PHYSIOLOGY

Role of Calcium and Potassium Channels in Effects of Hydrogen Sulfide on Frog Myocardial Contractility

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The effects of sodium hydrosulfide NaHS, a donor of hydrogen sulfide H_2S, on the force of muscle contraction were examined on isolated myocardial strips from frog ventricles. NaHS decreased the amplitude of muscle contractions in a dose-dependent manner under normal conditions and during inhibition of Ca channels with nifedipine. In contrast, under conditions of blockade of ATP-dependent potassium channels with glibenclamide, NaHS exerted a positive inotropic effect from the first minute of application. Neither blockade, nor activation of ATP-dependent K-channels with glibenclamide modulated the negative inotropic effect of NaHS. Inhibition of K-channels with tetraethylammonium (TEA) (3, 5, 10 mM) or 4-aminopyridine increased the amplitude of myocardial contractions. Preliminary application of 4-aminopyridine or TEA (3 mM) did not eliminate NaHS-induced negative inotropic effect, although higher TEA concentrations (5 or 10 mM) prevented it. The data indicate that the targets of H_2S in frog myocardium are ATP-dependent, Ca-activated, and voltage-dependent K-channels.

Key Words: *hydrogen sulfide*; *frog myocardium; calcium channels; ATP-dependent K-channels; voltage-dependent K-channels; Ca-activated K-channels*

Hydrogen sulfide (H_2S) is an endogenous gasotransmitter, which recently became known as an agent regulating the cardiovascular system along with NO and CO [3-5,8]. In various tissues, H_2S is synthesized from L-cysteine by cystathionine γ-lyase, cystathionine β-synthase, and 3-mercapto-sulfurtransferase [4,5]. Exogenous and endogenous H_2S affects the vascular system in virtually all classes of vertebrates attesting to ancestral phylogenetic origin of this agent as a gasotransmitter and to the universal character of its effects [10]. H_2S is involved in the regulation of various systems in living organism including central and peripheral nervous systems, as well as skeletal and smooth muscles [2-4,6,8,11]. There are data on cardioprotective role of H_2S manifested in alleviation of ischemia/reperfusion-induced damage to the myocardium in *in vitro* and *in vivo* experiments [5,7]. Some studies showed that H_2S shortens the length of action potential (AP) and reduces cardiac contractility in warm-blooded animals [1,7,12]. However, the data on cardiac control by H_2S gasotransmitter are scarce and fragmentary, and its molecular targets are still to be identified.

Our aim was to examine the effects of H_2S on contractility of *Rana ridibunda* myocardial strips and to reveal the role of calcium and potassium channels in the mechanism of its action.

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MATERIALS AND METHODS

The experiments with recording of contractility of the myocardial strips isolated from *Rana ridibunda* frogs were carried out during the autumn-winter period with a Power Lab workstation and a MLT 050/D force transducer (ADInstruments). Myocardial strips (4-6-mm long and 1 mm in diameter) were isolated from cardiac ventricles and placed into a 20-ml bath filed with Ringer solution for the cold-blooded animals (in mM): 118.0 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 10.0 Trizma (20°C, pH 7.3-7.4). The strips were stimulated via two silver electrodes with the rectangular pulses (10 V amplitude, 5 msec duration) applied at the rate of 0.1 Hz. The responses of the strips were normalized in percentage to the initial value. The data were processed statistically using Chart 5 software and Student's *t* test.

Sodium hydrosulfide NaHS was used as an H_2S donor, because this substance dissociates in aqueous solution to Na^+ and HS^-, thereafter HS^- binds H^+ to produce H_2S. In neutral solution, a third of sodium hydrosulfide is converted into H_2S gas, while the rest ($^2/_3$) assumes the form of HS^- [10]. This study used the following blockers and activators of the ionic channels: nifedipine, glibenclamide, Minoxidil, tetraethylammonium (TEA), and 4-aminopyridine (4-AP). The agents insoluble in water were dissolved in DMSO. The concentration of this solvent in the applied solutions was no more than 0.1%; at this concentration, DMSO *per se* produced no effect on myocardial contraction force. All reagents were from Sigma.

RESULTS

Application of NaHS (H_2S donor) at 100 μM decreased the amplitude of contraction of the myocardial strips. In control, the mean force of contraction was 0.82±0.11 g, while after 20 min it dropped to 0.58± 0.11 g or 68±4% of the initial value (n=16, p<0.05, Fig. 1, *a*). The decrease of contraction amplitude was accompanied by a decrease in contraction and relaxation rates to 50±4 and 53±10%, respectively (n=5, p<0.05). The effect of NaHS was reversible and dose-dependent with EC_{50}=38.4 μM (Fig. 1, *b*). Thus, H_2S produced a negative inotropic effect on the frog heart. Similar effect of this gas was observed in the isolated hearts of the warm-blooded animals [7,12]. In further experiments we used NaHS (100 μM) to analyze its effect under control conditions and in combination with various blockers and activators of different types of ionic channels.

Effect of NaHS under conditions of inhibition of voltage-dependent Ca-channels. Calcium ions play the key role in the regulation of myocardial contractility. Generally, depolarization of cardiomyocytic membrane leads to opening of voltage-dependent L-type Ca-channels, Ca^{2+} entry, release of Ca^{2+} from intracellular stores (via ryanodine receptors), and initiation of muscle contraction. However, mobilization of intracellular calcium in frog ventricular cardiomyocytes plays a minor role in excitation-contraction coupling because of poor developed sarcoplasmic reticulum, so the plasmalemmal Ca-channels are the main sources of intracellular calcium in these cells [14]. We hypothesized that the negative inotropic effect of NaHS results

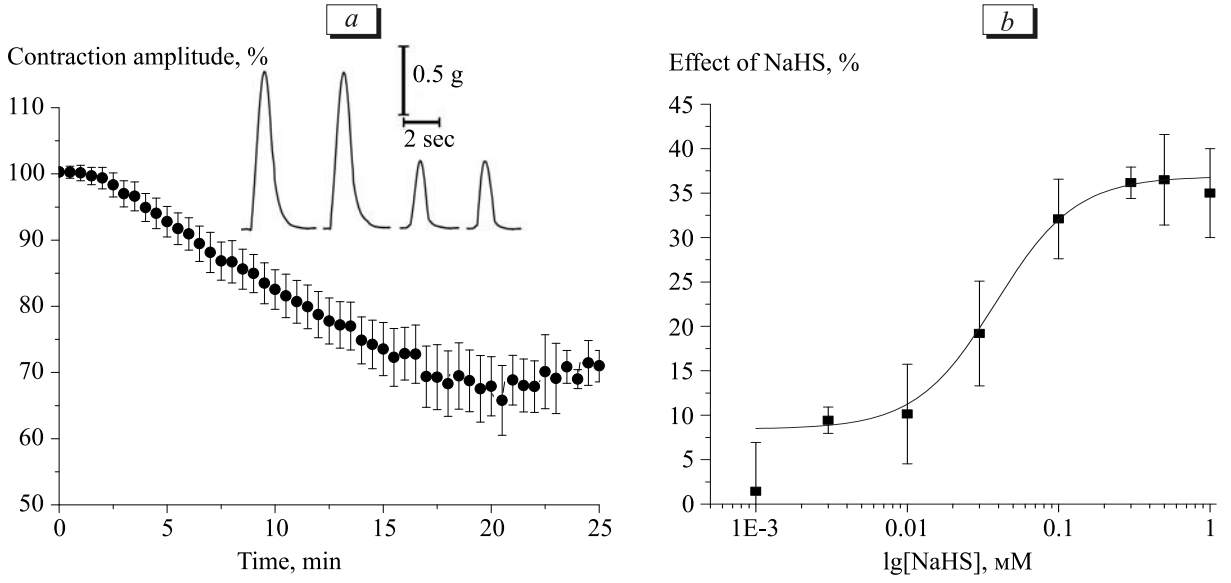

Fig. 1. Negative inotropic effect of NaHS in frog myocardial ventricle. *a*) dynamics of contraction amplitude of a myocardial strip under the action of NaHS (100 μM). The insert shows mechanograms in the control and on postapplication minute 20 (NaHS); *b*) dose-dependence of NaHS effect on contractile amplitude.

from inhibition of L-type Ca-channels; similar action of NaHS was observed in the rat cardiomyocytes [12]. As expected, inhibition of Ca-channels with nifedipine (10 μM) decreased the amplitude of contractions to 75±5% (n=5, p<0.05, Fig. 2). However, combined application of nifedipine with of NaHS produced virtually the same effect by decreasing the contraction amplitude to 72±2% (n=5, p<0.05, Fig. 2). Hence, the negative inotropic effect of NaHS in the frog heart is not related to inhibition of the inward calcium current.

Effect of NaHS under conditions of inhibition or activation of ATP-dependent K-channels (K(ATP) channels). K(ATP) channels are widely presented in the myocardium and their activation is an important endogenous mechanism of cardioprotection during ischemia/reperfusion injury and hypoxia [7]. In rat myocardium, NaHS shortened AP, and this effect was partially antagonized by glibenclamide, an inhibitor to K(ATP) channels [1]. In our experiments, glibenclamide (50 μM) produced no effect on the force of myocardial strip contraction (n=15) attesting to a negligible role of K(ATP) channels in maintaining the resting potential of cardiomyocytes. However, when NAHS was added to the glibenclamide-treated cells with blocked K(ATP) channels, it significantly increased the contraction amplitude on minute 3 to 111±2%, thereafter (on minute 20) it decreased this amplitude to 76±2% relatively to the initial value (n=13, p<0.05, Fig. 3, *a*). The initial increase in contraction amplitude can attest to contribution of activated K(ATP) channels to the development of the negative inotropic effect induced by NaHS; additionally, the positive inotropic effect of this substance could be manifested more strongly under conditions of K(ATP) channel blockade . Indeed, activation of K(ATP) channels with Minoxidil (100 μM) decreased the force of myocardial strip contraction to 51±7% (n=5, p<0.05), which is probably related to shortening of AP and inhibition of calcium entry through voltage-dependent Ca-channels [13]. The negative inotropic effect of NaHS preserved in the Minoxidil-treated cells: NaHS decreased the

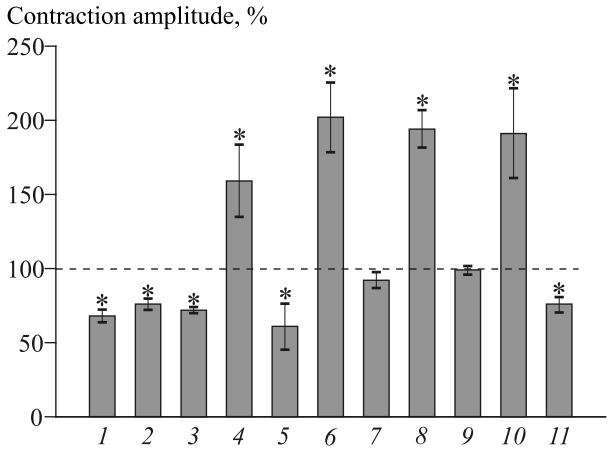

Fig. 2. Effect of NaHS on contraction amplitude of frog myocardial strips under conditions of inhibition of various types of K(Ca) channels with TEA, 4-AP, and nifedipine. During the analysis of the effects of NaHS, nifedipine, TEA, and 4-AP, the contraction amplitude in normal Ringer solution was taken as 100%; in the experiments with combined application of NaHS and nifedipine, TEA, or 4-AP, the contraction amplitudes recorded during individual application of, respectively, nifedipine, TEA, or 4-AP were taken as 100%. *p<0.05 relatively to contraction amplitude taken for 100%. *1*) NaHS; *2*) nifedipine (10 μM); *3*) nifedipine+NaHS; *4*) TEA (3 mM); *5*) TEA (3 mM)+NaHS; *6*) TEA (5 mM); *7*) TEA (5 mM)+NaHS; *8*) TEA (10 mM); *9*) TEA (10 mM)+NaHS; *10*) 4-AP (5 mM); *11*) 4-AP+NaHS.

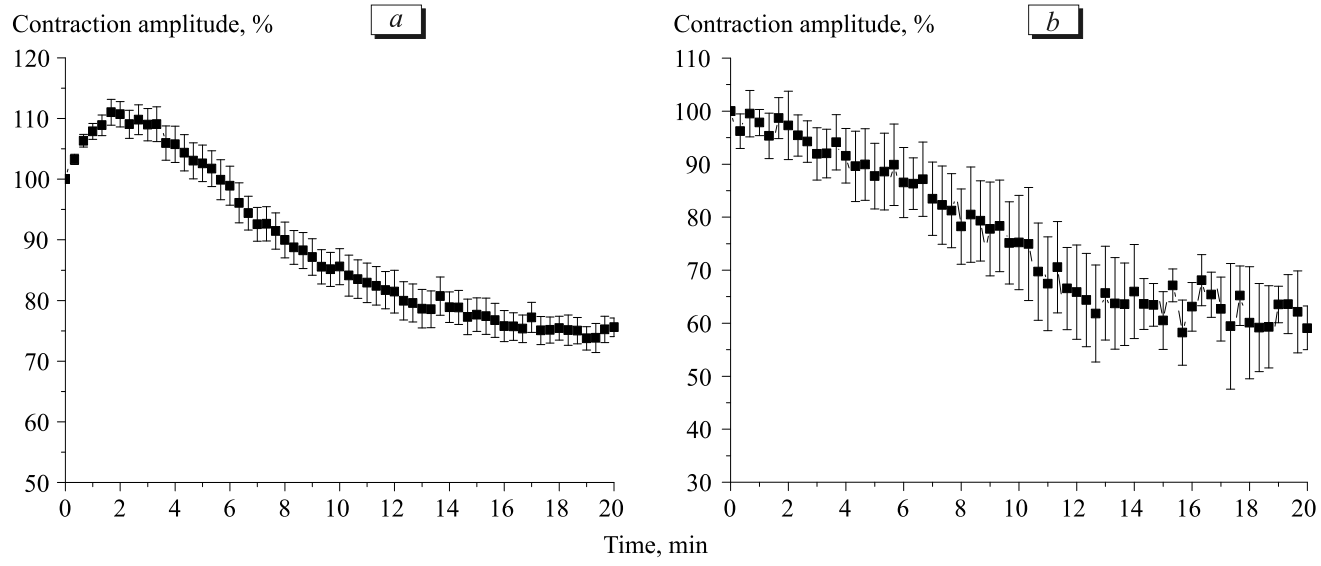

Fig. 3. The role of K(ATP) channels in the effects of NaHS on contractility of myocardial strips. Effect of NaHS (100 μM) on contraction amplitude of the myocardial strips under the action of glibenclamide (50 μM), a K(ATP) inhibitor (*a*) or during activation of K(ATP) channels with 100 μM Minoxidil (*b*).

contractile amplitude to 61±6% (n=5, p<0.05, Fig. 3, *b*). These data attest to the existence of additional targets for NaHS in frog myocardium in parallel to K(ATP) channels.

Effect of NaHS under conditions of inhibition of voltage-dependent and Ca-activated K channels (K(Ca) channels). The entire family of potassium channels is involved in shaping membrane repolarization in cardiomyocytes during various AP phases. Among these channels, the following are the most significant: two types of fast and slow activating and inactivating transient outward K-channels (Ito,f and Ito,s) and several types of delayed rectification potassium channels including IKr (rapid), IKs (slow), IKur (ultrarapid), *etc* [9,13]. In addition, K(Ca) channels recently found in the heart of warm-blooded animals can be involved in the control of AP duration and myocardial contractility [15]. There are various subtypes of K-channels differing by kinetic parameters, sensitivity to potassium channel blockers, and expression in the heart of various species and in different subdivisions of the heart [9]. In this study, TEA (an unspecific blocker of voltage-dependent and Ca-activated potassium channels) dose-dependently increased contraction force to 159±24% (n=5, p<0.05), 202±23% (n=7, p<0.05), and 194±13% (n=6, p<0.05) at concentrations of 3, 5, and 10 mM, respectively (Fig. 2). Combined application of NaHS and TEA (3 mM) decreased the contraction force to 61±16% (n=5, p<0.05), which did not differ from the individual (control) effect of NaHS (Fig. 2). However, higher concentrations of TEA (5 and 10 mM) completely prevented the negative inotropic effect of NaHS (Fig. 2). TEA is known as a unspecific blocker of potassium permeability; it dose-dependently blocks various types of voltage-dependent and Ca-activated K-channels in the heart and other excitable tissues [9,13].

4-AP is usually used for evaluation of the role of voltage-dependent K-channels in various excitable tissues. In the heart, 4-AP blocks fast transient outward K-channels (Ito) and delayed rectification K-channels (Ikur) [9,13]. In this study, 4-AP (5 mM) increased contraction force to 191±30% (n=7, p<0.05). When NaHS was applied in combination with 4-AP, it decreased contraction force to 76±5% (n=7, p<0.05, Fig. 2). It can be hypothesized that these types of ionic currents (Ito and IKur) are not involved in the effect of NaHS. Logically, the targets for H_2S in frog heart are potassium channels sensitive to blocking action of TEA and insensitive to 4-AP. These channels can belong to some subtypes of voltage-dependent K-channels responsible for currents shaping the late phase of AP repolarization in the myocardium, such as IKs, IKr, and IKss (steady-state potassium channels). In addition, this list can include K(Ca) channels; there are data on the activating effect of NaHS on high- and low-conductance KCa channels in various objects [6,11].

Our findings suggest that exogenous and probably endogenously synthesized H_2S plays an important role in the regulation of cardiac functions, while the targets of this gasotransmitter are ATP-dependent, Ca-activated, and voltage-dependent types of K-channels. Probably, H_2S-induced activation of potassium conductance shortens AP, inhibits inward calcium current, and finally moderates the force of myocardium contraction.

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