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Role of Cyclic Nucleotides in the Effect of Hydrogen Sulfide on Contractions of Rat Jejunum

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We studied the role of cyclic nucleotides in the influence of hydrogen sulfide (H_2S) donor, sodium hydrosulfide (NaHS, 200 μM), on motor activity of rat jejunum. NaHS reduced spontaneous and carbachol-induced contractions of rat jejunum segment, which suggests that H_2S can act through mechanisms involving muscarinic receptor activation. Against the background of a membrane-penetrating non-hydrolyzable cAMP analogue or under conditions of adenylate cyclase blockade, the inhibitory effect of NaHS on the carbachol-induced contractions was maintained. Against the background of elevated cGMP concentration or guanylate cyclase inhibition, the reduction of carbachol-induced contractions upon exposure to NaHS was less pronounced than in control. It was hypothesized that H_2S induces relaxation of carbachol-induced jejunum contractions, affecting protein kinase G targets or activating cGMP synthesis.

Key Words: *hydrogen sulfide; jejunum; carbachol-induced contractions; cAMP; cGMP*

Hydrogen sulfide (H_2S) was determined as a new type of gaseous transmitters along with NO and CO [1,3]. H_2S is endogenously synthesized in tissues of animals and humans and exerts various physiological effects in many body systems [3,5,14,15]. In the gastrointestinal tract (GIT), H_2S is produced by enzymes cystathionine-β-synthase and cystathionine-γ-lyase and sulfate-reducing bacteria that are a part of normal enterobacterial flora [7,8]. The data on the effect of H_2S on GIT motor activity are ambiguous: both relaxing and stimulating effects of this gas transmitter were revealed in different GIT compartments in different animal species [2,6,11,12]. The range of H_2S targets includes ion channels, receptors, intracellular enzyme systems [4,5,10,15]; the effects of this gas depend on protein target phosphorylation by various protein kinases [13].

The cyclic nucleotide system plays an important role in relaxation of smooth muscle cells of the intestine via activation of the corresponding protein kinases [9]. CAMP and cGMP are synthesized in response to vasointestinal peptide and nitric oxide release, respectively. Subsequent activation of protein kinases A and G leads to phosphorylation of various cellular targets, which causes receptor desensitization, inhibition of Ca^{2+} mobilization from intracellular stores, reduction of Ca^{2+} influx, and K-channel activation [9].

The aim of this study was to determine the role of cAMP and cGMP system in the influence of H_2S on carbachol-induced contractions of rat small intestine.

MATERIALS AND METHODS

Experiments were conducted in accordance with the Directive of the Council of the European Communities (86/609/EEC) and approved by the local ethics committee of the Kazan (Volga Region) Federal University (Protocol No. 8 issued May 05, 2015). The strength of contraction of 5-7-mm segments of rat jejunum was recorded under isometric conditions using a Biopac Systems instrument; contraction parameters were analyzed using AcqKnowledge 4.1 software. Throughout the experiment, the preparation was constantly perfused with Krebs solution of the following

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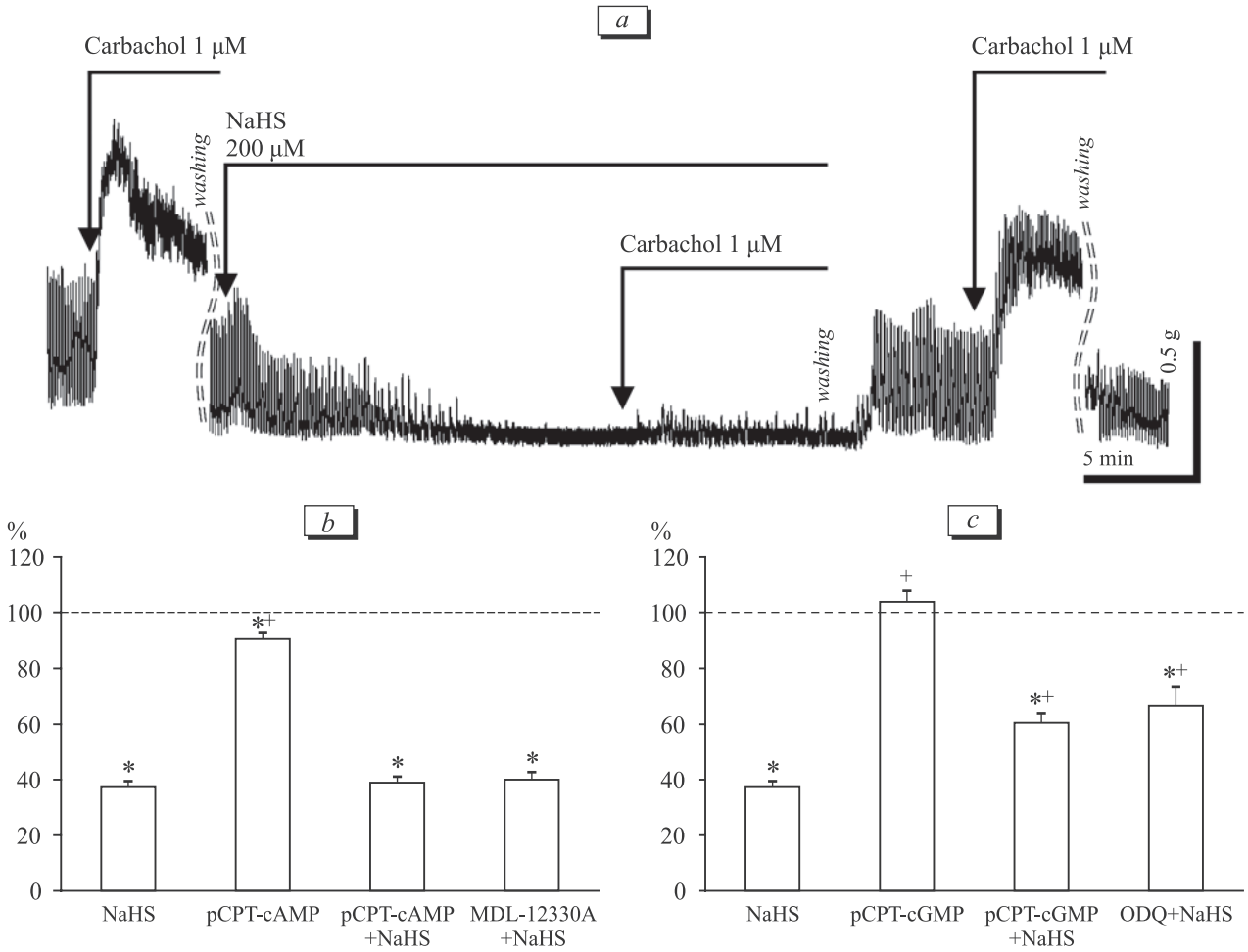

Fig. 1. Effect of NaHS on the carbachol-induced contractions of rat jejunum. *a*) Effect of NaHS (200 μM) on the carbachol-induced (1 μM) contractions. Arrow indicates the moment of drug application, horizontal line — duration of application. *b*) AUC for the carbachol-induced contraction under conditions of preliminary NaHS (200 μM) application, against the background of pCPT-cAMP (100 μM), pCPT-cAMP+NaHS, MDL-12330A (1 μM)+NaHS. *c*) AUC for the carbachol-induced contraction under conditions of preliminary NaHS (200 μM) application, against the background of pCPT-cGMP (100 μM), pCPT-cGMP+NaHS, ODQ (10 μM)+NaHS. The AUC for the carbachol-induced contraction in control was taken as 100%. $p<0.05$ in comparison with *control, $^{+}$NaHS application.

composition (in mM): 121 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 8 $C_6H_{12}O_6$ (pH 7.2-7.4) constantly aerated with 95% O_2 and 5% CO_2, the temperature was maintained at 37°C. After mounting, the preparation was kept for 60-90 min at a certain stretch until stable contractions were achieved. Then, spontaneous or carbachol-induced contractile activity was recorded in order to obtain control values, after that the drugs under study were applied.

Sodium hydrosulfide (NaHS) was used as H_2S donor producing H_2S in aqueous solutions. Taking into account pH and temperature, we found that only 11-13% of initial NaHS concentration yields H_2S in the solution [13]. Thus, when NaHS concentration is 200 μM, as it was in the experiments, 22-26 μM of H_2S is produced, which corresponds to physiological level.

The following drugs were used: membrane-penetrating non-hydrolysable cAMP and cGMP analogs 8-(4-chlorophenylthio)-adenosine-3,5-cyclic monophosphate (pCPT-cAMP) and 8 (4-chlorophenylthio)-guanosine-3,5-cyclic monophosphate (pCPT-cGMP), respectively, the adenylate cyclase inhibitor cis-N-(2-phenylcyclopentyl)azacyclotridec-l-en-amine hydrochloride (MDL-12330A), soluble guanylate cyclase inhibitor-1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-l-one (ODQ), acetylcholine receptor agonist carbachol (all reagents were from Sigma). The carbachol-induced (1 μM) contractions were analyzed by estimating the area under the curve (AUC) for a 2-min agonist application. Tonic tension and amplitude of spontaneous contractions in the control and after drug application were also analyzed.

In each series, *n* animals were used; from each animal, up to 4 preparations were obtained. Significance of differences was evaluated using paired Student's *t* test.

TABLE 1. Role of Cyclic Nucleotides in the Relaxation Effect of NaHS on Carbachol-Induced Contractions of Rat Jejunum Segment (*M*±*m*)

Index	Effect of carbachol	Carbachol+NaHS	Washing (Carbachol)
Control			
Tonic tension, g	0.80±0.13	0.31±0.02*	0.78±0.19
AUC, g×sec	237.51±12.33	88.13±7.98*	218.21±5.78
cAMP			
Tonic tension, g	1.48±0.09	0.69±0.04*	1.57±0.12
AUC, g×sec	257.10±8.91	96.65±4.98*	252.71±9.84
MDL-12330			
Tonic tension, g	1.18±0.13	0.75±0.07*	0.97±0.05
AUC, g×sec	208.71±14.31	79.34±2.11*	205.81±18.47
cGMP			
Tonic tension, g	1.33±0.21	0.79±0.17*	1.14±0.29
AUC, g×sec	232.14±13.46	134.07±10.14*	241.83±14.72
ODQ			
Tonic tension, g	1.67±0.08	0.96±0.06*	1.24±0.05
AUC, g×sec	185.95±17.10	115.38±10.47*	135.41±7.19

Note. *$p<0.05$ in comparison with the effect of carbachol in the control and after preliminary application of cAMP, MDL-12330A, cGMP, and ODQ.

RESULTS

Effect of NaHS on the carbachol-induced jejunum contractions. Preparations were stimulated with carbachol (1 μM) that causes smooth muscle cell contraction through activation of muscarinic cholinergic receptors [9]. Carbachol application caused a sharp increase in tonic tension from the control level that after reaching a maximum value (0.80±0.13 g, $n=4$, $p<0.05$) decreased and then plateaued (Fig. 1, *a*, Table 1). The preparation was then washed, and NaHS was applied for 6-10 min. NaHS reduced the amplitude of spontaneous contractions and tonic tension (Fig. 1, *a*; Table 2) as shown previously [12]. Subsequent carbachol addition showed that AUC for the induced contraction against the background of NaHS decreased to 37±2% of the control level (0.31±0.02 g, $n=4$; $p<0.05$) (Fig. 1; Table 1). After washing, the response of the preparation to carbachol was restored again (Fig. 1, *a*; Table 1). Thus, against the background of NaHS, effect of carbachol was reduced, which suggests that the signaling pathways triggered by the agonist participate in the effect of the gas. It is known that effects of H_2S in various systems can be associated with changes in concentration of cyclic nucleotides [4], therefore, we used agents that produce an increase and a decrease in the cAMP or cGMP level.

The role of the cAMP system in the effect of H_2S on carbachol-induced jejunum contractions. cAMP analogue pCPT-cAMP (100 μM) applied to the jejunum preparation reduced the amplitude of spontaneous contractions and tonic tension (Table 2), which confirms the role of cAMP as a relaxing agent in smooth muscle cells [9]. After preliminary application of pCPT-cAMP, carbachol produced less intensive contractions, which led to a decrease in AUC to 91±2% of that in control ($n=3$, $p<0.05$) (Fig. 1, *b*; Table 1). When pCPT-cAMP and NaHS were applied simultaneously, AUC for carbachol-induced contractions constituted 39±2% of the control level ($n=3$, $p<0.05$), which

TABLE 2. Effect of NaHS and Cyclic Nucleotides on the Amplitude of Spontaneous Contractions and Tonic Tension of Rat Jejunum Segment (*M*±*m*)

Experimental conditions	Amplitude, g	Tonic tension, g
Control	2.66±0.30	1.97±0.13
NaHS, 10 min	1.07±0.17*	0.78±0.08*
Control	2.45±0.16	1.50±0.07
cAMP, 10 min	0.65±0.09*	0.74±0.07*
Control	1.24±0.11	1.55±0.13
cGMP, 10 min	0.91±0.09*	0.80±0.03*

Note. *$p<0.05$ in comparison with the control.

did not differ from the effect of carbachol against the background of NaHS (Fig. 1, *b*; Table 1).

Adenylate cyclase was blocked by MDL-12330A (1 μM). After application of MDL-12330A+NaHS, the carbachol-induced contractions decreased to 40±3% of the control (n=13, p<0.05) (Fig. 1, *b*; Table 1), which also did not differ from the effect of carbachol against the background of NaHS. Thus, increased cAMP level insignificantly reduced the carbachol-induced contractions. However, the effect of NaHS on contractions caused by carbachol in presence of pCPT-cAMP or MDL-12330A was the same as in absence of these compounds. Hence, the adenylate cyclase system does not participate in the relaxing effect of NaHS on rat small intestine.

The role of the cGMP system in the effect of H_2S on carbachol-induced jejunum contractions. CGMP is produced by smooth muscles in response to nitric oxide release and guanylate cyclase activation. cGMP analogue pCPT-cGMP (100 μM) applied to the jejunum preparation reduced the amplitude of spontaneous contractions and tonic tension (Table 2). After preliminary pCPT-cGMP application, the effect of carbachol did not differ from that in the control and constituted 104±4% (n=3) (Fig. 1, *c*; Table 1). However, under conditions of simultaneous pCPT-cGMP and NaHS application, AUC for carbachol-induced contractions was 61±3% of the control level (n=3, p<0.05) (Fig. 1, *c*; Table 1), which significantly differed from that for the carbachol-induced contractions after NaHS application. Guanylate cyclase blockade with ODQ (10 μM) also reduced the effect of NaHS on carbachol-induced contractions (Fig. 1, *c*; Table 1).

Thus, the inhibitory effect of NaHS on carbachol-induced contractions is partly related to activation of cGMP targets or enhanced cGMP synthesis in smooth muscle cells. cGMP, in turn, activates protein kinase G that has specific phosphorylation targets, such as inositol-3-phosphate receptors, ryanodine receptors, and thus reduces calcium release from intracellular depots, which affects smooth muscle cell relaxation [9].

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