



# A Selective M<sub>1</sub> and M<sub>3</sub> Receptor Antagonist, Penehyclidine Hydrochloride, Exerts Antidepressant-Like Effect in Mice

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## Abstract

Recent studies indicate that anti-muscarinic receptor is a prospective strategy to treat depression. Although non-selective antagonist of muscarinic receptor scopolamine exhibits rapid and robust antidepressant-like effect, it still has various side effects including abuse risk. Penehyclidine hydrochloride (PHC) is a novel clinical anti-cholinergic drug derived from scopolamine in China, which selectively blocks M<sub>1</sub> and M<sub>3</sub> muscarinic receptor. Therefore, the objective of this study was to evaluate whether PHC would manifest antidepressant-like effects. Forced swim test (FST), tail suspension test (TST) and chronic unpredictable mild stress (CUMS) model of depression were explored to assess the antidepressant-like effect. Western blotting was further performed to detect the effects of PHC on the brain-derived neurotrophic factor (BDNF) signal cascade. Immunofluorescence was used to observe the activation of astrocyte. Moreover, different pharmacological inhibitors were applied to clarify the antidepressant-like mechanism. The results of the present experiments revealed that PHC decreased the immobility time of FST and TST in mice. In the CUMS model, PHC rapidly ameliorated anhedonia-like behavior (within 4 days), accompanying with the enhanced expression of BDNF and phosphorylation of extracellular signal-related kinase 1/2 (ERK1/2) in the hippocampus. In addition, blockade of the BDNF release by verapamil and activation of its Trk B receptor by K252a, rather than inhibition of opioid system by naloxone or sigma receptor by BD1047, abolished the antidepressant-like effects of PHC in mice. The findings suggest that PHC, an anti-muscarinic drug in clinical use, elicits rapid onset antidepressant-like effect, shedding light on the development of new antidepressants.

**Keywords** Penehyclidine hydrochloride · Muscarinic receptor · Depression · Antidepressant-like effect

## Introduction

Major depressive disorder (MDD) is a common recurring, debilitating mental illness that causes high morbidity even suicide. Since the low tolerability, slow onset, and low rates of efficacy of current antidepressants considered to act based

on the monoamine hypothesis, it is urgent need to uncover new biological mechanisms and therapies surmounting these drawbacks. Over the past decade, several promising antidepressants involved in glutamatergic system have been suggested as fast-acting and efficacious treatments, such as the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine and its metabolite (2R,6R)-hydroxynorketamine [1–3]. In addition, the cholinergic system has also been implicated in the development of antidepressants, with crucial evidence that the muscarinic acetylcholine (ACh) receptor antagonist scopolamine manifests rapid antidepressant-like effects [4–6].

Scopolamine is commonly used in the treatment of motion sickness, pregnancy-related vomiting and organic phosphorus poisoning [7]. Clinical studies reveal that the robust antidepressant effects of scopolamine are established 3 to 5 days following drug administration [6, 8]. The neural mechanisms underlying the antidepressant effects of ketamine still remain to be clearly elucidated.

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Although scopolamine is considered to non-selectively block  $M_1$ – $M_5$  muscarinic receptors, recent studies have demonstrated that the antidepressant-like effect appears to be mediated by  $M_1$  muscarinic receptors, which initiates the downstream molecular and cellular actions showing similarities with ketamine [9, 10]. For example, like ketamine, scopolamine rapidly increases transient burst of glutamate, mechanistic target of rapamycin complex 1 (mTORC1) signaling, and the activity-dependent release of brain-derived neurotrophic factor (BDNF) [4, 11]. However, another typical muscarinic receptors atropine lacks the antidepressant efficacy, particularly since atropine has poor penetration across the blood–brain barrier in its therapeutic dosage. Moreover, given the side effects including abuse and spatial learning and memory deficits caused by scopolamine [12], it still needs to search alternative muscarinic receptor antagonists as potential antidepressants.

Penehyclidine hydrochloride (PHC) or 3-(2'-phenyl-2'-cyclopentyl-2'-hydroxyl-ethoxy) quinuclidine is an anti-cholinergic drug derived from scopolamine in China, which selectively blocks  $M_1$  and  $M_3$  muscarinic receptor and passes through the blood–brain barrier [13, 14]. It is widely used in the clinical setting as a pre-anesthetic medication and a reversal agent in cases of organic phosphorus poisoning, conferring substantial advantages compared with other anticholinergic agents [15]. Therefore, based on the previous finding that PHC exerts robust pharmacologic properties by blocking muscarinic receptors, we hypothesized whether PHC would manifest antidepressant-like effects. The results of the present experiments reveal that PHC not only exerts antidepressant-like activities in the forced swimming test (FST) and tail suspension test (TST), but relatively fast ameliorates chronic unpredictable mild stress (CUMS)-induced depressive-like symptoms (within 4 days).

## Materials and Methods

### Experimental Animals

Male ICR mice (22–25 g, 8–10 weeks old) used in the study were obtained from the Animal Experiment Center of Xuzhou Medical University. The animals were housed five per cage under standard conditions (12:12 h light-dark cycle;  $23 \pm 1$  °C ambient temperature;  $55 \pm 10\%$  relative humidity) and allowed to acclimatize for a couple of days before use in all experiments. The experimental procedures were approved by the animal welfare committee of Xuzhou Medical University and followed the guidance of the NIH for the Care and Use of Laboratory Animals.

### Drugs and Administration

Penehyclidine hydrochloride (PubChem CID: 177923) was purchased from Chengdu List Pharmaceutical CO., Ltd. (Chengdu, China). Ketamine was purchased from Hengrui Medicine co., LTD. Verapamil hydrochloride and k252a (1 mM solution in dimethyl sulfoxide) were the products of Sigma-Aldrich (Saint Louis, MO, USA). Penehyclidine hydrochloride (0.3–3 mg/kg), verapamil hydrochloride (5 mg/kg) and ketamine (10 mg/kg) was dissolved in physiological saline and administered intraperitoneally (i.p.). k252a (0.5 µg/mouse) was prepared in 0.01 M phosphate buffer and administered intracerebroventricularly (i.c.v.) under anesthesia according to a previously described method [16].

### Forced Swimming Test (FST)

In the FST, mice were forced to swim individually in an acrylic cylinder (height: 20 cm, diameter: 10 cm) filled with water (depth: 15 cm) at a temperature of  $25 \pm 1$  °C for 6 min. The duration of immobility was recorded during the last 4 min of a 6 min observation period using ANY-maze software (Stoelting, Wood Dale, IL, USA). A mouse was judged to be immobile when it remained floating in an upright position with the head above the water level [17].

### Tail Suspension Test (TST)

The animals were suspended individually on a retort stand, placed 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded during the 6 min test using ANY-maze software. An animal was considered to be immobile when it did not show any movement of the body and hangs passively [18, 19].

### Open Field Test (OFT)

The open field arena (30 × 30 × 15 cm) was made of black plexiglas and a black bottom subdivided into 6 × 6 cm squares. The open field was used to evaluate the exploratory activity of the animal during 5 min. The observed parameters were the total distance and center time using ANY-maze software.

### Sucrose Preference Test

This test was performed as described previously [20]. Briefly, mice were placed individually into a two-bottle (1% sucrose solution or water), free-choice cage. After

adaptation, mice were deprived of water and food for 24 h, and then were allowed free access to the two bottles over a 1 h period. The sucrose preference (%) = sucrose consumption / (sucrose consumption + water consumption).

### Chronic Unpredictable Mild Stress (CUMS)

The mice were daily exposure to two or three of the following stressors in a random order over a 4-week period: crowd (15 mice) or single mice in a cage (24 h), cage shaking (2 h at 150 rpm), reversed light/dark cycles (24 h), placement in cold room (4 °C, 1 h), restraint in small tubes (50 ml centrifugal tube, 2 h), 45° cage tilt (14 h), wet cage (200 ml water in 100 g sawdust, 24 h), flashing light (120 flashes/min, 6 h), white noise (92 dB, 1500 Hz, 3 h), food deprivation for 24 h, water deprivation for 24 h, placement in an empty cage for 24 h. The same stressor was not carried out on consecutive days. The sucrose preference test, TST, FST and OFT were used to evaluate the depression-like behaviors caused by the CUMS.

### Western Blotting

Hippocampus tissues were lysed in RIPA buffer (Beyotime, Shanghai, China) for 30 min on ice. The protein concentrations were determined with a commercial kit (Beyotime, Shanghai, China). 40 µg of protein from each sample was separated by SDS-polyacrylamide gel electrophoresis (10% or 12%) and then transferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA). The membranes were blocked using 5% skim milk, and then subsequently probed with anti-GFAP (1:1,000, Cell Signaling Technology, Boston, MA, USA), anti-pERK1/2 (1:1,000, Cell Signaling Technology, Boston, MA, USA), anti-ERK1/2 (1:1,000, Cell Signaling Technology, Boston, MA, USA), anti-BDNF (1:1,000, Abcam, Cambridge, UK;) and anti- $\alpha$ -Tubulin (1:10,000, Sigma-Aldrich, St Louis, MO, USA) antibodies. Following incubation with the corresponding secondary antibodies (1:15,000, Odyssey, USA), the membranes were scanned, and the densitometry of bands was quantified with Odyssey Infrared Imaging System (Odyssey Sa, USA). Each experiment was replicated at least three times.

### Immunofluorescence Staining

Mice were transcardially perfused with ice-cold normal saline, followed by 4% paraformaldehyde (PFA) in 0.01 M phosphate buffered saline (PBS). After 12 h post-fixation in 4% PFA, the brain was immersed in a 30% sucrose solution in PBS overnight, and then was cut to a thickness of 15 µm. The slides were permeabilized with 0.01 M PBS containing 0.1% Triton X-100. After blocking with 10% goat serum, the slides were incubated with primary antibodies rabbit

anti-GFAP (1:300, Cell Signaling Technology, Boston, MA, USA). Alexa Fluor 448-conjugated goat anti-mouse antibody (1:1000, Abcam, Cambridge, MA, USA) were used as secondary antibody. Nuclei were stained with DAPI (Sigma-Aldrich, St Louis, MO, USA) before slides were viewed using a fluorescence microscope (Leica, Solms, Germany).

### Statistical Analyses

Data were expressed as mean  $\pm$  SD. One-way or two-way ANOVA followed by Tukey's multiple comparison test was performed using the GraphPad software Version 8.0 (GraphPad Software, Inc. La Jolla, CA, USA). Statistically significant level was set at  $P < 0.05$ .

## Results

### Penehyclidine Hydrochloride Reduces the Immobility Time in the FST and TST

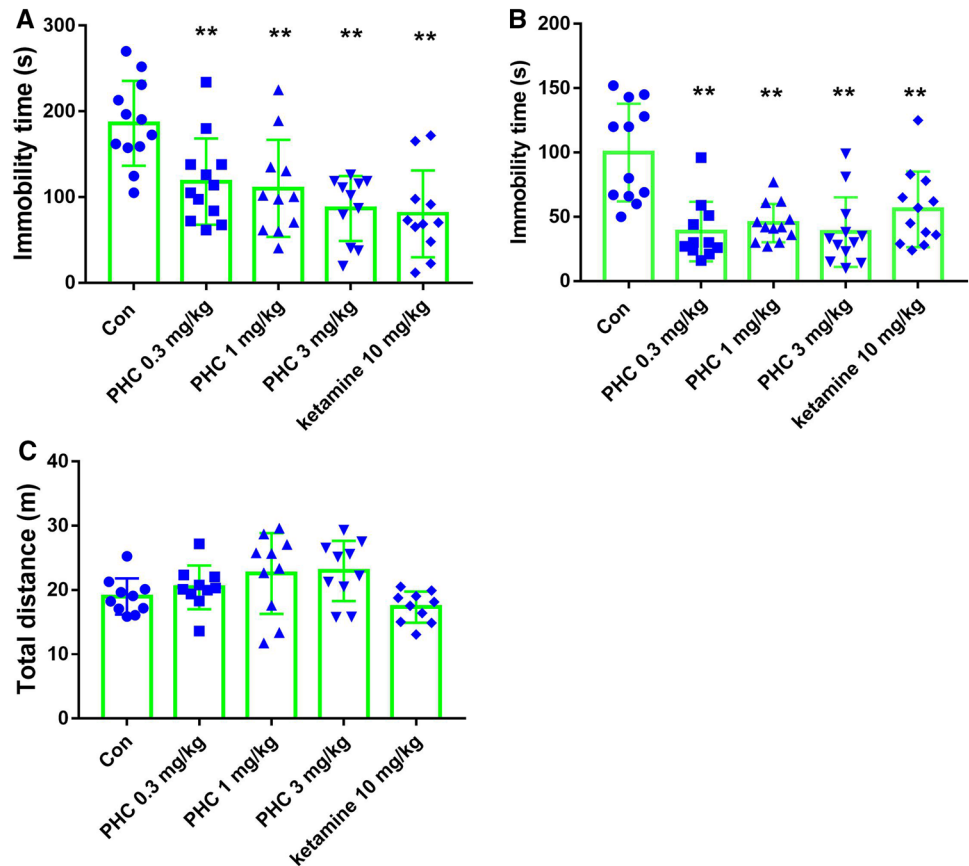
TST and FST are widely used to detect the potential antidepressant activity of different compounds. Here, we first evaluated the antidepressant-like activity of PHC in TST and FST assay using ketamine (10 mg/kg) as the positive control. As shown in the Fig. 1a, one-way ANOVA revealed a significant main effect of treatment [ $F(4, 52) = 8.45, P < 0.0001$ ]. Subsequent posthoc analysis indicated that similar with ketamine, PHC administration (0.3–3 mg/kg, i.p., 30 min) decreased the immobile time in the TST compared with the control group ( $n = 10–12$  in each group,  $P < 0.01$  respectively). We then detected the antidepressant effects of PHC in the FST. Similar to the TST results (Fig. 1b), one-way ANOVA revealed that there were significant difference between treatment groups: [ $F(4, 54) = 10.41, P < 0.0001$ ]. Post-hoc analysis by Tukey's test showed that injection of PHC (0.3–3 mg/kg, i.p.) significantly increased immobility time in the FST compared with control group ( $P < 0.01, n = 11–12$  in each group).

Figure 1C showed the effect of PHC on the locomotor activity of mice measured by the open field test for 5 min. The results showed that there was no significant difference between treatment groups. Thus, the behavioral tests indicated potential antidepressant-like activity of PHC.

### PHC Treatment Rescues the CUMS-Induced Depressive Symptoms

To further confirm the antidepressant effect and characterize the onset of PHC, we employed CUMS, which is currently the most commonly used, reliable, and effective rodent model of depression [21]. Anhedonia represents a core symptom of depression, which often measured by

**Fig. 1** Effects of penehyclidine hydrochloride (PHC) on behavioral action in the tail suspension test (TST) and forced swimming test (FST). **a** 30 min before TST, male ICR mice were injected i.p with saline, 0.3–3 mg/kg PHC and 10 mg/kg ketamine, respectively. **b** 30 min before FST, male ICR mice were injected i.p with saline, 0.3–3 mg/kg PHC and 10 mg/kg ketamine, respectively. **c** Effects of PHC and ketamine on total distance in the open field test. The data were expressed as mean  $\pm$  SD and analyzed with one-way ANOVA analysis. **\*\*** $P < 0.01$  versus control group



the preference for sucrose intake in depressive-like mice. As shown in Fig. 2a, one-way ANOVA revealed that there was significant difference between treatment groups: [F (4, 51) = 27.15,  $P < 0.0001$ ]. Post-hoc analysis by Tukey's test showed that injection of PHC (1 mg/kg, i.p.) for consecutive 4 days, but not 1 day, significantly reversed decreased sucrose preference compared with CUMS group ( $P < 0.01$ ,  $n = 11$ –12 in each group), suggesting a potential rapid onset of antidepressant activity. Similarly, as shown in Fig. 2b, c, one-way ANOVA revealed that there were significant differences between treatment groups: FST [F (4, 45) = 8.457,  $P < 0.001$ ], TST [F (4, 45) = 3.921,  $P < 0.01$ ]. Post-hoc analysis by Tukey's test showed that PHC (1 mg/kg, i.p., 4 days) significantly increased immobility time in the FST ( $P < 0.01$ ,  $n = 10$  in each group) and TST ( $P < 0.01$ ,  $n = 10$  in each group).

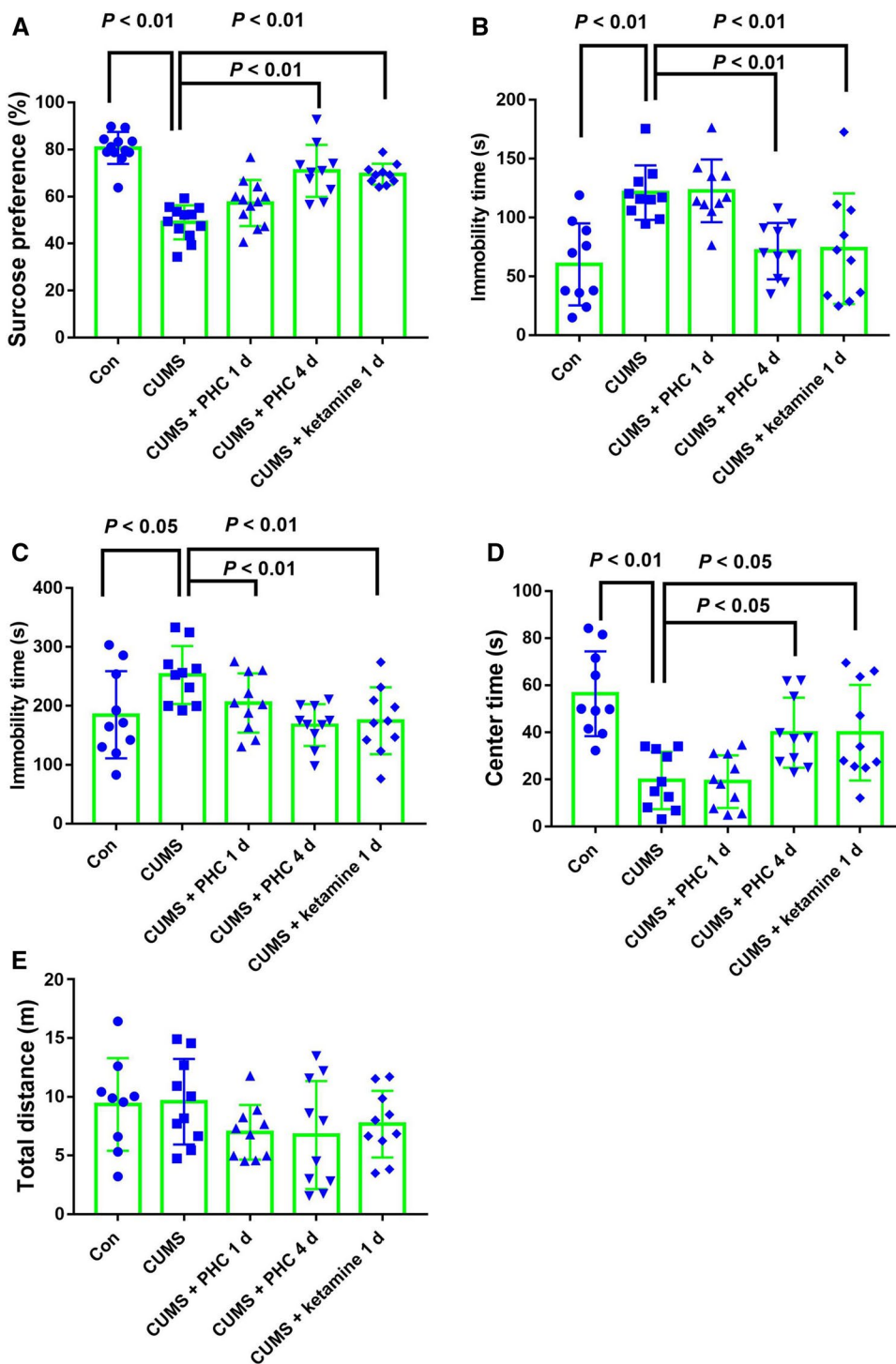
Depression is often accompanied by anxiety. In this study, as shown in Fig. 2d, e, CUMS caused decreases center time in the open field [F (4, 45) = 10.14,  $P < 0.001$ ; CUMS vs. control,  $P < 0.01$ ], and PHC treatment (1 mg/kg, i.p., 4 days) significantly reversed detrimental effects of CUMS ( $P < 0.01$ ), producing significant anxiolytic effects. The results revealed that PHC might possess promising antidepressant-like and anxiolytic-like effects.

### PHC Ameliorates the Deficit of BDNF-ERK1/2 Signal and Abnormal Glia Activation in the CUMS-Induced Depression

Since BDNF is considered an attractive candidate for predicting antidepressant therapeutic response [18], we next detected the effect of PHC on the BDNF expression and its related molecular signal extracellular regulated protein kinases 1/2 (ERK1/2) in the CUMS model. As shown in Fig. 3, one-way ANOVA revealed that there was significant difference between treatment groups: [for BDNF, F (3, 8) = 19.9,  $P < 0.001$ , for phospho-ERK1/2, F (3, 8) = 19.44,  $P < 0.001$ ]. Post-hoc analysis by Tukey's test showed that injection of PHC (1 mg/kg, i.p.) for consecutive 4 days significantly reversed decreased BDNF expression and phospho-ERK1/2 compared with CUMS group ( $P < 0.01$  respectively,  $n = 3$  in each group), revealing its potential antidepressant activity.

As the most abundant cell type in the brain, the deficit of astrocyte function is considered to participate in the pathological process of depression [22]. Additionally, astrocyte is also an important source of BDNF. Herein, to further confirm the antidepressant like effect of PHC, taken advantage of immunofluorescence for glial fibrillary acidic protein

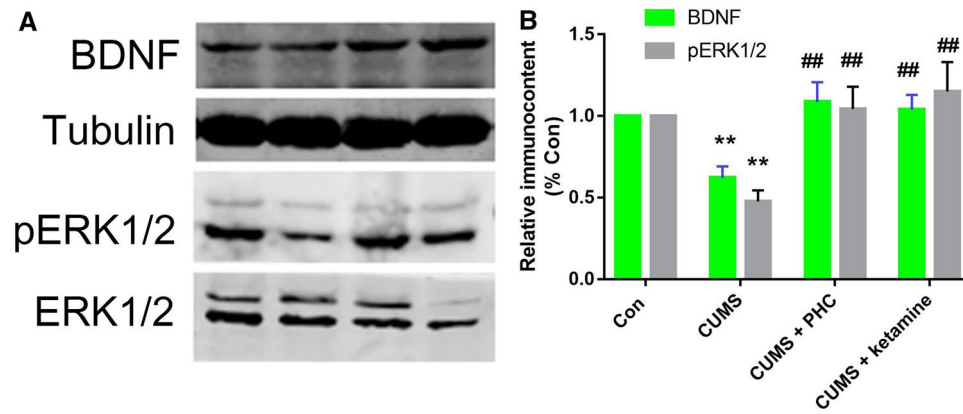
**Fig. 2** Effects of penehyclidine hydrochloride (PHC) on behavioral action in the chronic unpredicted mild stress (CUMS) model. Mice were exposure to CUMS as described in methods, and randomly divided into followed groups: Control, CUMS, CUMS + PHC 1 day, CUMS + PHC 4 days, CUMS + ketamine 10 mg/kg. **a** the sucrose consumption, **b** the immobility time of FST, **c** the immobility time of TST, **d** the time that mice stayed in the central field, **e** total distance in the locomotion test. The saline or drugs were administered i.p. once daily until the relief of depressive behaviors. The data were expressed as mean  $\pm$  SD and analyzed with one-way ANOVA analysis



(GFAP, a marker of astrocyte), we investigated whether PHC could improve the abnormal astrocyte activation in depression models. As shown in Fig. 4, CUMS caused the inhibition of astrocyte activation. However, the treatment of PHC for consecutive 4 days significantly increased the astrocyte activation. Taken together, these data indicated that PHC could restore depression symptom associated with restoration of BDNF signal.

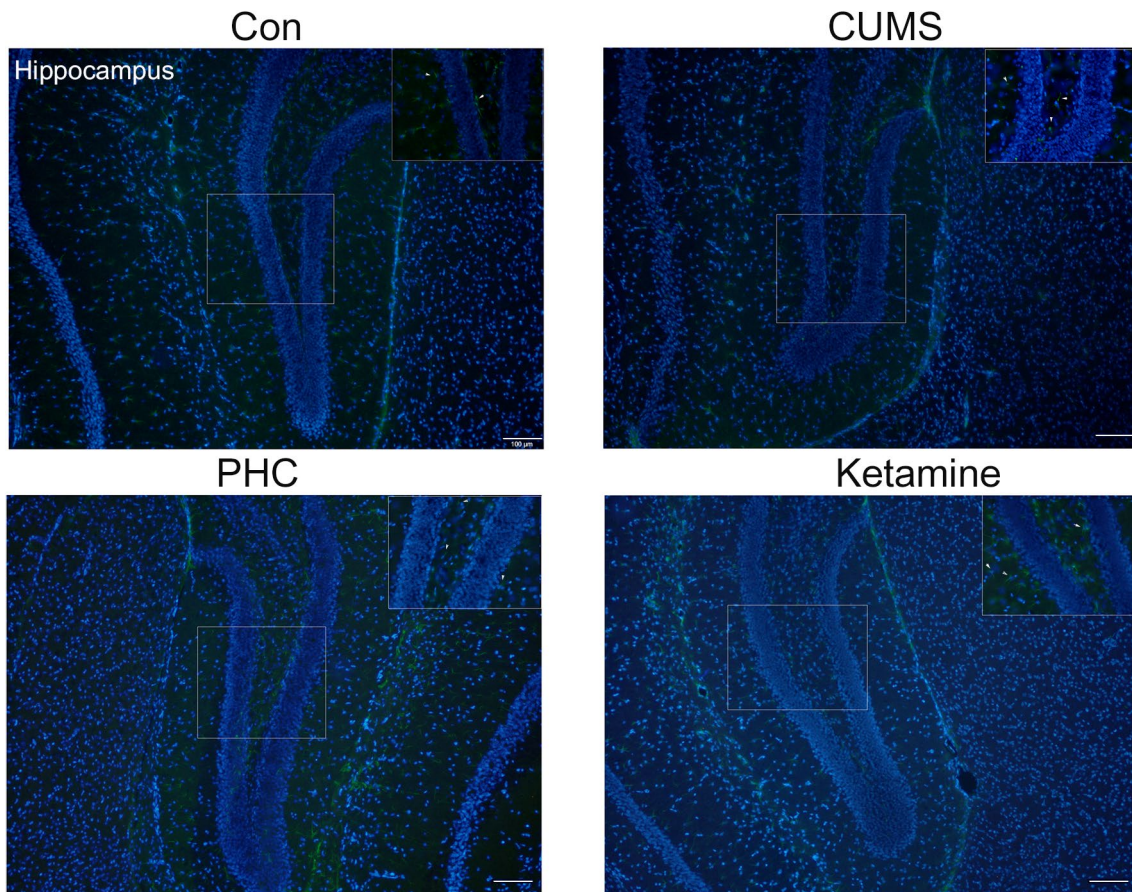
**Blockade of the BDNF Signal Suppresses the Antidepressant-Like Effects of PHC**

To investigate the potential mechanism underlying the antidepressant effects of PHC, firstly, in the test for single injection of PHC (1 mg/kg, 30 min, i.p.), K252a (an inhibitor of BDNF receptor), verapamil (a potent inhibitor of BDNF release), naloxone (an antagonist of opioid



**Fig. 3** Effects of penehyclidine hydrochloride (PHC) on the BDNF-ERK1/2 signal pathway in the CUMS-induced mice. Animals were subjected to CUMS as described in methods. Brain tissues were rapidly dissected and hippocampus were collected and kept for west-

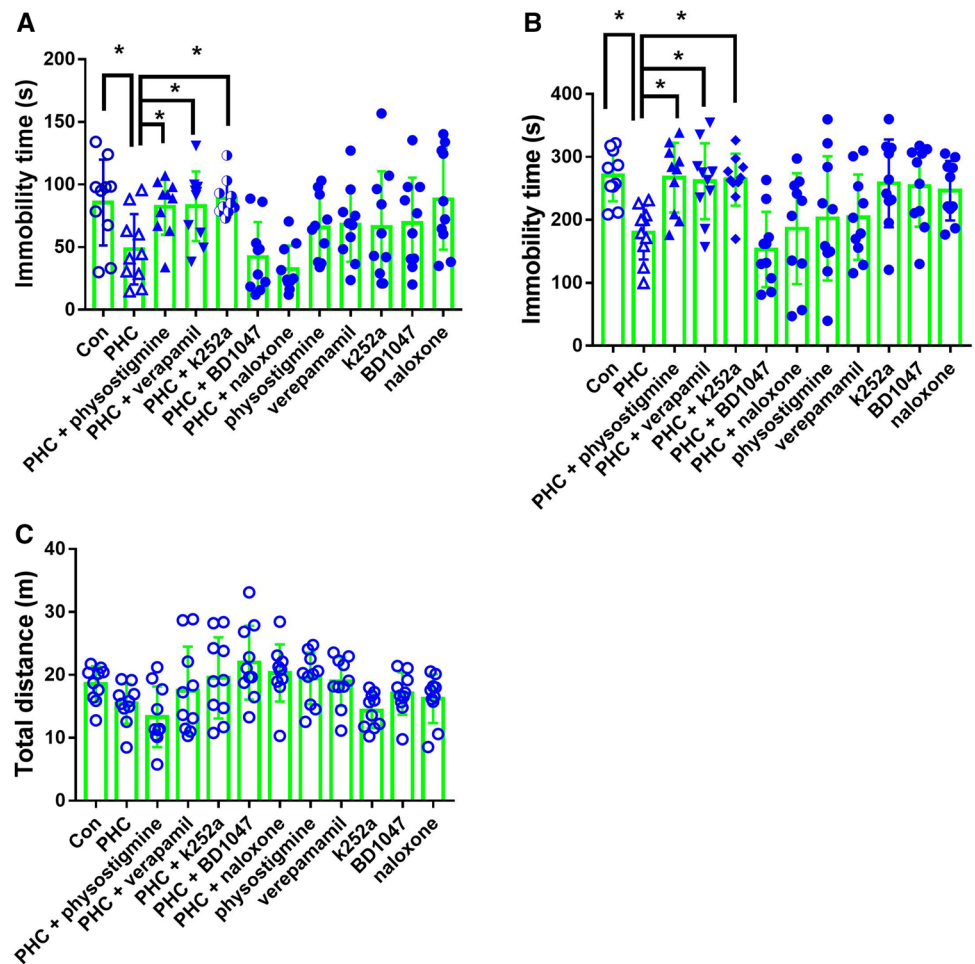
ern blotting assays as described in methods. The levels of phosphorylation of ERK1/2 were normalized by total ERK1/2. Data were expressed as mean  $\pm$  SD and analyzed with one-way ANOVA analysis. \*\* $P < 0.01$  versus control group, ## $P < 0.01$  versus CUMS group



**Fig. 4** Effects of penehyclidine hydrochloride (PHC) on the activation of astrocyte in the CUMS-induced mice. Morphological changes of astrocyte using immunofluorescence staining in the hippocampus. GFAP was marked in green, nuclei were marked in blue from a

DAPI staining. In the CUMS group, the arrow showed decreased the branch and volume of astrocyte. In the control and treatment group, the arrow showed normal branch and volume of astrocyte. The scale bar = 100  $\mu$ M (Color figure online)

**Fig. 5** Effects of various inhibitors on the single administration of PHC-induced antidepressant-like behaviors. Single injection of PHC (1 mg/kg, 30 min, i.p.), K252a (an inhibitor of BDNF receptor, 24 h before PHC), verapamil (a potent inhibitor of BDNF release, 30 min before PHC), naloxone (an antagonist of opioid receptor, 30 min before PHC), BD1047 (an antagonist of sigma receptor, 30 min before PHC), and physostigmine (an acetylcholinesterase inhibitor to increase Ach levels, 1 h before PHC) were employed in this test. **a** The immobility time of FST, **b** the immobility time of TST, **c** total distance in the locomotion test. The data were expressed as mean ± SD and analyzed with two-way ANOVA analysis



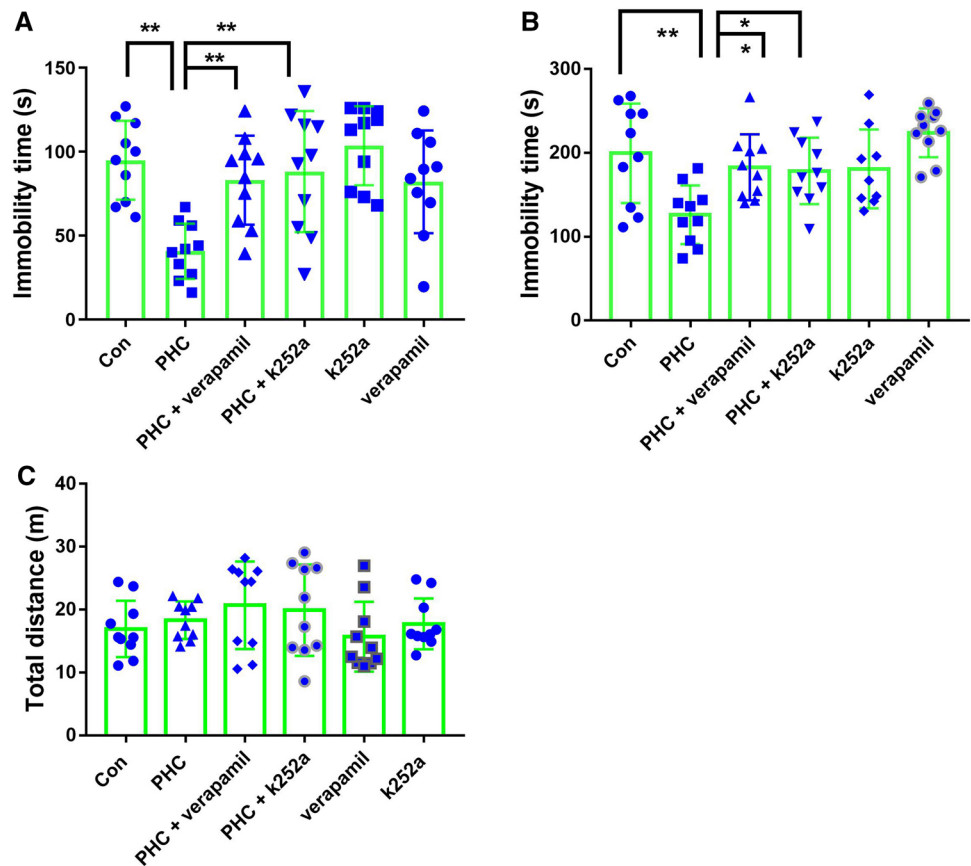
receptor), BD1047 (an antagonist of sigma receptor), and physostigmine (an acetylcholinesterase inhibitor to increase Ach levels) were employed [4]. In the FST (Fig. 5a), there was interaction effect between PHC and physostigmine treatment [F (1, 36)=9.27,  $P < 0.01$ ], verapamil treatment [F (1, 36)=7.504,  $P < 0.01$ ], k252a treatment F (1, 36)=8.693,  $P < 0.01$ ], but not BD1047 treatment [F (1, 36)=0.08963,  $P = 0.7664$ ] or naloxone treatment [F (1, 36)=0.6786,  $P = 0.4155$ ]. In the TST, as shown in Fig. 5B, the two-way ANOVA revealed a significant interaction between PHC and physostigmine treatment [F (1, 36)=14.83,  $P < 0.001$ ], verapamil treatment [F (1, 36)=18.65,  $P < 0.0001$ ], k252a treatment [F (1, 36)=9.279,  $P < 0.01$ ], but not BD1047 treatment [F (1, 36)=0.2538,  $P = 0.6175$ ] or naloxone treatment [F (1, 36)=0.8607,  $P = 0.3597$ ].

Post-hoc analysis by Tukey’s test showed that physostigmine blocked the PHC-induced decrease in the immobile time in the experiments of TST ( $P < 0.05$ ) and FST ( $P < 0.05$ ). It suggested that as an antagonism of Ach receptor, the antidepressant like effect of PHC indeed involved cholinergic system. Moreover, k252a (0.5 µg/mouse, i.c.v., pretreatment for 1 day) and verapamil (5

mg/kg, i.p., pretreatment for 30 min) almost completely reversed the antidepressant like effect of PHC ( $P < 0.05$  respectively), without change in locomotor activity (Fig. 5). However, neither BD1047 nor naloxone attenuated the PHC-decreased immobile time in TST and FST, pointing out that the antidepressant effect of PHC might be independent of sigma receptor or opioid receptor.

To further confirm whether BDNF signal could contribute to the antidepressant activities of PHC, the mouse were injected PHC for consecutive 4 days (1 mg/kg, i.p.) in the following study, which pretreated by k252a (0.5 µg/mouse, i.c.v., once daily for 2 days) and verapamil (5 mg/kg, i.p., once daily for 4 days). Results showed interaction effects of treatment in TST [for verapamil: F (1, 36) = 4.930,  $P < 0.05$ ; for k252a: F (1, 36) = 9.865,  $P < 0.01$ ] and FST [for verapamil: F (1, 36) = 12.37,  $P < 0.01$ ; for k252a: F (1, 36) = 5.626,  $P < 0.05$ ] (Fig. 6). Post-hoc analysis by Tukey’s test revealed that either k252a or verapamil weaken the PHC-decreased immobile time in TST and FST, showing participation of BDNF signal in the PHC-produced antidepressant effect.

**Fig. 6** Effects of verapamil and k252a on subchronic administration of PHC-induced antidepressant-like behaviors. Consecutive injection of PHC (1 mg/kg, 4 d, i.p.), K252a (once daily for 2 days) and verapamil (5 mg/kg, i.p., once daily for 4 days) were employed in this test. **a** The immobility time of FST, **b** the immobility time of TST, **c** total distance in the locomotion test. The data were expressed as mean  $\pm$  SD and analyzed with two-way ANOVA analysis



## Discussion

Recently, preclinical studies have confirmed that PHC possesses neuroprotective effects on various diseases such as neuroinflammation or ischemia injury [23–25]. One of the major contributions of the present study is the identification of the antidepressant-like effect of PHC, an antagonist of the muscarinic Ach receptor. To the best of our knowledge, this study demonstrated for the first time that PHC decreased the immobility time of mice in the FST and TST, as well as attenuated the anhedonia in the CUMS caused- depression model. Furthermore, this study also suggested that BDNF signal pathway may associate with the antidepressant effect of PHC.

Hyperactivity of brain cholinergic systems in the depression is gaining people's attention and regarded to contribute to the pathophysiology of depression. For example, a human imaging study suggests elevated ACh levels in actively depressed patients [26]. In addition, as an inhibitor of acetylcholinesterase increasing extracellular ACh level, physostigmine could trigger depressive symptoms in normal individuals and animals in previous research or our study [27]. The cholinergic receptor family is known to be divided into muscarinic and nicotinic receptors, and cholinergic antagonist specifically M

receptor blocker scopolamine can induce antidepressant-like responses. Interestingly, in this study, we described a clinical M receptor antagonist PHC as a novel potential fast-onset antidepressant (within 4 days). Despite PHC can't act as fast as ketamine in this study or scopolamine in previous research, the strengths of the former lie in safety, rare side effects and without addiction in clinical, suggesting the possibility of PHC as an alternative antidepressant instead of scopolamine even ketamine.

Muscarinic receptors consist of five distinct subtypes ( $M_1$ – $M_5$ ), which are expressed throughout the body. PHC is known to selectively act on  $M_1$  and  $M_3$ , and penetrate the blood–brain barrier, exerting potent peripheral and central anticholinergic effects. Given the insufficient affinity for  $M_2$  receptor, it is beneficial that PHC has no  $M_2$  receptor-associated cardiovascular side effects such as increased heart rate. On the other hand, blockage of  $M_1$  receptor recently has become attractive mechanism accounting for the rapid antidepressant actions of scopolamine [9, 10]. Although the exact location and functional role of all these subtypes has to date not been fully elucidated, the expression of  $M_1$  receptor is generally higher than that of  $M_3$  receptor in hippocampus, neocortex, amygdala [28]. On the mentioned above, it seems  $M_1$  receptor may mediate the antidepressant action of PHC but still need further experimental verification.



BDNF signal contributes to therapeutic effect of most rapid antidepressants and common antidepressants. Take the case of the muscarinic receptor blocker scopolamine, one hypothesis is that inhibition of muscarinic receptors on GABAergic interneurons (M1 receptors in particular) lead to disinhibition of pyramidal neurons, then increase glutamate transmission to activate  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, finally initiate BDNF release and its Trk B receptors activation [9]. It is worth noting that the release of BDNF also requires activation of L-type voltage-dependent calcium channels (VDCCs) [29]. Similarly, in this study, both the blocker of L-type VDCCs (verapamil) and the inhibitor of Trk B receptor (k252a) prevented the PHC-induced behaviors in FST and TST, suggesting the key role of BDNF signal in the antidepressant-like effect of PHC (Figs. 5, 6). Besides, studies have shown that sigma receptors or opioid receptors take part in the antidepressant effect of ketamine and several antidepressants [20, 30, 31]. However, the antagonists of sigma receptor and opioid receptors have no effect on the antidepressant-like effect of PHC (Fig. 5). It suggested the regulation of BDNF signal of PHC might not require opioid system or sigma receptors activation.

Collectively, these data demonstrated that PHC is a potential rapid onset antidepressant compound associated with restoration of BDNF signal. In light of pharmacological properties and fewer side effects of PHC in clinical, these findings may shed light on the development of new antidepressants.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare no conflict of interest.

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