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Hydrogen Sulfide Prevents ATP-induced Neurotoxicity *via* Inhibiting The NLRP1/caspase-1/gasdermin D-mediated Pyroptosis Pathway*

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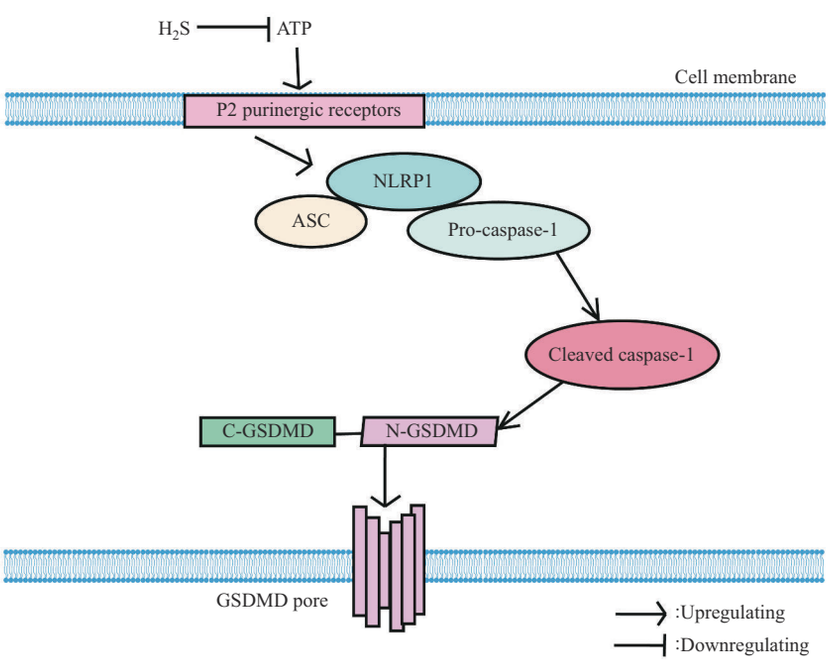
Graphical abstract



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Abstract **Objective** Stroke is a leading cause of death and disability worldwide, with ischemic stroke accounting for 80%−85% of cases. Despite the prevalence, effective treatments remain scarce. The compelling evidence suggest that high concentrations of ATP in the brain post-stroke can trigger irreversible neuronal damage and necrosis, contributing to a range of neurocellular dysfunctions. Pyroptosis, a recently identified form of programmed cell death, is characterized by caspase-1 activation and the action of the Gasdermin D (GSDMD) protein family, leading to cell perforation and inflammatory death. **Methods** In this study, human neuroblastoma SH-SY5Y cells were used to investigate the mechanisms of ATP-induced neurotoxicity and the protective effects of hydrogen sulfide (H_2S) against this toxicity through the antagonization of pyroptosis. We employed CCK-8 and LDH assays to assess cell viability. YO-PRO-1 fluorescent dyes and flow cytometry were conducted for detecting changes in cell membrane permeability. Western blot analysis was used to measure protein levels associated with cellular dysfunction. **Results** Our results indicate that high concentrations of ATP enhance cytotoxicity and increase cell membrane permeability in SH-SY5Y cells, that are mitigated by the H_2S donor NaHS. Furthermore, ATP was found to promote the activation of the NOD-like receptor pyrin domain-containing 1 (NLRP-1), caspase-1, and the cleavage of GSDMD, with NaHS significantly attenuating these effects. **Conclusion** Our research suggests that H_2S protects SH-SY5Y cells from ATP-induced neurotoxicity through a mechanism mediated by the NLRP1, caspase-1, and GSDMD pathway.

Key words stroke, ATP, H_2S, NLRP1, caspase-1, GSDMD, pyroptosis

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Stroke represents a principal cause of mortality and disability globally, posing significant threats to the health and well-being of middle-aged and elderly populations[1]. Ishemic stroke constitutes approximately 80%−85% of all stroke cases[2]. To date, tissue plasminogen activator (tPA) remains the only U. S. Food and Drug Administration (FDA) - approved therapeutic for ischemic stroke[3]. However, its efficacy is limited by a narrow therapeutic window and the potential for severe complications, restricting its benefit to a minority of patients[3]. Consequently, elucidating the pathophysiological mechanisms of ischemic brain injury and identifying novel therapeutic targets are imperative to enhance the treatment landscape for stroke worldwide.

ATP is well-known as a universal energy source for eukaryotic cells. Under physiological conditions, ATP concentrations remain low in the extracellular space, yet are maintained at high levels within the cytoplasm to support essential cellular functions. Under pathological conditions, ATP may be released into the extracellular space, where it plays a crucial role in disease progression by participating in signal transduction pathways. Typically, extracellular ATP enters adjacent cells by activating the P2 purinergic receptors, which include the ligand-gated ion channel P2X receptor and the G-protein-coupled P2Y receptor[4]. And this process is involved in tissue damage repair and the immune-inflammatory response. For example, studies have confirmed that under conditions of oxygen deprivation or hypoglycemia[5], hippocampal neurons release ATP and similarly, the striatum releases a substantial amount of ATP during cerebral ischemia[6]. Further research indicates that a high concentration of ATP in the brain alters the energy metabolism and inflammation levels in various neural cells (including neurons, microglia, and astrocytes), exacerbating the ischemic microenvironment and leading to neuronal death[7]. Therefore, exploring the mechanisms through which ATP induces neuronal death is crucial for understanding the pathophysiology of ischemic brain injury.

Prior to the 1980s, hydrogen sulfide (H_2S) was predominantly recognized as a toxic gas with potent reducing properties in nature. However, endogenous production of H_2S was later identified in several human organs, including the brain, liver, kidney, and vascular tissues[8]. Recent evidence increasingly substantiates the neuroprotective effects of H_2S[9]. For instance, H_2S has been shown to promote neurogenesis and ameliorate anxiety-like behaviors in diabetic rats[10]; it can also reverse oxidative stress-induced neuronal injury by modulating the mitogen-activated protein kinase signaling pathway[11]. Additionally, H_2S inhibits neuroinflammatory responses and neuronal death, which are pivotal in the pathology of ischemic stroke[12]. Post-intracerebral hemorrhage (ICH) interventions with H_2S significantly reduce the expression and activation of

the P2X7 receptor and its downstream inflammatory signaling pathway, the NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome, in microglia[13]. Recent studies from our group have further demonstrated that H_2S mitigates brain injury induced by middle cerebral artery occlusion/reperfusion (MCAO/R), reinforcing its protective role in neuronal damage[14]. Nevertheless, the capacity of H_2S to counteract neuronal damage induced by high concentrations of ATP and the underlying mechanisms involved remain to be elucidated.

In the present study, we explored the mechanisms underlying ATP-induced neurotoxicity and the neuroprotective effects of H_2S using SH-SY5Y neuroblastoma cells, which is widely employed to investigate the molecular mechanisms of neurodegeneration and cerebral ischemia-reperfusion injury[15]. Our findings indicate that H_2S mitigates neurotoxicity by inhibiting the ATP-induced NLRP1/caspase-1/GSDMD-dependent pyroptosis pathway. These results offer a novel perspective on the potential of H_2S to ameliorate neuronal damage in conditions such as ischemic stroke and other forms of traumatic brain injury.

1 Materials and methods

1.1 Reagents

NaHS was purchased from Sigma (St Louis, MO, USA, Cat#: 491-70-3), and dissolved in phosphate buffer solution (PBS) at 50 mmol/L as stock solutions. ATP was from Solarbio (Beijing Solarbio Science & Technology, China). Antibodies including rabbit anti-cleaved-caspase-1 (#4199T), anti-gasdermin D (#39754S) and NALP1 (#4990S) were from Cell Signaling Technology (Danvers, Massachusetts, USA). Rabbit anti-caspase-1 antibody (#AF5418) was from Affinity Biosciences (San Francisco, CA, USA). Antibodies including rabbit recombinant anti-cleaved N-terminal GSDMD antibody (ab215203), recombinant anti-GSDMD antibody (ab210070) were from Abcam. HRP-labeled goat anti-rabbit IgG antibody (#A0208) was from Beyotime (Shanghai, China). HRP-conjugated affinipure goat anti-rabbit IgG(H+L) (SA00001-2) was from Wuhan SANYING (Wuhan, China). LDH Cytotoxicity Assay Kit (#C0016) was from Beyotime (Shanghai, China). YO-PRO-1 (#Y3603) was from Invitrogen (USA). PVDF membranes were from Merk Millipore (Burlington, MA, USA). Cell Counting Kit-8 (#C0039) was from Beyotime (Shanghai, China).

1.2 Cell culture

SH-SY5Y cells were obtained from National Collection of Authenticated Cell Cultures (Shanghai, China) and were cultured in DMEM/high glucose medium (Hyclone) supplemented with 11% fetal bovine serum and double antibiotics (1%, 100 U/ml penicillin and 100 mg/L streptomycin and diluted at 100 times) at 37℃ under an atmosphere of 5% CO_2 and 95% air. The culture media were replaced every three days.

1.3 Cell viability assay

Cell viability was assessed using the CCK-8 kit according to the manufacturer's instructions. Cells were plated on 96-well plates with a cell density of 1.5×10^4/well and cultured for 24 h. Cells were treated with NaHS (250 μmol/L) for 30 min and then with indicated concentrations of ATP (5 mmol/L) for 24 h. After that, 10 μl CCK-8 solution was added into each well and incubated at 37℃ for 45 min. Absorbance was recorded at 450 nm using a microplate reader (Spectrophotometer-1510-01410; Thermo Fisher Scientific). The cell viability was calculated following the instructions. Cell viability= $(A_{\text{drug group}}-A_{\text{blank control}})/(A_{\text{control}}-A_{\text{blank control}})\times100$.

1.4 Lactate dehydrogenase activity assay

Lactate dehydrogenase (LDH) is an intracellular enzyme. The activity of intracellular LDH was assessed using LDH cytotoxicity assay kit[16]. Cells were plated on 96-well plates with a cell density of 1.5×10^4/well and cultured for 24 h. Cells were treated with indicated conditions for 24 h. After indicated treatment, cells were analyzed for the LDH activity according to the manufacturer instructions. Cells were incubated at 25℃ for 30 min in dark. At the end of reaction, formazan was formed as a chromogenic substance. Spectrophotometric absorbance at 490 nm were acquired using a microplate reader (Spectrophotometer-1510-01410. The ratio of LDH activity was determined, and cytotoxicity or mortality = $(A_{\text{experimental group}}-A_{\text{control group}})/(A_{\text{maximum enzyme activity of cells}}-A_{\text{control group}})\times100\%$.

1.5 Western blot

SH-SY5Y cells were grown in 25 cm^2 culture flasks up to 80%, treated with 0.25% EDTA/trypsin and seeded onto 100 mm Petri dish and cultured one

night. The cell density is approximately 60%. Cells were treated with NaHS (250 μmol/L) for 30 min and then with indicated concentrations of ATP (5 mmol/L) for 24 h. After that, cells were washed with PBS for 3 times and lysed in cell lysis buffer (400 μl) (Beyotime, Shanghai) . The cell lysate was collected by centrifugation (12 000 r/min) at 4℃ for 5 min, mixed with SDS loading buffer and denatured at 100℃ for 10 min. Protein samples of equal quantities were loaded into 12% SDS-PAGE gels (12 μl/well) and separated by electrophoresis. Proteins were transferred to the PVDF membranes (0.45 μm). The membranes after blocked with 5% skimmed milk powder at room temperature for 2 h, were incubated with one of the following rabbit primary antibody: GSDMD (1∶2 000), cleaved caspase-1 (1∶2 000), caspase-1 (1∶2 000), NALP1 (1∶2 000) and β-actin (1∶2 000) overnight at 4℃. After washed in TBS-Tween 3 times, 10 min each time, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1∶2 000) at room temperature for 2 h. The proteins were visualized using ChemiDocTMXRS+ (Bio-RAD). Image J software was used for analysis of the protein expression.

1.6 Cell membrane permeability

The cell membrane permeability was assessed by YO-PRO-1 using a protocol described previously[17]. YO-PRO-1 is a membrane-impermeable monomeric cyanine nucleic acid that stains double-stranded nucleic acids. YO-PRO-1 uptake by cells, indicative of cell membrane permeability, was examined using single cell imaging and flow cytometry. For single cell imaging, 3.5×10^4 cells were plated in each 10 mm culture dish and incubated for 24 h. Cells were treated with ATP (5 mmol/L) in the absence or presence of H_2S (250 μmol/L) for 24 h. YO-PRO-1 was added to culture media with a final concentration of 1 μmol/L and each sample was incubated with 1 μmol/L YO-PRO-1 and no light exposure for 45 min at 37℃. YO-PRO-1 fluorescence was determined using a fluorescence microscope (ZEISS, Germany).

For flow cytometry analysis, cells in 25 cm^2 culture flask that were treated by indicated treatment for 24 h, and then harvested by centrifugation. Cells were mixed with the 1 ml YO-PRO-1 (1 μmol/L) for 45 min at 37℃ in dark. YO-PRO-1 fluorescence was determined using flow cytometry (Beckman Coulter, USA).

1.7 Statistical analysis

All data were acquired from at least three independent experiments. Data are expressed as mean±SEM. Statistical analysis was performed using SPSS Statistics 19. The significance of inter-group differences was evaluated by one-way analyses of variance (ANOVA, least-significant difference’s test for post-hoc comparisons). Difference was considered to be significant when $P<0.05$.

2 Results

2.1 Treatment with NaHS protects SH−SY5Y cells from ATP−induced cytotoxicity

CCK-8 assay was conducted to examine the effects of ATP and NaHS, a H_2S donor, on the viability of SH-SY5Y neuroblastoma cells. Cells were exposed to different concentrations of ATP (1, 3, 5 and 7 mmol/L) for 24 h. Compared with cells under control conditions, cell viability significantly reduced after exposure to 5 and 7 mmol/L ATP (Figure 1a), demonstrating cytotoxicity at higher ATP concentrations. Conversely, NaHS treatment at concentrations of 200, 250 and 300 μmol/L during exposure to 5 mmol/L ATP significantly enhanced cell viability, particularly at 250 and 300 μmol/L (Figure 1b). NaHS did not significantly affect the viability of control or ATP-untreated cells. Correspondingly, intracellular lactate dehydrogenase (LDH) activity, a marker of cellular integrity, decreased following exposure to 5 mmol/L ATP for 24 h. However, this ATP-induced reduction in LDH activity was substantially mitigated by NaHS treatment (Figure 2). Treatment with NaHS alone did not alter LDH activity levels in control cells. Collectively, these results from the CCK-8 and LDH assays consistently demonstrate that NaHS treatment significantly mitigates ATP-induced cytotoxicity.

2.2 Treatment with NaHS inhibits ATP−induced cleavage of GSDMD and formation of large pores

N-gasdermin D (N-GSDMD) can oligomerize within plasma membranes to form membrane-spanning cytotoxic pores, consequently increasing cell membrane permeability and leading to pyroptotic cell death[18]. We investigated the cleavage of GSDMD, which produces pore-forming N-GSDMD proteins, in cells exposed to ATP with and without NaHS treatment. As depicted in Figure 3a, N-GSDMD protein levels, indicative of GSDMD cleavage, were

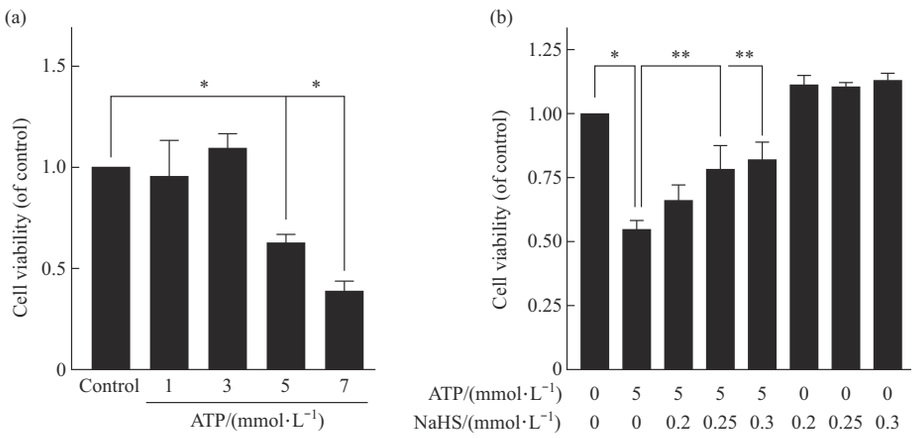

Fig. 1　Effects of treatment with NaHS on ATP−induced cytotoxicity in SH−SY5Y cells determined using the CCK−8 assay

(a) Cells were treated with indicated concentrations of ATP for 24 h. (b) Cells were treated with 5 mmol/L ATP in the absence or presence of indicated concentrations of NaHS for 24 h. Values are the mean±SEM (n=3). *P<0.05, compared to control cells; **P<0.05, compared to cells treated with ATP alone.

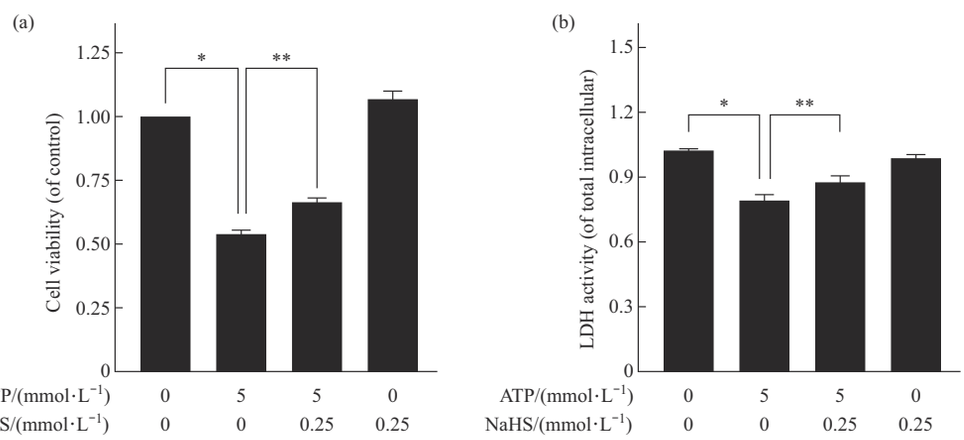

Fig. 2　Effect of treatment with NaHS on ATP−induced cytotoxicity in SH−SY5Y cells determined by the CCK−8 (a) and LDH assays (b)

Cells were treated with 5 mmol/L ATP in the absence or presence of 250 μmol/L NaHS for 24 h. Values are the mean±SEM (n=3−4). *P<0.05 compared to control cells; **P<0.05 compared to cells treated with ATP alone.

significantly elevated in cells treated with 5 mmol/L ATP. However, this effect was mitigated by NaHS treatment (Figure 3a). Furthermore, we analyzed total GSDMD protein expression. Notably, ATP treatment upregulated GSDMD levels, an effect that was largely inhibited by NaHS treatment (Figure 3b). Taken together, these findings indicate that GSDMD activation-dependent pyroptosis mediates ATP-induced cytotoxicity, and that NaHS effectively suppresses this ATP-induced, GSDMD-mediated pyroptotic pathway.

To further elucidate the role of pyroptosis in ATP-induced cytotoxicity, we assessed the formation of cytolytic pores in SH-SY5Y cells by analyzing YO-PRO-1 uptake through single-cell imaging and flow cytometry (Figure 4). Single-cell imaging revealed that ATP exposure increased both the number of cells exhibiting YO-PRO-1 uptake and the intensity of this uptake, indicating enhanced pore formation (Figure 4a). The ATP-induced increase in YO-PRO-1 uptake was attenuated by NaHS treatment (Figure 4a). Consistently, flow cytometry results showed a significant increase in the percentage of cells with YO-PRO-1 uptake following ATP exposure, an effect that

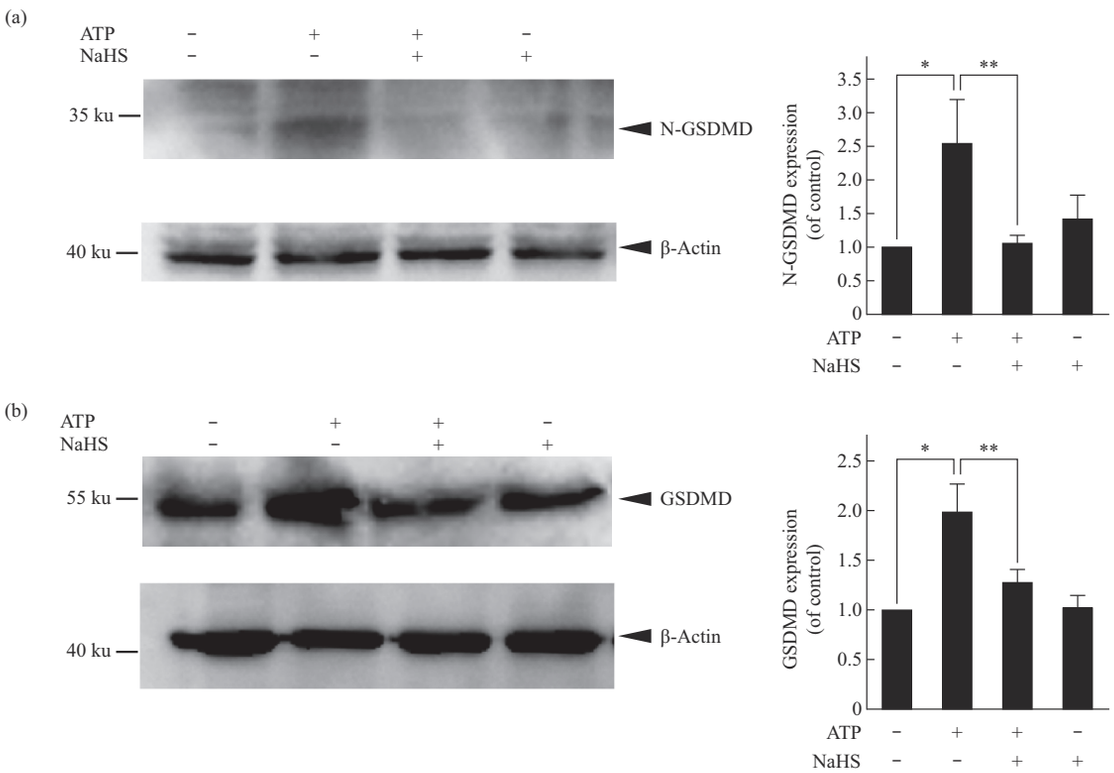

Fig. 3　Effects of treatment with NaHS on ATP−induced GSDMD cleavage and GSDMD expression in SH−SY5Y cells

Representative Western blots showing (left) and mean levels (right) of N-GSDMD (a) and GSDMD (b) in cells, following exposure to 5 mmol/L ATP for 24 h in the presence or absence of 250 μmol/L NaHS. Values are the mean±SEM (n=3). *P<0.05 compared to control cells; **P<0.05 compared to cells treated with ATP alone.

was also diminished by NaHS treatment (Figure 4b, c). These findings support the hypothesis that high concentrations of ATP induce GSDMD-mediated pyroptosis, and that NaHS can mitigate this ATP-triggered cell death pathway.

2.3　Treatment with NaHS reverses ATP−induced caspase−1 activation

Caspase-1 activation is crucial for the cleavage of GSDMD into its pore-forming component, N-GSDMD, which is essential for initiating pyroptosis[19]. To elucidate the impact of ATP and H_2S on caspase-1 activation, we analyzed levels of cleaved caspase-1, an indicator of activation, in SH-SY5Y cells *via* Western blot. Compared to control conditions, cells exposed to 5 mmol/L ATP exhibited a significant increase in cleaved caspase-1 levels, an effect that was inhibited by treatment with NaHS (Figure 5). Notably, ATP exposure also significantly enhanced caspase-1 protein expression, which was similarly curtailed by NaHS treatment (Figure S1). NaHS treatment alone did not affect the expression or activation of caspase-1. These findings indicate that ATP exposure stimulates the expression and activation of caspase-1, while H_2S effectively inhibits these ATP-induced changes in caspase-1.

2.4　Treatment with NaHS reverses ATP−induced upregulation of NALP1 expression

NLRP1, a key component of the inflammasome, exhibits distinct expression profiles in human tissues, with notably high levels in pyramidal neurons[20]. Next, we investigated the impact of ATP and NaHS treatments on NLRP1 expression using Western blot. As depicted in Figure 6, ATP exposure significantly increased NLRP1 expression, which was attenuated by NaHS treatment. Importantly, NaHS alone did not alter NLRP1 expression levels. These findings suggest that ATP-induced NLRP1 expression and activation are implicated in the initiation of caspase-1/GSDMD-mediated pyroptosis, which can be effectively mitigated by NaHS treatment.

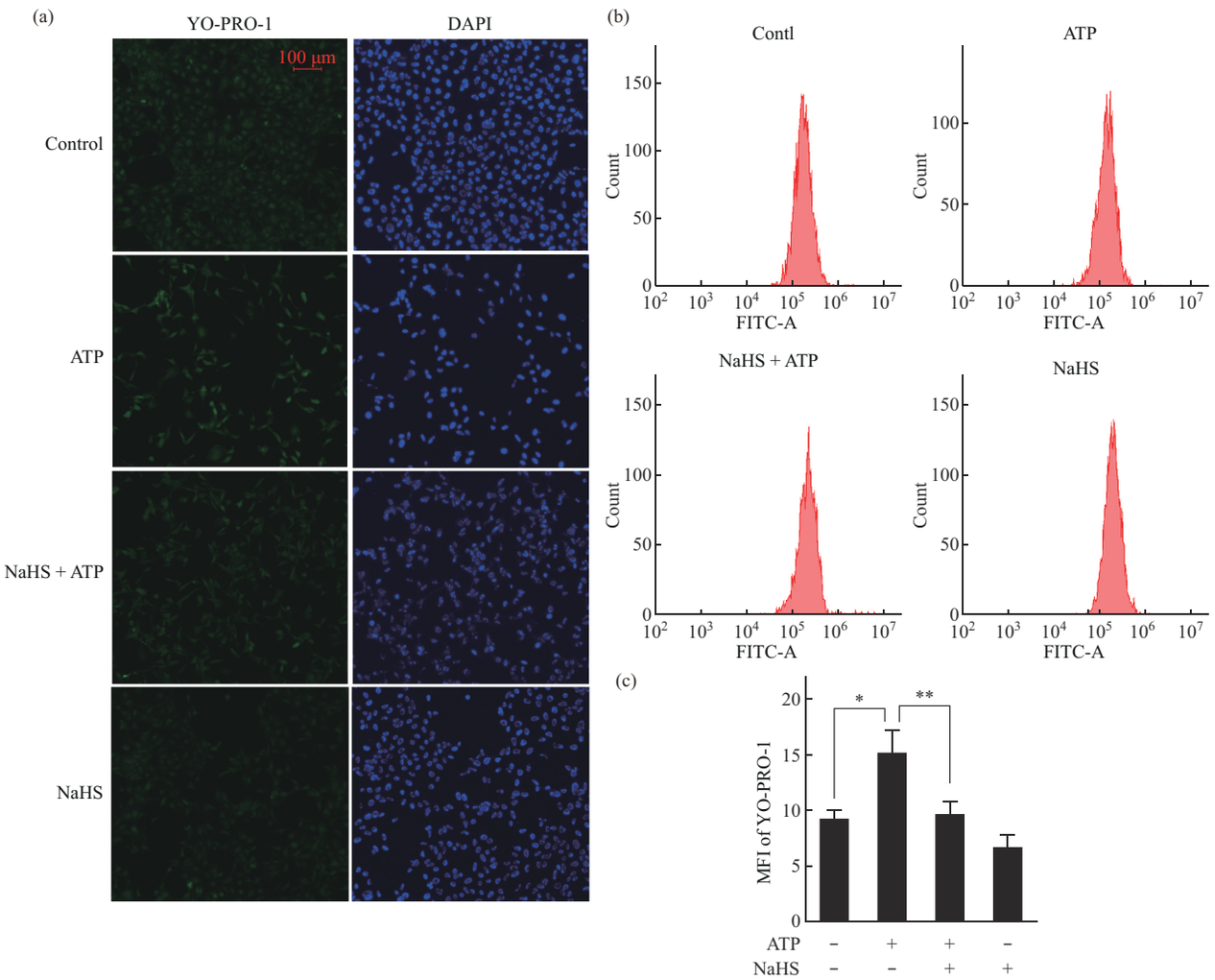

Fig. 4　Effects of treatment with NaHS on ATP−induced YO−PRO−1 uptake in SH−SY5Y cells

Cells were treated with 5 mmol/L ATP in the presence or absence of 250 μmol/L NaHS for 24 h, and YP-PRO-1 uptake was detected using a fluorescence microscope (a) and the mean fluorescence intensity (MFI) of YO-PRO-1 was determined using flow cytometry (b, c). Values are the mean±SEM (n=3). *P<0.05 compared to control cells; **P<0.05 compared to cells treated with ATP alone.

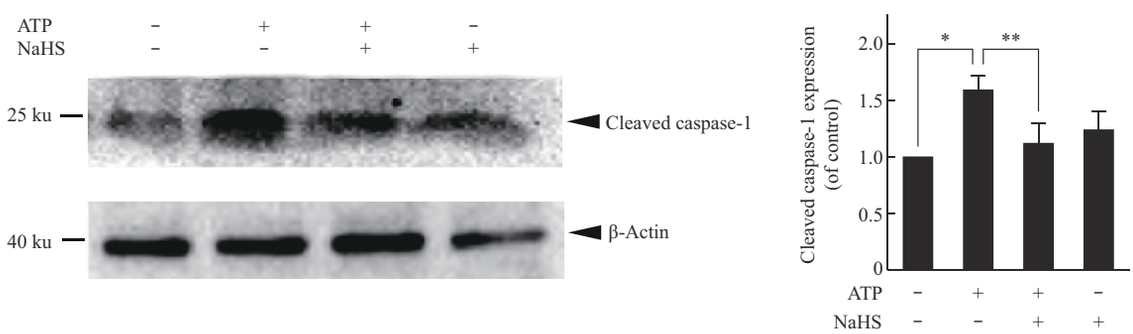

Fig. 5　Effects of treatment with NaHS on ATP−induced caspase−1 activation in SH−SY5Y cells

Representative Western blots showing (left) and mean levels (right) of cleaved-caspase-1 in cells, following exposure to 5 mmol/L ATP for 24 h in the presence or absence of 250 μmol/L NaHS. Values are the mean±SEM (n=3). *P<0.05 compared to control cells; **P<0.05 compared to cells treated with ATP alone.

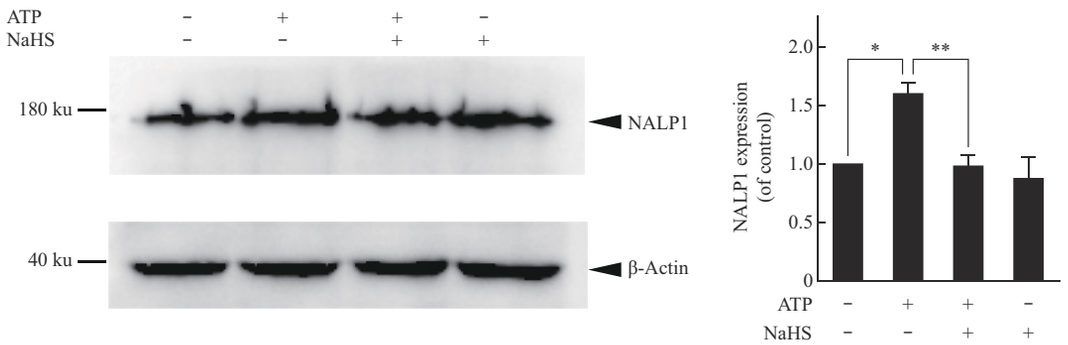

Fig. 6　Effects of treatment with NaHS on ATP−induced NALP1 expression in SH−SY5Y cells

Representative Western blots showing (left) and mean levels (right) of NALP1 expression in cells, following exposure to 5 mmol/L ATP for 24 h in the presence or absence of 250 μmol/L NaHS. Values are the mean±SEM (n=4). *P<0.05 compared to control cells; **P<0.05 compared to cells treated with ATP alone.

3　Discussion

In the present study, we showed that H_2S has a protective action against ATP-induced neurotoxicity in human SH-SY5Y neuroblastoma cells. More specifically, our study provides evidence to suggest that ATP induced cytotoxicity *via* the NLRP1/caspase-1/GSDMD-mediated pyroptosis pathway, which was inhibited by H_2S.

Neurologic deficits following ischemic stroke and other traumatic brain injury are primarily attributed to neuronal death, closely associated with high concentrations of extracellular ATP accumulation in damage regions[21]. Extracellular ATP is recognized as a damage-associated molecular pattern that potently triggers inflammation and activates the inflammasome *via* P2Y and P2X receptors[22]. Additionally, employing nucleoside triphosphate diphosphate hydrolase-1 to degrade high concentrations of ATP has been shown to effectively reduce neuroinflammation following ischemic brain injury[21]. These findings underscore the significant role of ATP in mediating inflammatory responses[23]. Consequently, mitigating the neurotoxic effects induced by high ATP concentrations could significantly improve outcomes after stroke-related brain injury. Previous research has documented the anti-inflammatory properties of H_2S in the brain and heart[24]. Consistent with these findings, our studies revealed that SH-SY5Y cells exposed to high ATP concentrations exhibit impaired activity (Figure 1a) and decreased intracellular LDH levels (Figure 2). However, treatment with NaHS effectively ameliorates ATP-induced toxicity (Figure 1b, 2), highlighting its potential as a therapeutic intervention.

To delineate the specific mechanisms of ATP-induced neurotoxicity, we focused on the pivotal role of cell pyroptosis. Pyroptosis, different from apoptosis and other regulatory cell death mechanisms such as ferroptosis, exhibit unique morphological and pathophysiological changes[25-26]. In brief, caspase-1 activation mediates the cleavage of GSDMD to produce its N-terminal fragment, N-GSDMD, which can oligomerize and form pores in the plasma membrane, leading to cell death and the release of pro-inflammatory cytokines IL-1β and IL-18[27]. Pyroptosis, characterized by these features, is a prevalent form of cell death in neurologic diseases and has been documented in ischemic stroke, cerebral hemorrhage[28]. In this study, we found that ATP exposure accelerated the cleavage of GSDMD to N-GSDMD in neural cells (Figure 3). Crucially, intervention with H_2S was able to halt this process and mitigate the activation of caspase-1 and the formation of membrane pores (Figure 4, 5).

Pyroptosis can be categorized into classical (caspase-1 mediated) and non-classical (caspase-4/5/9 mediated) pathways. The former is regulated by various inflammasomes including NLRP1, NLRP3, and AIM2[29]. Our previous research demonstrated that H_2S could mitigate cortical and retinal damage post-ischemic stroke by inhibiting the NLRP3/caspase-1/GSDMD pathway[14]. NLRP1, a critical component of the inflammasome complex alongside caspase-1[30], is

predominantly expressed in the brain, particularly in pyramidal neurons[20]. Prior studies have identified the involvement of the NLRP1 inflammasome and caspase-1 activation in neuronal pyroptosis triggered by amygdala kindling[31]. Additionally, we observed in SH-SY5Y cells that ATP exposure significantly elevated the expression levels of NLRP1 (Figure 6) and caspase-1 (Figure S1). Importantly, the ATP-induced activation of the NLRP1 inflammasome was mitigated by NaHS treatment. It has been proposed that the NF-κB pathway might regulate NLRP1 expression in primary cortical neurons under ischemic conditions[32]. Future investigations should explore these pathways further to elucidate the molecular mechanisms underlying ATP-induced pyroptosis and neuronal injury.

In summary, our study using SH-SY5Y cells provides evidence that H_2S mitigates ATP-induced neurotoxicity by inhibiting the NLRP1, caspase-1, and GSDMD-mediated pyroptosis pathway. These findings offer new insights into the mechanisms of ATP-induced neuronal cell death in stroke and other traumatic brain injuries, underscoring the potential of enhanced H_2S production as a neuroprotective strategy.

4 Conclusion

This study provides an evidence to suggest that H_2S is a neuroprotectant against ATP-induced neurotoxicity *via* inhibiting ATP-induced activation of the NLRP1/caspase-1/GSDMD pyroptosis pathway.

Supplementary Available online (http://www.pibb.ac.cn, http://www.cnki.net):
PIBB_20240117_Figure_S1.pdf

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硫化氢抑制NLRP1/caspase-1/gasdermin D通路对抗ATP神经毒性*

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摘要　目的　中风是导致世界范围内人类致死和致残的主要病因之一，在老年人中尤为突出，其中80%~85%的病例是缺血性脑卒中。已有大量文献表明，暴露于高浓度的ATP会导致神经元死亡，从而导致中风和其他创伤性脑损伤二次伤害。细胞焦亡是一种最新发现的细胞程序性死亡方式，主要通过激活包含caspase-1在内的多种caspase蛋白类型，造成包括GSDMD在内的多种Gasdermin家族成员发生剪切和多聚化，引发细胞穿孔，进而引起细胞死亡。本研究旨从细胞焦亡的角度探究高浓度ATP诱导的神经毒性作用和硫化氢（H_2S）的神经保护机制。**方法**　利用SH-SY5Y细胞建立ATP毒性损伤模型，检测H_2S对ATP诱导的神经毒性的影响，并探讨其潜在的分子机制。利用CCK-8细胞活性检测试剂盒和LDH检测试剂盒检测细胞活性。使用YO-PRO-1染色后，流式细胞仪测定法评估细胞膜渗透性。使用免疫印迹技术测定焦亡相关蛋白质水平。**结果**　SH-SY5Y细胞暴露于高浓度的ATP诱发了显著的细胞毒性和细胞膜通透性增加，这两者都被H_2S供体NaHS抑制。此外，暴露于ATP增强了NLRP-1、caspase-1的激活和N-GSDMD表达。通过NaHS预处理后，这些ATP诱导的作用显著减弱。**结论**　本实验研究结果表明，H_2S通过调控NLRP1/caspase-1/N-GSDMD通路抑制ATP诱导的细胞焦亡发挥神经保护作用。

关键词　中风，三磷酸腺苷，硫化氢，NLRP1，caspase-1，GSDMD，细胞焦亡
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