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Applying vinpocetine to reverse synaptic ultrastructure by regulating BDNF-related PSD-95 in alleviating schizophrenia-like deficits in rat

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ABSTRACT

Background: Schizophrenia is a mental disorder characterized by hyperlocomotion, cognitive symptoms, and social withdrawal. Brain-derived neurotrophic factor (BDNF) and postsynaptic density (PSD)-95 are related to schizophrenia-like deficits via regulating the synaptic ultrastructure, and play a role in drug therapy. Vinpocetine is a nootropic phosphodiesterase-1 (PDE-1) inhibitor that can reverse ketamine-induced schizophrenia-like deficits by increasing BDNF expression. However, the effects of vinpocetine on alleviating schizophrenia-like deficits via reversing the synaptic ultrastructure by regulating BDNF-related PSD-95 have not been sufficiently studied.
Methods: In this study, the schizophrenic model was built using ketamine (30 mg/kg) for 14 consecutive days. The effect of vinpocetine on reversing schizophrenia-like behaviors was examined via behavioral testing followed by treatment with certain doses of vinpocetine (20 mg/kg, i.p.). The BDNF and PSD-95 levels in the posterior cingulate cortex (PCC) were measured using biochemical assessments. In addition, the synaptic ultrastructure was observed using transmission electron microscopy (TEM).
Results: Ketamine induced drastic schizophrenia-like behaviors, lower protein levels of BDNF and PSD-95, and a change in the synaptic ultrastructure in the PCC. After treatment, the vinpocetine revealed a marked amendment in schizophrenia-like behaviors induced by ketamine, including higher locomotor behavior, lower cognitive behavior, and social withdrawal defects. Vinpocetine could increase the PSD-95 protein level by up-regulating the expression of BDNF. In addition, the synaptic ultrastructure was changed after vinpocetine administration, including a reduction in the thickness and curvature of the synaptic interface, as well as an increase in synaptic cleft width in the PCC.
Conclusion: Vinpocetine can reverse the synaptic ultrastructure by regulating BDNF-related PSD-95 to alleviate schizophrenia-like deficits induced by ketamine in rats.

1. Introduction

Schizophrenia is a severe and devastating mental disorder generally characterized by positive symptoms (e.g., hallucinations and delusions), negative symptoms (e.g., avolition and apathy), and cognitive impairments (e.g., impairments in memory, learning, and executive functioning) [1,2]. Schizophrenia is reported to affect around 1% of the world's population, with a high prevalence of 7.2/1000 individuals [3,4]. Existing standard-of-care antipsychotic medications, such as risperidone, are currently the first-line choice in the treatment of schizophrenic cognitive symptoms [5]. However, around 30% of schizophrenia patients will not respond satisfactorily to the use of antipsychotics [6].

Cognitive deficits are considered to be a core feature of schizophrenia, and depression is a complication [7]. The posterior cingulate cortex (PCC), which is a part of the prefrontal cortex (PFC), is implicated in the cognitive deficits of schizophrenic patients. Mitelman found that the metabolic activity and disruption of the default network mode in the PCC was decreased in schizophrenia [8], which may affect internally-directed cognitive processes [9].

The hypo-functionality of the dopamine D1 receptor in the PFC of schizophrenia patients is intimately involved in working memory and cognitive dysfunction [10,11]. Vinpocetine is a nootropic phosphodiesterase-1 (PDE-1) inhibitor that acts as an indirect dopamine D1 receptor agonist [12]. Vinpocetine pre-treatment revealed a marked amendment in the hyperlocomotion, anxiety, and short-term memory deficits in schizophrenia patients [13]. However, the underlying mechanisms of vinpocetine in improving several neurological and cognitive impairments in schizophrenia patients remain to be seen.

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that is involved in neuronal cell growth, survival, and synaptic plasticity [14]. In patients with schizophrenia, the reduced expression of BDNF and/or tropomyosin receptor kinase B (TrkB) has been found in the PFC

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[15]. There is also evidence for reduced BDNF levels in the peripheral blood of schizophrenia patients [16]. Cognitive defects, depression, and anxiety symptoms are related to the expression of BDNF in the PFC. Studies have found that decreased levels of BDNF were associated with schizophrenia, and after treatment, the serum BDNF levels in both groups increased [17,18]. Interestingly, vinpocetine can increase levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which eventually leads to the stimulation of cAMP- and cGMP-dependent protein kinases that in turn, and through other proteins, regulate the production of many genes, such as BDNF [19].

Moreover, BDNF has emerged as a major regulator of synaptic transmission and plasticity at adult synapses in many regions of the central nervous system [20], which may also be a possible underlying mechanism of schizophrenia [13]. Clinical, epidemiological, neuroimaging, and postmortem data all suggest that synaptic disturbances might play a critical role in the development of schizophrenia [21]. Current evidence suggests that the synaptic ultrastructure, synaptogenesis, and synaptic function are changed in the pathophysiology of schizophrenia disorders [22]. However, the molecular mechanism underlying these effects has not yet been fully elucidated.

The thickness of the PSD, which is localized at the postsynaptic membrane, is closely correlated with synaptic transmission efficiency. Abnormal alterations in the morphological structure of the PSD, especially the PSD-95 protein, may lead to disturbance in long-term synaptic plasticity and dysfunction in synaptic transmission, which may also be correlated with a change of the synaptic function in the pathophysiology of schizophrenia [23]. Decreased protein expression of PSD-95 has been observed in the cortex of postmortem schizophrenia patients [24]. Deletion of the DLG2 gene (which encodes PSD-95) causes a range of neuropsychiatric disorders, including schizophrenia [25]. Further evidence has revealed a significant decrease in PSD-95 mRNA expression levels in the dorsolateral and dorsomedial prefrontal cortex of postmortem schizophrenia patients, suggesting an association between PSD-95 dysfunction and schizophrenia [26]. Perhaps most interesting, an existing study suggests that BDNF signaling promotes PSD-95 expression in synapses and dendritic spines, and regulates the postsynaptic localization of PSD-95 via the TrkB signaling pathways [27]. Therefore, the synaptic ultrastructure regulated by the change in BDNF-related PSD-95 might be involved in schizophrenia.

The present study hypothesized that changes in the synaptic ultrastructure might be associated with schizophrenia, and that BDNF-related PSD-95 signaling pathways provide down-regulation in the development of schizophrenia. In an attempt to further understand this potential mechanism, the present study aimed to establish a schizophrenia rat model induced by ketamine. Schizophrenia-like behavior was detected using an open field test (OFT), Y-maze test (YMT), and social interaction test (SIT). BDNF and PSD-95 expression were measured using western blotting. In addition, immunohistochemistry and the synaptic ultrastructure were observed using transmission electron microscopy (TEM).

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley (SD) rats weighing 220–250 g (6 weeks old) were purchased from Beijing SPF Vital Laboratory Animal Technology Company (Beijing, China). Animals were housed in groups of four per cage (53.5 × 39 × 20 cm) under controlled 12-h light/12-h dark cycle conditions (lights on from 7 a.m. to 7 p.m.). The room controls were set to maintain a temperature of 22 ± 2 °C and a relative humidity of 55 ± 15%. The animals, which had free access to water and standard rat chow, were habituated to these conditions for seven days prior to the experimental procedures. All experiments were performed between 9 a.m. and 2 p.m. during the light cycle. The experiments were undertaken according to the National Institutes of Health Guidelines (Use of Laboratory Animals), and were approved by the Animal Care and Use Committee of Shiyan Renmin Hospital.

2.2. Experimental procedure

Ketamine (Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) was diluted in saline to 30 mg/ml and injected at a volume of 1 ml/kg of body weight. Rats ($n = 45$) were injected intraperitoneally twice daily (morning and late afternoon) with saline or ketamine (30 mg/kg) for 14 consecutive days [28]. Following ketamine exposure, the rats were left undisturbed for at least five days for drug treatment before behavioral training. The control group ($n = 15$) did not undergo any of the processing (Fig. 1).

2.3. Experimental design and pharmacological treatment

Ketamine-exposed rats were randomly assigned into one of three groups. The Ket group ($n = 15$) did not undergo any of the pharmacological treatments; the Ket-vertical group ($n = 15$) was treated with normal saline intraperitoneally for 7 consecutive days; the Ket-Vin group ($n = 15$) was treated with vinpocetine (20 mg/kg, i.p.) for 7 consecutive days. Behavioral tests were evaluated after the last injection of vinpocetine or saline (Fig. 1).

2.4. Behavioral tests

2.4.1. Open field test

An OFT is a mild stressful behavioral test that measure changes in locomotor activity and emotionality in rodents [29]. The open field area consisted of an enclosed square arena made of dark opaque Plexiglas (100 cm × 100 cm) surrounded by walls (30 cm). The field was divided into 25 squares with virtual grid lines for analysis using Smart 3.0 software. Each rat was gently placed into the same corner of the arena facing the same direction, and was allowed to freely explore the arena for 5 min. The surface of the open field arena was cleaned thoroughly with 70% ethanol between tests for different subjects. The rats' behaviors, including the numbers of crossings (with all four paws placed into a new square) and up-right postures (with both front paws raised from the floor) were recorded using a digital camera.

2.4.2. Y-maze test

YMTs are used to evaluate cognitive symptoms of schizophrenia [30]. The Y-maze apparatus consisted of three enclosed arms (50 cm × 16 cm) with geometric shapes on the walls (31 cm). The arms were labeled A, B, and C, and were positioned at equal angles. Rats were placed at the end of an arm, and allowed to freely explore the maze for 8 min. The series of arm entries were recorded using a digital camera. The surface of the Y-maze apparatus was cleaned thoroughly with 70% ethanol between tests for different subjects. An actual alternation was defined as a series of entries into all three arms on consecutive occasions. The percentage of alternation was calculated as the total of alternations (total arm entries - 2).

2.4.3. Social interaction test

SIT is utilized to assess a cluster of negative schizophrenia symptoms, such as social withdrawal [31]. The test chamber consisted of dark opaque Plexiglas (60 × 40 cm) divided into three compartments (A, B, and C), with a small opening (6 × 6 cm) in the dividers. An empty iron restraining cage or one with probe mice was placed in each of the two side chambers (A and C). During testing, a test rat was placed in the center chamber (chamber B) for 5 min, and other mice from the same dose group (but novel to each other; they were housed so as they had no opportunity to smell or see each other) were placed in one of two restraining cages. The time and number of interactions of a test rat with a novel rat were recorded using a digital camera.

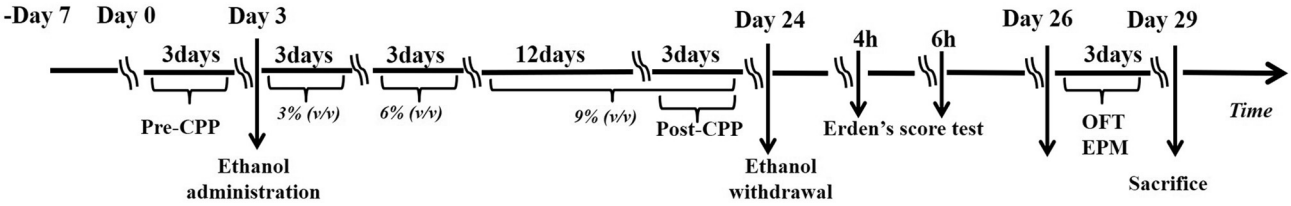

Fig. 1. Experimental schedule for developing the schizophrenic model and vinpocetine treatment. On day 0, rats were injected intraperitoneally with ketamine (30 mg/kg) for 14 consecutive days. After that, rats were left undisturbed for 5 days. On day 19, rats were treated with vinpocetine (20 mg/kg, i.p.) for 7 consecutive days. Behavioral tests were then performed on day 26, and rats were sacrificed on day 29.

2.5. Immunohistochemistry assay

Five rats in each group were anesthetized with 8% chloral hydrate (400 mg/kg, i.p.) and perfused transcardially with 200 ml of normal saline followed by 200–300 ml of 4% paraformaldehyde in a 0.1 M phosphate buffer (PB). Then, coronal cryotome sections (20 μm) were cut through the PCC using a cryostat and collected on poly-L-lysine-coated slides. The endogenous peroxidase of the sections was quenched with 0.3% H_2O_2 in methanol for 15 min. After being blocked with 4% goat serum in 0.3% Triton X-100 for 1 h at room temperature, sections were incubated with the primary antibody (anti-BDNF, 1:500, ab108319; anti-PSD-95, 1:500, ab18258; both from Abcam, Cambridge, MA, USA) for 24 h at 4 °C. Next, sections were incubated with the secondary antibody (Peroxidase-Conjugated AffiniPure Goat Anti-Mouse IgG (H + L), 1:200, ZB-2305; Peroxidase-Conjugated AffiniPure Goat Anti-Rabbit IgG (H + L), 1:200, ZB-2301; both from ZSGB-BIO, Beijing, China) for 2 h at room temperature. After that, sections were incubated in an ExtrAvidin peroxidase conjugate and reacted with a DAB kit (ZLI-0931, ZSGB-BIO) for color reaction. PCC sections were examined with an Olympus FluoView 1200 confocal microscope system (Olympus Corp., Tokyo, Japan), and photomicrographs of representative PCC areas were obtained.

2.6. Western blot assay

Five rats in each group were sacrificed under anesthesia. The PCC was dissected and the protein was extracted with a RIPA buffer (P0013B, Beyotime, Shanghai, China) containing the protease inhibitor cocktail (ST506, Beyotime, Shanghai, China) on ice. Tissue lysates were centrifuged at 14,000 rpm for 10 min at 4 °C to remove the insoluble fraction. Equal amounts of protein samples (30 μg) were denatured by heating at 95 °C for 10 min, and then separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred to nitrocellulose polyvinylidene fluoride (PVDF) membranes using a Trans-Blot wet transfer system (Bio-Rad Laboratories) with a transfer buffer. After being blocked by 5% non-fat milk, membranes were probed with the primary antibody (anti-BDNF, 1:1000, ab108319; anti-PSD-95, 1:1000, ab18258; both from Abcam, Cambridge, MA, USA) for 24 h at 4 °C. The PVDF membranes were then washed with Tris-buffered saline plus Tween 20, and then incubated with the secondary antibody (anti-BDNF: Peroxidase-Conjugated AffiniPure Goat Anti-Rabbit IgG (H + L), 1:200; anti-PSD-95: Peroxidase-Conjugated AffiniPure Goat Anti-Mouse IgG (H + L), 1:200) for 1 h at room temperature. The gray values of the bands were quantified with Image J software.

2.7. Transmission electron microscopy assay

PCC samples of 5 rats in each group were collected after being perfused transcardially with 200–300 ml of 4% paraformaldehyde. Immersion fixation was then completed at a size of around 1 mm^3. Samples were rinsed in cold phosphate-buffered saline (PBS) and placed in 2.5% glutaraldehyde at 4 °C for 2 h. After being post-fixed with 1% aqueous osmium tetroxide in a 0.2 M cacodylate buffer (pH 7.2) for 2 h, the tissue was rinsed with distilled water before dehydration in gradually-increasing ethanol concentrations, and infiltrated using a mixture of half acetone and half resin overnight at 4 °C. After that, the 70-nm-thick ultrathin sections were stained with 3% uranyl acetate for 20 min and 0.5% lead citrate for 5 min, and observed with the JEOL 1200EX at 120 kV (JEOL, Inc., Tokyo, Japan). Ultrastructural changes in the PCC synapses were observed under TEM (HT7700-SS, Hitachi, Tokyo, Japan). Bouton parameters were quantified using Image J software.

2.8. Statistical analysis

All measurements were obtained by an independent investigator blinded to the experimental conditions, and expressed as mean values ± standard deviation (SD). One-way ANOVA with post hoc test was used to compare the differences using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). The Pearson correlation between the BDNF protein and PSD-95 was tested using GraphPad Prism 6 software (GraphPad Software, Inc., CAPrism6, USA). A probability level of 0.05 was considered significant.

3. Results

3.1. Effect of vinpocetine on the schizophrenia-like behaviors induced by ketamine

The effect of vinpocetine treatment on the locomotor activity of rats with schizophrenia induced by ketamine was demonstrated by the OFT (ANOVA: $F = 3.771$, $P < 0.05$; Fig. 2A and B). The numbers of crossings and up-right postures in the Ket group were significantly higher compared to those of the control group (post hoc test: $P < 0.05$, Fig. 2A; post hoc test: $P < 0.05$, Fig. 2B). After treatment, the Ket+Vin group had a significant reversal in locomotor activity, including an increase in the number of crossings and up-right postures as compared with the Ket+Sal group (post hoc test: $P < 0.05$, Fig. 2A; post hoc test: $P < 0.05$, Fig. 2B).

Fig. 1C shows the spatial working memory results of the YMT (ANOVA: $F = 3.774$, $P < 0.05$). The percentage of alternation behavior was significantly lower in the Ket group than in the control group (post hoc test: $P < 0.001$, Fig. 2C). A significant change in the spatial working memory of the Ket+Vin group was observed as compared to the Ket+Sal group (post hoc test: $P < 0.001$, Fig. 2C).

As shown in Fig. 1D and E, a one-way ANOVA test revealed that the number and time of social interactions in the control group was significantly different compared with the Ket group (ANOVA: $F = 12.178$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 2D; post hoc test: $P < 0.05$, Fig. 2E). This ketamine-induced social withdrawal was reversed after

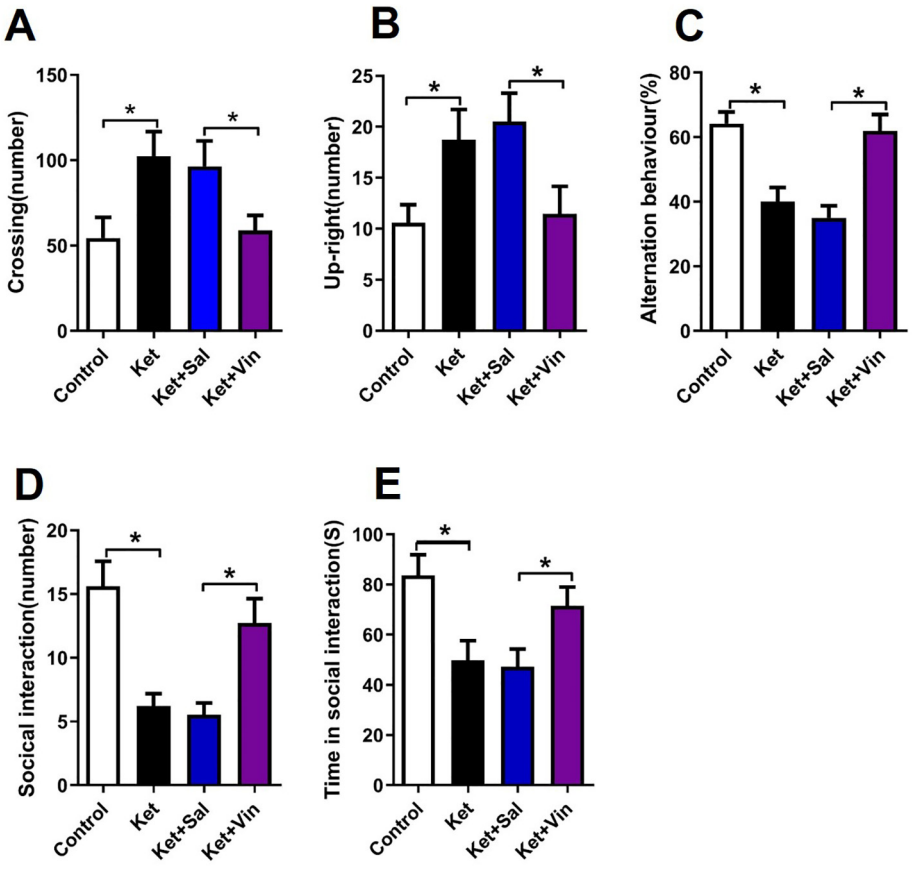

Fig. 2. Effect of vinpocetine on schizophrenic behaviors induced by ketamine. (A) Number of crossings in the OFT. (B) Number of up-right postures in the OFT. (C) Percentage of alternation behavior in the YMT. (D) Number of social interactions in the SIT. (E) Time of the social interactions in the SIT. Data represent the mean ± S.E.M. ($n = 60$). *$P < 0.05$ was significant.

vinpocetine treatment (post hoc test: $P < 0.05$, Fig. 2D; post hoc test: $P < 0.05$, Fig. 2E).

3.2. Effect of vinpocetine on change of BDNF and PSD-95 levels

As illustrated in Fig. 2, significant decreases in BDNF positive cells in the PCC were detected following ketamine administration (ANOVA: $F = 4.737$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 3C). After treatment with vinpocetine, the BDNF positive cells in the Ket+Vin group were significantly increased compared to the Ket +Sal group (post hoc test: $P < 0.05$, Fig. 3C). Statistical analysis revealed that the significant reduction in the number of PSD-95 positive cells in the PCC was induced by the ketamine (ANOVA: $F = 3.981$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 3D), while the vinpocetine could increase the number of PSD-95 positive cells (post hoc test: $P < 0.05$, Fig. 3D). Meanwhile, the number of PSD-95 positive cells was significantly correlated with the number of BDNF positive cells ($r^2 = 0.4766$, $P < 0.001$, Fig. 3E).

A lower level of BDNF protein was expressed in the control group compared to the Ket group (ANOVA: $F = 8.804$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 4A). The level of BDNF protein in the Ket+Vin group was significantly different compared to the Ket+Sal group (post hoc test: $P < 0.05$, Fig. 4A). The level of PSD-95 protein was significantly lower in the control group compared to the Ket group (ANOVA: $F = 5.005$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 4B). The vinpocetine significantly increased the number of PSD-95 positive cells in the PCC (post hoc test: $P < 0.05$, Fig. 4B). Through correlation analysis, it was found that there was a positive correlation between the level of BDNF protein and the level of PSD-95 protein in the PCC ($r^2 = 0.2530$, $P < 0.05$, Fig. 4C).

3.3. Correlation between protein level and behavioral tests

There was a negative correlation between the level of BDNF protein and the number of crossings or up-right postures in the OFT ($r^2 = 0.2513$, $P < 0.05$; $r^2 = 0.2918$, $P < 0.05$; Fig. 5A). There was also a negative correlation between the level of BDNF protein and both the YMT ($r^2 = 0.3253$, $P < 0.05$, Fig. 5B) and SIT (number of social interactions: $r^2 = 0.3145$, $P < 0.05$; time of social interaction: $r^2 = 0.5299$, $P < 0.05$; Fig. 5C) behavior tests. After Pearson analysis, it was found that there was a negative correlation between the level of BDNF protein and the number of crossings or up-right postures in the OFT ($r^2 = 0.2321$, $P < 0.05$; $r^2 = 0.2097$, $P < 0.05$; Fig. 5D). There was also a negative correlation between the level of BDNF protein and both the YMT ($r^2 = 0.4105$, $P < 0.05$, Fig. 5E) and SIT (number of social interactions: $r^2 = 0.4171$, $P < 0.05$; time of social interaction: $r^2 = 0.2806$, $P < 0.05$; Fig. 5F) behavior tests.

3.4. Effect of vinpocetine on change of synaptic ultrastructure

A reduction in the thickness and curvature of the synaptic interface and an increase in synaptic cleft width in the PCC were expressed in the Ket group compared to the control group (ANOVA: $F = 3.932$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 6B; ANOVA: $F = 4.812$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 6C; ANOVA: $F = 6.289$, $P < 0.05$; post hoc test: $P < 0.05$, Fig. 6D). Increased synaptic cleft width, as well as reduced postsynaptic

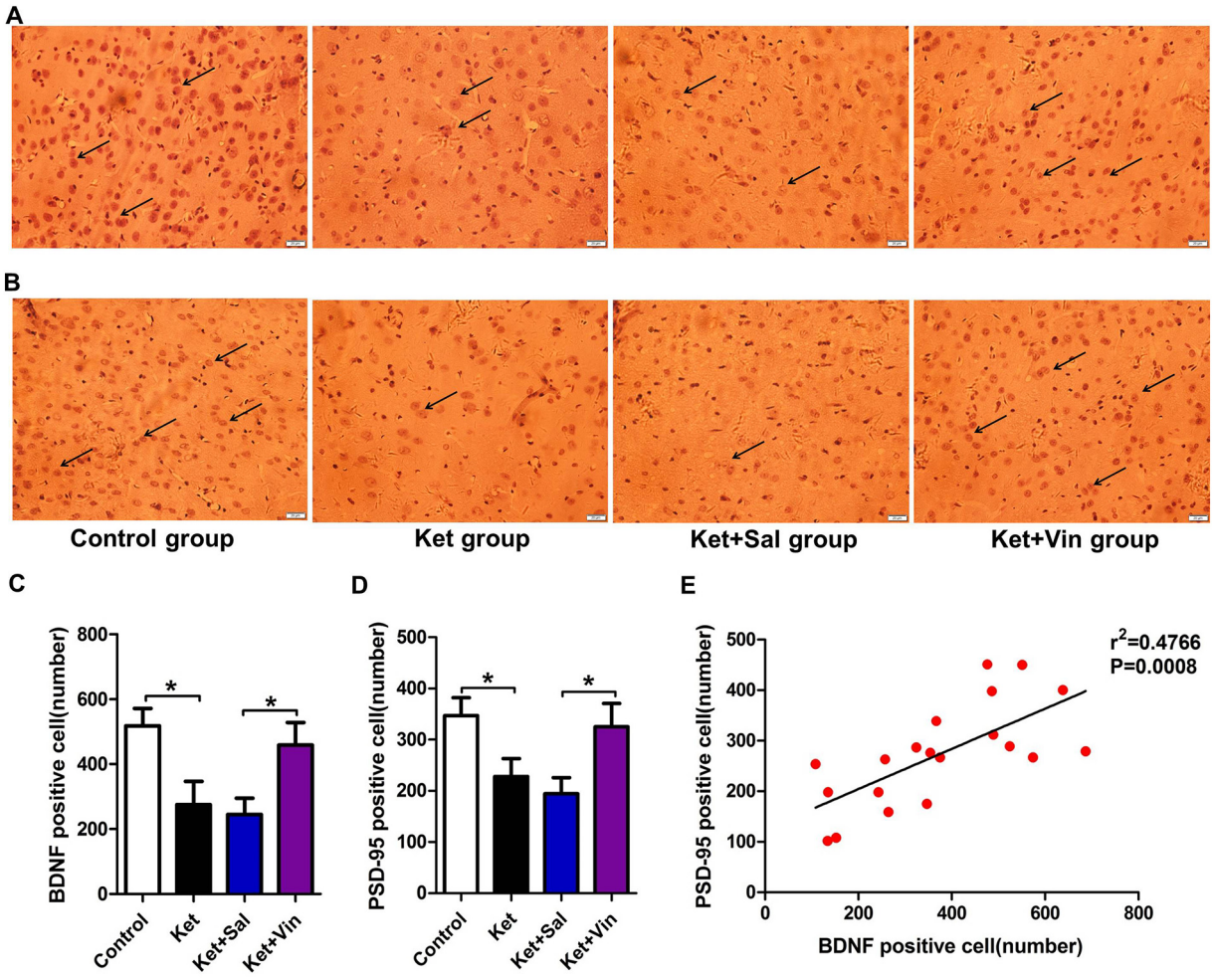

Fig. 3. Effect of vinpocetine on the changes of BDNF and PSD-95 positive cells in the PCC. (A) Representative images of BDNF positive cells in the PCC via photomicrographs (400×). (B) Representative images of BDNF positive cells in the PCC via photomicrographs (400×). (C) Number of BDNF positive cells in the PCC. (D) Number of PSD-95 positive cells in the PCC. (E) Correlation between PSD-95 positive cells and BDNF positive cells in the PCC. Data represent the mean ± S.E.M. ($n = 20$). *$P < 0.05$ was significant.

density thickness and synaptic curvature, was found in the Ket+Vin group compared to the Ket+Sal group (post hoc test: $P < 0.05$, Fig. 6B; post hoc test: $P < 0.05$ Fig. 6C; post hoc test: $P < 0.05$, Fig. 6D).

4. Discussion

The present study was designed to reveal the likely protective features and modulation of vinpocetine against ketamine-induced

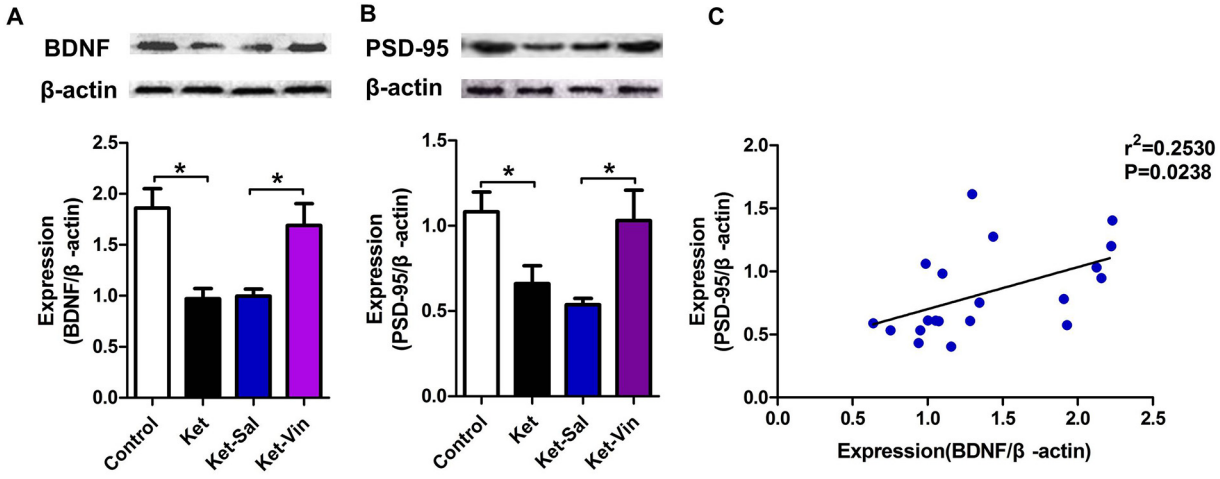

Fig. 4. Effect of vinpocetine on the changes of BDNF and PSD-95 protein levels in the PCC. (A) Quantitative analysis of BDNF protein expression in the PCC from the western blot. (B) Quantitative analysis of PSD-95 protein expression in the PCC from the western blot. (C) Correlation between PSD-95 protein level and BDNF protein level in the PCC. Data represent the mean ± S.E.M. ($n = 20$). *$P < 0.05$ was significant.

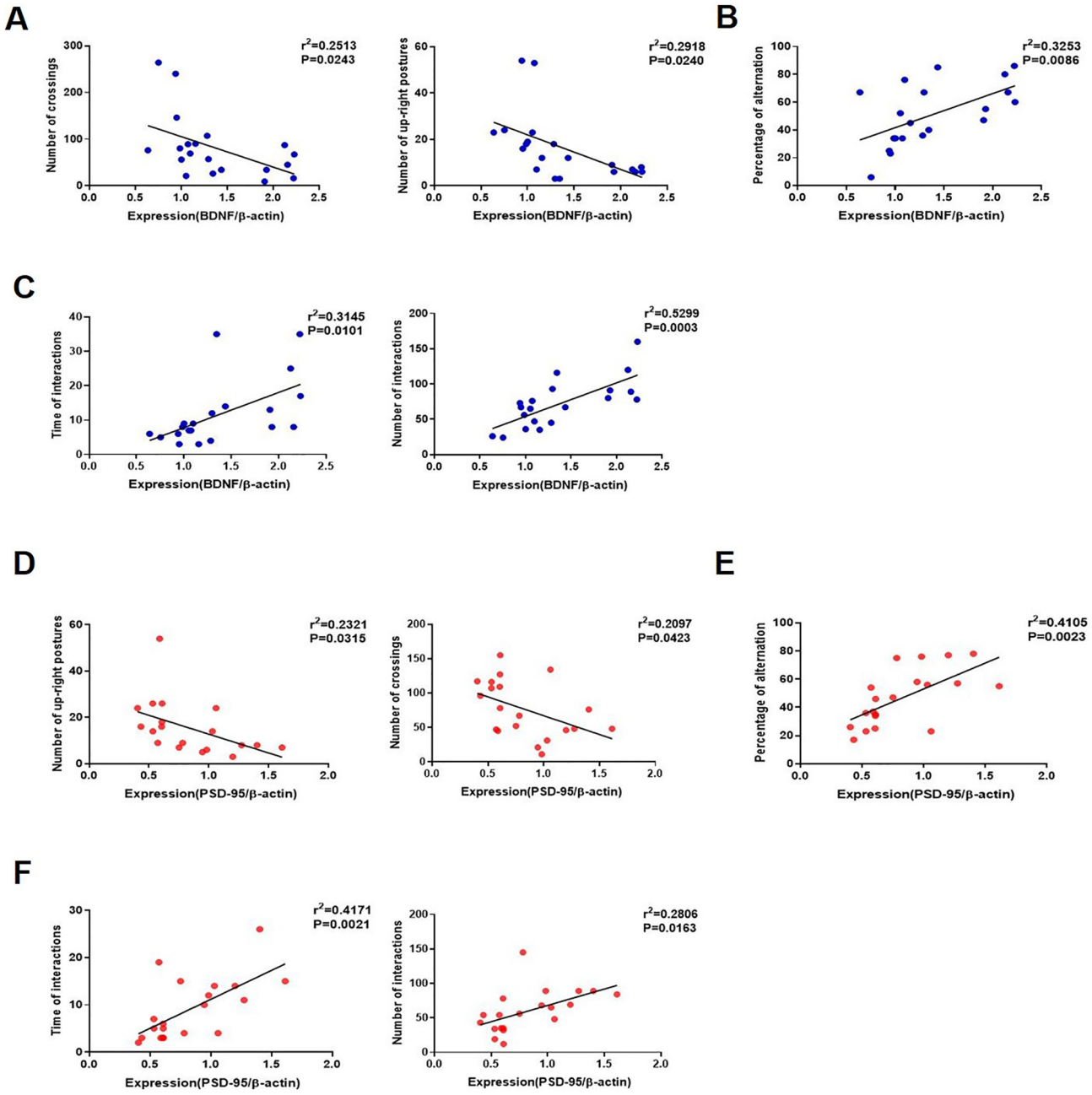

Fig. 5. Correlation between protein level and behavioral tests. (A) Correlation between BDNF protein level and OFT. (B) Correlation between BDNF protein level and Y-maze. (C) Correlation between BDNF protein level and SIT. (D) Correlation between PSD-95 protein level and OFT. (E) Correlation between PSD-95 protein level and Y-maze. (F) Correlation between PSD-95 protein level and SIT. Data represent the mean ± S.E.M. ($n = 20$). $^{*}P < 0.05$ was significant.

schizophrenia-like deficits in rats. The behavioral results demonstrated that after ketamine administration, the rats developed schizophrenia-like behaviors, including hyperlocomotion, short-term memory deficits, and social dysfunction. In contrast, vinpocetine administration for 7 consecutive days could reverse these symptoms. Vinpocetine could raise the levels of BDNF and PSD-95 in the PCCs of schizophrenic rats. A significant positive correlation between the levels of BDNF and PSD-95 proteins in the PCC was also discovered. Furthermore, there was a significant correlation between the protein level and behavior tests, which implies that vinpocetine can regulate the synaptic ultrastructure and alleviate ketamine-induced schizophrenia-like deficits in rats by up-regulating BDNF-related PSD-95 in the PCC.

N-methyl-D-aspartate receptors (NMDARs) have important and complex functions in psychotic disorders. Studies have found that NMDAR hypofunction contributes to the pathophysiology of schizophrenia [32,33]. Ketamine, an uncompetitive NMDA receptor antagonist, has important roles in synaptic plasticity processes [34]. Supporting evidence indicates that ketamine elicits negative cognitive symptoms and deficits in healthy men, similar to those found in schizophrenia [35,36]. In rodents, doses of ketamine (30 mg/ml) are commonly used to induced schizophrenia-like deficits [37,38]. Ketamine has been used to build the schizophrenic model in rodents and as a tool for the identification of potential therapies. In our study, after ketamine administration, the rats developed schizophrenia-like behaviors, including decreased locomotor behavior, an increase in the number of fecal grains and carding, increased alternation behavior, and social withdrawal. These are typical ketamine-induced schizophrenia-like behaviors, which indicate that the schizophrenic rat model was built effectively and successful.

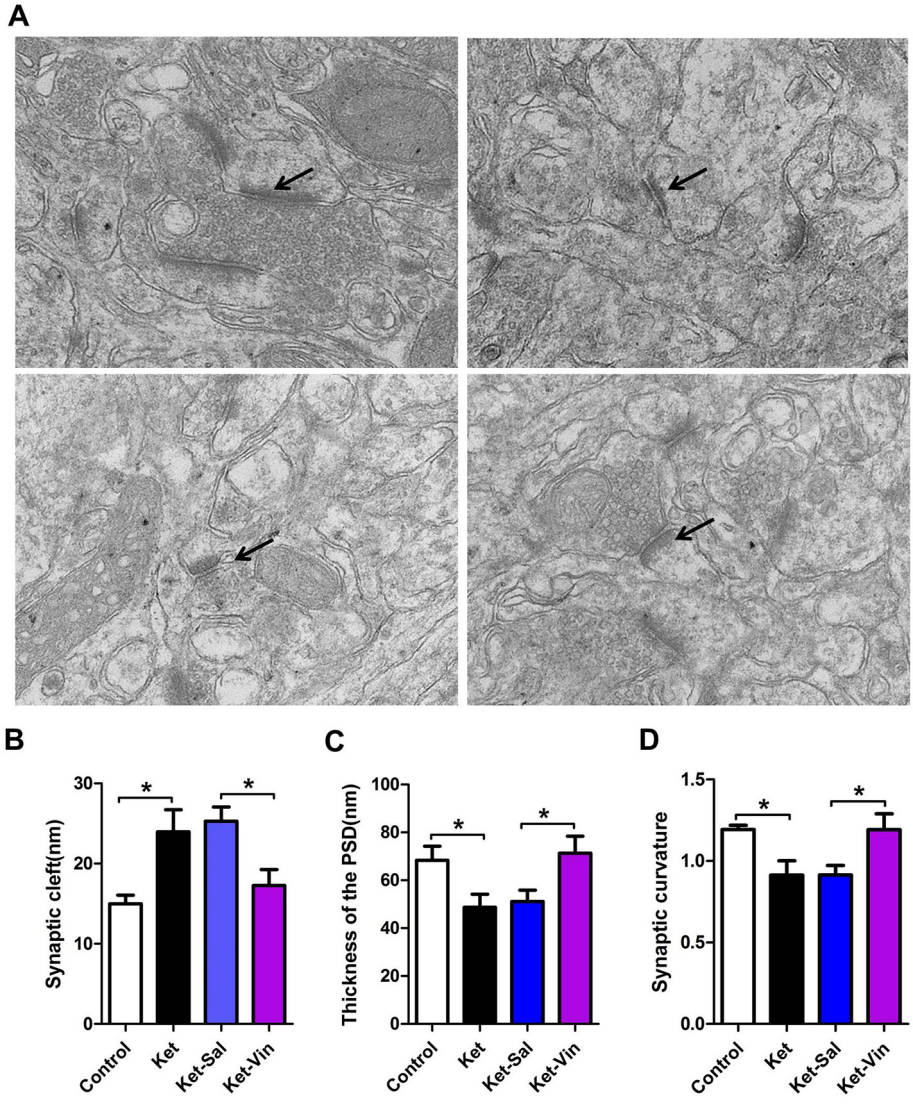

Fig. 6. Effect of vinpocetine on the change of synaptic ultrastructure in the PCC. (A) Representative images of the synaptic ultrastructure in the PCC under ×12,000 magnification. (B) Width of the synaptic cleft in the PCC. (C) Thickness of the PSD in the PCC. (D) Synaptic curvature in the PCC. Data represent the mean ± S.E.M. ($n = 20$). *$P < 0.05$ was significant.

BDNF is synthesized by neurons in the frontal cortex of rodents, and regulates synaptic density and cognition-like memory and social interaction [39], which are the two parameters that are significantly affected in patients with schizophrenia [40,41]. In this study, vinpocetine administration could reverse schizophrenia-like deficits induced by ketamine, and improve the level of BDNF protein in the PCC. These findings are in accordance with another study, which found that BDNF expression was improved by vinpocetine in the model of ketamine-induced schizophrenic rodents [13]. We speculate that ketamine could inhibit the activation of Ca^{2+}-CaM by decreasing the Ca^{2+} influx due to estrogen NMDAR [42]. We also speculate that calcium (Ca^{2+})-sensitive calmodulin (Ca^{2+}-CaM) can activate the phosphorylated cAMP-response element binding protein (CREB) by activating calmodulin kinase-II (CaMK-II), which could promote BDNF gene expression [43,44]. The underlying mechanism is that vinpocetine could increase cAMP and cGMP levels, thereby increasing the activity of cAMP and cGMP-dependent protein kinase A (PKA) and protein kinase G (PKG), which could promote the activation of P-CREB. P-CREB activation up-regulates the production of many genes, such as BDNF [13,45], and is a feasible molecular mechanism to explain why vinpocetine relieves schizophrenia-like behaviors.

Some studies have shown that BDNF activates the phosphatidylinositol 3-kinase (PI3K)-Akt pathway and triggers the synaptic delivery of PSD-95 via vesicular transport. BDNF-TrkB signaling also promotes the expression of PSD-95 in synapses [46,47]. The achieved results reveal that vinpocetine could improve the number of PSD-95 positive cells and the level of PSD-95 protein in the PCC, which is in accordance with a report by Bartolomeis [48]. In addition, the correlation analysis of BDNF and PSD-95 in this study demonstrated that there was a positive correlation between the levels of BDNF and PSD-95 in the PCC, which indicates that the vinpocetine could up-regulate PSD-95 expression by increasing the BDNF protein to alleviate the schizophrenia-like deficits.

PSD-95 is a key PSD molecule involved in the synaptic ultrastructure and contributes to activity-dependent synaptic plasticity, such as long-term potentiation, learning, and memory [49]. Furthermore, PSD-95 itself and its interaction with TrkB signaling have been implicated in schizophrenia [50]. Lower PSD-95 expression may be the key factor in arsenite-induced cognitive dysfunction, which causes the thickness of the PSD to increase significantly and the width of the synaptic cleft to decrease [51]. The results of the present study show that PSD thickness decreased significantly, and the width of the synaptic cleft, as well as the synaptic curvature, increased substantially after vinpocetine treatment.

The development of schizophrenia-like behaviors in this study has been correlated with changes in synaptic function, such as increased synaptic cleft width and reduced postsynaptic density thickness and

synaptic curvature. Studies have demonstrated that PSD-95 is involved in synaptic growth and plasticity, and strengthens synaptic transmission [52]. However, the effects of the synaptic cleft and synaptic curvature as they relate to synaptic functions in schizophrenia-like deficits, and the contact mechanism between the synaptic cleft and synaptic curvature with the PSD-95 protein, is still poorly understood. A curved synapse has more mitochondria than a straight synapse; therefore, we speculate that the curved synapse is more active, and the widening of the synaptic cleft could directly lead to the obstacle of synaptic neurotransmitter transmission, which might be why the rats exhibited schizophrenic symptoms [53]. The potential cause of the PSD-95 protein action on the synaptic cleft and synaptic curvature might be related to promoting synaptic growth and repair [54], which is an avenue for further research.

Despite the important role played by PSD-95 in mediating the ketamine-induced synaptic ultrastructure, BDNF protein expression could directly regulate synaptic density and synaptic growth [55]. In addition to BDNF, the signaling pathway also involved GSK-3β/β-catenin, which may also play a role in the possible mechanism of vinpocetine and contribute to the treatment efficiency that has been reported in the present study. This relationship will be explored further in future work.

5. Conclusions

This study provides novel research that analyses the influence of vinpocetine efficacy on a schizophrenic rat model induced by ketamine. This study indicated that vinpocetine could reverse the synaptic ultrastructure by up-regulating the PSD-95 protein levels via improving the expression of BNDF in the PCC to alleviate schizophrenia-like behaviors in the rat model induced by ketamine. Importantly, this may lead to a further understanding of the synaptic systems mediated by BDNF-related PSD-95 in schizophrenic therapies that include vinpocetine treatment.

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Ethical statement

All analyses were based on previous published studies; thus, no ethical approval or patient consent was required. In addition, all the authors declare no conflict of interest.

Declaration of competing interest

The authors have no financial interests or conflicts of interest to declare.

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