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The role of Na^+, K^+, $2Cl^-$-cotransport in the H_2S-dependent regulation of contractile activity of smooth muscle cells from rat pulmonary artery

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Abstract

Objective. Hydrogen sulfide (H_2S) is one of gasotransmitters that participate in the regulation of a large number of cellular functions. H_2S can also act as a pathological link in the development of vascular diseases, in particular hypertension. Na^+, K^+, $2Cl^-$-cotransporter (NKCC) might play an important role in vascular tone increasing due to involvement of chloride currents in the depolarization of smooth muscle cell membrane. Significant differences in the regulatory mechanisms of contractile properties of the vessels of systemic and pulmonary circulation might depend on the mechanisms of NKCC. So its role as a target for H_2S requires investigation. **Design and methods.** The changes in mechanical tension of ring segments from pulmonary artery (PA) of WKY and SHR rats under the action of the donor of H2S (L-cysteine) was studied by organ bath technique. **Results.** L-cysteine caused multidirectional effects on mechanical tension of PA smooth muscle cells from WKY rats precontracted with 30 mM KCl. Bumetanide (100 μM) suppressed the relaxation but not constriction of the intact and endothelium-denuded vascular segments caused by L-cysteine. In ring segments from PA of SHR rats, L-cysteine potentiated constriction in segments with intact endothelium but caused relaxation in endothelium-denuded segments.

Key words: smooth muscle cells, pulmonary artery, Na^+, K^+, $2Cl^-$-cotransport, hydrogen sulfide

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Роль Na^+, K^+, $2Cl^-$-котранспорта в H_2S-опосредованной регуляции сократительной активности гладкомышечных клеток легочной артерии крыс

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Резюме

Введение. Сероводород (H_2S) является представителем группы газовых посредников, которые участвуют в регуляции большого числа клеточных функций, а также может выступать патологическим звеном в развитии сосудистых заболеваний, в частности артериальной гипертензии. Предполагается, что Na^+, K^+, $2Cl^-$-котранспортер (NKCC) может играть важную роль в повышении сосудистого тонуса в связи с участием хлорных токов в индукции деполяризации мембраны гладкомышечных клеток (ГМК). Допускается, что существующие значительные отличия в механизмах регуляции сократительных свойств сосудов большого и малого кругов кровообращения могут зависеть от механизмов оперирования NKCC, что делает необходимым его изучение в роли мишени и для H_2S. **Материалы и методы.** Механографическим методом было исследовано действие донора H_2S (L-цистеина) на изменение механического напряжения (МН) сегментов легочной артерии (ЛА) крыс WKY и SHR. **Результаты.** На фоне предсокращения сосудистых сегментов крыс линии WKY гиперкалиевым раствором (30 мМ KCl) наблюдалось разнонаправленное действие L-цистеина на МН ГМК ЛА. Буметанид (100 мкМ) подавлял расслабляющее влияние L-цистеина на интактные и деэндотелизированные сосудистые сегменты, но не влиял на его констрикторные эффекты. При действии на ГМК ЛА крыс линии SHR с сохраненным эндотелием L-цистеин оказывал констрикторное действие, тогда как на деэндотелизированные сегменты — релаксирующее.

Ключевые слова: гладкомышечные клетки, легочная артерия, Na^+, K^+, $2Cl^-$-котранспорт, сероводород

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Introduction

Hydrogen sulfide (H2S), along with other gasotransmitters (NO, CO), participates in the regulation of a large number of cellular functions in various physiological and pathological processes [1–3]. At the present time, the H2S-dependent mechanisms of regulation of mechanical tension of vascular smooth muscles from systemic circulation. According to the available data [4–6], one of the main physiological effects of H2S is the relaxation of smooth muscle cells (SMC) through K_{ATP}-, K_{Ca}, K_v-channels, in particular. Despite of H2S is an effective relaxant, there is evidence of its constrictive action, especially in the range of low concentrations (10–100 μM) [2, 7, 8]. It is shown that this effect of H2S is due to the activation of Na+, K+,2Cl-cotransport (NKCC) [9, 13]. NKCC promotes an increase in the intracellular concentration of chloride ions, the depolarization of membrane of the SMC, the sarcolemma of which is enriched with anion channels, the opening of voltage-dependent Ca2+ channels, and the contraction of smooth muscles. This mechanism can underlie the development of arterial hypertension [10–16].

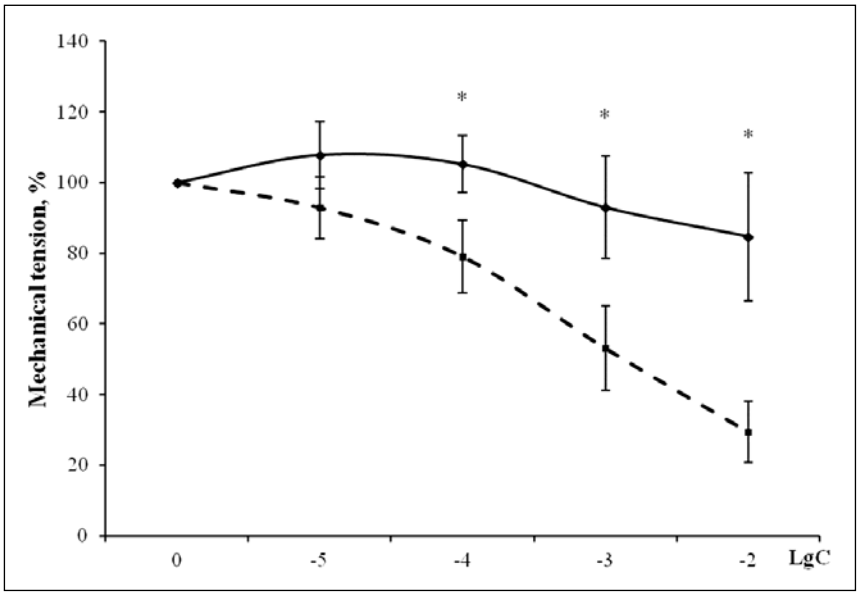
Taking into account the ability of H2S-donors to modulate the vascular tone, it seems promising to search for new drugs based on sulfur-containing compounds. However, it is necessary to take into account that significant differences in the regulatory mechanisms of the mechanical tension of the vessels from systemic and pulmonary circulation may occur. Considering this, the phenomenology of the H2S action on the tone of small-diameter vessels should be studied in details and targets of H2S should be revealed. The present study is devoted to the role of NKCC in the H2S-dependent mechanisms of regulation of pulmonary artery smooth muscles mechanical tension.

Material and methods

The study was performed on intact and endothelium-denuded smooth muscle segments of PA of male Wistar-Kyoto rats (WKY, 20 animals) and spontaneously hypertensive rats (SHR, 12 animals), which were sacrificed by a cervical dislocation under deep anesthesia (Nembutal 70 mg / kg, intraperitoneally). All manipulations were carried out in accordance with the "Rules for carrying out work using experimental animals".

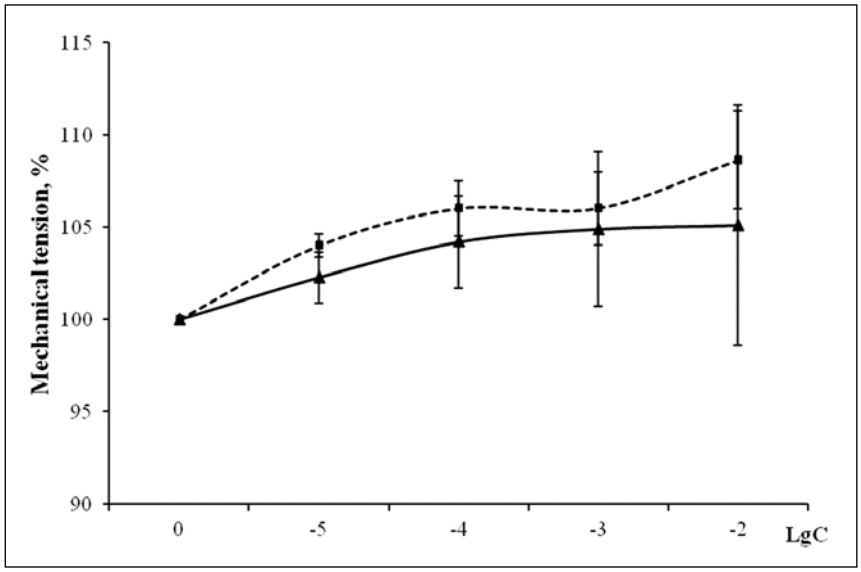
Prepared vascular segments were fixed in the organ bath chamber and incubated at 37,0 ± 0,5ºC and pH 7,35–7,40 for 40–50 min in Krebs physiological solution containing (in mM): 120,4 NaCl, 5,9 KCl, 2,5 $CaCl_2$, 1,2 $MgCl_2$, 5,5 glucose, 15 Tris buffer $NH_2C(CH_2OH)_3$). Mechanical tension (MT) of smooth muscle segments was recorded with the FT10G force sensor connected to a 14-bit A/D converter (L-791, L–CARD, Russia). The signal was

Figures. 1. L-cysteine influence on the mechanical tension of smooth muscles of the pulmonary artery of WKY rats precontracted with highpotassium Krebs solution



Note: Y-axis — mechanical tension (%); X-axis — the decimal logarithm of the concentration of L-cysteine; dotted line — endothelium-denuded segments; solid line — segments with intact endothelium; * — significant differences between segments with intact and denuded endothelium ($p < 0.05$).

Figures. 2. L-cysteine influence on the mechanical tension of the smooth muscles of the pulmonary artery of WKY rats, precontracted with highpotassium Krebs solution in the presence of bumetanide



Note: Y-axis — mechanical tension (%); X-axis — the decimal logarithm of the concentration of L-cysteine; dotted line — endothelium-denuded segments; solid line — segm ents with intact endothelium; * — significant differences between segments with intact and denuded endothelium ($p < 0.05$).

then recorded and processed using the L-Graph-II software (L–CARD, Russia).

The value of MT in highpotassium Krebs solution (KCl, 30 mM) was used as a control (100%).

Chemicals used: phenylephrine, bumetanide, L-cysteine (all Sigma Aldrich, USA).

Statistical analysis of the data was carried out using the SPSS Statistics 17.0.1 for Windows and nonparametric criteria: Mann-Whitney U test for independent samples and Wilcoxon T-test for dependent samples. Differences were considered reliable at a significance level of $p<0.05$.

Results

L-cysteine influence on the mechanical tension of pulmonary artery smooth muscle of normotensive rats precontracted with highpotassium Krebs solution

The sulfur-containing amino acid L-cysteine was used as a donor of endogenous H2S. In segments precontracted with highpotassium Krebs solution (KCl, 30 mM), L-cysteine (from 10 μM to 10 mM) caused a decrease of MT of endothelium-denuded smooth muscle segments of rat PA. In segments with intact endothelium precontracted with 30 mM KCl, addition of low concentrations of L-cysteine (10–100 μM) increased the MT, whereas 1–10 mM L-cysteine caused decrease of MT (Fig. 1).

We proposed that small concentrations (10–100 μM) of L-cysteine induce contractile responses of vascular SMCs by activation of NKCC.

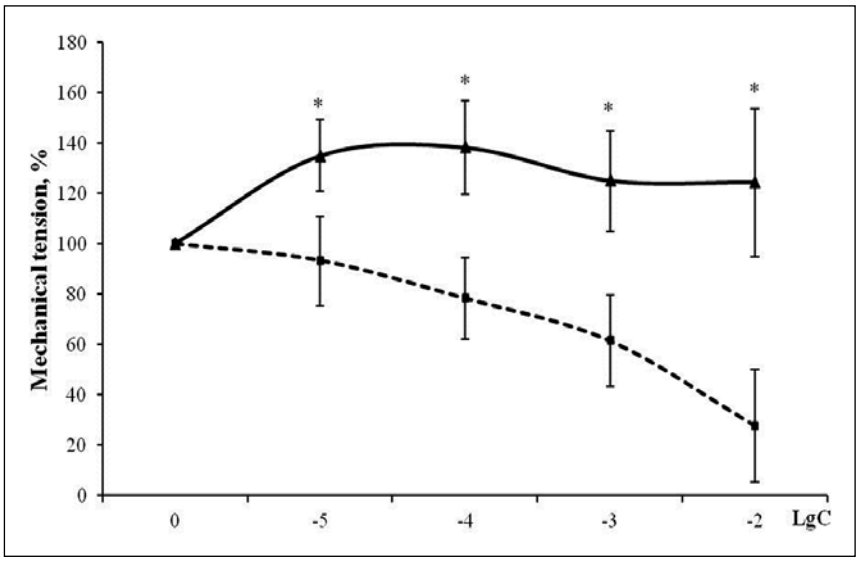
To investigate the role of NKCC in the mechanisms of L-cysteine action on the contractile activity of ring segments from rat PA selective inhibitor of NKCC bumetanide was used. Pretreatment with bumetanide (100 μM) of vascular segments with intact endothelium for 15 min did not eliminate the constrictive effect of low concentrations of L-cysteine. In addition, relaxing action of L-cysteine (1 mM — 10 mM) was reversed in the constrictive. Bumetanide changed relaxing action of L-cysteine (10 μM — 10 mM) to constrictive in endothelium-denuded segments of PA (Fig. 2).

The effect of L-cysteine on contractile activity of smooth muscles of pulmonary artery from hypertensive rats induced by highpotassium Krebs solution

Addition of L-cysteine (from 10 μM to 10 mM) caused dose-dependent increase in the MT of vascular segments of PA from SHR rats with intact endothelium precontracted with 30 mM KCl. But in endothelium-denuded segments, in contrast, L-cysteine (10 μM-10 mM) induced relaxation (Fig. 3).

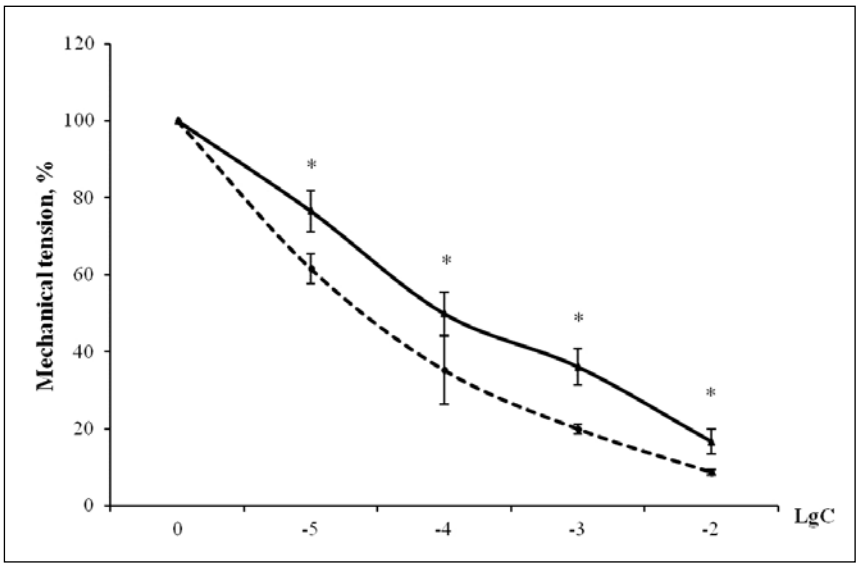
Pretreatment of intact smooth muscle segments with bumetanide (100 μM) reversed the constrictive

Figures. 3. L-cysteine influence on the mechanical tension of smooth muscles of the pulmonary artery of SHR rats precontracted with highpotassium Krebs solution



Note: Y-axis — mechanical tension (%); X-axis — the decimal logarithm of the concentration of L-cysteine; dotted line — endothelium-denuded segments; solid line — segments with intact endothelium; * — significant

Figures. 4. L-cysteine influence on the mechanical tension of the smooth muscles of the pulmonary artery of SHR rats, precontracted with highpotassium Krebs solution in the presence of bumetanide



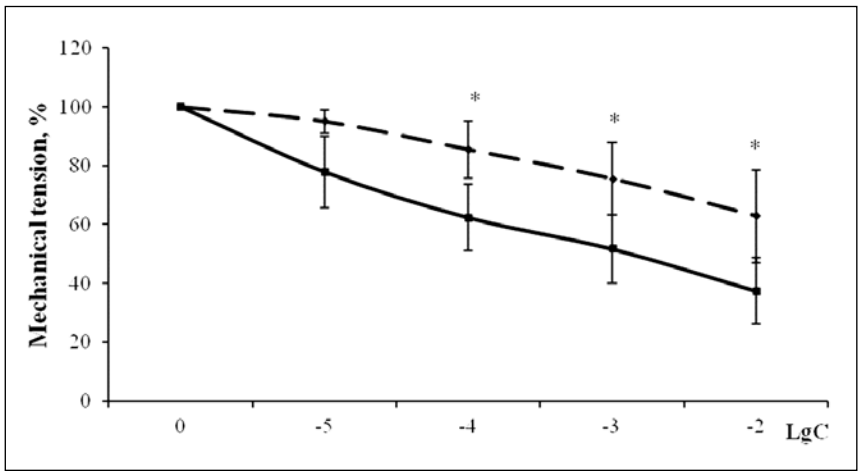
Note: Y-axis — mechanical tension (%); X-axis — the decimal logarithm of the concentration of L-cysteine; dotted line — endothelium-denuded segments; solid line — segments with intact endothelium; * — significant differences between segments with intact and denuded endothelium ($p < 0.05$).

action of the H2S donor, but enhanced its relaxing effect on endothelium-denuded segments (Fig. 4).

L-cysteine influence on the contractile activity of pulmonary artery smooth muscle of hypertensive rats, precontracted with phenylephrine

The effect of the phenylephrine (PE, 10 μM), an agonist of alpha1-adrenergic receptor, on vascular SMC led to the development of a contractile response, the amplitude of which was comparable to highpotassium contraction. In PE-induced contraction, the addition of L-cysteine (10 μM-10 mM) caused the relaxing effect on the endothelium-de-

Figures. 5. L-cysteine influence on the mechanical tension of the smooth muscles of the pulmonary artery of SHR rats, precontracted with PE (10 μM) in the presence of bumetanide



Note: Y-axis — mechanical tension (%); X-axis — the decimal logarithm of the concentration of L-cysteine; dotted line — L-cysteine influence on PE (10 μM) -induced contraction; solid line — the same but under the action of bumetanide (100 μM); * — significant differences between segments with intact and denuded endothelium ($p < 0.05$).

nuded segments both in the presence of bumetanide and without it (Fig. 5). At the same time, the greatest relaxation of the vascular segments occurred in the presence of the NKCC blocker.

Discussion

In our studies, a multidirectional action of the H2S donor on the MT of vascular segments of rat PA was determined and this action depended on the concentration of L-cysteine, the presence of the endothelium, and the nature of precontraction.

In experiments with normotensive rats, the sulfur-containing amino acid L-cysteine had only relaxing effect on endothelium-denuded segments of rat PA precontracted with 30 mM KCl at the whole concentration range (10 μM-10 mM). At the same time, in vascular segments with preserved endothelium low concentrations of L-cysteine (10 and 100 μM) had a constrictive effect on the smooth muscle segments, and relaxing at a higher concentration of the donor of H2S. It is possible that L-cysteine, affecting the endothelium layer, reduces the production of relaxing factors and / or releases constrictor factors [15, 17].

The blocker of NKCC bumetanide (100 μM) did not eliminate the constrictive effect of small doses, but reversed the effect of high concentrations of L-cysteine on SMC of PA.

The observed effect of the donor of H2S on the contractile activity of smooth muscles under the action of the NKCC blocker differs from the effect of H2S on the vascular wall of the rat aorta, where adding a donor at concentrations above 500 μM caused smooth muscle relaxation not eliminated by bumetanide [2]. This suggests that in pulmonary circulation the mechanisms of regulation of smooth muscles MT mediated by NKCC are differ significantly from those in the large circulatory system.

In studies with SHR rats, segments with intact endothelium responsed to the L-cysteine with a contraction, and in segments with both preserved and deleted endothelium endogenous donor of hydrogen sulfide caused relaxation in the presence of the NKCC blocker.

E. Akar in his experiments on rat aorta showed that inhibition of NKCC with bumetanide reduces the amplitude of contractions of aortic segments induced by phenylephrine and highpotassium Krebs solution [15]. In our experiments on hypertensive rats, the amplitude of contractions of smooth muscle segments induced by phenylephrine was lower in the presence of an inhibitor of NKCC. There are data on the involvement of transport in the pathogenesis of essential hypertension. Thus, genetically modified mice with a defective gene of Na+, K+,2Cl- cotransporter, (NKCC 1 – /– knockout mice) are characterized by a lowered blood pressure and decreased tone and the magnitude of contractile responses of vascular smooth muscle segments precontracted with phenylephrine [16]. Apparently, changes in the functioning of intracellular signaling systems

of smooth muscle cells from PA of SHR rats result in increased sensitivity to the action of intracellular H2S, and possibly increased NKCC activity not only in the SMC, but also in the endothelial cells, that changes the constrictive action of high concentrations of hydrogen sulfide on vasodilator after the pretreatment with bumetanide. Thus, arterial hypertension in SHR rats develops as a result of a violation of the function of 1–6 genes involved in the regulation of vascular tone. According to one of the hypotheses of increased blood pressure in SHR rats, there are hereditary defects of calcium and sodium ion channels located in the membrane of SMC of resistive arteries. There is an increased activity of the hydropyridine channels of the outer cell membrane and ryanodine receptors of the endoplasmic reticulum, that leads to an increase in the level of unbound intracellular calcium. This leads to an increase in the tone of the vessels and an increase in their sensitivity to pressor stimuli. The experimental data show a violation of the interaction between the Ca2 + input through the L-type channels and the release of it from the sarcoplasmic reticulum. In addition, SHR rats are characterized by a hereditarily induced decrease in endothelial NO production [17].

The revealed differences in the influence of the hydrogen sulfide donor on the mechanical tension of the vascular segments can be related to the different nature of these contractions. The increase in the mechanical tension of smooth muscle cells, caused by depolarization of the membrane with highpotassium Krebs solution is due to the operation of calcium-calmodulin (CAMK) branch of the calcium signaling system. While the induction and maintenance of the contraction caused by PE involves both CAMK and protein kinase C branches of the calcium signaling system [12]. This assumption is more important for normotensive rats. Possible factors of differences in the effects of L-cysteine on the mechanical tension of smooth muscles in hypertensive rats are more difficult to determine. Along with this, studies of the processes of excitation and contraction coupling in smooth muscle cells indicate the presence of additional factors affecting the final functional response of smooth muscle. One of these factors is Na+, K+,2Cl- cotransport.

Conclusion

Despite on some successes achieved in studying the mechanisms of regulation of vascular smooth muscle functions, many questions require further study. Obtained data can help to understand the effects of endogenous H2S and the role of NKCC, and contribute to development of approaches for the pharmacological correction of the cardiovascular system pathological conditions.

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Conflict of interest
The authors declare no conflict of interest.

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