Neuroscience Letters 563 (2014) 149–154

Contents lists available at ScienceDirect

Neuroscience Letters

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

Dose-dependent effect of sulfur dioxide on brain damage induced by recurrent febrile seizures in rats

Ying Han [1], Wenxia Yi [1], Jiong Qin *, Yang Zhao, Jing Zhang, Xingzhi Chang

Department of Pediatrics, Peking University First Hospital, Beijing 100034, China

HIGHLIGHTS

- The SO_2/AAT system is involved in febrile seizures (FS) and related toxicity injury.
- In a rat FS model, preconditioning with a low concentration of SO_2 alleviated neuronal damage and apoptosis.
- An AAT inhibitor or high concentration of SO_2 aggravated neuronal damage in FS rats.

ARTICLE INFO

Article history:
Received 23 October 2013
Received in revised form
15 December 2013
Accepted 18 December 2013

Keywords:
Sulfur dioxide
Aspartate aminotransferase
Febrile seizures
Brain damage

ABSTRACT

Sulfur dioxide (SO_2) regulates many physiological processes. Little is known about its roles in neurological disorders. In this study, we investigated the role of endogenous SO_2 in the development of febrile seizures (FS) and related brain damages. In the rat model of recurrent FS, we found that endogenous SO_2 in the plasma and hippocampus was increased, accompanied by upregulation of aspartate amino-transferase 1 (AAT1) and AAT2, and neuronal apoptosis and mossy fiber sprouting (MFS) in the hippocampus. Preconditioning with low concentration of SO_2 (1–10 μmol/kg) alleviated the neuronal damage, and attenuated neuronal apoptosis and MFS, whereas preconditioning with high concentration of SO_2 (100 μmol/kg) or inhibition of AAT aggravated the neuronal damage, and promoted neuronal apoptosis and MFS in hippocampus of rats with recurrent FS. These data indicate that endogenous SO_2 is involved in the development of FS and related brain damage. Preconditioning with low concentration of SO_2 may protect neurons from toxicity caused by FS.

1. Introduction

Febrile seizures (FS) are frequently encountered in pediatric populations, occurring in 2–4% of children between 6 months and 5 years of age [1]. Patient studies have suggested that recurrent FS may cause hippocampal damage, which is one of the most common neuropathological findings of temporal lobe epilepsy (TLE) [2]. It is important, therefore, to further investigate the pathogenesis of FS and related brain damage.

Sulfur dioxide (SO_2) is known to be an atmospheric pollutant, and studies concerning SO_2 have mainly focused on its toxicity in animals [3,4]. However, SO_2 has recently been shown to be produced endogenously by the normal mammalian metabolism of sulfur-containing amino acids [5], and L-cysteine in particular [6]. L-Cysteine is catalyzed by cysteine dioxygenase (CDO) to L-cysteinesulfinate, which converts to β-sulfinylpyruvate through transamination by aspartate aminotransferase (AAT); it then spontaneously decomposes to pyruvate and SO_2 [7]. Additionally, hydrogen sulphide (H_2S) can be catalyzed to SO_2 by nicotinamide adenine dinucleotide phosphate oxidase [8] or by the reduction of thiosulphate [7]. SO_2 is metabolized to sulfite and oxidized to sulfate by sulfite oxidase, and is finally excreted in urine [6]. The distribution of SO_2 and its generating enzymes, AAT and CDO, in different tissues of the rat, and the biological effects of endogenous SO_2 on cardiovascular diseases have recently been described [9–11]. By comparisons with other analogous endogenous gaseous molecules, such as nitric oxide (NO), carbon monoxide (CO) and H_2S, it has been suggested that SO_2 plays a regulatory role for physiological functions [9–11]. Recent studies demonstrated that NO, CO and H_2S play important roles in some of the nervous system diseases, including FS and related brain damage [12–17]. However, it remains unknown whether endogenous SO_2 is involved in the pathogenesis of FS and related brain injury. The present study was therefore intended to investigate the changes in SO_2 plasma and hippocampal levels in a rat model of recurrent FS,

Abbreviations: AAT, aspartate aminotransferase; FS, febrile seizures; HDX, L-aspartate-β-hydroxamate; MFS, mossy fiber sprouting.

* Corresponding author. Tel.: +86 13 701086050; fax: +86 10 66530532.
E-mail addresses: qinjiong@263.net, hanying@263.net (J. Qin).

[1] These authors contributed equally to this work.

0304-3940/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.12.042

and to evaluate the effects of endogenous SO_2 on FS-induced brain damage.

2. Materials and methods

Animal care and experimental protocols complied with the Animal Management Rule of the Ministry of Health, China and the Animal Care Committee of Peking University First Hospital, Beijing, China. Sprague–Dawley male rats were housed with their mothers under standard laboratory conditions until they were weaned at 21 days. Our studies started with animals aged 21 days. The FS model has been described previously in detail [15]. In brief, the control rats were placed into 37 °C water for 5 min, and the rats in the other seven groups were placed into 45.2 °C water until a seizure occurred. As described in detail previously [18], hyperthermia treatment initiated seizures in rats, manifested as facial clonus, head nodding, forelimb clonus, rearing of the animal to a standing posture aided by its tail and the laterally spreading of hindlimbs with increased tone, before falling back. Water immersion was carried out once every 2 days, for 10 times in total.

Rats were then randomly divided into eight groups that received the following treatments: (1) control group, preconditioning with normal saline 30 min before water immersion ($n = 24$); (2) FS group, hyperthermia treatment as described previously, preconditioning with normal saline 30 min before hyperthermia treatment ($n = 24$); (3) FS + S1 group, preconditioning with 1 μmol/kg of SO_2 ($n = 24$); (4) FS + S5 group, preconditioning with 5 μmol/kg of SO_2 ($n = 24$); (5) FS + S10 group, preconditioning with 10 μmol/kg of SO_2 ($n = 24$); (6) FS + S50 group, preconditioning with 50 μmol/kg of SO_2 ($n = 24$); (7) FS + S100 group, preconditioning with 100 μmol/kg of SO_2 ($n = 24$); (8) FS + HDX group, preconditioning with 3.7 mg/kg of L-aspartate-β-hydroxamate (HDX, an inhibitor of the enzyme responsible for endogenous generation of SO_2) 30 min before hyperthermia treatment ($n = 24$). For groups 3–7, a SO_2 donor ($NaHSO_3$ and Na_2SO_3, 1:3 MM ratio) was intravenously injected 30 min before each hyperthermia treatment. An equal volume of normal saline was injected intraperitoneally in control and FS groups. All rats were anaesthetized within 6 h after the final water immersion (Fig. 1B).

Rats were anesthetized with 4% chloral hydrate (600 mg/kg, intraperitoneally) and perfused through the heart with 0.9% saline followed by 3% paraformaldehyde (PFA) and 1% glutaraldehyde in phosphate-buffered saline (PBS). The brain was removed and the hippocampus was isolated and cut into approximately 1 mm^3 cubes after immersion in 3% glutaraldehyde in PBS. The tissue was washed three times in sucrose and post-fixed in 1% osmium tetroxide for 2 h, and then dehydrated in graded ethanol solutions and embedded overnight in Epon 812 at 37 °C. Semi-thin sections (2 μm) were stained with toluidine blue. These sections were used for selection of the centermost CA_1 and CA_3 subfields of the hippocampus. Ultrathin sections (60–90 nm) were stained with uranylacetate and lead citrate, and closely examined under a transmission electron microscope (JEM-100CX, JEOL, Japan).

SO_2 concentrations were measured using high-performance liquid chromatography (HPLC, Agilent 1200 series, Agilent Technologies, Palo Alto, CA, USA) [5]. The plasma and hippocampus samples for SO_2 determination were prepared in the same manner, as previously reported [5].

Total RNA in rat tissues was extracted using Trizol reagent and reverse-transcribed by oligo$(dT)_{15}$ primer and M-MLV reverse transcriptase. The reaction for the real-time PCR (final volume of 25 μL) was mixed with 2.5 μL of 10× PCR buffer, 1 μL of 7.5 μmol/L forward and reverse primer, 1 μL of 2.5 mmol/L dNTP mixture, 0.25 μL of Taq DNA polymerase, and 2 μL of rat tissue cDNA. PCR products were amplified again using the PCR primers (AAT1 forward: 5′-CCAGGGAGCTCGGATCGT-3′, reverse:

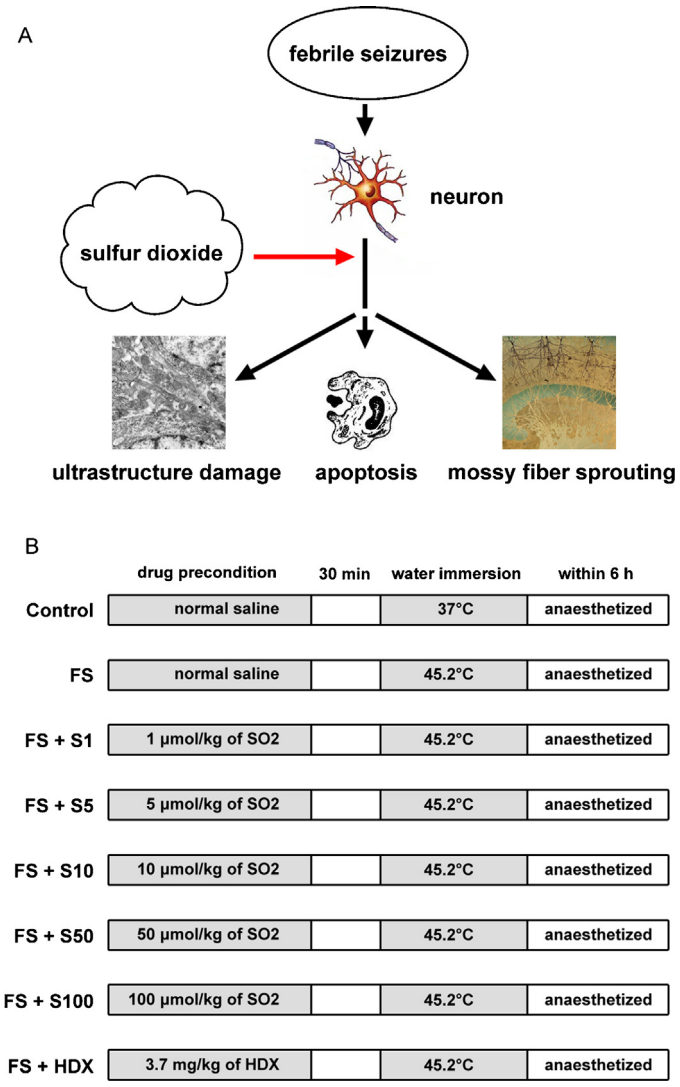

Fig. 1. Graphical abstract and flow chart of the experimental steps. (A) Graphical abstract. (B) Flow chart of the experimental steps.

5′-GCCATTGTCTTCACGTTTCCTT-3′, TaqMan probe: 5′-CCACCACCCTCTCCAACCCTGA-3′; AAT2 forward: 5′-GAGGGTCGGAGCCAGCTT-3′, reverse: 5′-GTTTCCCCAGGATGGTTTGG-3′, TaqMan probe: 5′-TTTAAGTTCAGCCGAGATGTCTTTC-3′; β-actin forward: 5′-ACCCGCGAGTACAACCTTCTT-3′, reverse: 5′-TATCGTCATCCATGGCGAACT-3′, TaqMan probe: 5′-CCTCCGTCGCCGGTCCACAC-3′). The PCR condition was set to predenaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. An extraction of the passaged viruses of all genotypes available was at 10-fold (1×10^{-1} to 1×10^{-6}) dilution and was analyzed by both real-time and reverse-transcribed PCR methods. β-Actin in each sample was used to calibrate the sample amount used for the determination.

Animals (six for each group) were perfused transcardially with saline followed by the 4% paraformaldehyde for 30 min. Brains were removed, maintained in the same fixative for 20 h, successively dehydrated overnight in 20 and 30% sucrose in PBS, frozen in liquid nitrogen, and then stored at −70 °C for further analysis. Brains coronal sections of 10 μm thickness were incubated with AAT1 or AAT2 antibodies (dilutions of 1:50 and 1:300, respectively) overnight at 4 °C, and then with a secondary biotinylated anti-rabbit or anti-mouse antibody (Zhongshan Goldenbridge Biotechnology, China) at 37 °C for 15 min (AAT1) or 60 min (AAT2). Signals were visualized by diaminobenzidine (DAB).