Biochemical and Biophysical Research Communications 446 (2014) 393–398

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

H_2S relaxes isolated human airway smooth muscle cells via the sarcolemmal K_{ATP} channel

Robert Fitzgerald [a], Breann DeSantiago [a,1], Danielle Y. Lee [a,1], Guangdong Yang [b], Jae Yeon Kim [a], D. Brian Foster [c], Yee Chan-Li [a], Maureen R. Horton [a], Reynold A. Panettieri [d], Rui Wang [e], Steven S. An [a,*]

[a] Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[b] Cardiovascular and Metabolic Research Unit, Lakehead University, Thunder Bay, ON, Canada
[c] Division of Cardiology, Johns Hopkins School of Medicine, Baltimore, MD, USA
[d] Division of Pulmonary, Allergy and Critical Care, Airways Biology Initiative, University of Pennsylvania Medical Center, Philadelphia, PA, USA
[e] Department of Biology, Lakehead University, Thunder Bay, ON, Canada

ARTICLE INFO

Article history:
Received 25 February 2014
Available online 12 March 2014

Keywords:
Asthma
Airway smooth muscle
Single cell contraction
H_2S
ATP-sensitive potassium channels

ABSTRACT

Here we explored the impact of hydrogen sulfide (H_2S) on biophysical properties of the primary human airway smooth muscle (ASM)–the end effector of acute airway narrowing in asthma. Using magnetic twisting cytometry (MTC), we measured dynamic changes in the stiffness of isolated ASM, at the single-cell level, in response to varying doses of GYY4137 (1–10 mM). GYY4137 slowly released appreciable levels of H_2S in the range of 10–275 μM, and H_2S released was long lived. In isolated human ASM cells, GYY4137 acutely decreased stiffness (i.e. an indicator of the single-cell relaxation) in a dose-dependent fashion, and stiffness decreases were sustained in culture for 24 h. Human ASM cells showed protein expressions of cystathionine-γ-lyase (CSE; a H_2S synthesizing enzyme) and ATP-sensitive potassium (K_{ATP}) channels. The K_{ATP} channel opener pinacidil effectively relaxed isolated ASM cells. In addition, pinacidil-induced ASM relaxation was completely inhibited by the treatment of cells with the K_{ATP} channel blocker glibenclamide. Glibenclamide also markedly attenuated GYY4137-mediated relaxation of isolated human ASM cells. Taken together, our findings demonstrate that H_2S causes the relaxation of human ASM and implicate as well the role for sarcolemmal K_{ATP} channels. Finally, given that ASM cells express intrinsic enzymatic machinery of generating H_2S, we suggest thereby this class of gasotransmitter can be further exploited for potential therapy against obstructive lung disease.

1. Introduction

H_2S, the most recently-discovered gasotransmitter after NO and CO, has been reported to exert many physiological effects [1,2]. H_2S acts as a neuromodulator and/or neuroprotectant in the central nervous system and is involved with long-term potentiation in the hippocampus [3]. H_2S has been shown to regulate insulin secretion [4,5], promote angiogenesis [6] and protect cardiac muscle from oxidative stress [7,8]. Among the many *physiologic* functions perhaps the most often reported is its mode of action on the vasculature [9–14]. Specifically, H_2S causes the relaxation of vascular smooth muscle via the ATP-sensitive potassium (K_{ATP}) channel [15].

In the lung, cystathionine-γ-lyase (CSE) is one of the major enzymes producing H_2S [16] and the deficiency of CSE in mice polarizes T cells that renders mice more susceptible to allergen-induced airway hyperresponsiveness (AHR) [17]. AHR is the excessive narrowing of airways and is a cardinal feature of asthma contributing to disease morbidity [18]. Toward this end, administration of H_2S donors has been shown to reduce the immune inflammatory response and AHR in animal models of asthma [17,19]. In patients with asthma, Tian and colleagues [20] have recently reported a positive correlation between decline in lung function and decreases in CSE expression and endogenous plasma H_2S concentration. Few studies have focused on the mechanistic actions of H_2S in the lung-resident cells. Even though the role of K_{ATP} channels in regulating airway functions has been reported [21–23], the effects of H_2S on airway smooth muscle (ASM), the end-effector of acute airway narrowing, are largely unexplored.

In this study, we explored the direct effects of GYY4137, an agent capable of generating H_2S, on the biophysical properties of

* Corresponding author. Address: Johns Hopkins University Bloomberg School of Public Health, 615 N Wolfe Street, Baltimore, MD 21205, USA. Fax: +1 410 955 0299.
E-mail address: san@jhsph.edu (S.S. An).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.02.129
0006-291X/

ASM using Magnetic Twisting Cytometry. Our findings showed that, at the single-cell level, GYY4137 causes ASM relaxation and that GYY4137-induced relaxation is mediated by H_2S that acts to hyperpolarize ASM via, in part, opening the sarcolemmal K_{ATP} channel. Given the need for efficacious bronchodilators for treating obstructive lung diseases, H_2S and its derived compounds may offer a promising new avenue for asthma therapy.

2. Materials and methods

2.1. Materials

DMEM-Ham's F-12 (1:1) was purchased from GIBCO (Grand Island, NY), and the synthetic arginine-glycine-aspartic acid (RGD) containing peptide was purchased from American Peptide Company (Sunnyvale, CA). Reagents were obtained from Sigma–Aldrich (St. Louis, MO) with the exception of GYY4137 and Glyburide (glibenclamide) which were purchased from Santa Cruz Biotechnology (Dallas, TX). All reagents ($Na_2S + 9H_2O$, GYY4137, glibenclamide, pinacidil, cromakalim, diazoxide, and propargylglycine) were reconstituted in either sterile distilled water or DMSO, frozen in aliquots, and diluted appropriately in serum-free media on the day of use.

2.2. ASM cell culture and characterization

Human bronchi were obtained from lungs unsuitable for transplantation in accordance with procedures approved by Committees on Studies Involving Human Beings from the University of Pennsylvania. Human ASM cells were prepared from these bronchi as described previously [24]. Unless otherwise specified, serum-deprived post-confluent cells were plated at 30,000 cells/cm^2 on plastic wells (96-well Removawell, Immunlon II: Dynetech) previously coated with type I collagen (Vitrogen 100; Cohesion, Palo Alto, CA) at 500 ng/cm^2. Cells were maintained in serum-free media at 37 °C in humidified air containing 5% CO_2 for 24 h prior to experiments. These conditions have been optimized for seeding cultured cells on collagen matrix and for assessing their mechanical properties [25–27].

2.3. Magnetic twisting cytometry (MTC)

Dynamic changes in cell stiffness were measured as an indicator of the single-cell contraction and relaxation of isolated human ASM cells using MTC as described by us in detail elsewhere [25–27]. In brief, RGD-coated ferrimagnetic microbeads (4.5 μm in diameter) bound to the cytoskeleton through cell surface integrin receptors were magnetized horizontally and then twisted in a vertically aligned homogeneous magnetic field that was varying sinusoidally in time. This sinusoidal twisting magnetic field caused both a rotation and a pivoting displacement of the bead: as the bead moves, the cell develops internal stresses which in turn resist bead motions [28]. Lateral bead displacements in response to the resulting oscillatory torque were detected with a spatial resolution of ~5 nm, and the ratio of specific torque to bead displacements was computed and expressed here as the cell stiffness in units of Pascal per nm (Pa/nm).

2.4. Immunoblotting

The expression levels of different proteins were determined by Western blot as described previously [29]. Cells were grown to near confluence in 6 well plates and growth-arrested as described above. Cells were lysed in 1× RIPA buffer (Upstate) containing protease inhibitors (Roche) by mechanical scraping, and total protein concentration was determined (BioRad Protein Assay Reagent). Equal amounts of lysates from each sample were resolved by SDS–PAGE, transferred to nitrocellulose membranes, and subsequently probed with the indicated primary antibody followed by HRP-conjugated anti-mouse (1:5000) or anti-goat (1:4000) antibody. Mouse anti-CTH (1:500; Santa Cruz Biotechnology) was used to detect cystathionine-γ-lyase (CSE) and goat anti-KIR6.1 (1:1000; Santa Cruz Biotechnology) was used to detect Kir6.1 subunit of the K_{ATP} channels in human ASM cells. Blots were developed using enhanced chemiluminescence and quantified using ImageJ (NIH).

2.5. H_2S measurements

To trap H_2S, zinc acetate (1% w/v) was added to media containing different concentration of GYY4137. After 5 min, the reaction was terminated with N,N-dimethyl-*p*-phenylenediamine sulfate (20 mM in 7.2 M HCl) and $FeCl_3$ (30 mM in 1.2 M HCl). H_2S in the sampled media interacts with N,N-dimethyl-*p*-phenylenediamine sulfate to form methylene blue. The absorbance of the resulting solution was determined at 670 nm after the mixture was kept in the dark for 20 min. H_2S concentration in the culture media was calculated against the calibration curve of standard Na_2S solutions.

2.6. Statistical analysis

Unless otherwise stated, we used Student's *t*-test and the Analysis of Variance (ANOVA) with adjusting for multiple comparisons by applying the Bonferroni's methods. To satisfy the normal distribution assumptions associated with ANOVA, cell stiffness data were converted to log scale prior to analyses. All analyses were performed in SAS V.9.2 (SAS Institute Inc., Cary, NC), and the 2-sided *P*-values less than 0.05 were considered significant.

3. Results

3.1. Na_2S causes acute relaxation of isolated human airway smooth muscle cells

We tested first the effects of a well-known H_2S donor, Na_2S, on the stiffness of isolated human ASM cells. Addition of Na_2S caused a rapid and dose-dependent decrease in cell stiffness (Fig. 1A). The onset of stiffness decreases occurred as early as 2 s following the addition of the highest dose of Na_2S (10 mM). Decreases were significant from the baseline after 2 s for 10 mM; 8 s for 5 mM; 120 s for 1 mM; and 178 s for 0.5 mM, and continued for the duration of Na_2S stimulation (Fig. 1A). Using a mixed effect model to control for random effect due to the repeated measurements, we found significant group (i.e. dose) differences at 600 s, except between 5 mM and 10 mM ($P = 0.139339$). For individual cells obtained from three additional lung donors, Na_2S (5 mM) markedly relaxed ASM ($P < 0.002$, Signed Rank Test), resulting in ~40–60% relaxation (Fig. 1B).

3.2. GYY4137 causes sustained relaxation of isolated human airway smooth muscle cells

We next tested the effects of a water-soluble agent capable of releasing H_2S, GYY4137 [30]. GYY4137 increased H_2S concentration in a dose- and time-dependent manner (Supplementary Fig. 1); GYY4137 (1–10 mM) acutely released ~10–275 μM of H_2S. H_2S released was sustained in culture over 24 h (data not shown). In isolated human ASM, GYY4137 decreased cell stiffness in a dose-dependent manner (Fig. 2). For acute exposure (Fig. 2A), stiffness decreases were significant from the baseline for all doses of GYY4137 tested, except 1.0 mM GYY4137, with