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Hydrogen sulfide inhibits ATP-induced neuroinflammation and $A\beta_{1-42}$ synthesis by suppressing the activation of STAT3 and cathepsin S

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ABSTRACT

Neuroinflammation and excessive β-amyloid$_{1-42}$ ($A\beta_{1-42}$) generation contribute to the pathogenesis of Alzheimer's disease (AD). Emerging evidence has demonstrated that hydrogen sulfide (H_2S), an endogenous gasotransmitter, produces therapeutic effects in AD; however, the underlying mechanisms remain largely elusive. In the present study, we investigated the effects of H_2S on exogenous ATP-induced inflammation and $A\beta_{1-42}$ production in both BV-2 and primary cultured microglial cells and analyzed the potential mechanism(s) mediating these effects. Our results showed that NaHS, an H_2S donor, inhibited exogenous ATP-stimulated inflammatory responses as manifested by the reduction of pro-inflammatory cytokines, ROS and activation of nuclear factor-κB (NF-κB) pathway. Furthermore, NaHS also suppressed the enhanced production of $A\beta_{1-42}$ induced by exogenous ATP, which is probably due to its inhibitory effect on exogenous ATP-boosted expression of amyloid precursor protein (APP) and activation of β- and γ-secretase enzymes. Thereafter, we found that exogenous ATP-induced inflammation and $A\beta_{1-42}$ production requires the activation of signal transducer and activator of transcription 3 (STAT3) and cathepsin S (Cat S) as inhibition of the activity of either proteins attenuated the effect of exogenous ATP. Intriguingly, NaHS suppressed exogenous ATP-induced phosphorylation of STAT3 and the activation of Cat S. In addition, we observed that NaHS led to the persulfidation of Cat S at cysteine-25. Importantly, mutation of cysteine-25 into serine attenuated the activity of Cat S stimulated by exogenous ATP and subsequent inflammation and $A\beta_{1-42}$ production, indicating its involvement in H_2S-mediated effect. Taken together, our data provide a novel understanding of H_2S-mediated effect on neuroinflammation and $A\beta_{1-42}$ production by suppressing the activation of STAT3 and Cat S.

1. Introduction

Alzheimer's disease (AD) is a major neurodegenerative disease, generally affecting people with age over 60 years. It is characterized by progressive cognitive decline and dementia (Maccioni et al., 2014). Despite decades of research, the treatment of this devastating disease remains largely lacking (Novak et al., 2017). Therefore, further studies are warranted to provide in-depth understating of the pathogenesis and to discover novel therapies.

Extensive evidence has indicated a pivotal role of neuroinflammation in the pathogenesis and progression of AD. Long standing anti-inflammatory drug therapy has shown beneficial effects against AD risk, symptomatic severity and overall disease progression (McGeer et al., 1996; Rich et al., 1995; Stewart et al., 1997). Microglia are the main inflammatory response cells in the central nervous system. In Alzheimer's disease brain, activated microglia are concentrated in regions of compact amyloid deposits (Chung et al., 1999). Together with neurons, glia cells are involved in Aβ deposition in Alzheimer's disease (Busciglio et al., 1993; Cras et al., 1990; Haass et al., 1991; Oberstein et al., 2015). Recent studies further demonstrated that a large portion of Aβ in the extracellular plaques of AD-brain parenchiyma is N-terminally truncated which are mainly released from microglia (Bayer and Wirths, 2014; Oberstein et al., 2015). These findings suggest that microglia-derived β-amyloid is an important player to drive AD pathogenesis.

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This study was therefore designed to investigate whether H_2S can inhibit Aβ formation and neuroinflammation and the underlying signaling mechanisms.

Emerging evidence has shown that extracellular ATP is a novel activator of microglial cells and it has been implicated in the pathogenesis of AD (Biber et al., 2007). ATP is stored within presynaptic vesicles and granules in healthy neurons and glial cells in millimolar concentrations (Le Feuvre et al., 2002); therefore, it can be abundantly released from damaged neurons and glial cells under neuropathological conditions like AD.

Extracellular ATP stimulates purinergic receptors on microglia and mediates the release of pro-inflammatory cytokines at the site of injury, thereby contributing to the neurodegeneration and behavioral disorders (Biber et al., 2007). There are two steps in ATP-induced inflammatory responses. The first step involves activation of nuclear factor-kappa B (NF-κB)-mediated signaling, by which the transcription of inflammasome-related components (e.g. inactive NACHT, LRR and PYD domains-containing protein 3 (NLRP3) and proIL-1β) is upregulated. In the second step, oligomerization and subsequent assembly of NLRP3, apoptosis-associated speck-like protein containing CARD domain (ASC), and procaspase-1 into a complex trigger the transformation of procaspase-1 to caspase-1, as well as the production and secretion of mature IL-1β (Shao et al., 2015). Additionally, NF-κB is known to stimulate the expression of many inflammatory mediators including cytokines, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Its activation was also found to upregulate BACE-1 expression, thereby increasing Aβ production (Na et al., 2007).

Signal transducer and activator of transcription 3 (STAT3) has also been demonstrated to be involved in neuronal differentiation, inflammation and Aβ pathogenesis (Wan et al., 2010; Wen et al., 2008). The involvement of STAT3 in P2 receptor-mediated inflammation and other transcriptional changes has been well documented (Cheng et al., 2013; Washburn and Neary, 2006). Along with these transcription factors, lysosome proteases play crucial roles in neuronal development, synaptic plasticity and neurodegeneration (Chen et al., 2008; Haque et al., 2008). Cathepsin S (Cat S), a lysosomal cysteine protease, is expressed predominantly in the microglia (Petanceska et al., 1996). P2 receptor activation stimulates Cat S (Clark et al., 2010), which regulates neuroinflammation and amylogenesis (Lemere et al., 1995; Wendt et al., 2008; Zhao et al., 2014b) and inhibition of Cat S can therefore produce neuroprotective effects (Xu et al., 2013). Moreover, Cat S has a definitive role in AD pathology (Lemere et al., 1995).

Hydrogen sulfide (H_2S), an endogenous gasotransmitter, has been shown to participate in neuronal modulation and protection in mammals in recent years (Cao et al., 2017). In brain physiology, it regulates the formation of long term potentiation by augmenting the activity of N-methyl-D-aspartate (NMDA) receptor (Kimura, 2013). Likewise, it also involves in the homeostasis of intracellular Ca^{2+} and H^+ (Cao et al., 2017; Nagai et al., 2004). Interestingly, H_2S also shows broad beneficial effects in various brain disorders such as ischemic stroke and Parkinson's disease (Hu et al., 2010; Lu et al., 2012; Zhang and Bian, 2014). H_2S was also found to ameliorate learning and spatial memory impairment in various AD animal models. Multiple mechanisms were reported. These include inhibition of mitogen-activated protein kinase (MAPK) and/or NF-κB pathways (Liu et al., 2015; Xuan et al., 2012), activation of Kelch-like ECH-associated protein 1–nuclear factor (erythroid-derived 2)-like 2 (Keap1-Nrf2) pathway (Liu et al., 2016), preservation of mitochondrial function (Zhao et al., 2016) and down-regulation of BACE1 and PS1 expression (He et al., 2016), etc. Given the significant role of exogenous ATP-induced neuroinflammation in AD, it is reasonable to postulate its involvement in H_2S-mediated protective effect in AD.

In fact, exogenous H_2S is recognized to possess anti-inflammatory effects though a consensus remains lacking regarding the pro- or anti-inflammatory role of endogenous H_2S. Both H_2S acute releaser NaHS and slow releaser GYY4137 were found to decrease lipopolysaccarides (LPS)-induced activation of NF-κB and release of TNF-α and IL-1β from macrophage (Hu et al., 2007a; Li et al., 2009). Moreover, NaHS can also attenuate LPS-induced p38 MAPK phosphorylation in microglial BV-2 cells resulting in an anti-inflammatory effect (Cao et al., 2017; Hu et al., 2007a). Nevertheless, whether exogenous H_2S affects exogenous ATP-induced neuroinflammation remains unclear. The current study was therefore designed to investigate the possible inhibitory effects of H_2S on exogenous ATP-induced inflammatory responses and $A\beta_{1-42}$ generation in microglial BV2 cells and mechanisms involved. Specifically, we analyzed the potential effects of H_2S on NF-κB signaling cascade and also determined the possible involvement of STAT3 and Cat S in observed effects. Moreover, we detected the persulfidation of Cat S and its possible contribution in observed effects of H_2S.

2. Materials and methods

2.1. Chemicals

Sodium hydrosulfide (NaHS), forskolin, 3-isobutyl-1-methylxanthine (IBMX), dichlorofluorescin diacetate and methylthiazolyl tetrazolium (MTT) were purchased from Sigma Aldrich (St. Louis, MO, USA). The specific Cat S inhibitor was ordered from Millipore (Catalog number: 219393; Cambridge, MA, USA). SC203282, SiRNA of STAT3 and Cat S was ordered from Santa Cruz Biotechnology (Santa Cruz, CA, USA). NaHS, ATP and MTT were dissolved in de-ionized water, while the specific Cat S inhibitor and SC203282 were dissolved in dimethylsulfoxide (DMSO). Primary antibodies against iNOS, COX-2, p-P65, p-IkBα, p-STAT3, Cat S and β-actin were purchased from Santa Cruz biotechnology (St. Louis, MO, USA). Monoclonal antibody against amyloid precursor protein (APP) (clone 22C11) and polyclonal antibody against APP C-terminus were from Millipore (Temecula, CA, USA).

2.2. Cell culture and treatments

The murine BV-2 microglial and human embryonic kidney 293 (HEK293) cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Both cell lines were cultured in 75 cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin (100 U)/streptomycin (100 mg/mL), and were maintained at 37 °C in an incubator under a humidified atmosphere of 95% air and 5% CO^2. Cells were split twice a week. For each experiment, confluent cells in 75-cm^2 flasks were seeded onto 35-mm dishes. Cells in culture dishes were used for experiments after reaching 80–90% confluence. Cells were either pretreated with NaHS for 30 min followed by ATP treatment for 24 h or pretreated with NaHS for 6 h followed by treatment with ATP for another 3 h.

2.3. Primary culture of mouse microglial cells

Primary astrocyte and microglia cultures were prepared from the cortex of newborn (1–2 days old) c57BL/6 mouse according to our previous literature (Hu et al., 2007b; Shigemoto-Mogami et al., 2001). In brief, cortical tissues were minced and digested with 0.2% trypsin. The cells were dissociated by mild mechanical trituration and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin and streptomycin, maintained at 37 °C. The medium was changed every 3 days. On reaching confluence (10–14 days), microglia were separated from the underlying astrocytic monolayer by gentle agitation. The floating microglia were collected and plated on sterile culture dishes. After 24-h culture, the microglial cells were ready for use.

2.4. Constructs and mutagenesis

The construct of Cat S was kindly provided by Dr. Hyun-Shik from