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Interplay Between Hydrogen Sulfide and Adrenergic and Muscarinic Receptors in the Mouse Atrium

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Abstract Hydrogen sulfide (H_2S) is synthesized endogenously, and it has a negative inotropic effect on myocardium of different animal species. Interaction between H_2S and adrenergic and muscarinic receptors in regulation of mouse atrium contractile function was investigated in this study. Sodium hydrosulfide (NaHS, 300 μM), the H_2S donor, decreased the contraction force of atrium. NaHS did not affect the positive inotropic effect of β-adrenoceptors (β-AR) activation by isoproterenol (ISO, 1 μM). The effect of the H_2S donor under β-AR stimulation showed no differences comparison to control values. The agonist of muscarinic receptors carbachol (1 μM) induced a negative inotropic effect and partially prevented the reduction of cardiac muscle contractility by NaHS. Moreover, the effect of carbachol was more pronounced after preliminary application of NaHS. At the same time, after inhibition of β-AR (propranolol, 1 μM) or muscarinic receptors (atropine, 1 μM), negative inotropic effect of NaHS was the same as in control conditions. These results suggest that the H_2S effects are mediated by intracellular signaling pathways activated by muscarinic receptors.

Keywords Hydrogen sulfide (H_2S) · Mouse atrium · β-Adrenoceptors · Muscarinic receptors

1 Introduction

Hydrogen sulfide (H_2S) as well as nitric oxide (NO) and carbon monoxide (CO) belongs to the gasotransmitters family. H_2S has important physiological effects on the gastrointestinal tract, nervous, and cardiovascular systems [1]. In the cardiovascular system, H_2S is synthesized endogenously from L-cysteine by cystathionine-γ-lyase (CSE) and 3-mercaptosulfotransferase (3-MST) [2] and induces negative chronotropic and negative inotropic effects in myocardium of different species [3–7]. Besides, H_2S has a cardio-protective role in various models of diseases, namely ischemia-reperfusion injury and chronic heart failure [2]. Cellular mechanisms of H_2S action include the activation of ATP-dependent K^+ channels, the inhibition of voltage-gated L-type Ca^{2+} channels, the activation of protein kinase C, the elevation of NO production, and the inhibition of phosphodiesterase (PDE) [2, 4–6, 8]. It was also suggested that H_2S can negatively modulate β-adrenoreceptor (β-AR) function via inhibition of the adenylyl cyclase activity [3]. Besides, it was shown that the suppression of H_2S synthesis downregulated the acetylcholine-induced vasorelaxation in mammalian vessels [8]. The aim of our study was to analyze the role of β-AR and acetylcholine receptors (AChR) in the inotropic effect of H_2S in the mouse atria.

2 Materials and Methods

Experiments were performed on laboratory white wild-type adult mouse. The work has been carried out in accordance with the EU Directive 2010/63/EU for animal experiments and approved by Local Ethical committee of Kazan Federal University (protocol no. 8 from 5.05.2015). Mice were decapitated under deep isoflurane anesthesia and dissection was performed. Isolated atria were used in the

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experiments. The samples were fixed in a 20-ml bath and perfused with Krebs solution of the following compositions (in mM): NaCl—137, KCl—5, $CaCl_2$—2, $MgSO_4$—1, $NaHCO_3$—11, glucose—11, ascorbic acid—0.3, NaH_2PO_4—1 ($t = 23$ °C); the solution was saturated with carbogen (95 % O_2, 5 % CO_2), pH 7.2–7.4 ($t = 23$ °C). Ascorbic acid was added to the working solution in order to increase the viability of the atria tissue. It is known that ascorbic acid may affect Ca(v)3.2 T-type Ca^{2+} channels [9]. However, in atria tissue, these channels involved mainly in pacemaker activity which was not the object of our study; moreover, in our preparation, the sinoatrial node was removed and preparation was stimulated externally. In control experiments, effects of NaHS, ISO, and carbachol on contractile response were not dependent on the presence of ascorbic acid in the Krebs solution.

The atrium was stimulated with two platinum electrodes (ESL-2 stimulator, Russia) with 0.1 Hz frequency, 40 mV amplitude, and 5 ms duration. Lower edge of the tissue was fixed to the bath base. Upper edge was linked to isometric tense transducer (MLT 050/D or TSD 125C, Biopac Systems Inc., USA) via a metal lath. The signals were recorded and analyzed using Elf (Zaharov A.V.) and Origin software. Contraction force was initially determined in volts and then the data was converted to grams. Sodium hydrogen sulfide (NaHS) was used as the H_2S donor. The following substances were also used: isoproterenol (ISO), carbamylchlorine (carbachol), propranolol, and atropine (Sigma, USA). The results of the experiments are shown as mean ± SE of mean, where n is the number of experiments, with statistical significance considered by the Student's t test. The significance level was defined at $p < 0.05$.

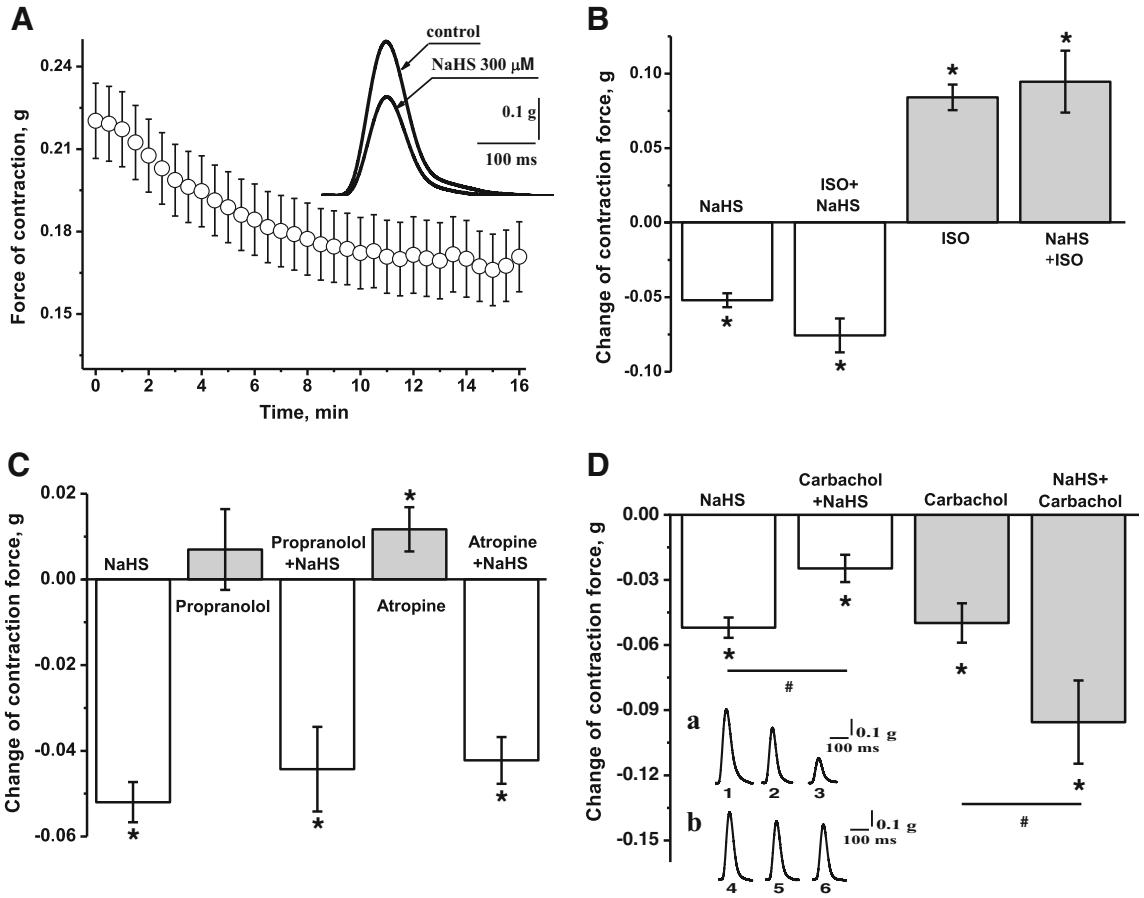

Fig. 1 The role of adrenergic and muscarinic receptors in the effects of sodium hydrosulfide (NaHS 300 μM) on the contractility of mouse atrium. **a** The dynamics of contraction force during bath application of NaHS 300 μM. On the top, the original recordings of contraction in control and by the sixteenth minute of NaHS exposure are shown. **b** The changes of contraction forces of mouse atrium after application of NaHS 300 μM in control and NaHS after preliminary exposure of atrium to isoproterenol (ISO, 1 μM); ISO in control and after preliminary exposure of atrium to NaHS. *$p < 0.05$—compare to control. **c** The changes of contraction forces of mouse atrium after application of NaHS 300 μM in control and NaHS after preliminary exposure of atrium to propranolol (1 μM) and atropine (1 μM). *$p < 0.05$—compare to control. **d** The change of contraction forces of mouse atrium after application of NaHS 300 μM in control and NaHS after preliminary exposure of atrium to carbachol, 1 μM; carbachol in control and after preliminary exposure of atrium to NaHS. *$p < 0.05$—compare to control; #$p < 0.05$ compare to the effect of NaHS or carbachol. In the *inset*, original signals of mouse atrium contractions are shown as follows: *a* in control (*1*), in the presence of NaHS (300 μM) (*2*), and subsequent application of carbachol (1 μM) (*3*); *b* in control (*4*), in the presence of carbachol (*5*), and subsequent application of NaHS (*6*)

3 Results and Discussion

Initially, the effect of exogenous H_2S on myocardial contractility was tested. Application of NaHS in concentration of 300 μM significantly decreased contraction force from 0.220 ± 0.014 g to 0.170 ± 0.014 g ($n = 46$, $p < 0.05$) (Fig. 1a). These results correspond to our previously obtained data [5]. It was shown that H_2S regulated cAMP levels, and it can negatively modulate β-AR function via inhibiting the adenylyl cyclase activity [3, 7]. β-ARs agonist ISO in our study enhanced contraction force of atrial myocardium by 0.084 ± 0.008 g ($n = 23$, $p < 0.05$). Subsequent application of NaHS (300 μM) induced the decrease of contraction force to the same extend as in control (0.076 ± 0.011 g, $n = 11$, $p < 0.05$) (Fig. 1b). The positive inotropic effect of ISO did not depend on the preliminary incubation of atrium in NaHS—the contraction force increased by 0.094 ± 0.020 g ($n = 10$, $p < 0.05$) from the initial level (Fig. 1b). The inhibitor of β-ARs propranolol (1 μM) did not change significantly the contraction force ($n = 10$, $p > 0.05$) and negative inotropic effect of NaHS was the same as in control conditions (0.044 ± 0.009 g, $n = 10$, $p < 0.05$) (Fig. 1c).

To analyze the interaction between H_2S and muscarinic AChR, carbachol was used in concentration of 1 μM. Carbachol reduced contraction force of atrium by 0.049 ± 0.009 g ($n = 13$, $p < 0.05$) from the initial level and partially prevented the negative inotropic effect of NaHS (0.025 ± 0.006 g, $n = 13$, $p < 0.05$) (Fig. 1d *b*). At the same time, we observed a significant increase of carbachol effect on the atrial contractility in the presence of NaHS (0.085 ± 0.018 g; n = 15, p <0.05) (Fig. 1d *b*). Atropine (1 μM) the inhibitor of muscarinic AChR increased the contraction force by 0.012 ± 0.005 g ($n = 5$, $p < 0.05$) and subsequent application of NaHS induced the decrease of contraction force to the same extend as in control (0.042 ± 0.005 g; $n = 10$, $p < 0.05$), which proposed that NaHS did not affect directly on the activity of muscarinic AChR (Fig. 1c).

This data suggest that intracellular signaling pathways activated by muscarinic AChR are involved in the negative inotropic effect of H_2S in the atrial myocardium of mouse. These pathways may include the muscarinic-2 AChR induced NO production and subsequent elevation of cGMP levels, stimulation of PDE2 and reduced cAMP synthesis [10, 11]. Moreover, it was shown that suppression of CSE induces the reduction of ACh effects in vascular tissue, whereas H_2S donor potentiates ACh effects. It was proposed that H_2S maintains a tonic inhibitory effect of PDE5 thereby delaying the degradation of cGMP [8] which can explain H_2S-dependent potentiation of carbacholine effect obtained in our study.

4 Conclusions

Taken together, the data suggest that there is no interaction between H_2S and ISO mediated effects in mouse atria under physiological conditions. However, activation of AChR reduced the negative inotropic effect of NaHS and vice versa; NaHS upregulated the negative inotropic effect of carbachol. The obtained data suggests that the negative inotropic effect of H_2S can be associated with the same intracellular signaling pathways which are triggered by the activation of muscarinic receptors. That regulation may occur due to changes in NO and cGMP levels which are involved in the negative inotropic effect of muscarinic receptors activation.

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