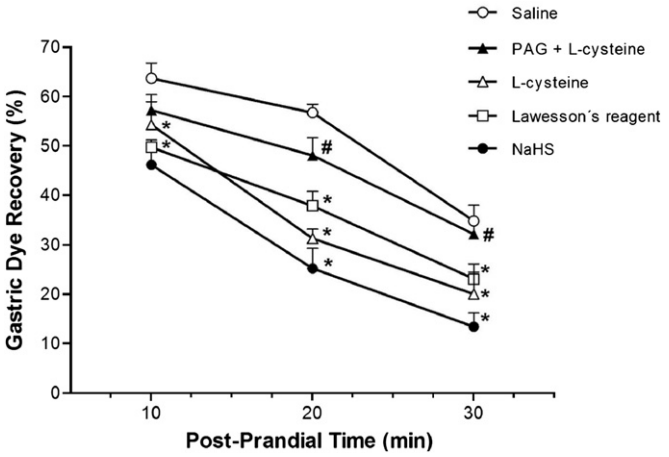

Fig. 2. Gastric dye recovery (%) obtained at 10, 20, or 30 min post-prandial intervals in awake mice treated with L-cysteine or H_2S donors. Each control and experimental subset consisted of 5–8 animals. First, animals were treated with saline (control), H_2S precursor (L-cysteine 100 mg/kg), H_2S donors (NaHS 50 μmol/kg and Lawesson's reagent 81 μmol/kg) or an inhibitor of CSE (PAG)+L-cysteine. Thirty min later, the mice were gavage-fed with a standard liquid test meal containing a nonabsorbable marker. After 10, 20 or 30 min, animals were sacrificed by cervical dislocation and gastric dye recovery assessment was performed by spectrophotometry. Results were expressed as mean ± S.E.M. $^*P < 0.05$, when compared to the saline group; $\#P < 0.05$, when compared the L-cysteine group. ANOVA and Newman–Keuls test.

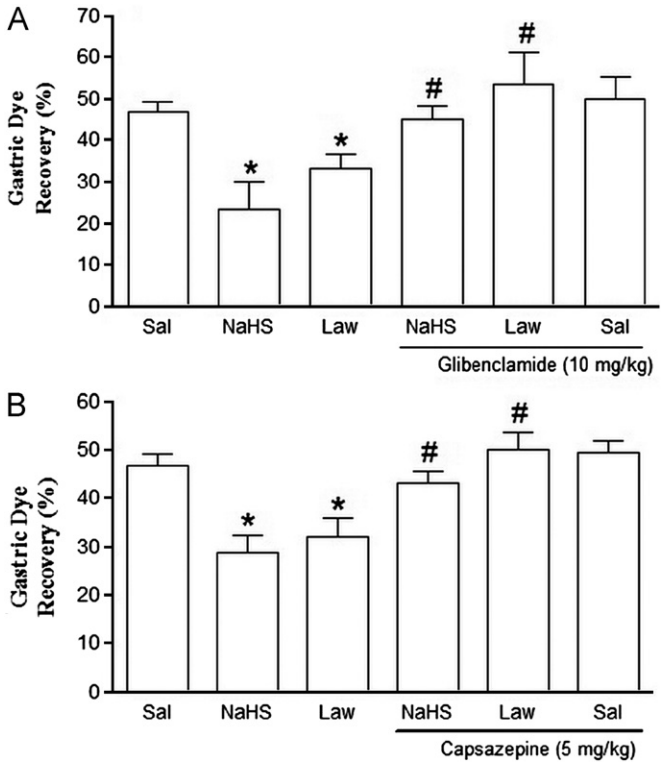

Fig. 3. Effects of K_{ATP} channels (panel A) and TRPV1 receptors (panel B) in H_2S donors accelerate gastric emptying of liquids in mice. Each control and experimental subset consisted of 5–8 animals. First, animals were pretreated with glibenclamide or capsazepine. After 1 h or 30 min, respectively, mice received intraperitoneally NaHS or Lawesson's reagent. Thirty min later, the mice were gavage-fed with a standard liquid test meal containing a nonabsorbable marker. After 20 min, animals were sacrificed by cervical dislocation and gastric dye recovery assessment was performed by spectrophotometry. Results are expressed as the means ± S.E.M. $^*P < 0.05$, when compared to the saline group; $\#P < 0.05$, when compared the NaHS or Lawesson's group. ANOVA and Newman–Keuls test.

TRPV1 antagonist, abolished the NaHS or Lawesson's reagent (panel B) induced decrease in gastric retention of liquids in mice.

We also observed that, in the pyloric sphincter, NaHS caused relaxation with a maximum value of 45.88 ± 2.97 (1000 μM) (Fig. 4, panels B and C) compared with papaverine (Fig. 4, panel A and C). However, the NaHS-induced pyloric sphincter relaxation (1000 μM NaHS) was abolished by glibenclamide 10 μM (Fig. 4, panel D) or capsazepine 3 μM (Fig. 4, panel E).

Table 1 shows that administration of L-cysteine, NaHS and Lawesson's reagent did not change the volume of gastric juice, the pH and the total acidity, as compared to the its respective values of the saline group. In contrast, the histamine treatment increased the volume and total acidity, while ranitidine (H_2 antagonist) decreased the volume and total acidity when compared to their respective values of saline group (Table 1).

4. Discussion

In the present study, we evaluated the effect of H_2S donors and the H_2S precursor on gastric emptying of liquid meals in awake mice and the involvement of K_{ATP} channels and TRPV1 receptors in this effect. Gastric emptying assessment, based on fractional dye retention, has been extensively used by others authors (Gondim et al., 1999; Sharma, 1983) and allowed us to evaluate gastric emptying in awake rats (Gondim et al., 2001), avoiding the anesthesia effects on the cardiovascular and autonomic functions and, thus, mimicking the clinical scenario with greater accuracy. Our results showed that NaHS, Lawesson's reagent and the L-cysteine accelerated the gastric emptying of liquids. Then we could infer that H_2S has prokinetic effects on gastric motility. Another possibility is a nonspecific effect of H_2S donors; however this seems improbable because PAG reversed the effect of L-cysteine on gastric emptying and PAG alone induced an increase in the gastric dye retention. PAG has been commonly used as a putative inhibitor of CSE and employed to inhibit H_2S production in cell homogenates at the tissue level and also in some *in vivo* experiments using animal models of diseases (Szabo, 2007). However, we cannot role out the possibility of non specific effect of PAG.

Since the phenol red assay employed is pH-dependent, an eventual gastric secretion change induced by H_2S donors or L-cysteine could have biased the gastric emptying measurements (Santos et al., 2007). However, this is not the case, as demonstrated in Table 1, which shows that L-cysteine or H_2S donors did not change the volume of gastric juice, pH and total acidity, as compared with the saline group. These results are in accordance with the literature (Wallace et al., 2007).

Several authors have demonstrated that H_2S may increase gastrointestinal muscle relaxation (Teague et al. 2002; Zhao et al. 2009). However, reports concerning the effects of H_2S on gastrointestinal motility *in vivo* are scarce. In our study we are reporting that H_2S donors could accelerate the gastric emptying of liquids in awake mice. This effect could be due to a decrease in gastric compliance and/or a decrease in antro- pyloric- duodenal resistance. In the present work, we showed that the decreases in pyloric resistance may be involved in this event. Since, the NaHS at concentrations of 1000 μM, induced a pyloric sphincter relaxation. These results are in accordance with the literature, Zhao et al., 2009 demonstrated that NaHS at concentrations of 300–1000 μM suppressed the amplitude of spontaneous contraction in the gastric antrum muscle, and Teague et al., 2002 showed that NaHS caused relaxation of the rabbit small intestine. Based of these findings, we propose that H_2S donors can decrease antro-pyloric- duodenal resistance by induced pyloric sphincter relaxation, which could explain the ability of H_2S donors to enhance gastric emptying.

Extensive experiments on cardiovascular tissues, including the isolated rat aorta (Zhao et al., 2001) and portal vein (Ali et al., 2006) and perfused rat mesenteric beds (Cheng et al., 2004), strongly suggest that H_2S induces vasorelaxation and decreases blood pressure (Zhao et al., 2001). The literature also shows that the blood volume retraction increases gastric emptying and decreases the mean arterial pressure (Gondim et al., 1998). Then, other possibility was that H_2S donor's prokinetic actions may be due to systemic effects on the cardiovascular system. However, in our opinion, this is not the case, because Zhao et al., (2001), demonstrated that intravenous bolus injection of H_2S decreased blood pressure of rats by 12–30 mmHg only transiently (29.5 ± 3.6 s), and our results showed that H_2S donors enhanced the gastric emptying for at least 30 min (Fig. 2). Then, it suggests that the H_2S donor's prokinetic effect results from a direct action in the gastrointestinal smooth muscle cells rather than a secondary hemodynamic effect.

Our results showed that glibenclamide, a K_{ATP} channel antagonist, reversed the prokinetic effect of H_2S donors on gastric motility. A number of studies have shown that H_2S activates K_{ATP} channels. Many effects of H_2S, including vasodilation, the inhibition of leukocyte adherence (Fiorucci et al., 2006) and analgesic effect (Distrutti et al., 2005) were inhibited by glibenclamide. In the stomach, glibenclamide has been shown to partially reverse the reduction in amplitude of the spontaneous contraction of the antrum smooth muscle induced by NaHS (Zhao et al., 2001). Our results show that, in the pyloric sphincter, glibenclamide

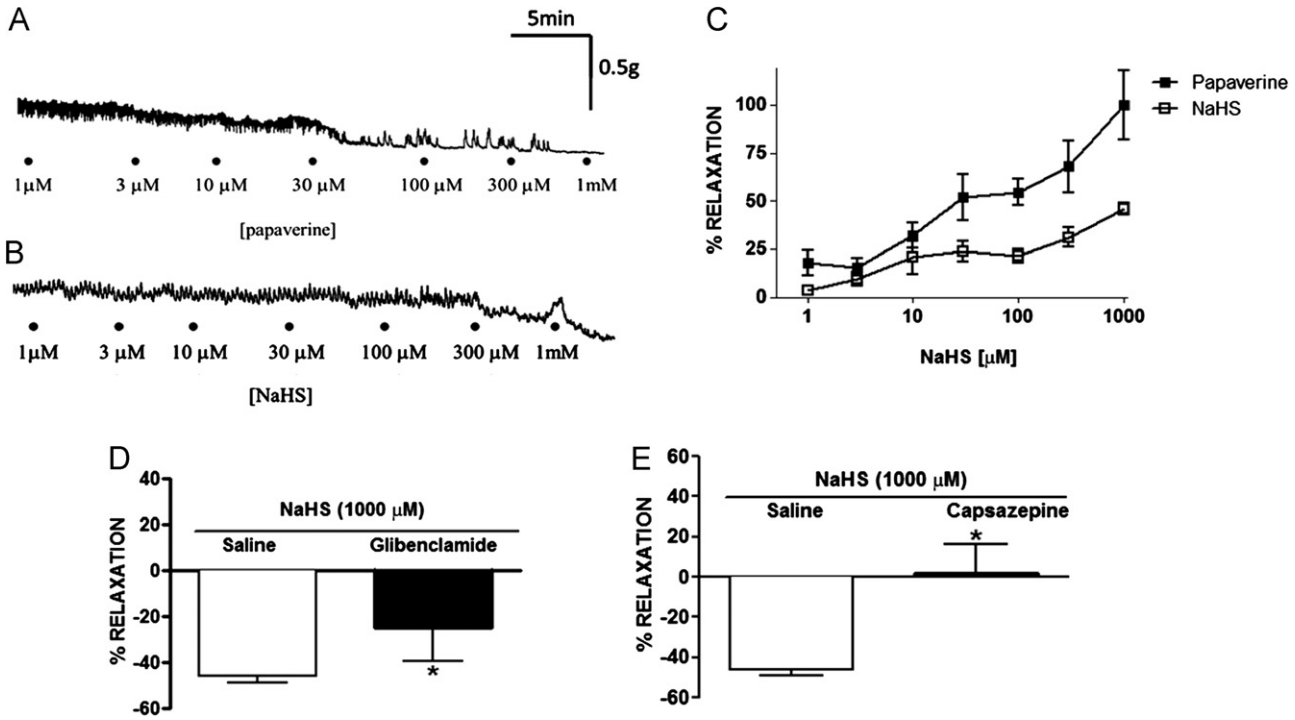

Fig. 4. Effects of K_{ATP} channels and TRPV1 receptors in NaHS induced pyloric sphincter muscle relaxation. After 1 h of equilibration, it was performed a cumulative curve concentration-response with NaHS (1–1000 μM). Glibenclamide and capsazepine was allowed to incubate for 30 min before the administration of NaHS (1–1000 μM). Panel A and B shows typical traces of the relaxation induced by papaverine and NaHS. Panel C, shows that NaHS at 1000 μM induced a pyloric sphincter relaxation, which was reversed by the pre-incubation with glibenclamide (panel D) or capsazepine (Papel E). Data show the mean ± S.E.M. from 4–9 mice. $^{*}P < 0.05$, when compared to the NaHS group, Student's *t*-test.

Table 1
Effects of L-cysteine or H_2S donors (NaHS or Lawesson's reagent) on gastric secretion of 4 h pylorus-ligated mice.

Experimental group ($N=6$)	Volume (μl)	pH	Total acidity (mEq[H^+]/l/4 h)
Saline	618 ± 76.6	1.9 ± 0.3	5.2 ± 0.5
L-cysteine (100 mg/kg)	565 ± 67.7	1.8 ± 0.2	6.9 ± 1.1
NaHS (50 μmol/kg)	492.5 ± 44.2	1.9 ± 0.3	5.6 ± 1.1
Lawesson's (81 μmol/kg)	594.3 ± 69.9	2.0 ± 0.2	5.8 ± 0.9
Histamine	1190 ± 163.6[a]	1.9 ± 0.03	12 ± 0.9[a]
Ranitidine	347.5 ± 91.3[a]	2.3 ± 0.03[a]	3.7 ± 0.5[a]

[a] $P < 0.05$, vs saline group; data shown are expressed as the means ± S.E.M. ANOVA and Newman–Keuls test.

decreased the NaHS-induced relaxation, infer that K_{ATP} activation was involved in this H_2S donor effect. Gallego et al. (2008) also demonstrated that K_{ATP} channels play an important role in the effect of H_2S in rat colon. On the other hand, Teague et al. (2002) demonstrated that the inhibitory effect of NaHS on the field-stimulated ileum was unaffected by pretreatment with glibenclamide and Nagao et al. (2011) showed that H_2S inhibited reversibly the spontaneous and cholinergically stimulated contractile activity in rat ileal longitudinal muscle and this effect was not mediated via K_{ATP} channels. Almost all the primary effects of action of H_2S in vascular smooth muscle are mediated by K_{ATP} channels, the opening of which induces hyperpolarization of the cell, closing of voltage-gated calcium channels, and muscular relaxation (Distrutti et al., 2005). In GI smooth muscle, the importance of K_{ATP} channels is more controversial and may vary with anatomic location.

Previous studies examining the urinary bladder muscle (Patacchini et al., 2005), intestinal secretions (Schicho et al., 2006) and gastric defense (Distrutti et al., 2005) had suggested a role for capsaicin sensitive nerves in the biological effects of H_2S. Other investigators have shown that capsaicin, which act via vanilloid receptors (Sterner and Szallasi, 1999), affect gastric and intestinal motility in vitro (Maggi et al., 1986). A recent report has shown that H_2S acts to increase mucosal secretion via stimulating TRPV-1 receptors on primary afferent nerve fibers (Schicho et al., 2006). Then, we hypothesized that TRPV1 receptors might be involved in the effects of H_2S donors on gastric emptying. Our findings demonstrated that enhancement of gastric emptying induced by H_2S donors were abolished by capsazepine, a TRPV1 receptors antagonist. TRPV1-dependent pathway has been shown to negatively influence the vagally-mediated muscle contractions in the gastrointestinal tract (Boudaka et al., 2007). It was demonstrated that capsaicin activates enteric cholinergic neurons in guinea-pig small intestine and induces contractile effect and your inhibition slower gastric emptying (Benko et al., 2005). Likewise, capsaicin induced a relaxation in precontracted muscle strips of the rat gastric fundus (Lefebvre et al., 1991). In the stomach, we also demonstrated that pretreatment with capsazepine abolishes the gastroprotective effects of L-cysteine, NaHS, or Lawesson's reagent on ethanol-induced gastric lesions (Medeiros et al., 2009). In our experiments, we also showed that capsazepine inhibit the NaHS-induced pyloric relaxation, suggests that the inhibitory effect of H_2S donor on contractile activity is, in part, mediated via activation of TRPV1 receptors. In contrast, Gallego et al. (2008) found that the relaxation effects of NaHS on the mouse jejunum and colon in TRPV1-deficient mice were almost identical to the wild-type mice. Nagao et al. (2011) also demonstrated there was no change of the inhibitory effect of H_2S on contractile activity

after pretreatment of capsaicin. The authors, however, not exclude the possibility that the decrease in baseline spontaneous contractile activity after exposure to the greater dose (10^{-4} M) capsaicin might have impacted some of the inhibitory effect of H_2S. Our results could not role out non-specific effects of capsazepine (Docherty et al., 1997, Liu and Simon, 1997).

Enteric neurons in the gastrointestinal tract are contacted by primary afferents of spinal and vagal origin (Mazzia and Clerc, 1997). Previous investigators have shown that chronic treatment with capsaicin (to ablate capsaicin-sensitive afferent neurons) does not affect the gastrointestinal propulsion in physiological states, whereas it reduces the inhibition of gastrointestinal transit due to surgical trauma or peritoneal irritation (Holzer et al., 1987; Holzer, 1991). A substantial number of these primary afferents have been shown to be immunoreactive to the TRPV1 (Holzer et al., 1987). These neurons can modulate gastrointestinal motility as they convey signals coming from the gastrointestinal tract to the central nervous system, and may simultaneously release transmitters that are able to affect enteric neurotransmission (Schicho et al., 2006). Medeiros et al. (2009) suggest that K_{ATP} and TRPV1 channels co-expressed on primary afferents neuron are involved in the gastric protection by H_2S. In porcine model of the metabolic syndrome, Bratz et al. (2008) showed that TRPV1 channels are functionally expressed in the systemic vessels and mediate endothelium-dependent vasodilation through a mechanism that involves nitric oxide and K^+ channels.

In summary, our results suggest that H_2S donors enhanced gastric emptying and induced a relaxation of pyloric sphincter muscle. Although there are many mechanisms by which this effect could occur, our data support the hypothesis that the activation of K_{ATP} channels and afferent neurons/TRPV1 receptors are of primary importance.

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