

Piperine Reverses Chronic Unpredictable Mild Stress-Induced Behavioral and Biochemical Alterations in Rats

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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, which was related to the serotonergic system. The present study aimed to examine the behavioral and biochemical effects of piperine in rats exposed to chronic unpredictable mild stress (CUMS). The results showed that CUMS caused depression-like behavior in rats, as indicated by the significant decrease in sucrose consumption and increase in immobility time in the forced swim test. In addition, it was found that serotonin (5-HT) and brain-derived neurotrophic factor (BDNF) contents in the hippocampus and frontal cortex were significantly decreased in CUMS-treated rats. Treating the animals with piperine significantly suppressed behavioral and biochemical changes induced by CUMS. The results suggest that piperine produces an antidepressant-like effect in CUMS-treated rats, which is possibly mediated by increasing 5-HT and BDNF contents in selective brain tissues.

Keywords Antidepressant · Brain-derived neurotrophic factor · Chronic unpredictable mild stress · Serotonin · Piperine

Introduction

Piperine, a major alkaloid of black pepper (*Piper nigrum* Linn.) and long pepper (*P. longum* Linn.), has been used extensively as condiment and flavoring for all types of savory dishes (Li et al. 2007b). In recent years, pharmacological studies have shown that piperine possesses analgesic, anti-inflammatory, anti-convulsant, anti-oxidant, and cognitive enhancing effects (Gupta et al. 2000; Selvendiran et al. 2003; Chonpathompikunlert et al. 2010). 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 (Kong et al. 2004; Lee et al. 2005; Li et al. 2007a). 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 (Li et al. 2007b). 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 (Mao et al. 2011a, b). 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 (Willner 1997, 2005). Therefore, in the present study, we examined whether long-term treatment with piperine can reverse CUMS-induced depressive-like behavior in rats. In order to investigate the mechanism(s) underlying the antidepressant-like action of piperine, we also measured serotonin (5-HT) and brain-derived

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neurotrophic factor (BDNF) contents in the hippocampus and frontal cortex of CUMS-treated rats, without or with piperine treatment.

Materials and Methods

Animals

Seven-week-old male Sprague–Dawley rats weighing 200–220 g were obtained from the Laboratory Animal Services Center, The Chinese University of Hong Kong, Hong Kong. Animals were housed in groups on a 12-hour light/dark cycle (lights on at 6:00 AM, lights off at 6:00 PM) under controlled temperature (22 ± 2 °C) and humidity (50 ± 10 %), and were given standard diet and water ad libitum. They were allowed to acclimatize for 7 days before use. 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). Effort was made to minimize the number and suffering of the animals.

CUMS Procedure

Rats were randomly assigned into four groups of ten individuals: control, CUMS plus vehicle (physiological saline), CUMS plus piperine (10 mg/kg), and CUMS plus imipramine (10 mg/kg). Piperine and imipramine (Sigma-Aldrich, St Louis, MO, USA, dissolved in saline containing 0.1 % Tween-80) were administered intraperitoneally (i.p.) in a volume of 10 ml/kg 30 min before each stressor once every day for 5 weeks. The CUMS procedure was performed as described by (Mao et al. 2010a, b), with a slight modification. Briefly, CUMS consisted of a variety of unpredictable stressors, namely, 48-h food deprivation, 24-h water deprivation, 5-min cold swimming (at 6 °C), 1-min tail pinch (1 cm from the end of the tail), physically restraint for 2 h, exposure to a foreign object (e.g., a piece of plastic) for 24 h, and overnight illumination. One of these stressors (in random order) was given every day between 9:30 am to 12:00 am for 5 weeks. Control (unstressed) animals were undisturbed except for necessary procedures such as routine cage cleaning.

Sucrose Preference Test

The sucrose preference test was carried out 24 h after the last drug treatment. The test was performed as described previously (Mao et al. 2010a, b). In the test, rats were individually housed. Before the test, the rats were trained to

adapt to sucrose solution (1 %, w/v) by placing two bottles of sucrose solution in each cage for 24 h; then one of the bottles was replaced with water for 24 h. After the adaptation procedure, the rats were deprived of water and food for 24 h. The sucrose preference test was conducted at 9:00 a.m. The rats were housed in individual cages and given free access to the two bottles containing 100 ml of sucrose solution (1 % w/v) and 100 ml of water, respectively. After 3 h, the volumes of consumed sucrose solution and water were recorded and the sucrose preference was calculated by the following formula:

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

Forced Swim Test

The forced swim test was carried out after the sucrose preference test. The test was performed according to the method by Porsolt et al. (1977) with minor modifications. As a pre-test, the rats were individually forced to swim for 15 min in a vertical plastic cylinder (diameter 21 cm, height 50 cm) containing 25 cm of water, maintained at 25 ± 1 °C. The rats were then removed and dried before being returned to cages. After 24 h, the rats were again placed in the cylinders in the same system depicted above. The total duration of immobility (seconds) was quantified during a test period of 5 min by two observers who were blind to the treatment given to each rat. A rat was considered immobile whenever it remained floating passively in a slightly hunched, but upright position with its head just above the surface.

Tissue Sample Collection

Twenty-four hours after the forced swim test, the rats were sacrificed by decapitation and the blood samples were collected in tubes. Whole brains were rapidly removed from rats and chilled in an ice-cold saline solution. 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.

Measurement of 5-HT Content

Hippocampus and frontal cortex samples were homogenized in 10 volumes of perchloric acid (0.2 N), and centrifuged at 10,000 g for 5 min. The supernatant was removed, neutralized with equal volume of 1.0 M borate buffer (pH 9.25), and centrifuged at 10,000 g for 1 min. The resulting supernatant was used for 5-HT assay by using

an immunoassay kit (Beckman Coulter, Fullerton, CA) according to the manufacturer's instructions. The absorbance was measured at 405 nm. The 5-HT level was expressed as ng/g wet weight of tissue.

Measurement of BDNF Content

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 by using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Chemicon International, Temecula, CA) according to the manufacturer's instructions. Briefly, samples and standards were applied in duplicate into 96-well immunoplates precoated with rabbit antihuman BDNF antibody and incubated on a shaker overnight at 4 °C. After washing four times, biotinylated mouse anti-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 HCl and absorbance recorded at 450 nm immediately. The values of standards and samples were corrected by subtracting the absorbance of nonspecific binding. The ranges of the calibration curve were 7.8–500 pg/ml. The intra-assay coefficient of variation was less than 5 %.

Statistical Analysis

Data are expressed as means \pm SEMs. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunnett's test, to detect inter-group differences. A difference was considered statistically significant when $p < 0.05$.

Results

The effect of piperine on the percentage of sucrose consumption in CUMS-treated rats is given in Fig. 1. One-way ANOVA showed a significant difference on the percentage of sucrose consumption among groups ($F(3, 36) = 25.82$, $p < 0.01$). The CUMS resulted in a significant decrease in the percentage of sucrose consumption ($p < 0.01$) compared with the controls. Treatment with piperine and imipramine significantly attenuated the decrease in the percentage of sucrose consumption of CUMS-treated rats ($p < 0.01$ and $p < 0.01$, respectively) compared with the CUMS-treated rats.

The effect of piperine on the immobility time of CUMS-treated rats in the forced swim test is given in Fig. 2. One-way ANOVA showed a significant difference on the immobility time among groups in the forced swim test ($F(3, 36) = 8.743$, $p < 0.01$). The CUMS resulted in a significant increase in the immobility time in the forced swim test ($p < 0.01$) compared with the controls. Treatment with piperine and imipramine significantly attenuated the increase in the immobility time of CUMS-treated rats ($p < 0.01$ and $p < 0.01$, respectively) in the forced swim test compared with the CUMS-treated rats.

The effect of piperine on 5-HT content in the hippocampus (Fig. 3a) and frontal cortex (Fig. 3b) of CUMS-treated rats is given in Fig. 3. One-way ANOVA showed a significant difference on 5-HT content in the hippocampus ($F(3, 20) = 11.27$, $p < 0.01$) and frontal cortex ($F(3, 20) = 11.95$, $p < 0.01$) among groups. The CUMS significantly decreased 5-HT content in the hippocampus ($p < 0.01$) and frontal cortex ($p < 0.01$) compared with the controls. Treatment with piperine and imipramine significantly increased 5-HT content in the hippocampus ($p < 0.05$ and $p < 0.01$, respectively) and frontal cortex ($p < 0.01$ and $p < 0.01$, respectively) of CUMS-treated rats compared with the CUMS-treated rats.

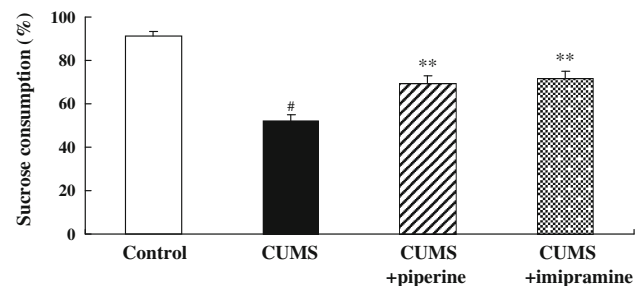


Fig. 1 Effect of piperine on the percentage of sucrose consumption in CUMS-treated rats. Values are given as mean \pm SEM ($n = 10$). [#] $p < 0.01$ compared with the control group; ^{**} $p < 0.01$ compared with the CUMS group

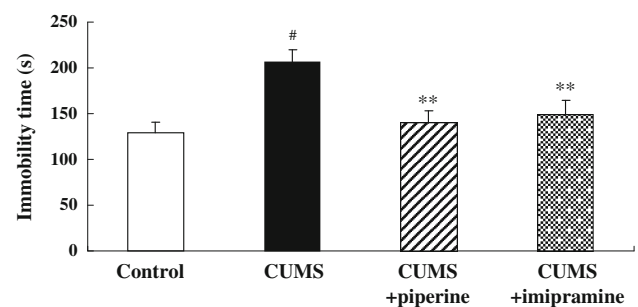


Fig. 2 Effect of piperine on the immobility time of CUMS-treated rats in the forced swim test. Values are given as mean \pm SEM ($n = 10$). [#] $p < 0.01$ compared with the control group; ^{**} $p < 0.01$ compared with the CUMS group

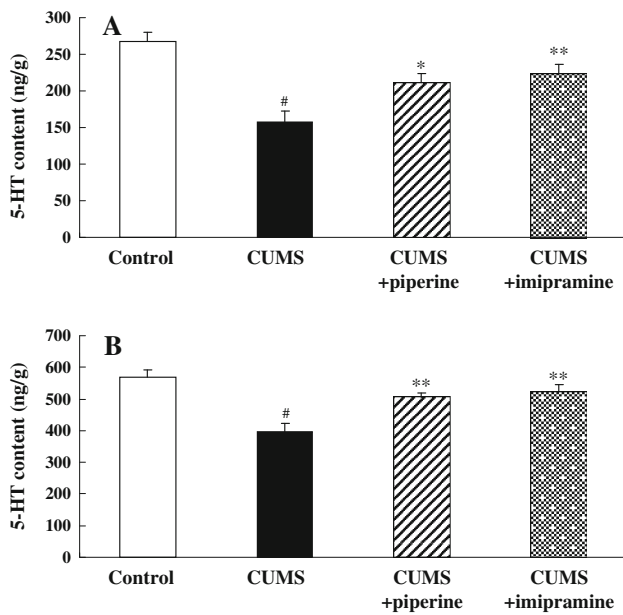


Fig. 3 Effect of piperine on 5-HT content in the hippocampus (a) and frontal cortex (b) of CUMS-treated rats. Values are given as mean \pm SEM ($n = 6$). [#] $p < 0.01$ compared with the control group; ^{*} $p < 0.05$; ^{**} $p < 0.01$ compared with the CUMS group

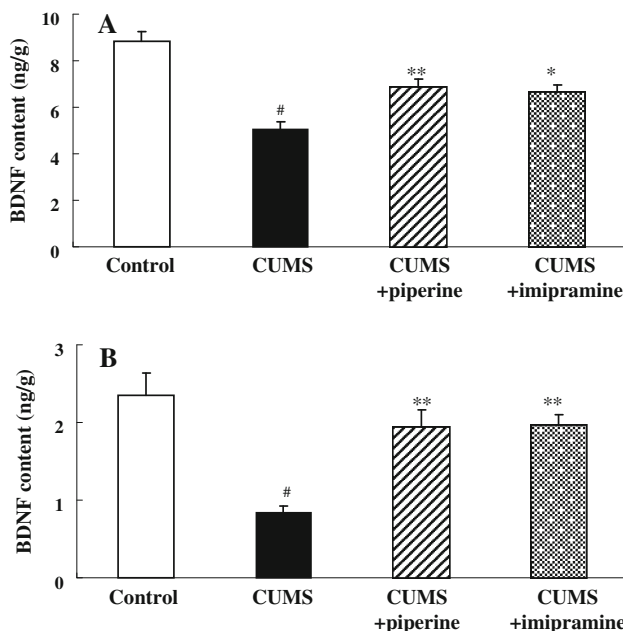


Fig. 4 Effect of piperine on BDNF content in the hippocampus (a) and frontal cortex (b) of CUMS-treated rats. Values are given as mean \pm SEM ($n = 6$). [#] $p < 0.01$ compared with the control group; ^{*} $p < 0.05$; ^{**} $p < 0.01$ compared with the CUMS group

The effect of piperine on BDNF content in the hippocampus (Fig. 4a) and frontal cortex (Fig. 4b) of CUMS-treated rats is given in Fig. 4. One-way ANOVA showed a significant difference on BDNF content in the hippocampus

($F(3, 20) = 18.19$, $p < 0.01$) and frontal cortex ($F(3, 20) = 7.83$, $p < 0.01$) among groups. The CUMS significantly decreased BDNF content in the hippocampus ($p < 0.01$) and frontal cortex ($p < 0.01$) compared with the controls. Treatment with piperine and imipramine significantly increased BDNF content in the hippocampus ($p < 0.01$ and $p < 0.05$, respectively) and frontal cortex ($p < 0.01$ and $p < 0.01$, respectively) of CUMS-treated rats compared with the CUMS-treated rats.

Discussion

It is generally believed that chronic stress is a key factor in the development and acceleration of affective disorders like depression (Willner 1997, 2005). In this regard, an animal model of CUMS-induced depression has been developed to simulate the pathogenesis of depression in humans. Several studies suggest that CUMS can induce behavioral and physiological changes resembling symptoms of clinical depression (Mao et al. 2010a, b; Willner 1997, 2005) and that CUMS-induced depression model can be used for evaluating the efficacy of antidepressant candidates through behavioral tests like sucrose preference test and forced swim test (Li et al. 2007b; Mao et al. 2009, 2010a, b). Sucrose preference test is an indicator of anhedonia-like behavioral change (Willner 1997, 2005). Anhedonia, a core symptom of human major depression, was modeled by inducing a decrease in responsiveness to rewards reflected by a reduced consumption and/or preference of sweetened solutions (Willner 1997, 2005). The results of present study showed that rats subjected to CUMS procedure consumed less sucrose solution when compared to non-stressed rats. Long-term treatment of piperine significantly suppressed this behavioral change which suggested the antidepressant-like action of piperine. To reduce the number of animals used, the effect of piperine on unstressed animals was not provided in this study. Antidepressants usually do not provide significant change on sucrose preference on unstressed animals in which depression has not been developed. In addition, the value of sucrose preference is capped at 100 % by definition. The value of unstressed control is usually over 90 % and this value is hard to be increased further by the drug treatment on unstressed animals. This phenomenon has been demonstrated in our previous study (Mao et al. 2010b).

The forced swim test has been widely used for assessing the effectiveness of candidate antidepressants. Consistent with previous findings (Zhou et al. 2008; Tõnissaar et al. 2008; Mao et al. 2009), in this study, the CUMS dramatically increased the immobility time of the rats in the forced swim test, indicating behavioral despair in these animals. Treatment with piperine significantly reversed the

CUMS-induced increase in the immobility time in rats. Each behavioral test may have its limitation and can be affected by the experimental conditions. For example, prior experience of forced swim in CUMS procedure may affect the behavior of animals in the FST although the methods have been used by many studies. To avoid the misinterpretation of the results, two or more behavioral tests should be conducted. In this study, both sucrose preference test and FST indicated that piperine treatment produced an antidepressant-like action in the CUMS-treated rats.

It has been shown that serotonergic system plays an important role in the pathogenesis of depression (Krishnan and Nestler 2008). The serotonergic hypothesis of depression (Schildkraut 1965) suggests that depressive symptoms are related to a reduced 5-HT concentration in the brain synapse and an enhancement in the concentration of this neurotransmitter is able to induce antidepressive action. Our previous studies have demonstrated that the antidepressant-like effect of piperine in the mouse forced swim test and tail suspension test may be mediated by the activation of serotonergic system (Mao et al. 2011a, b). In the present study, we found that the CUMS caused a significant decrease in 5-HT content in the hippocampus and frontal cortex of CUMS-treated rats. Piperine treatment significantly increased 5-HT content in the hippocampus and frontal cortex of CUMS-treated rats, further indicating serotonergic system may be involved in the antidepressant-like of piperine.

The role of BDNF in the pathogenesis of depression and in the mechanism of action of antidepressants has been well appreciated. It has been shown that BDNF protein and mRNA were decreased in depressive animals, which could be reversed by long-term antidepressant treatment (Li et al. 2007b; Mao et al. 2010a, b, 2012). In addition, Monteggia et al. (2007) showed that conditional BDNF knockout mice displayed an enhancement of depression-like behavior, as assessed by forced swim and sucrose preference tests. Ibarguen-Vargas et al. (2009) also demonstrated that BDNF deficit can dampen the effects of antidepressants in mice exposed to chronic unpredictable mild stress. Consistent with these findings, in the present study, the CUMS was found to significantly decrease BDNF content in the hippocampus and frontal cortex of CUMS-treated rats, while piperine treatment significantly reversed the CUMS-induced changes in BDNF expression. A previous study (Li et al. 2007b) has also demonstrated that piperine significantly increased BDNF protein levels in the hippocampus of chronic stress-treated mice, further suggesting BDNF may be involved in the antidepressant-like effect of piperine.

Several studies have shown that increased levels of 5-HT could induce BDNF expression in the hippocampus and cerebral cortex (Ivy et al. 2003). 5-HT has also been found to be able to potently and transiently increase BDNF

cellular content in rat neonatal astrocytes in vitro (Juric et al. 2006). In addition, the activation of 5-HT receptors coupled to cAMP production and CREB activation can induce BDNF gene transcription (Mattson et al. 2004). On the other hand, BDNF has been showed to produce potent neurotrophic effects on serotonergic neuronal populations (Huang and Reichardt 2001). So, it could be hypothesized that piperine might exert antidepressant-like activity by regulating the interaction between 5-HT and BDNF.

In conclusion, piperine treatment was found to alleviate CUMS-induced depressive-like behavior. The mechanism underlying the antidepressant-like action of piperine may be mediated by increasing 5-HT and BDNF contents in the hippocampus and frontal cortex.

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Conflict of interest The authors declare that they have no conflict of interest.

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