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THE ROLF. OF POTASSIUM CHANNELS IN THE EFFECT OF HYDROGEN SULFIDE ON THE SPONTANEOUS CONTRACTILE ACTIVITY OF RAT JEJUNUM

C.I. Sabirullina, M.U. Shafigullin, N.N. Khaertdinov, G.F. Sitdikova

Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kremlevskaya st., 18, Kazan, 420008, Russia

Hydrogen sulfide (H_2S) a gas, well-known for its toxic effects associated with impaired oxidative phosphorylation in the cell. H_2S endogenously produced in mammalian tissues and plays a major role in physiological and pathological processes. Traditionally known as a toxic gas it is also an important gaseous messenger [1,2]. Like other gasotransmitters, H_2S has a relaxing effect on the smooth muscle in the cardiovascular system, gastrointestinal tract, reproductive system [3,4]. In a number of studies the relaxing effect of H_2S in various parts of the gastrointestinal tract in different animal species was found [5-7]. However, it was also shown that H_2S could produce different effects on smooth muscle motility dependent on concentration [8].

The aim of our study was to determine the effect of H_2S on contractile activity in smooth muscle of rat intestine and identify the role of voltage-dependent and ATP-dependent potassium channels in the effects of H_2S.

Materials and methods

Experiments were performed on isolated segments of jejunum from Rattus norvegicus on the equipment of Biopac Systems, Inc. (USA). Animals were anesthetized by using 5% isoflurane (Abbott Laboratories, North Chicago, IL. USA). The abdomen was opened and the mid jejunum was removed and placed in oxygenated Krebs solution. The 7 mm long muscle strip is suspended vertically in a 20-ml tissue chamber, the lower end fixed to the rubber block and the other end was connected to a force transducer (TSD125C, Biopac Systems, Inc USA). During the experiment, the muscle strip was continuously washed with 37°C Krebs solution and aerated with 95% oxygen and 5% carbon dioxide.

Registration and analysis of the parameters of the muscle contraction was performed using the program AcqKnowledge 4.1. Amplitude (force in grams), frequency and the baseline tone were analyzed. The baseline tone was accessed using values of maximum relaxation between contractions.

Sodium hydrosulfide (NaHS) was used as H_2S donor. H_2S in an aqueous solution dissociates to ions (Na^+) and anion hydrosulphide (HS^-), which reacts with protons (H^+) to form H_2S [4]. In aqueous solution, approximately one third of the gas in the nondissociated form. It is known that at pH 7.4 and a temperature of 37°C, only 18% of NaHS is presented in the form of a gas - H_2S [9]. The following drugs were used: L-cysteine, β-cyano-L-alanine, 4-aminopyridine, glibenclamide, diazoxide (Sigma, USA). Substances, insoluble in water, were dissolved in dimethylsulfoxide (DMSO), which at the concentration used (up to 0.01%) had no effect on spontaneous contractile activity of the jejunum.

Results and discussion

In control the jejunum segment spontaneously contracted with average frequency 0,45 ± 0,01 Hz and amplitude - 0,57 ± 0,5 *g* ($n = 20$).

To analyze the mechanisms of NaHS action according our previous data we used concentration - 200 μM, which reduced amplitude of contractions to 19,6 ± 2,8% ($n = 20$, $p < 0,05$), baseline tone to 87,7 ± 2,5% ($n = 20$, $p < 0,05$), frequency to 90,08 ± 2,17% ($n = 21$, $p < 0,05$) compare to control.

Endogenous H_2S is produced from L-cysteine by two enzymes, cystathionine (β) synthase and (CSB) and cystathionine (γ) lyase (CSE). For detection of endogenous synthesis of hydrogen sulfide in the intestinal cells, we used its endogenous donor - L-cysteine and inhibitor of CSE - β-cyano-L-alanine.

L-cysteine in the cumulative addition of 10, 50, 100, 200 μM resulted in dose-dependent decrease of amplitude (after 200 μM) to 80.5 ± 3% (n − 8, p <0,05), baseline tone - 74.13 ± 7% (и = 6, p <0.05) frequency - 97.11 ± 1.7% (n −5, p> 0,05).

β-cyano-L-alanine at 200 μM showed not significant changes in the parameters of spontaneous activity: to 30 minutes of application amplitude of spontaneous contraction was 114.7 ± 9.3% (n =4, p> 0,05), frequency - 101.7 ± 0.4% (и − 4, p> 0,05), baseline tone - 97.48 ± 5.2% (n = 4, p> 0,05). These results show that the substrate of synthesis of the H_2S — L-cysteine reduced the spontaneous contraction parameters as H2S donor, probably due to endogenous synthesis of gas. Inhibitor of enzyme that catalyzing the synthesis of H_2S - β-cyano-L-alanine resulted in no significant effect on the contractile activity, which can probably be attributed to the presence of other enzymes of the gas synthesis in the tissue or insufficiently concentration of inhibitor.

It is known that K^+-channels play a key role in maintaining of the tone of smooth muscles, are involved in the control of gastrointestinal smooth muscle contraction, affecting the membrane potential, slow waves of depolarization, duration of the action potential (Horowitz, 1999). K -channels may be the targets of the effects of hydrogen sulfide.

We investigated the role of voltage-dependent and ATP-dependent potassium channels in our experiments. Previously, we studied the role of Ca^{2+}-activated and voltage-dependent K -channels, in which we used nonspecific blocker of these channels tetraethylammonium (TEA) and it was shown that after TEA application the effects of NaHS on the amplitude, baseline tone and frequency were the same as in control.

Inhibitor of voltage-dependent potassium channels 4-aminopyridine (4-AP) at concentrations 200 цМ increased the amplitude to 121,4 ± 4,8 % (n = 7, p <0.05) relative to a control, wherein the baseline tone and frequency were not changed. In the background 4-AP effect of NaHS on the amplitude baseline tone fully preserved, but the frequency of contractions increased - 103,3 ± 4,9 (n=5, p<0.05).

It has been shown, that in vascular smooth muscle cells the effects of NaHS were mediated through activation of $K_{(ATP)}$-channels. In our study to determine the role of $K_{(ATP)}$-channels we evaluated the effects of hydrogen sulfide donor after inhibition or activation of these channels.

Inhibitor of $K_{(ATP)}$-channels by glibenclamide at the concentration 50 цМ decreased the amplitude of contractions to 63,84 ± 5,93% (n = 10, p <0,05)and frequency to 90,73 ± 1,91% (n = 10, p<0.05), baseline tone not significantly changed (103,52 ± 3,07%) (л = 10, p> 0.05). On the

background of the glibenclamide, effect of NaHS on the amplitude and frequency of contractions completely preserved (22,60 ± 4,39% and 86,88 ± 1,99%, respectively), while the baseline tone significantly increased to 111,65 ± 3,83% (n − 10, p <0.05).

$K_{(ATP)}$-channels were activated using diazoxide (100 μM). Application of diazoxide caused the decrease of the force of contraction to 65 ± 5,1% (n = 5, p <0.05) from the control values without changing the frequency of spontaneous contractions and baseline tone. Adding NaHS on the background of the diazoxide decreased the amplitude and frequency of the same abbreviations as in the control, but the effect to baseline tone not manifested.

The results of our study showed that donor H_2S - NaHS endogenously synthesized in the intestinal cells and causes a reduction of the spontaneous contractile activity of rat jejunum segment, reducing the amplitude, frequency and the base tone. The findings suggest that the effects of NaHS on the amplitude of the contractions arc not related to its effect on the voltage-dependent potassium and $K_{(ATP)}$-channels, effects to baseline tone mediated by $K_{(ATP)}$-channels of smooth muscle cells, and a decrease in the frequency - by voltage-gated potassium channels of neuronal cells or muscle cells of intestine.

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