

Interplay of Hydrogen Sulfide and Nitric Oxide on the Pacemaker Activity of Interstitial Cells of Cajal from Mouse Small Intestine

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We studied whether nitric oxide (NO) and hydrogen sulfide (H₂S) have an interaction on the pacemaker activities of interstitial cells of Cajal (ICC) from the mouse small intestine. The actions of NO and H₂S on pacemaker activities were investigated by using the whole-cell patch-clamp technique and intracellular Ca²⁺ analysis at 30°C in cultured mouse ICC. Exogenously applied (±)-S-nitroso-N-acetylpenicillamine (SNAP), an NO donor, or sodium hydrogen sulfide (NaHS), a donor of H₂S, showed no influence on pacemaker activity (potentials and currents) in ICC at low concentrations (10 μM SNAP and 100 μM NaHS), but SNAP or NaHS completely inhibited pacemaker amplitude and pacemaker frequency with increases in the resting currents in the outward direction at high concentrations (SNAP 100 μM and NaHS 1 mM). Co-treatment with 10 μM SNAP plus 100 μM NaHS also inhibited pacemaker amplitude and pacemaker frequency with increases in the resting currents in the outward direction. ODQ, a guanylate cyclase inhibitor, or glibenclamide, an ATP-sensitive K⁺ channel inhibitor, blocked the SNAP+NaHS-induced inhibition of pacemaker currents in ICC. Also, we found that SNAP+NaHS inhibited the spontaneous intracellular Ca²⁺ ([Ca²⁺]_i) oscillations in cultured ICC. In conclusion, this study describes the enhanced inhibitory effects of NO plus H₂S on ICC in the mouse small intestine. NO+H₂S inhibited the pacemaker activity of ICC by modulating intracellular Ca²⁺. These results may be evidence of a physiological interaction of NO and H₂S in ICC for modulating gastrointestinal motility.

Key Words: *Interstitial cells of Cajal (ICC); Intestinal motility; Pacemaker currents; Nitric Oxide; Hydrogen sulfide*

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INTRODUCTION

Gasotransmitters are small molecules of endogenous gases and have important physiological functions. The current known gasotransmitters include nitric oxide (NO), carbon monoxide, hydrogen sulfide (H₂S), and others.¹⁻³ It is well known that NO has an inhibitory action in the gastrointestinal (GI) tract. NO is produced by NO synthase (NOS) and plays specific roles in the modulation of contractile functions in GI smooth muscle.^{4,5}

H₂S is the most recently identified gaseous mediator. The physiological and pathological functions of H₂S have recently attracted intense research interest. Much of this

interest stems from the discovery of endogenous H₂S generation by the pyridoxal-5'-phosphate-dependent enzymes cystathionine beta-synthase and cystathionine gamma-lyase in mammalian tissues.^{6,7} In general, H₂S has inhibitory effects in the GI tract. H₂S results in relaxation of isolated ileal muscle strips⁸ and inhibits the motor patterns in the colon and jejunum of various species.⁹

The motility of the GI tract is generated by periodic electrical activity called slow waves, and it is widely accepted that the slow waves originate from the interstitial cells of Cajal (ICC) and are propagated to nearby muscle cells via the gap junction.¹⁰ Because of the crucial role of ICC on GI motility, ICC can be a regulatory target for many hor-

mones, neurotransmitters, and various endogenous compounds for modulating GI motility.

The interaction between NO and H₂S is an interesting area of study. Many reports have suggested that the effect of H₂S is, at least partially, via the activation of NOS.¹¹⁻¹³ Also, it was shown that NO donors enhance H₂S production and increase cystathionine gamma-lyase expression in cultured smooth muscle cells.¹⁴ These reports suggest that H₂S and NO may interact with each other.

Recently, we reported the effects of NO and H₂S on the electrical activity of ICC in mouse small intestine^{15,16} but did not check the interaction of NO and H₂S in ICC. In this study, therefore, we determined the interaction of NO and H₂S in small intestinal ICC.

MATERIALS AND METHODS

1. Animal and tissue preparation

Balb/c mice (3-7 days old) of either sex were anesthetized with diethyl ether and sacrificed by cervical dislocation. All animals were treated ethically according to the guiding principles for the care and use of animals in the field of physiological sciences approved by the Institutional Animal Use and Care Committee at Chosun University College of Medicine. The small intestine was excised 1 cm below the pyloric ring to the cecum and opened along the mesenteric border. The luminal contents were washed away with Krebs-Ringer bicarbonate solution.

2. ICC culture

The isolated tissue was pinned to the base of a Slygard dish, and the mucosa was removed by sharp dissection. Small strips of intestinal muscle were equilibrated in calcium-free Hank's solution with the following constituents (in mM): KCl, 5.36; NaCl, 125; NaOH, 0.336; Na₂HCO₃, 0.44; glucose, 10; sucrose, 2.9; and HEPES, 11. The pH was adjusted to 7.4 with Tris for 30 minutes. Cells were dispersed by incubating for 15 minutes at 37°C in an enzymatic solution containing collagenase (1.3 mg/ml; Worthington Biochemicals, Lakewood, NJ, USA), bovine serum albumin (2 mg/ml; Sigma, St. Louis, MO, USA), trypsin inhibitor (2 mg/ml; Sigma), and ATP (0.27 mg/ml). The cells were finely chopped and placed onto sterile glass coverslips coated with poly-L lysine in 35-mm culture dishes and incubated at 37°C in a 95% O₂ and 5% CO₂ incubator in smooth muscle growth medium (Cambrex Bio Science, Walkersville, MD, USA) supplemented with 2% antibiotics/antimycotics (Gibco, Grand Island, NY, USA) and murine stem cell factor (5 ng/ml; Sigma).

3. Patch-clamp experiments

The whole-cell configuration of the patch-clamp technique was used to record membrane currents (voltage clamp) and membrane potentials (current clamp) from the cultured ICC. Currents or potentials were amplified by using an Axopatch 1-D (Axon Instruments, Foster City, CA, USA). Command pulse was applied by using an IBM-com-

patible personal computer and pClamp software (version 9.2; Axon Instruments). The data were filtered at 5 KHz. All experiments were carried out at 30°C.

4. Solutions and drugs

The cells were bathed in a standard solution containing the following (in mM): KCl, 5; NaCl, 135; CaCl₂, 2; glucose, 10; MgCl₂, 1.2; and HEPES, 10. The standard solution was adjusted to pH 7.4 with Tris. The pipette solution contained the following (in mM): K-aspartate, 120; KCl, 20; MgCl₂, 5; K₂ATP, 2.7; Na₂GTP, 0.1; creatine phosphate disodium, 2.5; EGTA, 0.1; and HEPES, 5. The solution was adjusted to pH 7.4 with Tris.

All drugs in this study were purchased from Sigma Chemical Co. and were dissolved in appropriate solvent as mentioned in the product information. NaHS was dissolved in water and was used fresh in every experiment.

5. Measurement of the intracellular Ca²⁺ concentration

Changes in the intracellular Ca²⁺ concentration ([Ca²⁺]_i) were monitored by using fluo-3/AM, which was initially dissolved in dimethyl sulfoxide and stored at -20°C. The cultured ICC on coverslips (25 mm) were rinsed twice with a bath solution. The coverslips were then incubated in the bath solution containing 1 μM fluo-4 with 5% CO₂ at 37°C for 5 min, rinsed 2 more times with the bath solution, mounted on a perfusion chamber, and scanned every 0.4 s with a Nikon Eclipse TE200 inverted microscope equipped with a Perkin-Elmer Ultraview confocal scanner and a Hamamatsu Orca ER 12-bit CCD camera (×200). Fluorescence was excited at a wavelength of 488 nm, and emitted light was observed at 515 nm. During scanning of the Ca²⁺ imaging, the temperature of the perfusion chamber containing the cultured ICC was kept at 30°C. The variations of intracellular Ca²⁺ fluorescence emission intensity were expressed as F1/F0, where F0 is the intensity of the first imaging.

6. Statistical analysis

Data are expressed as means±standard errors. Differences in the data were evaluated by a Student's t-test. A p value < 0.05 was taken as a statistically significant difference. The n values reported in the text refer to the number of cells used in the patch-clamp experiments.

RESULTS

1. Effect of NO+H₂S on pacemaker potentials generated by ICC

To investigate the effect of NO+H₂S, we performed electrophysiological recording from cultured ICC under the current clamp mode (*I*=0), in which spontaneous depolarization (pacemaker potentials) was generated by ICC. The resting membrane potential was -61±2 mV and the amplitude of the pacemaker potential was 29.5±4 mV. Treatment with a low concentration of (±)-S-nitroso-N-acetylpenicillamine (SNAP; an NO donor; 10 μM) did not influ-

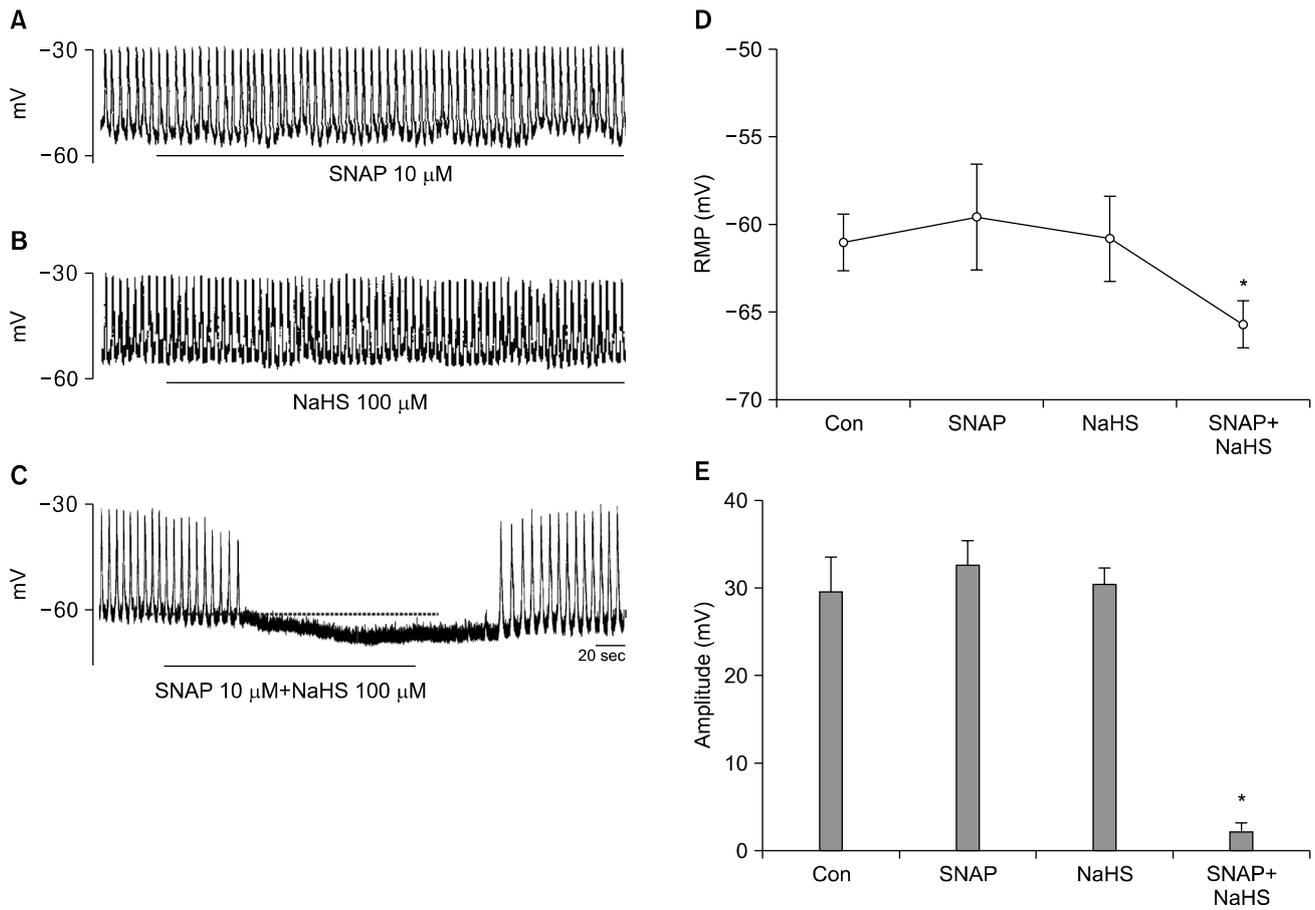


FIG. 1. Effects of $\text{H}_2\text{S}+\text{NO}$ on pacemaker potentials recorded in ICC. (A) Pacemaker potentials of ICC exposed to SNAP ($10\ \mu\text{M}$) in the current clamping mode ($I=0$). (B) Pacemaker potentials of ICC exposed to NaHS ($100\ \mu\text{M}$) in the current clamping mode ($I=0$). (C) Pacemaker potentials of ICC exposed to SNAP ($10\ \mu\text{M}$)+NaHS ($100\ \mu\text{M}$) in the current clamping mode ($I=0$). The dotted lines indicate the control resting membrane potentials. Responses to SNAP+NaHS are summarized in (D, E). Bars represent the means \pm SE. *Asterisks indicate significantly different from controls ($p < 0.05$). Con: control, RMP: resting membrane potentials.

ence pacemaker potentials in ICC according to the membrane potentials (Fig. 1A); $100\ \mu\text{M}$ NaHS (a donor of H_2S) also did not have an effect (Fig. 1B). However, co-treatment with SNAP ($10\ \mu\text{M}$)+NaHS ($100\ \mu\text{M}$) produced membrane hyperpolarization and decreased the amplitude of the pacemaker potentials (Fig. 1C). In the presence of SNAP and NaHS, the resting membrane was hyperpolarized to $-64.8 \pm 1\ \text{mV}$ ($n=3$; Fig. 1D) and the amplitude of the pacemaker potentials decreased to $2.1 \pm 1.1\ \text{mV}$ ($n=3$; Fig. 1E).

2. Effect of $\text{NO}+\text{H}_2\text{S}$ on pacemaker currents generated by ICC

To examine the role of $\text{NO}+\text{H}_2\text{S}$ on pacemaker currents in ICC, we tested under a voltage clamp at a holding potential of $-70\ \text{mV}$. In the control condition, ICC generated pacemaker currents. Treatment with a high concentration of SNAP ($100\ \mu\text{M}$) or NaHS ($1\ \text{mM}$) decreased both the frequency and the amplitude of the pacemaker currents and the resting currents were increased in the outward direction (Fig. 2A, B). The application of a low concentration of SNAP ($10\ \mu\text{M}$) or NaHS ($100\ \mu\text{M}$), however, did not show any influence on pacemaker currents in ICC (Fig. 2C, D).

However, co-treatment with a low concentration of SNAP ($10\ \mu\text{M}$)+NaHS ($100\ \mu\text{M}$) inhibited the frequency and amplitude similar to the effect of a high concentration of SNAP or NaHS on pacemaker currents in ICC (Fig. 2E). The value obtained after co-treatment with low concentrations of SNAP and NaHS was significantly different from that obtained after single treatment with low concentrations of SNAP or NaHS ($n=3$; Fig. 2F-H).

3. Involvement of ATP-sensitive K^+ channels and cyclic GMP on $\text{NO}+\text{H}_2\text{S}$ -induced effects on pacemaker currents generated by ICC

To determine the role of ATP-sensitive K^+ (K_{ATP}) channels or cyclic guanosine monophosphate (cGMP) on $\text{NO}+\text{H}_2\text{S}$ -induced action on pacemaker currents, we used glibenclamide, an inhibitor of K_{ATP} channels, and ODQ, an inhibitor of guanylate cyclase. Treatment with ODQ ($10\ \mu\text{M}$) or glibenclamide ($10\ \mu\text{M}$) alone had no effect on pacemaker currents, but pre-treatment with ODQ or glibenclamide completely blocked the SNAP ($10\ \mu\text{M}$)+NaHS ($100\ \mu\text{M}$)-induced effects on ICC (Fig. 3A, B). The value obtained after co-treatment with a low concentration of SNAP and NaHS

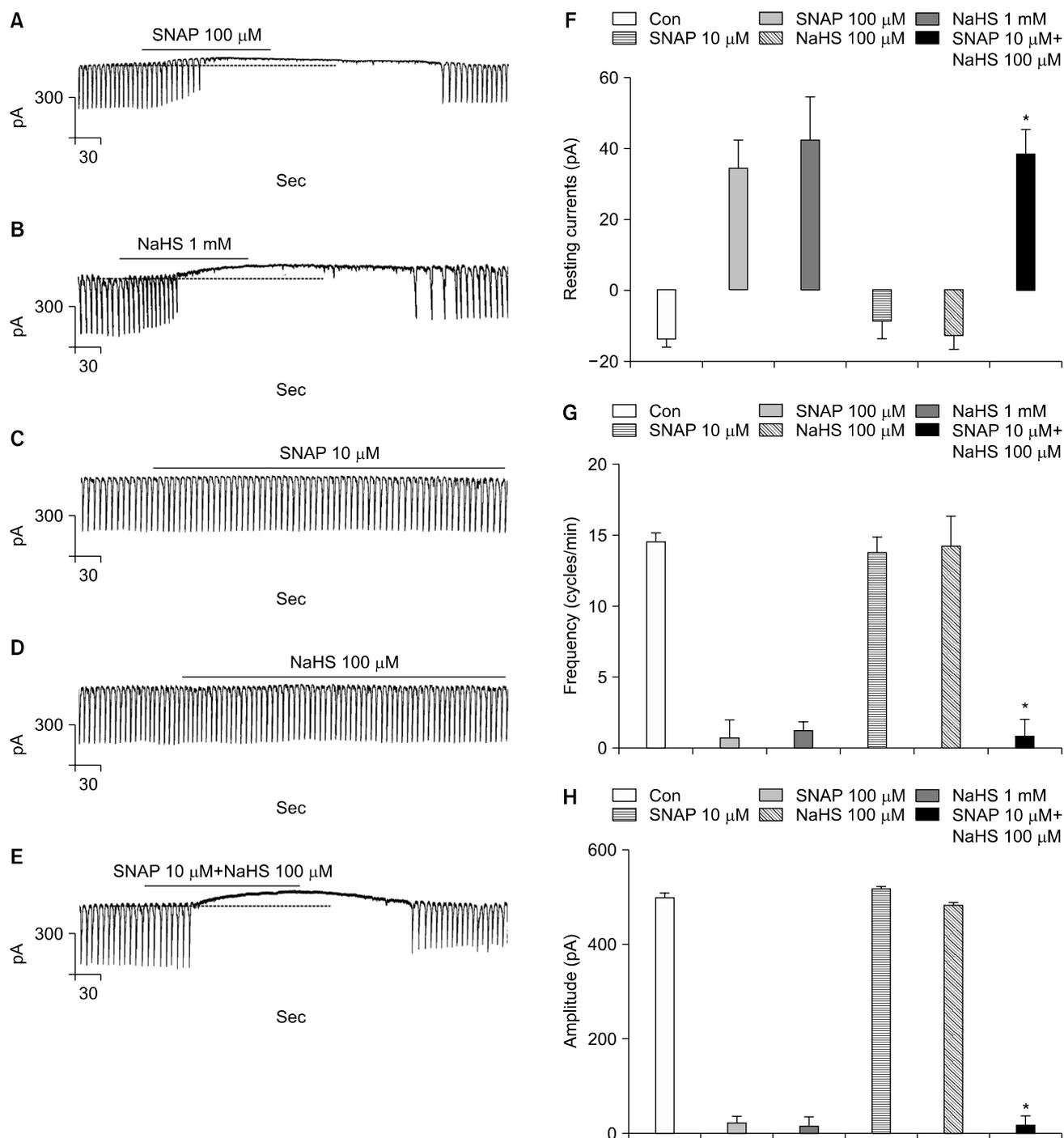


FIG. 2. Effects of H₂S+NO on pacemaker currents recorded in cultured ICC. (A) Pacemaker currents of ICC exposed to SNAP (100 μM) at a holding potential of -70 mV. (B) Pacemaker currents of ICC exposed to NaHS (1 mM) at a holding potential of -70 mV. (C) Pacemaker currents of ICC exposed to SNAP (10 μM) at a holding potential of -70 mV. (D) Pacemaker currents of ICC exposed to NaHS (100 μM) at a holding potential of -70 mV. (E) Pacemaker potentials of ICC exposed to SNAP (10 μM)+NaHS (100 μM) at a holding potential of -70 mV. The dotted lines indicate the control resting current levels. (F-H) summarize the effects of SNAP+NaHS on pacemaker currents in ICC. Bars represent the means±SE. *Asterisks indicate significantly different from single treatment of SNAP (10 μM) or NaHS (100 μM) ($p < 0.05$). Con: control.

in the presence of ODQ or glibenclamide was significantly different from that obtained in the absence of ODQ or glibenclamide ($n=3$; Fig. 3C-E).

4. Inhibition of intracellular Ca²⁺ oscillation by NO+H₂S

To investigate the role of intracellular Ca²⁺ ([Ca²⁺]_i) in the NO+H₂S-induced action on pacemaker currents, we examined the effect of SNAP (10 μM) + NaHS (100 μM) on [Ca²⁺]_i

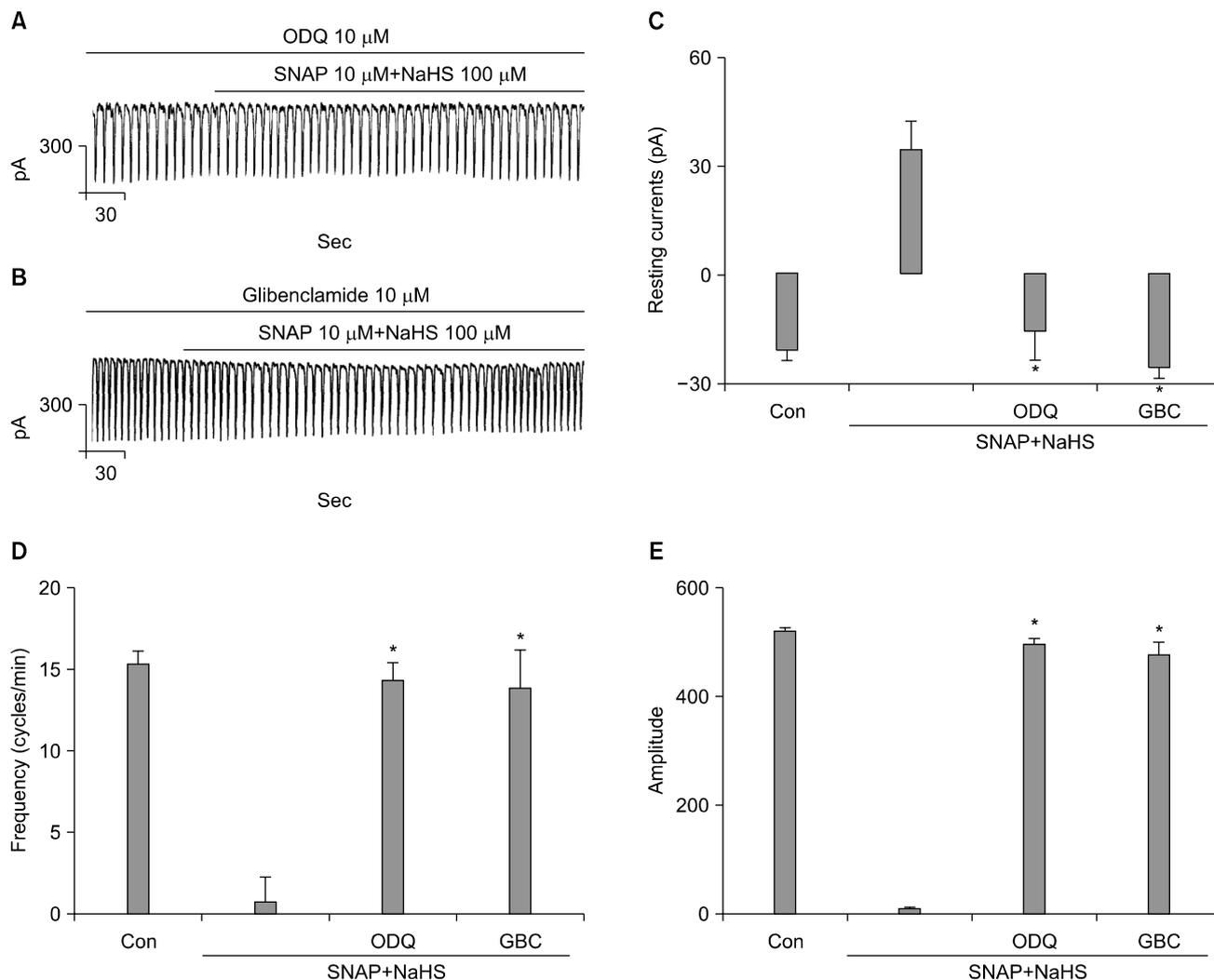


FIG. 3. Effects of ODQ or glibenclamide on NO+H₂S-induced effects in cultured ICC. (A) Pacemaker currents of ICC exposed to SNAP (10 μ M)+NaHS (100 μ M) in the presence of ODQ (10 μ M). (B) Pacemaker currents of ICC exposed to SNAP (10 μ M)+NaHS (100 μ M) in the presence of glibenclamide (10 μ M). Responses to SNAP+NaHS in the presence of ODQ or glibenclamide are summarized in (C-E). Bars represent the mean values \pm SE. The dotted lines indicate the zero current levels. *Asterisks indicate significantly different from SNAP+NaHS ($p < 0.05$). Con: control, GBC: glibenclamide.

oscillations in ICC. In this study, we measured spontaneous [Ca²⁺]_i oscillations of ICC, which are connected with cell clusters. Spontaneous [Ca²⁺]_i oscillations were observed in many ICC that were loaded with fluo4-AM. Treatment with SNAP (10 μ M) or NaHS (100 μ M) did not show any influence on spontaneous [Ca²⁺]_i oscillations of ICC ($n=4$; Fig. 4A, C). However, in the presence of co-application of SNAP (10 μ M)+NaHS (100 μ M), the spontaneous [Ca²⁺]_i oscillations of ICC rapidly declined ($n=4$; Fig. 4E, F). The time series data are shown in Fig. 4B, D, and G.

DISCUSSION

The main objective of this study was to investigate the interaction between H₂S and NO in ICC. By electrophysiological study with cultured ICC, we found that 100 μ M NaHS or 10 μ M SNAP alone had a negligible effect in

ICC, which is consistent with our previous report.^{15,16} However, unexpectedly, when these two donors were mixed together, we saw a marked inhibition of pacemaker currents in ICC. Because stimulating endogenous NO production with L-arginine and exogenous application of NO donors produced similar effects, the possibility that the effect resulted merely from the chemical reaction of the two donors can be excluded.

In our previous reports, we showed that 100 μ M SNAP or 1 mM NaHS inhibits pacemaker activity in ICC.^{15,16} However, to study the interaction of NaHS and SNAP, we needed to find a concentration of NO or NaHS that had no action on ICC; we found that 100 μ M NaHS or 10 μ M SNAP alone had no effect. It was reported previously that the mixture of NO donors and H₂S potentiated the relaxation effect of the NO donors in aortic rings in vitro.¹⁷ Also, many reports have suggested that NO donors stimulate H₂S pro-

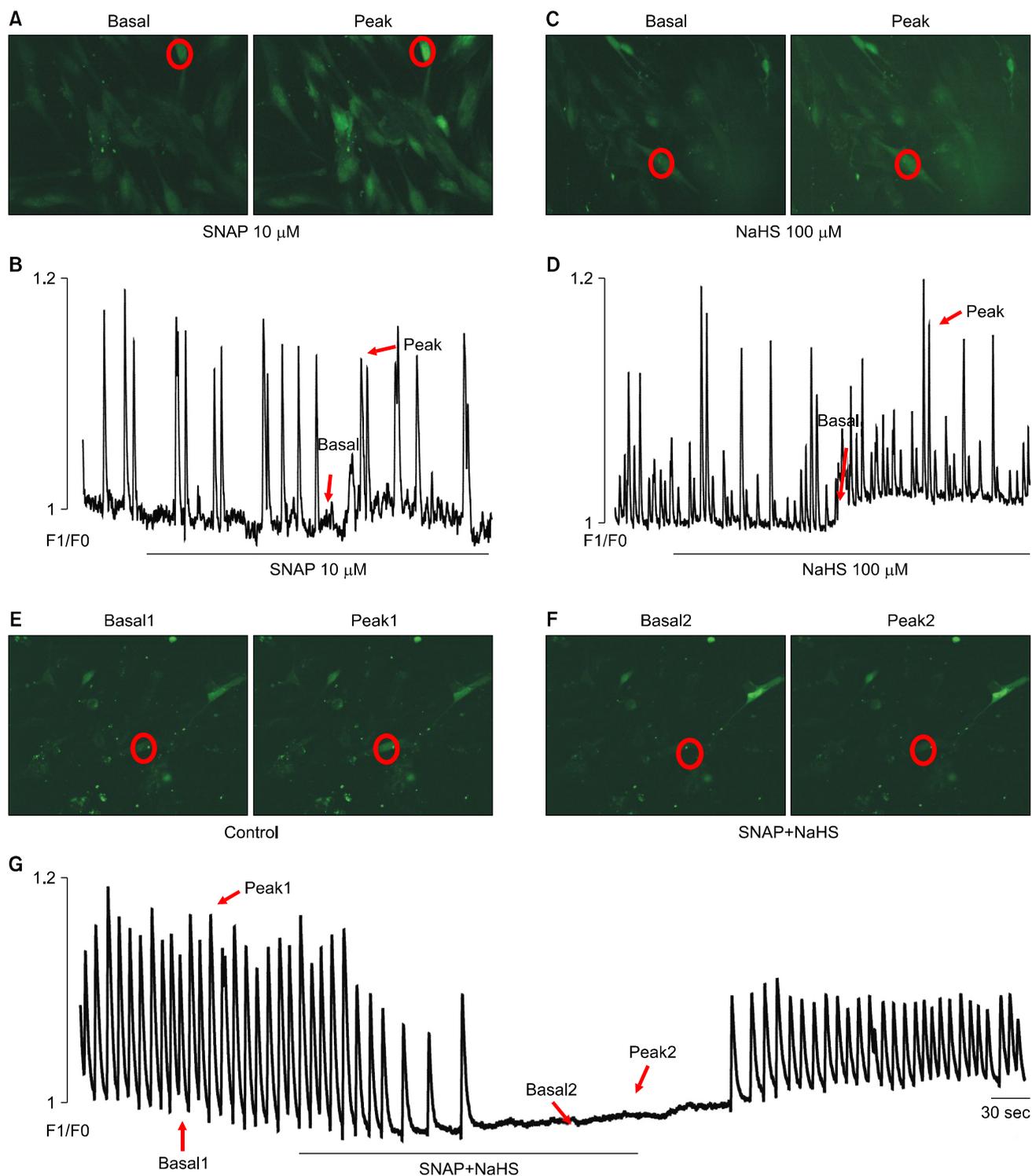


FIG. 4. Effects of NO+H₂S on [Ca²⁺]_i oscillation in cultured ICC. (A) Basal and peak point of the ICC image in the presence of SNAP (10 μM). (B) The sequential fluorescence intensity change plotted in (A) is shown in red. (C) Basal and peak point of the ICC image in the presence of NaHS (100 μM). (D) The sequential fluorescence intensity change plotted in (C) is shown in red. (E) Basal and peak point of the ICC image in the control condition. (F) Basal and peak point of the ICC image in the presence of SNAP (10 μM)+NaHS (100 μM). (G) The sequential fluorescence intensity change plotted in (E, F) is shown in red. The interval of the representative frame was 1 s and the exposure time of each frame was 500 ms.

duction¹⁴ and that H₂S enhances the activation of NOS.¹¹⁻¹³ These reports indicate that H₂S and NO can influence the production of each other. Therefore, H₂S and NO interact

with each other in a number of ways.¹⁸ In this study, we also showed that H₂S and NO interacted with each other to inhibit the pacemaker activity in ICC.

Our next finding was that the concentrations of NaHS and NO that had no effect on pacemaker activity alone could enhance the inhibitory action by co-treatment in ICC. To determine this, we focused on K_{ATP} channels. In our previous report, we showed the existence of K_{ATP} channels in ICC with pinacidil, a K_{ATP} channel opener,¹⁹ and the localization of K_{ATP} channels Kir 6.2 and SUR 2B in cultured ICC.²⁰ Also, we reported that SNAP inhibited pacemaker currents that were blocked by an inhibitor of K_{ATP} channels in ICC.¹⁶ However, we found that glibenclamide could not block the NaHS-induced effect.¹⁵ In this study, we found that glibenclamide blocked the effects in ICC induced by NO+H₂S. These findings suggest that the enhanced effect of H₂S+NO on ICC is by stimulating NO action by H₂S. Furthermore, the major second messenger of NO is cGMP by activation of guanylate cyclase in various cells.²¹ In ICC in particular, some reports have shown that NO inhibits electrical activity by cGMP regulation.^{16,22} In this study, we found that a guanylate cyclase inhibitor blocked the NO+H₂S-induced effects in ICC. This result supports our suggestion that the effect of NO+H₂S on ICC is via the stimulation of NO action by H₂S.

To understand how NO+H₂S inhibits pacemaker activity in ICC, we checked $[Ca^{2+}]_i$ by using live cell imaging. It is well known that the periodic pacemaker activity of ICC is dependent on $[Ca^{2+}]_i$ oscillation, and that this pacemaker mechanism is initiated by release of Ca²⁺ from the endoplasmic reticulum through the inositol triphosphate receptor and is followed by re-uptake of Ca²⁺ into the mitochondria.^{23,24} Furthermore, many reports have shown that the opening of K_{ATP} channels decreases $[Ca^{2+}]_i$ levels by inhibiting external Ca²⁺ influx or release from intracellular store.^{25,26} If the NO+H₂S-induced inhibitory action on ICC is via an enhancement of NO, this mixture should inhibit the $[Ca^{2+}]_i$ oscillation. In fact, we found that NO+H₂S inhibited the $[Ca^{2+}]_i$ oscillation. Interestingly, our previous report showed that FCCP, an inhibitor of mitochondrial Ca²⁺ uptake, blocked the pacemaker activity of ICC, similar to the action induced by a high concentration of NaHS.¹⁵ Namely, in ICC, NO may stimulate K_{ATP} channels and then decrease the $[Ca^{2+}]_i$, but NaHS blocks mitochondrial Ca²⁺ uptake.

In conclusion, the results of the present study indicate that H₂S and NO interact in ICC and this interaction results in the inhibition of pacemaker activity. Also, the interaction of H₂S and NO is via the stimulation or enhancement of NO. However, further study is needed to understand whether H₂S stimulates NO production or increases NO efficacy.

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REFERENCES

1. Goodwin LR, Francom D, Dieken FP, Taylor JD, Warenycia MW,

- Reiffenstein RJ, et al. Determination of sulfide in brain tissue by gas dialysis/ion chromatography: postmortem studies and two case reports. *J Anal Toxicol* 1989;13:105-9.
2. Warenycia MW, Goodwin LR, Benishin CG, Reiffenstein RJ, Francom DM, Taylor JD, et al. Acute hydrogen sulfide poisoning. Demonstration of selective uptake of sulfide by the brainstem by measurement of brain sulfide levels. *Biochem Pharmacol* 1989; 38:973-81.
3. Savage JC, Gould DH. Determination of sulfide in brain tissue and rumen fluid by ion-interaction reversed-phase high-performance liquid chromatography. *J Chromatogr* 1990;526:540-5.
4. Sanders KM, Ward SM. Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. *Am J Physiol* 1992;262: G379-92.
5. Ueno T, Duenes JA, Zarroug AE, Sarr MG. Nitroergic mechanisms mediating inhibitory control of longitudinal smooth muscle contraction in mouse small intestine. *J Gastrointest Surg* 2004;8:831-41.
6. Bukovska G, Kery V, Kraus JP. Expression of human cystathionine beta-synthase in *Escherichia coli*: purification and characterization. *Protein Expr Purif* 1994;5:442-8.
7. Erickson PF, Maxwell IH, Su LJ, Baumann M, Glode LM. Sequence of cDNA for rat cystathionine gamma-lyase and comparison of deduced amino acid sequence with related *Escherichia coli* enzymes. *Biochem J* 1990;269:335-40.
8. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 1997;237:527-31.
9. Gallego D, Clavé P, Donovan J, Rahmati R, Grundy D, Jiménez M, et al. The gaseous mediator, hydrogen sulphide, inhibits in vitro motor patterns in the human, rat and mouse colon and jejunum. *Neurogastroenterol Motil* 2008;20:1306-16.
10. Ward SM, Burns AJ, Torihashi S, Harney SC, Sanders KM. Impaired development of interstitial cells and intestinal electrical rhythmicity in steel mutants. *Am J Physiol* 1995;269: C1577-85.
11. Yong QC, Lee SW, Foo CS, Neo KL, Chen X, Bian JS. Endogenous hydrogen sulphide mediates the cardioprotection induced by ischemic postconditioning. *Am J Physiol Heart Circ Physiol* 2008;295:H1330-H40.
12. Minamishima S, Bougaki M, Sips PY, Yu JD, Minamishima YA, Elrod JW, et al. Hydrogen sulfide improves survival after cardiac arrest and cardiopulmonary resuscitation via a nitric oxide synthase 3-dependent mechanism in mice. *Circulation* 2009;120: 888-96.
13. Pan TT, Feng ZN, Lee SW, Moore PK, Bian JS. Endogenous hydrogen sulfide contributes to the cardioprotection by metabolic inhibition preconditioning in the rat ventricular myocytes. *J Mol Cell Cardiol* 2006;40:119-30.
14. Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H₂S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J* 2001;20:6008-16.
15. Parajuli SP, Choi S, Lee J, Kim YD, Park CG, Kim MY, et al. The inhibitory effects of hydrogen sulfide on pacemaker activity of interstitial cells of cajal from mouse small intestine. *Korean J Physiol Pharmacol* 2010;14:83-9.
16. Park CG, Kim YD, Kim MY, Kim JS, Choi S, Yeum CH, et al.

- Inhibition of pacemaker currents by nitric oxide via activation of ATP-sensitive K⁺ channels in cultured interstitial cells of Cajal from the mouse small intestine. *Naunyn Schmiedebergs Arch Pharmacol* 2007;376:175-84.
17. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 1997;237:527-31.
 18. Li L, Hsu A, Moore PK. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation--a tale of three gases! *Pharmacol Ther* 2009;123:386-400.
 19. Jun JY, Choi S, Chang IY, Yoon CK, Jeong HG, Kong ID, et al. Deoxycholic acid inhibits pacemaker currents by activating ATP-dependent K⁺ channels through prostaglandin E2 in interstitial cells of Cajal from the murine small intestine. *Br J Pharmacol* 2005;144:242-51.
 20. Choi S, Yeum CH, Chang IY, You HJ, Park JS, Jeong HS, et al. Activating of ATP-dependent K⁺ channels comprised of K(ir) 6.2 and SUR 2B by PGE2 through EP2 receptor in cultured interstitial cells of Cajal from murine small intestine. *Cell Physiol Biochem* 2006;18:187-98.
 21. Lincoln TM, Cornwell TL. Intracellular cyclic GMP receptor proteins. *FASEB J* 1993;7:328-38.
 22. Sergeant GP, Johnston L, McHale NG, Thornbury KD, Hollywood MA. Activation of the cGMP/PKG pathway inhibits electrical activity in rabbit urethral interstitial cells of Cajal by reducing the spatial spread of Ca²⁺ waves. *J Physiol* 2006;574:167-81.
 23. Suzuki H, Takano H, Yamamoto Y, Komuro T, Saito M, Kato K, et al. Properties of gastric smooth muscles obtained from mice which lack inositol trisphosphate receptor. *J Physiol* 2000;525:105-11.
 24. Ward SM, Ordog T, Koh SD, Baker SA, Jun JY, Amberg G, et al. Pacemaking in interstitial cells of Cajal depends upon calcium handling by endoplasmic reticulum and mitochondria. *J Physiol* 2000;525:355-61.
 25. Brayden JE. Functional roles of KATP channels in vascular smooth muscle. *Clin Exp Pharmacol Physiol* 2002;29:312-6.
 26. Small RC, Berry JL, Foster RW. Potassium channel opening drugs and the airways. *Braz J Med Biol Res* 1992;25:983-98.