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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