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CELL BIOPHYSICS

The Effects of Carbon Monoxide and Hydrogen Sulfide on Transmembrane Ion Transport

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Abstract—The activity of electroneutral ion transport in response to the effect of the gasotransmitters carbon monoxide and hydrogen sulfide was investigated. It was shown that phenylephrine, an activator of receptor-regulated calcium uptake, enhanced the relaxing action of carbon monoxide and hydrogen sulfide. In contrast, inhibition of the membrane potassium conductance, especially its voltage-dependent component, decreased the myogenic effects of carbon monoxide in the smooth muscles. The effects of hydrogen sulfide depended on its concentration and the means of activation of the cell transport systems. Furthermore, sodium-dependent components of the membrane conductivity are also involved in the effects of this gasotransmitter on ion transport systems in addition to the calcium and potassium conductance. This expands the range of the potential gasotransmitter-affected targets of signaling pathways, which may result in either activation or inhibition of cell functions. The consequences of such impacts on the functionally significant responses of cells, organs, and systems should be taken into account in various physiological and pathological states.

Keywords: carbon monoxide, hydrogen sulfide, potassium conductance of membrane, $Na^+,K^+,2Cl^-$ cotransporter, smooth muscle cells

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The group of gasotransmitters continues to grow and currently includes, in addition to nitrogen monoxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), other gases (CH_4 and NH_3), and some molecules of a broad group of reactive oxygen species. Gasotransmitters are produced by virtually all cells and due to their high lipophilicity are involved in many mechanisms of intra- and intercellular communication, which indicates the importance of these agents in the regulation of vital functions of cells, tissues, and the all body [1–3]. Significant progress in the studies of the reactions mediated by gasotransmitters has been made due to the discovery of the ability of certain chemical compounds to mimic the effects of these signaling molecules, acting as their donors.

Key membrane and molecular systems that implement the transmission of such signals, mechanisms and ways of interaction of gasotransmitters with "classical" mediators (Ca^{2+} and cyclic nucleotides), except perhaps NO, are in the early stages of study.

Abbreviations: SMC, smooth muscle cells; PE, phenylephrine; CORM-2, tricarbonyldichlororuthenium(II) dimer; AP, action potential; NKCC, $Na^+,K^+,2Cl^-$ cotransporter.

In recent years, close attention has been paid to the identification of ion-transport cell systems involved in the effects of gasotransmitters [4–6]. As an example, it was shown for smooth muscle cells (SMCs) of blood vessels and visceral organs that one can affect their contractile properties by modulating the ion channels of cell membranes directly via chemical modification of the channel proteins or indirectly via the involvement of secondary mediators [7, 8]. It has been noted that CO and H_2S, like NO, increase the permeability of cell membranes for K^+ ions by activating various components (potential-dependent, calcium-activated, and ATP-sensitive) of potassium conductance of the SMC membrane [7, 9, 10], but the contribution of each of these in the mechanisms that regulate the coupling of excitation–contraction in smooth muscles has been studied insufficiently. There are also data that indicate that CO and H_2S are able to reduce calcium currents through L-type Ca^{2+}-channels [11–13]. However, there is a lack of clarity in defining the role of electroneutral ion transport caused by the operation of the $Na^+,K^+,2Cl^-$ and K^+,Cl^- cotransporters, whose main function is the regulation of cell volume and

maintaining intracellular homeostasis of monovalent ions. It was shown on rat endothelial cells that H_2S reversed the work of the Na^+,Ca^{2+} exchanger, causing Ca^{2+} entry into the cell [14], and KB-R7943 an inhibitor of this exchanger, in turn, eliminated such a stimulatory effect of NaHS [15].

Thus, despite a certain success in understanding intracellular signaling mediated by gas mediators, a number of unresolved questions remain about the mechanisms of the CO and H_2S effects on ion transport systems of cytoplasmic membranes.

MATERIALS AND METHODS

The study of the contractile reactions of smooth muscle in blood vessels was performed on segments of the thoracic aorta with the removed endothelial layer of adult male rats of the Wistar line (40 individuals) that were killed by the method of cervical dislocation under deep anesthesia (nembutal at a dose of 70 mg/kg, intraperitoneally) in accordance with the requirements of the Rules for conducting work using experimental animals (Order of MH USSR No. 755 of July 12, 1977). Recording the mechanical stress of the aorta segments was performed in the isometric mode using a Myobath II mechanography device and a LAB-TRAX-4/16 complex (Germany). The preparations were fixed in aerated chambers filled with standard physiological Krebs solution (in mM: NaCl, 120.4; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; glucose, 5.5; Tris, 15) at 37°C, pH 7.35–7.40. After 40–50 min of incubation in normal Krebs solution the contraction of vascular segments was induced by high potassium Krebs solution (equimolar substitution of 30 mM NaCl for KCl), and in some experiments, by phenylephrine (PE, 1 μM); the amplitude of the contractile responses were considered as the control (100%). The tricarbonyldichlororuthenium(II) dimer (CORM-2) and sodium hydrosulfide (NaHS) were used as donors of CO and H_2S, respectively [5, 7].

The changes of the electrical and contractile activity parameters of smooth muscles were investigated via the method of a double sucrose bridge on isolated ureter smooth muscle preparations of guinea pigs (30 animals). This technique allows one to simultaneously record the electrical stimulus evoked action potential (AP) and the contraction of SMCs. The ureter segments were fixed in the chamber of the instrument, perfused by physiological Krebs solution and limited from both sides by sucrose sections (0.3 M solution of sucrose). The electrical potentials were determined using non-polarizable electrodes. The mechanical stress of smooth muscle preparations was recorded by an FT10G isometric force sensor connected to a 14-bit ADC L-791 (L-CARD, Russia). The received signals were processed using appropriate software (LGraph2, L-CARD, Russia). The values of the AP parameters (the amplitude spike component and the plateau duration) and the SMC contraction amplitudes under the action of depolarization stimulus in the Krebs solution were use as the control (100%).

The activity of the Na^+,K^+,2Cl^- cotransporter (NKCC) was studied using the radionuclide method on isolated rat aorta SMCs, which was used in three to eight passages (Lonza, United States). The NKCC activity was investigated as the bumetanide-sensitive $^{86}Rb^+$ influx component. The cells were seeded onto 24-well plates and washed twice with 2 mL aliquots of medium containing 150 mM NaCl and 10 mM HEPES-Tris-buffer (pH 7.4). The medium was then removed and 0.25 mL of solution containing 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose, 20 mM HEPES-Tris-buffer (pH 7.4) and the tested compounds was added. After 10 min of incubation at 37°C in 0.25 mL of the medium containing 1–2 μCi/mL of $^{86}Rb^+$ in the presence or absence of bumetanide (20 μM) the absorption of the isotope was stopped by adding 2 mL of ice-cold medium containing 100 mM $MgCl_2$ and 10 mM HEPES-Tris-buffer (pH 7.4). The cells were washed three times and lysed (1% SDS/4 mM EDTA). The radioactivity of the incubation medium and cell lysate were measured using a liquid scintillation analyzer. The $^{86}Rb^+$ (K^+) influx rate V (nmol per mg protein in 10 min) was calculated as $V = a/A \cdot mt$, where A is the radioactivity of the cell lysate (cpm), a is the radioactivity of $^{86}Rb^+$ in the incubation medium (cpm/nmol), m is the protein content in cell lysate (mg), and t is the time of incubation of cells with the isotope (10 min).

The study of the Ca^{2+}-dependent potassium permeability (Gardos channels) of the erythrocyte membrane was performed using the potentiometric method of continuous registration of the cell membrane potential changes via the pH of the medium measurements, based on the fact that in the presence of the protonophore CCCP (carbonylcyanide-*m*-chlorophenylhydrazon, 20 μM) the H^+ distribution depends on the membrane potential according to $E_m = (pH_i - pH_o) \cdot RT/F$, where pH_i and pH_o are pH values of the cytoplasm and incubation medium, respectively. To obtain the Ca^{2+}- and redox-induced hyperpolarization response, the Ca^{2+}-ionophore A23187 (0.5 μM) or the artificial electron donor system ascorbate (10 mM)–phenazine metosulfate (0.1 mM) were added to the incubation medium, respectively. In both cases the hyperpolarization response of the red blood cell membrane developed, whose amplitude reflected the activity of Ca^{2+}-dependent potassium channels (K_{Ca}-channels). NaHS was added 5 min before adding agents that cause the hyperpolarization response to the suspension of red blood cells.

Statistical processing of the obtained data was performed using SPSS Statistics 17.0.1 for Windows. The significance of the differences was determined by the non parametric criteria of the Mann–Whitney U test

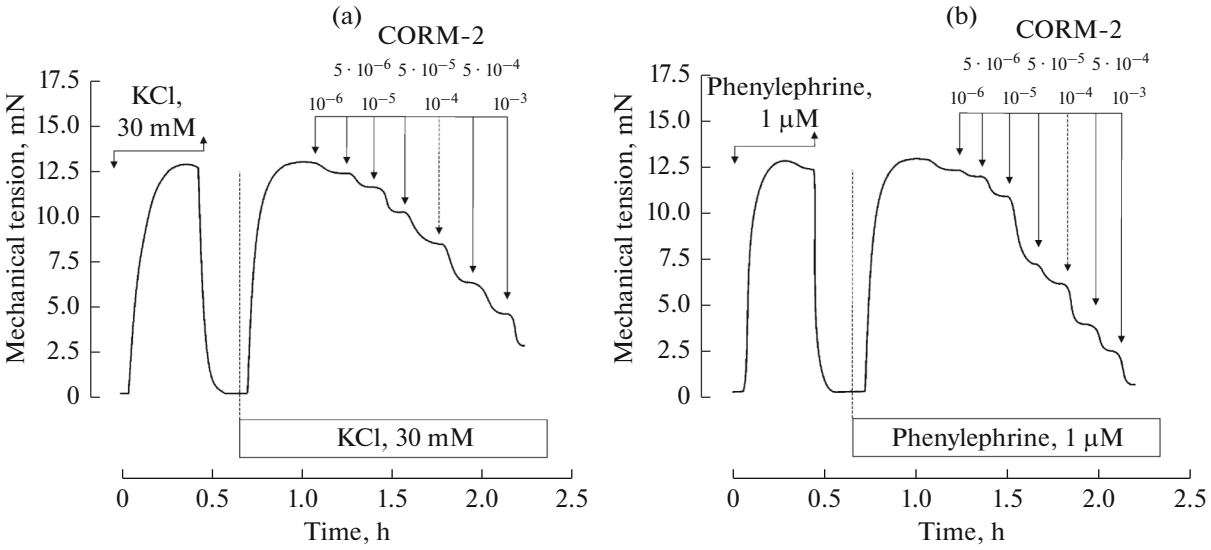

Fig. 1. The effect of CORM-2 on the contraction of the rat aorta SMCs induced by high potassium Krebs solution (a) and phenylephrine (b). *Y* axis, mechanical tension (mN), *X* axis, time (hours). The arrows indicate the addition and removal of the respective solutions.

for independent and Wilcoxon *T* criterion for dependent samples. Data are presented as the median (Me) and the interquartile range (Q_1; Q_3).

RESULTS

The influence of carbon monoxide on the electrical and contractile properties of the vessel and ureter SMCs. The CO donor CORM-2 at concentrations from 1 to 1000 μM had no effect on the initial mechanical tension of smooth muscle of the rat aorta segments. However, the application CORM-2 in the same range of concentrations to the SMCs contracted by high potassium led to a dose-dependent reduction of the mechanical tension of the vascular segments (Fig. 1a). Relaxing to close to half of the maximum was observed in response to the action of 100 μM CORM-2; the amplitude of the contractile response of SMC was 57.1 (53.9; 61.2)% ($n = 10$, $p < 0.05$) of the hyper potassium contraction control. The addition of CORM-2 (1–1000 μM) to the rat aorta segments contracted via stimulation of α_1-adrenergic receptors by PE (1 μM) led to a dose-dependent decrease in the contractile reaction of the vascular SMCs (Fig. 1b). Relaxing to close to half of the maximum was observed in response to the action of 10 μM CORM-2; amplitude reduction was 58.4 (52.6; 67.4)% ($n = 10$, $p < 0.05$) of the PE-induced control. Thus, using the α_1-adrenoceptor agonist PE as a contraction inducing agent, the CORM-2 concentration that causes the half maximum relaxing effect was tenfold lower.

Upon addition of CORM-2 at concentrations 1, 10 and 100 μM into the perfusion solution, initial parameters of the electrical and contractile activity of smooth muscle strips of the ureter did not changed. However, the presence of the donor in the Krebs solution after application of the depolarizing stimulus caused a dose dependent decrease of the contraction amplitude of SMC, the amplitude and duration of the plateau of the AP compared with the control responses to the stimulus in saline (Table 1). In the conditions of the effect of 10 μM PE, there was significant enhancement of the inhibitory effects of the CO donor on the electrical and contractile properties of the SMC of the ureter ($p < 0.05$).

The influence of hydrogen sulfide on the electrical and contractile properties of the vessel and ureter smooth muscles. NaHS, a donor of H_2S at concentrations from 5 to 1000 μM had no effect on the initial mechanical tension of vascular segments; however, it had opposite effects on their contractile responses. At concentrations of 5–50 μM NaHS caused an additional increase of the mechanical tension and the relaxation of vascular SMCs at concentrations of 500 and 1000 μM. Under the action of 100 μM NaHS a biphasic response was observed, the amplitude of the high potassium induced SMC contraction initially increased and then was inhibited (Fig. 2a). In subsequent experiments changes of the vasorelaxing effect of NaHS (500 μM), which caused a decrease in the amplitude of the high potassium-induced contractions to 63.3 (50.2; 69.7)% ($n = 6$, $p < 0.05$) compared to the control, were studied. NaHS (1–1000 μM) treatment of the rat aorta SMCs contracted by PE induction led to dose dependent inhibition of the contractile activity of the vessel smooth muscle segments (Fig. 2b). A close to half maximum relaxing effect was observed in response to the action of 100 μM NaHS, the amplitude of the contractions of the SMCs was

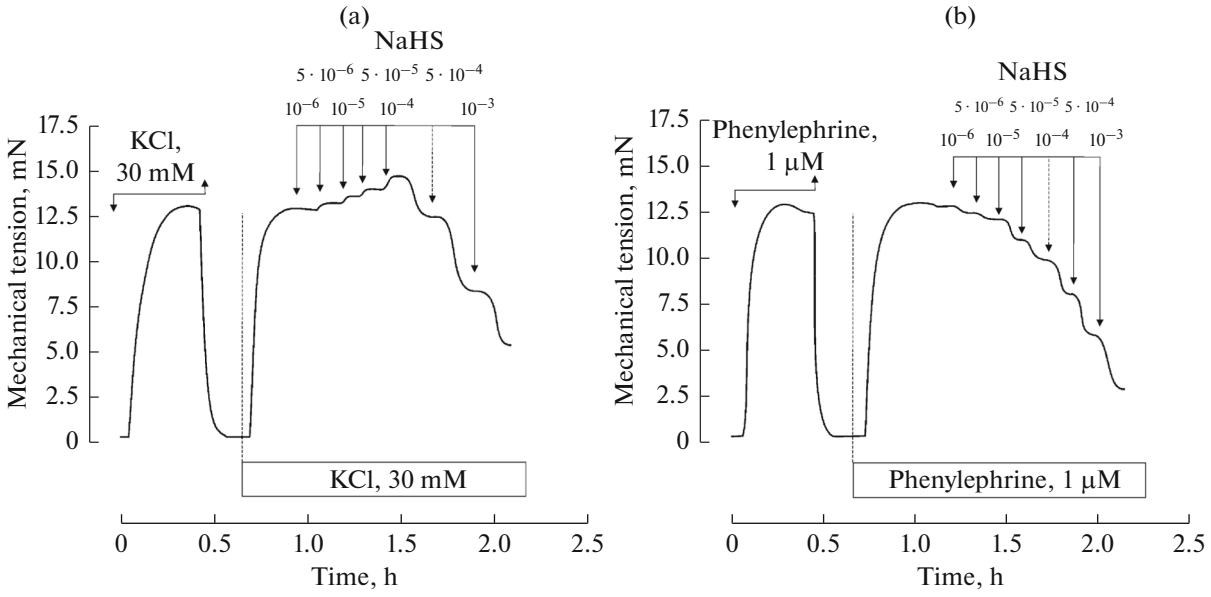

Fig. 2. The effect of NaHS on contraction of the rat aorta SMC induced by high potassium Krebs solution (a) and phenylephrine (b). Symbols as in Fig. 1.

58.6 (51.6; 60.4)% ($n = 6$, $p < 0.05$) of the control PE-induced contraction. Thus, the characteristics of the mechanical tension changes of the smooth muscle of the rat aorta segments depended not only on the effective concentration of the H_2S donor, but also on the method of contraction generation.

A donor of H_2S at concentrations of 10, 100, and 1000 μM had an activating influence on the electrical and contractile properties of smooth muscles of the ureter, causing a dose-dependent increase of the contraction amplitude and of the AP indicators (Table 1). In the presence of 10 μM PE, the H_2S donor had increased stimulating effects ($p < 0.05$) at the concentrations under study.

The role of potassium conductance of the SMC membrane in the effects of gasotransmitters. Interaction of gasotransmitters with ion channels in the membrane is considered as an important mechanism of their action on the contractile and electrical properties of SMCs. It is assumed that the effects of these gases can be associated not only with influence on calcium conductance, but also with changes of the potassium conductance of the SMC membrane.

In the presence of tetraethylammonium (10 mM) an unspecific potassium channel blocker, the relaxing effect of CORM-2 on vascular segments was attenuated after preliminary contraction by 30 mM KCl or PE by 24.3% ($n = 8$, $p < 0.05$) and 21% ($n = 8$, $p < 0.05$), respectively, in comparison with the value of the amplitude in the absence of the blocker. After a 10-minutes pretreatment of the vascular segments by 1 mM 4-aminopyridine (a potential-dependent potas-

Table 1. The effect of CORM-2 and NaHS on the electrical and contractile activity of the guinea pig ureter smooth muscle, Me (Q1; Q3)

The group	CORM-2			NaHS		
	1 μM	10 μM	100 μM	10 μM	100 μM	1000 μM
Control, %	100	100	100	100	100	100
Contraction amplitude, %	98.6 (92.1; 102.8)	81.5* (76.3; 90.6)	73.3* (68.5; 81.1)	121.9* (110.4; 132.5)	143.1* (130.4; 156.5)	154.7* (142.4; 168.9)
AP amplitude, %	89.2* (80.2; 105.0)	82.9* (78.7; 93.1)	70.8* (65.4; 83.2)	103.6 (96.4; 110.2)	107.1* (98.8; 113.3)	111.5* (91.4; 123.1)
Plateau duration AP, %	96.7 (92.4; 105.7)	81.7* (77.6; 92.7)	69.8* (61.5; 74.2)	105.7 (95.7; 114.4)	109.3* (95.7; 115.9)	115.9* (99.8; 126.6)

*$p < 0.05$ compared to the control.

Table 2. The effect of CORM-2 and NaHS on the electrical and contractile properties of the ureter SMC when the potassium channels are blocked, Me (Q1; Q3)

The group	CORM-2, 10 μM	CORM-2 + tetraethylammonium, 5 mM	CORM-2 + 4-aminopyridine, 1 mM	100 μM	NaHS + tetraethylammonium, 5 mM	NaHS + 4-aminopyridine, 1 mM
Control, %	100	100	100	100	100	100
Contraction amplitude, %	81.5* (76.3; 90.6)	91.2*# (82.8; 99.2)	93.8*, # (86.6; 98.5)	143.1* (130.4; 156.5)	139.4*, # (128.2; 147.4)	125.1*, # (108.7; 135.9)
AP amplitude, %	82.9* (78.7; 93..1)	90.7*# (88.3; 97.1)	95.1*, # (90.1; 103.5)	107.1* (98.8–113.3)	102.9# (92.1–109.3)	97.2# (84.6–111.5)
Plateau duration AP, %	81.7* (77.6; 92.7)	94.6*, # (83.2;100.9)	93.8*, # (89.4; 98.7)	109.3* (95.7; 115.9)	108.5* (91.9; 117.7)	96.8# (85.6; 115.5)

* $p < 0.05$ compared to the control; #, $p < 0.05$ compared to the action of CORM-2 or NaHS in the absence of the inhibitor.

sium channel blocker), the magnitude of relaxation caused by the addition of CORM-2 for the high potassium or PE-induced contraction was significantly reduced by 29% ($n = 8$, $p < 0.05$) and 25% ($n = 8$, $p < 0.05$), respectively. Addition to the solution of 5 mM tetraethylammonium or 1 mM 4-aminopyridine also led to a significant weakening of the influence of 10 μM CORM-2 on the AP parameters and the amplitude of contractions of the guinea pig ureter SMCs under the action of depolarization stimulus.

Upon treatment by 10 mM tetraethylammonium or 1 mM 4-aminopyridine, the relaxing effect of 500 μM NaHS on the vascular SMCs preliminarily contracted by 30 mM potassium chloride, was significantly reduced by 13.9% ($n = 6$, $p < 0.05$) and 19.5% ($n = 6$, $p < 0.05$), respectively, in comparison with the effects of the donor in the absence of the blocker. The magnitude of the relaxation of the rat aorta segments, which were preliminarily contracted by PE in response to the action of 100 μM NaHS, significantly decreased in the presence of tetraethylammonium (10 mM) by 13.8% ($n = 6$, $p < 0.05$) and in the presence of 4-aminopyridine by 16.2% ($n = 8$, $p < 0.05$). At the same time, tetraethylammonium at a concentration of 5 mM did not significantly change the activating action of 100 μM NaHS on the SMC contraction and the AP parameters of the guinea pig ureter. Pretreatment by 4-aminopyridine (1 mM) caused a significant decrease of the effects of the H_2S donor on the ureter smooth muscle (Table 2).

The ambiguity of the influence of NaHS on the potassium conductance of the SMC membrane required clarification; therefore, the research was conducted on suspensions of red blood cells because their membrane contains only K_{Ca}-channels [16].

Adding to the cell incubation medium of NaHS at concentrations ranging from 0.005 mM to 0.3 mM led to a change in the amplitude of the hyperpolarization response of the erythrocyte membrane induced by either the calcium ionophore A23187 or the ascorbate–phenazine metosulfate redox system. In the absence of the donor, the amplitude of the A23187- and redox-dependent hyperpolarization response was 22.1 (20.8; 22.6) mV ($n = 25$, $p < 0.01$) and 49.8 (44.6; 50.7) mV ($n = 25$, $p < 0.01$), respectively. Incubation of erythrocytes with various concentrations of NaHS led to unequal changes of the hyperpolarization response amplitude. Thus, in the presence of 0.005 mM NaHS, the amplitude of the A23187-dependent hyperpolarization response significantly increased, while the redox-dependent hyperpolarization response decreased compared with control values ($p < 0.01$). Introduction into the incubation medium of erythrocytes at higher concentrations of NaHS (0.01 to 0.3 mM) led to a decrease in the amplitude of the hyperpolarization response caused by both means. It should be noted that the suppression of the A23187-dependent hyperpolarization response in the presence of 0.15–0.3 mM NaHS was more pronounced than suppression of the redox-dependent response. These data indicate that there is a different influence of hydrogen sulfide on the activity of these channels stimulated by different means.

The study of the influence of gasotransmitters on the activity of the $Na^+,K^+,2Cl^-$ cotransporter. Pretreatment by bumetanide (100 μM), which is an inhibitor of the $Na^+,K^+,2Cl^-$ cotransporter, for 15 min led to inhibition of the relaxing action of the CO donor on the rat aorta segments that were preliminarily contracted by 30 mM KCl or PE, by 10.5% ($n = 6$, $p < 0.05$) and 8.4% ($n = 6$, $p < 0.05$), respectively. In the presence of 100 μM bumetanide, the addition of CORM-2 (10 μM) caused a significant inhibition of the contraction amplitude by 13.3% and of the AP amplitude and the duration of the plateau in the ureter smooth muscle by 14.2 and 10.3% ($n = 8$, $p < 0.05$), respectively, compared to values in the absence of the inhibitor.

Pretreatment of the rat aorta segments by bumetanide (100 μM) also eliminated the constriction

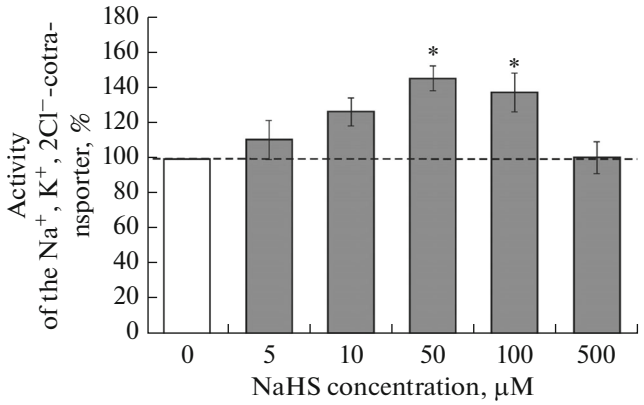

Fig. 3. The effect of sodium hydrogen sulfide on the activity of the Na^+,K^+,$2Cl^-$ cotransporter; *$p < 0.05$ compared to the absence of NaHS in the incubation medium. Data were obtained in three independent experiments performed on four samples.

effect of low concentrations of NaHS (5–100 μM) on the vascular SMCs that were preliminarily contracted by the hyper potassium solution (n = 7, p < 0.05). The study of the NKCC activity as a bumetanide-sensitive 86Rb+ influx component allowed us to establish that the addition into the incubation medium of 5, 10 and 500 μM NaHS had no statistically significant effect on the activity of this transporter. However, in the presence of 50 and 100 μM NaHS, NKCC activity significantly increased (Fig. 3). Inhibition of NKCC by bumetanide (100 μM) significantly reduced the effect of NaHS (100 μM) on the electrical and contractile activity of the ureter SMCs ($p < 0.05$) as well.

The effect of NaHS on the hyperpolarization response amplitude of red blood cells in the presence of bumetanide (5 μM) was also dependent on the method of stimulation. Thus, the amplitude of the A23187-dependent hyperpolarization response under the combined action of NaHS and bumetanide was significantly higher in comparison to that obtained in the absence of the inhibitor ($p < 0.01$). In contrast, the amplitude of the redox-stimulated hyperpolarization response of erythrocytes significantly decreased compared to the values in the absence of bumetanide.

DISCUSSION

One of the central problems in the study of the regulation of cell function is the identification of systems that cause the effects of perturbations in intracellular communication. The main role in these processes is attributed to potential-dependent and receptor-depended (low selective) ion channels of the membrane.

We have found a dose-dependent inhibitory effect of CO on the electrical and contractile activity of SMCs of the rat aorta and guinea pig ureter, which is probably related to the changes of ion conductivity of their membranes and/or to the involvement of one or more links in the network of intracellular regulatory systems. It is known that a characteristic feature of excitation–contraction coupling is the inflow of extracellular calcium ions [17, 18]. An increase of their concentration within the cell can be reproduced by physiologically active substances via α_1-adrenergic effects, for example. By binding to their receptor in the plasma membrane of smooth muscle, they not only provide receptor-controlled Ca^{2+} influx into the cell, but also activate the C-kinase branch of the calcium signaling system and promote the release of Ca^{2+} from the depot. In fact, preliminarily contracted ring segments by an α_1-adrenomimetic PE increased the CORM-2 effects on the electrical and the contractile properties of smooth muscles of the aorta segments and of the ureter. This is probably connected with the peculiarities of the influence on the C-kinase branch of the calcium regulation of the electrical and contractile activity parameters of SMCs and the inclusion of this intracellular signal transmission pathway becomes an additional target for CO.

Blocking of potassium channels of the SMC membrane led to a decrease in myogenic effects, indicating the participation of the membrane potassium conductivity in the mechanisms of action of this gasomediator [10], and mainly of its potential-dependent component.

The direction of the vasoactive effects of H_2S, as we have established, depended on the effective concentration of hydrogen sulfide and of characteristics of the factor that caused the SMC contractile response. Thus, in the case of the receptor-controlled Ca^{2+} influx activation by phenylephrine, the H_2S donor (5–1000 μM) caused dose-dependent relaxation of vascular SMC, while under the action of 30 mM potassium chloride a similar effect of NaHS was observed at concentrations over 100 μM. In fact, the bumetanide sensitive component of the $^{86}Rb^+$ influx into the isolated vascular SMC was significantly increased in the presence of exactly 50 and 100 μm NaHS. Blocking of potassium channels by tetraethylammonium or 4-aminopyridine weakened the vasorelaxing action of H_2S, confirming the role of potential-dependent potassium channels in the effects of H_2S, along with the already known K_{ATP} channels [7].

In contrast to the vascular SMCs, the ureter SMCs responded to NaHS by a dose-dependent increase in the values of the parameters of the electrical and contractile activity caused, as suggested, by a decrease in cAMP-dependent regulation of the potassium permeability of the SMC membranes [17, 19]. The ambiguity of the effect of different concentrations of H_2S on the potassium conductance of the cell membrane was reflected in the inhibition of the activity of K_{Ca}-channels of the erythrocyte membranes stimulated by the calcium ionophores A23187 or phenazine metosulfate.

The inhibition of the $Na^+,K^+,2Cl^-$ cotransporter confirmed the participation of sodium ions in their influence on the effects of CO and H_2S via the ability to form gradients. When the activity of this transporter was inhibited by bumetanide, the influence of CO and NaHS on the contraction of the rat aorta vascular segments and on the indicators of the electrical and contractile activity of the ureter SMCs decreased. Previously, we have shown that the impact on the contractile activity of the electroneutral NKCC is related to the transfer of chloride ions (with Na^+ and K^+) across the membrane into the cell and maintaining the intracellular concentration of chloride ions in SMC above the equilibrium [20–22]. As a result of the increase in chloride conductance and depolarization of the SMC membrane additional potential-dependent Ca^{2+}-channels of the L-type are opened and the intracellular concentration of Ca^{2+} and contractile response of smooth muscle increase [18, 23]. This is an additional indication that chloride currents are involved in the regulation of excitation–contraction coupling in SMCs.

CONCLUSIONS

Intracellular signal transduction mediated by gasotransmitters along with "classical" mediators can be one of the candidates for participation in the regulation of the functional properties of various cells, tissues, and organs. These results indicate that gas intermediaries exert an influence on the mechanisms of electrogenesis and maintain muscle cell contraction by modulating the work of ion transport systems, in particular potassium channels and the $Na^+,K^+,2Cl^-$ cotransporter.

The elucidation of the effector targets of the gas mediator action and the nature of the interaction with them is essential not only from the perspective of obtaining fundamental knowledge on the principles of intra- and intercellular signaling, but can also be used in the development of molecular technologies to control cellular behavior by modifying gas communication. The question of the interaction of the NO-, CO-, and H_2S-dependent signaling pathways, whose crossing at various levels of the signaling cascades can lead to their mutual activation or inhibition, deserves special attention. The result will be a change in functionally important reactions of individual cells, organs, and systems under various physiological and pathological conditions.

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