Hydrogen sulphide increases pulmonary veins and atrial arrhythmogenesis with activation of protein kinase C

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Abstract
Hydrogen sulphide (H₂S), one of the most common toxic air pollutants, is an important aetiology of atrial fibrillation (AF). Pulmonary veins (PVs) and left atrium (LA) are the most important AF trigger and substrate. We investigated whether H₂S may modulate the arrhythmogenesis of PVs and atria. Conventional microelectrodes and whole-cell patch clamp were performed in rabbit PV, sinoatrial node (SAN) or atrial cardiomyocytes before and after the perfusion of NaHS with or without chelerythrine (a selective PKC inhibitor), rottlerin (a specific PKC α inhibitor) or KB-R7943 (a NCX inhibitor). NaHS reduced spontaneous beating rates, but increased the occurrences of delayed afterdepolarizations and burst firing in PVs and SANs. NaHS (100 μmol/L) increased I_{KATP} and I_{NCX} in PV and LA cardiomyocytes, which were attenuated by chelerythrine (3 μmol/L). Chelerythrine, rottlerin (10 μmol/L) or KB-R7943 (10 μmol/L) attenuated the arrhythmogenic effects of NaHS on PVs or SANs. NaHS shortened the action potential duration in LA, but not in right atrium or in the presence of chelerythrine. NaHS increased PKC activity, but did not translocate PKC isoforms α, ε to membrane in LA. In conclusion, through protein kinase C signalling, H₂S increases PV and atrial arrhythmogenesis, which may contribute to air pollution-induced AF.

KEYWORDS
atrial fibrillation, hydrogen sulphide, protein kinase C

1 | INTRODUCTION

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, increases the incidences of heart failure, stroke and mortality.¹-⁴ Air pollution increases the risk of AF.⁵,⁶ Each 6.0 μg/m³ increase in PM₂.₅ increases the risk of AF by 26%.⁵ The mechanisms underlying air pollution-induced arrhythmogenesis remain unclear. Air pollution is associated with autonomic tone changes,⁷ inflammation⁸ and cardiac ischaemia.⁹ Hydrogen sulphide (H₂S) is one of the most common toxic air pollutants.¹⁰ H₂S is produced by the anaerobic...
bacterial breakdown of sulphur-containing matter and can be found in various natural environments and industrial settings, such as spas, sewers, landfills, waste water plants and oil refineries. Increases in the H₂S concentration are associated with daily all-natural-cause mortality and cardiovascular hospitalization. However, it is not elucidated whether H₂S may play a role in the pathophysiology of air pollution-induced AF.

Pulmonary veins (PVs) and left atrium (LA) are the most important AF triggers and substrates. Calcium dysregulation plays a critical role in the occurrences of AF and PV arrhythmogenesis. The activation of Na⁺/Ca²⁺ exchangers (NCXs) induces delayed afterdepolarizations (DADs) and increases PV arrhythmogenic activity. Protein kinase C (PKC)-mediated signalling plays an important role in NCX activation. H₂S was known to activate PKC, thus H₂S may increase lnCX and increase PV arrhythmogenesis leading to AF genesis. Moreover, sinoatrial node (SAN) dysfunction plays an important role in AF pathophysiology and increases PV arrhythmogenesis. H₂S reduces the electrical activity of SANs, which may modulate the arrhythmogenesis of PVs and increase the risk of AF genesis.

H₂S plays a critical role in cell signalling and attenuates ischaemia-reperfusion injury by activating the ATP-sensitive potassium channel (KATP). The activation of the KATP channel shortens the action potential duration (APD), which may increase the risk of AF by facilitating the genesis of re-entry circuits. Previous studies have revealed that IₖATP differentially regulates the electrical activity of right atrium (RA) and LA. The different effects of IₖATP on APD shortening in the LA and RA can increase the risk of cardiac arrhythmias due to increasing interatrial dispersion. Accordingly, H₂S may modulate the electrical activity of PVs, atria and SANs and increase the risk of air pollution-induced AF. This study explored the effects of H₂S on PVs, atria and SANs, and investigated the potential underlying mechanisms.

## 2 METHODS

### 2.1 Animal and tissue preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Taipei Medical University and conformed to the Institutional Guidelines for the Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals published by the United States National Institute of Health. As described previously, male rabbits (1.5-2 kg) were anaesthetized with an intravenous injection of sodium pentobarbital (100 mg/kg). The adequacy of the anaesthesia was confirmed by the lack of corneal reflexes and motor responses to pain stimuli induced using a scalpel tip. The heart and lungs were rapidly excised following midline thoracotomy. For SAN tissue preparation, SANs were isolated from the RA and superior vena cava. PVs were separated from the atria at the LA-PV junction and from the lungs at the end of PV myocardial sleeves. One end of the preparation was pinned to the bottom of a tissue bath using needles, and the other end was connected to a Grass Instruments FT03C force transducer (MA, USA) using silk thread. Tissue preparations were superfused with normal Tyrode’s solution composed of NaCl (137 mmol/L), KCl (4 mmol/L), NaHCO₃ (15 mmol/L), NaH₂PO₄ (0.5 mmol/L), MgCl₂ (0.5 mmol/L), CaCl₂ (2.7 mmol/L) and dextrose (11 mmol/L) at a constant rate of 3 mL/min at 37°C as described previously. NaH(Sigma, MO, USA) was used as a donor of H₂S. PVs, atria and SANs were exposed to different concentrations of NaHS (1, 10, and 100 μmol/L) in Tyrode’s solution for 40 minutes to investigate the electrophysiological effects of H₂S.

### 2.2 Electrophysiological and pharmacological studies

The transmembrane APs of PVs, SANs and atria were recorded using machine-pulled glass capillary microelectrodes filled with 3 mol/L KCl; the microelectrodes were connected to a World Precision Instrument model FD223 electrometer (FL, USA) under a tension of 150 mg. The electrical and mechanical events were simultaneously displayed on a Gould 4072 oscilloscope (OH, USA) and Gould TA11 recorder. Electrical stimuli were applied using a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. For PVs with spontaneous activity and SANs, the APs were recorded for 20 minutes. For the LA and RA, the AP parameters were measured with 2-Hz electrical stimuli. The AP amplitude (APA) was determined by measuring the difference between the resting membrane potential (RMP) and the peak of AP depolarization. The APD at repolarization extents of 90%, 50% and 20% of the APD were measured and designated APD₉₀, APD₅₀ and APD₂₀, respectively. Burst firing was defined as the occurrence of accelerated spontaneous activities (faster than the basal rate) with sudden onset and termination. DAD was defined as the presence of a spontaneous depolarization of the impulse after complete repolarization. The electrical and mechanical events (contractility and diastolic tension) were continuously and simultaneously displayed and recorded during all aforementioned procedures. To investigate the electrophysiological effects of H₂S, a physiological concentration of NaHS (100 μmol/L) was administered with or without KB-R7943 (a NCX inhibitor, 10 μmol/L), chelerythrine (a selective PKC inhibitor, 3 μmol/L) or rottlerin (a specific PKC δ inhibitor, 10 μmol/L) in PVs, atria and SANs.

### 2.3 Electropharmacological studies in isolated single PV and atrial cardiomyocytes

Pulmonary vein and atria cardiomyocytes from rabbits were enzymatically dissociated, as previously described. The whole-cell patch clamp technique was performed in the PV and atrial cardiomyocytes with pacemaker activity before and after the administration of NaHS with or without chelerythrine; the APs were recorded using an Axopatch 1D amplifier (Axon Instruments, California, USA) at 35°C ± 1°C. The ionic currents were recorded in the voltage clamp mode. For the lnCX, PV and atrial cardiomyocytes were perfused with an external solution containing NaCl (140 mmol/L), CaCl₂ (2 mmol/L), MgCl₂ (1 mmol/L), glucose (10 mmol/L) and HEPES
(5 mmol/L) (pH adjusted to 7.4 with NaOH/HCl). Micropipettes were filled with a solution containing NaCl (20 mmol/L), CsCl (110 mmol/L), MgCl₂ (0.4 mmol/L), CaCl₂ (1.75 mmol/L), TEACl (20 mmol/L), BAPTA (5 mmol/L), glucose (5 mmol/L), MgATP (5 mmol/L) and HEPES (10 mmol/L) (pH adjusted to 7.25 with CsOH). The I\text{NCX} was elicited through depolarization in 10-mV steps from a holding potential of −40 mV to test potentials from −100 to +100 mV for 300 mseconds at a frequency of 0.1 Hz. The I\text{NCX} amplitudes were measured as 10-mmol/L nickel-sensitive currents. For the I\text{KATP}, PV and atria cardiomyocytes were perfused with an external solution containing NaCl (135 mmol/L), KCl (5.4 mmol/L), MgCl₂ (1.0 mmol/L), CaCl₂ (1.0 mmol/L), NaH₂PO₄ (0.33 mmol/L), HEPES (10 mmol/L) and glucose (10 mmol/L) (pH adjusted to 7.4 with NaOH). CdCl₂ (0.2 mmol/L) and 4-aminopyridine (2 mmol/L) were added to the external solution to inhibit Ca²⁺ and transient outward currents, respectively.³¹ Micropipettes were filled with a solution containing KCl (140 mmol/L), MgCl₂ (1.0 mmol/L), HEPES (10 mmol/L), EGTA (5 mmol/L) and GTP (0.1 mmol/L) (pH adjusted to 7.3 with KOH).

2.4 | Cell fractionation and Western blot for translocated PKC

Membrane and cytosol fractions were performed from LA tissues using Mem-PER Plus Membrane Protein Extraction Kit (Thermo Scientific, Waltham, MA) according manufacturer’s instructions.

**FIGURE 1** Effects of NaHS on the spontaneous activity of pulmonary veins (PVs) and sinoatrial nodes (SANs). Representative recordings and average data of beating rates in PVs (n = 10, panel A) and SANs (n = 9, panel B) before and after superfusion with different concentrations of NaHS (1, 10, and 100 μmol/L) with NaOH. CdCl₂ (0.2 mmol/L) and 4-aminopyridine (2 mmol/L) were added to the external solution to inhibit Ca²⁺ and transient outward currents, respectively.³¹ Micropipettes were filled with a solution containing KCl (140 mmol/L), MgCl₂ (1.0 mmol/L), HEPES (10 mmol/L), EGTA (5 mmol/L) and GTP (0.1 mmol/L) (pH adjusted to 7.3 with KOH).
Briefly, LA tissues with or without NaHS (100 μmol/L) incubation for 40 minutes were homogenized in Permeabilization Buffer on for 10 minutes on ice with agitation. The cell lysate was centrifuged at 16,000 g at 4°C for 15 minutes, and the supernatant was saved as cytosolic protein. The pellets were resuspended in Solubilization Buffer at 4°C for 30 minutes with constant shaking and were then centrifuged at 16,000 g at 4°C for 15 minutes. The resulting supernatants were collected as membrane fraction.

For immunoblotting of PKC proteins, 70 μg of cytosolic and 100 μg of membrane proteins were separated on 8% SDS-PAGE and transferred by electrophoresis onto an equilibrated polyvinylidene difluoride membrane. Blots were probed with primary antibodies against PKC α (GeneTex), PKC ε (Abcam), GAPDH and secondary antibodies conjugated with horseradish peroxidase (HRP). Bound antibodies were detected with an enhanced chemiluminescence detection system and analysed with AlphaEase FC software. All targeted bands were normalized to GAPDH to confirm equal protein loading.

2.5 Protein kinase C activity assay

Protein kinase C activity was assayed using PKC Kinase Activity Assay Kit (Abcam) as manufacturer’s instructions. Briefly, total proteins from LA tissues with or without NaHS (100 μmol/L) incubation for 40 minutes were added to PKC substrate coated wells of a 96-well microtitre plate, and reactions were initiated by adding ATP. The phosphorylated substrates were recognized by a phospho-PKC substrate-specific antibody and a secondary antibody conjugated with HRP. Bound antibodies were detected with TMB substrate, and the absorbance was measured at OD450 nm. Relative kinase activity was calculated from standard curve and normalized to individual control.

2.6 Measurement of intracellular reactive oxygen species

Pulmonary vein cardiomyocytes were treated with NaHS (100 μmol/L) for 40 minutes, and the reactive oxygen species (ROS) sensitive fluorescent probe CellROX Deep Red reagent (5 μmol/L, Life Technologies) was added 30 minutes before the end of the treatment. The fluorescent signals were detected on a laser scanning confocal system (Zeiss LSM 510, Carl Zeiss) equipped with the inverted microscope (Axiovert 100, Carl Zeiss) using a 60×1.4 numerical aperture oil immersion objective as described previously.

**Figure 2** NaHS-induced triggered activity or burst firing in pulmonary vein (PV) and sinoatrial node (SAN) preparations. A, Representative recordings of delayed after depolarization (↓) and burst firing (*) in PV preparations superfused with NaHS (100 μmol/L). B, Representative recordings of delayed after depolarization (↓) and burst firing (*) in SAN preparations superfused with NaHS (100 μmol/L). C, KB-R7943 (10 μmol/L) suppressed NaHS (100 μmol/L)-induced burst firing (*) and delayed afterdepolarization (↓) in PVs. D, KB-R7943 (10 μmol/L) suppressed NaHS (100 μmol/L)-induced delayed afterdepolarization (↓) and burst firing (*) in SANs.
Fluorescent images were analysed using Image-Pro Plus 6.0 and SigmaPlot 12.3 software.

2.7 | Statistical analyses

All continuous variables are expressed as mean ± standard error of mean. One-way repeated-measures analysis of variance followed by Bonferroni’s analysis was used to compare the differences in PVs, SANs and LA before and after drug administration. The chi-square analysis with Fisher’s exact test was used to compare the incidences of DADs and burst firing in PVs and SANs before and after drug administration. P < .05 was considered statistically significant. Statistical analysis was performed using SigmaPlot 12 (Systat software).

3 | RESULTS

3.1 | Effects of H₂S on the electrical activity of PVs, and SANs

As shown in Figure 1A, NaHS (1, 10, and 100 μmol/L) significantly reduced the PV beating rates in a concentration-dependent manner. However, as shown in Figure 2A, NaHS (1, 10 and 100 μmol/L) induced the occurrences of DADs in 6 PVs (60% vs 0% at baseline, P < .05) and induced burst firing in 5 PVs (50% vs 0% at baseline, P < .05). Similarly, NaHS (1, 10, and 100 μmol/L) concentration dependently reduced SAN beating rates (Figure 1B). Furthermore, NaHS (1, 10, and 100 μmol/L) induced the occurrences of DADs in 3 SANs (33% vs 0% at baseline, P > .05) and burst firing in 2 SANs (22% vs 0% at baseline, P > .05; Figure 2B). NaHS reduced SAN and PV beating rates to a similar extent (22% vs 23%, P > .05) but induced higher arrhythmogenicity in PVs than in SANs. Figures 2C,D show the effects of KB-R7943 on NaHS-induced PV and SAN arrhythmogenesis. In 6 of PVs with NaHS-induced triggered activity, KB-R7943 (10 μmol/L) reduced the occurrences of triggered DADs (100% vs 0%, P < .05) but did not change PV beating rates. Moreover, in 3 PVs with NaHS-induced burst firing, KB-R7943 reduced the occurrences of burst firing (100% vs 0%, P > .05). Similarly, in 3 SANs with NaHS-induced triggered activity, KB-R7943 reduced the occurrences of the triggered DADs (100% vs 0%, P > .05). In 2 SANs with NaHS-induced burst firing, KB-R7943 reduced the occurrence of burst firing (100% vs 0%, P > .05) but did not change SAN beating rates.

As shown in Figure 3, in the presence of chelerythrine (3 μmol/L), NaHS (100 μmol/L) did not change the beating rates or induce triggered activity and burst firing in PVs and SANs. Similarly, NaHS (100 μmol/L) did not change the beating rates, triggered activity and burst firing in PVs in the presence of rottlerin (10 μmol/L).

3.2 | Effects of H₂S on atrial electrical activity

NaHS at 100 μmol/L, but not at 1 and 10 μmol/L, significantly shortened APD₉₀ and reduced the contractility of the LA (Figure 4A).
However, NaHS did not change the APA, RMP, APD20, APD50, APD90 and contractility of the RA (Figure 4B). In LA tissues pretreated with chelerythrine (3 μmol/L), NaHS (100 μmol/L) did not shorten APD90 or reduce contractility (Figure 4C).

3.3 | Effects of H2S on IKATP and INCX

We investigated the effects of NaHS on IKATP and INCX in isolated single PV and atrial cardiomyocyte. As shown in Figure 5, NaHS (100 μmol/L) significantly increased the IKATP and the forward mode of the INCX. However, in the presence of chelerythrine (3 μmol/L), NaHS did not change the IKATP and INCX in PV cardiomyocytes.

We compared the effects of NaHS on IKATP and INCX in LA and RA cardiomyocytes. As shown in Figure 6, NaHS significantly increased the INCX and IKATP in LA cardiomyocytes, but not in RA cardiomyocytes.

3.4 | Effect of NaHS on translocation of PKC isoforms, PKC activity and ROS

As shown in Figure 7A,B, NaHS did not change the membrane to cytosol ratios of PKC α and ε in LA. However, NaHS-treated LA had larger PKC activity than those without treatment. As shown in 7C, NaHS-treated PV cardiomyocytes had lower ROS in cytosol than did control PV cardiomyocytes.

4 | DISCUSSION

Air pollution is caused by multiple air pollutants, including H2S. This study is the first to report that H2S induces the occurrences of DADs and burst firing in PVs and SANs. H2S-induced PKC activation is reported to play a role in regulating intracellular calcium handling by facilitating cytosolic calcium clearing through NCX channel in the development of calcium overloading and cardiomyocyte hypercontraction induced by ischaemic-reperfusion insults.26 In single-cell experiments, we revealed that H2S increased the forward mode of the INCX in the PV cardiomyocytes, which was attenuated by PKC inhibition. In addition, we observed that KB-R7943 and chelerythrine suppressed NaHS-induced PV and SAN arrhythmogenesis. These results suggest that H2S-induced PKC signalling increases PV and SAN arrhythmogenesis with the activation of NCX.

Hydrogen sulphide has been reported to exert a negative chronotropic effect in SANs; this effect is inhibited by the KATP channel.
blocker, glibenclamide. Similarly, this study found that H$_2$S significantly reduced SAN beating rates, and this effect was attenuated by PKC inhibition. In single-cell experiments, we revealed that NaHS increased the $I_{\text{KATP}}$ in PV cardiomyocytes, which was attenuated by PKC inhibition. Because PKC activation is required for $K_{\text{ATP}}$ channel opening, these results indicate that H$_2$S modulates SAN function by activating PKC and $K_{\text{ATP}}$ channels. SAN dysfunction plays an important role in AF pathophysiology and increases PV arrhythmogenesis. Accordingly, H$_2$S may modulate SAN function and result in PV arrhythmogenesis and AF occurrence.

In the present study, NaHS increased $I_{\text{KATP}}$ and $I_{\text{NCX}}$ in PV cardiomyocytes, which were attenuated by chelerythrine (a selective PKC inhibitor). Additionally, chelerythrine and rottlerin (a specific PKC δ inhibitor) attenuated the arrhythmogenic effects of NaHS, thereby suggesting that PKC may mediate the effects on membrane ion currents caused by H$_2$S. H$_2$S diffuses through the cell membrane directly because the H$_2$S molecule is very small and non-polar. H$_2$S has been shown to activate different PKC isoforms directly. Protein kinase-catalysed phosphorylation can regulate the activity of ion channels, including the $K_{\text{ATP}}$ and NCX channel. The activation of PKC increases the open probability of $K_{\text{ATP}}$ channel and acts via phosphorylation of a specific, conserved threonine residue in the $K_{\text{ATP}}$ channel. In addition, PKC directly phosphorylates NCX channel, which significantly enhances $I_{\text{NCX}}$.

Protein kinase C exists as several different isoforms and six isoforms (α, β, δ, ε, η, and ζ) were detected in hearts, among which PKC isoforms α, δ and ε are the prominent isoforms expressed in the heart. Moreover, chelerythrine is well-known to inhibit PKC α, β1, γ and δ. Therefore, PKC α and PKC δ are more likely to be essential to NaHS-mediated arrhythmogenesis. We found that NaHS did not induce translocation of PKC isoforms α, ε from cytosol to membrane but did increase PKC kinase activity. In the presence of

**FIGURE 5** Effects of NaHS on $I_{\text{NCX}}$ and $I_{\text{KATP}}$ in PV cardiomyocytes. A. The tracings and current-voltage relationship of $I_{\text{NCX}}$ in PV cardiomyocytes before and after NaHS (100 μmol/L) with (n = 8) and without (n = 9) chelerythrine (3 μmol/L). B. The tracings and current-voltage relationship of $I_{\text{KATP}}$ in PV cardiomyocytes before and after NaHS (100 μmol/L) with (n = 8) and without (n = 7) chelerythrine (3 μmol/L). The insets in the current traces show the various clamp protocols.

*P < .05, **P < .01, ***P < .005 vs baseline
rottlerin at 10 μmol/L, NaHS did not change PV electrical activity. These findings suggested that H2S activates PKC δ and results in its arrhythmogenesis.

The present study revealed that H2S differentially changed the cardiac electrophysiology of the LA and RA, whereas H2S significantly shortened the APD and reduced the contractility of the LA but not of the RA. These effects were attenuated by chelerythrine, suggesting that PKC signalling plays a vital role in the effects of H2S. The different effects of H2S on APD shortening in the LA and RA increase the dispersion of the APD, facilitating the maintenance of cardiac arrhythmias. Nevertheless, the mechanisms underlying the different effects of H2S in the LA and RA are unclear. A previous study reported that the higher expression of heat stress protein 70 in the RA may attenuate the response of the RA to the activation of the KATP channel by hypoxia and reperfusion.40,41 Previous studies have shown that LA plays a critical role in AF genesis compared to RA. Therefore, H2S may have different electrophysiological effects on RA and LA cardiomyocytes. We found that NaHS significantly increased the lNCX and lKATP in LA cardiomyocytes but not in RA cardiomyocytes, which may result in the shortening of APD in NaHS-treated LA.

Air pollutant is known to increase oxidative stress. We evaluated the effects of H2S on oxidative stress in PV cardiomyocytes by measurement of intracellular ROS using a laser scanning confocal microscope and found that NaHS-treated PV cardiomyocytes had lower cytosol ROS than did control PV cardiomyocytes. Similarly, previous study has shown that H2S reduces oxidative stress in mouse model.42 These findings suggested that oxidative stress does not underlie the effects of H2S on cardiomyocytes, and H2S may activate PKC through its direct chemical effects, leading to the increases in lNCX and lKATP.

The effects of H2S has been extensively studied as an environmental pollutant.43,44 Although H2S has been widely recognized as a cardioprotective agent for majority of cardiac disorders such as

**FIGURE 6** Effects of NaHS on lNCX and lKATP in atrial cardiomyocytes. A, The tracings and current-voltage relationship of lNCX in left atrial (LA, n = 8) and right atrial (RA, n = 9) cardiomyocytes before and after NaHS (100 μmol/L). B, The tracings and current-voltage relationship of lKATP in LA (n = 9) and RA (n = 8) cardiomyocytes before and after NaHS (100 μmol/L). The insets in the current traces show the various clamp protocols. *P < .05, **P < .01 vs baseline.
myocardial infarction/reperfusion injury, cardiac hypertrophy, myocardial fibrosis and heart failure, acute exposures of H2S may cause cardiac arrhythmia. Inhaled H2S induces sinus bradycardia and sinus arrest. Circulating halogen reactants cause cardiac injury by damaging important intracellular Ca2+ regulators. Although we do not provide a direct relationship between H2S and AF, our works suggest that H2S increases PV and SAN arrhythmogenesis and regulates atrial electrophysiology which contribute to AF.

This study should be interpreted with caution due to the potential limitations. Air pollutants may trigger the occurrence of AF via direct and/or indirect effects on the atrial myocardium. In this study, we found that H2S has direct electrophysiological effects on AF substrates and triggers, supporting that H2S may contribute to air pollution-induced AF at least in part. However, simply investigating H2S may not fully uncover the mechanisms of polluted air-induced PV and atrial arrhythmogenesis as air pollution contains multiple pollutants in addition to H2S. In addition, this study found that chelerythrine or rottlerin had inhibitory effects on NaHS-induced arrhythmogenesis, and NaHS-treated atrium had larger PKC activity than those without treatment, suggesting that PKC pathway plays a crucial role in H2S-mediated arrhythmogenesis. Nevertheless, chelerythrine is an inhibitor with multiple functions, it is also an antagonist of G-protein-coupled CB1 receptors. Studies have suggested that activation of CB1 receptors promotes activation of mitogen-activated protein kinases p38 and JNK. Mitogen-activated protein kinase is known to be functional in cardiomyocytes and is activated in response to stress, reactive oxygen species and inflammation. Rottlerin, a PKC δ inhibitor, is also an uncoupler of mitochondrial oxidative phosphorylation. Therefore, the electrophysiological data in this study did not exclude the possibility that several signalling pathways may involve the effects of H2S-mediated arrhythmogenesis. The precise signalling underlying the effects of H2S may not be fully elucidated.

5 | CONCLUSION

Hydrogen sulphide increases the arrhythmogenesis of PVs and SANs and differentially regulates the cardiac electrophysiology of the LA and RA. The activation of PKC signalling and increases in the I_{KATP} and I_{NCX} induced by H2S in PV and SAN cardiomyocytes may contribute to air pollution-induced AF.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to disclose.

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REFERENCES

1. Kannel WB, Abbott RD, Savage DD, McNamara PM. Epidemiologic features of chronic atrial fibrillation: the Framingham study. N Engl J Med. 1982;306:1018-1022.
2. Wolf PA, Abbott RD, Kannel KB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke. 1991;22:983-988.
3. Krahn AD, Manfreda J, Tate RB, Mathewson FA, Cuddy TE. The natural history of atrial fibrillation: incidence, risk factors, and prognosis in the Manitoba follow-up study. Am J Med. 1995;98:476-484.
4. Benjamin EJ, Wolf PA, D’Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation. 1998;98:496-502.
5. Link MS, Gibson HL, Schwartz J, et al. Acute exposure to air pollution triggers atrial fibrillation. J Am Coll Cardiol. 2013;62:816-825.
6. Rich DQ, Mittleman MA, Link MS, et al. Increased risk of paroxysmal atrial fibrillation episodes associated with acute increases in ambient air pollution. Environ Health Perspect. 2006;114:120-123.

7. Gold DR, Litonjua A, Schwartz J, et al. Ambient pollution and heart rate variability. Circulation. 2000;101:1267-1273.

8. Peters A, Frolkich M, Doring A, et al. Particulate air pollution is associated with an acute phase response in men: results from the MONICA-Augsburg study. Eur Heart J. 2001;22:1198-1204.

9. Pekkanen J, Peters A, Hoek G, et al. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the exposure and risk assessment for fine and ultrafine particles in ambient air (ULTRA) study. Circulation. 2002;106:933-938.

10. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for hydrogen sulfide and carbonyl sulfide. U.S. Department of Health and Human Services.

11. Weil ED, Sandler SR, Gernon M. Sulfur compounds. In: Kirk-Othmer Encyclopedia of Chemical Technology, 5th edn. Hoboken, NJ: John Wiley and Sons; 2006:23:9-16.

12. Pouliquen F, Blanc C, Arretzc E, et al. Hydrogen sulfide. In: Elvers B, ed. Ullmann’s Encyclopedia of Industrial Chemistry. Volume A13: High-performance Fibers to Imidazole and Derivatives. Deerfield Beach, FL: VCH Publishers; 1989;A12:467-485.

13. Finnbjörnsdottir RG, Oudin A, Elvarsson BT, Gislason T, Rafnsson V. Environmental epidemiology. European Heart Journal. 2002;23:9-16.

14. Vaziri SM, Larson MG, Benjamin EJ, Levy D. Echocardiographic predictors of nonrheumatic atrial fibrillation: the Framingham heart study. Circulation. 1997;85:793-798.

15. Reiffenstein RJ, Hulbert WC, Roth SH. Toxicology of hydrogen sulfide: an endogenous gaseous signaling molecule in cardiovascular disease. Circ Res. 2008;79:632-641.

16. Zhang Z, Huang H, Liu P, Tang C, Wang J. Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening KATP channels. Can J Physiol Pharmacol. 2007;85:1248-1253.

17. Lin YK, Lai MS, Chen YC, et al. Hypoxia and reoxygenation modulate the arrhythmogenic activity of the pulmonary vein and atrium. Clin Sci. 2012;122:121-132.

18. Chen YC, Chen SA, Chen YJ, Chang MS, Chan P, Lin CI. Effects of thyroid hormone on the arrhythmogenic activity of pulmonary veincardiomyocytes. J Am Coll Cardiol. 2002;39:366-372.

19. Likhaga B, Chang SL, Chen YC, et al. Histone deacetylase inhibition reduces pulmonary vein arrhythmogenesis through calcium regulation. Int J Cardiol. 2014;177:983-989.

20. Wongcharoen W, Chen YC, Chen YJ, et al. Effects of a Na+/Ca2+ exchange inhibitor on pulmonary vein electrical activity and ouabain-induced arrhythmogenicity. Cardiovasc Res. 2006;70:497-508.

21. Hu K, Duan D, Li GR, Nattel S. Protein kinase C activates ATP-sensitive K+ current in human and rabbit ventricular myocytes. Circ Res. 1996;78:492-498.

22. Viatchenko KS, Kornyeyev D, El-Bizri N, et al. Intracellular Na+ overload causes oxidation of CaMKII and leads to Ca2+ mishandling in isolated ventricular myocytes. J Mol Cell Cardiol. 2014;76:247-256.

23. M Suzuki, WU YM, LI Q, LI S, LI Q, HE RR. Electrophysiological effects of hydrogen sulfide on human atrial fibers. Chin Med J. 2011;123:3455-3459.

24. Abramochkin DV. Modulation of sinoatrial node pacemaker activity by carbon monoxide and hydrogen sulfide. Dokl Biol Sci. 2013;453:338-341.

25. de Sisti A, Leclercq JF, Fiorello P, Manot S, Halimi F, Attuel P. Electrophysiological characteristics of the atrium in sinus node dysfunction: atrial refractoriness and conduction. J Cardiovasc Electrophysiol. 2000;11:30:33.

26. Chang HY, Lin YJ, Lo LW, et al. Sinus node dysfunction in atrial fibrillation patients: the evidence of regional atrial substrate remodel- ing, Europace. 2013:15:205-211.

27. Levin IB. Modulation of ion channels by protein phosphorylation and dephosphorylation. Annu Rev Physiol. 1994;56:193-212.

28. Light PE, Bladen C, Winkfein RJ, Walsh MP, French RJ. Molecular basis of protein kinase C-induced activation of ATP-sensitive potassium channels. Proc Natl Acad Sci USA. 2000;97:9058-9063.

29. Ferreira JC, Mohly-Rosen D, Boutjdir M. Regulation of cardiac excitability by protein kinase C isozymes. Front Biosci. 2012:4:532-546.

30. Gray CC, Amrani M, Yacoub MH. Heat stress proteins and myocardial protection: experimental model or potential clinical tool? Int J Biochem Cell Biol. 1999;31:559-573.

31. Armstead WM, Hecker JG. Heat shock protein modulation of KATP and KCa channels cerebrovasodilation after brain injury. Am J Physiol Heart Circ Physiol. 2005;289:H1184-H1190.

32. Al-Magableh MR, Kemp-Harper BK, Hart JL. Hydrogen sulfide treatment reduces blood pressure and oxidative stress in angiotensin II-induced hypertensive mice. Hypertens Res. 2015;38:13:20.

33. Beauchamp RO, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. Crit Rev Toxicol. 1984:13:25-97.

34. Reiffenstein RJ, Hultberg WC, Roth SH. Toxicology of hydrogen sulfide. Annu Rev Pharmacol Toxicol. 1992;32:109-134.

35. Shen Y, Shen Z, Luo SS, Guo W, Zhu YZ. The cardioprotective effects of hydrogen sulfide in heart diseases: from molecular mechanisms to therapeutic potential. Oxid Med Cell Longev. 2015;2015:925167.

36. Agency for Toxic Substances and Disease Registry (ATSDR). Medical management guidelines for hydrogen sulfide. U.S. Department of Health and Human Services.

37. Ahmad S, Ahmad A, Hendry-Hofer TB, et al. Sarcoendoplasmic reticulum Ca2+ ATPase: a critical target in chlorine inhalation-induced cardiotoxicity. Am J Respir Cell Mol Biol. 2015;52:492-502.
48. Volpato GP, Searles R, Yu B, et al. Inhaled hydrogen sulfide: a rapidly reversible inhibitor of cardiac and metabolic function in the mouse. *Anesthesiology*. 2008;108:659-668.

49. Whitsel EA, Avery CL. The environmental epidemiology of atrial arrhythmogenesis. *J Epidemiol Community Health*. 2010;64:587-590.

50. Dhopheshwarkar AS, Jain S, Liao C, Ghose SK, Bisset KM, Nicholson RA. The actions of benzophenanthridine alkaloids, piperonyl butoxide and (S)-methoprene at the G-protein coupled cannabinoid CB1 receptor in vitro. *Eur J Pharmacol*. 2011;654:26-32.

51. Mukhopadhyay P, Rajesh M, Bátikai S, et al. CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. *Cardiovasc Res*. 2010;85:773-784.

52. Pearson G, Robinson F, Gibson TB, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev*. 2001;22:153-183.

53. Gschwendt M, Müller HJ, Kielbassa K, et al. Rottlerin, a novel protein kinase inhibitor. *Biochem Biophys Res Commun*. 1994;199:93-98.

54. Soltoff SP. Rottlerin is a mitochondrial uncoupler that decreases cellular ATP levels and indirectly blocks protein kinase C delta tyrosine phosphorylation. *J Biol Chem*. 2001;276:37986-37992.

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