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Research Paper

Electrophysiological effects of hydrogen sulfide on pacemaker cells in sinoatrial nodes of rabbits

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Abstract: The cardiac electrophysiological effects of hydrogen sulfide (H_2S) on pacemaker cells in sinoatrial (SA) nodes of rabbits were examined using intracellular microelectrode technique. The results obtained were as follows: (1) The velocity of diastolic (phase 4) depolarization (VDD) and rate of pacemaker firing (RPF) in normal pacemaker cells in SA nodes were decreased by NaHS (H_2S donor) (50, 100, 200 μmol/L) in a concentration-dependent manner; (2) ATP-sensitive K^+ (K_{ATP}) channel blocker glybenclamide (Gli, 20 μmol/L) blocked the effect of NaHS (100 μmol/L) on pacemaker cells; (3) Pretreatment with CsCl (2 mmol/L), a blocker of pacemaker current (I_f), did not affect the effect of NaHS (100 μmol/L) on SA node pacemaker cells; (4) *DL*-propargylglycine (PPG, 200 μmol/L), an inhibitor of cystathionine γ-lyase (CSE), did not affect the parameters of action potentials in pacemaker cells in SA nodes. All these results suggest that H_2S exerts a negative chronotropic action on pacemaker cells in SA nodes of rabbits. These effects are likely due to an increase in potassium efflux through opening K_{ATP} channels; I_f is unlikely to play a major role in these effects. In our study, there was no evidence for the generation of endogenous H_2S by CSE in SA node pacemaker cells.

Key words: electrophysiology; hydrogen sulfide; action potential; sinoatrial node

硫化氢对家兔窦房结起搏细胞的电生理效应

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摘 要：本研究应用细胞内微电极技术，观察硫化氢(hydrogen sulfide, H_2S)对家兔窦房结起搏细胞的电生理效应。结果表明：(1) NaHS (H_2S 供体) 50、100、200 μmol/L 浓度依赖地降低家兔窦房结起搏细胞 4 相去极化速率及起搏放电频率。(2) ATP 敏感性钾(ATP-sensitive K^+, K_{ATP})通道阻断剂格列苯脲(glybenclamide, Gli, 20 μmol/L)阻断 NaHS (100 μmol/L)的电生理效应。(3)预先应用起搏离子流(pacemaker current, I_f)通道阻断剂氯化铯(CsCl, 2 mmol/L)对 NaHS (100 μmol/L)的电生理效应无影响。(4)胱硫醚-γ裂解酶(cystathionine γ-lyase, CSE)的不可逆抑制剂 *DL*-propargylglycine (PPG, 200 μmol/L)对家兔窦房结起搏细胞的动作电位参数无影响。以上结果提示，H_2S 对家兔窦房结起搏细胞有负性变时作用，这些效应可能与其开放 K_{ATP} 通道，增加 K^+ 外流有关，与 I_f 无关。本实验没有发现窦房结起搏细胞内有 CSE 催化产生的内源性 H_2S 的合成。

关键词：电生理；硫化氢；动作电位；窦房结
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Hydrogen sulfide (H_2S), which was traditionally considered to be a toxic gas in contaminated environments playing a neurotoxic role and inhibiting the respiratory system, has been proved to be the third endogenous signaling gasotransmitter, besides nitric oxide (NO) and carbon monoxide (CO), with important physiological functions[1-4].

It is now clear that H_2S has the vasorelaxant function. In vascular smooth muscle cells (VSMCs), the opening of ATP-sensitive potassium (K_{ATP}) channels and the entrance of extracellular calcium were reported to be involved in

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H_2S actions, but the cGMP and Ca^{2+}-dependent potassium channel pathways were not included[5,6].

H_2S is the first identified gaseous opener of the K_{ATP} channels in VSMCs[5] and the K_{ATP} channels are widely distributed in heart cells. It is known that the opening of K_{ATP} channels in myocardium is an important endogenous cardioprotective mechanism[7]. Recently, H_2S has been found to play a negative inotropic role in the heart and could be endogenously produced by the cardiac tissues as a physiological cardiac function regulator. And this effect of H_2S is mediated by the K_{ATP} channel pathway[8]. Endogenous H_2S can be generated from *L*-cysteine catalyzed by two pyridoxal-5'-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), in mammalian tissues[9-11]. The expressions of these two enzymes are tissue-specific[4]. H_2S is directly produced in myocardial tissues, arterial and venous tissues by CSE, which can be inhibited by *DL*-propargylglycine (PPG)[5,8,12,13]. Our previous study had demonstrated that H_2S facilitated carotid sinus baroreflex through the opening of K_{ATP} channels and further closing calcium channels[14], and that H_2S concentration-dependently decreased the action potential duration (APD) in guinea pig papillary muscles[15].

The aim of our study was to observe the electrophysiological effects of H_2S on pacemaker cells in sinoatrial (SA) nodes of rabbits and elucidate the mechanism(s) involved.

1 MATERIALS AND METHODS

1.1 *Electrophysiological measurements*

Rabbits of either sex [(2.2±0.2) kg, Grade II, Certificate No. 04037, provided by Experiment Animal Center of Hebei Province] were killed with a single blow on the head and the hearts were quickly excised. The region of the right atrium bounded by the crista terminalis and the superior and inferior vena cava, and the interatrial septum were dissected free from the adjacent tissues[16] in Krebs-Henseleit (KH) solution (0-4 °C). The preparations were pinned down on a thin silicon disc on the base of a perfusion chamber and equilibrated for 1 h. The KH solution was prepared with deionized, distilled water and composed of (in mmol/L): NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 1.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, and glucose 11.1. It was oxygenated with 95% O_2 and 5% CO_2 and maintained at (36.0±0.5) °C with pH of 7.40±0.03. The transmembrane potentials were recorded by KCl (3 mol/L)-filled micropipettes (tip diameter less than 0.5 μm), coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to the A/D converter and processed by a microcomputer. The maximal diastolic potential (MDP), amplitude of action potential (APA), APD at 90% repolarization (APD_{90}), maximal rate of depolarization (V_{max}), rate of pacemaker firing (RPF), and velocity of diastolic (phase 4) depolarization (VDD) were analyzed with the system of sampling and processing cardiac transmembrane potential designed by our department[17].

1.2 *Experimental protocols*

1.2.1 *Electrophysiological effects of NaHS on SA node pacemaker cells*

The experiment started after the preparation was equilibrated for 60 min in the KH solution at a perfusion rate of 4 mL/min. The effects of H_2S on action potentials (APs) were studied in a non-cumulative manner. Only one concentration of NaHS was given to a preparation. After recording of 3 control APs, NaHS at 50, 100, 200 μmol/L was separately applied. APs were then recorded at 1, 5, 10, 20, 30 and 40 min after application of NaHS. The preparation was washed with KH solution to observe the recovery of APs.

1.2.2 *Effects of glybenclamide (Gli) on NaHS-induced changes in APs in SA node pacemaker cells*

The effects of NaHS alone were observed firstly after application for 25 min. Then after superfusion of Gli (20 μmol/L) for 15 min, NaHS (100 μmol/L) was added and APs were recorded.

1.2.3 *Effects of CsCl on NaHS-induced changes in APs in SA node pacemaker cells*

The effects of NaHS alone were observed firstly after application for 25 min. Then after pretreatment with CsCl (2 mmol/L) for 15 min, NaHS (100 μmol/L) was added and APs were recorded.

1.2.4 *Effects of PPG on APs in SA node pacemaker cells*

After recording of 3 control APs, PPG (200 μmol/L) was applied. APs were then recorded at 5 min interval lasting 120 min after application of PPG.

1.3 *Reagents*

NaHS, CsCl and PPG were purchased from Sigma. Gli (a K_{ATP} channel blocker) was purchased from Tianjin Institute of Medical and Pharmaceutical Industry. NaHS was used as a donor of H_2S. NaHS was employed in our experiments for a better definition of H_2S concentration in solution than bubbling H_2S gas. NaHS dissociates to Na^+ and HS^- in solution. Then HS^- associates with H^+ and H_2S is produced. About one-third of the H_2S in solution exists as undissociated form (H_2S), and two-thirds as HS^- which is at equilibrium with H_2S[12]. Gli was initially dissolved in

dimethylsulfoxide (DMSO, 100 μmol/L). The final concentration of DMSO in the KH solution was 0.04% (*V*/*V*). CsCl [an inhibitor of pacemaker current (I_f)]was dissolved in superfusate. PPG was dissolved in distilled water.

1.4 *Statistics*

All data were presented as mean±SD. The differences in the parameters between pre- and post-application of reagents were analyzed by paired Student's *t* test. Differences between groups were assessed by one-way ANOVA and unpaired *t* test. Statistical significance was set at $P<0.05$.

2 RESULTS

2.1 Effects of H_2S on transmembrane APs

Compared with the control group, NaHS (50-200 μmol/L) significantly decreased VDD and RPF ($P<0.05$, $P<0.01$) in a concentration-dependent manner but had no significant effects on the other parameters of APs (Table 1, Fig.1). The changes in RPF induced by NaHS paralleled to those of VDD. The above effects occurred after 5-10 min of NaHS superfusion and reached the peak within 25-30 min.

2.2 Effects of Gli on NaHS-induced changes in APs in SA node pacemaker cells

NaHS (100 μmol/L) significantly decreased VDD and RPF compared with that in the control group ($P<0.01$). Gli (20 μmol/L) alone had no significant effects on APs. After pretreatment with Gli, the electrophysiological effects of NaHS (100 μmol/L) were inhibited ($P<0.01$). But VDD and RPF were not significantly different from those in the control group (Table 2).

2.3 Effects of CsCl on NaHS-induced changes in APs in SA node pacemaker cells

CsCl (2 mmol/L) decreased VDD and RPF ($P<0.05$). After pretreatment with CsCl, the electrophysiological effects of NaHS (100 μmol/L) were not inhibited. NaHS continued to decrease VDD and RPF on the base of the chronotropic effects of CsCl, and VDD and RPF were significantly decreased compared with those in the control group ($P<0.01$) and those in CsCl group ($P<0.05$) (Table 2).

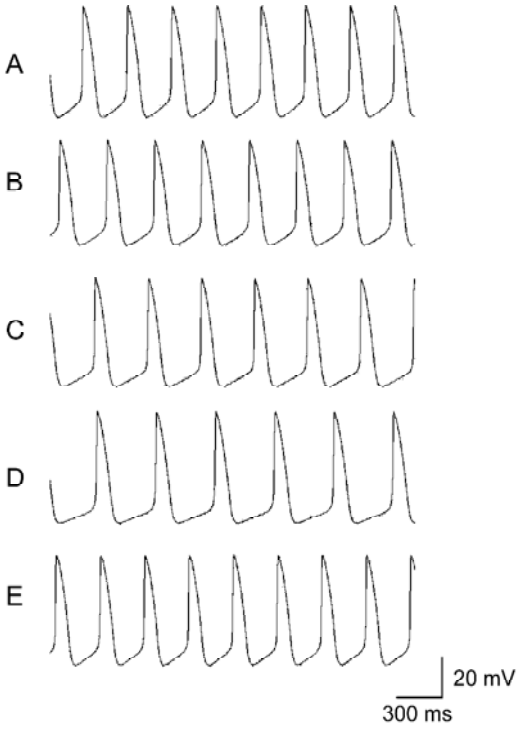

Fig. 1. Effects of H_2S on transmembrane action potentials in rabbit sinoatrial node pacemaker cells. *A*: Control. *B*: 50 μmol/L NaHS. *C*: 100 μmol/L NaHS. *D*: 200 μmol/L NaHS. *E*: Washout.

2.4 Effects of PPG on APs in SA node pacemaker cells

Continuous superfusion of PPG (200 μmol/L) had no effects on APs in the normal SA node pacemaker cells (Table 3).

As we need quite a long time to observe the changes in APs in SA node pacemaker cells using intracellular microelectrodes after administration of several reagents, we had ever specially beheld the APs in SA node pacemaker cells

Table 1. Effects of H_2S on transmembrane action potentials in rabbit sinoatrial node pacemaker cells

	MDP (mV)	APA (mV)	V_{max} (V/s)	VDD (mV/s)	APD_{90} (ms)	RPF (beats/min)
Control	-57±6	65±4	7.2±1.9	63±12	163±18	179±14
50 μmol/L NaHS	-56±5	63±3	6.9±2.1	49±13 *	165±19	163±13 *
100 μmol/L NaHS	-56±7	63±5	7.0±2.3	38±10 $^{**+}$	164±21	151±10 $^{**+}$
200 μmol/L NaHS	-54±6	62±5	6.7±1.8	22±11 $^{**++\#}$	167±23	134±16 $^{**++\#}$
Washout	-56±7	64±3	7.1±2.2	64±11	165±21	180±12

n=6. $^{*}P<0.05$, $^{**}P<0.01$ *vs* control; $^{+}P<0.05$, $^{++}P<0.01$ *vs* 50 μmol/L NaHS; $^{\#}P<0.05$ *vs* 100 μmol/L NaHS. MDP, maximal diastolic potential; APA, amplitude of action potential; V_{max}, maximal rate of depolarization; VDD, velocity of diastolic (phase 4) depolarization; RPF, rate of pacemaker firing; APD_{90}, action potential duration at 90% repolarization.

Table 2. Effects of Gli (20 μmol/L) and CsCl (2 mmol/L) on NaHS (100 μmol/L)-induced changes in transmembrane action potentials in rabbit sinoatrial node pacemaker cells

	MDP (mV)	APA (mV)	V_{max} (V/s)	VDD (mV/s)	APD_{90} (ms)	RPF (beats/min)
Control	-59±8	67±3	6.8±2.2	66±10	145±13	181±15
NaHS	-59±7	65±4	6.6±2.1	42±12 **	147±14	153±14 **
Gli	-58±7	67±5	6.9±2.3	67±13	147±12	180±14
Gli + NaHS	-57±9	65±6	6.7±1.9	62±15 $^{++}$	147±11	175±15 $^{++}$
Washout	-59±9	67±5	6.7±2.1	64±12	147±13	178±13
Control	-51±4	62±5	4.5±2.1	70±12	143±11	169±11
NaHS	-50±4	61±3	4.5±1.9	43±13 **	144±12	145±13 **
CsCl	-50±5	60±6	4.3±2.2	55±11 $^{*+}$	143±10	156±12 $^{*+}$
CsCl + NaHS	-49±3	60±5	4.2±1.9	40±10 $^{**\#}$	143±12	139±11 $^{**\#}$
Washout	-50±4	61±5	4.3±2.1	68±12	143±11	168±13

n=12. $^{*}P$<0.05, $^{**}P$<0.01 *vs* control; ^{+}P<0.05, ^{++}P<0.01 *vs* NaHS; $^{\#}P$<0.05 *vs* CsCl. MDP, maximal diastolic potential; APA, amplitude of action potential; V_{max}, maximal rate of depolarization; VDD, velocity of diastolic (phase 4) depolarization; RPF, rate of pacemaker firing; APD_{90}, action potential duration at 90% repolarization.

Table 3. Effects of PPG (200 μmol/L) on transmembrane action potentials in rabbit sinoatrial node pacemaker cells

	MDP (mV)	APA (mV)	V_{max} (V/s)	VDD (mV/s)	APD_{90} (ms)	RPF (beats/min)
Control	-49±9	58±12	4.0±1.4	57±8	152±9	190±21
PPG	-48±10	60±11	4.2±1.2	56±10	150±12	187±19

MDP, maximal diastolic potential; APA, amplitude of action potential; V_{max}, maximal rate of depolarization; VDD, velocity of diastolic (phase 4) depolarization; RPF, rate of pacemaker firing; APD_{90}, action potential duration at 90% repolarization.

for 100 min without any disposal to cells. And we didn't find any change in APs in SA node pacemaker cells. Therefore, in our experiments, the changes in APs in SA node pacemaker cells were due to the effects of reagents.

3 DISCUSSION

The present study showed that H_2S concentration-dependently decreased VDD and RPF but had no significant effects on the other parameters of APs in SA node pacemaker cells. The changes in RPF induced by H_2S paralleled to those of VDD. It is well known that RPF is determined by MDP, level of threshold and VDD. The major ionic currents responsible for diastolic depolarization are the decaying potassium currents (I_K), increasing slow inward calcium currents ($I_{Ca\text{-}L}$ and $I_{Ca\text{-}T}$) and increasing inward sodium currents (I_f)[18,19]. Therefore, any factors promoting potassium efflux or/and inhibiting calcium or/and sodium influx may decrease VDD.

In our study, H_2S had no significant effects on V_{max} or APA in SA node pacemaker cells. It suggests that the decrease in calcium influx may not be related to the effects of H_2S.

It has been reported that H_2S is the first identified gaseous opener of the K_{ATP} channels in VSMCs[5] and the K_{ATP} channels are widely distributed in heart cells[20-22]. Geng *et al*. recently reported that H_2S could be endogenously produced by heart tissues, as a physiological cardiac function regulator, and mediated by the K_{ATP} channel pathway[8]. Thus, we observed the effects of K_{ATP} channel blocker Gli on H_2S-induced changes in APs. Gli could inhibit the electrophysiological effects of H_2S. The results indicate that the effects of H_2S on VDD may be due to the enhancement of potassium efflux through the opening of K_{ATP} channels.

In order to examine whether decrease in sodium influx was involved in the effects of H_2S, we used the I_f blocker CsCl[23]. CsCl could not inhibit the electrophysiological effects of H_2S, indicating that I_f may not be involved in the effects of H_2S.

The results so far only discussed the effects of exogenous H_2S. In our previous study, it was demonstrated that endogenous H_2S generated by papillary muscles might

play an important role in APs in guinea pig papillary muscles[15]. To determine whether endogenous H_2S could be generated in SA nodes and the function of it, moreover considering that CSE, but not CBS plays a major role in generating H_2S in cardiovascular tissues under physiological conditions[4,11,12,24], PPG (an inhibitor of CSE) was used in our experiment. Zhao *et al*. reported that PPG might be a membrane-permeable reagent, and that it had the potential to be used to study the physiological function of endogenously produced H_2S[13]. In the present study, after pretreatment with PPG (200 μmol/L), the APs had no significant changes compared with that in the normal SA node pacemaker cells. The results indicate that in SA node pacemaker cells endogenous H_2S may not be generated by CSE.

Although it has not been found that endogenous H_2S is generated in SA node pacemaker cells, H_2S is directly produced by CSE, but not CBS, in other cardiovascular tissues, including myocardial tissues, arterial and venous tissues[4,8,12,13,24]. In addition, it has been demonstrated that H_2S concentration in rat serum is approximately 46 μmol/L[5]. Therefore, under physiological and/or pathologic conditions, H_2S from blood and other cardiovascular tissues could affect SA node to exert the regulative function.

Recently, some reports indicated that H_2S exerts cardiovascular protective function. Preconditioning with NaHS significantly decreased the duration and severity of ischemia/reperfusion-induced arrhythmias in the isolated heart while increased cell viability[25]. In our experiment, H_2S decreased VDD and RPF that shorten the work time of heart and protect the heart. The negative chronotropic role of H_2S in the heart may be one of mechanisms that H_2S exerts cardioprotective effects during ischemia/reperfusion injury.

In summary, our observation demonstrates that H_2S exhibits electrophysiological effects on pacemaker cells in SA nodes of rabbits. H_2S exerts a negative chronotropic action. These effects may be attributed to an increase in potassium efflux through the opening of K_{ATP} channels. In our study, there was no evidence for the generation of endogenous H_2S by CSE in SA node pacemaker cells.

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