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Research report

Imbalance of leptin pathway and hypothalamus synaptic plasticity markers are associated with stress-induced depression in rats

Jin-Fang Ge[a,b], Cong-Cong Qi[b], Jiang-Ning Zhou[b,*]

[a] School of Pharmacy, Anhui Medical University, Hefei, Anhui, 230032, China
[b] CAS Key Laboratory of Brain Function and Diseases, School of Life Science, University of Science and Technology of China, Hefei, Anhui, 230027, China

HIGHLIGHTS

- Depressive behavior can be induced by chronic unpredictable mild stress (CUMS).
- CUMS rats showed reductions of serum leptin and hypothalamus LEPR mRNA expression.
- Hypothalamic synaptotagmin I and synapsin I mRNA expressions increased in CUMS rats.
- Serum CORT concentration was inversely related to LEPR mRNA expression.
- LEPR mRNA expression was inversely related to synaptotagmin I but not synapsin I.

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ABSTRACT

Increasing evidences have indicated that chronic stress is a contributing risk factor in the development of psychiatric illnesses including depression. The mechanisms of their psychopathology are multifaceted and include, besides others, alterations in neuroendocrine function and brain plasticity. In the present study, we investigated the behavior of stressed animals by the sucrose preference test, open field test (OFT), forced swimming test (FST), and tail-suspension test (TST). The response of hypothalamic–pituitary–adrenal (HPA) axis, leptin pathway, and synaptic plasticity markers in the hypothalamus were also detected. Our data demonstrated that chronic unpredictable mild stress (CUMS) could induce depression-like behavior in rat model, accompanied with the hyperactivity of HPA axis. The serum leptin level and hypothalamic mRNA expression of leptin receptor (LEPR) were both decreased. Results of Pearson test showed that the decreased serum leptin level was negatively related with the locomotion and rearing frequency in the open-field test, and the hypothalamic mRNA expression of LEPR was inversely related to serum CORT concentration. Moreover, our results showed that the mRNA expression of synaptotagmin I and synapsin I was both increased in the hypothalamus of CUMS rats, providing new evidence for the synaptic plasticity change in the hypothalamus of depressive rats. Furthermore, our results demonstrated that the mRNA expression of synaptotagmin I, but not synapsin I, was correlated with the depression-like behaviors and HPA axis hyperactivity in CUMS rats. Together with our previous results, the current findings suggested that a CUMS rat model could be effectively used to study molecular mechanisms underling the depressive symptomatology.

1. Introduction

Depression is the most prevalent psychiatric disorder, with a 17% lifetime prevalence and is a major cause of morbidity, disability and mortality [1,2]. Evidence form animal and human studies showed that stressful life events and the consequent hypothalamic–pituitary–adrenal (HPA) axis hyperactivity are among the most potent factors that trigger depressive episodes [3,4]. Studies targeting at the neurobiological mechanism underlying the association between stress and depression are now emerging in both preclinical and clinical reports [5,6]. However, currently available anti-depressants are only effective in small parts of depression patients, with weeks to months taking to produce a response. The mechanisms underlying the pathogenesis of depression and the therapeutic actions of antidepressants remain poorly understood.

Leptin is a 16-kD hormone secreted by adipose tissue, which plays a key role in regulating energy intake and expenditure. Since

Abbreviations: CUMS, chronic unpredictable mild stress; OFT, open field test; FST, forced swimming test; TST, tail-suspension test; HPA, hypothalamic–pituitary–adrenal axis; LEPR, leptin receptor.

* Corresponding author at: Department of Neurobiology and Biophysics, School of Life Science, University of Science and Technology of China, 443 Huang-Shan Road, Hefei, Anhui 230027, China. Tel.: +86 551 3607658; fax: +86 551 3600408.
E-mail address: jnzhou@ustc.edu.cn (J.-N. Zhou).

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the initial discovery of leptin [7], its extra-metabolic effects have become increasingly appreciated [8,9]. The observation of leptin in several brain areas related to affect and cognition, including the hypothalamus and hippocampus, has sparked increasing interest in various psychiatric illnesses including depression [10–12]. Studies have demonstrated that low leptin level was associated with major depression disorder (MDD) [11,12], suicidality [13], and resistance to antidepressant treatment [14] in humans, with negative relationships between leptin and severity of both depression and anxiety symptoms [13,15]. Leptin receptor knockout mice shows depression-like behavioral deficits, and leptin could improve depression-like behaviors and reverse impaired hippocampal neurogenesis under chronic stress or excessive glucocorticoid conditions [16]. Conversely, higher leptin levels with depressive disorders were also reported by other studies [15–17].

Increasing evidence has demonstrated the connection of synaptic dysfunction with depression [18], and synaptic vesicle associated proteins have been identified as possible factors involved in the pathophysiology of depression [5,6,19–21]. Synaptotagmin I is one of the major integral proteins of the synaptic vesicle membrane and is required for vesicle fusion and neurotransmitter release [22], and synapsin I is a regulator of synaptic transmission and plasticity. [23] Differential expression of these two proteins after chronic stress may contribute to the molecular basis of stress-induced changes in synaptic plasticity in the hippocampus or cortex [20,24], as well as associated behavioral and cognitive alterations [5,6]. However, little is known about their changes in hypothalamus, which is one of the most important brain involved in the pathophysiology of depression.

To gain further insights into the association of stress with depression, we replicated the chronic unpredictable mild stress (CUMS) rat model and observed the depression-like behaviors in the present study. The responses of HPA axis and leptin pathway to CUMS were measured through the detection of serum corticosterone (CORT) and leptin concentrations and hypothalamic mRNA expression of corticotropin-releasing hormone (CRH) and leptin receptor (LEPR). Stress-induced alterations in synaptic plasticity were investigated through the gene expressions of Synaptotagmin I and synapsin I after CUMS in rat hypothalamus.

2. Materials and methods

2.1. Animals

Fourteen male Sprague-Dawley rats, aging 2 months, were purchased from Anhui Experimental Animal Center of China. The rats were divided into control and CUMS group and maintained under a 12:12 h light/dark cycle (lights on 07.00 h). The ambient temperature was maintained at 21–22 °C with 50–60% relative humidity. The rats in control group were housed 3–4 per cage with access to food and water available ad lib., the rats in CUMS group were raised solitarily and received stress according to the CUMS procedure. All experimental procedures in this study were approved by Animal Care and Use Committee at University of Science and Technology of China, which complies with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985).

2.2. CUMS Procedure

The CUMS paradigm consisted of daily exposure to alternating stressors along with occasional overnight stressors for consecutive 3 weeks. Stressors consisted of (1) 24 h wet cage; (2) 1 h warm swim at 30 °C, after which they were toweled dry; (3) 24 h tilt cage with 30 degree from the horizontal; (4) 5 min cold swim at 8–10 °C, after which they were toweled dry; (5) 2 min tail pinch; (6) 24 h deprivation of food and water; (7) 5 min hot stress in oven at 42 °C. The different stressors were distributed randomly at an interval of at least 7 days. All stressors were administered three times within 21 days.

2.3. Behavioral tests

Behavioral tests were performed in a soundproof room with neutral environment. Each test was carried out during the light phase of the light/dark cycle. Sucrose preference and open-field tests were conducted every week during CUMS, and tail suspension test (TST) and forced swimming test (FST) were conducted after CUMS finished.

2.3.1. Sucrose preference test

After a 24 h period of food and water deprivation, animals were given free access to two bottles containing water and 2% sucrose solution, respectively. 6 h later, the volumes of water and sucrose consumed were measured. The percentage of sucrose solution from the total liquid ingested was used as a measure for rats' sensitivity to reward.

2.3.2. Open-field test

The open-field apparatus consisted of a black square arena 100 cm × 100 cm, with a black wall 30 cm high. The floor was marked with a grid dividing the floor into 16 equal-size squares. During a 5-min observation period, the rat was placed at one corner of the apparatus facing the wall. Horizontal locomotion (number of total squares crossed) and the frequencies of rearing (defined as it standing upright on its hind legs) and grooming (protracted washing of the coat) were recorded [25].

2.3.3. Tail suspension test (TST)

The TST was carried out according to the method of Yamawaki et al. [26] with a little modification. Briefly, rats were suspended by the bands and hung from a mounted hook 50 cm above the floor for 6 min. Time spent immobile during the last 4-min testing period was measured. Immobility time was defined as lack of all movement except for whisker movement and respiration.

2.3.4. Forced swimming test (FST)

The FST was carried out according to the method in our previous study [27]. The behavioral cylinder was 60 cm high and 25 cm in diameter maintained at 24–25 °C, filled with 30 cm of water, so that rats could not support themselves by touching the bottom with their paws or tail. The FST paradigm includes 2 sections: an initial 15-min pre-test followed by a 5-min test 24 h later. After each session, the rats were removed from the cylinders, dried with towels and placed into heated cages for 15 min, and then returned to their home cages. Rats were considered to be immobile when they did not make any active movements. Struggling was considered when the rats make active movements with their forepaws in and out of the water along the side of the swim chamber. Swimming was considered when the rats make active swimming or circular movements.

2.4. Measurement of serum corticosterone (CORT) and leptin

24 h after the last behavioral test, rats were deeply anesthetized with chloral hydrate and the blood was taken from the abdominal aorta. The serum was collected and the concentrations of CORT and leptin were measured using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Enzo Life Sciences, Inc., USA) according to the manufacturer's instruction.

2.5. RNA isolation and real time PCR

Hypothalamus were rapidly dissected and frozen quickly in liquid nitrogen, and stored at −80 °C. Total RNA was extracted using the Trizol (Invitrogen, Carlsbad, CA) method. cDNA was synthesized using reverse transcriptase (Promega, Wisconsin, USA). Q-PCR was performed using SYBR Green PCR Kit (Applied Biosystems, USA) and an ABI Prism 7000 Sequence Detector system in 25 μL volume for 40 cycles (15 s at 95 °C; 60 s at 62 °C for rat β-actin, CRH, LEPR, synaptotagmin I, and synapsin I. The following primers used in our study were as follows: rat β-actin 5′-TTGCTGACAGGATGCAGAA-3′ and 5′-ACCAATCCACACAGAGTACTT-3′; rat CRH 5′-CAGAACAACAGTGCGGGCTCA-3′ and 5′-AAGGCAGACAGGGCGACAGAG-3′; rat LEPR 5′-TGT CAG AAA TTC TAT GTG GTT TTG T-3′ and 5′- TTG GAT AGG CCA GGT TAA GTG-3′; rat synaptotagmin I 5′-CTTGAGCAACCAACATCCGC-3′ and 5′-ATGACTGGCACTCACCATTTTT-3′; rat synapsin I 5′-GTTCTTCGGAATGGGGTCAAA-3′ and 5′-GAACCATCTGGGCAAACACC-3′. The relative amount of target gene was calculated using the $2^{-\Delta\Delta Ct}$ method. The relative amplification efficiencies of the primers were tested and shown to be similar.

2.6. Statistical analysis

All statistical analyses were performed using SPSS (version 12.0.1, SPSS Inc., Chicago, IL, USA). Data are expressed as means ± S.E.M. and $P < 0.05$ was considered statistically significant. The difference between control and CUMS groups was tested by student's *t*-test. The correlation analysis was performed by Pearson correlation test.

3. Results

3.1. Depression-like behavior and hyperactivity of HPA axis induced by CUMS

Fig. 1 shows the changes of bodyweight and behavior tests induced by CUMS. The CUMS rats gain less bodyweights during the 3-weeks period stress than the control rats (Fig. 1A). The sucrose