Acta Physiologica Sinica, April 25, 2007, 59 (2): 215-220
http://www.actaps.com.cn

Research Paper

Electrophysiological effects of hydrogen sulfide on guinea pig papillary muscles *in vitro*

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Abstract: The cardiac electrophysiological effects of hydrogen sulfide (H_2S) were examined in guinea pig papillary muscles *in vitro* using intracellular microelectrode technique. The results obtained were as follows: (1) the duration of action potential (APD) in the normal papillary muscles was decreased by NaHS (H_2S donor, 50, 100, 200 μmol/L) in a concentration-dependent manner; (2) in partially depolarized papillary muscles, 100 μmol/L NaHS not only reduced APD, but also decreased the amplitude of action potential (APA), overshoot (OS) and maximal velocity of depolarization at phase 0 (V_{max}); (3) pretreatment with ATP-sensitive K^+ (K_{ATP}) channel blocker glibenclamide (20 μmol/L) partially blocked the effects of NaHS (100 μmol/L); (4) pretreatment with L-type Ca^{2+} channel agonist Bay K8644 (0.5 μmol/L) also partially blocked the effects of NaHS (100 μmol/L); (5) pretreatment with Ca^{2+}-free Krebs-Henseleit solution containing glibenclamide (20 μmol/L) completely blocked the effects of NaHS (100 μmol/L); (6) APD in the normal papillary muscles was increased by *DL*-propargylglycine (PPG, an inhibitor of cystathionine γ-lyase, 200 μmol/L). All these results suggest that the electrophysiological effects of H_2S on papillary muscles in our study are due to an increase in potassium efflux through the opening of K_{ATP} channels and a decrease in calcium influx. Endogenous H_2S may act as an important regulator in electrophysiological characters in papillary muscles.

Key words: electrophysiological; hydrogen sulfide; action potential; papilary muscles

硫化氢对离体豚鼠乳头状肌的电生理效应

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摘 要：应用细胞内微电极技术，观察硫化氢(hydrogen sulfide, H_2S)对离体豚鼠乳头状肌细胞的电生理效应。结果表明：(1) NaHS (H_2S 的供体，50、100、200 μmol/L)可浓度依赖地缩短正常乳头状肌的动作电位时程。 (2)对部分去极化乳头状肌，NaHS (100 μmol/L)除缩短动作电位时程外，还降低动作电位幅值和超射值，减慢零相最大上升速度。(3)预先应用 ATP 敏感性钾(ATP-sensitive K^+, K_{ATP})通道阻断剂格列苯脲(glibenclamide, Gli, 20 μmol/L)，可部分阻断 NaHS (100 μmol/L)的电生理效应。 (4)预先应用 L 型钙通道开放剂 Bay K8644 (0.5 μmol/L)，可部分阻断 NaHS (100 μmol/L)的电生理效应。 (5)预先应用含 Gli (20 μmol/L)的无钙 Krebs-Henseleit 液灌流标本，可完全阻断 NaHS (100 μmol/L)的电生理效应。 (6) *DL*-propargylglycine (PPG，一种胱硫醚 -γ-裂解酶的不可逆抑制剂，200 μmol/L)可延长正常乳头状肌的动作电位时程。以上结果提示，H_2S 可能通过兴奋 K_{ATP} 通道促进 K^+ 外流，同时抑制 Ca^{2+} 内流，进而影响豚鼠乳头状肌电生理效应。乳头状肌中内源性 H_2S 可能发挥重要的电生理作用。

关键词：电生理；硫化氢；动作电位；乳头状肌
中图分类号：Q463

Hydrogen sulfide (H_2S) was only recognized as a kind of toxic gas in contaminated environments with a strong odor of rotten eggs for a long time, and its major effects were the intoxication of the central nervous system and the inhibition of the respiratory system[1-3]. But increasing lines of evidence proves that H_2S might be the third endogenous signaling gasotransmitter, besides nitric oxide (NO) and carbon monoxide (CO), and has important physiological

Received 2006-11-02 Accepted 2006-12-29
This work was supported by the National Science Foundation of Hebei Province (No. C200700821).
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functions[4].

Endogenous H_2S may be generated by two pyridoxal-5'-phosphate-dependent enzymes — cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) in mammalian tissues which use *L*-cysteine as the main substrate[5-7]. The expressions of these two enzymes are tissue-type specific[4]. H_2S is directly produced in myocardial tissues, arterial and venous tissues by CSE[8-10]. *DL*-propargylglycine (PPG) is a specific inhibitor of CSE, which can suppress endogenous H_2S production[11].

H_2S has vasorelaxant function and the underlying mechanism is being studied. 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 H_2S actions, while cGMP and Ca^{2+}-dependent potassium channel pathways were not included[9,12]. H_2S is the first identified gaseous opener of K_{ATP} channels in VSMCs[9]. K_{ATP} channels are widely distributed in the myocardium and opening of them is an important endogenous cardioprotective mechanism[13]. H_2S plays a negative inotropic role in the heart and could be endogenously produced by the cardiac tissues as a physiological cardiac function regulator, which is mediated by K_{ATP} channel pathway[8]. In addition, our laboratory demonstrated that H_2S could facilitate carotid sinus baroreflex through the opening of K_{ATP} channels and further closing calcium channel[14]. Therefore, H_2S also palys an important role in the modulation of blood pressure. The present study was undertaken to investigate the electrophysiological effects of H_2S on guinea pig papillary muscles *in vitro* and the underlying mechanism(s).

1 MATERIALS AND METHODS

1.1 *Electrophysiological measurement*

Guinea pigs of either sex (weighing 300-400 g, provided by the Experimental Animal Center of Hebei Province) were killed with a single blow on the head and the hearts were removed and placed in cold (0-4 °C) Krebs-Henseleit (K-H) solution. The papillary muscles were cut from the right ventricle and then pinned on a thin silicon disc at the bottom of a perfusion chamber. The preparation was perfused (4 mL/min) with K-H solution of the following composition (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 at (35.0±0.5) ºC. K-H solution was saturated by a mixture of 95% O_2 and 5% CO_2 and pH was 7.39±0.03. The papillary muscles were paced by square pulse (1 Hz, 1 ms, 1.5 times threshold) provided by a simulator (SEN-3201, Nihon Kohden). The transmembrane action potentials (APs) were recorded by 3 mol/L KCl-filled micropipettes (a tip resistance of 10-20 MΩ) coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to an A/D converter and processed by a microcomputer. Resting potential (RP), overshoot (OS), amplitude of AP (APA), maximal rate of depolarization at phase 0 (V_{max}), duration of AP (APD), 50% of APD (APD_{50}) and 90% of APD (APD_{90}) were analyzed, and plateau period duration (PPD) was calculated from regression analysis of repolarization at phase 2 and phase 3[15,16].

1.2 *Experimental protocols*

1.2.1 *Electrophysiological effects of H_2S on normal papillary muscles*

APs were recorded after equilibrium for 1 h. After recording of 3 control APs, NaHS (50, 100, 200 μmol/L) were applied respectively. APs were then recorded at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 min after application of NaHS. The preparation was washed with K-H solution to observe the recovery of AP.

1.2.2 *Electrophysiological effects of H_2S on partially depolarized papillary muscles*

The preparations were equilibrated for 1 h in the normal K-H solution. Then slow response AP was induced in the papillary muscles by exposing the preparation to K-H solution containing KCl (18 mmol/L) and isoprenaline (1.5 μmol/L). After recording of 3 control APs, NaHS (100 μmol/L) was administered and APs were recorded.

1.2.3 *Effects of K_{ATP} channel blocker glibenclamide (Gli) on H_2S-induced changes of AP in papillary muscles*

The effects of NaHS (100 μmol/L) alone were observed. Then after pretreatment with Gli (20 μmol/L) for 20 min, NaHS (100 μmol/L) was added and APs were recorded.

1.2.4 *Effects of L-type calcium channel agonist Bay K8644 on H_2S-induced changes of AP in papillary muscles*

The effects of NaHS (100 μmol/L) alone were observed. Then after pretreatment with Bay K8644 (0.5 μmol/L) for 15 min, NaHS (100 μmol/L) was added and APs were recorded.

1.2.5 *Effects of H_2S on AP in normal papillary muscles superfused with Ca^{2+}-free K-H solution containing Gli*

The effects of NaHS (100 μmol/L) alone were observed firstly. Then after superfusion with Ca^{2+}-free K-H solution instead of the normal K-H solution for 50 min, the preparation continued to be exposed to Ca^{2+}-free K-H so-

lution containing Gli (20 μmol/L) for 20 min. And then NaHS (100 μmol/L) was added and APs were recorded.

1.2.6 *Electrophysiological effects of CSE inhibitor PPG on normal papillary muscles*

APs were recorded after equilibrium for 1 h. After recording of 3 control APs, PPG (200 μmol/L) was applied. APs were then recorded at 5 min interval lasting for 150 min after application of PPG.

1.3 *Biochemicals and reagent*

NaHS, Bay K8644 and PPG were purchased from Sigma. Gli was purchased from Tianjin Institute of Medical and Pharmaceutical Industry. NaHS was used as a donor of H_2S. NaHS was employed in these experiments for a better definition of H_2S concentration in solution than bubbling H_2S gas. NaHS dissociates to Na^+ and HS^- in solution. Thereafter HS^- associates with H^+ and produces H_2S. Approximately one-third of H_2S in aqueous solution exists in the undissociated form (H_2S), whilst the remaining two-thirds of H_2S exists as HS^- which is in equilibrium with H_2S[10]. Gli was initially dissolved in dimethylsulfoxide (DMSO, 100 μmol/L). The final concentration of DMSO in the K-H solution was 0.04% (*V*/*V*). Bay K8644 was dissolved in 99% ethyl alcohol. PPG was dissolved in distilled water.

1.4 *Statistics*

All data were presented as means±SEM. The differences of the parameters between before and after chemical application 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 AP in normal papillary muscles

Compared with that in the control group, PPD, APD_{50}, APD_{90} and APD were decreased by NaHS (50-200 μmol/L) in a concentration-dependent manner. The effects occurred after 5-20 min of superfusion of NaHS and reached the peak within 30-45 min. NaHS (50, 100 μmol/L) significantly decreased PPD, APD_{50}, APD_{90} and APD ($P<0.05$, $P<0.01$) but had no significant effect on the other parameters of AP ($P>0.05$). High concentration of NaHS (200 μmol/L) decreased not only PPD, APD_{50}, APD_{90} and APD ($P<0.001$), but also APA ($P<0.05$) (Table 1, Fig.1*A*).

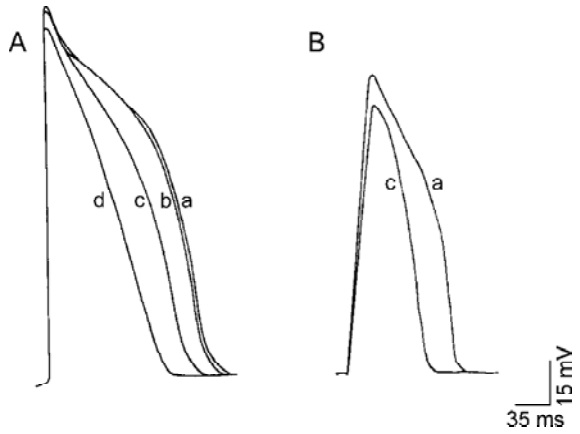

Fig.1. Effects of NaHS on the action potential in normal and partially depolarized guinea pig papillary muscles. *A*: Normal papillary muscle. a: control; b: 50 μmol/L NaHS; c: 100 μmol/L NaHS; d: 200 μmol/L NaHS. *B*: Partially depolarized papillary muscle. a: control; c: 100 μmol/L NaHS. The records in *A* or *B* were from the same cell. Similar results were obtained in others cells.

2.2 Effects of H_2S on AP in partially depolarized papillary muscles

In partially depolarized papillary muscles induced by high K^+, NaHS (100 μmol/L) not only decreased PPD, APD_{50}, APD_{90} and APD ($P<0.05$, $P<0.01$), but also reduced APA, OS and V_{max} ($P<0.01$, $P<0.001$) (Table 2, Fig.1*B*).

Table 1. Effects of H_2S on the parameters of action potential in guinea pig papillary muscles

Group	RP (mV)	OS (mV)	APA (mV)	V_{max} (V/s)	PPD (ms)	APD_{50} (ms)	APD_{90} (ms)	APD (ms)
Control	-84.2±2.3	34.9±3.8	119.0±3.1	185.9±14.2	120.6±3.1	157.2±3.8	179.3±3.6	190.1±4.1
NaHS 50 μmol/L	-83.9±2.0	35.0±3.6	118.8±2.9	189.5±18.0	109.9±3.3**	144.7±3.9**	167.6±2.9**	179.2±4.6*
NaHS 100 μmol/L	-83.3±2.7	34.6±2.9	117.9±2.8	184.9±13.1	97.2±3.8^{**++}	129.0±4.5^{**++}	153.8±4.1^{**++}	166.2±3.7^{**+}
NaHS 200 μmol/L	-84.1±1.8	32.1±2.6	116.3±2.3^{*+}	181.8±17.8	71.7±3.6$^{***+++\#}$	98.3±4.2$^{***+++\#}$	122.8±3.8$^{***++\#}$	135.2±3.5$^{***++\#}$
Washout	-83.8±1.6	35.2±4.0	119.2±3.8	188.8±19.2	122.5±4.5	159.5±5.4	181.5±4.3	191.7±4.9

RP, resting potential; OS, overshoot; APA, amplitude of action potential; V_{max}, maximal rate of depolarization at phase 0; PPD, plateau period duration; APD, duration of action potential; APD_{50}, 50% of APD; APD_{90}, 90% of APD. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* control; $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ *vs* NaHS (50 μmol/L); $^{\#}P<0.05$ *vs* NaHS (100 μmol/L). $n=6$.

Table 2. Effects of H_2S on the parameters of action potential in partially depolarized papillary muscles

Group	RP (mV)	OS (mV)	APA (mV)	V_{max} (V/s)	PPD (ms)	APD_{50} (ms)	APD_{90} (ms)	APD (ms)
Control	-55.7±2.3	35.8±3.1	91.3±2.8	31.8±5.1	98.2±4.2	130.1±5.6	142.3±5.4	154.5±5.3
NaHS	-54.6±2.7	25.9±3.8***	80.4±2.1**	17.5±4.5**	78.4±4.3**	106.3±5.7**	119.0±6.2*	127.9±4.9**
Washout	-56.1±2.6	36.2±4.1	92.2±3.7	28.3±5.7	96.2±4.9	127.7±6.1	140.4±6.7	154.3±6.2

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* control. $n=6$.

2.3 Effects of Gli on H_2S-induced changes of AP in papillary muscles

PPD, APD_{50}, APD_{90} and APD were significantly decreased by NaHS (100 μmol/L) compared with that in the control group ($P<0.01$). K_{ATP} channel blocker Gli (20 μmol/L) alone had no significant effect on AP ($P>0.05$). After pretreatment with Gli, the electrophysiological effects of NaHS (100 μmol/L) were partially inhibited. PPD, APD_{50}, APD_{90} and APD were significantly different from those in the control group ($P<0.05$, $P<0.01$) and those in NaHS group ($P<0.05$, $P<0.01$) (Table 3).

2.4 Effects of Bay K8644 on H_2S-induced changes of AP in papillary muscles

L-type calcium channel agonist Bay K8644 (0.5 μmol/L) markedly increased PPD, APD_{50}, APD_{90} and APD ($P<0.05$, $P<0.01$, $P<0.001$), but had no significant effect on RP, OS, APA and V_{max} ($P>0.05$). After pretreatment with Bay K8644, the electrophysiological effects of NaHS (100 μmol/L) were partially inhibited. PPD, APD_{50}, APD_{90} and APD were significantly different from those in the control group ($P<0.05$, $P<0.01$, $P<0.001$) and those in NaHS group ($P<0.05$, $P<0.01$, $P<0.001$) (Table 4).

2.5 Effects of H_2S on AP in normal papillary muscles superfused with Ca^{2+}-free K-H solution containing Gli

Superfusion with Ca^{2+}-free K-H solution instead of the normal K-H solution significantly shortened PPD, APD_{50}, APD_{90} and APD ($P<0.001$), and decreased OS and APA ($P<0.05$). After pretreatment with Ca^{2+}-free K-H solution containing Gli (20 μmol/L), the electrophysiological effects of NaHS (100 μmol/L) were completely inhibited. PPD, APD_{50}, APD_{90} and APD were not significantly different from those in Ca^{2+}-free K-H solution ($P>0.05$) (Table 5).

2.6 Effects of PPG on AP in normal papillary muscles

PPG (200 μmol/L) significantly increased PPD, APD_{50}, APD_{90} and APD ($P<0.01$), but had no significant effect on the other parameters of AP ($P>0.05$). The effects occurred after 30-40 min of superfusion of PPG and reached the peak within 90-120 min (Table 6).

Table 3. Effects of Gli (20 μmol/L) on H_2S-induced changes of action potential in guinea pig papillary muscles

Group	RP (mV)	OS (mV)	APA (mV)	V_{max} (V/s)	PPD (ms)	APD_{50} (ms)	APD_{90} (ms)	APD (ms)
Control	-82.1±3.1	36.7±2.5	118.8±2.4	176.3±15.3	77.6±2.6	105.2±3.1	131.3±3.3	143.1±3.5
NaHS	-80.7±3.2	35.8±2.8	116.4±2.7	173.9±18.2	55.4±3.2**	78.6±4.0**	105.1±5.1**	118.0±4.6**
Gli	-82.2±2.9	35.5±3.1	117.8±2.9	175.3±20.1	78.4±3.3^{++}	106.3±4.2^{++}	131.6±5.3^{++}	143.0±4.8^{++}
Gli+NaHS	-81.9±3.0	35.4±3.1	117.5±2.8	174.7±21.3	65.8±3.1$^{**+\#\#}$	91.1±3.9$^{**+\#\#}$	119.1±4.6$^{**+\#}$	132.3±5.1$^{*++\#}$
Washout	-81.8±2.2	36.2±3.8	118.1±3.3	177.4±22.3	79.6±3.4	107.7±4.3	132.3±5.1	144.5±4.9

$^{*}P<0.05$, $^{**}P<0.01$ *vs* control; $^{+}P<0.05$, $^{++}P<0.01$ *vs* NaHS; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* Gli. $n=6$.

Table 4. Effects of Bay K8644 (0.5 μmol/L) on H_2S-induced changes of action potential in guinea pig papillary muscles

Group	RP (mV)	OS (mV)	APA (mV)	V_{max} (V/s)	PPD (ms)	APD_{50} (ms)	APD_{90} (ms)	APD (ms)
Control	-83.3±2.3	33.7±3.8	116.9±2.6	223.0±23.1	92.6±4.7	123.3±5.1	154.1±4.9	169.7±4.3
NaHS	-82.9±3.7	33.1±4.2	115.8±4.3	218.2±30.6	68.3±3.7**	94.1±4.5**	126.3±3.1***	141.0±2.8***
Bay K8644	-83.1±4.3	34.2±5.2	117.4±3.3	235.8±40.0	106.5±2.9^{*+++}	140.1±3.6^{*+++}	171.7±4.3^{***+++}	186.2±3.2^{**+++}
Bay K8644 + NaHS	-82.8±4.2	33.6±4.9	116.5±4.2	221.2±36.1	83.2±3.5$^{*+\triangle\triangle}$	112.0±4.4$^{*+\triangle\triangle}$	143.0±3.8$^{**++\triangle\triangle}$	157.3±3.1$^{***+++\triangle\triangle\triangle}$
Washout	-83.9±4.3	33.9±5.1	118.0±4.8	228.1±24.8	93.5±3.8	124.5±4.7	155.3±3.9	169.9±4.4

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* control; $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ *vs* NaHS; $^{\triangle\triangle}P<0.01$, $^{\triangle\triangle\triangle}P<0.001$ *vs* Bay K8644. $n=6$.

Table 5. Effects of H_2S on the parameters of action potential in normal papillary muscles superfused with Ca^{2+}-free K-H solution containing Gli (20 μmol/L)

Group	RP (mV)	OS (mV)	APA (mV)	V_{max} (V/s)	PPD (ms)	APD_{50} (ms)	APD_{90} (ms)	APD (ms)
Control	-80.3±2.5	31.7±3.4	111.9±2.8	220.0±23.6	96.6±4.9	129.3±5.5	165.1±4.7	181.7±3.3
NaHS	-79.9±3.7	31.1±4.0	111.5±3.5	216.2±32.6	70.3±3.7**	101.1±4.8**	137.3±4.1**	153.5±4.8**
Ca^{2+}-free	-80.1±4.3	25.2±3.2*+	103.4±3.8*+	216.9±37.9	20.5±3.9***+++	38.1±4.6***+++	71.7±3.8***+++	84.2±3.3***+++
Ca^{2+}-free + Gli	-80.8±4.2	24.6±2.9*+	103.5±4.1*+	215.2±29.4	20.2±3.5***+++	38.3±4.4***+++	72.0±3.6***+++	83.8±2.9***+++
Ca^{2+}-free + Gli + NaHS	-81.8±3.5	24.3±3.3*+	102.9±3.7*+	216.2±35.2	20.0±3.6***+++	37.9±4.0***+++	71.4±3.7***+++	82.7±2.1***+++
Washout	-80.9±3.3	31.9±4.6	112.3±3.8	221.1±34.5	97.5±4.3	130.5±4.8	167.3±4.9	183.9±4.0

*P<0.05, **P<0.01, ***P<0.001 *vs* control; +P<0.05, +++P<0.001 *vs* NaHS. n=6.

Table 6. Effects of PPG (200 μmol/L) on the parameters of action potential in guinea pig papillary muscles

Group	RP (mV)	OS (mV)	APA (mV)	V_{max} (V/s)	PPD (ms)	APD_{50} (ms)	APD_{90} (ms)	APD (ms)
Control	-78.0±2.6	31.6±2.4	109.4±2.9	196.2±20.3	101.6±4.1	134.3±5.2	160.5±5.9	174.7±5.5
PPG	-81.5±3.1	30.6±3.9	112.0±3.0	198.5±21.1	110.2±4.2**	144.7±5.4**	170.0±6.2**	184.5±6.0**

**P<0.01 *vs* control. n=6.

3 DISCUSSION

The present study showed that H_2S could concentration-dependently decrease PPD, APD_{50}, APD_{90} and APD of AP in the guinea pig papillary muscles. It has been widely accepted that the APD is mainly dependent on PPD, which is influenced by potassium efflux and calcium influx. Therefore, any factor promoting potassium efflux or/and inhibiting calcium influx may decrease PPD.

The slow response AP was induced in the papillary muscles by exposure to K-H solution containing KCl (18 mmol/L) and isoprenaline (1.5 μmol/L). Under these conditions, calcium currents play an important role in the depolarization of AP, while both potassium currents and calcium currents play important roles in the repolarization of AP. H_2S significantly reduced not only APA, OS and V_{max}, but also PPD, APD_{50}, APD_{90} and APD of slow response AP. It's suggested that the decrease in calcium influx may be related to the effects of H_2S.

H_2S is the first identified gaseous opener of K_{ATP} channels in VSMCs[9] and K_{ATP} channels widely distribute in myocardium[17-19]. Geng *et al*. recently reported that H_2S could be endogenously produced by heart tissues, as a physiological cardiac function regulator, and mediated by K_{ATP} channel pathway[8]. So we observed the effects of K_{ATP} channel blocker Gli on H_2S-induced changes of AP. Gli could partially inhibit the electrophysiological effects of H_2S. The results indicate that the effects of H_2S on APD are in part due to the enhancement of potassium efflux through the opening of K_{ATP} channels.

In order to examine the effect of H_2S on calcium influx, we used the L-type Ca^{2+} channel agonist Bay K8644. Bay K8644 also partially inhibited the electrophysiological effects of H_2S. The results indicate that the reduction of calcium influx may also contribute to the effects of H_2S.

Moreover, to further analyze the mechanisms involved, we observed the effects of H_2S on AP in the normal papillary muscles in Ca^{2+}-free K-H solution containing Gli. Pretreatment with Ca^{2+}-free K-H solution containing Gli completely blocked the effects of H_2S. The results indicate that the effects of H_2S on APD are due to the changes of potassium and calcium currents.

In addition, it was observed that high concentration of H_2S (200 μmol/L NaHS) decreased APA, suggesting a possible inhibition of sodium channel or/and a reduction of calcium influx by H_2S.

The results so far only discussed the effects of exogenous H_2S. To determine the function of endogenous H_2S, PPG (an inhibitor of CSE) was used in our experiment. Zhao *et al*. reported that PPG might be membrane-permeable, and that it had the potential to be used to study the physiological function of endogenously produced H_2S[11]. In the present study, after pretreatment with PPG (200 μmol/L), PPD, APD_{50}, APD_{90} and APD were significantly increased compared with that in the control papillary muscles. These results indicate that endogenous H_2S generated by cardiac tissues may play an important role in regulating AP in guinea pig papillary muscles.

Recently, some reports indicated that H_2S exerted cardiovascular protective function. The reduced production

of endogenous H_2S is an essential factor in the development of spontaneous hypertension[20]. Moreover, Zhang *et al*. reported that endogenous H_2S was involved in the pathogenesis of rat's hypoxic pulmonary hypertension (HPH) and exogenously applied H_2S could exert protective effect during HPH [21]. In our research, H_2S decreased calcium influx, as a result, intracellular Ca^{2+} concentration declined and myocardial contractility weakened. The negative inotropic role of H_2S in the heart, which was also observed by Geng *et al*.[8], may be one of mechanisms by which H_2S exerts protective effect during spontaneous hypertension and HPH. In addition, we found H_2S decreased APD in papillary muscles, that is to say, H_2S could shorten the work time of papillary muscles and thus exerted a cardioprotective function.

In summary, our observations demonstrate that H_2S exhibits electrophysiological effects on guinea pig papillary muscles. These effects may be attributed to an increase in potassium efflux through the opening of K_{ATP} channels and a decrease in calcium influx. Endogenous H_2S may act as an important regulator in electrophysiological characters of papillary muscles.

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