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Bidirectional effects of hydrogen sulfide via ATP-sensitive K^+ channels and transient receptor potential A1 channels in RIN14B cells

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ABSTRACT

Hydrogen sulfide (H_2S) reportedly acts as a gasotransmitter because it mediates various cellular responses through several ion channels including ATP-sensitive K^+ (K_{ATP}) channels and transient receptor potential (TRP) A1 channels. H_2S can activate both K_{ATP} and TRPA1 channels at a similar concentration range. In a single cell expressing both channels, however, it remains unknown what happens when both channels are simultaneously activated by H_2S. In this study, we examined the effects of H_2S on RIN14B cells that express both K_{ATP} and TRPA1 channels. RIN14B cells showed several intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) responses to NaHS (300 μM), an H_2S donor, i.e., inhibition of spontaneous Ca^{2+} oscillations (37%), inhibition followed by $[Ca^{2+}]_i$ increase (24%), and a rapid increase in $[Ca^{2+}]_i$ (25%). K_{ATP} channel blockers, glibenclamide or tolbutamide, abolished any inhibitory effects of NaHS and enhanced NaHS-mediated $[Ca^{2+}]_i$ increases, which were inhibited by extracellular Ca^{2+} removal, HC030031 (a TRPA1 antagonist), and disulfide bond-reducing agents. NaHS induced 5-hydroxytryptamine (5-HT) release from RIN14B cells, which was also inhibited by TRPA1 antagonists. These results indicate that H_2S has both inhibitory and excitatory effects by opening K_{ATP} and TRPA1 channels, respectively, in RIN14B cells, suggesting potential bidirectional modulation of secretory functions.

1. Introduction

Hydrogen sulfide (H_2S) reportedly acts as a gasotransmitter, like nitric oxide, carbon monoxide and ammonium (Wang, 2014). In mammals, H_2S is enzymatically synthesized from cysteine in various tissues and also produced by enterobacteria in the gastrointestinal tract (Carbonero et al., 2012; Kamoun, 2004). Many reports indicate that H_2S plays an important role in various physiological functions (Predmore et al., 2012). In addition, therapeutic drugs releasing H_2S as anti-inflammatory have been developed (Chan and Wallace, 2013; Szabó, 2007).

Various ion channels are reported as targets of H_2S (Tang et al., 2010). In sensory neurons, H_2S acts on transient receptor potential (TRP) V1 and voltage-dependent T-type Ca^{2+} channels, leading to bladder contraction and hyperalgesia, respectively (Maeda et al., 2009; Patacchini et al., 2005). We previously reported that H_2S causes excitation of sensory neurons via Ca^{2+}-permeable TRPA1 channels (Miyamoto et al., 2011), which are a member of the TRP family of non-selective cation channels activated by cold temperatures and several pungent irritants (Bandell et al., 2004; Jordt et al., 2004; Story et al., 2003). In addition to sensory neurons, TRPA1 channels are expressed in some secretory cells, such as enteroendocrine cells and pancreatic β cells (Cao et al., 2012; Cho et al., 2014; Nozawa et al., 2009; Purhonen et al., 2008). The increase in intracellular Ca^{2+} through TRPA1 channels is thought to be important for triggering secretion of hormones and autacoids in these cells.

Furthermore, some secretory cells also express another target molecule of H_2S, ATP-sensitive K^+ (K_{ATP}) channels (Basavappa et al., 1994; Inagaki et al., 1995), which are inward rectifying K^+ channels composed of four Kir6.*x* subunits (forming the pore of the channel) and four sulfonylurea receptors (SUR*x*; auxiliary proteins). Their activity is regulated by intracellular nucleotides (ADP and ATP) and their opening results in hyperpolarization. Thus, activation of K_{ATP} channels by H_2S causes inhibitory effects, such as relaxation in the smooth muscle of blood vessels and gastrointestinal tract, and decrease insulin release from pancreatic β cells (Yang et al., 2005; Zhao et al., 2001).

TRPA1 and K_{ATP} channels are activated by H_2S at a similar concentration range (micromolar levels) (Miyamoto et al., 2011; Yang et al., 2005; Zhao et al., 2001). However, there are no reports examining cellular responses to H_2S in single cells expressing both channels. Although H_2S reportedly exhibits excitatory and inhibitory effects on duodenal motility through different channels on different cells (Lu et al., 2014), it remains unknown what happens when different channels in a single cell are simultaneously activated by H_2S. In this study, we investigated the effects of NaHS, an H_2S donor, on

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RIN14B cells, a rat pancreatic δ cell line, because these cells express TRPA1 channels (Nozawa et al., 2009) and may also express K_{ATP} channels as suggested by an electrophysiological study (Bränström et al., 1997).

2. Materials and methods

2.1. Cell culture

RIN14B cells were purchased from DS Pharma Biomedical (Osaka, Japan) and cultured in RPMI 1640 medium (Gibco/Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Gibco/Life Technologies) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

2.2. Ca^{2+} imaging

RIN14B cells were placed on coverslips coated with poly-D-lysine and cultured for 24 h. Cells were then incubated with 10 μM fura-2 acetoxymethylester (Dojindo, Kumamoto, Japan) and 0.002% cremophor EL (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at room temperature in normal solution (140 mM NaCl, 3.3 mM KH_2PO_4, 0.8 mM K_2HPO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, and 20 mM HEPES, pH adjusted to 7.4 with NaOH). Fura-2 fluorescence was measured using an inverted microscope (Diaphot 300, Nikon, Tokyo, Japan) with a fluorescence ratio imaging system (ORCA-ER, Hamamatsu Photonics, Shizuoka, Japan). Cells were continuously superfused with normal solution and illuminated at 340 and 380 nm for 61.1 ms at 5 s intervals. The respective fluorescence signals (F_{340} and F_{380}) were detected at 500 nm. The intracellular calcium concentration ($[Ca^{2+}]_i$) was calculated using a Calcium Calibration Buffer Kit (Invitrogen/Life Technologies). All experiments were performed at room temperature (22–25 °C).

2.3. RT-PCR

Total RNAs were extracted from RIN14B cells and rat heart as a positive control which expresses all subunits of K_{ATP} channel (Morrissey et al., 2005; Zhou et al., 2007) using TRI Reagent (Sigma-Aldrich). RIN14B cells and rat heart tissue were homogenized with TRI Reagent. Lysates were centrifuged at 12,000*g* for 10 min at 4 °C. Supernatants were mixed with chloroform and centrifuged at 12,000*g* for 15 min at 4 °C. The aqueous layers were collected and isopropanol was added for precipitation. After centrifugation at 12,000*g* for 10 min at 4 °C, the pellet was washed with 75% ethanol and dissolved in RNase-free distilled water.

RNA samples were treated with DNase I (Invitrogen/Life Technologies) for 15 min at room temperature. The reaction was stopped by adding EDTA and heating at 65 °C. Samples were reverse-transcribed using ReverTra Ace with Oligo dT primers (TOYOBO, Osaka, Japan).

PCR was performed with Taq DNA polymerase (Roche, Basel, Switzerland). Template cDNAs (75 ng/reaction) were mixed with dNTP (200 μM) and each primer (0.5 μM). The following primers designed to detect K_{ATP} channel subunits were used: 5′-AAGCGCAACTCTATGAGAAG-3′ (forward) and 5′-ACCAGAACTCAGCAAACTGT-3′ (reverse) for Kir6.1 (product size: 212 bp), 5′-CGCATGGTGACAGAGGCAATG-3′ and 5′-GTGGAGAGGCACAACTTCGC-3′ for Kir6.2 (297 bp), 5′-TGCCAGCTCTTTGAGCATTG-3′ and 5′-AGGATGATACGGTTGAGCAGG-3′ for SUR1 (558 bp), 5′-TTGTTCGAAAGAGCAGCATAC-3′ and 5′-GCCCGCATCCATAATAGAGG-3′ for SUR2A (155 bp), 5′-TTGTTCGAAAGAGCAGCATAC-3′ and 5′-AGCAGTCAGAATGGTGTGAACC-3′ for SUR2B (152 bp), and 5′-TGTCACCAACTGGGACGATA-3′ and 5′-ACCCTCATAGATGGGCACAG-3′ for the housekeeping gene β-actin (280 bp). Thermal cycles were performed using a PC320 system (ASTEC, Fukuoka, Japan). Samples were incubated for 1 min at 94 °C (for initial denaturation) followed by 30 cycles of denaturation (30 s, 94 °C), annealing (30 s, 55 °C for Kir6.1 and SUR2A, or 58 °C for Kir6.2 and SUR2B, or 60 °C for SUR1 and β-actin), and elongation (30 s, 72 °C). The final elongation was 4 min at 72 °C and products were cooled to 4 °C. RNAs without RT were used as a negative control to examine DNA contamination.

PCR products were separated on a 1.5% agarose gel and visualized with ethidium bromide under UV illumination.

2.4. Immunostaining

RIN14B cells were placed on coverslips and cultured for 24 h. Cells were then rinsed in PBS (137 mM NaCl, 2.68 mM KCl, 10.1 mM Na_2HPO_4, and 1.76 mM KH_2PO_4, pH adjusted to 7.4 with NaOH) and fixed with 4% paraformaldehyde for 1 h at room temperature. Cells were permeabilized with 0.3% Triton X-100 in PBS for 15 min, and blocked with 10% normal donkey serum in PBS for 1 h. Cells were then incubated with a rabbit anti-TRPA1 antibody (1:200; Novus Biologicals, Littleton, CO, USA) in 2% normal donkey serum in PBS for 1 h, and further incubated with an Alexa Fluor 488-conjugated donkey anti-rabbit antibody (H+L) (1:1000; Invitrogen/Life Technologies) in PBS in the dark. Coverslips were mounted on glass slides with Dapi-Fluoromount G (SouthernBiotech, Birmingham, AL, USA). Images were captured with a fluorescence microscope (LSM 700, Carl Zeiss, Oberkochen, Germany).

2.5. 5-Hydroxytryptamine (5-HT) release

RIN14B cells were seeded in 60 mm dishes at a density of $2–5 \times 10^5$ cells/ml and cultured for 48–72 h at 37 °C/5% CO_2. Once the cells reached confluence, the culture medium was removed and the cells were pre-incubated with normal solution containing 2 μM fluoxetine (5-HT reuptake inhibitor) for 1 h, and then incubated with 2 ml normal solution containing stimulants for 15 or 30 min at 37 °C. The secretory responses were terminated by placing the dishes on ice. Culture supernatants were collected and centrifuged for 2 min at 800*g* to remove any detached cells. Perchloric acid at a final concentration of 0.4 N was added to the supernatants and the culture dishes, and the cells remaining in the dishes were scraped and collected. After centrifugation, the acidified samples were neutralized with K_2HPO_4. After the removal of potassium percolate, clear supernatants were analyzed on a high-performance liquid chromatography (HPLC) system equipped with an electrochemical detector. The mobile phase consisted of a citric acid buffer (0.1 M citric acid, 0.1 M sodium acetate; pH 3.5), 19% methanol, 5 mg/l EDTA–2Na, and 190 mg/l 1-octanesulfonic acid. The mobile phase was degased and perfused at 0.5 ml/min. Samples (50 μl) were injected with an autosampler (Model 33, System Instruments, Tokyo, Japan), and the 5-HT in the sample was separated on an octadecylsilane column (EICOMPAK SC-5ODS, 3.0×150 mm^2, EICOM, Kyoto, Japan) kept at 30 °C. Detection of 5-HT was performed at +450 mV with an electrochemical detector (ECD-300, EICOM), and the area under the 5-HT peak was measured. The ratio of 5-HT release (%) was determined by dividing the amount of 5-HT in the supernatant by the total 5-HT (5-HT in the supernatant plus the 5-HT in the cells).

2.6. Materials

Sodium hydrosulfide (NaHS) was purchased from Strem Chemicals (Newburyport, MA, USA). Allyl isothiocyanate (mustard oil), dithiothreitol (DTT), and ruthenium red were from Wako Pure Chemical Industries (Osaka, Japan). HC030031, iodoresiniferatoxin, SB366791, and fluoxetine hydrochloride were from Tocris (Bristol, UK). Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) was from Nacalai Tesque (Kyoto, Japan). ω-Conotoxin GVIA and ω-agatoxin IVA were from