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# The gaseous mediator, hydrogen sulphide, inhibits *in vitro* motor patterns in the human, rat and mouse colon and jejunum

D. GALLEGO,*,† P. CLAVÉ,†,‡,§ J. DONOVAN,¶ R. RAHMATI,** D. GRUNDY,¶ M. JIMÉNEZ*,† & M. J. BEYAK††

*Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain
†Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Instituto de Salud Carlos III, Barcelona, Spain
‡Department of Surgery, Hospital de Mataró, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain
§Fundació de Gastroenterologia Dr. F. Vilardell, Barcelona, Spain
¶Department of Biomedical Science, University of Sheffield, Sheffield, UK
**Golestan University of Medical Sciences, Gorgan, Iran
††Gastrointestinal Diseases Research Unit (GIDRU), Departments of Medicine and Physiology, Queen's University, Kingston, ON, Canada

***Abstract*** *Hydrogen sulphide ($H_2S$) has been recently proposed as a transmitter in the brain and peripheral tissues. Its role in the gastrointestinal tract is still unknown despite some data which suggest an involvement mediating smooth muscle relaxation. The aim of this study was to investigate the effect of this gas on intestinal segments from mouse jejunum and colon, and muscular strips from the human and rat colon. In isolated segments of mouse colon and jejunum, bath applied sodium hydrogen sulphide (NaHS) (a $H_2S$ donor) caused a concentration-dependent inhibition of spontaneous motor complexes (MCs) ($IC_{50}$ 121 μmol $L^{-1}$ in the colon and 150 μmol $L^{-1}$ in the jejunum). This inhibitory effect of NaHS on MCs was (i) unaffected by tetrodotoxin (TTX), capsaicin, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate and N-nitro-L-arginine suggesting a non-neural effect and (ii) significantly reduced by apamin 3 μmol $L^{-1}$. NaHS concentration-dependently inhibited the spontaneous motility in strips from human colon ($IC_{50}$ 261 μmol $L^{-1}$) and rat colon ($IC_{50}$ 31 μmol $L^{-1}$). The inhibitory effect of NaHS on colonic strips was (i) unaffected by the neural blocker TTX (1 μmol $L^{-1}$) with $IC_{50}$ 183 μmol $L^{-1}$ for the human colon and of 26 μmol $L^{-1}$ for the rat colon and (ii) significantly reduced by glybenclamide (10 μmol $L^{-1}$), apamin (3 μmol $L^{-1}$) and TEA (10 mmol $L^{-1}$) with $IC_{50}$ values of 2464, 1307 and 2421 μmol $L^{-1}$ for human strips, and 80, 167 and 674 μmol $L^{-1}$ for rat strips respectively. We conclude that $H_2S$ strongly inhibits in vitro intestinal and colonic motor patterns. This effect appears to be critically dependent on K channels particularly apamin-sensitive SK channels and glybenclamide-sensitive K (ATP) channels.*

***Keywords*** *cystathionine β-synthase, cystathionine γ-lyase, gastrointestinal tract, hydrogen sulphide, potassium channels, smooth muscle.*

*Address for correspondence*
Professor Marcel Jiménez, VMD, PhD, Department of Cell Biology, Physiology and Immunology. Edifici V, Universidad Autonoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
Tel: +34 93 581 1566; fax: +34 93 581 2006;
e-mail: marcel.jimenez@uab.es
Assistant Professor Michael J. Beyak, BSc, MD, FRCP (C), Gastrointestinal Diseases Research Unit (GIDRU), GIDRU Wing, Kingston General Hospital, 76 Stuart St, Kingston, K7M 2V7 Ontario, Canada.
Tel: +613 549 6666, ext. 6517; fax: +613 548 2426;
e-mail: beyakm@queensu.ca
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## INTRODUCTION

The foul smelling gas, hydrogen sulphide ($H_2S$) has been proposed as a third gaseous neuromodulator, after nitric oxide (NO) and carbon monoxide (CO). $H_2S$ can be produced via the reduction of the amino acid cysteine through two endogenous enzyme systems. The first enzyme is cystathionine β-synthase (CBS) which is present in brain and peripheral tissues, including the

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gut.[1] The second enzyme is cystathionine $\gamma$-lyase (CSE), found only in peripheral tissues.[2,3] Both CBS and CSE have been found in enteric neurons.[4] In addition, a third, non-pyridoxal-phosphate-dependent enzyme, mercaptopyruvate sulfurtransferase, has been proposed as a hydrogen sulfide-generating enzyme.[5] $H_2S$ could be generated through non-enzymatic reactions as it has been shown that washed human erythrocytes incubated with glucose and elemental sulphur produce $H_2S$ at a constant rate. As sulphur and glucose are both available in circulating blood, this is a possible pathway of production *in vivo*.[6] In addition, large quantities of $H_2S$ are produced by endogenous sulphate reducing bacteria in the gastrointestinal (GI) tract, and concentrations can reach 3 mmol $L^{-1}$ in the colon.[7]

Emerging evidence indicates that $H_2S$ can have major effects on excitable tissues, such as nerves and smooth muscle.[8–11] In the urinary bladder, Pataccini *et al.*[12] have demonstrated an excitatory action of $H_2S$ on bladder contraction, which was mediated by capsaicin sensitive nerves. In contrast, $H_2S$ caused relaxation in isolated ileal muscle strips.[13] In vascular smooth muscle, $H_2S$ has been shown to facilitate the release of NO, acting in synergy with endogenously released NO to cause relaxation.[14] Recent work by Shichio *et al.* [4] has demonstrated a pro-secretory effect of $H_2S$ in the human colon. This effect is mediated by capsaicin sensitive nerves, suggesting it activates extrinsic afferents to mediate secretion via an axon reflex. These data show that $H_2S$ can cause either contraction or relaxation and the actions of $H_2S$ might be nerve mediated or alternatively a putative effect on smooth muscle could be postulated.

The effects of $H_2S$ on an integrated motor pattern such as peristalsis have not yet been examined, nor have the effects on human GI smooth muscle been identified. In the present experiments, we examined the effects of $H_2S$ on distension-induced motor patterns in the mouse jejunum and colon, and subsequently characterized the effects and the mechanism of $H_2S$ induced relaxation in spontaneous contractions in the rat and human colon.

## MATERIALS AND METHODS

### Mouse tissue preparation

Experiments were performed on isolated segments of jejunum and colon from C57Bl6 mice of either sex. To examine the role of transient receptor potential of the 'vanilloid' subfamily (TRPV1), TRPV1−/− animals were used.[15] Animals were killed by overdose of sodium pentobarbital (300 mg $kg^{-1}$; Sagatal, Rhone Poulenc Fr., Dagenham, UK) followed by exsanguination. All experiments were conducted according to the requirements of the United Kingdom Animals (Scientific Procedures) Act (1986). The abdomen was opened and the mid jejunum and distal colon were removed, flushed of their contents and placed in oxygenated Krebs solution.

### Rat tissue preparation

Male Sprague–Dawley rats (Charles River, Lyon, France), 8–10 weeks old and weighing 300–350 g, were used ($n$ = 7). They were kept under conventional conditions in an environmentally controlled room (20–21 °C, 60% humidity, 12 : 12 h light–dark cycle) in groups of three animals and had unlimited access to water and food. Before the *in vitro* studies, rats were kept individually and fasted for 16–18 h with *ad libitum* access to water. Animals were decapitated and bled. All the experimental protocols were approved by the ethical committee of the Universitat Autònoma de Barcelona (Spain).

The entire colon was carefully removed and placed in a dissection dish containing carbogenated Krebs solution. The mesenteric fat was removed and the colon was opened along the mesenteric border. A small segment of mid colon was pinned to a Sylgard base with the mucosa facing upwards and the mucosal and submucosal layers were gently removed to study circular muscle strips.

### Human tissue preparation

Specimens of distal and sigmoid colon were obtained from patients (aged 47–78 years, $n$ = 15) during colon resections for neoplasm. Colon segments from macroscopically normal regions were collected and transported to the laboratory in cold saline buffer. The tissue was placed in Krebs solution on a dissection dish and the mucosal layer was gently removed. Circular muscle strips, $10 \times 4$ mm, were cut. The patients provided informed consent and experimental procedure was approved by the ethics committee of the Hospital of Mataró (Barcelona, Spain).

### Mechanical experiments

*Isolated mouse colon and jejunum* In the experiments with isolated mouse intestine, a 3-cm segment of gut was placed in an organ bath (10 mL) perfused with warm (36 °C) oxygenated Krebs solution and was cannulated at both ends, with a pressure transducer at the aboral end. The lumen was distended with saline to

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a pressure of 2–3 cmH2O, and spontaneous motor complexes (MCs) were recorded. Signals were amplified using a Neurolog NL108 Digitimer, (Welwyn Garden City, UK) pressure amplifier, digitized at 100 Hz using a CED 1401 interface (Cambridge Electronic Designs, Cambridge, UK) and displayed on a PC running SPIKE2 sofware package.

Drugs were added directly to the bath solution, except for a few cases when luminal application of $H_2S$ was examined. Agonists were perfused for 10 min followed by a washout period of 30 min prior to further agonist application. Antagonists were added to the bath solution 30 min prior to agonist application. The roles of endogenous adenosine triphosphate (ATP) and NO release were examined using pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate (PPADS, 30 $\mu$mol $L^{-1}$) and $N_e$-nitro-L-arginine methyl ester (100 $\mu$mol $L^{-1}$) respectively. The potential role of TRPV1 receptors was assessed using TRPV1−/− transgenic mice. To allow the examination of direct effects on smooth muscle contraction, TTX (1 $\mu$mol $L^{-1}$) was used as it blocks all neurally mediated MCs in this preparation.

*Colonic strips* Human and rat tissue muscle strips were examined in a 10 mL organ bath filled with Krebs solution at 37 ± 1 °C. An isometric force transducer (Harvard VF-1 Harvard Aparatus Inc, Holliston, MA, USA) connected to an amplifier was used to record the mechanical activity. Data were digitized (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 human tissue and of 1 g to the rat tissue and they were allowed to equilibrate for 1 h. After this period, strips displayed spontaneous phasic activity. To estimate the responses to the drugs, the area under the curve (AUC) of spontaneous contractions from the baseline was measured before and after drug addition. To normalize data, the value of AUC obtained before the treatment was considered 100 and the percentage of inhibition of the spontaneous motility was estimated with the AUC obtained after the treatment.

## Solutions and drugs

The composition of the Krebs solution was (in mmol $L^{-1}$) 10.10 glucose, 115.48 NaCl, 21.90 $NaHCO_3$, 4.61 KCl, 1.14 $NaH_2PO_4$, 2.50 $CaCl_2$ and 1.16 $MgSO_4$ bubbled with a mixture of 5% $CO_2$ : 95% $O_2$ (pH 7.4). The following drugs were used: sodium hydrogen sulphide (NaHS), apamin, glybenclamide, tetraethylammonium chloride (TEA), capsaicin, ATP, $N_e$-nitro-L-arginine methyl ester (L-NAME), bethanechol chloride (Sigma Chemicals, St Louis, MO, USA) tetrodotoxin (TTX) (Latoxan, Valence, France), PPADS (Tocris, Bristol, UK). Stock solutions were made by dissolving drugs in distilled water except for glibenclamide which was dissolved in DMSO and capsaicin which was dissolved in a solution of 80% DMSO/20% Tween 80.

## Data analysis and statistics

In the mouse-isolated intestine experiments, MCs were analysed with respect to peak amplitude (relative to basal pressure). The mean amplitude of the five contractions before drug application was considered as baseline, and inhibitory effects were expressed as % change from baseline. In the human and rat muscle strip experiments, cumulative concentration–response curves of $H_2S$ using NaHS as a donor were calculated to estimate the $IC_{50}$. To normalize data, we calculated the percentage inhibition by the drugs considering the AUC before the addition of the $H_2S$ donor as 100%. $H_2S$ dose–response curves were performed in control, in the presence of TTX and in the presence of neural blockade plus potassium channel antagonists. Because of the difficulty to perform all protocols with the same tissue, protocols were randomly performed without repetition of the same protocol in the same animal/patient. The differences between groups were compared by two-way analysis of variance (two-way ANOVA). A $P < 0.05$ was considered statistically significant. '*n*' values indicate the number of animals and patients. Statistical analysis was performed with GraphPad Prism version 4.00 (GraphPad Software, San Diego CA, USA). Data are expressed as mean ± SEM.

# RESULTS

In both the mouse colon and jejunum, bath-applied NaHS caused an inhibition of spontaneous MCs (Fig. 1A). This effect was dose-dependent, with $IC_{50}$ values of 121 $\mu$mol $L^{-1}$ in the colon and 150 $\mu$mol $L^{-1}$ in the jejunum, and maximal (100%) inhibition occurring between 300 and 1000 $\mu$mol $L^{-1}$ (Fig. 1B). Luminal application of NaHS also caused an inhibition of motility but the effect was less consistent and required higher concentrations compared to bath application. For all further experiments, NaHS was directly applied to the bath.

## Role of TRPV1 receptors

As previous work on urinary bladder muscle and intestinal secretion has suggested a role for capsaicin sensitive nerves in the effects of $H_2S$, we hypothesized

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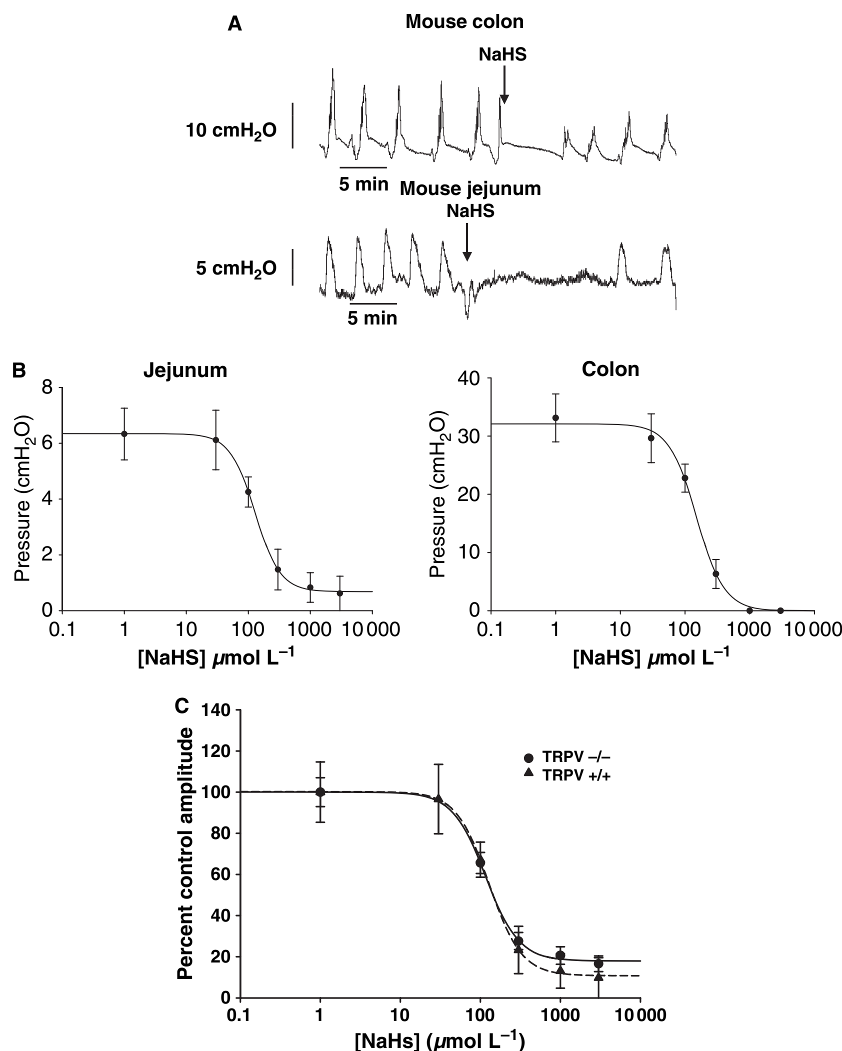


**Figure 1** (A) Recordings of spontaneous MCs in the isolated mouse jejunum and colon. NaHS (300 $\mu$mol $L^{-1}$) causes a significant inhibition of the amplitude of these MCs in both the colon (top trace) and jejunum (bottom trace). (B) Concentration–response relationship for NaHS in mouse jejunum and colon. NaHS caused a concentration-dependent inhibition with similar $IC_{50}$ values in colon and jejunum. (C) Concentration–response curves to NaHS in TRPV+/+ and −/− mice. Concentration–response relationships to NaHS do not differ significantly between the two groups. Data are expressed as mean ± SEM.

that TRPV1 channels may be involved in the inhibitory effects of NaHS on spontaneous MCs. In the mouse jejunum and colon, capsaicin (100 nmol $L^{-1}$) caused an inhibition of MCs. In contrast, this effect was absent in intestine from TRPV1−/− animals. However, in both TRPV1+/+ and −/− intestine, NaHS caused a similar concentration-dependent inhibition with nearly identical concentration–response relationships ($n$ = 6 of each) (Fig. 1C).

## Role of purinergic receptors

As ATP is an important inhibitory neurotransmitter in the intestine, and our own preliminary experiments have indicated a role for P2 receptors in $H_2S$-mediated excitation of visceral afferent nerves in the mouse (unpublished observations), we sought to examine the role of endogenously released ATP in the $H_2S$-mediated relaxation of jejunal and colonic motility. ATP (300 $\mu$mol $L^{-1}$) caused inhibition of MCs, an effect that was prevented by 30 $\mu$mol $L^{-1}$ PPADS. In contrast, the effect of NaHS (300 $\mu$mol $L^{-1}$) was not significantly inhibited by PPADS (30 $\mu$mol $L^{-1}$) (80.1 ± 8.8% *vs* 76.33 ± 9.3% inhibition $P > 0.05$, $n$ = 5).

## Role of NO release

As NO is an important inhibitory neurotransmitter in the gut, and because of the evidence, at least in some vascular smooth muscles, that NO may mediate some of the effects of $H_2S$, we examined the role of NO release using the NO-synthase blocker, L-NAME. In the mouse jejunum, L-NAME (100 $\mu$mol $L^{-1}$) increased MC amplitude and frequency, while in the colon, MC amplitude was inhibited. However in neither region was the inhibitory effect of NaHS (300 $\mu$mol $L^{-1}$) attenuated, MC amplitude inhibition being similar in both regions (74.3 ± 8.9% control *vs* 80.3 ± 10.1% L-NAME, $n$ = 6).

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## Non-neurnal effect of $H_2S$

To examine the effects of NaHS on smooth muscle contraction, independent of neural influences, we blocked intrinsic neurotransmission using TTX (1 $\mu$mol $L^{-1}$). This abolished spontaneous MCs and increased tone. Under these conditions, NaHS resulted in a reduction in basal tone (3.5 ± 0.4 *vs* 1.2 ± 0.22 $cmH_2O$; $P < 0.05$, $n = 5$). To study the effect of NaHS on a pharmacologically induced contraction, we examined the effect of perfusing 300 $\mu$mol $L^{-1}$ NaHS on the contraction induced by 30 $\mu$mol $L^{-1}$ bethanechol under control conditions. In the presence of TTX, bethanechol induced a contraction that partially relaxed during a 5-min perfusion. The amplitude of this contraction was significantly attenuated in the presence of 300 $\mu$mol $L^{-1}$ NaHS. (25.8 ± 6.6 *vs* 10.2 ± 3.5; $P < 0.05$, $n = 6$). Contraction amplitude was restored after washout of NaHS (Fig. 2).

## Effects of NaHS on human and rat colon muscle strips

Sodium hydrogen sulphide concentration-dependently inhibited spontaneous motility. In the human colon, $IC_{50}$ was 261 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50} = -3.58 \pm 0.05$; $n = 5$) and in the rat colon $IC_{50}$ was 31 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50} = -4.5 \pm 0.04$, $n = 6$). No major differences were found when dose–response curves were performed in the presence of the neural blocker TTX (1 $\mu$mol $L^{-1}$). In the human, $IC_{50}$ was 183 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50} = -3.73 \pm 0.08$; $n = 5$, ns) and in the rat colon, $IC_{50}$ was 26 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50} = -4.57 \pm 0.04$; $n = 6$, ns) (Fig. 3).

## Role of potassium channels

Available evidence suggests that some of the effects of $H_2S$ may be mediated via activation of one or more potassium channel subtypes. We therefore designed a number of experiments to examine the role of K channels in the actions of $H_2S$ in the GI tract. In the presence of TTX, TEA (10 mmol $L^{-1}$), a K-channel blocker, significantly reduced the inhibitory effect induced by $H_2S$. The NaHS $IC_{50}$ was reduced to 2421 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50} = 2.6 \pm 0.06$; $n = 5$, $P < 0.0001$) for the human colon and 674 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50} = -3.1 \pm 0.04$; $n = 6$, $P < 0.0001$) for the rat colon.

In the presence of TTX (1 $\mu$mol $L^{-1}$), the ATP sensitive channel blocker glybenclamide (10 $\mu$mol $L^{-1}$) significantly reduced the inhibitory effect of $H_2S$.

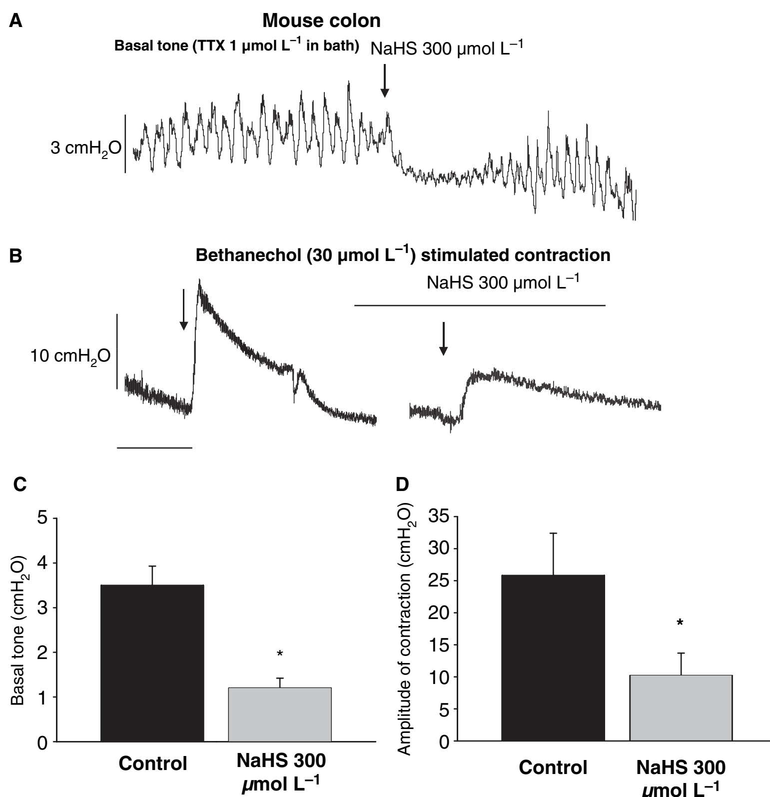


**Figure 2** NaHS directly inhibits smooth muscle contraction in the mouse colon. In the presence of TTX, MCs are abolished and only oscillating, slow, wave-like activity is observed. NaHS causes a fall in basal (myogenic) tone (A). When a contraction is stimulated by 30 $\mu$mol $L^{-1}$ bethanechol, the amplitude is significantly reduced in the presence of NaHS (B). Summary of the effect of NaHS on basal tone (C) and bethanechol contraction amplitude (D). Data are expressed as mean ± SEM. *$P < 0.05$

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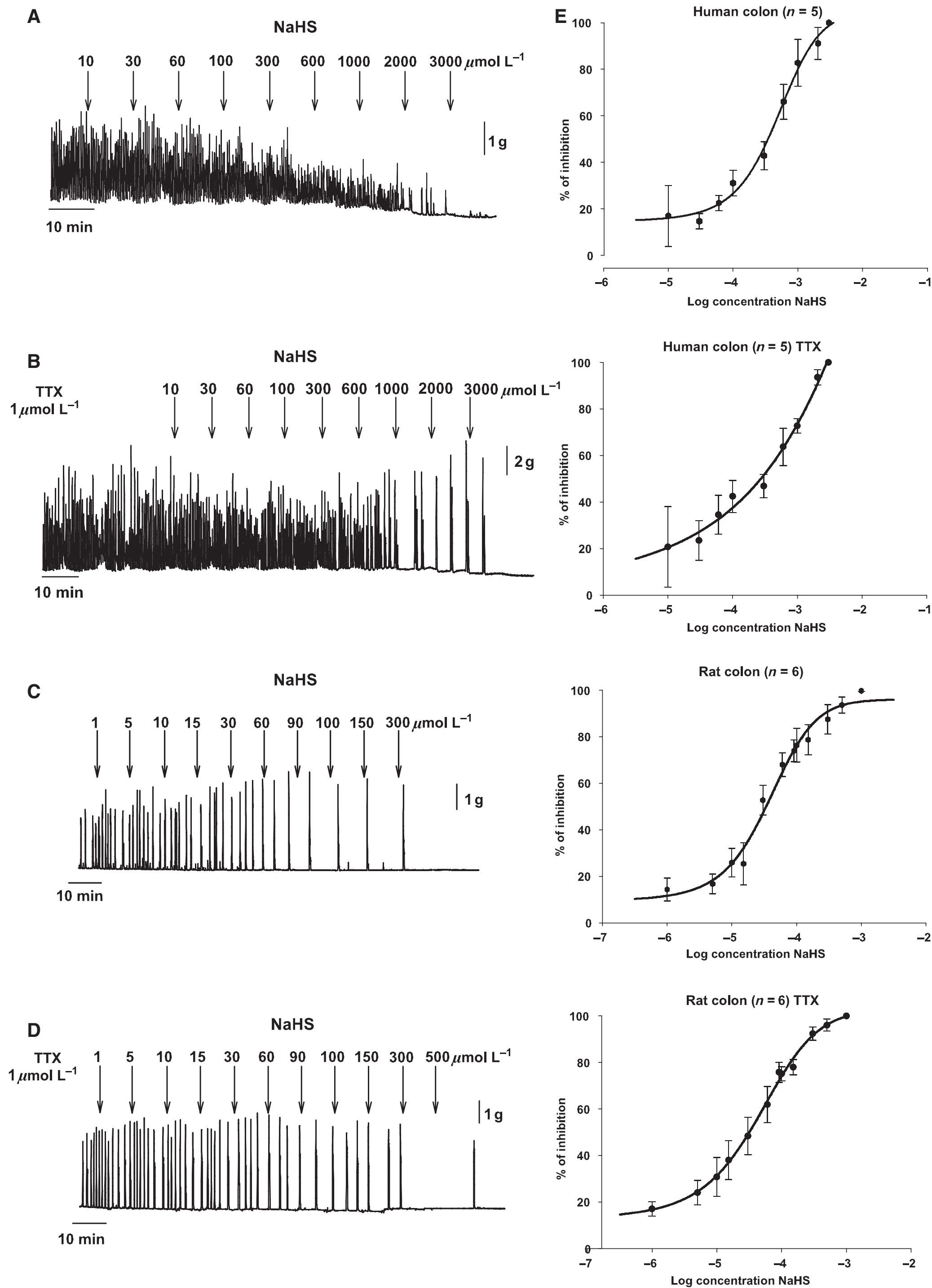


**Figure 3** (A) Mechanical recordings showing the inhibitory effect of cumulative doses of NaHS, a $H_2S$ donor (10–3000 $\mu$mol $L^{-1}$) in control conditions and (B) after the incubation with TTX (1 $\mu$mol $L^{-1}$) in the human colon. (C) Mechanical recordings showing the inhibitory effect of cumulative doses of NaHS, a $H_2S$ donor (1–1000 $\mu$mol $L^{-1}$) in control conditions and (D) after the incubation with TTX (1 $\mu$mol $L^{-1}$) in the rat colon. (E) Dose–response curves. Data are expressed as mean ± SEM.

Glybenclamide increased the NaHS $IC_{50}$ to 2464 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50}$ = −2.6 ± 0.1; $n = 5$, $P < 0.0001$) for the human colon and to 80 $\mu$mol $L^{-1}$ (95% confidence interval log $IC_{50}$ = −4.0 ± 0.04; $n = 6$, $P < 0.0001$) for the rat colon (Fig. 4).

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In the presence of TTX, the small conductance calcium-activated potassium channel blocker apamin (3 $\mu$mol $L^{-1}$) significantly reduced the inhibitory effect of $H_2S$. Apamin increased the NaHS $IC_{50}$ to 1307 $\mu$mol $L^{-1}$ (95% confidence interval $\log IC_{50} = -2.88 \pm 0.07$; $n = 5$, $P < 0.0001$) in the human colon and to 167 $\mu$mol $L^{-1}$ (95% confidence interval $\log IC_{50} = -3.77 \pm 0.06$, $n = 6$, $P < 0.0001$) in the rat colon (Fig. 5). Based on these results, we examined the effect of apamin on the NaHS-induced inhibition of MCs in the mouse colon. In the mouse colon preparation, the inhibitory effect of NaHS on spontaneous MCs was significantly attenuated by apamin (3 $\mu$mol $L^{-1}$). In some cases (Fig. 6), the inhibitory effect was converted to the excitation in the presence of apamin, suggesting a crucial role for SK channels in the effects of NaHS on colonic motility.

In the presence of TTX, glybenclamide (10 $\mu$mol $L^{-1}$) and apamin (3 $\mu$mol $L^{-1}$) had a cumulative effect reducing the inhibition caused by NaHS. The $IC_{50}$ was 3294 $\mu$mol $L^{-1}$ (95% confidence interval $\log IC_{50} = -2.51 \pm 0.08$; $n = 5$, $P < 0.0001$) for the human colon and 263 $\mu$mol $L^{-1}$ (95% confidence interval $\log IC_{50} = -3.57 \pm 0.05$; $n = 6$, $P < 0.0001$) for the rat colon. These results were statistically different from those obtained with apamin and glybenclamide alone.

## DISCUSSION

The toxic gas, hydrogen sulphide, has recently been described in a number of tissues as a novel gaseous mediator. $H_2S$ has been shown to have effects on a variety of smooth muscle preparations, including vascular, bladder and intestine. $H_2S$ might have several putative origins in the GI tract. Enteric neurons expressing CBS and CSE have been described[4] and therefore $H_2S$ can be a neurotransmitter in the GI tract. However, the release of $H_2S$ after neuronal stimulation has not still been demonstrated in enteric neurons although $H_2S$ causes the relaxation of GI smooth muscle.[16] Other putative sources of $H_2S$ is the blood or the vascular tissues.[1] Moreover, large amounts of $H_2S$ are present in the intestine and colon. In this study, we sought to examine the effects of the $H_2S$ donor, NaHS on motor patterns in isolated jejunum and colon. Our experiments clearly demonstrate that

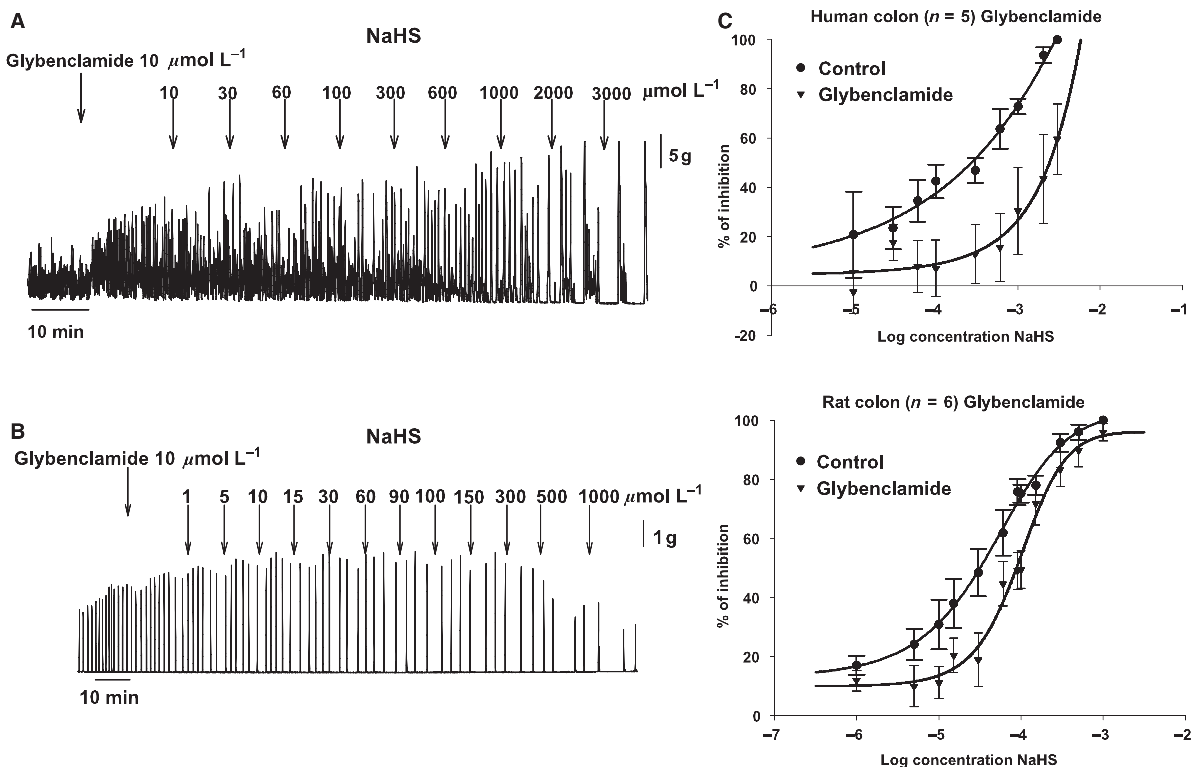


**Figure 4** (A) Mechanical recordings showing the inhibitory effect of cumulative doses of NaHS, a $H_2S$ donor (10–3000 $\mu$mol $L^{-1}$) after the incubation with glybenclamide (10 $\mu$mol $L^{-1}$) in the human colon. (B) Mechanical recordings showing the inhibitory effect of cumulative doses of NaHS, a $H_2S$ donor (1–1000 $\mu$mol $L^{-1}$) after the incubation with glybenclamide (10 $\mu$mol $L^{-1}$) in the rat colon. (C) Dose–response curves. Data are expressed as mean ± SEM.

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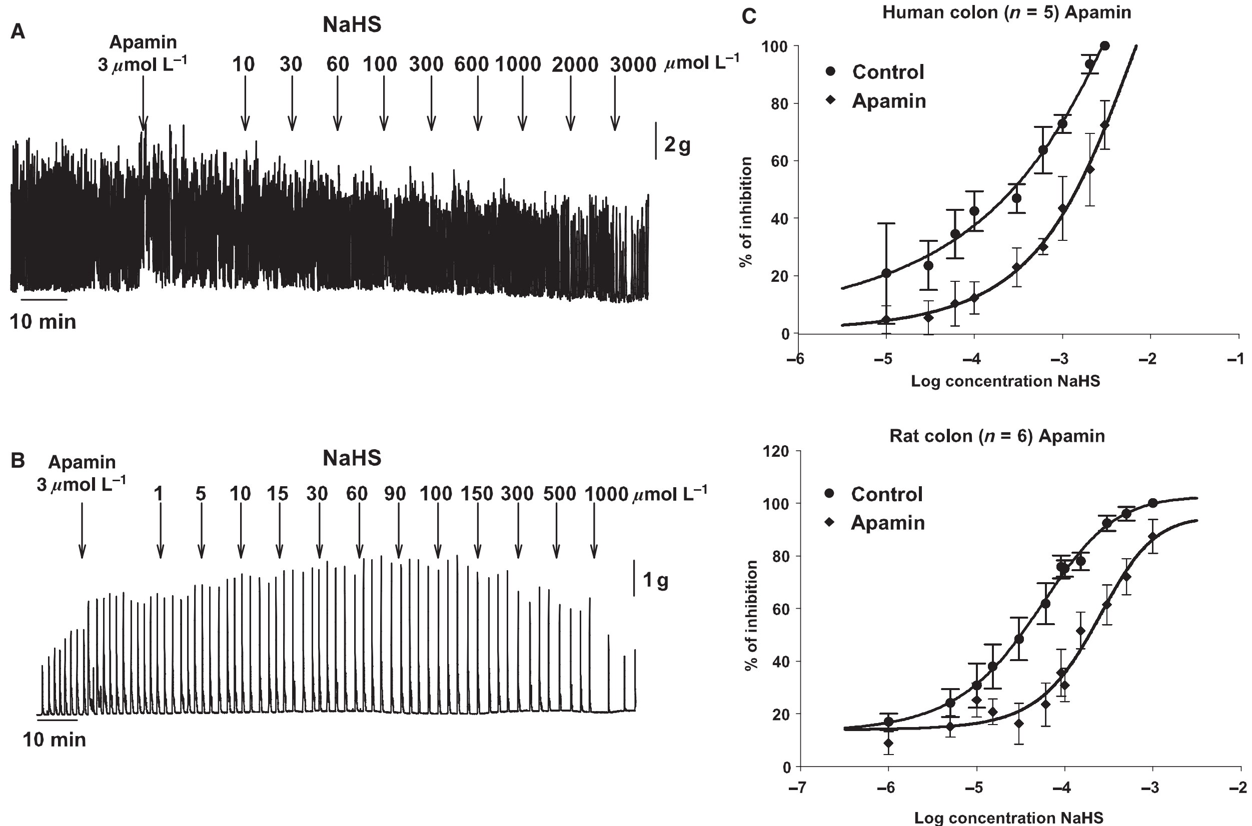


**Figure 5** (A) Mechanical recordings showing the inhibitory effect of cumulative doses of NaHS, a $H_2S$ donor (10–3000 $\mu$mol $L^{-1}$) after incubation with apamin 3 $\mu$mol $L^{-1}$ in the human colon. (B) Mechanical recordings showing the inhibitory effect of cumulative doses of NaHS, a $H_2S$ donor (1–1000 $\mu$mol $L^{-1}$) after incubation with apamin (3 $\mu$mol $L^{-1}$) in the rat colon. (C) Dose–response curves. Data are expressed as mean ± SEM.

NaHS, at physiologically relevant concentrations, inhibits spontaneous MCs in the isolated jejunum and colon. These effects are not mediated by TRPV1 receptors on capsaicin sensitive nerves (as suggested in bladder, and GI secretory effects) nor are they exclusively dependent on endogenous inhibitory reflexes utilizing NO or ATP. We have however shown that NaHS inhibits motility largely through an action on multiple potassium channels.

Work by Pattachini *et al.*[12] has demonstrated that in isolated urinary bladder muscle NaHS caused contraction that was abolished by capsaicin pretreatment. Furthermore, the effects of NaHS were similar to that of capsaicin, and could be blocked by neurokinin antagonists. These observations taken together were interpreted to suggest that NaHS stimulated endogenous capsaicin sensitive nerves, resulting in tachykinin release and subsequent smooth muscle contraction. Recent work by Schichio *et al.*[4] has also demonstrated a pro-secretory effect of $H_2S$ in the rodent and human colon as well as stimulation of enteric nerves. Capsaicin sensitive nerves were also implicated in these effects. This raised the possibility that NaHS might act via the capsaicin receptor, TRPV1. Indeed, in our experiments, capsaicin inhibited MCs, in a similar way to NaHS. However, utilizing TRPV1-deficient mice, we found that the effects of NaHS were almost identical to the wildtype controls. Experiments performed by Pataccini *et al.*[17] showed that the effects of NaHS in bladder could not be blocked by the TRPV1 antagonists capsazepine and SB366791, however, the effect of NaHS was antagonized with ruthenium red. The authors suggested that this raised the possibility of an action on TRPV1 receptors at a novel site or other TRP channels. In a separate study, TRPV1 antagonists attenuated NaHS-induced neurogenic inflammation in the lung.[18] However, our experiments with transgenic mice effectively rule out a role for the TRPV1 receptor in mediating NaHS inhibition of motility, at least in the mouse intestine; our results here point to other mechanisms of action.

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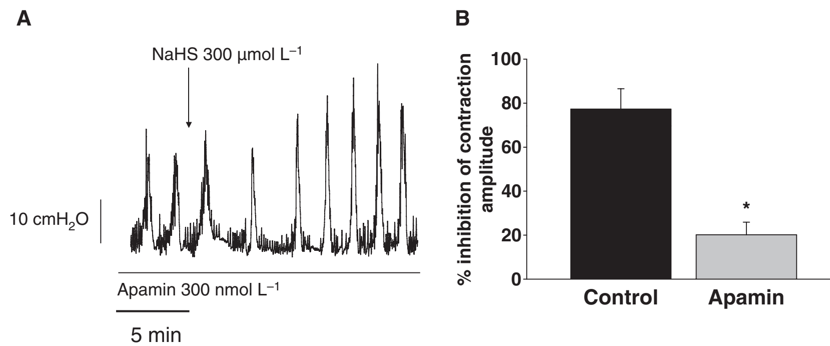


**Figure 6** Mechanical recordings showing reversal of the inhibitory effect of NaHS (300 $\mu$mol $L^{-1}$) in the MCs by apamin (3 $\mu$mol $L^{-1}$) (A) and percentage of inhibition induced by NaHS in the absence and presence of apamin (B). Data are expressed as mean ± SEM. **P* < 0.05

Previous work on vascular smooth muscle has suggested that NO may mediate or synergize with the effects of $H_2S$.[19] Given that NO is one of the most important inhibitory neurotransmitters in the gut, we examined its role in the inhibition of peristalsis induced by NaHS. At a concentration that effectively blocked endogenous NO synthase, L-NAME altered peristaltic contractions in the jejunum and colon in different ways. In the jejunum, contraction amplitude and frequency were increased, as was basal tone. In contrast, in the colon, contraction amplitude was decreased. The reasons for these regional differences are not clear. Powell *et al.*[20] have made a similar observation in the mouse colon using another NOS inhibitor. It may be that in the jejunum NO plays a predominantly inhibitory role while in the colon NO may activate some excitatory neural circuits itself, or mediate a postinhibitory 'rebound' contraction. Another possibility is that NO excites the longitudinal muscle, as has been demonstrated in the oesophagus.[21] Nonetheless, despite alterations in their basal motility patterns, L-NAME failed to prevent the inhibition of peristalsis by NaHS, suggesting that endogenous NO does not play a major role in NaHS-induced inhibition.

ATP is increasingly recognized as an important inhibitory neurotransmitter in the gut. Acting through P2Y receptors it mediates a fast inhibitory junction potential.[22–24] Furthermore, ATP release can be stimulated by a variety of noxious stimuli in the viscera (e.g. distention, hypoxia, etc.).[25] Our own results show that exogenous ATP can inhibit MCs in the jejunum and colon. Additional preliminary data suggest that $H_2S$ can excite intestinal afferent fibres, in part via ATP release, and an action on PPADS sensitive receptors. We therefore examined the effects of PPADS on NaHS inhibition of MCs. At concentrations that prevent the inhibition of MCs by exogenous ATP, NaHS had no inhibitory effect on MCs suggesting that ATP does not mediate the inhibitory effects.

There is a recent literature suggesting that $H_2S$ may directly inhibit the contraction in a variety of smooth muscle such as bladder, vascular, and in ileal smooth muscle strips.[13] To examine this possibility in our preparation, we blocked neurally mediated MCs with TTX, thus permitting examination of the direct effects on smooth muscle. Under these conditions, $H_2S$ inhibited basal tone and the contraction evoked by the muscarinic agonist bethanechol. Subsequently, utilizing muscle strips from the rat and human colon, we used a number of pharmacological approaches to examine the ionic mechanism of action of $H_2S$ on these tissues. Blockade of potassium channels with the non-selective potassium channel blocker TEA resulted in significant attenuation of the inhibitory effect of NaHS. This led us to utilize more selective blockers of specific potassium channel families. The selective calcium activated SK-channel blocker apamin resulted in significant inhibition of the relaxant effects of NaHS. In some cases, the inhibitory effect was converted by apamin to an excitatory one. In addition, glybenclamide, a KATP channel antagonist, also significantly diminished the inhibitory effect of NaHS. The interpretation of these results should be cautious because potassium channels are also involved in the control of the resting membrane potential and an increase in motility is often observed after potassium channel blockade. The displacement of the curve might also be attributable to the modification of the resting conditions. In this sense, the effect of glybenclamide is greater in human than in rat tissue and this might be related to the higher increase in spontaneous motility observed in human than in rats. However, when TTX is added or NOS is inhibited by L-NAME an increase in motility is usually observed (more prominent in rats) and the effect of $H_2S$ is not modified. Moreover, the important shift of the curves found in presence of TEA or the combination of both apamin and glybenclamide strongly suggest the involvement of potassium channels on the $H_2S$ response. These results agree with those in vascular smooth muscle and in the rabbit ileum.[13] It appears from our experiments here that the relaxant

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effect of $H_2S$ in human and rodent GI smooth muscle is direct via the activation of K channels possibly located in smooth muscle.[26,27] The reduction in the frequency of spontaneous contraction after NaHS addition suggests a putative action of $H_2S$ in K channels located in interstitial cells of Cajal (ICC),[28,29] an alternative which should be considered in future experiments. Interestingly, in the isolated mouse colon preparation, in some cases, in the presence of apamin, an excitatory effect of NaHS was unmasked. The mechanism remains to be determined, however, the action of NaHS may depend critically on the relative balance between inhibitory and excitatory actions. The finding of an important role for calcium-activated potassium channels suggests that increase in intracellular calcium may be important and may explain the finding of excitation in the presence of apamin. The source of this intracellular calcium rise remains to be determined. Further experiments using intracellular microelectrode and patch clamp techniques are needed to characterize fully the ionic mechanisms underlying $H_2S$-induced smooth muscle relaxation and putative prejunctional or postjunctional actions of $H_2S$ including the effect on ICCs and smooth muscle should be further studied with electrophysiological techniques.

The finding that $H_2S$ is a potent inhibitor of motility is perhaps surprising, given the large quantities present in the GI tract. Our experiments, however, demonstrated that luminal application of NaHS inhibited motility far less effectively and consistently than bath application. This suggests that, even in the jejunum, the mucosa serves as a particularly effective barrier to the diffusion of $H_2S$ across to the muscle layers. Indeed, the colonic mucosa is endowed with an efficient $H_2S$-detoxifying mechanism, oxidizing more than 300 $\mu$mol of $H_2S$ to thiol compounds daily in the rat colon.[7] However, when this barrier is broken down, such as in severe colitis, a greater amount of $H_2S$ may access the muscle layers and inhibit motility, possibly becoming clinically relevant in conditions such as toxic megacolon, which can complicate severe colitis. It is of particular interest that $H_2S$ has been implicated in the pathogenesis of acute pancreatitis and lipopolysaccharide-induced systemic inflammation, two conditions known to be associated with increased gut permeability and bacterial translocation.[14,30,31] Recent work has also demonstrated an antinociceptive effect of NaHS in the colon.[32] How much of this is due to muscle relaxation is not clear. These investigators did not find a change in compliance of the bowel but did not carry out detailed investigation of the biomechanical properties.

In summary, our studies have demonstrated a novel inhibitory action of $H_2S$ on rodent and, now for the first time, human colonic motility. The inhibitory action of $H_2S$ appears to be critically dependent on K channels, particularly apamin-sensitive SK channels and glybenclamide-sensitive KATP channels. Our results are obtained from the small intestine and colon and it is important to perform future studies in other areas of the GI tract where $H_2S$ might be a putative neurotransmitter or alternatively where sources of luminal $H_2S$ are important. These observations may have physiological and pathophysiological relevance in conditions where $H_2S$ production is increased or where barrier or detoxification mechanisms are impaired.

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