Physiology & Behavior 167 (2016) 145–153

Contents lists available at ScienceDirect

Physiology & Behavior

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

The mechanism of 5-lipoxygenase in the impairment of learning and memory in rats subjected to chronic unpredictable mild stress

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HIGHLIGHTS

- Chronic unpredictable mild stress (CUMS) resulted in learning and memory dysfunction in rats.
- CUMS-treated rats showed increased expression of 5-lipoxygenase in the hippocampus.
- 5-lipoxygenase inhibition enhances synaptic plasticity of hippocampal neurons and improves learning and memory function.

ARTICLE INFO

Article history:
Received 19 March 2016
Received in revised form 20 July 2016
Accepted 13 September 2016
Available online 14 September 2016

Keywords:
5-Lipoxygenase
Inflammation
Cognitive impairment
Synaptic plasticity

ABSTRACT

Objectives: To examine the mechanism of 5-lipoxygenase (5-LO) in the learning and memory dysfunction in rats subjected to chronic unpredictable mild stress (CUMS).
Methods: Eighty rats were divided into eight groups: the 0.5% sodium carboxymethyl cellulose solution (NaCMC)-treated group, empty vector (LV-Mock)-treated group, CUMS + NaCMC-treated group, CUMS + sertraline-treated group, CUMS + caffeic acid (10 mg/kg)-treated group, CUMS + caffeic acid (30 mg/kg)-treated group, CUMS + LV-Mock-treated group, and CUMS + 5-LO-silencers lentiviral vectors (LV-si-5-LO)-treated group, n = 10. Sucrose preference tests were performed to assess depression-like behavior. The Morris water maze and step-down tests were used to evaluate learning and memory performance. The levels of inflammatory cytokines, malondialdehyde, and the activity of superoxide dismutase (SOD) were detected to estimate inflammation and oxidative stress. Changes in 5-LO mRNA and protein were detected using reverse transcription polymerase chain reaction and Western blotting. The expression of synaptophysin, postsynaptic density-95 (PSD-95), and brain-derived neurotrophic factor (BDNF) in the hippocampus were measured using immunohistochemical staining.
Results: Treatment with caffeic acid or LV-si-5-LO increased sucrose consumption, decreased escape latency and increased the number of platform crosses in the Morris water maze test, and decreased the number of errors and prolonged the latency in the step-down test. We observed a decreased expression of 5-LO, and levels of malondialdehyde, leukotriene-B4, tumor necrosis factor-α, and interleukin-6, while the protein levels of synaptophysin, PSD-95, BDNF, and the activity of SOD were increased in the hippocampus of the CUMS-treated rats.
Conclusions: CUMS-induced impairment in learning and memory could be triggered by an inflammatory response in the rat hippocampus, which results in oxidative stress injury and impacts the synaptic plasticity of hippocampal neurons. Inhibition of the activity or expression of 5-LO could suppress hippocampal inflammation, enhance synaptic plasticity, and improve learning and memory function in depressed rats.

1. Introduction

Depression is a chronic, highly prevalent, and recurring mood disorder. In addition to the common symptoms of a mood disorder, most patients exhibit cognitive dysfunction, such as memory deficits, poor sustained attention, and reduced executive function; however, there are few effective treatments for these symptoms [1,2]. Growing evidence implicates neuroinflammation [3,4], oxidative stress [5,6], and

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http://dx.doi.org/10.1016/j.physbeh.2016.09.010
0031-9384/

neurotrophic factors [7,8] as key mediators in the pathophysiology of mood disorders and cognitive impairment in depressed patients. Among these mediators, inflammation plays an increasingly prominent role in the pathogenesis of depression and cognitive impairment [9–11]. Patients with major depression who are otherwise medically healthy have activated inflammatory pathways as exhibited by an up-regulation in the expression of pro-inflammatory cytokines, acute-phase proteins, chemokines, and adhesion molecules [12]. Antidepressants can significantly reduce depressive symptoms through their anti-inflammatory effects [13,14]. In animal models of depression, several pro-inflammatory cytokines (e.g., interleukin (IL)-1, tumor necrosis factor (TNF)-α, and interferon-gamma) influence neuronal functions through their involvement in apoptosis, excitotoxicity, oxidative stress, and metabolic derangement. Pro-inflammatory cytokines are up-regulated in patients with severe depression, and cytokine immunotherapy elicits depressive symptoms that are amenable to antidepressant treatment [15–17]. It is suggested that stressors and inflammation share a common ability to impair neurons and alter neurotransmission, ultimately contributing to depression and cognitive impairment.

5-Lipoxygenase (5-LO), a lipid-peroxidizing enzyme involved in the conversion of arachidonic acid into leukotrienes (LTs) by inserting two molecules of oxygen into fatty acids, is widely expressed in central nervous system (CNS) neurons where its expression and activity are enhanced in an age-dependent manner [18,19]. 5-LO may play a role in the occurrence of neurological and psychiatric disorders. 5-LO levels are up-regulated in the brains of suicide victims [20], and inhibition of 5-LO has potent anti-depressant effects in mice [21]. Absence of the 5-LO gene prevents stress-induced memory deficits, synaptic dysfunction and tauopathy activation in a mouse model of Alzheimer's disease [22]. Currently, the U.S. Food and Drug Administration (FDA) is investigating whether the use of antagonists of the downstream products of 5-LO is associated with mood changes and suicide. Regardless, the relationship between 5-LO antagonists and suicide in patients with depression is not clear. The other targets of 5-LO antagonists might contribute to these mechanisms [20]. These studies imply a possible role of 5-LO in the cognitive deficits observed in depressed patients. However, the link between 5-LO and cognitive dysfunction induced by depression is unknown.

Caffeic acid (3,4-dihydroxycinnamic acid) is a natural compound that inhibits 5-LO and exerts potent anti-inflammatory and antioxidant properties. Recently, Takeda et al. reported that caffeic acid provided neuroprotective and anti-depressive activities [23]. Sertraline, a selective serotonin reuptake inhibitor (SSRI), can improve cognitive deficits [24]. Therefore, in this study we used sertraline as a positive control and caffeic acid and 5-LO-silencer lentiviral vectors to inhibit the activities or suppress the expression of 5-LO to investigate the mechanism and effects of 5-LO on depressive behavior and learning and memory deficits in the chronic unpredictable mild stress (CUMS) rat model.

2. Materials and methods

2.1. Animals

Sprague-Dawley rats (180–200 g, 8-weeks-old) were purchased from the Animal Experimental Center of Chongqing Medical University (CQMU). Rats subjected to CUMS were singly housed in standard rat cages, while the control group was housed in groups of 5 per cage. Animals were housed in an air-conditioned room at 25 ± 2 °C and 60–70% humidity on a 12-h light/dark cycle with food and water available ad libitum. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and approved by the Animal Care and Use Committee of CQMU. Rats were housed and acclimatized for 7 days prior to experimental manipulation.

2.2. Experimental design

As shown in Fig. 1, a total of 150 rats were randomly divided into a control (n = 20) and a CUMS group (n = 130). The control rats were treated with an empty vector (LV-Mock) via an intracerebroventricular injection (15 μl, 10^8 TU/ml, n = 10) or with a 0.5% sodium carboxymethyl cellulose (NaCMC) solution (2 ml/kg/day orally for 28 days, n = 10) 6 weeks after the start of the CUMS procedure. After 6 weeks of CUMS, the CUMS-exposed rats with significant differences in the sucrose preference test were further subdivided into six subgroups, each with 10 animals. The remaining 70 animals without significant differences in the sucrose preference test were not enrolled in the following test. Subgroup one received a 0.5% NaCMC solution (2 ml/kg/day) orally for 28 days, subgroup two was treated with sertraline (5 mg/kg/day for 28 days, through gavage), subgroups three and four were treated with 10 or 30 mg/kg/day of caffeic acid to inhibit the activity of 5-LO for 28 days through gavage, and subgroups five and six were treated with 5-LO-silencer lentiviral vectors (LV-si-5-LO) and the empty vector (LV-Mock), respectively, through intracerebroventricular injection (15 μl, 10^8 TU/ml).

2.3. CUMS paradigm

Rats were subjected to a series of unpredictable and mild stressors as previously described [25] with slight modifications: tail pinch (1 cm from the end of the tail) for 1 min, cage tilting (45°) for 24 h, cold swim at 4 °C for 5 min, wet bedding for 24 h, thermal environment (47 °C) for 5 min, noise (92 dB, 1500 Hz) for 2 h, overnight illumination, and water deprivation for 24 h. Rats were individually exposed to the randomized stressors once a day for 42 days. The same stressor was never performed successively. The control rats were housed in a separate room and were not subjected to any stressors.

2.4. Sucrose preference test

Anhedonia induced by the CUMS protocol was assessed by the sucrose preference test. Rats were habituated to a 1% sucrose solution prior to the test. For the sucrose preference test, two bottles of sucrose solution were placed in each cage for 24 h, and one bottle of sucrose was replaced with water for the subsequent 24 h. Sucrose and water consumption was measured by comparing the weights of the bottles before and after the 1 h testing window after 24 h of fasting.

2.5. Morris water maze

The Morris water maze was used to test spatial learning and memory based on reported methods [26,27]. The training was conducted in two steps. In Step 1 (day 1), the rats were placed on the platform for 1 min and then allowed to swim freely to the platform. If a rat did not reach the platform within 180 s, it was guided to the platform by the researchers. On days 2–4, the training consisted of four sessions per day. A different entry site was used for each daily session, and the rats were placed in the water and allowed to search for the platform. If a rat did not reach the platform within 180 s, it was guided to the platform by the researchers and allowed to stay on the platform for 10 s. The maximum search time was set as 180 s. In Step 2 (day 5), the platform was removed, and the rats were placed in the water at the entry site where the last training was performed. The latency to escape (with a maximum of 180 s) and the number of crosses in the quadrant where the platform was located were recorded.

2.6. Step-down-type passive avoidance test

The step-down-type passive avoidance test consisted of two sessions as previously described with minor modifications [28]. In the training session, each rat was placed in a cage for 3 min and allowed