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Antidepressant-like effect of n-3 PUFAs in CUMS rats: role of tPA/PAI-1 system

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HIGHLIGHTS

- Chronic stress altered the tPA/PAI-1 system in CUMS rats.
- N-3 PUFAs and sertraline might be involved in modulating the tPA/PAI-1 system.
- TPA/PAI-1 system might be a potential target for treatment of depression.

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ABSTRACT

BDNF is strongly implicated in the development of depression. Recent evidence has indicated that tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) are related to the cleavage of pro-brain-derived neurotrophic factor (BDNF) into its mature form. Chronic unpredicted mild stress (CUMS) is widely used to induce depressive behaviors in rodents. Therefore, we investigated the effects of PUFAs and sertraline on tPA/PAI-1 system in CUMS rats. After 5 weeks of CUMS procedures, the rats were induced to a depressive-like state. The expressions of PAI-1 and proBDNF were increased in the prefrontal cortex and hippocampus of CUMS rats. N-3 polyunsaturated fatty acids (PUFAs) or sertraline administration reversed the changes in behavioral test and induced the expression of tPA in certain brain areas, but failed to restore the CUMS-induced PAI-1 expression. Meanwhile, the antidepressant treatment also accelerated the extracellular conversion of proBDNF into mature BDNF in CUMS rats. Our results firstly showed the synchronously altered balance of tPA/PAI-1 system in the prefrontal cortex and hippocampus of CUMS rats, which was partly ameliorated by PUFAs and sertraline medication, providing new evidence for the involvement of tPA/PAI-1 system in the progression and treatment of depression.

1. Introduction

Depression is associated with decreased neuroplasticity, neuronal atrophy and aberrant neurogenesis which is related to inadequete neurotrophins formation in limbic system [1]. Brain-derived neurotrophic factor (BDNF) is a pivotal neurotrophin that has been extensively investigated. Well-documented evidence suggested that the down-regulation of BDNF is involved in the pathophysiology of depression [2]. BDNF is originally synthesized as a glycosylation precursor protein proBDNF. In the secretion process, the precursor protein is converted into mature BDNF by proteases. Pro- and mature BDNF have their own preferred cognate receptors. ProBDNF binding with high affinity to p75NTR leads to cell apoptosis, whereas mature BDNF preferentially activates Trk receptors to bring about survival [3].

As proBDNF and mature BDNF have opposing biological effects [4], the regulation on the cleavage of proBDNF becomes critical in controlling BDNF functions. Interestingly, the traditional fibrinolytic factor, tissue plasminogen activator (tPA), is effective in converting the inactive precursor plasminogen to plasmin, which then facilitates proBDNF cleavage into mature BDNF [5]. TPA is a serine protease produced and secreted not only in endothelial cells but also in neurons and glia of the central nervous system, exerting important function in the brain parenchyma. However, plasminogen activator inhibitor-1 (PAI-1), the major endogenous inhibitor for tPA, is a member of the serpin family proteinase inhibitors. Combination of PAI-1 to tPA terminates tPA enzymatic activity in the extracellular space [3]. Despite well-documented data indicated that tPA/PAI-1 system is crucial in BDNF cleavage, the evidence concerning tPA/PAI-1 system in animal model of depression remains blank.

Dietary intake of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid

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(DHA, 22:6, n-3) is known to be beneficial for mental health [6,7]. Recent meta-analysis has shown the antidepressant effect of n-3 PUFA supplementation on depression patients [8]. Besides, diminished level of BDNF in different brain structures, such as frontal cortex and hippocampus, is associated with reduced content of n-3 PUFAs [9]. In addition, a study showed that DHA dietary supplementation increases the level of mature BDNF in the hippocampus of rats [10]. Interestingly, EPA treatment significantly and dose-dependently increased the plasma level of tPA and decreased PAI-1 level in rats [11]. Recent clinical trial also demonstrated that n-3 PUFA supplementation augments endothelial t-PA release [12]. Based on these findings, it is therefore possible that PUFAs may be involved in enhancing pro-BDNF cleavage into mature BDNF through the influence on the tPA/PAI-1 system, thus resulting in an antidepressant-like effect.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (Male, 150–180 g; The Experimental Animal Center of the Second Xiangya Hospital) were initially housed in groups in a temperature-controlled environment under a 12/12 h light/dark cycle with free access to food and water except during experimental procedures. This study was approved by the Animal Care & Use Committee of Central South University. All experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals (Chinese Council).

2.2. Drug treatment and CUMS procedures

Rats were randomly assigned to six groups ($n = 9$): control, control + PUFAs, control + sertraline, CUMS, CUMS + PUFAs, CUMS + sertraline. The PUFA groups received daily gavage of 1.5 g/kg n-3 PUFAs (EPA34%, DHA24%, Sheng Tianyu Biotechnology, China) 2 weeks before the CUMS procedures started. The sertraline groups received daily gavage of 8 mg/kg sertraline (Eastbang Pharmaceuticals, China) at day 1 when the CUMS procedures started. The doses of n-3 PUFAs and sertraline were based on previous studies [13,14]. Both n-3 PUFAs and sertraline were solubilized in saline containing 0.5% Tween. The control and CUMS groups received same volume of saline (Fig. 1.).

The rats of CUMS groups were housed in a separate cage (cage size: 26 × 19 × 15 cm), while others shared one cage (three per cage, cage size: 90 × 45 × 25 cm). After acclimatized to the laboratories for 5 days, and 2 weeks gavage of n-3 PUFAs or saline respectively, the CUMS groups received random unpredictable stress for next 5 consecutive weeks [14]. Stress stimuli included: cage tilting for 24 h; damp bedding for 24 h; fasting for 24 h; water deprivation for 24 h, finally with 1 h an empty bottle; light-dark-cycle reversal (12 h/12 h), behavior restriction for 2 h; 30 min noise and 5 min tail pinch. Rats received one of these stressors per day and same stressor was not applied in 2 consecutive days. The rats were weighted every 3 days and the doses were adjusted to its weight gain.

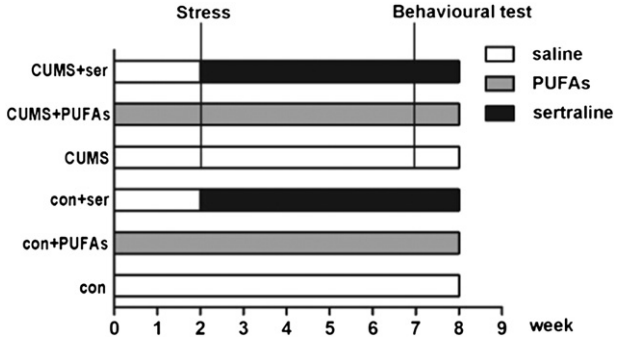

Fig. 1. Drug treatments and chronic unpredicted mild stress procedures. con, control; ser, sertraline.

2.3. Forced swimming test (FST)

FST is one of the behavioral tests widely employed to screen antidepressant efficacy and depressive-like behavior in rodents. Increasing immobility time is the indicator for depressive-like symptom. The test was performed as previously reported [15]. In brief, each rat was placed in a plastic drum (45 cm height, 25 cm diameter) containing approximately 35 cm of water (24 ± 1 °C) for a 15-min pretest. After swimming, rats were dried with towels and placed back in their home cage. Twenty-four hours later, the rat was exposed to the same experimental conditions outlined above for a 5-min FST. Immobility was defined as floating passively and only making slight movements to keep the head above water. Each test session was videotaped and the duration of immobility was scored by an experienced observer blind to the experiment design.

2.4. Sucrose preference test (SPT)

Depressive-like phenotype was assayed by sucrose preference test (SPT) for anhedonia-like reactivity [16]. The whole test took 3 days. Briefly, rats were placed in individual cages for habituation to the sucrose solution (1%, *w/v*): two bottles of 1% sucrose solution were placed in the cage for the first 32 h, then after 16 h water deprivation, one bottle of 1% sucrose solution was replaced with water for 1 h. The rats were permitted free access to the two bottles. The volumes of consumed sucrose solution and water were recorded. Sucrose preference which represents the anhedonia was defined as the ratio of the volume of sucrose vs total volume of sucrose and water consumed.

2.5. Open field test (OFT)

Spontaneous locomotor and rearing activity were measured in the open-field test performed as described previously [14] with minor modifications. Briefly, the test chamber consisted of a square arena (90 cm × 90 cm × 40 cm).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 move freely. The number of crossing (segments crossed with all four paws) and number of rearing (standing on their hind paws) were scored over a 5-minute period. The apparatus was cleaned with 75% ethanol prior to each test session to eliminate odors.

2.6. Western blot analysis

After CUMS procedures and behavioral test, the rats were anesthetized with 10% chloral hydrate (4 ml/kg) and tissues were rapidly collected. Protein extracts of tissues (10 μg) were mixed with gel loading buffer and separated on 12% SDS-PAGE gels. After electrophoresis, the proteins were transferred onto PVDF membranes and then blocked with 5% nonfat dry milk in Tris-buffered saline (TBS). Membranes were incubated with the following primary antibodies: anti-BDNF (1:200, Santa Cruz, USA), anti-PAI-1 (1:500, Santa Cruz, USA), anti-tPA (1:2000, Abcam, USA) and anti-β-actin (1:200, Santa Cruz, USA). After incubation with the primary antibodies, membranes were washed with Tris-buffered saline containing 0.05% Tween-20 (TBST), and incubated with appropriate horse radish peroxidase (HRP)-conjugated secondary antibodies (1:2000). The film signal was digitally scanned and then quantified using Image J software.

2.7. Statistical analysis

All statistical procedures were performed on SPSS version 19. Data were expressed as mean ± SEM, and analyzed statistically by one-way analysis of variance (ANOVA) for multiple comparisons followed