The Effect of Hydrogen Sulfide on Electrical Activity of Rat Atrial Myocardium

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Changes in the configuration of action potentials and in the frequency of pacemaker discharges in a preparation of isolated rat right atrium under the effect of sodium hydrosulfide degrading in water solution with hydrogen sulfide release were studied by intracellular recording of action potentials in the myocardium. Sodium hydrosulfide in concentrations of 100-500 μM markedly reduced the duration of action potentials at the level of 50 and 90% repolarization and decelerated the sinus rhythm. Moreover, sodium hydrosulfide shortened action potentials in the preparations working in the forced rhythm. Glybenclamide (potassium ATP-dependent channel blocker; 10 μM) reduced the effect sodium hydrosulfide (200 μM) by more than 60%, which suggested the involvement of potassium ATP-dependent current in the realization of the effect of hydrogen sulfide on configuration of action potentials. Hence, hydrogen sulfide, recently described as a signal compound, modulates many electrophysiological parameters of the myocardium.

Key Words: *hydrogen sulfide; atria; action potential; potassium ATP-dependent channels; glybenclamide*

Studies of the mechanisms of physiological action of gaseous signal compounds are now in progress. Along with the well-known gaseous regulator nitrogen oxide (NO), this group of substances includes carbon monoxide (CO), which causes vasodilatation [7,14], facilitates acetylcholine secretion in the neuromuscular synapse [11], changes in contractile activity of the heart [2], and some other physiological effects [2,7]. Persuasive evidence of the existence of one more gaseous signal compound, hydrogen sulfide (H_2S), was published recently. Though this substance is better known as a toxic gas with unpleasant smell, it can be produced in the body from L-cysteine in the reaction catalyzed by cystationine-γ-liase or cystationine-β-synthase [5,10]. Many physiological effects of H_2S are described: amplification of NMDA receptor stimulation [1,8], effect on long-term potentiation [1], a strong vasodilating effect [5].

Cystationine-γ-liase capable of producing H_2S was detected in the myocardium [3,10]. Negative inotropic and chronotropic effect of H_2S in the isolated heart was described [3]. Studies on isolated heart, isolated cardiomyocytes, and *in vivo* demonstrated cardioprotective effects of H_2S [4,9,13]. As for the mechanisms of H_2S effects, the majority of authors hypothesize activation of potassium ATP-dependent current (I_{KATP}) [3,6,14]. A recent report describes suppression of the L-type calcium current under the effect of H_2S [12]. On the other hand, the effects of H_2S on the parameters of electrical activity in the myocardium remain not studied.

We studied the effect of H_2S on configuration of action potentials (AP) and frequency of pacemaker discharges in a preparation of isolated rat right atrium and evaluated the possibility of involvement of potassium ATP-dependent channels in the realization of H_2S effects.

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MATERIALS AND METHODS

The study was carried out on 20 male outbred rats. The animals were decapitated, the thoracic cage was directly opened, and the heart was removed. The heart was washed in Tyrode's solution containing (in mmol/liter) 133.47 NaCl, 4.69 KCl, 1.35 $NaH_2PO_4 \times 2H_2O$, 16.31 $NaHCO_3$, 1.18 $MgSO_4 \times 7H_2O$, 2.5 $CaCl_2 \times 2H_2O$, and 7.77 glucose) saturated with carbogen (95% O_2 and 5% CO_2). The right atrium with the sinoatrial node was resected, placed in a 3-ml box, and superfused with Tyrode's solution at 38°C and 10 ml/min flow rate. The isolated myocardial fragment was fixed to the bottom of the box with its endocardial side up. The intervenous area with the sinoatrial node was removed for experiments in which the preparation worked in the forced rhythm.

The effect of H_2S on electrical activity was studied using sodium hydrosulfide (NaHS; Sigma). This substance dissolved in water dissociates into Na^+ and HS^- ions; these latter ones react with proton, which results in the formation of H_2S dissolved in water [3]. The gas is gradually released from the solution, and hence, NaHS solutions were prepared directly before putting them into experimental box. In order to detect the dose dependence of H_2S effects, NaHS concentrations of 50, 100, 200, 300, and 500 μM were tested (10 min each) in each experiment. The significance of NaHS effects in different concentrations was evaluated in comparison with the control values of electric activity (before NaHS treatment). In experimental series I (evaluation of dose dependence of H_2S effects), the preparation worked in its intrinsic rhythm (n=7), in series II in forced rhythm at a frequency of 6 Hz (n=7), and in experimental series III (evaluation of the effect of glybenclamide (potassium ATP-dependent channel blocker; Sigma) on the intensity of H_2S effects) the preparation also worked in forced rhythm at a frequency of 6 Hz (n=7).

Action potentials were recorded by the method of intracellular shunting of bioelectrical activity with glass microelectrodes of 15-30 MΩ resistance. The signal was digitally processed on an E14-140 analog digital transformer (L-Card) and recorded in a computer using L-Graph v. 1.0 software (L-Card).

The data were processed using MiniAnalysis c. 3.0.1 software (Synaptosoft). The length of AP at the level of 50 and 90% repolarization of cardiomyocyte membrane was evaluated. In addition, the frequency of AP (sinus rhythm) was evaluated in experiments with the intrinsic rhythm. The results were statistically processed using Statistica 6.0 software. The significance of differences was evaluated using Wilcoxon's test.

RESULTS

The effects of different concentrations of NaHS in preparations working in their intrinsic rhythms are presented in Fig. 1, *a*. In a concentration of 50 μM, NaHS caused no appreciable changes in the studied

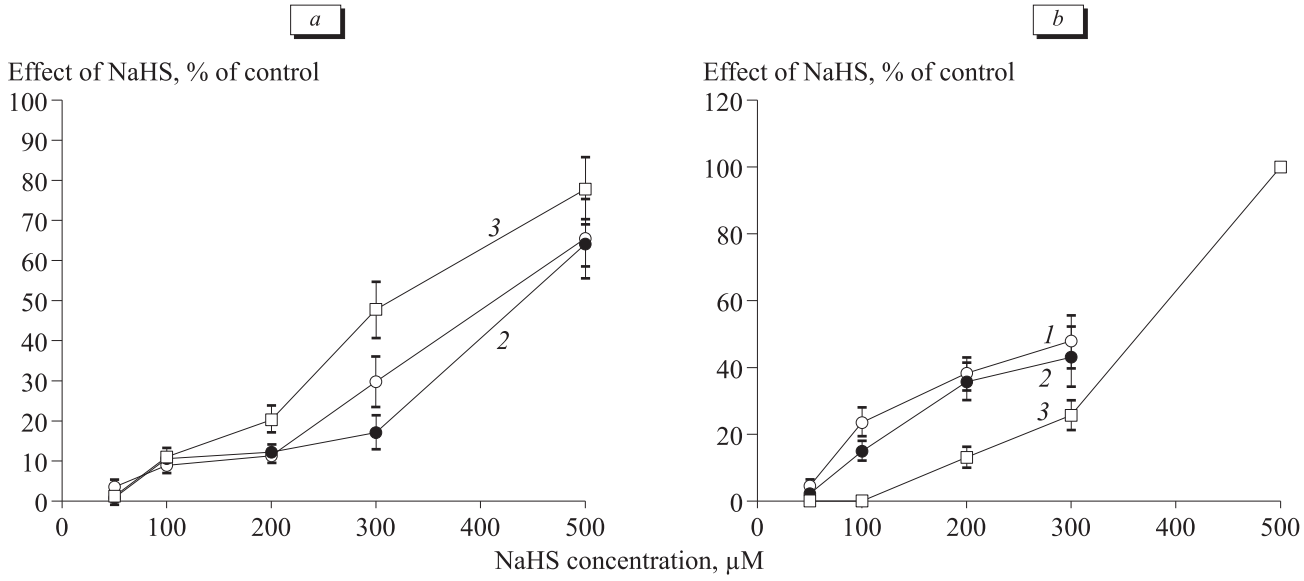

Fig. 1. Dose dependent hydrogen sulfide effects. Ordinate: NaHS effect. *a*) curves for preparations working in their intrinsic rhythm. *1*) shortening of AP duration at the level of 50% repolarization; *2*) shortening of AP duration at the level of 90% repolarization; *3*) prolongation of the cycle (rhythm deceleration). All values significant in comparison with the control (Wilcoxon test, $p<0.05$) except NaHS concentration of 50 μM. *b*) curves for preparations working in the forced rhythm. *1*) shortening of AP duration at the level of 50% repolarization; *2*) shortening of AP duration at the level of 90% repolarization; *3*) reduction of AP amplitude. All values are significant in comparison with the control (Wilcoxon test, $p<0.05$) except the values for NaHS concentration of 50 μM and the reduction of amplitude for 100 and 200 μM NaHS.

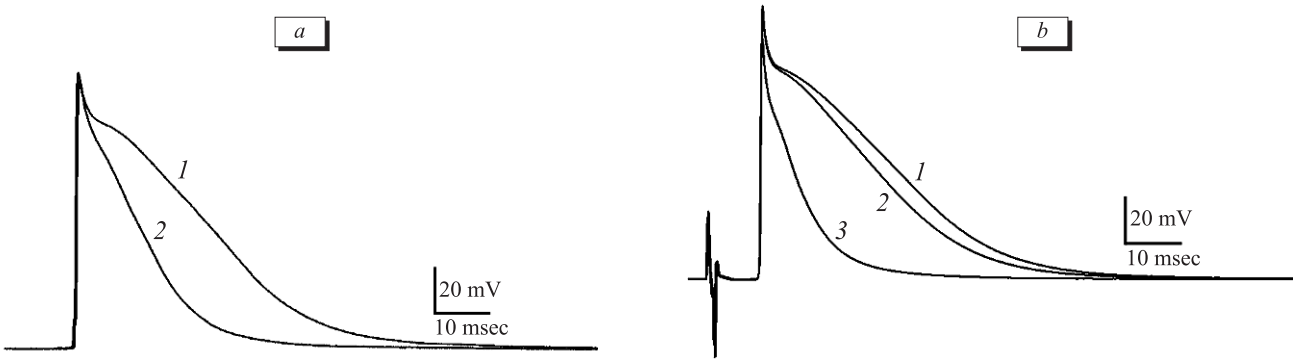

Fig. 2. Original records of AP. *a*) preparation working in its intrinsic rhythm, recording of one cell. *1*) AP in the control; *2*) AP during minute 10 of treatment by 300 μM NaHS. *b*) preparation working in forced rhythm, recording in one cell. *1*) AP in the control; *2*) AP during minute 10 of treatment by 100 μM NaHS; *3*) AP during minute 10 of treatment by 300 μM NaHS.

parameters. NaHS in all other tested concentrations significantly reduced AP duration at 50 and 90% repolarization and prolonged the cardiac cycle (decelerated sinus rhythm). No appreciable changes in AP amplitude were detected. The configurations of AP in the control and under the effect of 200 μM NaHS were compared (Fig. 2, *a*).

The result indicates that H_2S in concentrations close to those detected in body tissues (more than 50 μM in the plasma [14]) modifies electrical activity of the rat right atrium. Inhibition of the rhythm under the effect of H_2S is obviously caused by its effect on electrical activity of sinoatrial node cells.

In order to demonstrate the relationship between the observed shortening of AP and H_2S treatment of the working atrial myocardium (but not its effect on the rhythmogenic structures), we experimented with forced rhythm on atrial preparations from which the intervenous region was resected. In the preparations working in the forced rhythm NaHS also significantly reduced AP length at 50 and 90% repolarization in all tested concentrations except 50 μM (Fig. 1, *b*, 2, *b*). In addition, NaHS in concentrations of 300 and 500 μM significantly reduced AP amplitude; moreover, perfusion with 500 μM NaHS led to complete suppression of electrical activity by the 4th minute.

Hence, H_2S significantly modifies AP configuration in the working myocardium and decelerates the sinus rhythm, while in higher concentrations it suppresses electrical activity.

It was interesting to evaluate the mechanisms underlying the effect of H_2S on AP configuration. Activation of I_{KATP} is the most common explanation of H_2S effects [3,6]. It is well known that I_{KATP} activation under conditions of ischemia leads to pronounced shortening of AP at the expense of repolarization stimulation. In order to check, whether this current mediates the effect of H_2S on AP configuration, a series of experiments with glybenclamide (selective blocker of potassium ATP-dependent channel) was carried out.

The effect of 200 μM H_2S in the control was compared with the effect after treatment with 10 μM glybenclamide in each experiment. The effect of H_2S dropped more than 3-fold in the presence of glybenclamide (Fig. 3). Hence, presumably, potassium ATP-dependent channels are involved in mediation of H_2S effects.

It is noteworthy that glybenclamide does not completely eliminate the effect of H_2S. Presumably, there are also other mechanisms of H_2S effect on AP configuration, for example, recently demonstrated inhibitory effect of H_2S on L-type calcium current.

It remains unclear in what way H_2S modifies potassium ATP-dependent channels: directly or through other systems of intracellular signaling. This problem is interesting for further studies.

Hence, we showed that H_2S, a gaseous signal compound, in concentrations above 100 μM causes chang-

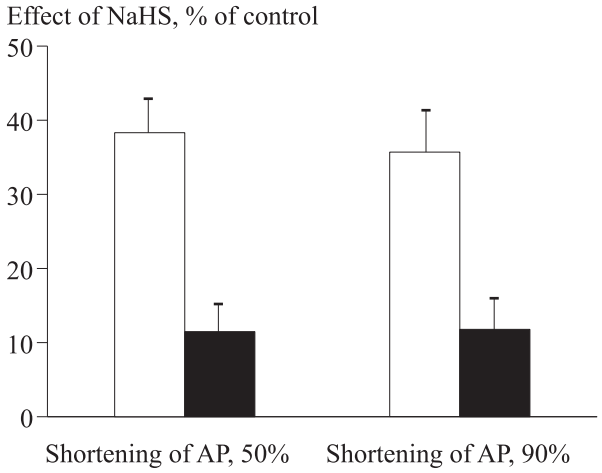

Fig. 3. Reduction of AP duration at the level of 50 and 90% repolarization under the effect of NaHS in parallel with 10 μM glybenclamide and without it. Ordinate: NaHS effect. All differences from the control are significant (Wilcoxon test, $p<0.05$). The difference between NaHS effect in the presence of glybenclamide and without it is also significant for AP shortening at the level of 50 and 90% repolarization (Wilcoxon test, $p<0.05$). Light bars: effect of 200 μM NaHS; dark bars: effect of 200 μM NaHS in the presence of 10 μM glybenclamide.

es in electrical activity of the rat atrial myocardium, for example, reduction of AP length in the working myocardium fibers. This effect is largely mediated through activation of I_{KATP} current. It is noteworthy that the described shortening of AP under the effect of H_2S can indicate arrhythmogenic effects of this substance towards the supraventricular compartments.

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