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Hydrogen sulfide attenuates lipopolysaccharide-induced cognitive impairment: A pro-inflammatory pathway in rats

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ABSTRACT

The present study investigated the effect of sodium hydrosulfide (NaHS), a H_2S donor, on cognitive impairment and neuroinflammatory changes induced by bilateral intracerebroventricular injections of LPS at a dose of 10 μg/rat. Rats received 5 mg/kg NaHS or volume-matched vehicle administration by intraperitoneal injection 3 days before LPS injection then for 9 days once daily. Morris water maze was used to detect the cognitive function. Compared to the sham-treated rats, LPS injection significantly prolonged the mean escape latency in the navigation test ($P<0.05$) and shortened the adjusted escape latency by approximately 30% ($P<0.05$). Meanwhile, LPS injection decreased H_2S level but increased pro-inflammatory mediators (i.e., TNF-α, TNFR1, degradation of IκB-α and thereafter activation of NF-κB) in hippocampus. However, these effects of LPS were significantly ameliorated with NaHS treatment ($P<0.05$ *vs* vehicle-treated group). The present data suggest that H_2S attenuates LPS-induced cognitive impairment through reducing the overproduction of pro-inflammatory mediators via inhibition of NF-κB pathways in rats. This study sets the stage for exploring a novel H_2S releasing agent for preventing or retarding the development or progression of neurological disorders such as Alzheimer's disease.

1. Introduction

A recent study suggested that neuroinflammation plays a major role in the pathogenesis of a number of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. AD is clinically characterized by progressive memory loss and cognitive decline. Although the etiology of AD in humans is still unknown, experimental and clinical evidence suggests a close association between neuroinflammation and AD pathogenesis (Akiyama et al., 2000, Loh et al. 2006). It is well known that microglia activation normally is a neuroprotective process, e.g., phagocytosis and clearance of amyloid and other exogenous substances, but overproduction of inflammatory mediators in the brain occurs when microglia, which are often found in close physical association with amyloid plaques in AD brains, become chronically activated (Versijpt et al., 2003; Venneti et al., 2009). It has been proposed that elevated levels of pro-inflammatory cytokines, including tumor necrosis factor (TNF), may inhibit phagocytosis of Aβ in AD brains thereby hindering efficient plaque removal by resident microglia (Koenigsknecht-Talboo and Landreth, 2005). Clinically, TNF-driven processes have been implicated in AD pathology and may contribute to cognitive dysfunction and accelerate progression of AD (Tobinick et al., 2006; Alvarez et al., 2007; Tan et al., 2007). In support of the idea that inflammatory disease may accelerate AD pathogenesis, the bacterial endotoxin lipopolysaccharide (LPS), a potent trigger of inflammation that elicits production of TNF and other cytokines, can accelerate the appearance and severity of AD pathology in several animal models of AD (Qin et al., 2007), intracentrically microinjection or chronic infusion (Flick and Gifford, 1986; Kitazawa et al., 2005). It is widely used to produce neuroinflammation, either by systemic injection (Qin et al., 2007), intracentrically microinjection or chronic infusion (Hauss-Wegrzyniak et al., 1998), or by incubation with brain cells (Kim et al., 2000).

Hydrogen sulfide (H_2S), recently hypothesized as the third 'gasotransmitter' alongside nitric oxide and carbon monoxide, has attracted extensive attention because of its multiple physiological and pathophysiological roles in various body systems (Wang, 2003; Kimura et al., 2005; Li et al., 2006). Endogenous H_2S is formed from cysteine by the action of either cystathionine β-synthase (CBS) or cystathionine c-lyase (CSE). CBS, the primary H_2S-producing enzyme in the brain, is highly expressed in the hippocampus (Abe and Kimura, 1996). Moreover, relatively high concentrations of H_2S (50–160 μmol/L) have been observed in brain tissues of a number of species including humans, rats and bovines (Goodwin et al., 1989; Warenycia et al., 1989; Savage and Gould, 1990). H_2S has been reported to be cardioprotective (Zhu et al., 2007; Chuah et al., 2007) and anti-inflammation (Li et al., 2005). In the central nervous system, it is reported that H_2S protects neurons against oxidative stress (Kimura and Kimura, 2004), attenuates LPS-induced neuroinflammation in microglia (Hu et al., 2007), and

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physiological concentrations of H_2S specifically potentiate the activity of the N-methyl-D-aspartate receptor and enhance the induction of hippocampal long-term potentiation (Abe and Kimura, 1996), which is associated with learning and memory. Whether there is any direct relationship between H_2S and neuroinflammation in vivo is still not known. Though two manuscripts reported that: 1) H_2S obviously diminished $A\beta_{25-35}$-induced apoptosis in PC12 cells (Tang et al., 2008); 2) H_2S alteration in level may be associated with the severity of AD (Liu et al., 2008). These findings suggest that endogenous H_2S might be involved in the pathogenesis of neuroinflammatory disease such as AD.

Therefore the present study was designed to investigate 1) whether sodium hydrosulfide (NaHS), a H_2S donor, prevents LPS-induced cognitive impairment in rats; and 2) what are the possible mechanisms?

2. Materials and methods

2.1. Animals

Male SPF Sprague–Dawley (SD) rats (8–10 weeks old weighing 250–350 g) were used in the present study. The rats were obtained from the Animal Center of the Third Military Medical University (Chongqing, China) (Certificate No. SCXK 20020003). The animals were acclimatized for 4 days at 22 ± 1 °C with a 12-h light–dark cycle, and were allowed free access to food and tap water throughout the experiment. All efforts were made to minimize the number of animals used, and all animal studies were performed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China (November 14, 1988) and the Institutional Review Committee for the use of Animal.

2.2. Drug administration and LPS intracerebroventricular injections

56 rats were randomly assigned to five groups: sham ($n=10$), sham + NaHS ($n=10$), LPS ($n=12$), LPS + ibuprofen (IBU, $n=12$) and LPS + NaHS ($n=12$). Rats in sham and LPS groups were given normal saline (NS) by intraperitoneal injection (i.p.) for 12 days, rats in LPS + IBU (Sigma, St. Louis, MO) groups were administrated with IBU by gavage at the dose of 40 mg/kg, and rats in sham + NaHS and LPS + NaHS groups were administered with NaHS (Sigma, St. Louis, MO) resolution by i.p. at the dose of 5 mg/kg (0.09 mM/kg), three days before surgery and thereafter continuously for 9 days. Three days after treatment with NaHS, NS or IBU, rats were anesthetized with 40 mg/kg chloral hydrate (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) by i.p., and secured in a stereotaxic apparatus(SR-6N, Narishige, Japan) with rectal temperature maintained at 37 °C using a heating pad. An area of skin on top of the skull was shaved and sterilized conventionally. Two small holes for needle insertion were drilled in the parietal bone posterior to bregma on either side of the midline (coordinates: posterior −0.8 mm, medial/lateral ±1.5 mm relative to bregma, dorsal/ventral −3.8 mm below dura; Paxinos and Watson, 1997). LPS (5 μg in 5 μL sterile NS) was injected into bilateral intracerebroventricular in the LPS, IBU and NaHS groups via a stainless steel needle using a microinjector. LPS solution was injected into each of the lateral cerebral ventricles over a 5-min period with a 5-min waiting period between the two injections. The sham and sham + NaHS group underwent all surgical steps, with the exception that saline was administered rather than LPS. Following wound suturing, all rats received an intramuscular injection of 40,000 U/0.25 mL of the antibiotic penicillin (Harbin Pharmaceutical Group Co., Ltd., General Pharmaceutical Factory, Harbin, China). Rats were closely monitored during recovery and kept in a room at 22 °C. Animal body weights were recorded daily, and their general behaviors were monitored. The rectal temperature was monitored by a clinical thermometer after surgery daily to ensure rats in health condition.

2.3. Morris water maze test

Cognitive function was evaluated by Morris water maze (Morris, 1984). The apparatus and test procedure have been described elsewhere (Gong et al., 2005). The Morris water maze test began on the 5th day after LPS injection. The procedure included two steps. The first step was the place navigation test from Days 1 to 4, in which the escape latency was used to evaluate learning and memory function. The second step was the spatial probe test on Day 5 after removal of the platform after the space navigation test, which was performed to test the ability of rats to find the removed platform by memory.

2.4. Neuronal ultrastructure observation in CA1 region of hippocampus

For neuronal ultrastructure observation in CA1 region of hippocampus, after the behavioral experiments three rats from each group were anesthetized by an overdose of 7% chloral hydrate (50 mg/kg) and were perfused transcardially with 0.1 M phosphate buffer with 0.4% heparin, immediately followed by 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.38). Brains were removed and cut into 50 mm sections with a Vibratome. Vibratome sections were routinely embedded in glycide ether for ultrastructural examination. After light microscopic examination of the embedded Vibratome sections, selected CA1 regions of the dorsal hippocampus were collected. Next, these tissue masses were retained in glutaraldehyde (2.5%) for 24 h. Then semi-thin sections were made, which would be stained with the Toluidine blue so as to locate pyramidal layer of hippocampal CA1 region, after a series of process of dehydration, infiltration, embedment, and polymerization. Finally, ultra-thin sections (50 nm) were cut and taken at the interval of six sections. Two copper screens were observed in compatible times, and three photos were taken from each rat at least for transmission electron microscopy (TEM).

2.5. Measurement of H_2S level in hippocampus

The remaining rats from each group after the behavioral experiments were euthanized by rapid decapitation, and the left hippocampus was removed and flash-frozen with liquid nitrogen, then homogenized in ice-cold 50 mM/L potassium phosphate buffer, pH 8.0 (12% wt/vol), with a Polytron homogenizer. The homogenate was centrifuged (47,000 g; 10 min; 4 °C) and the supernatant (75 μL) was mixed with 0.25 mL Zn acetate (1%) and 0.45 mL water for 10 min at room temperature. TCA (10%; 0.25 mL) was then added, centrifuged (14,000 g; 10 min; 4 °C), and the clear supernatant was collected and mixed with *N*,*N*-dimethyl-p-phenylenediamine sulfate (20 mM/L; 133 μL) in 7.2 M/L HCl and $FeCl_3$ (30 mM/L; 133 μL) in 1.2 M/L HCl. After 20 min, absorbance at 670 nm was measured with a microplate reader. The calibration curve was linear from 0 to 320 μM/L NaHS or 96 μM/L H_2S (Wang et al., 2009; Qu et al., 2006).

2.6. Real time RT-PCR analysis

The remaining rats from each group after the behavioral experiments were euthanized by rapid decapitation, and four right hippocampi were removed and placed into the tube containing Trizol (Huashun Bioengineering Co, Shanghai, China). Total RNA was isolated and purified with RNeasy Mini Kit (Qiagen, Valencia, CA). The forward and reverse primer sequences for selected genes were designed with the ABI Primer Express software (Foster City, CA) and listed in Table 1. The Power SYBR Green Master Mix (Applied Biosystems, Cheshire, UK) was used for real time PCR analysis. The relative differences in expression between groups were expressed using cycle time (Ct) values as follows: the Ct values of the interested genes were first normalized with GAPDH of the same sample, and then the relative differences between control and treatment groups

Table 1
Primer sequences for real time RT-PCR analysis.

Gene	Genbank accession#	Forward	Reverse
TNF-α	L19123	GTGATCGGTCCCAACAAGGA	CTCCCACCCTACTTTGCTTGTG
TNFR1	NM_013091	GCTGCACCAAGTGCCACAA	TCACACACCTCGCAGACTGTTTC
GAPDH	NM_017008	CAGTGCCAGCCTCGTCTCA	TAACCAGGCGTCCGATACG

were calculated and expressed as relative increases, setting control as 100%.

2.7. Western blot analysis

The remaining rats from each group after the behavioral experiments were euthanized by rapid decapitation, and three right hippocampi were removed and rapidly frozen at −80 °C. The frozen tissues were cut into small pieces, homogenized in 0.5 mL of RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% sodium dodecylsulfate, 50 mM Tris–hydrochloric acid, 2 mM phenylmethylsulfonyl fluoride pH 7.4), and incubated at 4 °C overnight. The dissolved proteins were collected after centrifugation at 10,000 *g* for 30 min, and the supernatant was then collected. Protein concentrations were determined using the enhanced BCA protein assay kit (Biocolor Biotechnology, Shanghai, China). The proteins were then separated by SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane, then the membrane was incubated with a primary antibody (Ab) against TNF-α (1:1500, Cell Signaling Technology, Inc., Boston, USA), TNF-α receptor 1 (TNFR1, 1:3000, Abcam, Inc., Cambridge, USA), IκB-α (1:1500, Cell Signaling Technology, Inc., Boston, USA) and GAPDH (1:50,000, Kangcheng Inc., Shanghai, China), and then it was incubated with the appropriate secondary horseradish peroxidase-conjugated anti-rabbit IgG antibodies(1:40,000, Santa Cruz Biotechnology, Inc., Santa Cruz, USA). The membranes were visualized using an ECL system and then developed on Hyperfilm (Amersham). Immunoreactive proteins were visualized using the ECL western blotting detection kit (Thermo Fisher Scientific Inc., Boston, USA) (Liu et al., 2009).

2.8. Statistical analysis

All values were presented as means and standard error. One-way analysis of variance (ANOVA) was used to examine statistical comparisons between groups. The statistical significance of difference between two groups was determined using two-tailed Student's *t*-test. All analyses were performed using SPSS 12.0. A probability value of <0.05 was taken to indicate statistical significance.

3. Results

3.1. Effect of NaHS on LPS-induced cognitive impairment

LPS injection into bilateral ventricle was tolerated by majority of rats, with less than 5% mortality. Initially, all the rats lost a few grams of body weight within 2 days after surgery. However, all of the rats had regained weight and continued to grow normally for the duration of the study. From the 5th day after surgery, all the rats received 5-day Morris water maze test. The Morris water maze test showed that NaHS treatment in sham group did not affect the rat's cognitive function, while the learning and memory abilities of LPS-treated rats were significantly impaired compared with sham group. In the navigation test, the mean escape latency was tripled ($P<0.05$, Fig. 1A), while in the spatial exploring test, the adjusted escape latency was shortened by approximately 30% ($P<0.05$, Fig. 1B), indicative of cognitive impairment. However, LPS-induced learning and memory deficits were significantly alleviated by treatment with NaHS as well as IBU compared with LPS group ($P<0.05$, Fig. 1A–B).

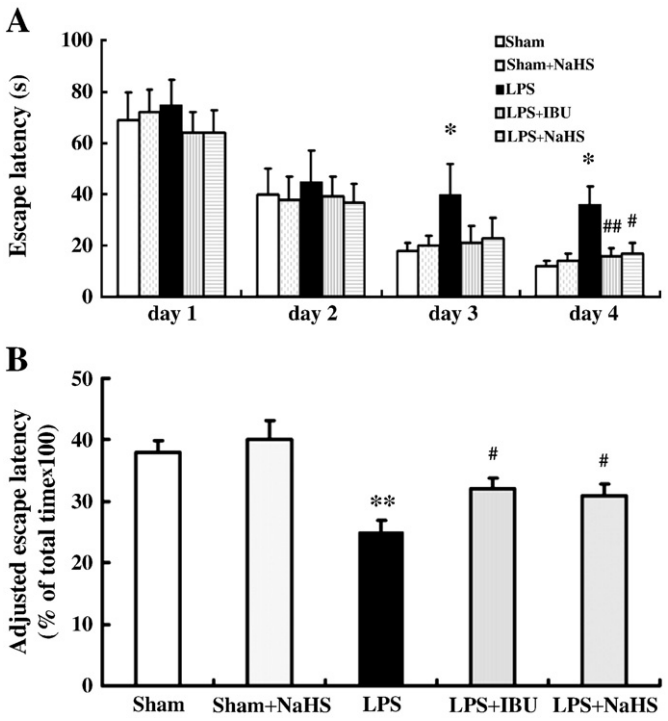

Fig. 1. Effect of NaHS on LPS-induced cognitive impairment. LPS (5 μg/5 μL) or NS was slowly injected into bilateral ventricle, and subjected to the Morris water maze test 5 days later. A) The escape latency in the navigation test. B) The adjusted escape latency in the spatial exploring test. Data presented as mean ± SEM, $n=10$–12. $^{*}P<0.05$, $^{**}P<0.01$ *vs* sham; $^{\#}P<0.05$, $^{\#\#}P<0.05$ *vs* LPS alone.

3.2. Effect of NaHS on LPS-induced neuronal ultrastructure alteration

The neuronal ultrastructure in hippocampal CA1 regions was observed by TEM after the behavioral experiments. TEM evidence showed that the nucleus of hippocampal neurons in CA1 regions were big, round, or oval. The euchromatin distributed homogeneously, the structures of intracytoplasmic mitochondria and rough endoplasmic reticulum were clear, and the ribosomes were abounded (Fig. 2A), and the neurons in sham + NaHS group were closely similar to those in sham group. On the contrary, the nucleus of hippocampal neurons subjected to LPS were irregular, a chromatin mass formed, the perinuclear space obviously became thicker, mitochondria swelled and vacuolized, cristae arrangement became disordered, mitochondria membranes were ruptured, and the endocytoplasmic reticula were expanded. Some neurons were even obviously broken to pieces and the membrane dissolved (Fig. 2B). However, treatment with IBU could obviously relieve the neuronal damage compared with those of LPS alone. Most of the nuclear chromatins distributed homogeneously, the ultramicrostructure was similar to those of neurons in sham group with swollen mitochondria mitigated, lamellar cristae in mitochondria became clear, and the ribosomes became more abundant (Fig. 2C). Treatment with NaHS also relieved neuronal morphological damage compared with those of LPS alone, and the ultramicrostructure was similar to those of the IBU group (Fig. 2D).

3.3. Alteration of H_2S level in hippocampus

After the behavioral experiments, H_2S level in hippocampus was assayed. We found that administration of NaHS to rats in sham group did not affect the hippocampal H_2S concentration, but LPS injection decreased H_2S level in hippocampus compared with sham group

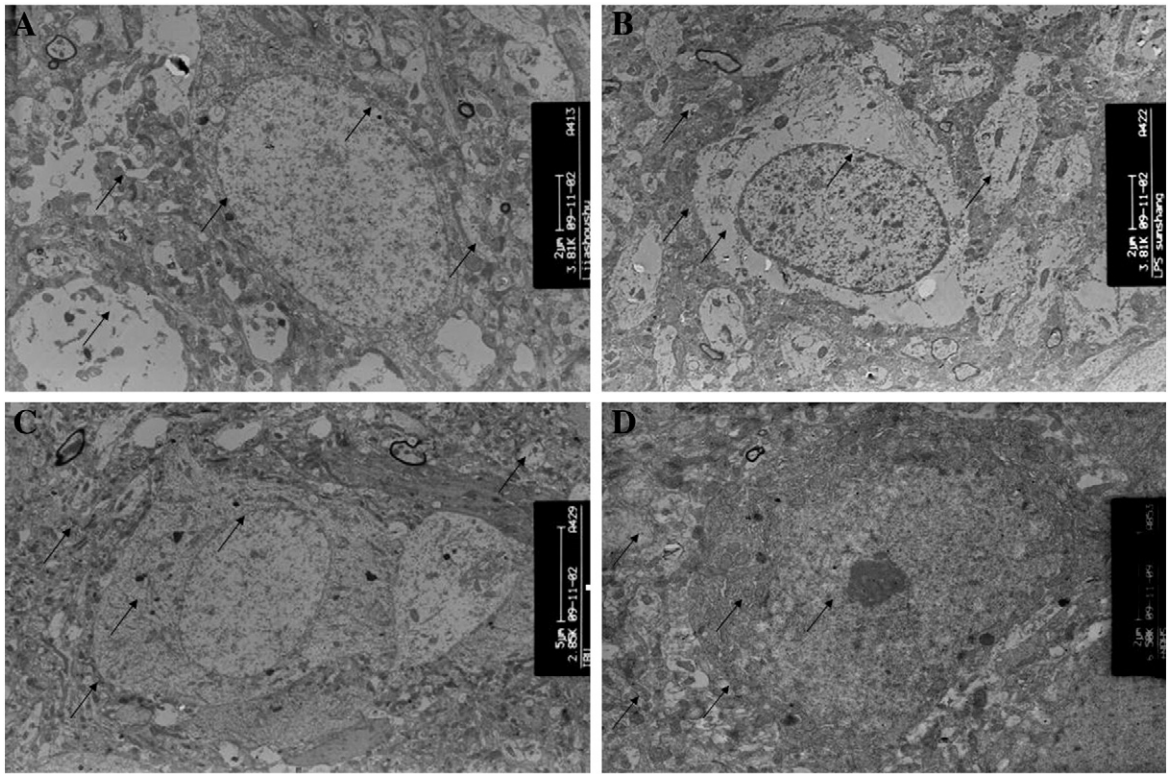

Fig. 2. Effect of NaHS on LPS-induced neuronal ultrastructure alteration. The neuronal ultrastructure in hippocampal CA1 regions was examined by TEM, and all the ultrastructure changes were indicated by arrows. A: Sham (×3810) and B: LPS (×3810) injection showed remarkable ultrastructure damages of hippocampal neurons in CA1 regions. Treatment with C: IBU (×2850), as well as D: NaHS (×6500) significantly attenuated the damages subjected to LPS.

($P<0.05$, Fig. 3). In contrast, treatments with IBU or NaHS significantly increased the hippocampal H_2S level compared with LPS group ($P<0.05$, Fig. 3).

3.4. Effect of NaHS on the expressions of TNF-α and TNFR1 mRNA

After the behavioral experiments, the expressions of TNF-α and TNFR1 mRNA in hippocampus of four rats from each group were detected by real time RT-PCR analysis. Compared with sham group, NaHS did not affect the expressions of TNF-α and TNFR1 mRNA, LPS injection into bilateral ventricle significantly increased the expressions of TNF-α and TNFR1 mRNA, which were approximately 3.3-fold and 1.8-fold, respectively ($P<0.01$, Fig. 4A–B). However, compared with LPS group, treatments with IBU or NaHS significantly decreased the mRNA expressions of these selected genes ($P<0.05$, Fig. 4A–B).

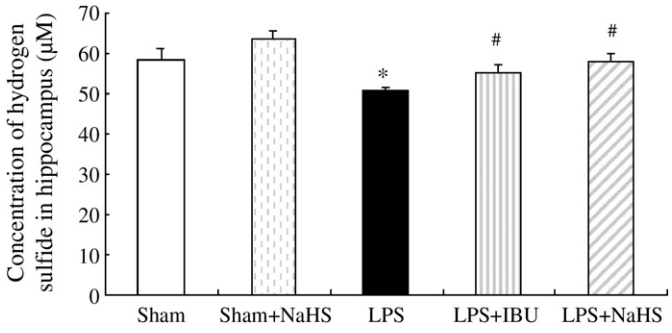

Fig. 3. Alteration of H_2S level in rat hippocampus. Administration of NaHS to rats in sham group did not affect the hippocampal H_2S concentration, but LPS injection decreased H_2S level in hippocampus compared with sham group. Treatments with IBU or NaHS significantly increased the hippocampal H_2S level compared with LPS group. Data presented as mean ± SEM, $n=7$–9. $^{*}P<0.05$ *vs* sham; $^{\#}P<0.05$ *vs* LPS alone.

3.5. Effect of NaHS on the expressions of TNF-α and TNFR1 protein

To explore the mechanism of action of NaHS on amelioration of LPS-induced cognitive impairment, the expressions of TNF-α and TNFR1 protein were determined by Western blot. Consistent with the mRNA expressions, NaHS did not affect the expressions of TNF-α and TNFR1 protein, but LPS injection into bilateral ventricle significantly increased the expressions of TNF-α and TNFR1 protein ($P<0.01$, Fig. 5A–C). However, after treatment with IBU or NaHS, the TNF-α and TNFR1 expressions significantly decreased compared with LPS group ($P<0.05$, $P<0.01$, Fig. 5A–C).

3.6. Protection by NaHS against LPS toxicity involved IκB-α degradation and NF-κB p65 phosphorylation

To further explore the mechanisms underlying the inhibitory effect of NaHS on the expressions of TNF-α and TNFR1, IκB-α and phosphorylation of NF-κB p65 were analyzed by Western blot. NF-κB is inactive in the cytosol because it is bound to IκB, and becomes active after IkB has been phosphorylated and subsequently degraded. NaHS was found not to affect the IκB-α protein levels in sham group. LPS injection into bilateral ventricle significantly decreased the IκB-α protein levels ($P<0.01$, Fig. 5A, D). However, treatment with IBU or NaHS was found significantly to repress the LPS-induced IκB-α degradation ($P<0.01$, Fig. 5A, D). RelA/p65 is a subunit of the NF-κB transcription complex, which plays a crucial role in inflammatory and immune responses. After the LPS-induced phosphorylation and degradation of IκB-α, p65, a part of p65/p50 heterodimer of NF-κB, translocates to the nucleus and then phosphorylates. Accordingly, NaHS was found not to affect the NF-κB p65 phosphorylation in sham group. LPS injection into bilateral ventricle significantly enhanced the NF-κB p65 phosphorylation ($P<0.01$, Fig. 5A, E). However, treatment with IBU or NaHS was found significantly to decrease the LPS-induced

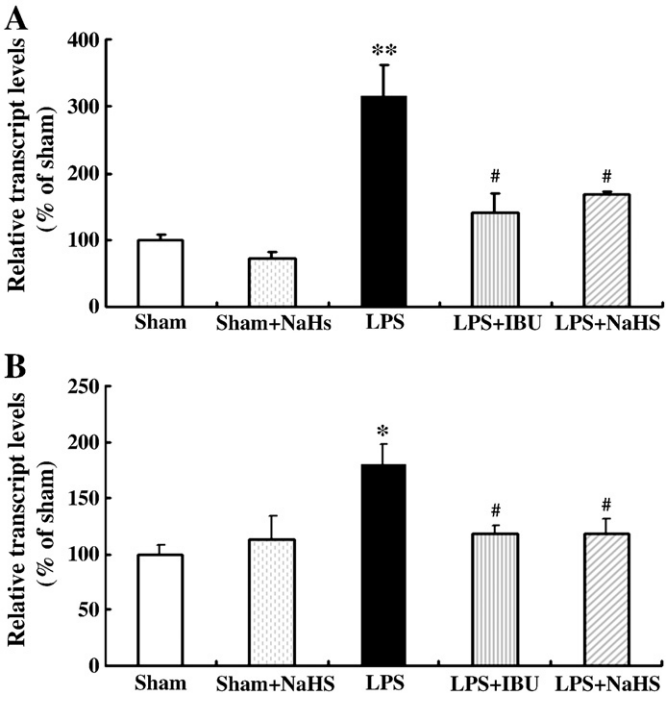

Fig. 4. Effect of NaHS on the expressions of TNF-α and TNFR1 mRNA. The expressions of TNF-α and TNFR1 mRNA in hippocampus were detected by real time RT-PCR. NaHS did not affect the expressions of TNF-α and TNFR1 mRNA, but LPS injection into bilateral ventricle significantly increased the expressions of TNF-α and TNFR1 mRNA. Treatments with IBU or NaHS significantly decreased the mRNA overexpressions of these selected genes. A) The expressions of TNF-α mRNA. B) The expressions of TNFR1 mRNA. Data are mean ± SEM, $n=4$. $^{*}P<0.05$, $^{**}P<0.01$ *vs* sham; $^{\#}P<0.05$ *vs* LPS.

NF-κB p65 phosphorylation ($P<0.01$, Fig. 5A, E). Together, these findings indicate that the inhibition of LPS-induced production of TNF-α and TNFR1 by NaHS or IBU is mediated by suppressing LPS-induced IκB-α degradation and thereafter activation of NF-κB.

4. Discussion

The present study clearly demonstrated that (1) a single injection of LPS into bilateral ventricles produced cognitive impairment in rats, and neuronal ultrastructure damage, with concomitant production of TNF-α and TNFR1, and these effects occurred via IκB-α degradation and thereafter activation of NF-κB in rat's hippocampus; (2) Pre- and after LPS injection treatment with NaHS, a H_2S donor, significantly attenuated LPS-induced cognitive deficits, and neuronal ultrastructure damage, with concomitant inhibitions of TNF-α and TNFR1 production, as well as repressed LPS-induced IκB-α degradation and thereafter activation of NF-κB.

In the present study, NaHS administration by i.p. at the dose of 0.09 mM/kg is safe consistent with the reported (Qu et al., 2006). However, NaHS significantly attenuated LPS-induced learning and memory deficits and alleviated neuronal ultrastructure damage. NaHS was used as a H_2S donor, which is dissolved in water, HS^- is released and forms H_2S with H^+. This provides a solution of H_2S at a concentration that is about 33% of the original concentration of NaHS (Reiffenstein et al., 1992). Consistent with the rat's cognitive function, NaHS did not increase hippocampal H_2S level in sham group, LPS injection decreased H_2S level in hippocampus, while treatments with IBU or NaHS significantly increased the hippocampal H_2S level. Under physiologic conditions, H_2S level in hippocampus may keep balance by its autoregulation. However, H_2S production is suppressed by inflammatory stimulation of microglia and astrocytes, and this suppression reduces the natural anti-inflammatory effect of H_2S (Lee et al., 2009). Although H_2S level by treatments with IBU or NaHS is similar, it is concluded that the mechanisms are different. IBU, a typical nonsteroidal anti-inflammatory drug, cannot cause a release of H_2S for its structure without sulfur-containing group. Hence it is thought that IBU attenuates the activation of microglia and astrocytes induced by LPS, and thereafter maintains H_2S level in hippocampus. On the whole, the present study indicated that H_2S is involved in attenuation of LPS-induced neuroinflammation in vivo.

Hippocampus is the brain region known to be particularly susceptible to AD, and has an important role in learning and memory functions (Rosi et al., 2005; Tanaka et al., 2006). Learning and memory deficits occur when neuroinflammation affects hippocampus function (Rosi et al., 2005; Tanaka et al., 2006; Lee et al., 2008), even under conditions of no apparent neuron death in hippocampus (Tanaka et al., 2006), and this study further demonstrated that bilateral ventricle injection of LPS, with hippocampus as a target, can also produce learning and memory deficits and neuronal ultrastructure damage, and this cognitive impair is related to hippocampal inflammation and its neuronal damage (Hauss-Wegrzyniak et al., 1998; Rosi et al., 2005).

It has been proposed that elevated levels of pro-inflammatory cytokines, including TNF-α, may inhibit phagocytosis of Aβ in AD brains thereby hindering efficient plaque removal by resident microglia (Koenigsknecht-Talboo and Landreth, 2005). In the present study, we found that a single injection of LPS into bilateral ventricles significantly enhanced the production of TNF-α and TNFR1 mRNA and protein, and accompanied by a decrease of H_2S levels. However, administration of NaHS significantly decreased the production of TNF-α and TNFR1 mRNA and protein, and accompanied by an increase of H_2S levels. Cells can express two different TNF receptors, TNFR1 and TNFR2. TNFR2 is mainly expressed in immune cells. Both receptors are very similar in their extracellular domains, but TNFR2 does not contain a death domain, which is an important protein–protein interaction domain. Activation of the TNFR1 by binding of TNF-α can lead to a multitude of cellular responses, including inflammation (McAlpine and Tansey, 2008). Accordingly, TNFR1-deficient mice exhibit some of the same phenotypes as TNF-α knockout mice, including the poor response to infection (Wajant et al., 2003). Consistent with the role of TNF-α in regulation of APP processing, genetic deletion of the TNF-α receptor gene *Tnfrsf1a* in the APP 23 transgenic mouse model reduced both the number of amyloid plaques and the cognitive deficits in these mice (He et al., 2007).

Among the several transcriptional factors activated by inflammatory responses during viral and bacterial infections, NF-κB is known to up-regulate the expressions of cytokines, chemokines, adhesion molecules, acute phase proteins, and inducible effector enzymes that are involved in innate immune response (Ghosh and Karin, 2002). NF-κB usually exists as a homodimer or heterodimer, and a heterodimer of p50 and one of Rel family p65 is known to mediate the expression of genes related with innate immune response (Sha, 1998). In unstimulated cells, NF-κB dimers are bound to inhibitory κB (IκBs), and as a result are retained in the cytoplasm. However, when the cells are stimulated with pro-inflammatory stimuli, IκBs are rapidly phosphorylated and degraded via IκB kinase complex, and the free NF-κB is translocated to the nucleus, where it binds to target sites and induces the transcriptions of pro-inflammatory mediators (Li and Verma, 2002). The present study revealed that NaHS inhibited the LPS-induced degradation of IκB-α, and the subsequent phosphorylation of the p65 subunit of NF-κB in the nucleus. It can be concluded that the free NF-κB is translocated to the nucleus, where it binds to target sites and induces the transcriptions of pro-inflammatory mediators, including TNF-α and TNFR1. However, treatment with NaHS inhibits the degradation of IκB and the subsequent phosphorylation of the p65 subunit of NF-κB in the nucleus, by which the generations of TNF-α and TNFR1 are repressed.

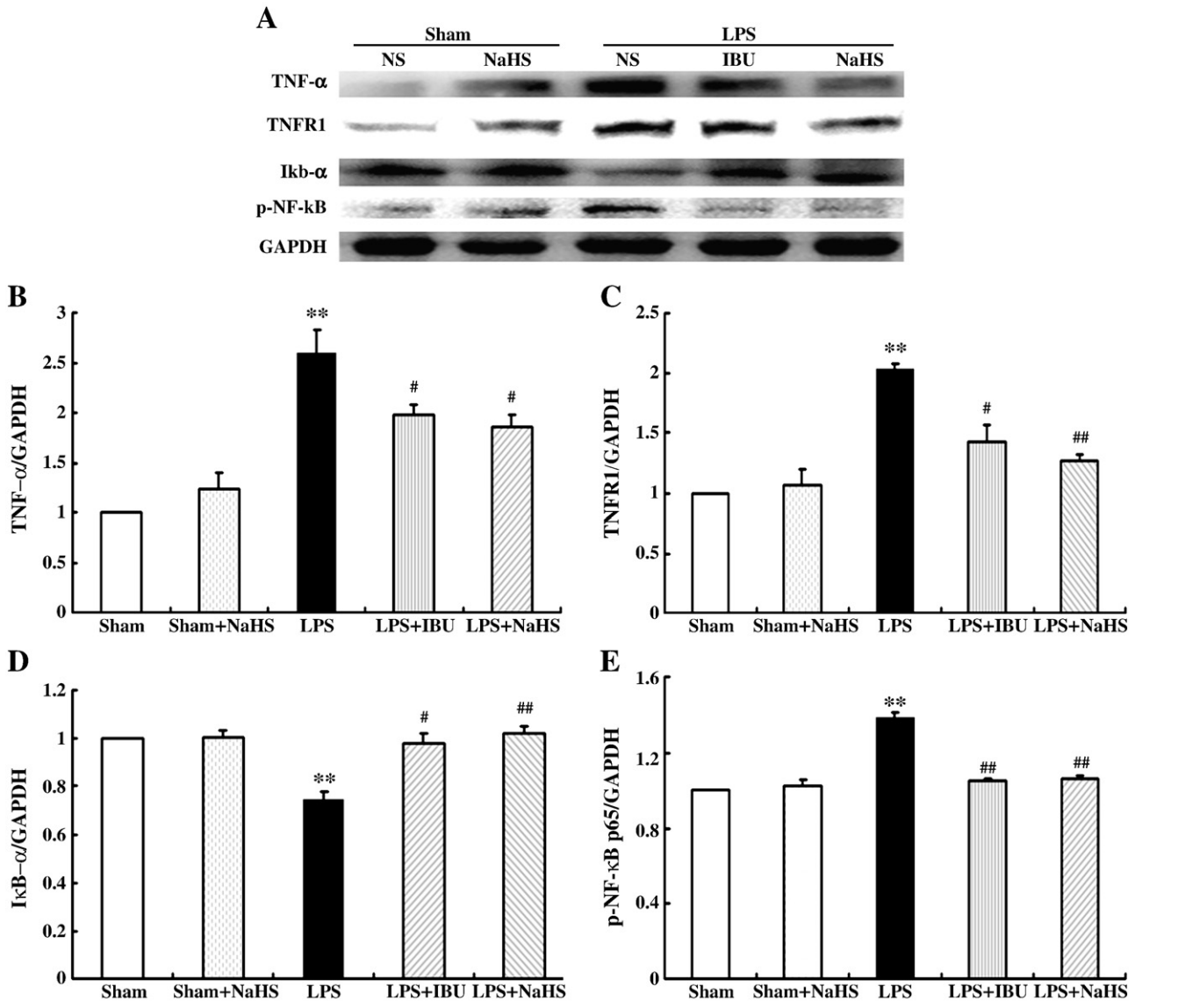

Fig. 5. Western blotting analysis of the relative protein contents. A) Western blot of various protein contents. The relative optical density was normalized to GAPDH. B) TNF-α and C) TNFR1 proteins, D) IκB-α degradation and E) NF-κB p65 phosphorylation. Data are mean ± SEM, $n = 3$. $^{**}P<0.01$ *vs* sham; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* LPS.

In summary, the present study evaluated the activity of H_2S to inhibit LPS-induced cognitive impairment and neuronal ultrastructure damage in rats for the first time. After that, we also investigated the mechanism of H_2S inhibitions of TNF-α and TNFR1 generations, and the related degradation of IκB-α and activation of NF-κB. This study sets the stage for exploring a novel H_2S releasing agent for preventing or retarding the development or progression of neuroinflammatory disease such as AD.

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