Aims Hydrogen sulphide (H2S) is an endogenously generated gaseous transmitter that has recently been suggested to regulate cardiovascular functions. To date, there is no direct evidence for a potential role of H2S in regulating calcium channels in the heart. The present study aims to examine the hypothesis that H2S is a novel inhibitor of the L-type calcium channel current (ICa,L).

Methods and results Electrophysiological measurements were performed in cardiomyocytes isolated from Wistar-Kyoto and spontaneously hypertensive rats. Bath application of 100 \( \mu \text{M} \) NaHS (a H2S donor) significantly reduced the time required for the repolarization of the action potential. Inhibition of the peak ICa,L by NaHS was determined to be concentration-dependent (25, 50, 100, 200, and 400 \( \mu \text{M} \)). NaHS inhibited the recovery from depolarization-induced inactivation. Electric field-induced [Ca2+]i transients and contraction of single cardiomyocytes and isolated papillary muscles were reduced by NaHS treatment. In contrast, caffeine induced an increase in [Ca2+]i that was not altered by NaHS. NaHS had no effect on the KATP current or on the levels of cAMP and cGMP in the current study.

Conclusion H2S is a novel inhibitor of L-type calcium channels in cardiomyocytes. Moreover, H2S-induced inhibition of [Ca2+]i appears to be a secondary effect owing to its initial action towards ICa,L. The inhibitory effect of H2S on ICa,L requires further investigation, particularly in the exploration of new pathways involved in cardiac calcium homeostasis and disease pathology.

KEYWORDS Calcium; Ion channels; Myocytes

1. Introduction

Hydrogen sulphide (H2S) is an endogenously generated gaseous transmitter that has recently been suggested to regulate cardiovascular functions. Exogenous administration of H2S (in the form of NaHS—a water-soluble H2S donor) has been shown to decrease blood pressure in acute and chronic experimental rat models. The vasodilator effect of H2S has been ascribed to its ability to open ATP-sensitive potassium channels (KATP channels) in vascular smooth muscle cells (VSMCs). Furthermore, H2S has been shown to be an Akt-dependent pro-angiogenic factor in both in vitro and in vivo experiments.

Exogenous administration of H2S has been shown to be cardioprotective in various disease models. For example, administration of NaHS significantly decreases the duration and severity of ischaemia/reperfusion-induced arrhythmias and increases the viability of cardiomyocytes in isolated perfused rat hearts. NaHS treatment can also reduce infarct size in a rat model of coronary artery ligation. In addition, chronic treatment with NaHS for 3 months decreases medial thickening of intramyocardial coronary arteries, interstitial fibrosis, and reactive oxygen species production in spontaneously hypertensive rats (SHR). H2S has been shown to exert a negative inotropic effect in isolated perfused rat hearts and papillary muscles. These findings suggest that the negative inotropic effects of H2S are mediated via the opening of KATP channels and by reducing calcium influx. To date, no experimental evidence has been presented describing the mechanisms by which H2S mediates these cardiac effects.

Calcium homeostasis plays a pivotal role in myocardial physiology and diseases. The influx of calcium through the L-type calcium channels is a trigger for ryanodine-dependent calcium release from the sarcoplasmic reticulum, which plays an essential role in the excitation/contraction coupling in cardiomyocytes. Alterations in the density and function of L-type calcium channels have been implicated in a variety of cardiovascular diseases such as atrial fibrillation, cardiac hypertrophy, heart failure, and ischaemic heart disease. Moreover, administration of L-type calcium
channel blockers is recognized as an effective therapeutic for hypertension, cardiac hypertrophy, arrhythmia, congestive heart failure, and myocardial ischemia. To date, there is no direct evidence about the potential role of H$_2$S in regulating calcium channels in the heart. We hypothesize that H$_2$S may regulate cardiac function by modulating calcium channels in cardiomyocytes. Therefore, in the present study, we have determined the role that H$_2$S plays in the regulation of the L-type calcium current ($I_{Ca,L}$), intracellular calcium transient ([Ca$^{2+}$])i, and contraction in rat cardiomyocytes. Since H$_2$S has been shown to open K$_{ATP}$ channels in VSMCs, the potential role of H$_2$S on K$_{ATP}$ channels in cardiomyocytes was also examined. In addition, cAMP and cGMP, the well-known intracellular secondary messengers that regulate $I_{Ca,L}$ in cardiomyocytes, were also measured. To assess whether H$_2$S exerts differential effects in regulating $I_{Ca,L}$ and contraction in normal and hypertrophied cardiac myocytes, we determined the effects of H$_2$S in cardiomyocytes isolated from both Wistar-Kyoto (WKY) rats and SHR.

2. Methods

2.1 Animals

WKY rats and SHR were obtained from the Department of Experimental Animals, Chinese Academy of Sciences. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH) in the USA and was approved by the Ethics Committee of Experimental Research, Fudan University Shanghai Medical College.

2.2 Isolation of cardiomyocytes

Ventricular myocytes were obtained from the hearts of male WKY rats and SHR (200–250 g) by enzymatic dissociation according to the method described by Zhang et al. Isolated hearts were perfused using Langendorff apparatus with a buffer containing 1 mg/mL collagenase type II at 37°C to isolate the cells (see Supplementary material online).

2.3 Electrophysiological measurements

Cardiomyocytes were placed in a chamber on the stage of an inverted microscope (Leica, DMIL, Wetzlar, Germany) and continuously perfused at a constant rate (1.8 mL/min) with Tyrode’s solution (maintained at 35°C). The cells were patch-clamped in the whole-cell configuration using a patch-clamp amplifier (Axopatch 200B, Axon instruments, Burlingham, CA, USA). Signals were recorded and analysed using pClamp 6.0 and Clampfit 9.0 software (Axon instruments). Briefly, patch electrodes were fabricated from borosilicate glass tubes and filled with a pipette solution containing (in mM) CsCl 120, MgCl$_2$ 2, CaCl$_2$ 1, Na$_2$ATP 5, EGTA 11, and HEPES 10 (pH adjusted to 7.4 with CsOH). Filled electrodes had a tip resistance of 3–5 MΩ.

The $I_{Ca,L}$, action potential, and $K_{ATP}$ current were measured as described. $I_{Ca,L}$ was induced by repeatedly applying a single 200 ms depolarizing pulse to 0 mV from a holding potential of −40 mV at 6 s intervals. Action potentials were recorded under a current clamp mode with a two-fold threshold stimulation. The $K_{ATP}$ current was measured with a 9 s depolarizing ramp voltage clamp pulse from −120 to +60 mV (see Supplementary material online).

2.4 Measurement of [Ca$^{2+}$]i

[Ca$^{2+}$]i was determined in isolated cardiomyocytes loaded with fluo-3/AM using confocal microscopy as described. The [Ca$^{2+}$]i transient was elicited by application of a two-fold threshold stimuli (1 ms, 0.5 Hz), and the fluorescent signals were obtained using the excitation and emission wavelengths of 488 and 535 nm (see Supplementary material online).

2.5 Measurement of single cardiomyocyte contraction

Cardiomyocytes were perfused with Tyrode’s solution at 35°C and electrically stimulated (0.5 Hz). Contractions were monitored using an optical edge-tracking method using an inverted microscope and image-analysing software as described (see Supplementary material online).

2.6 Measurement of isolated papillary muscle contraction

Papillary muscles were mounted vertically between two spring clips in a 20 mL chamber containing Tyrode’s solution and electrically stimulated at a frequency of 12 pulses/min. Muscle contraction signals were recorded via a transducer and analysed with a computerized recording system as described (see Supplementary material online).

2.7 Measurement of cAMP and cGMP levels

cGMP and cAMP levels were determined using a commercially available enzyme immunoassay kit following the manufacturer’s instructions.

2.8 Statistical analysis

All experimental data are presented as means ± SEM. Differences between groups were analysed by one-way ANOVA followed by post hoc Tukey’s test where applicable. Significance was established at the P < 0.05 level.

3. Results

3.1 H$_2$S donor inhibits the time for repolarization of action potential

As shown in Figure 1, bath application of NaHS (100 μM) significantly reduced the time to 25, 50, and 90% repolarization (P < 0.05). This effect could be washed out. The amplitude of the action potentials were 93.00 ± 11.68, 95.45 ± 8.02, and 91.79 ± 11.4 mV at baseline, following the application of NaHS and the washout period in cardiomyocytes isolated from WKY rats, respectively. Amplitudes of the action potentials were 97.50 ± 6.83, 99.35 ± 6.77, and 97.80 ± 7.81 mV at baseline, following the application of NaHS and the washout period in cardiomyocytes isolated from SHR, respectively. The H$_2$S donor was found not to exert an effect on the amplitude of the action potentials, nor on the resting potentials. No significant differences were observed in the H$_2$S donor-induced decrease in repolarization time in cardiomyocytes isolated from either WKY rats or SHR.

3.2 H$_2$S donor inhibits $I_{Ca,L}$ in cardiomyocytes

As shown in Figure 2A and B, exposure of cardiomyocytes to 100 μM NaHS resulted in a 15.00 ± 2.08 and a 19.61 ± 2.18% decrease in peak $I_{Ca,L}$ in cardiomyocytes isolated from WKY rats and SHR, respectively (P < 0.05 vs. control). True values of $I_{Ca,L}$ were −3.21 ± 0.13 and −2.72 ± 0.24 pA/pF, respectively, before and after NaHS application in cardiomyocytes isolated from WKY rats. Similarly, in cardiomyocytes of SHR, $I_{Ca,L}$ values were −3.32 ± 0.16 and −2.67 ± 0.68 pA/pF,
was blunted by NaHS. Likewise, the inhibitory effects of NaHS towards WKY cardiomyocytes (Figure 2E) were comparable with that of SHR (Figure 2F). Figure 2G shows the concentration response relationship of NaHS-induced inhibition on peak \(I_{\text{Ca,L}}\) in response to NaHS application at concentrations of 25, 50, 100, 200, and 400 \(\mu M\) \((P < 0.05\) vs. control; \(K_d\) 87 ± 4 \(\mu M\) for WKY and 84 ± 6 \(\mu M\) for SHR). The inhibitory effects of NaHS on peak \(I_{\text{Ca,L}}\) in cardiomyocytes isolated from WKY rats were comparable with that of SHR. There were no significant differences in NaHS-induced inhibition on the peak of \(I_{\text{Ca,L}}\) in cardiomyocytes isolated from WKY rats and SHR. Figure 2H shows that NaHS and a H\(_2\)S gas solution exerted a similar concentration-dependent inhibitory effect on peak \(I_{\text{Ca,L}}\) at concentrations of 25, 50, 100, 200, and 400 \(\mu M\) \((K_d\) 87 ± 4 \(\mu M\) for NaHS and 84 ± 8 \(\mu M\) for H\(_2\)S).

The effect of NaHS on the \(-V\) relationship of \(I_{\text{Ca,L}}\) is shown in Figure 3A. Bath application of NaHS (100 and 400 \(\mu M\)) showed a concentration-dependent suppression on the peak of the \(-V\) curve without altering the reversal potential and the voltage dependence of peak \(I_{\text{Ca,L}}\). The effects of NaHS on the steady-state activation of \(I_{\text{Ca,L}}\) are shown in Figure 3B. These curves were fitted using the Boltzmann equation: \(G/G_{\text{max}} = 1/[1 + \exp(V_T - V_{1/2}/\kappa)]\), where \(G/G_{\text{max}}\) represents a ratio of conductances to the maximum conductance, and \(V_T\) represents the values of the depolarizing pulses. \(V_{1/2}\) (a half-maximum inactivation potential) values of the curves were –18.93 ± 1.47 and –18.53 ± 1.67 mV in the control and the NaHS-treated groups, respectively. \(K\) values were 7.53 ± 1.33 and 8.69 ± 1.54 in the control and NaHS-treated groups. There was no shift in the steady-state activation curve of \(I_{\text{Ca,L}}\) in cardiomyocytes treated with NaHS (100 \(\mu M\)). The effects of NaHS on the steady-state inactivation of \(I_{\text{Ca,L}}\) are shown in Figure 3C. These curves were fitted using the Boltzmann equation: \(I/I_{\text{max}} = 1/[1 + \exp(V_T - V_{1/2}/\kappa)]\), where \(I/I_{\text{max}}\) represents a ratio of currents to the maximum current, and \(V_T\) represents the values of the depolarizing potential of the pre-pulses. \(V_{1/2}\) values were –23.26 ± 0.79 and –22.55 ± 0.84 mV in the control and the NaHS-treated groups. \(K\) values were 6.55 ± 0.69 and 7.19 ± 0.74 in the control and the NaHS-treated groups. Bath application of NaHS (100 \(\mu M\)) did not cause any significant shift in the steady-state inactivation curve of \(I_{\text{Ca,L}}\). Figure 3D shows the effects of NaHS on the kinetics of recovery of \(I_{\text{Ca,L}}\) from inactivation. An increase in the interval between the conditioning pulse and the test pulse resulted in a recovery of \(I_{\text{Ca,L}}\) which can be described by the exponential equation: \(I/I_{\text{max}} = 1 - \exp(-t/\tau)\), where \(\tau\) represents the values of the interval, and \(\tau\) represents the time constant of \(I_{\text{Ca,L}}\) recovery from inactivation. The \(\tau\) value was 70.56 ± 4.43 and 73.21 ± 3.46 ms in the control group with a holding potential of –70 and –80 mV, respectively. Bath application of NaHS (100 \(\mu M\)) induced a shift in the recovery curve of \(I_{\text{Ca,L}}\) with a \(\tau\) value of 91.86 ± 6.81 and 94.12 ± 5.68 ms and a holding potential of –70 or –80 mV, respectively \((P < 0.05)\) vs. control. As the time interval increased to 50–200 ms, the \(I/I_{\text{max}}\) values in the NaHS-treated group were significantly decreased in comparison with that of the control group \((P < 0.05)\).

### 3.3 The effect of H\(_2\)S donor on \([\text{Ca}^{2+}]_i\)

The electrically induced transient increase in the integral of \([\text{Ca}^{2+}]_i\) was significantly inhibited by NaHS (100 \(\mu M\)) by
**Figure 2** H\(_2\)S and its donor inhibit \(I_{Ca,L}\). (A and B) The effects of NaHS (100 μM) on peak \(I_{Ca,L}\) as determined in isolated cardiomyocytes derived from WKY rats (\(n = 6\)) and SHR (\(n = 6\)), with a gradual washout period following the application of the compound tested. (C and D) The effects of NaHS on peak \(I_{Ca,L}\) in cardiomyocytes isolated from WKY rats (\(C, n = 6\)) and SHR (\(D, n = 6\)) without a washout period after application of the test compound. (E and F) The effects of NaHS on peak \(I_{Ca,L}\) in isolated cardiomyocytes of WKY rats (\(E, n = 6\)) and SHR (\(F, n = 6\)) that were stimulated with isoprenaline. (G) A concentration response relationship of NaHS-induced inhibition on \(I_{Ca,L}\). NaHS (25, 50, 100, 200, and 400 μM) caused a concentration-dependent inhibition on peak \(I_{Ca,L}\) at 1 min after application of the H\(_2\)S donor. The inhibitory effect of NaHS on peak \(I_{Ca,L}\) in cardiomyocytes isolated from WKY rats was comparable with that of SHR (\(n = 7\) for each group). (H) The inhibitory effects of NaHS and H\(_2\)S on \(I_{Ca,L}\) were comparable (\(n = 6\) for each group). Values are the means ± SEM; *\(P < 0.05\) vs. WKY control; #\(P < 0.05\) vs. SHR control.
3.4 H2S donor exerts a negative inotropic effect in isolated single cardiomyocytes and papillary muscles

As shown in Figure 5A and B, bath application of NaHS (100 μM) resulted in a decrease in cell shortening and in the maximal velocity of cell shortening (−dI/dt) and relaxation (−dL/dt) (P < 0.05 vs. control). NaHS (100 μM) caused a decrease in the shortening of cardiomyocytes in ~1 min. The observed effect peaked within ~2 min and lasted for ~3 min (Figure 5C, P < 0.05 vs. control). The inhibitory effects of NaHS (25, 50, and 100 μM) on cell shortening were concentration-dependent (Figure 5D). However, there was no significant difference in NaHS-induced inhibition of contraction in single cardiomyocytes isolated from WKY rats and SHR.

Bath application of NaHS (135 μM) rapidly caused a decrease in the active tension of the papillary muscles at 3 min. This effect peaked at 10 min and lasted for 20 min (Figure 5C, P < 0.05 vs. control). NaHS (135, 270, and 540 μM) concentration dependently caused a reduction in the active tension (Figure 5D) as well as in the maximal velocities of the contraction and relaxation, and electromechanical delay (Table 1). The time to peak tension and the time to 50% relaxation were not altered by NaHS (Table 1). Likewise, the NaHS-induced negative inotropic effects observed in the papillary muscles isolated from WKY rats were comparable with that of SHR except for electromechanical delay. NaHS was effective in decreasing the electromechanical delay in SHR but not in WKY rats at a concentration of 135 μM.

3.5 The effect of H2S donor on the KATP channels

As shown in Figure 6, the KATP channel opener, pinacidil (100 μM), significantly increased the KATP current elicited by a ramp voltage clamp pulse from −120 to +60 mV with an ATP concentration of 1 or 3 mM in the pipette. NaHS did not induce such a current at concentrations of 50 and 100 μM, suggesting that this compound was not able to open the KATP channels. At a higher concentration of 300 μM, NaHS exerted an effect in increasing the current

82.63 ± 4.04 and 83.03 ± 3.09% in cardiomyocytes isolated from WKY rats and SHR, respectively (P < 0.05). These observations suggest an inhibitory role of NaHS on calcium-induced calcium release in rat cardiomyocytes (Figure 4A and B). There was no significant difference in the inhibitory effects of NaHS on the integral of [Ca2+]i in cardiomyocytes isolated from either WKY rats or SHR.

The upper panel of Figure 4C shows that the response elicited by caffeine (10 mM) was ~30-fold greater than that elicited by electrical stimulation which was coupled with mechanical contraction. The lower panel in Figure 4C shows that a second application of caffeine did not cause a further release of calcium, suggesting that the first caffeine application had induced a complete release of calcium from internal stores. As shown in Figure 4D and E, a bath application of caffeine (10 mM) caused a transient increase in [Ca2+]i in cardiomyocytes of WKY rats and SHR. Furthermore, the observed caffeine-induced calcium release could not be inhibited by NaHS (100 μM) treatments.
induced by the aforementioned ramp voltage clamp pulse \( P, 0.05 \) vs. control.

3.6 \( \text{H}_2\text{S} \) donor has no effect on the intracellular cAMP and cGMP levels

Bath application of isoproterenol (10 \( \mu \)M) caused an increase in cAMP but not in cGMP levels. However, NaHS (100 \( \mu \)M) did not alter the levels of cAMP and cGMP in cardiomyocytes isolated from WKY rats and SHR in the presence or absence of isoproterenol (Table 2).

4. Discussion

The present study provides the first piece of evidence that \( \text{H}_2\text{S} \) and its donor NaHS are inhibitors of \( I_{\text{Ca,L}} \) in cardiomyocytes. Compared with \( \text{H}_2\text{S} \), NaHS has been shown to exert similar cardiovascular effects such as an opening of
KATP channels in VSMCs, while also having a pro-angiogenic effect in vascular endothelial cells. NaHS has been used in numerous studies to investigate the biological roles of H2S. In the present investigation, we show that NaHS has a similar inhibitory effect on I\textsubscript{Ca,L} in cardiomyocytes compared with gaseous H2S. Thus, NaHS was used as a donor of H2S in the present study.

We found that the I-V relationship curve was not shifted upon NaHS treatment. This suggests that the voltage dependence of the cardiac L-type calcium channels was not modified by an interaction with the H2S donor. Moreover, the H2S donor did not cause any change in the curves of the steady-state activation and inactivation. Our data highlight that there is no differential interaction of the H2S donor with the rested, inactivated, and activated L-type calcium channels. In contrast, we found that the H2S donor inhibited the recovery from depolarization-induced inactivation. This observation suggests that H2S does not rapidly dissociate from the L-type calcium channels.

**Table 1**  Effect of H2S on the contraction parameters of the isolated papillary muscles

<table>
<thead>
<tr>
<th>Concentration of NaHS ((\mu M))</th>
<th>(dT/dt) (%)</th>
<th>(-dT/dt) (%)</th>
<th>EMD (ms)</th>
<th>TPT (ms)</th>
<th>RT1/2 (ms)</th>
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<tbody>
<tr>
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<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
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<tr>
<td>Control</td>
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<td>100 ± 1</td>
<td>18 ± 1</td>
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<td>26 ± 2</td>
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<td>88 ± 6**</td>
<td>19 ± 1</td>
<td>28 ± 2**</td>
<td>144 ± 9</td>
</tr>
<tr>
<td>270</td>
<td>72 ± 6*</td>
<td>69 ± 3*</td>
<td>21 ± 1*</td>
<td>29 ± 2*</td>
<td>139 ± 9</td>
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<td>540</td>
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<td>41 ± 4**</td>
<td>28 ± 3*</td>
<td>38 ± 2**</td>
<td>139 ± 9</td>
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</table>

*Values are means ± SEM. \(dT/dt\), maximal rate of rise of tension, expressed as the percentage of baseline contraction; \(-dT/dt\), maximal rate of decrease of tension, expressed as the percentage of baseline contraction; EMD, electromechanical delay; TPT, time to peak tension; RT1/2, time to 50% relaxation. *\(P < 0.05\) vs. WKY control; **\(P < 0.05\) vs. SHR control.

**Figure 5**  H2S exerts a negative inotropic effect in isolated single cardiomyocytes and papillary muscles. (A) Time course of NaHS (100 \(\mu M\))-induced inhibition of cell shortening in cardiomyocytes isolated from WKY rats and SHR. (B) Concentration-dependent inhibition by NaHS (25, 50, and 100 \(\mu M\)) on cell shortening in isolated cardiomyocytes. Values are the means ± SEM; n = 13 in the WKY group and n = 14 in the SHR group; *\(P < 0.05\) vs. WKY control; #\(P < 0.05\) vs. SHR control. (C) The negative inotropic effects of NaHS (135 \(\mu M\)) on the active tension of isolated papillary muscles as a function of time; n = 13 in the WKY control group and n = 17 in the WKY NaHS-treated group; n = 13 in the SHR control and in the SHR NaHS-treated group. (D) The concentration-dependent inhibition of a cumulative bath application of NaHS (135, 270, and 540 \(\mu M\)) on the active tension of isolated papillary muscles; n = 8 in the WKY group and n = 7 in the SHR group. Values are the means ± SEM. *\(P < 0.05\) vs. WKY control/baseline; #\(P < 0.05\) vs. SHR control/baseline.
**H₂S inhibits the L-type calcium channels**

L-type calcium channels. However, the H₂S donor exerted no effect on the amplitude of the action potential. Whether H₂S is able to exert a direct action on sodium channels remains to be determined.

\[ \text{ICa,L} \] triggers an opening of the calcium-releasing channels located in the sarcoplasmic reticulum that leads to a release of calcium that can bind with troponin and induces the contraction of cardiomyocytes.⁹ In the present study, [Ca²⁺]i and cardiomyocyte contractions were also reduced in the presence of H₂S donor. Whereas, the caffeine-induced increase in [Ca²⁺]i was not inhibited. Though caffeine has been shown to be a phosphodiesterase inhibitor which also blocks the ryanodine receptors, this substance acts as a ryanodine receptor agonist at a concentration of 10 mM.²⁵ It is well established that caffeine at a 10 mM concentration induces an increase in [Ca²⁺]i by stimulating calcium release from the sarcoplasmic reticulum. Therefore, the present data suggest that H₂S blocks the L-type calcium channels in the cell membrane of cardiomyocytes but not the calcium-releasing channels in the sarcoplasmic reticulum. The inhibitory effects of H₂S on electrically induced [Ca²⁺]i may be an effect secondary to H₂S-induced inhibition of the initial transmembrane \[ \text{ICa,L} \]. In this context, the inhibition of H₂S on electrically induced contraction of cardiomyocytes can also be ascribed to an inhibition of \[ \text{ICa,L} \]. The inhibitory effect of H₂S on \[ \text{ICa,L} \] or [Ca²⁺]i, and contraction of the cardiomyocyte provides a new mechanism to regulate calcium homeostasis and cardiac function. An abnormal increase in [Ca²⁺]i in cardiomyocytes has been reported to be deleterious to the cells. Calcium overload leads to altered mitochondria function and promotes apoptosis by activation of calpain in cardiomyocytes. Chronic increases in [Ca²⁺]i also result in cardiomyocyte hypertrophy.¹² Whether the H₂S-induced decrease in \[ \text{ICa,L} \] and [Ca²⁺]i is capable of protecting cardiomyocytes against apoptosis and hypertrophy remains to be determined. The inhibitory effect of the H₂S donor on cardiomyocyte contraction identified in the present study suggests that the negative inotropic effects of H₂S may contribute to the decrease in blood pressure observed in SHR.³

The inhibitory effect of H₂S or its donor on \[ \text{ICa,L} \] was found to occur at concentrations of 25, 50, 100, 200, and 400 μM. The physiological levels of plasma H₂S have been reported to be ~50 μM in rats, ~34 μM in mice, and ~44 μM in human.²⁹ The localized tissue levels of H₂S are known to be higher than its circulatory levels. For instance, the physiological concentration of H₂S in brain tissues has been reported to be 50–160 μM.³⁶ When NaHS was dissolved in saline, about one-third of the H₂S exists as an un-dissociated anion.³¹ Therefore, the physiologically relevant concentrations of the H₂S donor (25–100 μM) that are effective at blocking the L-type calcium channels in vitro are likely attainable in vivo.

In the present study, the effects of the H₂S donor on \[ \text{ICa,L} \] and [Ca²⁺]i and contractions were examined in cardiomyocytes isolated from both SHR and WKY rats. Apart from differences in the electromechanical delay, the inhibitory effects of NaHS on the other parameters examined were comparable in cardiomyocytes of SHR and WKY rats. It has been shown that there is no difference in the molecular structure of the L-type calcium channels between SHR and WKY rats.³⁷ This may explain why the inhibitory effects of the H₂S donor on \[ \text{ICa,L} \] in SHR and WKY rats are comparable. Since

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**Figure 6** The effects of H₂S on K̄ATP channels. (A and B) Representative superimposed K̄ATP current traces recorded in the absence and presence of NaHS (50, 100, and 300 μM; n = 9 in each group), pinacidil (100 μM, n = 8 for the 1 mM ATP group and n = 7 for the 3 mM ATP group), and glybenclamide (30 μM, n = 6 in each group) with an ATP concentration of 1 mM (A) or 3 mM (B) in the pipette. (C) Graphs showing the mean values of the K̄ATP current in the absence and presence of NaHS (50, 100, and 300 μM), pinacidil (100 μM), and glybenclamide (30 μM). Values are the means ± SEM. *P < 0.05 vs. control (1 mM ATP in the pipette); #P < 0.05 vs. control (3 mM ATP in the pipette).

The current experiments showed that the time to repolarization of the action potential was decreased in the presence of the H₂S donor, suggesting that H₂S blocks the
ICa,L is a trigger of cell shortening and papillary muscle contraction, the comparable effect of the H2S donor on these parameters in SHR and WKY rats may also be ascribed to the similar action of this gaseous molecule on the L-type calcium channels. Indeed, these data are not sufficient to suggest a possible role of H2S in the pathogenesis of hypertension in SHR by exerting different actions on cardiomyocytes of normal and hypertensive rats. In this context, the vasodilator effect of this compound on peripheral resistance vessels may play a more important role in blood pressure regulation. However, the inhibitory effect of H2S on L-type calcium channels and cardiomyocytes and papillary muscle contraction suggest that this gaseous molecule plays a role in the regulation of calcium homeostasis and cardiac function in both normal and hypertensive rats. In subjects with severe heart failure, H2S may aggravate the disease by reducing cardiac output. In contrast, L-type calcium channel inhibitors have been shown to exert beneficial effects such as a reduction of myocardial hypertrophy and cardiomyocyte apoptosis in hypertrophied hearts without severe heart failure. Therefore, the inhibitory effect of H2S on L-type calcium channels may be beneficial for hypertrophied hearts without heart failure. The significance of the cardiac role of H2S in the pathogenesis of hypertension and cardiac hypertrophy remains to be investigated in various disease models. In such experiments, the role of endogenously generated H2S, specifically that locally generated in the heart and peripheral resistance vessels, should be examined.

In the cardiovascular system, H2S is endogenously generated from cysteine by cystathionine γ-lyase. Changes in H2S levels are associated with several pathologies such as hypertension in rats and inflammation in mice and in patients of septic shock. The results of the present study suggest that a change in H2S levels in diseases may result in an alteration in ICa,L, [Ca2+]i, and contraction of cardiomyocytes. An activation of the cAMP/PKA pathway causes an increase in intracellular calcium in cardiomyocytes of SHR. However, in the current study, the inhibitory effect of the H2S donor on ICa,L was not associated with a decrease in cAMP levels. These findings do not support a role of cAMP in mediating the inhibitory effect of the H2S donor on ICa,L. This is in accord with our previous study in that H2S caused a pro-angiogenic effect by modulation of an Akt-dependent pathway without any changes in cAMP and cGMP in vascular endothelial cells. Whether H2S interacts with multiple intracellular targets such as NO remains unknown. Moreover, it remains to be determined whether H2S exerts a direct or indirect effect on calcium-dependent enzymes required for the synthesis or breakdown of these second messengers.

Here we found that the H2S donor had no effect on electromechanical delay at a concentration that was effective in reducing active tension in cardiac papillary muscles of WKY rats. This observation suggests that this compound had no effect on intercellular coupling in normal myocardium. Interestingly, the H2S donor increased electromechanical delay in the myocardium of SHR, suggesting an inhibition on the intercellular coupling in hypertrophied myocardium. The pathophysiological role of this different action in normal and hypertrophied myocardia remains to be determined.

H2S and its donor have been shown to activate the KATP channels in VSMCs. In the present study, the H2S donor (50 and 100 µM) did not exert an effect similar to that elicited by the known KATP channel opener pinacidil, suggesting that this H2S donor does not open cardiac KATP channels at physiologically relevant concentrations. The mechanisms underlying the increase in such a current, elicited by NaHS at a concentration of 300 µM, remain to be investigated.

In conclusion, as summarized in Supplementary material online, Figure S1, H2S is a novel inhibitor of L-type calcium channels in cardiomyocytes. H2S-induced inhibition of ICa,L is a secondary effect due its initial inhibitory effects towards ICa,L rather than a direct action on the calcium-releasing channels located in the sarcoplasmic reticulum. The effect of H2S is not associated with any alterations in cAMP and cGMP levels. H2S-induced inhibition of ICa,L is associated with decreased contractility of isolated cardiomyocytes and papillary muscles. The effect of H2S as an L-type calcium channel blocker requires further investigation, particularly in the exploration of new pathways involved in cardiac calcium homeostasis and diseases.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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H₂S inhibits the L-type calcium channels

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