Effects of a Hydrogen Sulfide Donor on Spontaneous Contractile Activity of Rat Stomach and Jejunum

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We studied the effect of sodium hydrosulfite (NaHS), a donor of hydrogen sulfide (H_2S), on spontaneous contractive activity of isolated preparations of rat stomach and jejunum under isometric conditions. NaHS in concentrations of 10-200 μM reduced the amplitude, tonic tension, and frequency of contractions of the preparations. Blockade of K^+ channels with a non-specific antagonist tetraethylammonium (10 mM) increased contraction amplitude in the stomach strip and jejunum segment. The effects of NaHS on all parameters of contractile activity of the stomach and jejunum were fully preserved against the background of tetraethylammonium application. These data suggest that H_2S in physiologically relevant concentrations inhibited spontaneous contractile activity of smooth muscle cells in rat stomach and jejunum by reducing the amplitude and frequency of contractions and decreased tonic tension without affecting the function of voltage- and calcium-dependent K^+ channels.

Key Words: *hydrogen sulfide; smooth muscle cells; stomach; jejunum; potassium channels*

It is now established that hydrogen sulfide (H_2S) is a gasotransmitter that participates in intra- and intercellular signaling [2,6]. H_2S is endogenously synthesized in animal and human tissues and produces a variety of physiological effects [2,6,15]. It has been shown that H_2S is synthesized in different parts of the GIT of various animal species in reactions catalyzed by cystathionine-β-synthase and cystathionine-γ-lyase, and produced by sulfate-reducing bacteria, a component of normal flora of the large intestine [9,10]. H_2S synthesis enzymes were found in all parts of GIT, in epithelial cells, muscle layer, in the enteric nervous system, as well as in the interstitial cells of Cajal [9,12]. The data on the effect of H_2S on motor activity of GIT are scarce and contradictory: both relaxing and stimulating effects of this gasotransmitter were revealed in different parts of GIT in different animal species [3,5,8,11,12].

Here we studied of the influence of H_2S on spontaneous activity of rat stomach and jejunum and the role of K^+-channels in H_2S effects.

MATERIALS AND METHODS

Analysis of spontaneous contractile activity was carried out on preparations of rat stomach and jejunum by means of Biopac Systems, Inc. device. Animal treatment was conducted in accordance with ethical norms. The animals were anesthetized with 5% isoflurane (Abbott Laboratories) before the experiment. For contractile activity analysis, a 5-7-mm strip (thickness 2 mm) was excised along the greater curvature of the stomach, or an 8-mm jejunum segment were used. The preparation was mounted vertically in a 20-ml bath, the lower end rigidly fixed to the block, the other end connected to a strain gauge transducer (TSD125C; Biopac Systems, Inc.). Contractile activity was recorded using an amplifier, further analysis of the parameters of

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contractions was performed using the AcqKnowledge 4.1 software. The following parameters were analyzed: amplitude (strength of contraction, g), tonic tension (g), and frequency of contractions (Hz). Tonic tension of the preparation between contractions was assessed during the periods of maximum relaxation.

Throughout the experiment, the preparation was washed with Krebs solution of the following composition: 121.0 mM NaCl, 5.9 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25.0 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 8.0 mM $C_6H_{12}O_6$ (pH 7.2-7.4) aerated with a mixture of 95% $O_2$and 5% CO_2; the temperature was maintained at 37°C. After mounting, the preparation was maintained under certain stretching for 60-90 min until stable contractions were achieved. The preparations that did not develop stable spontaneous contractile responses were excluded from the study. The strength and frequency of contractions and tonic tension of the preparations in response to administration of the test substances were determined. Significance of differences was determined using Student's *t* test.

For evaluation of the effects of exogenous H_2S, we used sodium hydrosulfide (NaHS), a H_2S donor that dissociates in solution to sodium ion (Na^+) and hydrosulfide anion (HS^-), which produces H_2S by reacting with a proton. It is known that 18% NaHS is in the form of H_2S gas at pH 7.4 and 37°C [14]. The antagonist of various types of K^+-channels, tetraethylammonium (TEA; Sigma) was also used in the experiments.

To analyze the effect of NaHS, the substance was cumulatively applied to the bath with the preparation to concentrations of 10, 50, 100, and 200 μM, each concentration was maintained for 10 min. The influence of the substance on the strength, frequency of contraction, and tonic tension was calculated in relation to the initial (control) level in percent. When TEA was used, the parameters of contractile activity against the background of the preliminary application of TEA were taken as 100%.

RESULTS

In the control, the amplitude of spontaneous contractions of the stomach strip was 0.51±0.13 g, the frequency of contractions was 0.07±0.01 Hz, and tonic tension was 0.79±0.07 g (n=9). Application of NaHS in concentrations of 10, 50, and 100 μM produced no changes in the parameters of contractile activity (Table 1). In a concentration of 200 μM, NaHS significantly reduced the amplitude of contractions to 54±10%, frequency of contractions to 69±8%, and tonic tension to 91±3% (n=9, p<0.05; Table 1, Fig. 1, *a*).

The amplitude of spontaneous contractions of the isolated jejunum segment was 0.57±0.50 g (n=20), frequency of its spontaneous contractions 0.45±0.01 Hz, and tonic tension 0.640±0.035 g. Against the background of NaHS application, a dose-dependent decrease in the amplitude of contractions was observed (Table 2). NaHS in a concentration of 200 μM reduced the amplitude of contractions to 11±2% (n=9, p<0.05; Table 2, Fig. 1, *c*). A significant decrease in the frequency of contractions occurred at NaHS concentration of 200 μM and tonic tension reduction was observed at all tested concentrations (Table 2).

Further increase in NaHS concentration to 300 μM led to complete blockade of contractile activity of both preparations. The effect of NaHS on contractile activity of both stomach and intestine was reversible, and all the studied parameters rapidly returned to baseline after replacing bathing solution with the control one. Repeated application of NaHS had similar effects. All these facts suggest that the applied concentrations of H_2S donor were not toxic. Taking into account the

TABLE 1. Effect of NaHS on Spontaneous Contractile Activity of Rat Stomach Strip and against the Background of Preliminary TEA Application

Parameter		Concentration of NaHS			
		10 μM	50 μM	100 μM	200 μM
Amplitude	control	97.96±3.47	98.71±5.53	90.91±10.24	54.37±9.82*
	TEA	101.47±2.03	102.62±7.82	83.37±16.06	24.62±5.04*
Frequency	control	96.91±11.40	89.87± 4.57	86.77±7.44	69.28±6.79*
	TEA	92.54±6.61	97.14±7.43	97.14±8.15	89.63±4.35*
Tonic tension	control	94.82±4.19	83.12±7.37	82.42±10.27	91.32±3.47*
	TEA	101.34±2.43	98.78±5.18	88.71±8.91	65.11±8.65*

Note. Here and in Table 2: effects of NaHS are presented as % of contractile activity in the control or after preliminary TEA application. Significance of difference (*p<0.05) is shown in comparison with the initial level in the control or upon TEA exposure, n=9.

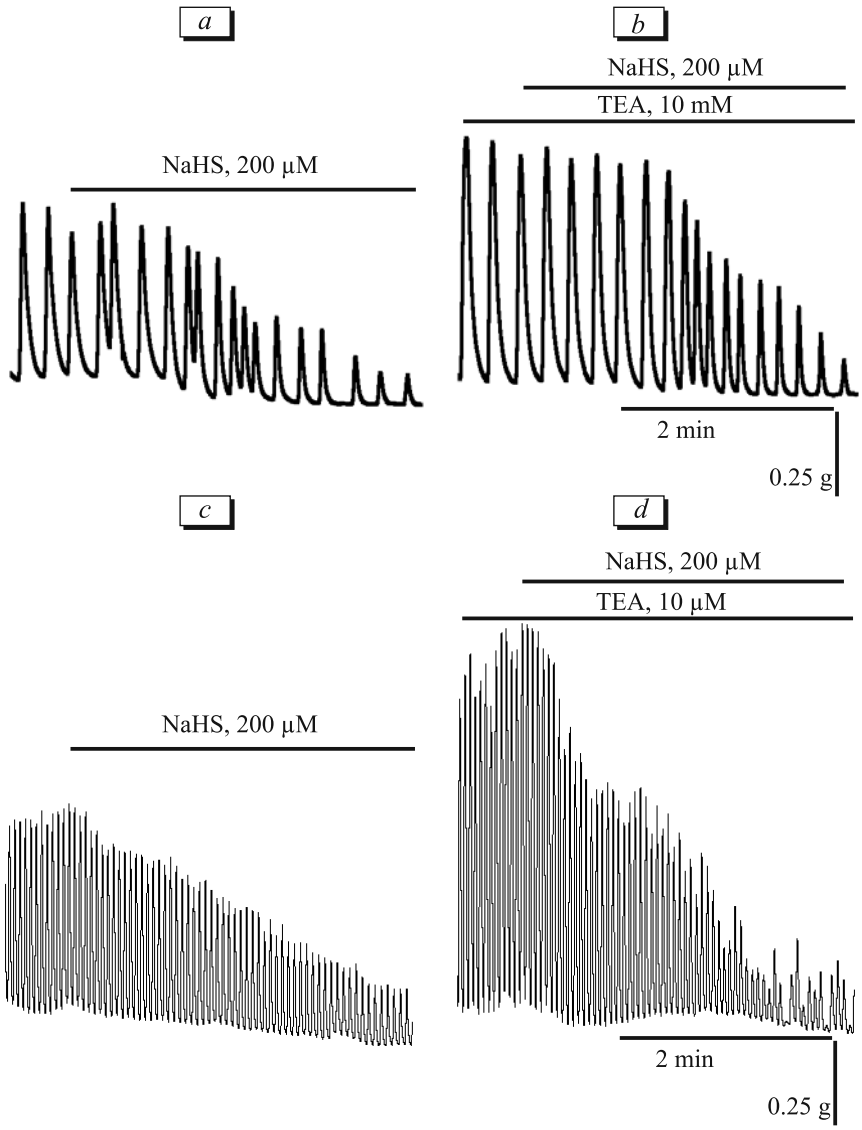

Fig. 1. Mechanograms of spontaneous contractile activity of a stomach strip (*a*, *b*) and a jejunum fragment (*c*, *d*) upon exposure to NaHS (200 μM) in control (*a*, *c*) and against the background of TEA (10 mM; *b*, *d*).

fact that under our experimental conditions only 18% of NaHS form H_2S, effective concentrations of H_2S were between 1.8 and 36 μM. Although the blood and tissue H_2S concentrations were within the nanomolar range [4], it is believed that local gas concentrations close to places of synthesis and targets might be much higher [9]. Therefore, effective concentrations of H_2S in our experiments were close to physiological.

Our experiments indicate that the stomach preparation demonstrated lower sensitivity to NaHS than the jejunum one. This can be explained by differences in the expression of H_2S synthesis enzymes and endogenous gas concentrations in the studied regions. Indeed, the level of cystathionine-γ-lyase expression was similar in the stomach wall and in the jejunum, whereas cystathionine-β-synthase expression prevails in the stomach, and H_2S synthesis is more intense in the stomach than in the jejunum [10]. Moreover, molecular targets of H_2S may vary throughout the GIT. It is known that motor activity regulating mechanisms can vary in different parts of GIT (stomach, small and large intestine), and even in different muscle layers (longitudinal or circular) of the wall in the same GIT portion [11].

The strength of smooth muscle contraction directly depends on intracellular calcium concentration, which is determined by Ca^{2+} entry from the extracellular medium through L-type Ca^{2+} channels and its outward current through Ca^{2+} channels of ryanodine and inositol-3-phosphate receptors of the sarcoplasmic reticulum. The entry of extracellular calcium occurs during depolarization of the plasmalemma in the course of action potential and intracellular calcium release occurs both during action potentials and between them [7]. It is beyond doubt that the inhibiting effect of H_2S on the strength of contractions and tonic tension observed in our experiments is related to the dynamics of intracellular calcium concentration, both during contractions and between them.

TABLE 2. Effect of NaHS on Spontaneous Contractile Activity of Rat Jejunum Fragment in the Control and against the Background of Preliminary TEA application

Parameter		Concentration of NaHS			
		10 μM	50 μM	100 μM	200 μM
Amplitude	control	89.32±5.28*	83.26±9.09*	62.26±10.18*	10.62±2.09*
	TEA	86.05±9.90	85.11±7.15	52.94±19.01*	7.30±2.42*
Frequency	control	100.10±0.50	99.33±0.61	95.04±2.72	75.71±6.64*
	TEA	103.16±0.84	106.99±3.61	102.68±2.11	85.69±4.47*
Tonic tension	control	96.61±1.30*	92.48±2.78*	91.21±3.80*	83.57±6.67*
	TEA	101.00±0.43	100.30±0.49	99.34±1.48	91.59±2.83*

K^+ channels are known to play the key role in tonus maintaining and in the control of GIT smooth muscle contraction, they affect resting potential, slow depolarization waves, action potential duration and dynamics of intracellular Ca^{2+} concentration [7]. Therefore, we studied the role of K^+ channels in NaHS effects using a nonspecific antagonist of these channels TEA; according to some data, mechanisms of H_2S effects may be associated with activation or inhibition of K^+ channels of various types [5,14].

Application of TEA for 20 min significantly increased the strength of contraction of the stomach strip to 161±17% (n=9; p<0.05) and jejunum segment to 152±9% (n=9, p<0.05) relative to the control (Fig. 1, *b*, *d*). The frequency of contractions and tonic tension remained practically unchanged in both preparations. The increase of contraction strength upon K^+ channel blockade was probably related to longer repolarization phase of the action potential in smooth muscle cells and enhanced Ca^{2+} entry triggering the contraction [8]. The effects of NaHS on the amplitude, frequency of contractions, and basal tone were fully preserved in preparations of stomach and jejunum under conditions of K^+ channel blockade with TEA (Tables 1 and 2, Fig. 1, *b*, *d*). Since TEA inhibits voltage-dependent and calcium-activated K^+ channels of high conductivity, our results indicate that these two types of K^+ channels are not involved in the effects of H_2S on spontaneous contractile activity of the stomach and jejunum, which is confirmed by the data of other investigators [12].

What are the molecular mechanisms that provide the inhibitory effect of H_2S on GIT contractile activity? Several assumptions can be made.

First, we can not exclude the influence of H_2S on apamin-sensitive Ca^{2+}-activated K^+ channels of low conductance (not inhibited by TEA), which may participate in the regulation of contraction amplitude by means of both hyperpolarization of smooth muscle cell membrane and regulation of neurotransmitter release in the enteric nervous system [5,12].

Second, a direct effect of H_2S on voltage-dependent Ca^{2+} channels of the plasma membrane and Ca^{2+} channels of the intracellular depots is possible, bearing in mind that the role of H_2S in the regulation of Ca-homeostasis has been shown in glia, neurons, and cardiomyocytes [1,2,6].

Third, ATP-dependent K^+ channels (K(ATP) channels) could be the target of H_2S, and their activation, according to some researchers, can mediate the inhibitory effects of NaHS on the amplitude of smooth muscle cell contractions [12]. Other researchers have not observed the participation of K(ATP) channels in the effects of NaHS [3,8,12].

Fourth, a direct effect of H_2S on the contractile proteins of smooth muscle cells cannot be ruled out. It was shown that inhibition of myosin light chain phosphatase partially prevented the inhibitory effect of NaHS on the contraction strength in mouse stomach preparations [3] and in rat jejunum [11].

As for reduction of the frequency of spontaneous contractions upon exposure to NaHS, it can be explained by the effects of H_2S on the mechanisms of generation of slow depolarization waves in interstitial cells of Cajal, which determine GIT automatic activity. It is known that pacemaker activity in Cajal cells depends on oscillations of intracellular calcium concentration and is triggered by calcium release from endoplasmic reticulum through inositol-3-phosphate receptors [7]. Indeed, cell culture studies of mouse small intestine smooth muscle cells have shown that NaHS blocked Ca-oscillations and spontaneous electrical activity of Cajal cells [13].

Our data indicate that H_2S in physiologically relevant concentrations decreases spontaneous contractile activity of rat stomach and jejunum smooth muscle cells by reducing the amplitude, frequency, and tone and without affecting the function of voltage-depen-

dent and Ca-activated K^+ channels. Molecular mechanisms of effects of H_2S on contractile activity of GIT require further study.

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