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Research report

Neotrofin reverses the effects of chronic unpredictable mild stress on behavior via regulating BDNF, PSD-95 and synaptophysin expression in rat

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HIGHLIGHTS

- CUMS caused depressive-like behavior in rats.
- Neotrofin ameliorated the depressive-like behavior induced by CUMS.
- Neotrofin reversed the decreased expression of BDNF induced by CUMS in amygdala.
- Neotrofin reversed the decreased expression of PSD-95 induced by CUMS in amygdala.
- Neotrofin reversed the decreased expression of synaptophysin induced by CUMS in amygdala.

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ABSTRACT

Depression is one of the most common neuropsychiatric disorders and has been associated with a wide range of neuronal structural changes in brain regions. Neotrofin, a neurotrophin agonist, has been demonstrated to exhibit neuroprotection in various in vivo and in vitro studies. The present study aimed to investigate the neuroprotective and ameliorating effects of neotrofin treatment in a rat model of chronic unpredictable mild stress (CUMS) induced depression. The results showed that CUMS was effective in producing depression-like behavior in rats as indicated by decreased responses in the sucrose preference test, and locomotor activity in the open-field test. Moreover, the expression of brain-derived neurotrophic factor (BDNF), PSD-95 and synaptophysin were decreased in the amygdala of CUMS rats. Chronic administration of neotrofin (60 mg/kg, i.p., 5 weeks) significantly ameliorated all these behavioral and biochemical alterations associated with CUMS induced depression, which demonstrated that the expression changes of BDNF, PSD-95 and synaptophysin were correlated with the depression-like behaviors of CUMS rats. Taken together, the results of the present study highlight that neotrofin exhibits neuroprotective and antidepressant-like effects against CUMS induced depression, and suggest a possible mechanism for this protection via changes in synaptic plasticity within the amygdala. These findings reveal the therapeutic potential of neotrofin for use in clinical trials in the treatment of neuronal deterioration in depression.

1. Introduction

Depression is a major life-threatening psychiatric disorder and imposes a substantial health burden [1–3]. Increasing evidence has indicated that depression is related to structural changes resulting from synaptic plasticity in specific brain regions [4–7]. Although the basic signaling mechanisms underlying synaptic plasticity have been widely studied, their potential for application in the clinical treatment of depression has received little attention. Therefore, investigations directed toward this area are urgently needed to identify more effective agents for the treatment of depression.

Neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) are proteins that play important roles in promoting neuronal growth and survival during development, and are crucial for protecting and regulating the integrity and function of neurons throughout life [8]. Recently, there is growing evidence indicating that neurotrophic factors may function as attractive candidates for several neuropsychiatric diseases including depression

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; BDNF, brain derived neurotrophic factor; CUMS, chronic unpredictable mild stress; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; PSD-95, postsynaptic density protein 95.

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[9,10]. For example, the neurotrophin BDNF, and transcripts for interneuron-related peptides, were observed to be decreased in the amygdala of female patients with major depressive disorder [11]. The involvement of neurotrophins as related to depressive-like behaviors may be localized to selective brain areas as neuroimaging studies have suggested that the amygdala, prefrontal cortex and hippocampus are associated with the pathophysiology of depression [12]. However, the mechanisms underlying the antidepressant effects related to BDNF in the amygdala remain poorly understood.

Neotrofin (AIT-082, leteprinim potassium), a purine hypoxanthine derivative neurotrophin agonist, is currently being investigated as a potential neuroprotective agent for Alzheimer's disease and other neurological disorders [13,14]. Previous reports have shown that neotrofin may enhanced neurite outgrowth in PC12 cells [15], as well as induce neurogenesis in the hippocampal dentate gyrus of adult animals [16,17]. Some recent data suggested that neotrofin has neuroprotective effects in the neonatal hypoxic ischemic brain injury model by decreasing apoptotic cell death [18]. In addition, neotrofin has also been shown to ameliorate brain injury in endotoxin-induced periventricular white matter injury of the neonatal rat model [19]. Interestingly, molecular and cellular evidence has indicated that neotrofin exerts its neuroprotective effects mainly by modulating the expression of neurotrophic growth factors in the brain. For example, neotrofin was able to increase mRNA levels of multiple neurotrophic factors in various brain regions [20,21], and results in significant increases in BDNF and NGF concentrations in the adult rat [22]. The neuroprotective effects of neurotrophins might result from the secondary regulation of synapse-associated signaling systems in neurons. Results obtained from in vitro studies indicated that treatment with neotrofin also significantly increased synaptophysin levels in PC12 cells [23]. However, the effects of neotrofin and neurotrophic factors on the regulation of presynaptic and postsynaptic proteins and associated structural changes in depression as assessed in vivo have not been explored in any detail. It is reasonable to hypothesize that neurotrophic factors may regulate the downstream expression of such factors as PSD-95 and/or synaptophysin in a way that can lead to structural changes and/or alterations in neurotransmission as associated with depression.

Therefore, the present study was designed to investigate the neuroprotective and ameliorating effects of neotrofin treatment in a rat model of CUMS-induced depression-like behavior. To further examine the underlying mechanisms of neotrofin, the role of BDNF and synaptic plasticity-associated proteins upon neotrofin-induced antidepressant-like effects were also assessed in the amygdala, a specific brain region associated with depression.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 180–200 g were obtained from the Shandong University Animal Centre. All procedures were approved by the Shandong University Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Animals were housed in standard plastic cages and maintained on a 12 h light/dark cycle (lights on 0630 h) at room temperature (22–24 °C). Standard laboratory food and water were freely available except during experimental procedures. The rats were allowed to acclimatize to laboratory conditions for at least one week prior to use. Behavioral tests were performed in the light phase of the light/dark cycle. All efforts were made to minimize pain and the numbers of the animals used in these experiments.

2.2. Drugs and treatment groups

Neotrofin (Santa Cruz Biotechnology, Santa Cruz, CA) was dissolved in normal saline at a concentration of 10 mg/ml. Animals were randomly allocated to one of the following four groups with $N = 12$/group: (a) control (non-stressed group), (b) CUMS, (c) neotrofin treatment (60 mg/kg) followed by CUMS, (d) vehicle treatment (physiological saline 10 ml/kg) followed by CUMS. Dose and route of neotrofin administration used in the present experiment were chosen as based on previous results [24,25]. In all experiments, neotrofin or saline were administered intraperitoneally (i.p.) in a constant volume at 30 min prior to stress exposure. All treatments consisted of an intraperitoneal injection between 0900 and 1000 h administered once a day for 5 weeks. To habituate to intraperitoneal injection, all rats were administered saline (10 ml/kg) daily for three days prior to the experiment.

2.3. CUMS procedure

The CUMS procedure was performed as described previously [26], with minor modifications. Briefly, rats were exposed to a weekly stress regime consisting of 48 h food deprivation, 24 h water deprivation, 1 min tail pinch (1 cm from the end of the tail), 45° cage tilt, 5 min cold swimming (at 4 °C), 2 h physically restraint and overnight illumination. One of these stressors was applied individually each day in a random order for 5 consecutive weeks. The non-stressed control animals were housed in a separate room and had no contact with the stressed groups.

2.4. Sucrose preference test

The sucrose preference test was performed after the 5 weeks of CUMS exposure as described previously [26] with minor modifications. Briefly, the animals were placed individually in cages for ~~a~~ habituation ~~of~~ to the sucrose solution (1%, w/v) to be used in the test: two bottles of 1% sucrose solution were placed in the cage for the first 24 h, then one bottle of 1% sucrose solution was replaced with tap water for the second 24 h period. After this adaptation protocol, rats were deprived of water and food for 24 h. The test was then performed at 0900 h when the rats were housed in individual cages and were permitted free access to the two bottles, one containing 100 ml of 1% sucrose solution and the other 100 ml of tap water. After 3 h, the volumes of consumed sucrose solution and water were recorded. The sucrose preference which represents the anhedonia was then calculated as: sucrose consumption/[water consumption + sucrose consumption] × 100%.

2.5. Open-field test

Spontaneous locomotor activity was measured in the open-field test performed as described previously [27] with minor modifications. Briefly, the test chamber consisted of a square plywood arena (100 cm × 100 cm × 40 cm) with a black surface covering the inside walls. The floor was divided into 25 equal squares by black lines. The rat was placed into the center of the open field and allowed to explore freely. The horizontal locomotor activities (segments crossed with all four paws) and vertical exploratory activities (number of rearings defined as standing on their hind paws) were scored over a 5 min period. The apparatus was cleaned with detergent prior to each test session to remove any olfactory cues.

2.6. Western blot analysis

The western blot analysis was performed as described previously [28] with minor modifications. Briefly, rats were decapitated 24 h after behavioral testing and the amygdala was carefully dissected from the brain. Then the amygdala was immediately homogenized in 600 μl ice-cold lysis buffer and incubated on ice for 30 min. The homogenate was centrifuged (14,000 rpm) at 4 °C for 10 min and supernatants collected. The protein concentration was measured with the BCA protein assay kit (Beyotime, Jiangsu, China). The lysate (50 μg per lane) was separated by 12% SDS-PAGE gels. After electrophoresis, the separated proteins were transferred onto the PVDF membrane (Millipore) and blocked with PBST (5% nonfat dried milk in PBS containing 0.1% Tween-20) for 1 h at room temperature. Subsequently, the membranes were incubated with the appropriate primary antibodies overnight at 4 °C: polyclonal rabbit anti-BDNF (1:500, Santa Cruz Biotechnology, Santa Cruz, CA), monoclonal rabbit anti-PSD-95 (1:800, Santa Cruz Biotechnology, Santa Cruz, CA), monoclonal rabbit anti-synaptophysin (1:1000, Cell Signaling Technology, Beverly, MA) and polyclonal rabbit anit-β-actin (1:5000, Santa Cruz Biotechnology, Santa Cruz, CA). Following three washes with TBST buffer, the blots were incubated with secondary horseradish peroxidase-conjugated antibodies (1:5000, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h followed by three washings. The blots were detected using the enhanced chemiluminescence method, and the signal was visualized with Amersham Hyperfilm ECL (GE Healthcare, Buckinghamshire, UK). Protein band densities were quantified by Image-J software (NIH) and were expressed as a percentage of the control.

2.7. Data analysis

All statistical procedures were performed on SPSS version 13.0. All data were expressed as mean ± SEM. The data were analyzed statistically by one-way analysis of variance (ANOVA) for multiple comparisons followed by the Newman–Keuls post hoc test. A value of $p < 0.05$ was considered statistically significant.