European Journal of Pharmacology 616 (2009) 223–228

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, Gastrointestinal and Urogenital Pharmacology

Dual effect of exogenous hydrogen sulfide on the spontaneous contraction of gastric smooth muscle in guinea-pig

Peng Zhao [a], Xu Huang [a], Zuo-yu Wang [a], Zhang-xun Qiu [a], Yan-fei Han [a], Hong-li Lu [a], Young-chul Kim [b], Wen-xie Xu [a,*]

[a] Department of Physiology, Shanghai Jiaotong University School of Medicine, 800 Dongchuan Road, 328# Wenxuan Medical Building, Shanghai 200240, China
[b] Department of Physiology, Chungbuk National University College of Medicine, 12 Gaeshin-dong, Hungduk-gu, Cheongju, Chungbuk, 361-763, Korea

ARTICLE INFO

Article history:
Received 14 February 2009
Received in revised form 4 May 2009
Accepted 18 May 2009
Available online 23 May 2009

Keywords:
Hydrogen sulfide
Gastric smooth muscle
ATP-sensitive K^+ channel
Voltage–gated K^+ channel

ABSTRACT

Hydrogen sulfide (H_2S) is produced endogenously in mammalian tissues and is important in both physiological and pathological processes. Despite its importance, little is known regarding the effect of H_2S on gastrointestinal motility. We evaluated the effect of H_2S on the spontaneous contraction of gastric antrum smooth muscle in the guinea pig (*Cavia porcellus*) using a physiograph. In addition, we investigated whether the effect of H_2S was mediated by ionic channels by recording membrane currents in freshly dispersed gastric antrum myocytes using a whole-cell patch clamp. Sodium hydrogen sulfide (NaHS), an H_2S donor, had a dual effect on the spontaneous contraction of gastric antrum muscle strips. At high concentrations (0.3–1.0 mM), NaHS suppressed the amplitude of spontaneous contraction. At low concentrations (0.1–0.3 mM), NaHS enhanced the resting tension of muscle strips while slightly reducing the contractile amplitude. The excitatory effect on spontaneous contraction, caused by low concentrations of NaHS, was abolished when the muscle strips were pretreated with 10 mM tetraethylammonium (TEA), a nonselective potassium channel blocker, or 0.5 mM 4-Aminopyridine (4-AP), a voltage-gated K^+ channel blocker. However, the excitatory effect of NaHS was not completely blocked by low concentrations of TEA (1 mM). Pretreatment with both TEA (1 mM) and 4-AP (0.5 mM) completely abolished the excitatory effect. The dose-response curve for the inhibitory effect of NaHS on the spontaneous contraction of gastric smooth muscle was shifted significantly to the left by TEA and 4-AP. Both Pinacidil, a K_{ATP} channel opener, and NaHS significantly inhibited TEA-potentiated spontaneous contraction. Glibenclamide, a K_{ATP} channel blocker, partially, but significantly, reversed the reduction in amplitude. NaHS enhanced the amplitude of the K_{ATP} current, but inhibited the voltage-gated K^+ channel current (IK_V) in a dose-dependent manner. NaHS had no effect on STOC at low concentrations (0.1–1.0 mM) but significantly inhibited STOC at high concentrations (4–10 mM). Our results suggest that H_2S has multiple actions during the regulation of gastric motility in the guinea-pig. An excitatory effect is mediated via inhibition of the voltage-gated K^+ channel and an inhibitory effect is mediated via activation of the K_{ATP} channel.

1. Introduction

Hydrogen sulfide (H_2S), traditionally known as a toxic gas, is also an important gasotransmitter. H_2S is generated in mammalian cells during L-cysteine metabolism. Its production is catalyzed primarily by 3 enzymes: cystathionine β-synthetase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptosulfurtransferase (Beauchamp et al., 1984; Guidotti, 1996; Hosoki et al., 1997; Moore et al., 2003; Kimura et al., 2005; Tang et al., 2006). A number of studies suggest that H_2S has many physiological functions including the regulation of vascular tone, myocardial contractility, neurotransmission, and insulin secretion (Xu et al., 2008a; Yang et al., 2005; Lowicka and Bełtowski, 2007; O'Sullivan, 2006). Administration of H_2S or sodium hydrogen sulfide (NaHS), an H_2S donor, results in vascular smooth muscle relaxation and blood vessel dilation (Wang et al., 2008; Zhao et al., 2001; Tang et al., 2005), as well as an increase in insulin release (Yang et al., 2005). H_2S also has a negative chronotropic and inotropic effect in the heart, which is thought to be protective (Xu et al., 2008a; Geng et al., 2004).

Importantly, H_2S also plays a significant role in regulating the gastrointestinal system. Both CBS and CSE are found in guinea-pig (*Cavia porcellus*) and human colonic submucosal and myenteric nerve plexuses. Serosal application of NaHS or L-cysteine stimulates luminal chloride secretion in guinea-pig and human colonic tissues (Schicho et al., 2006). Together, these data suggest that H_2S is generated in the enteric nervous system and participates in the regulation of gut secretory function. Several studies also have demonstrated that H_2S is involved in the regulation of gastrointestinal motility. For example, H_2S inhibits spontaneous or acetylcholine-induced contraction of the ileum in a number of animal species, including the rabbit and guinea-pig (Hosoki et al., 1997; Teague et al., 2002). In addition,

* Corresponding author. Tel.: +86 21 34205639.
E-mail address: wenxiexu@sjtu.edu.cn (W. Xu).

0014-2999/$ – see front matter.
doi:10.1016/j.ejphar.2009.05.014

intraperitoneal administration of NaHS causes relaxation of the rat colon *in vivo* (Distrutti et al., 2006). The majority of studies suggest that at physiological concentrations H_2S induces relaxation of smooth muscle. This effect has been observed in the respiratory tract, the vascular system, the intestine, and the bladder (Wang et al., 2008; Zhao et al., 2001; Tang et al., 2005; Kubo et al., 2007; Wang, 2002). However, Ali et al. (2006) reported that low concentrations of H_2S enhanced vascular tension caused by phenylephrine (PE) or high levels of KCl.

We investigated the effect of exogenous H_2S on gastric antrum smooth muscle in the guinea-pig. In addition, we evaluated the effect of H_2S on three potassium channels: an ATP-sensitive K^+ channel (K_{ATP} channel), a voltage-gated K^+ channel (delayed rectifier potassium channel, I_{KV} channel), and a Ca^{2+}-activated K^+ channel to determine whether they were involved in mediating the effects of H_2S.

2. Material and methods

2.1. Tissue preparation and isometric measurement

We used male and female EWG/B guinea-pigs (mean weight: 300 ± 50 g) provided by the Experimental Animal Center of the Chinese Academy of Sciences, Shanghai. The animals were euthanized using a lethal dose of intravenous pentobarbital sodium (50 mg/kg). The whole stomach was excised and placed in a pre-oxygenated physiological salt solution (PSS) containing 126 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 14 mM glucose, and 10.5 mM Hepes (pH adjusted to 7.4 with Tris) at room temperature. The corporal region was removed and cut in the longitudinal direction, along the lesser curvature. The contents of the stomach were removed, and we obtained patches of the muscle coat by removing the mucosal layer in Tyrode's solution. We prepared muscle strips (2 × 10 mm) from the gastric antrum and circular smooth muscle from the main body of the stomach by cutting along the vertical direction of the longer axis of the stomach.

We recorded the isometric contraction in a vertical chamber (5 ml capacity). The isolated gastric smooth muscle strip was fixed in a tissue chamber containing 5 ml CO_2/bicarbonate-buffered Tyrode's solution containing 116 mM NaCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, 24 mM $NaHCO_3$, and 5 mM glucose (pH 7.3–7.4, bubbled with 5% CO_2/95%O_2). The chamber was maintained at 37 °C using a water jacket. One end of the chamber was attached to an isometric force transducer (RM6240C, Chengdu Instrument Factory, China) to record the contraction. The muscle strip was incubated at the appropriate tension, and then exposed to NaHS at a range of concentrations (0.1 mM, 0.3 mM, 0.5 mM, and 1 mM).

All experimental protocols included in this manuscript were approved by the local animal care committee and conform to the Guide for the Care and Use of Laboratory Animals published by the Science and Technology Commission of P.R.C. (STCC Publication No.2, revised 1988).

2.2. Cell preparation and electrophysiological recording

We isolated the gastric myocytes using collagenase, as described previously (Xing et al., 2007). The main body of the stomach was rapidly cut and the mucosal layer was separated from the muscle layers in a Ca^{2+}-free physiological salt solution (Ca^{2+}-free PSS). The circular muscle layer was dissected from the longitudinal layer using fine scissors, and cut into small segments (2 × 3 mm). These segments were incubated in a medium modified from Kraft–Bruhe (K–B) solution (0.5 mM EGTA, 10 mM Hepes, 3 mM $MgCl_2$, 50 mM KCl, 10 mM glucose, 20 mM KH_2PO_4, 20 mM Taurine, and 50 mM L-Glutamic acid, adjusted to pH 7.4 with KOH) for 30 min at 4 °C. Following this, the segments were incubated for 20–30 min at 36 °C in digestion medium Ca^{2+}-free PSS, containing collagenase (2 mg/ml, Worthington), trypsin inhibitor (0.5 mg/ml, Amresco) and bovine serum albumin (55 mg/ml, Biotech Grade). After digestion, the supernatant was discarded and the softened muscle segments were transferred into the modified K–B solution. The single cells were dispersed by gentle trituration using a wide-bore fire-polished glass pipette. The isolated gastric myocytes were incubated in a modified K–B solution at 4 °C. Several drops of the cell suspension were dropped into a perfusion bath, which was fixed on the stage of an inverted phase-contrast microscope for 15–20 min prior to the start of the experiment. The cells were then perfused with PSS at a rate of ~1.5 ml/min. We used a single 4-channel perfusion system (BPS-4, ALA, USA) to exchange the solution.

We used a conventional whole-cell patch clamp configuration to record the K_{ATP} channel current (I_{KATP}). The membrane potential was clamped at −60 mV. The pipette solution consisted of: 107 mM KCl,

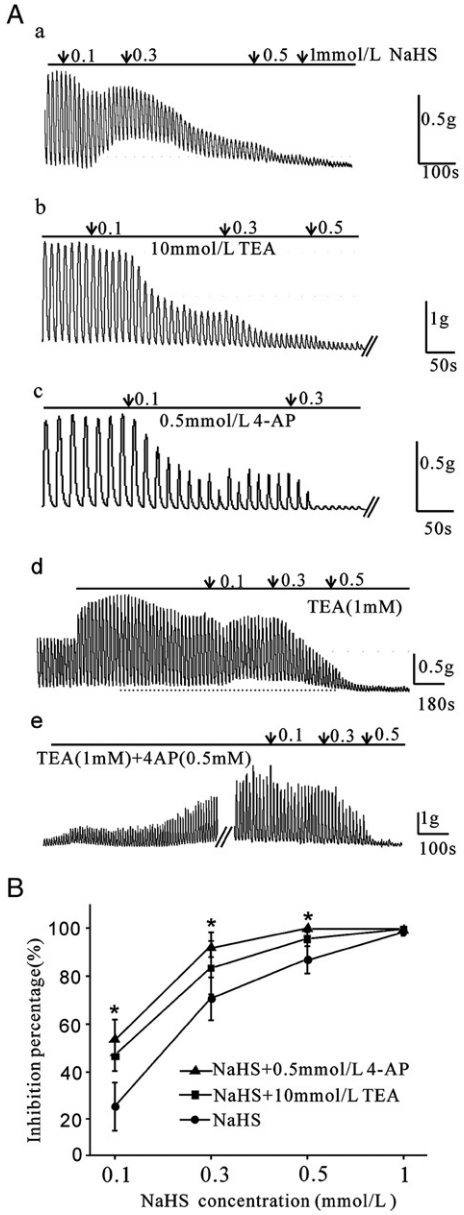

Fig. 1. Effect of NaHS on spontaneous contraction of gastric smooth muscle. Aa. shows representative effects of NaHS at different concentrations on spontaneous contraction of gastric smooth muscle. Ab,c. shows representative effects of different concentrations of NaHS on spontaneous contraction of gastric smooth muscle when the muscle strip was pretreated with TEA (a nonselective potassium channel blocker) and 4-Aminopyridine (a specific voltage-gated K^+ channel blocker). Ad,e. show representative effects of different concentrations of NaHS on spontaneous contraction of gastric smooth muscle when the muscle strip was pretreated with low concentration TEA only and combined with 4-AP. B. shows dose-dependent inhibition curves of NaHS on spontaneous contraction in control and under the condition of pretreatment with TEA or 4-AP. $n = 5$, $^*P < 0.05$ *vs* TEA and 4-AP groups.