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Short communication

Brain-derived neurotrophic factor signalling mediates the antidepressant-like effect of piperine in chronically stressed mice

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HIGHLIGHTS

- Piperine reverses depressive-like behavior in CUMS-treated mice.
- Piperine increases BDNF content in the hippocampus and frontal cortex of CUMS-treated mice.
- Treatment of K252a abolishes the antidepressant-like effect of piperine in CUMS-treated mice.

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ABSTRACT

Previous studies in our laboratory have demonstrated that piperine produced antidepressant-like action in various mouse models of behavioral despair. This study aimed to investigate the role of brain-derived neurotrophic factor (BDNF) signalling in the antidepressant-like effect of piperine in mice exposed to chronic unpredictable mild stress (CUMS). The results showed that CUMS caused depression-like behavior in mice, as indicated by the significant decrease in sucrose consumption and increase in immobility time in the forced swim test. It was also found that BDNF protein expression in the hippocampus and frontal cortex were significantly decreased in CUMS-treated mice. Chronic treatment of piperine at the dose of 10 mg/kg significantly ameliorated behavioural deficits of CUMS-treated mice in the sucrose preference test and forced swim test. Piperine treatment also significantly decreased immobility time in the forced swim test in naive mice. In parallel, chronic piperine treatment significantly increased BDNF protein expression in the hippocampus and frontal cortex of both naive and CUMS-treated mice. In addition, inhibition of BDNF signalling by injection of K252a, an inhibitor of the BDNF receptor TrkB, significantly blocked the antidepressant-like effect of piperine in the sucrose preference test and forced swim test of CUMS-treated mice. Taken together, this study suggests that BDNF signalling is an essential mediator for the antidepressant-like effect of piperine.

1. Introduction

Depression is a commonly occurring, debilitating, and life-threatening psychiatric disorder. Traditionally, decreased monoamine function in the brain is considered to be the cause of depression and monoamine-based antidepressants are the most widely used antidepressants in clinic [16]. Further studies have shown a causal relationship between the incidence of major depressive disorders and the dysregulation of hypothalamic-pituitary-adrenal (HPA) axis, with the latter being characterized by elevated levels of circulating glucocorticoids and impaired glucocorticoid receptor-mediated negative feedback on the function of HPA axis [38]. Recently, a leading hypothesis of depression suggests that neurotrophic factors play critical roles in the pathogenesis of depression [6,9,16]. The brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor family, is highly expressed in hippocampus and cortex [29]. Postmortem analyses have revealed lower levels of BDNF in patients with major depression [4], while BDNF infusion into the brain has been found to produce antidepressant-like action [36]. Clinical studies have found decreased BDNF levels in the blood of depressive patients [1,2,14,15], while antidepressant treatment seems to be able to normalize BDNF levels [3,34]. BDNF is thus an attractive topic in research into the pathophysiology of depression and the mechanism of action of antidepressants.

Piperine, a major alkaloid of black pepper (*Piper nigrum* Linn.) and long pepper (*Piper longum* Linn.), has been used extensively as condiment and flavoring for all types of savory dishes [21]. In recent years, pharmacological studies have shown that

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piperine possesses analgesic, anti-inflammatory, anti-convulsant, anti-oxidant, and cognitive enhancing effects [5,8,32]. Moreover, piperine is reported to inhibit monoamine oxidase activity, increase monoamine neurotransmitters levels, and thus produce antidepressant-like activity in various mouse models of behavioral despair [18,20]. The antidepressive effect of piperine has also been observed in mice exposed to chronic mild stress, which were related to up-regulation of hippocampal progenitor cell proliferation [21]. Studies from our laboratory also showed that treating mice intraperitoneally with piperine caused a significant reduction of immobility time in both forced swim and tail suspension tests, which was related to the serotonergic system [27,28]. However, the molecular mechanism(s) underlying the antidepressant-like action of piperine remains unclear. Chronic unpredictable mild stress (CUMS)-induced depression is generally thought to be the most promising and valuable depressive model in animals and has been widely used for investigating the pathophysiology of depression and the associated therapeutic interventions [39,40]. Therefore, in the present study, firstly, we examined the effect of piperine (10 mg/kg) on depressive-like behavior and BDNF protein expression in the hippocampus and frontal cortex of CUMS-treated mice; secondly, we used K252a, an inhibitor of the BDNF receptor TrkB, to further investigate the direct link between BDNF signalling and the antidepressant-like effect of piperine in CUMS-treated mice.

2. Materials and methods

Male ICR mice weighing 20–25 g were obtained from the Laboratory Animal Services Center, The Chinese University of Hong Kong, Hong Kong. The animals were maintained on a 12 h light–dark cycle under regulated temperature ($22 \pm 2\,^{\circ}C$) and humidity ($50 \pm 10\%$) and fed with standard diet and water *ad libitum*. They were allowed to acclimate 3 days before use. Two experiments were performed in this study, with ten mice used per group ($n = 10$). The first experiment had two variables: stress condition (CUMS and non-stressed) and drug treatment (piperine and vehicle). The second experiment, in which all mice were subjected to the CUMS procedure and also had two variables: injection treatment (K252a and DMSO) and drug treatment (piperine and vehicle). Behavioural tests were performed during the light phase of light–dark cycle. The experiments on animals have been approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong and conformed to the guidelines of the "Principles of Laboratory Animal Care" (NIH publication No.80–23, revised 1996).

Piperine and K252a were purchased from Sigma (St. Louis, MO, USA). The repeated drug treatment of CUMS animals was performed once daily at 10:00 a.m.–12:00 p.m. during the last 3 weeks. Piperine (10 mg/kg, dissolved in saline containing 0.1% Tween-80) and K252a (25 μg/kg, dissolved in 0.1% DMSO) were administered intraperitoneally (i.p.) in a volume of 10 ml/kg. The doses chosen were based on previous reports [13,27,28,41]). Controlled animals were given the corresponding vehicle, also in the same volume.

The CUMS procedure was performed as described by Mao et al. [24]. In brief, the CUMS protocol consisted of the sequential application of a variety of mild stressors: (1) food deprivation for 24 h, (2) water deprivation for 24 h, (3) exposure to a empty bottle for 1 h, (4) cage tilt (45°) for 7 h, (5) overnight illumination, (6) soiled cage (200 ml water in 100 g sawdust bedding) for 24 h, (7) forced swimming at 8 °C for 6 min, (8) physically restraint for 2 h, and (9) exposure to a foreign object (e.g., a piece of plastic) for 24 h. These stressors were randomly scheduled over a one-week period and repeated throughout the 6-week experiment (Table 1). Non-stressed animals were left undisturbed in their home cages except during housekeeping procedures such as cage cleaning.

Sucrose preference test was carried out 1 day after CUMS. The test was performed as described previously [24]. Briefly, 72 h before the test, rats were trained to adapt 1% sucrose solution (w/v): two bottles of 1% sucrose solution were placed in each cage, and 24 h later 1% sucrose in one bottle was replaced with tap water for 24 h. After the adaptation, rats were deprived of water and food for 24 h. Sucrose preference test was conducted at 9:00 a.m. 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 1 h, the volumes of consumed sucrose solution and water were recorded and the sucrose preference was calculated by the following formula:

$$\text{Sucrose preference} = \frac{\text{sucrose consumption}}{\text{water consumption+sucrose consumption}} \times 100\%.$$

The forced swim test was carried out 2 day after CUMS. The test was performed according to the method of Porsolt and colleagues [30]. Briefly, mice were forced to swim in a transparent glass vessel (25 cm high, 14 cm in diameter) filled with 10 cm of water at 24–26 °C. The total duration of immobility (seconds) was measured during the last 4 min of a single 6 min test session. Mice were considered immobile when they made no attempts to escape except the movements necessary to keep their heads above the water.

Twenty-four hours after the forced swim test (3 day after CUMS), the mice were sacrificed by decapitation. Whole brains were rapidly removed from mice and chilled in ice-cold saline. Various brain areas, including hippocampus and frontal cortex, were dissected on a cold plate and frozen in liquid nitrogen immediately. The tissue samples were stored at −80 °C until assay. Hippocampus and frontal cortex samples were weighed and homogenized in tenfold volume of lysis buffer. The homogenate was then centrifuged at 10,000 *g* for 30 min at 4 °C and supernatants were used for BDNF assays. Protein levels of BDNF were measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Chemicon International, Temecula, CA) according to the manufacturer's instructions. Briefly, samples were applied into 96-well immunoplates precoated with rabbit anti-human BDNF antibody and incubated on a shaker overnight at 4 °C. After washing four times, biotinylated mouse anti-human BDNF antibody was added and incubated for 3 h at room temperature. Then streptavidin-HRP conjugate solution was added and incubated at room temperature for 1 h after washing. TMB/E substrate was added and incubated at room temperature for 15 min. The reaction was stopped with 1 M hydrochloric acid and absorbance recorded at 450 nm immediately. The values of standards and samples were corrected by subtracting the absorbance of non-specific blinding. The ranges of the calibration curve were 7.8–500 pg/ml. The BDNF content was expressed as ng/g wet weight of tissue.

Data were expressed as mean ±SEM. Statistical analysis was performed using two-way ANOVA followed by Bonferroni test. The GraphPad Prism software was used to perform the statistics (version 5.0; GraphPad Software, Inc., San Diego, CA). The difference was considered statistically significant when $p < 0.05$.

3. Results

Chronic treatment of piperine produced an antidepressant-like effect in both sucrose preference test and forced swim test of CUMS-treated mice (Fig. 1). For sucrose preference test (Fig. 1a), the interaction between stress condition (CUMS or non-stressed) and drug treatment (piperine or vehicle) was significant [$F_{(1,36)} = 7.57$, $p < 0.01$]. The effect of stress was significant [$F_{(1,36)} = 12.55$, $p < 0.01$], indicating CUMS resulted a significant reduction in the percentage of sucrose consumption in mice. The effect of drug was also significant [$F_{(1,36)} = 34.08$, $p < 0.01$]. Post hoc analysis indicated that CUMS-treated mice receiving piperine show a significant increase