Neuroscience Letters 584 (2015) 208–213

Contents lists available at ScienceDirect

Neuroscience Letters

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

Chronic unpredictable stress impairs endogenous antioxidant defense in rat brain

Yi Che [a], Zhu Zhou [a,b,c], Yuxin Shu [a], Chao Zhai [a], Yuanyuan Zhu [a], Songjie Gong [a], Yonghua Cui [a], Jun-Feng Wang [b,c,d,*]

[a] Medical College of Soochow University, Suzhou, PR China
[b] Kleysen Institute for Advanced Medicine, Health Sciences Centre, Winnipeg, Canada
[c] Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada
[d] Department of Psychiatry, University of Manitoba, Winnipeg, Canada

HIGHLIGHTS

- Chronic unpredictable stress induced depressive-like behaviors.
- Chronic unpredictable stress increased lipid peroxidation in rat brain.
- Chronic unpredictable stress decreased endogenous antioxidant defense.

ARTICLE INFO

Article history:
Received 5 September 2014
Received in revised form 8 October 2014
Accepted 16 October 2014
Available online 24 October 2014

Keywords:
Chronic unpredictable stress
Depression
Lipid peroxidation
Glutathione peroxidase
Catalase
Antioxidant capacity

ABSTRACT

Many studies have shown that chronic stress can cause neuronal damage and depression, but this exact mechanism still remains unknown. Neurons are vulnerable to lipid peroxidation-induced damage because the major part of neuronal cell membrane is polyunsaturated fatty acids that are substrate for reactive oxygen species. Since endogenous antioxidant defense systems normally eliminate production of reactive oxygen species, deficient antioxidant defense can cause oxidative stress-induced damage. In the present study, to understand the role of endogenous antioxidant defense in chronic stress-induced neuronal damage, we analyzed lipid peroxidation, total antioxidant capacity, and activities of catalase and glutathione peroxidase in frontal cortex, hippocampus and striatum of rats exposed to chronic unpredictable stress. We found that chronic unpredictable stress for four weeks in rats induced depressive-like behaviors such as anhedonia, despair and decreased exploration. Malondialdehyde, a lipid peroxidation product, is increased, but total antioxidant capacity, glutathione peroxidase activity and catalase activity are decreased in brain of rats exposed to chronic unpredictable stress. Our findings suggest that down regulation of endogenous antioxidant defense induces lipid peroxidation contributing a role to chronic stress and depression.

1. Introduction

Stress is the normal physical response of humans and other organisms to stimulation by environmental challenges, both external and internal. While mild acute stress can be beneficial, particularly as part of an organism's response to critical threats, chronic stress can be physically detrimental and have negative impacts on health both physiologically and psychologically. Studies have shown that chronic stress can impair many cellular functions such as neurotransmitter release, receptor binding and neuronal outgrowth in animal brains [3,6,16,17,22,28,30,32]. Chronic stress can also lead to serious mental problems in humans and contributes significantly to the aetiologies of anxiety and depression [20,25,33]. Despite findings that chronic stress can cause neuronal damage and mental problems, the precise mechanisms still remain unknown.

Oxygen is essential to life, but the process of oxygen metabolism in an organism also forms partially reduced reactive oxygen species (ROS) that can cause oxidative stress, a potential threat to regular cellular functioning. A series of natural antioxidant defense systems normally eliminate ROS production and limit tissue damage.

* Corresponding author at: Kleysen Institute for Advanced Medicine, Health Sciences Centre, SR436 – 710 William Avenue, Winnipeg, MB R3E 0Z3, Canada.
Tel.: +1 204 975 7705.
E-mail address: jun-feng.wang@med.umanitoba.ca (J.-F. Wang).

http://dx.doi.org/10.1016/j.neulet.2014.10.031
0304-3940/

If production of ROS exceeds the cellular antioxidant capacity, excessive ROS can result in oxidative stress. Therefore oxidative stress can be induced by either increased ROS production, reduced antioxidant defense, or both. ROS can react with many biological molecules including lipids, proteins and DNA, subsequently causing lipid peroxidation, protein cleavage and DNA mutation. The brain cells are vulnerable to oxidant stress because although the brain constitutes less than 2% of total body weight, it consumes approximately 20% of total body oxygen. The brain is particularly susceptible to lipid peroxidation because the major constitute of neuronal cell membranes is polyunsaturated fatty acids that are substrate for free radicals [19,35]. Chronic stress has been found to increase malondialdehyde and protein carbonyl levels in various brain regions [1,7,18,24]. Previously we also found that chronic treatment with stress hormone corticosterone increased protein carbonylation in cultured neuronal cells [34]. These results suggest that chronic stress may increase oxidative damage to lipid and protein. The chronic unpredictable stress (CUS) animal model has been considered as a clinically relevant model for anxiety and depression with good face validity [4,8,11,14]. In the present study, to understand the effect of CUS on endogenous antioxidant defense in the brain, we analyzed lipid peroxidation, total antioxidant capacity, and activities of catalase and glutathione peroxidase in frontal cortex, hippocampus and striatum of rats exposed to chronic unpredictable stress for four weeks. Frontal cortical, hippocampus and striatum regions were chosen, because frontal cortex plays a key role in processes that control mood, cognition and motor behavioral functions, and hippocampus is involved in emotion, motivation, and emotional association with memory, while striatum is closely linked with the limbic system, which involves emotion and motivation [15,27,29,36]; and because these regions have all shown impairment in patients with depression [5,21,23].

2. Methods

2.1. Chronic unpredictable stress model

Male Sprague-Dawley rats (the Animal Center of Soochow University) weighing approximately 200–220 g at the beginning were used. All experiments followed the guidelines of Soochow University Animal Experimental Committee. Rats were divided into two groups including non-stressed (control) and CUS groups ($n = 6$ each). Rats in the control group were kept undisturbed in their home cages, while rats in the CUS group were exposed to various stress conditions for 28 days. These stressors include bright light stimulation, water or food deprivation, acoustic stimulation, inverse light dark cycles, foot shock, tail pinch, and others as indicated in Table 1. To avoid predictability, rats were exposed to these stressors at different times on each day. Behavioral tests were started at day 29, 24 h after the last foot shock. After behavioral tests, rat frontal cortex, hippocampus and striatum were isolated for biochemical analysis.

2.2. Open field test

A black Plexiglas square box (100 cm × 100 cm × 50 cm) was used for the open field test. The box was divided into two areas: a peripheral area and a square center area (70 cm × 70 cm). Rats were placed in the center of the testing box and allowed to explore freely for 5 min. Rat activity was recorded with a video camera connected to the computer. The distance of moving, number of rearings and frequency of center entries were analyzed.

Table 1
Daily schedules for the chronic unpredictable stress.

Day of treatment	Stressor used
Day 1	Bright light (30 min)
Day 2	Food deprivation (24 h)
Day 3	Acoustic stimulation (120 db, 30 min)
Day 4	Inverse light dark cycle
Day 5	Inverse light dark cycle
Day 6	Foot shock (25 V, 30 time)
Day 7	Hot environment (40 °C) and overcrowding (30 min)
Day 8	Foot shock (25 V, 30 time)
Day 9	Tail pinch (1 min, 1 cm from the end of the tail)
Day 10	Bright light (30 min)
Day 11	Water deprivation (24 h)
Day 12	Food deprivation (24 h)
Day 13	Cold swimming (4 °C, 5 min)
Day 14	Acoustic stimulation (120 db, 30 min)
Day 15	Foot shock (25 V, 30 time)
Day 16	Hot environment (40 °C) and overcrowding (30 min)
Day 17	Tail pinch (1 min, 1 cm from the end of the tail)
Day 18	Inverse light dark cycle
Day 19	Food deprivation (24 h)
Day 20	Bright light (30 min)
Day 21	Cold swimming (4 °C, 5 min)
Day 22	Cold swimming (4 °C, 5 min)
Day 23	Acoustic stimulation (120 db, 30 min)
Day 24	Water deprivation (24 h)
Day 25	Tail pinch (1 min, 1 cm from the end of the tail)
Day 26	Bright light (30 min)
Day 27	Hot environment (40 °C) and overcrowding (30 min)
Day 28	Foot shock (25 V, 30 time)

2.3. Sucrose preference test

This procedure consisted of overnight water deprivation and then free access to two bottles containing water and 1% sucrose solution for 3 h. Rats were trained to adapt to 1% sucrose solution 48 h before testing. Two bottles of 1% sucrose solution were placed in each cage, and 24 h later one of these bottles was replaced with tap water for 24 h. After the adaptation, rats were deprived of water and food for 24 h and then given free to access to two bottles containing 200 ml of 1% sucrose solution and 200 ml of water respectively. 3 h later, the volumes of consumed sucrose solution and water were recorded. The sucrose preference was calculated by the following formula: Sucrose preference = sucrose consumption × 100%/(water consumption + sucrose consumption).

2.4. Forced swim test

Rats were individually placed in a glass cylinder (40 cm tall and 25 cm in diameter) containing water at 25 °C to a height of 30 cm. The testing procedure included a 15-min pre-test followed by a 5-min test on a second day. Rats were placed in water for 15 min, and the following day placed in water for another 5 min. Immobility behavior was recorded and analyzed during this 5 min. Immobility was defined as floating passively and not making any active movement.

2.5. Analyses of lipid peroxidation, total antioxidant capacity, catalase activity and glutathione peroxidase activity

Rat frontal cortex, hippocampus and striatum were isolated, homogenized in 10:1 (vol/wt) ice-cold phosphate buffered saline and then centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was used for subsequent analyses of lipid peroxidation, total antioxidant capacity, glutathione peroxidase activity and catalase activity, measured with a spectrophotometer. These analyses were performed using kits according to the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). Briefly, lipid peroxidation was analyzed by measuring