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Signaling effects of sodium hydrosulfide in healthy donor peripheral blood mononuclear cells

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ABSTRACT

Hydrogen sulfide (H_2S) is an endogenous gasotransmitter in human physiology and inflammatory disease, however, with limited knowledge of how signal transduction pathways are involved in immune cells. To examine the effects of sulfide on relevant intracellular signaling in human peripheral blood mononuclear cells (PBMCs), we stimulated healthy donor PBMCs with sodium hydrosulfide (NaHS, 1–1000 μM) to mimic H_2S stimulation, and analyzed phosphorylation of p38 mitogen activated protein kinase (MAPK) (pT180/pY182), NF-κB p65 (pS529), Akt (pS473) and CREB/ATF1 (pS133/pS63) with flow and mass cytometry. In contrast to transient effects in subsets of lymphocytes, classical monocytes demonstrated sustained phosphorylation of p38, Akt and CREB/ATF1. NaHS induced calcium dependent phosphorylation of p38, Akt and CREB, but not NF-κB, and the phosphorylation of Akt was partly dependent on p38, indicative of p38-Akt crosstalk. Attenuation of these effects by molecules targeting p38 and Hsp90 indicated Hsp90 as a possible target for H_2S-induced activation of p38. These results provide a description of a NaHS-induced signal transduction pathway in human primary immune cells that may have relevance for the role of sulfides in inflammation.

Abbreviations: H_2S, hydrogen sulfide; PBMCs, peripheral blood mononuclear cells; NaHS, sodium hydrosulfide; MAPK, mitogen activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; CREB, cAMP response element-binding protein; ATF1, activating transcription factor 1; Hsp90, heat shock protein 90; PKC, protein kinase C; LPS, lipopolysaccharide; MFI, median (or mean) fluorescent intensity; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; BAPTA, 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; PMA, phorbol 12-myristate 13-acetate; GFP, green fluorescent protein.

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1. Introduction

Hydrogen sulfide (H_2S) is a colorless malodorous gas that has received a substantial interest as an important gasotransmitter in regulation of inflammation [1–3]. H_2S is produced endogenously through the transsulfuration or 3-mercaptopyruvate sulfurtransferase/H_2S pathway [4–7], and the concentration of free H_2S in plasma is below 1 μM [8,9]. The concentration of endogenous H_2S is increased during sepsis, hemorrhagic shock and in cardiovascular disease [10–12]. Higher levels of H_2S are also measured in synovial fluid of chronically inflamed joints in rheumatoid arthritis patients [13,14]. As an exogenous source, inhalation of higher than ambient concentrations of H_2S is estimated to lead to an increase in plasma sulfide concentration [15]. Further, H_2S-releasing drugs are also developed and used for their anti-inflammatory properties in treatment of gastrointestinal disorders [16]. However, the role of H_2S as a possible mediator during inflammation remains elusive, with disparate inflammatory effects attributed to this gasotransmitter [10,11,13,14,17–21].

The transcription factor NF-κB is considered a key regulator of inflammation [22,23], and stimulation of the tumor necrosis factor receptors (TNFRs) or Toll like receptors (TLRs) typically activate both NF-κB p65 and p38 [24,25]. The p38 MAPK signaling pathway is involved in several aspects of inflammation, such as production of both pro- and anti-inflammatory cytokines, prostaglandin synthesis and inducible nitric oxide synthase (iNOS) production [26–30]. H_2S is known to modulate NF-κB activity through sulfhydration [31,32], and to increase phosphorylation of p38 in beta cells [33], vascular smooth muscle cells [34], endothelial cells [35] and glioma cells [36] but decrease phosphorylation in neutrophils [37]. Activation of protein kinase C (PKC) or the calcium/calmodulin dependent signaling cascade can both lead to p38 phosphorylation [30,38–40]. Preconditioning of cardiomyocytes with H_2S leads to cardioprotection and PKC activation, and this cardioprotection is attenuated by the PKC inhibitor chelerythrine [41]. Activation of conventional PKC isoforms is dependent on calcium [42], and H_2S is also reported to influence calcium homeostasis in excitable cells [41,43]. Whether H_2S activates p38 in PBMCs and if PKC or calmodulin dependent signaling is involved is not known.

Crosstalk between p38 and the proliferative Akt pathway during stimulation with lipopolysaccharide (LPS) or formyl-methionyl-leucyl-phenylalanine (fMLP) has been described to take place via the MAPK-activated protein kinases MK2/3 in macrophages [44] and neutrophils [45], respectively. H_2S treatment alone increases phosphorylation of Akt (S473) in rat myocardium tissue [46], pancreatic acinar cells [47] and endothelial cells [48], but only in endothelial cells have H_2S-induced Akt phosphorylation been shown to be p38 dependent, indicating p38-Akt crosstalk [48]. Akt plays a role in polarization of macrophages differentiation [49], but whether p38-Akt crosstalk takes place in primary monocytes during treatment with H_2S is, however, unknown. The use of comprehensive high dimensional single cell signaling assessment to describe the effects of H_2S on signal transduction in primary immune cells is so far in its infancy [50], and conventional flow cytometry and mass cytometry is a particular powerful technique suitable for delineating effects of small molecular inhibitors or activators in complex mixtures of blood cells [51–53]. To better understand how this gasotransmitter influence immune cells, we investigated how sodium hydrosulfide affects p38 MAP kinase and NF-κB intracellular signaling systems of PBMCs.

2. Materials and methods

2.1. Chemicals

Sodium hydrosulfide (NaHS) was acquired from Sigma-Aldrich (St. Louis, MO, USA), and a fresh stock was prepared prior to each experiment by dissolving it in saline at 0.2 M. The molecules ryanodine, 2-aminoethoxydi-phenyl borate (2-APB), W-7, KN-93, geldanamycin and ethylene glycol-bis(2-aminoethyl)-*N,N,N,N*-tetraacetic acid (EGTA) were purchased from Sigma-Aldrich. BAPTA-AM was purchased from Thermo Fisher Scientific (Waltman, MA, USA). The molecules RO318220, GÖ6976, LY333531, chelerythrine and PKC-412 were purchased from Santa Cruz (Dallas, TX, USA). BIRB796 and PH-797804 were acquired from Selleckchem (Houston, TX, USA). All molecules except EGTA were dissolved in DMSO. EGTA was dissolved in double distilled water (ddH_20) adjusted to pH 7.5. Paraformaldehyde (16%) was purchased from Thermo Fisher Scientific. Molecules used in the automated screen were selected from libraries from Selleckchem, Enzo Life Sciences (Farmingdale, NY, USA), Tocris Bioscience (Bristol, UK) or Sigma (details in Supplemental Table S2).

2.2. Culturing of THP-1 and Jurkat cells and isolation of PBMCs

The T-cell leukemia cell line Jurkat (Clone E6-1) and the myeloid cell line THP-1 were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Dr. Vibeke Andresen (University of Bergen, Norway) kindly provided the NF-κB/Jurkat/GFP™ Transcriptional Reporter Cell Line (System Biosciences, Palo Alto, CA, USA). All cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich, Inc. St. Louis, MO, USA) with 10% heat-inactivated Fetal Bovine Serum (PAA Laboratories GmbH, Austria), 1% L-glutamine (Sigma-Aldrich) and 1% Penicillin/Streptomycin (Sigma-Aldrich). Cells were cultured at 5% CO_2 humidified atmosphere at 37 °C. PBMCs were acquired from random healthy blood donors from the Department of Immunology and Transfusion Medicine, Haukeland University Hospital, Bergen, Norway. The local research ethics committee at Haukeland University Hospital approved the study (Regionale komiteer for medisinsk og helsefaglig forskningsetikk, REK), and the donors provided informed consent. Blood was collected in accordance with the Helsinki Declaration. Blood collected in EDTA tubes was diluted in saline (1:2) prior to density gradient separation (Lymphoprep™, Stemcell Technologies). PBMCs were isolated and washed two times in sterile saline prior to resuspension at 0.5×10^6 cells/ml in complete RPMI medium. PBMCs were kept in an incubator at 5% CO_2 humidified atmosphere at 37 °C for at least 60 min prior to experiments.

2.3. In vitro treatment of cells

For phospho flow cytometry experiments, PBMCs or cell lines were transferred to polypropylene flow cytometry tubes purchased from Sarstedt (Germany). After treatment of cells for the desired treatment time, cells were fixed by adding 16% paraformaldehyde to a final concentration of 4%. The fixation process was carried out at room temperature for 15 min prior to washing with saline. Cells were centrifuged at 1200 rpm for 5 min and subsequently resuspended in ice-cold methanol. Cells were immediately stained for flow cytometry or stored at −80 °C until staining. For mass cytom-