Research report

Effects of curcumin on learning and memory deficits, BDNF, and ERK protein expression in rats exposed to chronic unpredictable stress

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HIGHLIGHTS

- Curcumin prevented the CUS-induced cognitive impairment.
- Curcumin prevented hippocampal BDNF protein levels in stressed rats.
- Curcumin prevented CUS-induced hippocampal ERK phosphorylation.

ABSTRACT

Accumulating evidence suggests that cognitive processes, such as learning and memory, are affected in depression and antidepressant treatment may ameliorate cognitive impairments. Recent studies have shown that curcumin exhibits antidepressant-like effects. The aim of the present study was to determine whether curcumin administration influences chronic unpredictable stress (CUS)-induced cognitive deficits and explores underlying mechanisms. Male Wistar rats were subjected to CUS protocol for a period of 5 weeks to induce depression. The depressive-like behavior was tested using sucrose preference test, open field test and Morris water maze test. Effects of curcumin on brain-derived neurotrophic factor (BDNF) and extracellular signal-regulated kinase (ERK) levels in the hippocampus were also examined. Chronic treatment with curcumin significantly reversed the CUS-induced behavioral and cognitive parameters (reduced sucrose preference and impaired learning and memory function) in stressed rats. Additionally, CUS reduced hippocampal BDNF and ERK levels, while curcumin effectively reversed these alterations. Taken together, our results indicate that the antidepressant-like effects of curcumin in CUS rats are related to its aptitude to promote BDNF and ERK in the hippocampus.

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1. Introduction

Depression is a chronic, recurring and potentially life-threatening mood disorder that has been estimated to affect 21% of the world population [1]. Although depression is a leading cause of disability worldwide and a serious health problem, mechanisms underlying its pathophysiology and antidepressant remain unclear. There is strong evidence that impaired cognition, such as learning and memory is a core element of major depression, and

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2. Materials and methods

2.1. Animals

Male Wistar rats (200–250 g, 6 weeks old) were purchased from Laboratory Animal Center, Shandong University. Upon arrival, the animals were housed under standard laboratory conditions (temperature 20 ± 2 °C, 12 h:12 h light/dark cycle, lights on 8 A.M.), had free access to food and water and were allowed to habituate to the novel environment for 1 week. In the handling and care of all animals, the International Guiding Principles for Animal Research, as stipulated by the World Health Organization and as adopted by the Laboratory Animal Center, Shandong University were followed. During the study, the number of animals used and their suffering was minimized.

2.2. Drug and drug administration

Curcumin was purchased from Sigma Chemical Co. (USA). Curcumin (10 mg/kg), freshly suspended in water, was administrated by gavage once a day for 5 weeks.

2.3. Body weight

Rats were weighted weekly throughout the experiment but the measure was never recorded after the food or water deprivation.

2.4. Chronic unpredictable stress (CUS) procedure

Rats were subjected to CUS for 5 weeks. The procedure of CUS was performed as previously described [26], with minor modifications. In brief, the CUS protocol consisted of a variety of mild stressors: (1) level shaking for 10 min, (2) forced swimming at 4 °C for 6 min, (3) restrain for 4 h, (4) cage tilt (45°) for 7 h, (5) overnight illumination, (6) soiled cage for 24 h, (7) nip tail for 1 min. Stressors were administered in a semi-random manner, at any time of day. In this respect, the stress sequence was changed every week in order to make the stress procedure unpredictable. These stressors were randomly scheduled over a one-week period and repeated throughout the 5-week experiment. Control animals were housed in a separate room and had no contact with the stressed animals.

2.5. Sucrose preference test

Sucrose preference test was carried out at the end of 5-week CUS exposure. The test was performed as described previously [27] with minor modifications. Briefly, before the test, rats were trained to adapt to sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After adaptation, rats were deprived of water and food for 24 h. Sucrose preference test was conducted in which rats were housed in individual cages and were free to access to two bottles containing 100 ml of sucrose solution (1%, w/v) and 100 ml of water, respectively. After 24 h, the volumes of consumed sucrose solution and water were recorded and the sucrose preference was calculated as the sucrose preference (%) = sucrose consumption/(sucrose consumption + water consumption).

2.6. Locomotor activity test

The open field apparatus consisted of a 90 cm × 90 cm gray wooden box with 45 cm high boundary walls, divided into 36 equal squares by red lines marked on the floor. Each animal was placed in the central square and observed for 5 min. After each trial, the apparatus was cleaned with 5% ethanol. The following behaviors were recorded: the number of crossing (the number of squares crossed); rearing (the frequency of standing on hind limbs).

2.7. Morris water maze test

The Morris water maze was used to examine the learning and memory ability after tail suspension test. A cylindrical tank (120 cm in diameter) was filled with water (21–24 °C). The tank was divided into 4 quadrants and a circular escape platform 10 cm in diameter was placed at a fixed position in the center of one of the four quadrants, the target quadrant. The platform was set 2 cm below the water level where rats could not see it directly. A digital camera was positioned above the center of the tank and linked to a tracking system in order to record the performance of rats (SMART polyvalent video-tracking system, Panlab, Spain). Rats were allowed to swim freely for 60 s to become acclimatized to the apparatus before the test. From the next day, in the hidden platform trials, four acquisition trials were carried out each day for 5 days. In each trial, rats were trained to learn to find the hidden platform, based on several cues external to the maze. The time taken to escape onto the hidden platform was measured. Rats were given 60 s to find the hidden platform during each acquisition trial. If it failed to locate the platform within 60 s, it was guided onto the platform. The rat was allowed to stay on the platform for 20 s. Performance was tested 24 h after the final training day in a probe trial during which the platform was removed, the rat was placed in the start point and its
behavior was monitored for 60 s. Time spent in the target quadrant was recorded.

2.8. Tissue sampling

At the end of the experiment the animals were sacrificed under deep anesthesia. Brains were rapidly removed, and the hippocampus was isolated. The samples were stored at −80°C until processed for biochemical estimations.

2.9. Western blotting analysis

Protein from hippocampus was loaded onto a 12% SDS-PAGE gel, electrophoretically transferred to polyvinylidene difluoride membrane. The primary antibodies used were as follows: BDNF (1:1000, Santa Cruz Biotechnology Inc.), ERK1/2 (1:1000; Cell Signaling Tech.), and phospho-ERK1/2 (1:1000, Cell Signaling Tech.). β-Actin (1:2000; Sigma–Aldrich) was used as an internal control. Secondary antibodies were horseradish peroxidase conjugated to goat/mouse anti-rabbit IgG (1:8000, Sigma–Aldrich). The membranes were developed using an enhanced chemiluminescence detection system (Pierce, Rockford, IL). The intensity of bands was determined using the Image-Pro Plus 6.0 software.

2.10. Statistical analysis

Quantitative data were presented as the mean ± SEM. Statistical analysis of data was carried out by one-way analysis of variance (ANOVA), followed by post hoc Tukey’s multiple comparison test. To analyze water-maze test, the average escape latency of 4 trials per day per animal was calculated and evaluated by repeated measures ANOVA. Differences were considered statistically significant if the p value was <0.05.

3. Results

3.1. Effects of curcumin on body weight gain

Body weight was measured before the onset of the CUS regimen and then weekly until the end of the CUS procedure 5 weeks later (Fig. 1). There was no statistically significant difference between the body weight gain of all groups throughout the CUS regimen (p > 0.05).

3.2. Effects of curcumin on sucrose preference test

As shown in Fig. 2, after 5-week CUS exposure, the percentage of sucrose consumption was significantly diminished in the stressed rats compared to the control animals, while chronic treatment with curcumin at daily dose of 10 mg/kg significantly increased the percentage of sucrose consumption, as compared to the stressed rats [F(3, 36) = 9.999, p < 0.001]. In addition, curcumin alone had no effect on the sucrose consumption in rats.

3.3. Effects of curcumin on locomotor activity test

As shown in Fig. 3, the results indicated that the rats in all groups had no difference in the numbers of crossing and rearing in the open-field test (p > 0.05).

3.4. Effects of curcumin on learning and memory functions

Fig. 4A showed the average escape latency of the four groups of animals during acquisition training of the Morris water maze test. In all the groups, escape latency became progressively shorter across training sessions (F(3, 36) = 74.91, p < 0.001. Repeated measures ANOVA). Repeated measures ANOVA revealed no interaction between training days and groups (F(3, 36) = 0.22, p > 0.05). A further analysis revealed that there are significant differences in escape latency at day 5 (p < 0.05) between CUS and the control group, while curcumin administration significantly improved such deficiencies (p < 0.05).

To determine the degree of memory, on the sixth day the animal was placed in the pool for 60 s without the escape platform and the time the animal spent in the target quadrant was recorded. As shown in Fig. 4B, rats exposed to CUS spent less time in the target quadrant compared to the control animals, while treatment with curcumin reversed these changes.

3.5. Effects of curcumin on hippocampal BDNF protein levels

As shown in Fig. 5, CUS procedure resulted in a significant decrease in the protein expression of BDNF in hippocampus compared to the control animals, while curcumin dramatically inhibited CUS-induced decrease of protein expression of BDNF in stressed rats [F(3, 16) = 23.17, p < 0.001].

3.6. Effects of curcumin on hippocampal ERK protein levels

As shown in Fig. 6, CUS reduced phosphorylated protein levels of ERK in the rat hippocampus, and curcumin normalized its levels [F(3, 16) = 11.48, p < 0.01].

4. Discussion

In the present study, we demonstrated that chronic administration of curcumin exhibited antidepressant-like activities in CUS model of depression in rats. Moreover, curcumin treatment
Fig. 3. Effects of chronic mild stress (CUS) and of curcumin treatment on behavior changes in open field test. Data represent mean ± S.E.M.

Fig. 4. Effects of chronic mild stress (CUS) and curcumin treatment on behavior changes in Morris water maze (MWM) test. (A) Latency to escape onto the platform during learning sessions in the MWM test in different groups. (B) Time spent in the target quadrant by mice in different groups during water maze probe test. N = 10 rats/group. Data represent mean ± S.E.M. *p < 0.05 compared with sham group, #p < 0.05 compared with hypoxia group.

significant prevented the stress-induced alterations of BDNF and ERK protein expression in the rat hippocampus.

CUS procedure has long been used as a model of depression and most effects of CUS can be reversed by chronic administration of antidepressant agents [19]. The effects of stress and antidepressants on body weight are still controversial. In the present study, no change in body weight gain due to CUS or curcumin was found throughout the experimental process. This result is in line with previous studies [28–30]. Additionally, body weight gain was not influenced by the administration of curcumin, suggesting that curcumin has no anorectic effect which is one marked feature of the selective serotonin reuptake inhibitors [31,32].

Anhedonia, one of the core criteria for major depression diagnosis, has been defined as a decrease in responsiveness to rewards reflected by a reduced intake of palatable sweet solutions. Sucrose preference test has been interpreted in the literature as an index of anhedonia-like behavioral change [25]. In the present study, we found that a decrease of sucrose preference induced by CUS procedure was significantly reversed by chronic treatment with curcumin, which was consistent with a previous study [33]. Therefore, curcumin exhibited antidepressant-like properties based on the results of these tests.

The MWM is one of the most widely used tests to measure hippocampal-dependent spatial-based learning and memory [34,35]. Effects of stress on MWM performance were contradicting: some studies showed that stress could impair cognitive function [36–38], while others showed improvement [23,39]. These discrepant findings might be due to variables such as stress procedure,

Fig. 5. Effects of chronic mild stress (CUS) and curcumin treatment on BDNF levels in the hippocampus of rats. (A) The left panel is a representative immunoblot made from hippocampal tissues of rats. (B) The right panel is a histogram showing the quantification of BDNF/β-actin ratio levels in the Western blots. N = 4 rats/group. Data represent mean ± S.E.M. ***p < 0.001 compared with control; **p < 0.01 vs CUS alone.

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strain of animals and experimental manipulation. Our results demonstrated that CUS caused significant learning and memory deficits in the MWM test, while curcumin treatment could dramatically reverse these cognitive alterations. In addition, there was no difference in locomotor activity between all groups in the open field test, suggesting the observed impairment of memory of the rats induced by CUS was not attributed to the differences in their locomotion activities.

BDNF is widely distributed in the brain, and its expression is present in high concentration in hippocampus and cerebral cortex [40]. BDNF has been proposed to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses [41]. Exposure to stress has been shown to decrease the expression of BDNF in animal model of depression and chronic administration of antidepressants increase BDNF expression in the hippocampus [42]. Moreover, it is well known that BDNF is implicated in long-term potentiation, which is considered as a potential cellular mechanism underlying learning and memory [43,44]. Our study showed that chronic treatment with curcumin reversed the CUS-induced decrease in BDNF expression in hippocampus, consistent with previous research [15,17,18]. However, the underlying mechanism by which curcumin increases hippocampal BDNF expression need to be further investigated.

Although there is substantial literature on the effects of stress on memory from behavioral and pharmacologic perspectives, the exact molecular mechanisms involved in the modulation of learning and memory by stress is still elusive. ERK is one of the most important and best-studied intracellular signaling pathways and it is highly sensitive to stress and closely associated with cognitive and mood processing [45,46]. The present results further confirmed that exposure to stress was able to inactivate the signaling and transcription factor ERK 1/2 in the hippocampus through phosphorylation. Our study also found that administration of curcumin was able to prevent stress-induced decrease in phosphorylation of ERK 1/2. Additionally, trained stressed rats treated with curcumin displayed better performances in both the navigation task and theprobe trail, suggesting that the phosphorylation of ERK might be essential for learning and memory in the Morris water maze task.

In conclusion, the present study explored the antidepressant-like effects and mechanisms of curcumin in the CUS model of Wistar rats. Curcumin significantly reversed the CUS-induced behavioral and cognitive alterations in stressed rats. In addition, curcumin effectively prevented BDNF and ERK decrease in the hippocampus. These results suggest the involvement of BDNF and ERK in the antidepressant-like effects of curcumin.

Conflict of interest

The authors have no conflict of interest to declare.

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