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Inhibitory action of hydrogen sulfide on esophageal striated muscle motility in rats

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ABSTRACT

Hydrogen sulfide (H_2S) is recognized as a gaseous transmitter and has many functions including regulation of gastrointestinal motility. The aim of the present study was to clarify the effects of H_2S on the motility of esophageal striated muscle in rats. An isolated segment of the rat esophagus was placed in an organ bath and mechanical responses were recorded using a force transducer. Electrical stimulation of the vagus nerve evoked contractile response in the esophageal segment. The vagally mediated contraction was inhibited by application of an H_2S donor. The H_2S donor did not affect the contraction induced by electrical field stimulation, which can excite the striated muscle directly, not via vagus nerves. These results show that H_2S has an inhibitory effect on esophageal motility not by directly attenuating striated muscle contractility but by blocking vagal motor nerve activity and/or neuromuscular transmissions. The inhibitory actions of H_2S were not affected by pretreatment with the transient receptor potential vanniloid-1 blocker, transient receptor potential ankyrin-1 blocker, nitric oxide synthase inhibitor, blockers of potassium channels, and ganglionic blocker. RT-PCR and Western blot analysis revealed the expression of H_2S-producing enzymes in esophageal tissue, whereas application of inhibitors of H_2S-producing enzymes did not change vagally evoked contractions in the esophageal striated muscle. These findings suggest that H_2S, which might be produced in the esophageal tissue endogenously, can regulate the motor activity of esophageal striated muscle via a novel inhibitory neural pathway.

1. Introduction

Hydrogen sulfide (H_2S) is recognized as a gaseous transmitter and a biological mediator (Kimura, 2014a, 2014b; Olson et al., 2012; Vandiver and Snyder, 2012). H_2S is endogenously produced by three enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST) (Kimura, 2014a, 2014b; Olson et al., 2012; Vandiver and Snyder, 2012). Gut bacteria can also produce H_2S (Pimentel et al., 2013). H_2S has many functions in the control of cardiovascular, immune, and gastrointestinal systems (Farrugia and Szurszewski, 2014; Jimenez, 2010; Krueger et al., 2010; Vandiver and Snyder, 2012). H_2S can regulate gastrointestinal motility (Farrugia and Szurszewski, 2014; Jimenez, 2010). Excitatory and inhibitory effects of H_2S on smooth muscle motility of the stomach (Dhaese and Lefebvre, 2009; Huang et al., 2013; Liu et al., 2014; Medeiros et al., 2012; Zhao et al., 2009), duodenum (Lu et al., 2014), jejunum (Gallego et al., 2008; Kasparek et al., 2012; Lu et al., 2014), ileum (Hosoki et al., 1997; Lu et al., 2014; Teague et al., 2002) and colon (Gallego et al., 2008; Gil et al., 2011, 2013; Lu et al., 2014) have been reported. The inhibitory effects are mediated via opening of potassium channels on smooth muscle cells (Gallego et al., 2008; Lu et al., 2014; Medeiros et al., 2012; Zhao et al., 2009), and the excitatory effects are expressed by inhibition of potassium channels on smooth muscle cells (Liu et al., 2014; Lu et al., 2014; Zhao et al., 2009) and activation of transient receptor potential vanniloid-1 (TRPV1) on sensory nerves (Lu et al., 2014). In vascular smooth muscle, on the other hand, H_2S has been shown to cause relaxation by opening of potassium channels (Zhao et al., 2001), enhancing the effects and production of nitric oxide (NO) (Hosoki et al., 1997; Kida et al., 2013), and activating transient receptor potential ankyrin-1 (TRPA1) on sensory nerves (Pozsgai et al., 2012; White et al., 2013). In addition, H_2S can induce contraction in the urinary bladder by activating capsaicin-sensitive nerves (Patacchini et al., 2004). These findings suggest that H_2S has dual effects on smooth muscle motility, which are mediated by various factors including ion channels and receptors on neurons and muscle cells.

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In contrast to the tunica muscularis of the stomach, small intestine and large intestine, the external muscle layer of the mammalian esophagus contains not only smooth muscle fibers but also striated muscle fibers (Neuhuber et al., 2006; Wörl and Neuhuber, 2005). Peristalsis of the striated muscle esophagus is regulated by vagal neurons (Bieger and Neuhuber, 2006; Clouse and Diamant, 2006). In addition, a local neural pathway involving capsaicin-sensitive sensory neurons and intrinsic nitrergic neurons regulates esophageal striated muscle in the rat, mouse, hamster and suncus (Boudaka et al., 2007; Izumi et al., 2003; Shiina et al., 2012, 2006). Capsaicin-activated primary afferent neurons act on myenteric nitrergic neurons, which release NO, and then NO can inhibit the release of neurotransmitters from vagal motor neurons in the striated muscle esophagus (Boudaka et al., 2007; Izumi et al., 2003; Shiina et al., 2012, 2006). Although inhibitory effects of H_2S on esophageal motility in fish (Dombkowski et al., 2011) and the echiuran worm (Julian et al., 1998) have been shown, it is not clear whether H_2S is involved in the regulation of striated muscle motility in the mammalian esophagus.

Therefore, the aim of the present study was to clarify the effects of H_2S on motility of the esophageal striated muscle in the rat. Firstly, we investigated whether the effect of H_2S on esophageal motility is mediated via a neurogenic pathway. We then examined the characteristics of the H_2S signal pathway in the esophagus pharmacologically by comparing them with those of the H_2S signal pathway in other organs.

2. Materials and methods

2.1. Animals

Male Wistar rats (*Rattus norvegicus*, 10–15 weeks of age, 250–300 g in weight) were obtained from Japan SLC (Shizuoka, Japan). They were maintained in plastic cages at 24 ± 2 °C with a 12:12-h light-dark cycle (light on at 08:00–20:00 h) and given free access to laboratory chow and water. The Animal Care and Use Committee of Gifu University approved the animal experiments.

2.2. Esophageal tissue preparations

Animals were anesthetized with isoflurane and were exsanguinated via axillary arteries. A 1-cm-long segment from the middle thoracic part of the esophagus was dissected out. The segment of the esophagus was immediately immersed in Krebs' solution (see below) at room temperature, and the intraluminal contents of the excised segment were flushed using a small cannula containing Krebs' solution.

2.3. Recording of mechanical activity in esophageal segments

The whole segment was transferred to a 10-ml thermostatically controlled (35 °C) organ bath containing Krebs' solution bubbled with 95% O_2+5% CO_2 gas mixture and maintained at pH 7.4. Contractile activity was measured in the circular direction so as to avoid contamination from contractions of the longitudinally oriented smooth muscularis mucosae. Two L-shaped stainless-steel pins were introduced into the esophageal lumen; one pin was fixed to the bottom of the organ bath and the other was connected to the bar of an isometric force transducer (T7-8-240; Orientec, Tokyo, Japan). Contractile responses were recorded isometrically on a PowerLab system (AD Instruments, Bella Vista, NSW, Australia) through an AC amplifier (AS1202, NEC, Tokyo, Japan). An initial resting tension of 1.0 g was applied to the preparations, which were subsequently allowed to equilibrate for at least 30 min.

2.4. Electrical stimulations

For inducing muscle contractile response, electrical stimulations were applied to esophageal preparations. In experiments using vagal stimulation, the end of the vagus nerve trunk was drawn into a bipolar suction electrode and the electrode was immersed together with the esophagus preparation in the bath. For avoiding interference with the tissue movement, the electrode was fixed loosely. Electrical field stimulation (EFS) was also applied through a pair of platinum electrodes each placed on either side of the tissue for direct stimulation to the esophageal striated muscle. The vagus nerve and the tissue were stimulated using an electronic stimulator (model SEN-3201, Nihon Kohden, Tokyo, Japan) connected to the electrodes. For stimulation of vagus nerves to evoke twitch contractions, single square-wave pulses of supramaximal intensity (80 V) and 100 μs in duration were applied at intervals of 1 min. For EFS, single square-wave pulses of supramaximal intensity (80 V) and 10 ms in duration were applied at intervals of 2 min.

2.5. Solutions and drugs

During experiments, tissues were maintained in Krebs' solution consisting of (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.7. D-tubocurarine, N^G-nitro-L-arginine methyl ester (L-NAME) and DL-propargylglycine (PAG) were obtained from Sigma-Aldrich (St Louis, MO, USA). Tetrodotoxin, tetraethylammonium chloride (TEA), (aminooxy)acetic acid (AOAA), sodium monohydrogensulfide (NaHS) and hexamethonium bromide were obtained from Wako (Osaka, Japan). Glibenclamide, 4-(3-Chloro-2-pyridinyl)-*N*-[4-(1,1-dimethylethyl) phenyl]-1-piperazinecarboxamide (BCTC), and 2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)-*N*-(4-isopropylphenyl)acetamide (HC030031) were obtained from Tocris Bioscience (Bristol, UK). Apamin was obtained from Peptide Institute (Osaka, Japan). Tetrodotoxin was dissolved in citrate solution. BCTC, HC030031 and glibenclamide were dissolved in DMSO. Other drugs were dissolved in distilled water. The highest concentration of vehicles for the drugs alone had no effect on the basal tone and contractile responses at the concentrations used. The concentrations of drugs given were final concentrations in the bath solution.

2.6. RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

The expression of H_2S-producing enzyme gene mRNA was assessed by RT-PCR. Total cellular RNA was extracted from tissue homogenates of the rat esophagus, stomach, ileum, and colon using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). First-strand cDNA was synthesized from 2 μg of total RNA by using SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) and Random primers (Thermo Fisher Scientific). The absence of PCR-amplified DNA fragments in the samples without reverse transcription indicated the isolation of RNA free from genomic DNA contamination. The PCR was performed with platinum Taq DNA polymerase (Thermo Fisher Scientific). The primer sets were as follows: CBS sense 5′- CCA GAA AAA GGG CAA CTG GA -3′ and anti-sense 5′- GGA TCT ACA CCG ATG ATT TT -3′ (predicted size=770 bp); CSE sense 5′- AAT GGA GTT CGC GTG CTG TG -3′ and anti-sense 5′- ATA AAT AAC CTT TTC TAC CC -3′ (predicted size=782 bp); 3MST sense 5′- TGG ACG CCC GCG CAG CTG GC -3′ and anti-sense 5′- TGT GTC CTT CAC AGG GTC TTC -3′ (predicted size=361 bp); and β-actin sense 5′- TGA CCC TGA AGT ACC CCA TTG-3′ and anti-sense 5′- TCA GGA TCT TCA TGA GGT AG -3′ (predicted size=387 bp). All primers were purchased from