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Potential role of the gaseous mediator hydrogen sulphide (H_2S) in inhibition of human colonic contractility

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ABSTRACT

Background: Hydrogen sulphide (H_2S) is an endogenous signalling molecule that might play a physiologically relevant role in gastrointestinal motility. Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) are two enzymes responsible for H_2S production. D,L-Propargylglycine (PAG) is a CSE inhibitor whereas both aminooxyacetic acid (AOAA) and hydroxylamine (HA) are CBS inhibitors. The characterization of H_2S responses and its mechanism of action are crucial to define H_2S function.
Methods: Human colonic strips were used to investigate the role of H_2S on contractility (muscle bath) and smooth muscle electrophysiology (microelectrodes). NaHS was used as a H_2S donor.
Results: Combination of PAG and AOAA depolarized the smooth muscle (5–6 mV, $n=4$) and elicited a transient increase in tone (260.5 ± 92.8 mg, $n=12$). No effect was observed on neural mediated inhibitory junction potential or relaxation. In the presence of tetrodotoxin 1 μM, NaHS concentration-dependently inhibited spontaneous contractions ($EC_{50}=329.2$ μM, $n=18$). This effect was partially reduced by the guanylyl cyclase inhibitor ODQ 10 μM ($EC_{50}=2.6$ μM, $n=12$) and by L-NNA 1 mM ($EC_{50}=1.4$ mM, $n=8$). NaHS reversibly blocked neural mediated cholinergic ($EC_{50}=2$ mM) and tachykinergic ($EC_{50}=5.7$ mM) contractions. NaHS concentration-dependently reduced the increase in spontaneous mechanical activity (AUC) induced by carbachol ($EC_{50}=1.9$ mM) and NKA ($EC_{50}=1.7$ mM AUC).
Conclusions: H_2S might be an endogenous gasomediator regulating human colonic contractility. Its inhibitory effect is observed at high concentrations and could be mediated by a direct effect on smooth muscle with a possible synergistic effect with NO, as well as by an interaction with the cholinergic and tachykinergic neural mediated pathways.

Introduction

Hydrogen sulphide (H_2S) is nowadays recognized as the third gasotransmitter along with nitric oxide (NO) and carbon monoxide (CO). H_2S fulfils, at least in part, the criteria to be considered a gasotransmitter with relevant functions in the gastrointestinal (GI) tract [1,2].

H_2S is synthesized mainly by the two pyridoxal phosphate dependent enzymes cystathionine β-synthase (CBS; EC 4.2.1.22) and cystathionine γ-lyase (CSE; EC 4.4.1.1) which use L-cysteine as substrate [3,4]. In addition, a third non-pyridoxal-phosphate-dependent enzyme, 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2), has been proposed as a H_2S generating enzyme in combination with cysteine aminotransferase (EC 2.6.1.3) [3,5,6]. Due to bacterial production, H_2S is present in the lumen of the human large intestine at high concentrations (range of millimolar). However, the amount of H_2S that reaches the subepithelial space is very low due to the capability of faecal components to bind the sulphide as well as to colonic epithelial cells metabolic barrier [7]. This H_2S

Abbreviations: H_2S, hydrogen sulphide; GI, gastrointestinal; GC, guanylyl cyclase; NKA, neurokinin A; NaHS, sodium hydrogen sulphide; TTX, tetrodotoxin; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; NO, nitric oxide; CO, carbon monoxide; SMCs, smooth muscle cells; ICCs, interstitial cells of cajal; RMP, resting membrane potential; IJP, inhibitory junction potential; EJP, excitatory junction potential; AOAA, aminooxyacetic acid; PAG, D,L-propargylglycine; HA, hydroxylamine; EFS, electrical field stimulation; AUC, area under curve.

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source could exert significant effects when the epithelial barrier is dysfunctional or damaged [7–9].

H_2S endogenous production has been demonstrated in the GI tract and both CBS and CSE have been localized along the gut [1,10,11]. CBS and CSE have been detected in enteric neurons, interstitial cells of Cajal (ICCs) [9] and smooth muscle cells (SMCs) [11,12]; therefore it is possible that different cell types are able to produce H_2S.

Studies performed with the H_2S donor NaHS have demonstrated that H_2S has pro-secretory effects in several species including the human and rat colon [9,12]. NaHS presynaptically potentiates fast excitatory cholinergic nicotinic post-synaptic potentials (fEPSPs) in splanchnic nerves which might contribute to the inhibition of colonic motility in mice [13]. NaHS also causes concentration-dependent relaxation of smooth muscle contractility and inhibition of peristalsis [4,11,14–17], and might interact directly with SMCs causing hyperpolarization and therefore smooth muscle relaxation [16]. Studies performed with laboratory animals have demonstrated that NaHS is able to interact with the cholinergic pathway. Recently, a reduction of cholinergic excitatory junction potential (EJP) induced by NaHS has been demonstrated in the rat colon [18]. In the respiratory system, H_2S reversibly inhibited acetylcholine (ACh)-induced calcium oscillations responsible for the contraction of airway SMCs [19]. Similar findings have been reported in guinea-pig ileum, rat jejunum and rabbit stomach, where H_2S significantly reduced cholinergic mediated contractions [15,20,21].

Scarce data is available about the possible role of endogenous H_2S in the GI tract. A role for endogenous H_2S in smooth muscle inhibitory tone has been recently proposed, as both CBS and CSE inhibitors were able to depolarize resting membrane potential (RMP) and to increase spontaneous motility in the rat colon [11]. Endogenous H_2S might also participate in the CO mediated transwall gradient of the RMP in the mouse colon [22].

The vast majority of the papers refer to the potential role of H_2S in animal models. However, little is known about the potential role of H_2S in the human GI tract [9,16]. Accordingly, in the present work we have investigated the potential role of H_2S in human colonic excitability and contractility using inhibitors of CSE and CBS. Moreover, we have investigated the inhibitory effect of NaHS on spontaneous and neural mediated contractions.

Methods

Human tissue preparation

Tissue specimens of human sigmoid colon were obtained from patients undergoing colon resections for neoplasm. Colon segments from macroscopic marginal regions were collected and transported in cold saline buffer. Tissue was placed in Krebs solution on a dissection dish and the mucosal layer was carefully removed. Muscle strips (10 mm × 4 mm) were cut oriented in the circular direction. Patients provided informed consent and all the experimental procedures were approved by the ethics committee of the Hospital of Mataró (Barcelona, Spain) CEIC code 04/09.

Mechanical studies

Mechanical activity was studied in a 10 mL organ bath. Circularly-oriented preparations were tied to an isometric force transducer (Harvard VF-1 Harvard Apparatus Inc., Holliston, MA, USA) using 2/0 silk thread. The isometric force transducer was connected to an amplifier to record the mechanical activity. Data were digitalized (25 Hz) using Datawin1 software (Panlab-Barcelona, Spain) coupled to an ISC-16 A/D card installed in a PC computer. A tension of 4 g was applied to the tissue and was allowed to equilibrate for 1 h. After this period, strips displayed spontaneous phasic activity.

Electrical field stimulation (EFS) was applied through two platinum electrodes (0.7 cm apart) placed on the support holding the tissue. Different parameters of EFS and different pharmacological conditions were used to reveal inhibitory and excitatory neural mediated responses. To study the inhibitory neuromuscular transmission, preparations were studied in classical "non-adrenergic, non-cholinergic conditions" (NANC). In this case, Krebs containing atropine, propranolol and phentolamine (all at 1 μM) was used and EFS was applied for 2 min (pulse duration 0.4 ms, frequency 2 Hz, and amplitude 50 V) to inhibit spontaneous myogenic contractions. In contrast, in order to study the excitatory neuromuscular transmission, tissue was incubated with "non-nitrergic, non-purinergic" conditions. In this case Krebs solution contained L-NNA (1 mM) and MRS2500 (1 μM) to avoid both NO and purinergic neural mediated inhibitory responses [23–26]. To study the cholinergic component, EFS was applied for 1 s (pulse duration 0.4 ms, frequency 50 Hz, amplitude 50 V). To study the tachykinergic component, "non-nitrergic, non-purinergic" conditions were also used and atropine (10 μM) was added to avoid neural mediated cholinergic responses. In this case EFS was applied for 10 s (pulse duration 0.4 ms, frequency 50 Hz, amplitude 50 V).

Cumulative concentration–response curves of NaHS (10, 30, 60, 100, 300, 600, 1000, 2000 and 3000 μM) were performed in the presence of the neural blocker tetrodotoxin (TTX) at 1 μM and after incubation with ODQ (10 μM) and L-NNA (1 mM). Cumulative concentration–response curves of HA (0.01, 0.1, 1, 10 and 100 μM) were performed in control conditions and in the presence of TTX (1 μM) and the guanylyl cyclase (GC) inhibitor ODQ (10 μM). Carbachol-induced responses were studied by means of a cumulative concentration–response curve (0.01, 0.1, 1, 3 and 10 μM) in control conditions and after incubating with NaHS or atropine both in normal and in calcium-free Krebs solution. Neurokinin A (NKA) effects were also studied by performing a cumulative concentration response curve (0.1, 1, 10 and 100 nM) in control conditions and after incubation with NaHS or GR159897 (NK_2 receptor antagonist).

Intracellular microelectrode recordings

Muscle strips were pinned to the base of a Sylgard coated chamber and continuously perfused with Krebs solution at 37 °C. Strips were allowed to equilibrate for approximately 1 h before recording. Circular SMCs were impaled with sharp glass microelectrodes filled with 3 M KCl (30–60 MΩ). Membrane potential was measured using standard electrometer Duo773 (WPI Inc., Sarasota, FL, USA). Tracings were displayed on an oscilloscope 4026 (Racal-Dana Ltd., Windsor, UK) and simultaneously digitalized (100 Hz) using PowerLab 4/30 system and Chart 5 software for Windows (all from ADInstruments, Castle Hill, NSW, Australia). Nifedipine (1 μM) was used to abolish the mechanical activity and obtain stable impalements. EFS were applied using two silver chloride plates placed perpendicular to the longitudinal axis of the preparation and 1.5 cm apart. Train stimulation had the following parameters: total duration, 100 ms; frequency, 30 Hz; pulse duration, 0.3 ms, and increasing amplitude strengths of 5, 10, 12, 15, 17, 20, 25, 30 and 50 V. RMP was measured before and after drug addition. The amplitude of IJP was measured in control conditions and after infusion of drugs.

Data analysis and statistics

Cumulative concentration–response curves of H_2S using NaHS as a donor were performed in order to calculate the EC_{50}. To