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Original article

Hippocampal BDNF signaling is required for the antidepressant effects of perillaldehyde

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ABSTRACT

Background: Perillaldehyde is one of the main components in perilla. Previous studies have shown that perillaldehyde exerted an antidepressant effect in mice, some of which is mediated through regulation of the anti-inflammatory system and the monoamine system. The primary objective of this study was to investigate the possible effects of perillaldehyde on the neurotrophic system and to elucidate whether its antidepressant effect requires brain-derived neurotrophic factor (BDNF) signaling.
Methods: Mice were exposed to chronic unpredictable mild stress (CUMS) and orally administrated with perillaldehyde for 4 weeks for behavioral testing.
Results: Perillaldehyde not only reversed the decrease in sucrose preference but also attenuated the increase in feeding latency. In addition, perillaldehyde can attenuate the reduction of CUMS-induced hippocampal BDNF levels. Our further study found that the BDNF receptor tropomyosin receptor kinase B (TrkB) antagonist K252a completely blocked the antidepressant effect of perillaldehyde in mice. Biochemical analysis showed that K252a pretreatment completely prevented the improvement of BDNF, extracellular signal–regulated kinase (ERK) phosphorylation and synaptic protein.
Conclusions: These results indicated that activation of BDNF-ERK signaling in the hippocampus was required, at least in part for the antidepressant effects of perillaldehyde.

Introduction

Depression is a common mental disease which can be described as sadness, lost of interest, low emotion and lacking positive thoughts. Over the past 50 years, the monoamine deficiency hypothesis has been mostly investigated among all the hypotheses of pathogenesis in depression [1]. However, it is accepted that the pathophysiology of depression is not well understood and needs to be further elucidated [2]. Recently, some researchers analyzed the role of several neurotrophic factors in depression and proposed that the brain-derived neurotrophic factor (BDNF), primarily involved in the regulation of maintenance, proliferation and survival of neurons in the brain, can elaborate depression [3]. This study provided the first evidence for the role of decreased BDNF expression in the pathophysiology of depression and also demonstrated that antidepressants can exert their function through promoting the expression of BDNF. As a result, BDNF is considered as a potent target for the antidepressants development.

Perillaldehyde is a monoterpenoid abundant in the herb perilla such as *Perilla frutescens* [4], which is frequently included in traditional Chinese decoctions used for the treatment of major depression [5,6]. More importantly, perillaldehyde has been previously reported to produced an antidepressant-like effect in mice exposed to chronic mild stress for ten days [7]. In addition, perillaldehyde also exhibited the antidepressant-like effect in lipopolysaccharide-induced mice likely through the regulation of anti-inflammatory and monoaminergic systems [8]. However, it remains unclear whether the neurotrophic system such as the BNDF signaling is involved in the antidepressant effect of the natural product perillaldehyde.

In this study, based on the neurotrophic hypothesis of depression, we therefore investigated whether BDNF played a key role in the antidepressant-like effects of perillaldehyde in a mouse depressive-like model, chronic unpredictable mild stress (CUMS). To further confirm that the effect of perillaldehyde was

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mediated by BDNF signaling, a tropomyosin receptor kinase B (TrkB) receptor antagonist K252a was used to block BDNF signaling before perillaldehyde administration in mice.

Materials and methods

Animals

Eight-weeks old male ICR mice (25 ± 2 g) were purchased from Shanghai Slac Animal Center, PR China. Animals were housed five per cage (320 × 180 × 160 cm) under a normal 12-h/12-h light/dark schedule (lights on at 07:00 a.) except during the CUMS procedure. The animals were allowed 1 week to adapt before the beginning of the experiments. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%. Animals have free access to food and water except during the sucrose preference test. The animal experiments complied with the ARRIVE guidelines and were approved by the University. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care.

Reagents

Perillaldehyde was purchased from Aladdin Biotechnology Co. (Shanghai, PR China). Fluoxetine hydrochloride and primary β-actin antibody were purchased from Sigma (St. Louis, USA). K252a was purchased from Alomone Laboratories (Jerusalem, Israel). The anti-BDNF antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). The antibody for synapsin I was purchased from Millipore Corporation (Billerica, USA). The antibodies for extracellular signal–regulated kinase (ERK)1/2, phospho-ERK1/2 and postsynaptic density protein 95 (PSD95) were purchased from Cell Signaling Technology (Beverly, USA).

Drug administration

The mice were randomly divided into eight groups with treated by vehicle, fluoxetine or perillaldehyde in both Control and CUMS exposure (n = 10). Both perillaldehyde and fluoxetine were diluted/dissolved in vehicle, 0.5% carboxymethyl cellulose-Na. During the whole drug treatment, Vehicle, perillaldehyde (60 and 120 mg/kg) and fluoxetine (20 mg/kg) were orally administered once daily for 4 weeks. The doses of the drugs used were selected on the basis of the literature data and our preliminary experiment [8,9].

Then, additional experiment was performed to confirm the role of BDNF in the antidepressant-like effects of perillaldehyde. In this experiment, the mice were randomly divided into five groups which included Control, CUMS, CUMS-K252a, CUMS- perillaldehyde and CUMS-K252a-perillaldehyde. K252a (25 μg/kg) was dissolved in saline with 0.1% DMSO and injected intraperitoneally 1 h prior to perillaldehyde administration (60 mg/kg).

During the whole experiments, the body weights of the animals were recorded once per week.

CUMS

The CUMS procedure is performed as previously described [9]. In detail, several stressors were applied individually and continuously throughout the experiment, including stripping, dirty cage exposure, light/dark continuous (2 h), empty bottle exposure, 45° cage tilt, space reduction, predator sound and night illumination. Control animals were housed in separate rooms and were not in contact with the stressed group. To avoid the habits to the regular order of stressors, all stressed sources are randomly applied. The entire CUMS takes place over 8 weeks.

Sucrose preference test

Sucrose training were performed prior to the experiment to monitor the normal sucrose preferences of the animals. Formal testing was performed at the end of 4 and 8 weeks of CUMS exposure [10]. Briefly, mice were trained to adapt to sucrose solution (1%, w/v) prior to testing. After adaptation, the mice were deprived of water for 12 h. During the test, the mice were housed in separate cages and each was free to obtain two bottles containing the sucrose solution or water. After 24 h, the weight of the consumed solution was calculated as sucrose preference.

Novelty-suppressed feeding test

The novelty-suppressed feeding test was performed 24 h after the last sucrose preference test. The test device consisted of a plastic box (50 × 50 × 20 cm). Food was detained from the mice for 24 h prior to testing. At the beginning of the test, individual food pellets were placed on a white paper platform located in the center of the box. Then place the mouse in the corner of the maze and start the stopwatch immediately. The score of interest is measured until the mouse reaches the food with the front paws and begins to eat. The food consumption in cage within 5 min was measured immediately after the test as a reference value.

Real-time PCR

Total RNA was isolated using Trizol reagent according to the manufacturer's instructions. Reverse transcription of cDNA synthesis was performed using M-MLV reverse transcriptase. Real-time PCR reactions were performed by using a SYBR Premix Ex Taq Kit. BDNF (forward 5′-TTATTTCATACTTCGTTGC-3′; reverse 5′-TGTCAGCCAGTGATGTCG-3′) and internal control GAPDH (forward 5′-GGGGTGTGAACCACGAGAAAT-3′; reverse 5′-GGAAGAATGGTT GGCTGGGT-3′) primers were used. The results were analyzed by $2^{-\Delta\Delta CT}$ method. The cycle of BDNF is standardized through the cycle of GAPDH.

Western blot

The brain tissue was homogenized in a lysis buffer and incubated on ice for 30 min, and the homogenate was centrifuged at 4 °C for 15,000 x 20 min, and the supernatant was collected. The protein concentration was determined by the BCA method. The protein was separated by SDS-PAGE and transferred to a PVDF membrane. After blocking for 1 h at room temperature at 5% BSA/TBST, the membrane was incubated with primary antibody at 4 °C (anti-BDNF: 1:600, anti-TrkB: 1:1000, anti-pTrkB: 1:1000, anti-synaptic I: 1:1000, anti-PSD 95:1:1000, anti-ERK: 1:1000, anti-PERK: 1:1000, anti-β-Actin: 1:5000). The membrane is then incubated with the appropriate secondary antibody and finally detected by enhanced chemiluminescence.

Statistical analyses

All data are expressed as mean ± SEM. The data were analyzed by two-way or one-way ANOVA, followed by Tukey's *post hoc* test. Values of $p < 0.05$ were statistically significant in the analysis.

Results

Perillaldehyde reversed the anhedonia in sucrose preference test

According to two-way ANOVA (Fig. 1), sucrose preference was decreased significantly in CUMS mice compared to normal control group [$F(1,72) = 15.51$, $p < 0.001$], suggesting that CUMS induced a

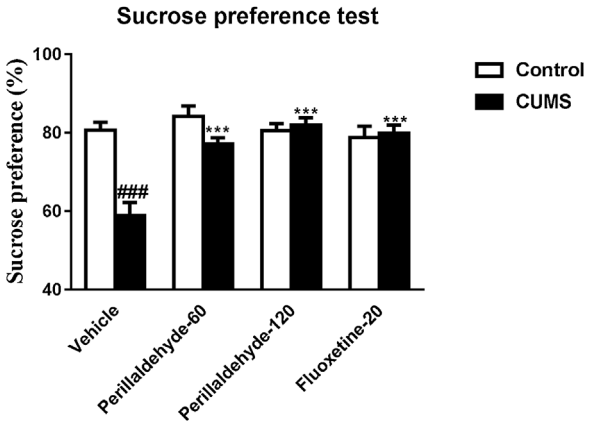

Fig. 1. Perillaldehyde (60 and 120 mg/kg) and fluoxetine (20 mg/kg) reversed the anhedonia in sucrose preference test (n = 10); $^{###}p < 0.001$ *vs.* Control-vehicle group, $^{***}p < 0.001$ *vs.* CUMS-vehicle group.

significant anhedonia in mice mimicking depression in human. Treatment factor (fluoxetine and perillaldehyde) [$F(3,72) = 10.50$, $p < 0.001$] and the interaction [$F(3,72) = 10.57$, $p < 0.001$] between CUMS and treatment were also significant. Both perillaldehyde (60 and 120 mg/kg) and fluoxetine (20 mg/kg) dramatically elevated the sucrose preference [$p < 0.001$, $p < 0.001$, $p<0.001$, respectively] in CUMS mice. In addition, neither fluoxetine nor perillaldehyde change the sucrose preference (about 80%) in normal groups.

Perillaldehyde attenuated the feeding latency in novelty-suppressed feeding test

According to two-way ANOVA (Fig. 2), CUMS increased the feeding latency [$F(1,72) = 9.63$, $p < 0.001$] when compared with normal group in the novelty-suppressed feeding test. In contrast, the treatment factor (fluoxetine and perillaldehyde) [$F(3,72) = 2.05$, $p > 0.05$] and the interaction [$F(3,72) = 2.56$, $p > 0.05$] between CUMS and treatment did not reach the significance. Tukey's *post-hoc* test showed that both perillaldehyde (60 and 120 mg/kg) and fluoxetine (20 mg/kg) dramatically reduced the feeding latency [$p < 0.05$, $p < 0.05$, $p<0.05$, respectively] in CUMS mice.

In addition, the effect of treatment factor (fluoxetine and perillaldehyde) on the total food consumption was significant according to two-way ANOVA [$F(3,72) = 3.22$, $p < 0.05$]. However, the CUMS factor [$F(1,72) = 1.97$, $p > 0.05$] and the interaction [$F(3,72) = 2.52$, $p > 0.05$] between CUMS and treatment did not reach significance. The total food consumption was decreased by administration with perillaldehyde at 60 mg/kg [$p < 0.05$]. In this way, the decrease of feeding latency induced by perillaldehyde and fluoxetine was not due to the increase of food consumption. On the other hand, perillaldehyde did not change behaviors of normal animals in novelty-suppressed feeding test.

Perillaldehyde increased hippocampal BDNF levels in CUMS mice

Both mRNA and protein levels of BDNF in the hippocampus were measured in our study (Fig. 3). Two-way ANOVA showed that BDNF mRNA expression was affected by CUMS factor [$F(1,56) = 5.22$, $p < 0.05$], treatment factor (fluoxetine and perillaldehyde) [$F(3,56) = 6.06$, $p < 0.01$] and their interaction [$F(3,56) = 3.67$, $p < 0.05$]. On the other hand, BDNF protein levels were affected by CUMS [$F(1,32) = 17.77$, $p < 0.001$], treatment factor [$F(3,32) = 7.96$, $p < 0.001$] but not their interaction [$F(3,32) = 2.76$, $p > 0.05$]. *Post-hoc* test indicated that perillaldehyde (60 and 120 mg/kg), as well as fluoxetine (20 mg/kg) reversed the reductions of mRNA expression [$p < 0.05$, $p < 0.01$, $p<0.001$, respectively] and

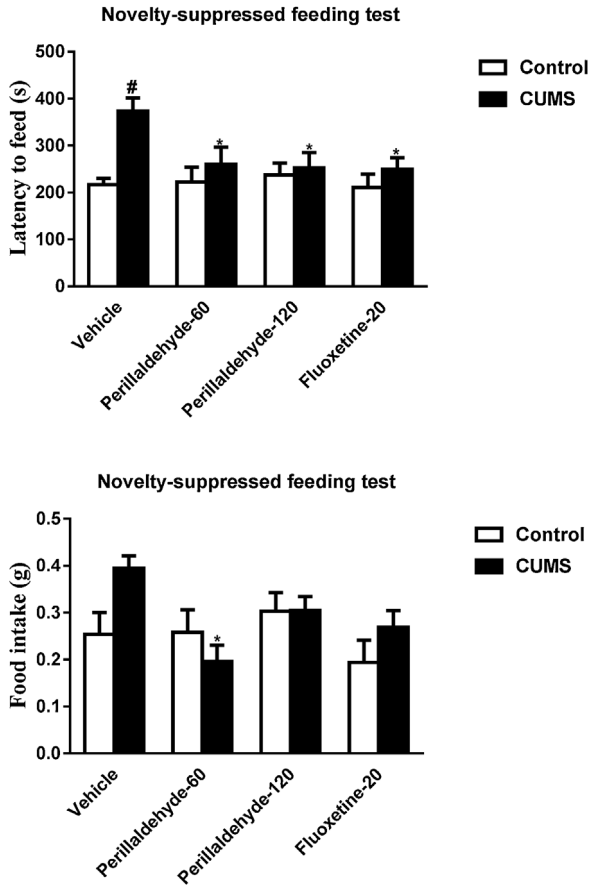

Fig. 2. Effects of perillaldehyde (60 and 120 mg/kg) and fluoxetine (20 mg/kg) on the feeding latency (A) and total food consumption (B) in novelty-suppressed feeding test (n = 10); $^{#}p < 0.05$ *vs.* Control-vehicle group, $^{*}p < 0.05$ *vs.* CUMS-vehicle group.

protein expression [$p < 0.01$, $p < 0.01$, $p<0.001$, respectively] after 4 weeks administration.

In addition, only perillaldehyde at 120 mg/kg partly increased BDNF protein expression [$p = 0.08$] in normal animals.

Pharmacological inhibition of the BDNF signaling blocks the antidepressant effects of perillaldehyde in sucrose preference tests

To determine whether increased BDNF expression mediates the anti-depressant-like effect of perillaldehyde in CUMS mice, we pretreated the CUMS mice with K252a, a selective inhibitor of BDNF signaling (refs), before the administration of perillaldehyde. CUMS decreased the sucrose preference [$F(1,18) = 15.05$, $p < 0.01$]. The two-way ANOVA indicated that there were significant perillaldehyde factor [$F(1,36) = 4.98$, $p < 0.05$], K252a factor [$F(1,36) = 4.35$, $p < 0.05$] and their interaction [$F(1,36) = 8.68$, $p < 0.01$] on the sucrose preference. Tukey's test showed that perillaldehyde reversed the reduction [$p < 0.01$] of sucrose preference. Treatment with K252a alone did not alter sucrose preference in the CUMS mice, however, it completely abolished the effect of perillaldehyde on sucrose preference [$p < 0.01$] in CUMS mice (Fig. 4).

Pharmacological inhibition of the BDNF signaling blocks the antidepressant effects of perillaldehyde in novelty-suppressed feeding test

CUMS induced an increase of feeding latency [$F(1,18) = 14.74$, $p < 0.01$] in novelty-suppressed feeding test. The two-way ANOVA

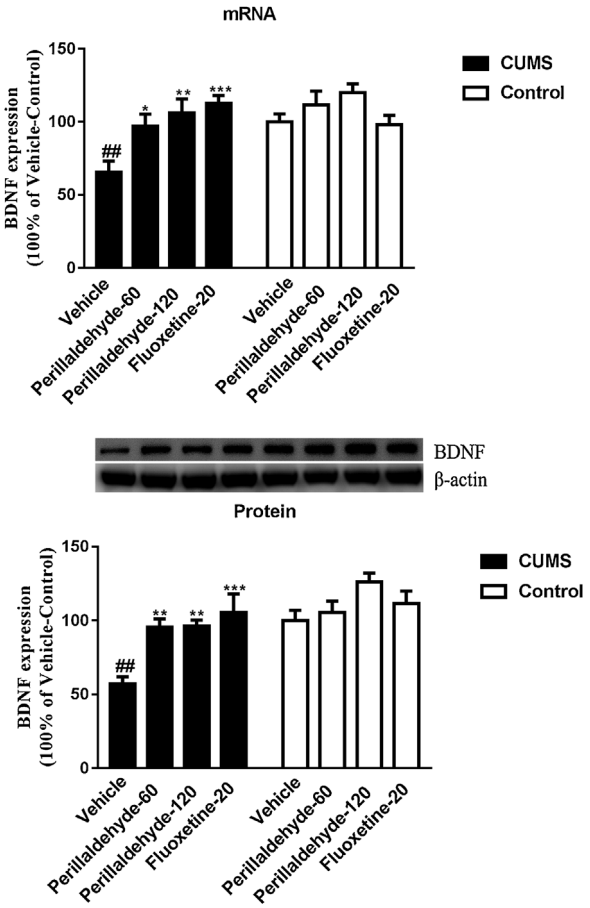

Fig. 3. Effects of perillaldehyde (60 and 120 mg/kg) and fluoxetine (20 mg/kg) on the hippocampal BDNF levels in CUMS mice (n = 5 or 8); $^{\#\#}p < 0.01$ *vs.* Control-vehicle group, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* CUMS-vehicle group.

indicated that there were significant perillaldehyde factor [$F(1,36) = 4.46$, $p < 0.05$] and their interaction [$F(1,36) = 7.79$, $p < 0.01$] on feeding latency. However, the K252a factor [$F(1,36) = 2.82$, $p > 0.05$] was not significant. Tukey's test showed that perillaldehyde reversed the increase [$p < 0.01$] in CUMS mice. Treatment with K252a alone did not alter both behaviors in the CUMS mice (Fig. 5), but completely abolished the effect of perillaldehyde on feeding latency [$p < 0.05$] when co-administration with perillaldehyde.

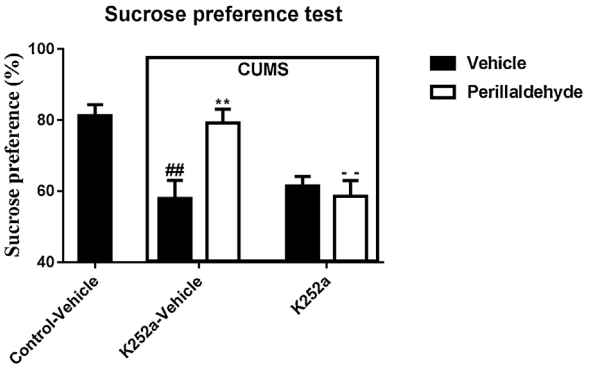

Fig. 4. Pharmacological inhibition of the BDNF signaling blocks the antidepressant effects of perillaldehyde (60 mg/kg) in sucrose preference tests (n = 10); $^{\#\#}p < 0.01$ *vs.* Control-vehicle group, $^{**}p < 0.01$ *vs.* CUMS-vehicle group, $^{--}p < 0.01$ *vs.* CUMS-perillaldehyde group.

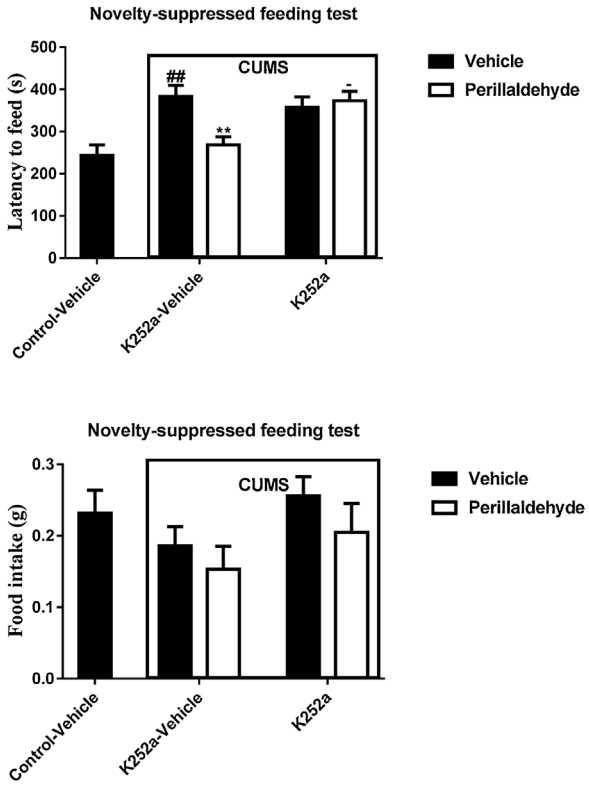

Fig. 5. Pharmacological inhibition of the BDNF signaling blocks the antidepressant effects of perillaldehyde (60 mg/kg) in novelty-suppressed feeding test (n = 10); $^{\#}p < 0.05$ *vs.* Control-vehicle group, $^{*}p < 0.05$ *vs.* CUMS-vehicle group, $^{-}p < 0.05$ *vs.* CUMS-perillaldehyde group.

In addition, CUMS did not affect the total food consumption in novelty-suppressed feeding test [$F(1,18) = 1.22$, $p > 0.05$]. There was no significant difference of perillaldehyde factor [$F(1,36) = 1.72$, $p > 0.05$], K252a factor [$F(1,36) = 3.62$, $p > 0.05$] and their interaction [$F(1,36) = 0.08$, $p > 0.05$] based on Two-way ANOVA.

K252a blocks the effects of perillaldehyde on BDNF-TrkB signaling

CUMS not only decreased BDNF levels [$F(1,8) = 9.50$, $p < 0.05$], but also inhibited TrkB phosphorylation [$F(1,8) = 9.82$, $p < 0.05$] in the hippocampus (Fig. 6). Perillaldehyde factor [$F(1,16) = 14.42$, $p < 0.01$; $F(1,16) = 14.00$, $p < 0.01$, respectively], K252a factor [$F(1,16) = 16.40$, $p < 0.001$; $F(1,16) = 9.70$, $p < 0.01$, respectively] and their interaction [$F(1,16) = 10.06$, $p < 0.01$; $F(1,16) = 6.66$, $p < 0.05$, respectively] on BDNF levels and TrkB phosphorylation were significant according to the Two-way ANOVA. Tukey's test indicated that the administration of perillaldehyde significantly reversed these abnormalities [$p < 0.001$, $p < 0.01$, respectively], however, K252a fully blocked its effects on BDNF-TrkB signaling [$p < 0.001$, $p < 0.01$, respectively] in CUMS mice.

K252a blocks the effects of perillaldehyde on ERK phosphorylation

ERK phosphorylation was decreased [$F(1,8) = 16.27$, $p < 0.01$] in the hippocampus after CUMS exposure (Fig. 7). The significant perillaldehyde factor [$F(1,16) = 26.12$, $p < 0.001$], K252a factor [$F(1,16) = 35.39$, $p < 0.001$] and their interaction [$F(1,16) = 14.59$, $p < 0.01$] on ERK phosphorylation were found. Administration of perillaldehyde significantly increased ERK phosphorylation [$p < 0.001$], while K252a fully prevented the effect of perillaldehyde on ERK phosphorylation [$p < 0.001$] in CUMS mice.

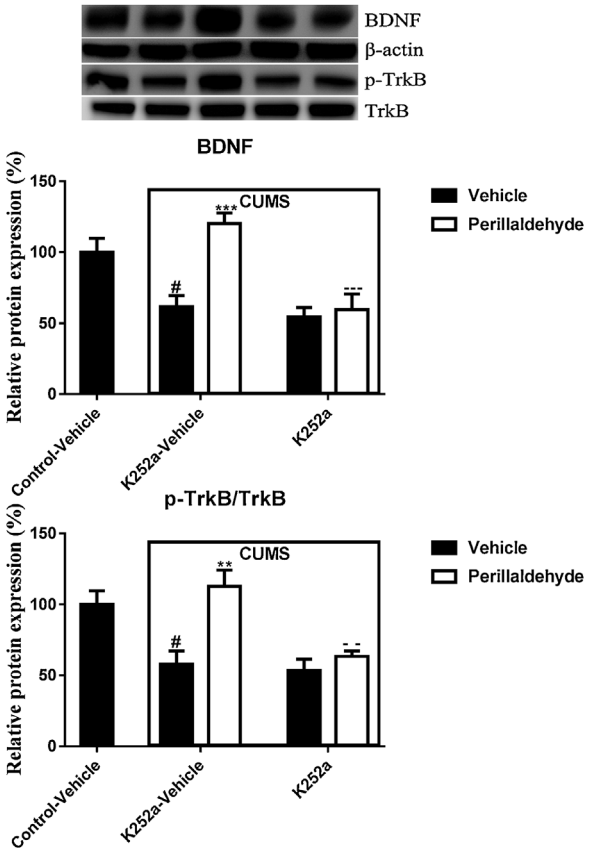

Fig. 6. K252a blocks the effects of perillaldehyde (60 mg/kg) on BDNF expression and TrkB phosphorylation in the hippocampus (n = 15); $^{\#}p < 0.05$ *vs.* Control-vehicle group, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* CUMS-vehicle group, $^{--}p < 0.01$ and $^{---}p < 0.001$ *vs.* CUMS-perillaldehyde group.

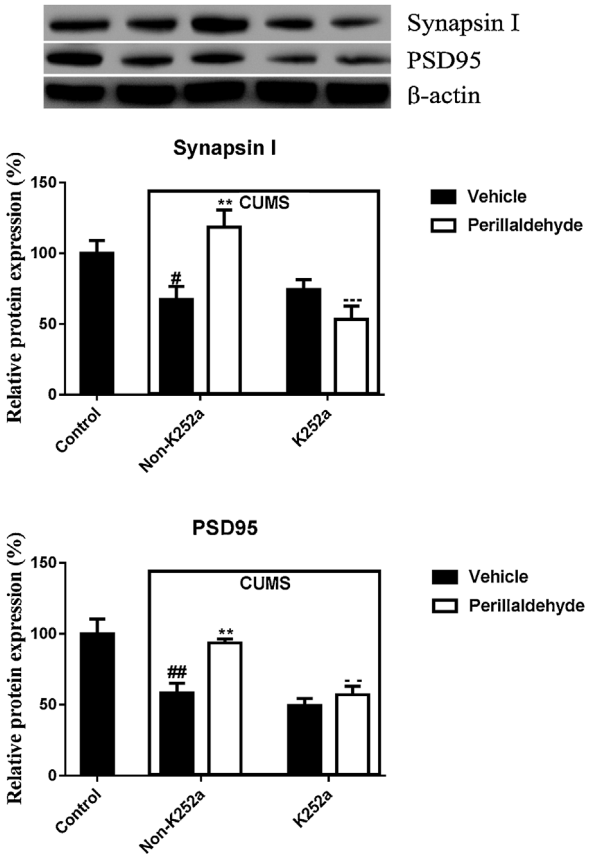

Fig. 8. Pharmacological inhibition of the BDNF signaling blocks the effects of perillaldehyde (60 mg/kg) on synaptic proteins in mice induced by CUMS (n = 5); $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* Control-vehicle group, $^{**}p < 0.01$ *vs.* CUMS-vehicle group, $^{--}p < 0.01$ and $^{---}p < 0.001$ *vs.* CUMS-perillaldehyde group.

Pharmacological inhibition of the BDNF signaling blocks the effects of perillaldehyde on synaptic proteins in mice induced by CUMS

The levels of synaptic proteins such as synapsin I [$F(1,8) = 6.40$, $p < 0.05$] and PSD95 [$F(1,8) = 11.15$, $p < 0.01$] were markedly reduced by CUMS (Fig. 8). The two-way ANOVA indicated that there were significant K252a factor [$F(1,16) = 9.29$, $p < 0.01$] and their interaction [$F(1,16) = 14.21$, $p < 0.01$] on synapsin I levels. On the contrary, the perillaldehyde factor [$F(1,16) = 2.51$, $p > 0.05$] was not significant. On the other hand, the two-way ANOVA indicated that there were significant perillaldehyde factor [$F(1,16) = 15.79$, $p < 0.01$], K252a factor [$F(1,16) = 17.60$, $p < 0.001$] and their interaction [$F(1,16) = 6.43$, $p < 0.05$] on PSD95 levels. Perillaldehyde significantly reversed these down-regulations [$p < 0.01$, $p < 0.01$, respectively], while K252a totally blocked the effects of perillaldehyde on the levels of synapsin I [$p < 0.001$] and PSD95 [$p < 0.01$] in CUMS mice.

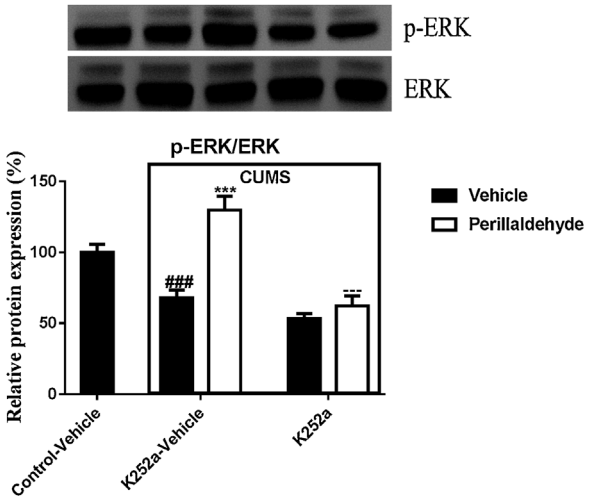

Fig. 7. K252a blocks the effects of perillaldehyde (60 mg/kg) on ERK phosphorylation in the hippocampus (n = 5); $^{\#\#}p < 0.01$ *vs.* Control-vehicle group, $^{***}p < 0.001$ *vs.* CUMS-vehicle group, $^{---}p < 0.001$ *vs.* CUMS-perillaldehyde group.

Discussion

In the present study, CUMS was used to induce depression in mice because CUMS mimics chronic stress in human daily life. At the end of the experiment, we tested sucrose preference because the reduction in sucrose preference could be considered an indicator of anhedonia, one of the core symptoms of human depression [11]. The results showed that CUMS reduced the preference of sucrose, while 4-week administration of perillaldehyde (60 and 120 mg/kg) reversed this effect. Subsequently, we further evaluated the anti-depressant effect of perillaldehyde using a novelty-suppressed feeding test, an increasingly popular anxiety model for anti-depression research [12]. The results indicated that perillaldehyde (60 and 120 mg/kg) reversed the increase of feeding latency in CUMS mice. Consistent with the previous two studies in which administration of perillaldehyde for nearly 10 days shortened the immobility time in the forced

swimming test [7,8], the behavioral results of our study confirmed the antidepressant-like effects of perillaldehyde without affecting the behavioral of control animals, indicating that perillaldehyde did not affect normal behavior. In addition, it is worth noting that perillaldehyde at 60 mg/kg significantly reduced the total food consumption in the novelty-suppressed feeding test. We speculated that the increase in total food consumption of CUMS-vehicle animals might be due to excessive powder dropping in the test. In general, most publications take less time, 5–15 min to detect the food consumption, but prolonging the consumption time will reduce the sensitivity of the evaluation. Therefore, dropping or particles may increase the weighing error in short-term experiments. Furthermore, the total food consumption is measured by excluding the stomach and hunger of the animal [13]. Therefore, an increase in food consumption means that the increase in the feeding latency of CUMS vehicle animals is not due to stomach and hunger problems. On the other hand, the data showed that there was no significant change in body weight when compared between each animal group (data not shown), which is consistent with previous studies [14].

In addition to abnormal behavioral, recent research has improved our understanding of the molecular mechanisms of antidepressants. In order to address the limitations of the monoamine hypothesis in explaining the hysteresis after antidepressant treatment, neurotrophic hypotheses were proposed [15,16]. This hypothesis suggests that the lagging effect of antidepressants is due to the delayed expression of BDNF in the central nervous system. BDNF is a major neurotrophic factor that mediates neuronal proliferation, differentiation, and survival [17] and participates in the processes of memory and cognition [18]. A growing number of clinical studies have shown that BDNF levels were reduced in plasma or brain tissues in patients with depression. In contrast, BDNF levels were elevated after effective treatment including antidepressant drugs or electroconvulsive therapy in both clinical trials [19,20] and preclinical studies [14,21]. In agreement with these findings, our study also found that CUMS induced down-regulation of hippocampal BDNF and perillaldehyde (60 and 120 mg/kg) increased the mRNA and protein levels of BDNF in hippocampus of CUMS mice, indicating that BDNF may be involved in the antidepressant effect of perillaldehyde. In contrast, perillaldehyde did not significantly increase BDNF levels in the control group. This may be due to the high level of BDNF expression in normal brain that cannot be further increased upon perillaldehyde treatment. Alternatively, as we measured BDNF expression two days after the last drug administration these two wash days may normalize the expression of BDNF in normal animals, which is supported by one previous study that reported a significant reduction in BDNF after 1 week wash period compared to treatment without wash phase [22].

Signal transduction pathway is considered to be the core problem of depression-like research and is also a core issue based on receptor/ligand theory. Next we used the TrkB antagonist K252a to detect whether BDNF signaling activation is required for the antidepressant-like effects of perillaldehyde. K252a blocks BDNF signaling and disrupts the effects of BDNF-dependent drugs [23]. Therefore, mice were co-injected with K252a and perillaldehyde for 4 weeks. The results showed that K252a eliminated the antidepressant-like effect of perillaldehyde in the sucrose preference test and the novelty-suppressed feeding test because the combined administration of K252a and perillaldehyde did not reverse the depression-like behaviors. There was no significant change in BDNF levels and TrkB phosphorylation after combination administration, suggesting that BDNF-TrkB signal transduction was completely inhibited, and K252a was effective in this study. In addition to BDNF-TrkB signaling, we also investigated changes in ERK phosphorylation, as the MEK/ERK cascade is a downstream regulator of BDNF-TrkB signaling, and ERK phosphorylation is also thought to mediate neurogenesis and intracellular signal transduction mechanisms of cognitive function [24,25]. Pretreatment with K252a abolished the effect of perillaldehyde on ERK phosphorylation, suggesting that the hippocampal BDNF-TrkB-ERK signaling pathway is involved in the antidepressant effect of perillaldehyde.

BDNF enhances excitatory synaptic transmission and is associated with synaptic function [26]. The expression of synaptic-associated proteins is inhibited upon deletion of BDNF gene [27]. Thus, we measured the levels of synapsin I and

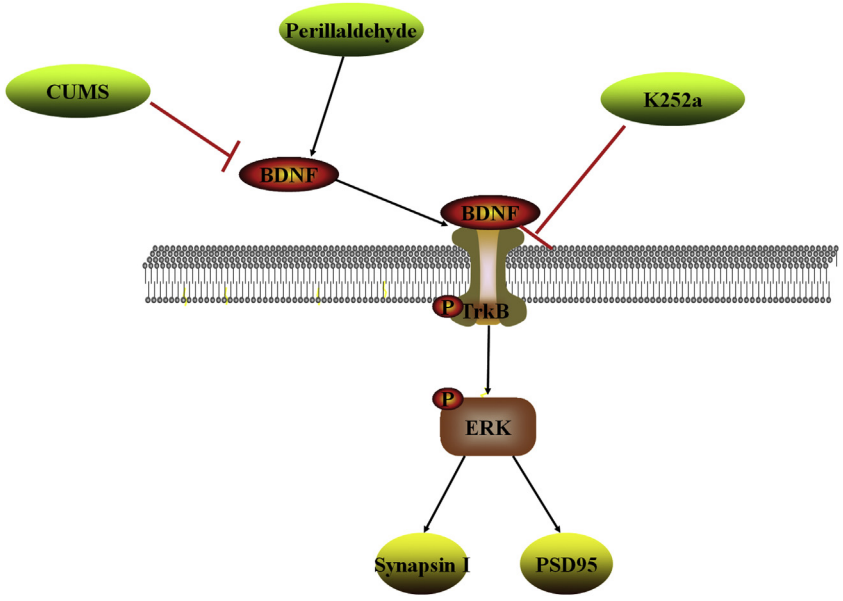

Fig. 9. Involvment of BDNF-TrkB-ERK signaling in the antidepressant-like mechanism of perillaldehyde.

PSD-95, the two major synaptic proteins from presynaptic and postsynaptic, respectively, in the brain of CUMS treated with K252a. Consistent with changes in BDNF and TrkB phosphorylation, K252a inhibited the levels of hippocampal synapsin I and PSD95 induced by perillaldehyde in CUMS mice. Combined with the results above, it indicated that perillaldehyde promoted hippocampal synaptic protein levels in a BDNF-TrkB-ERK-dependent manner (Fig. 9). On the other hand, it should be noted that we have only measured the role of perillaldehyde in the hippocampus. Previous studies showed that BDNF signaling pathway in the prefrontal cortex, amygdala or hypothalamus also mediated the actions of antidepressant [28–30]. In this way, further research is needed to perform this effect in other brain regions such as the prefrontal cortex, amygdala, and hypothalamus.

Moreover, although K252a is known to antagonize TrkA, TrkB, and TrkC, it is clear that the activation of NGF, BDNF and NT-3 is preferentially mediated by TrkA, TrkB and TrkC, respectively (refs). In addition, neurotrophin-4 (NT-4) is another neurotrophic factor that activates the TrkB receptor. TrkB binds BDNF and NT-4 more strongly than it binds NT-3. TrkC binds NT-3 more strongly than TrkB. However, recent neurotrophic hypothesis about depression only indicates that BDNF is the major factor involved in the pathophysiology of depression and the treatment of antidepressants [3]. This is why we only detect BDNF in the hippocampus. Therefore, although K252a may also inhibit NGF-TrkA and NT-3-TrkC signaling pathways, these signaling pathways are not associated with the pathophysiology of depression and would have limited contribution to the interference of K252a in perillaldehyde-mediated antidepressant effect. However, although the antidepressant-like effects of perillaldehyde likely depend on the hippocampal BDNF signaling pathway, it remains unknown whether other potential tyrosine kinase receptors targets of K252a may play a role too.

Last, it cannot deny that the theory of monoamine deficiency not only accelerates the understanding of depression pathophysiology but also contributes to the development of antidepressants. However, considering that the pathophysiology of depression is complicated, we could not evaluate the antidepressant-like mechanism from all the related aspects such as monoamines, the hypothalamic-pituitary-adrenal (HPA) axis, neuroinflammation and neurotrophins at one time. Therefore, it might be one of the limitations of the present study. Also, it is no doubt that further study needs to elucidate whether perillaldehyde could modulate monoamines, the HPA axis and cytokines.

Taken together, our results provide direct evidence that perillaldehyde has an antidepressant effect in CUMS mice, and these effects appear to be mediated, at least in part, by restoring the hippocampal BDNF-TrkB-ERK signaling pathway. This study provides further understanding of the mechanism underlying the putative therapeutic effect of perillaldehyde in the treatment of depression.

Conflict of interests

The authors declare that they have no conflicts of interest.

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