Neuroscience 330 (2016) 90–99

IRON-INDUCED NEURONAL DAMAGE IN A RAT MODEL OF POST-TRAUMATIC STRESS DISORDER

MING ZHAO,[a†] ZHIBO YU,[a,b†] YANG ZHANG,[a] XUELING HUANG,[c] JINGMING HOU,[d] YANGANG ZHAO,[e] WEI LUO,[a] LIN CHEN,[a] LAN OU,[a] HAITAO LI[a*] AND JIQIANG ZHANG[e*]

[a] *Department of Radiology, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China*

[b] *Department of Medical Imaging, PLA No.324 Hospital, Chongqing 400020, PR China*

[c] *Chongqing Three Gorges Medical College, Chongqing 404100, PR China*

[d] *Department of Rehabilitation, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China*

[e] *Department of Neurobiology, Chongqing Key Laboratory of Neurobiology, Third Military Medical University, Chongqing 400038, PR China*

Abstract—Previous studies have shown that iron redistribution and deposition in the brain occurs in some neurodegenerative diseases, and oxidative damage due to abnormal iron level is a primary cause of neuronal death. In the present study, we used the single prolonged stress (SPS) model to mimic post-traumatic stress disorder (PTSD), and examined whether iron was involved in the progression of PTSD. The anxiety-like behaviors of the SPS group were assessed by the elevated plus maze (EPM) and open field tests, and iron levels were measured by inductively coupled plasma optical emission spectrometer (ICP-OES). Expression of glucocorticoid receptors and transferrin receptor 1 (TfR1) and ferritin (Fn) was detected by Western blot and immunohistochemistry in selected brain areas; *TfR1* and *Fn mRNA* expression were detected by quantitative-polymerase chain reaction (Q-PCR). Ultrastructures of the hippocampus were observed under a transmission electron microscope. Our results showed that SPS exposure induced anxiety-like symptoms and increased the level of serum cortisol and the concentration of iron in key brain areas such as the hippocampus, prefrontal cortex, and striatum. The stress induced region-specific changes in both protein and mRNA levels of *TfR1* and *Fn*. Moreover, swelling mitochondria and cell apoptosis were observed in neurons in brain regions with iron accumulation. We concluded that SPS stress increased iron in some cognition-related brain regions and subsequently cause neuronal injury, indicating that the iron may function in the pathology of PTSD.

Key words: post-traumatic stress disorder, cognition, iron, ferritin, transferrin receptor.

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a disabling neuropsychiatric disorder characterized by intrusion of the event, avoidance of reminding it, alterations in cognition and mood, and hyperarousal based on the Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition, DSM-V). The overall lifetime prevalence rate of PTSD is approximately 13% of women and 6% of men in the United States, making it one of the most common psychiatric disorders (Spoont et al., 2015). Despite high morbidity associated with PTSD, the pathophysiology of PTSD remains largely unclear. Previous animal studies have shown that abnormalities of the hippocampus, prefrontal cortex, and amygdala may contribute to the pathogenesis of PTSD (Pitman et al., 2012). A recent study indicated that the hippocampus was critical for regulation of stress responses and was especially vulnerable to elevated glucocorticoids (Kim et al., 2015). Prior work has suggested that hippocampal atrophy was affected by the neurotoxicity of excitatory amino acids and intracellular calcium overloading (Han et al., 2013; Gao et al., 2014); however, additional studies are necessary to elucidate changes in other brain areas during the pathogenesis of PTSD.

Maintenance of functional activity of the central nervous system requires the participation of many metal ions, and the function of iron is most prominent (Crichton et al., 2011). Although the activity mediated by iron is very important, cells require highly sophisticated and precise regulatory mechanisms due to the high oxidative activity of iron, potentially resulting in cytotoxic effects (Hare et al., 2013). In recent years, many studies have shown the redistribution and deposition of iron in the brains of some neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease and multiple sclerosis (Zhu et al., 2009; Hare et al., 2013; Wieler et al., 2015). Recently, iron accumulation was observed prior to plaque formation in an animal model of AD (Leskovjan et al., 2011).

*Corresponding authors. Tel: +86-13883818704 (H. Li), +86-13228686343 (J. Zhang).

E-mail addresses: liihait@163.com (H. Li), zhangjqtmmu@yahoo.com (J. Zhang).

† These authors contributed equally to this work.

Abbreviations: AD, Alzheimer's disease; EPM, elevated plus maze; Fn, ferritin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ICP-OES, inductively coupled plasma optical emission spectrometer; IHC, Immunohistochemistry; PD, Parkinson's disease; PTSD, post-traumatic stress disorder; SPS, single prolonged stress; TfR1, transferrin receptor 1.

http://dx.doi.org/10.1016/j.neuroscience.2016.05.025
0306-4522/

Moreover, gene mutations were discovered in neuroferritinopathy (Curtis et al., 2001). These studies suggest that elevated iron level may be one of the initial factors contributing to neuronal death.

Two key components of the iron regulatory system in the brain are transferrin receptor 1 (TfR1) and ferritin (Fn). Studies have shown that iron crosses the blood–brain barrier and neuronal cellular membranes primarily through a transferrin receptor-mediated endocytotic mechanism (Hare et al., 2013). Some researchers have found that the high density of TfR1 in the hippocampus and basal ganglia region indicated sensitivity to degeneration in AD (Morris et al., 1994a; Sumbria et al., 2013). TfR1 expression has been reported to change in animal models of PD and is involved in cellular apoptosis (Kalivendi et al., 2003). Fn is a main intracellular protein storing excess iron within its shell, and is considered a pro-oxidant as well as an antioxidant (Arosio et al., 2009). Alterations in levels of Fn have been reported in the brains of subjects with AD and PD, and are associated with neurodegeneration (Connor et al., 1995; Grunblatt et al., 2011). Recently, Fn in mitochondrion was observed to be increased and act as an important protective protein in AD (Gao and Chang, 2014). During oxidative stress, Fn is the main source of iron and reactive oxygen species; However, whether iron, TfR1 and Fn are involved in the progression of PTSD remains unclear.

Some studies have recently shown a disorder of iron metabolism in the serum and brains of rats with psychological stress (Wang et al., 2008; Chen et al., 2009; Yu et al., 2011). Notably, the iron level has been shown to be significantly higher in areas of the brain that are very similar to brain regions affected by PTSD, such as the prefrontal cortex, hippocampus, and striatum (Wang et al., 2008). Therefore, we hypothesized that abnormal metabolism of iron in the brain may exist in rats after strong traumatic stress. To address this question, we assessed iron level and iron-related proteins using a single prolonged stress (SPS)-induced paradigm, which is a reliable model and has been extensively applied in the investigation of PTSD (Yamamoto et al., 2009; Lee et al., 2016; Wu et al., 2016b).

EXPERIMENTAL PROCEDURES

Animals and establishment of SPS model

All animal-related procedures in this study were conducted in strict compliance with approved institutional animal care and use protocols of Third Military Medical University. Specific-pathogen-free (SPF) grade Sprague–Dawley rats were purchased from the Experimental Animal Center of this University, group housed, given free access to water and *lab chow*, and kept on a 12-h light/dark cycle. A total of 120 male rats (10–12 weeks age, 200–220 g) were randomly divided into a control group (n = 60) or SPS group (n = 60). The rat model of PTSD was established using SPS as determined by international PTSD Scientific Meetings in Japan (Liberzon et al., 1997). The procedure (as indicated in Fig. 1) was as follows: The SPS rats were bound for two hours using the plastic rigid bound device (diameter: 6 cm, length: 20 cm) and forced swimming was performed for 20 min (20 °C, deep: 50 cm). Next, rats had a rest for 15 min and were then anesthetized with ether to a deep coma. Finally, they were placed in their home cages without disturbance for 7 days. The rats in the control group remained in their home cages as routine without any treatment.

Elevated plus maze (EPM) test

On the eighth day, animals from each group (n = 10) were placed in the center of the EPM (Arm length: 50 cm, arm width: 10 cm, height of baffle plate: 40 cm, Height of device: 1.5 m) and oriented into one of the open arms. A digital camera was used to record the activity of rats for 5 min (min). The percentage (%) of time spent in the open arms and distance traveled during the 5 min were recorded. In addition, the number of entries into the open arms as a percentage (%) of the total number of entries into all arms was recorded and analyzed by a computer.

Open field test

The animals from each group (n = 10) were placed in the central area of the open field (diameter: 100 cm, baffle high: 40 cm) and a digital camera was used to record the rats' activity over a 15 min period. The site was cleaned using a cloth with 70% ethanol after each experiment. Activity parameters including movement, distance, and time of each interval were analyzed by a computer. The crossing distance and time in the central area were considered measures of anxiety (Prut and Belzung, 2003).

Enzyme-linked immunosorbent assay

The rats from each group (n = 10) were intraperitoneally injected with 4% chloral hydrate, and blood was collected from the aortaventralis. The samples were placed at room temperature and centrifuged, and then the supernatant liquid was collected. The standard reagent (k7430, Biovine, USA) was diluted as 120 μg/L, 80 μg/L, 40 μg/L, 20 μg/L and 10 μg/L, and then 50 μL was added to each well along with the serum samples. After incubation for 30 min at 37 °C, the liquid was removed and washed 5 times. The detection regent (k7430, Biovine) was added in each well to incubate and was then washed again. Fifty microliters of substrate solution was added to each well and incubated for 15 min at room temperature, followed by 50 μL of stop solution. The optical density of each well was determined within 15 min using a microplate reader (iMark, BIO-RAD) set at 450 nm.

Inductively coupled plasma optical emission spectrometer (ICP-OES)

Samples from the hippocampus, striatum and prefrontal cortex from each group (n = 10) were dissected and weighted, diluted to a ratio of 1:20 with HEPES buffer (20 mmoL/L) (weight/volume) and then homogenized. Each sample was mixed with an equal volume of ultrapure nitric acid, digested in a 50 °C warm water