

# Dihydromyricetin exerts a rapid antidepressant-like effect in association with enhancement of BDNF expression and inhibition of neuroinflammation

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Received: 20 June 2017 / Accepted: 11 October 2017 / Published online: 20 October 2017  
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## Abstract

**Rationale** Major depressive disorder (MDD) is a highly prevalent illness that affects large populations across the world, and increasing evidence suggests that neuroinflammation and levels of brain-derived neurotrophic factor (BDNF) are closely related to depression. Dihydromyricetin (DHM) is a kind of flavonoid natural product that has been reported to display multiple pharmacological effects, including anti-inflammatory and anti-oxidative properties, and these may contribute to ameliorate MDD.

**Objective** This study investigated the effect of DHM on depression-related phenotypes in various experimental animal models. **Methods** The antidepressant-like effect of DHM was validated via depression-related behavioral tests in naïve male C57BL/6 mice, as well as in the acute lipopolysaccharide-induced mouse model of depression. The chronic unpredicted mild stress (CUMS) mouse model of depression was also used to assess the rapid antidepressant-like effect of DHM by tail suspension

test (TST), forced swimming test (FST), locomotor activity, and sucrose preference test (SPT). The expression of BDNF and inflammatory factors were determined through Western blotting and enzyme-linked immunosorbent assay, respectively.

**Results** DHM reduced immobility time in the TST and FST both in mice and the acute LPS-induced mouse model of depression. Seven days of DHM treatment ameliorated depression-related behaviors induced by CUMS, whereas similar treatment with the typical antidepressant venlafaxine did not. DHM activated the ERK1/2-CREB pathway and increased glycogen synthase kinase-3 beta (GSK-3 $\beta$ ) phosphorylation at ser-9, with upregulation of BDNF expression, in both hippocampal tissues and cultured hippocampal cells.

**Conclusion** The present data indicate that DHM exerts a more rapid antidepressant-like effect than does a typical antidepressant, in association with enhancement of BDNF expression and inhibition of neuroinflammation.

**Keywords** Dihydromyricetin · Antidepressant activity · Brain-derived neurotrophic factor · ERK1/2 · Glycogen synthase kinase-3 $\beta$

Zhaoxiang Ren and Pengju Yan contributed equally to this work.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00213-017-4761-z>) contains supplementary material, which is available to authorized users.

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## Introduction

Major depressive disorder (MDD) is a highly prevalent illness that affects large populations across the world (Fang et al. 2013; Pesarico et al. 2016; Tao et al. 2016). Although the pathobiological basis of MDD is not understood, increasing evidence suggests that neuroinflammation and levels of brain-derived neurotrophic factor (BDNF) in the hippocampus are closely related to depression-like behavior (Chan et al. 2016; Wohleb 2016). Structural and functional changes in the hippocampus may play a critical role in the development of MDD (Dale et al. 2016; Harrisberger et al. 2015); decreased BDNF

expression is evident in MDD patients (Nuernberg et al. 2016), and antidepressant drugs can restore expression of BDNF in either patients with MDD or experimental models of depression (Cheng and Salton 2013; Dwivedi 2013; Gimenez-Cassina et al. 2012; Manosso et al. 2015). The prefrontal cortex (Zhang et al. 2014) and nucleus accumbens (Yang et al. 2016) may also play a role in such experimental models. In addition, numerous studies have demonstrated that neuroinflammation plays an important role in the development of MDD (Eyre et al. 2016; Kaufmann et al. 2017; Vogelzangs et al. 2014). Some inflammatory mediators may also constitute biomarkers and indicate therapeutic response to antidepressant treatment (Hughes et al. 2016; O'Connor et al. 2009; Rapaport et al. 2015). Therefore, regulation of neuroinflammation has also been considered as a potential therapeutic target for MDD.

Dihydromyricetin (DHM) is a flavonoid extracted from *Ampelopsis grossedentata* in southern China (Chen et al. 2015; Tong et al. 2015) that has been reported to display multiple pharmacological effects, including anti-tumor, anti-inflammatory, and anti-oxidative properties (Jiang et al. 2014; Kao et al. 2017; Liu et al. 2016; Tang et al. 2016; Xie et al. 2015). We have previously shown that DHM protects dopaminergic neurons against MPTP-induced neurodegeneration in a mouse model of Parkinson's disease by suppressing the activity of glycogen synthase kinase-3 beta (GSK-3 $\beta$ ) (Ren et al. 2016) and protects neurons from ischemia-reperfusion-induced apoptosis by activating ERK1/2 and the cAMP response element-binding (CREB) pathway (Zhao et al. 2017) in vivo and in vitro. In addition to direct protection of neurons, we have recently shown that DHM inhibited microglia-mediated inflammation in affording protection from ischemic insult (Zhao et al. 2017). In agreement with our observation, recent studies also showed that DHM suppresses inflammatory responses in RWA 264.7 cells through inhibiting activation of NF- $\kappa$ B and MAPK signaling pathways (Hou et al. 2015) and ameliorates behavioral deficits in animal models of Alzheimer's disease (Liang et al. 2014). In addition, activities against alcohol intoxication and to maintain glucose homeostasis have also been reported (Chen et al. 2015; Le et al. 2016; Shen et al. 2012).

Given the evidence for important neuroprotective, anti-inflammatory, and anti-oxidative effects in MDD treatment, we investigated the potential therapeutic effects of DHM, a substance that readily crosses the blood-brain barrier (Shen et al. 2012; Youdim et al. 2003), on depression-related phenotypes in experimental mouse models. Our results indicate that DHM treatment ameliorates depression-related behavior in both the acute lipopolysaccharide (LPS)-induced and the chronic, unpredicted mild stress (CUMS)-induced models of depression in mice. We further demonstrate that DHM activates the MAPK/CREB signaling pathway and inhibits the activity of GSK-3 $\beta$ , which resulted in upregulation of BDNF both in vivo and in cultured hippocampal neurons.

## Materials and methods

### Animals

Male C57BL/6J mice (age 6–8 weeks;  $22 \pm 3$  g) were purchased from Shanghai SLAC Laboratory Animals Co. Ltd. (Shanghai, China) and housed in groups of 4–6 per cage in a humidity- and temperature-controlled vivarium on a 12:12 h light/dark cycle (lights on at 8:00 a.m.). Food and water were available ad libitum except during weeks when animals were trained to perform the sucrose preference test (Wang et al. 2016). All animal protocols were proved by Animal Care and Use Committee of Soochow University and were in compliance with the Guidelines for the Care and Use of Laboratory Animals (Chinese-National-Research-Council, 2006) and the “ARRIVE” (Animals in Research: Reporting In Vivo Experiments) guidelines. Every effort was made to minimize animal suffering and to reduce the number of animals used in the experiments (Ren et al. 2016).

### Drugs and reagents

DHM ((2R,3R)-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-4-one; catalog number ST03840120MG; purity by HPLC  $\geq 98\%$ ) was obtained from Shanghai Standard Biotech Co., Ltd., Shanghai, China; it was initially dissolved in dimethylsulfoxide (DMSO) for stock solutions and diluted with saline for intraperitoneal (i.p.) injection (final trace concentration of DMSO  $\leq 0.2\%$ ) (Ren et al. 2016) at an injection volume of 1 ml/kg body weight. DHM powder was stored in brown bottle covered by silver paper at 4 °C, away from light and kept dry. The DHM solution was stored at  $-40$  °C away from light. Both DHM powder and solution were prepared immediately prior to use. For cell culture, DHM was dissolved in DMSO to 100 mM as stock solution and diluted with cell culture medium to concentrations from 10 to 50  $\mu$ M. Lipopolysaccharide (LPS), venlafaxine hydrochloride, and U0126 were purchased from Sigma-Aldrich, Co. (Darmstadt, Germany) and LY294002 (10 mg/ml) was purchased from Beyotime (Shanghai, China). LPS and venlafaxine hydrochloride were dissolved in PBS or saline, and U0126 was dissolved in DMSO for stock solutions.

### LPS-induced model of depression

Mice were randomly divided into 4 groups of 12 animals each: saline, LPS (i.p., 0.83 mg/kg), LPS + DHM 10.0 mg/kg, and LPS + DHM 20.0 mg/kg. In DHM treatment groups, 10.0 or 20.0 mg/kg DHM was administered i.p. once daily for 3 days before LPS treatment. On the last day, 0.83 mg/kg LPS was injected 30 min after DHM administration; 24 h later, the tail suspension test (TST) and forced swim test (FST) were performed and locomotor activity was measured (Jangra et al.

2016; Wang et al. 2010; Zhu et al. 2016). Also, the body weight of mice was measured before and 24 h post-injection of LPS.

### Chronic unpredicted mild stress model of depression

The CUMS procedure was carried out as described previously (Wang et al. 2016). Mice were randomly divided into five groups of at least 12 mice each: saline, CUMS, CUMS + DHM 10.0 mg/kg, CUMS + DHM 20.0 mg/kg, and CUMS + venlafaxine 10.0 mg/kg. In brief, mice were subjected to a variety of stresses for 8 weeks, with the stressors and stressor sequences applied at random each week to ensure unpredictability. Additionally, mice were subjected daily to two of the following stressors: light/dark cycle inversion (for 48 h in a different room), damp sawdust (over-night), sawdust-free cage (over-night), white noise (2 h), movement restraint (in a small tube), 45° cage tilting (over-night), cold or hot water swimming (5 min), water deprivation (24 h), tail pinch (2 min), overcrowding cage (over-night), and electric stimulus (0.5 mA, 30 times in 15 s). In the CUMS model, stressors were applied over 8 weeks and then mice in treatment groups were administered DHM 10.0 or 20.0 mg/kg i.p. or venlafaxine 10.0 mg/kg i.p. once daily for 7 days; the TST and FST were performed on the third and seventh days, respectively (Abe-Higuchi et al. 2016; Mao et al. 2017).

### Behavioral tests

**Tail suspension test** Mice were subjected to the TST as described previously (Pesarico et al. 2016; Tao et al. 2016; Wang et al. 2016). Briefly, animals were suspended at least 80 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail, under both acoustic and visual isolation. The total TST procedure was 6 min, and immobility time was recorded during the last 4 min; mice were considered immobile only when hanging passively and motionless.

**Forced swim test** Mice were subjected to the FST as described previously (Pesarico et al. 2016; Tao et al. 2016; Wang et al. 2016), subject to minor modifications. Briefly, each mouse was placed individually into a 4000 ml Pyrex glass beaker containing 3000 ml of warm water (temperature  $25 \pm 1$  °C). The total FST procedure was 6 min, and immobility time was recorded during the last 4 min; mice were considered immobile only when motionless or floating with only small movements necessary to keep the head above water.

**Locomotor activity** An infrared photobeam activity cage system (Jiliang Ltd., Shanghai, China) was used to record and analyze mouse horizontal locomotor activity. Mice were kept in the test room for 30 min before the experiment to habituate to the new environment. Mice were then placed individually in a  $25 \times 25 \times 30$  cm plexiglass/

polyvinyl arena. Total distance traveled (mm) and time (s) spent in the center area were counted for 20 and 5 min, respectively, using videotracking software (ANY-maze; Stoelting, IL, USA) (Pesarico et al. 2016; Ren et al. 2016; Tao et al. 2016; Wang et al. 2016).

**Sucrose preference test** This test was performed as described previously (Wang et al. 2016), subject to minor modifications. Briefly, mice were placed individually into a two-bottle, free-choice cage. One of the bottles contained water and the other 1% (w/v) sucrose solution. Prior to initiation of the test, all mice were acclimatized to 1% sucrose solution and drinking water for 48 h. Before the sucrose preference test, mice were deprived of water and food for 24 h, after which they were allowed free access to the two bottles over a 2-h period. The sucrose preference test was performed weekly during the CUMS procedure, with sucrose preference (%) = sucrose consumption/(sucrose consumption + water consumption).

### Primary neuron culture

Primary mouse neurons were derived from the hippocampus of C57BL/6 mice at embryonic day 17 or 18, as described previously (Wang et al. 2016). Dissected hippocampus tissue was digested with 2% trypsin and DNase-I for 30 min in 37 °C and resuspended in Neurobasal medium (Gibco, NY, USA) containing 2% B-27. After filtration with a 70- $\mu$ m sieve, collected cells were seeded in poly-D-lysine-coated plates and cultured at 37 °C in a 5% CO<sub>2</sub> incubator for 14 days. Culture medium was replaced every other day.

### Western blotting

Hippocampus tissue and cultured hippocampal neuron were lysed in RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 1% Na deoxycholate, 1% Triton X-100, 1 mM PMSF, EDTA, and protease inhibitor) for 30 min on ice and then incubated at 95 °C for 5 min (Ren et al. 2016). Western blotting was performed using standard protocols, and blots were probed with anti-pCREB (S133) (1:800; 9198S, Santa Cruz), anti-CREB (1:1000; 9197S, Santa Cruz), anti-pERK1/2 (1:1000; 4370S, Cell Signaling Technology), anti-ERK1/2 (1:1000; 4695S, Cell Signaling Technology), anti-BDNF (1:200; SC-546, Santa Cruz), anti-pGSK3 $\beta$  (S9) (1:1000; 9323S, Cell Signaling Technology), anti-GSK3 $\beta$  (1:1000; 9832S, Cell Signaling Technology), and anti- $\alpha$ -tubulin (1:10,000; T6074, Sigma-Aldrich) antibodies. Data were analyzed and quantified by ChemiScope 3300 Mini (CLINX, Shanghai, China) and densitometry with ImageJ software. Phosphorylated ERK, GSK3 $\beta$ , and CREB were related to tubulin. Each experiment was replicated at least three times

and signals normalized to control in each independent experiment.

### RNA extraction and quantitative real-time PCR

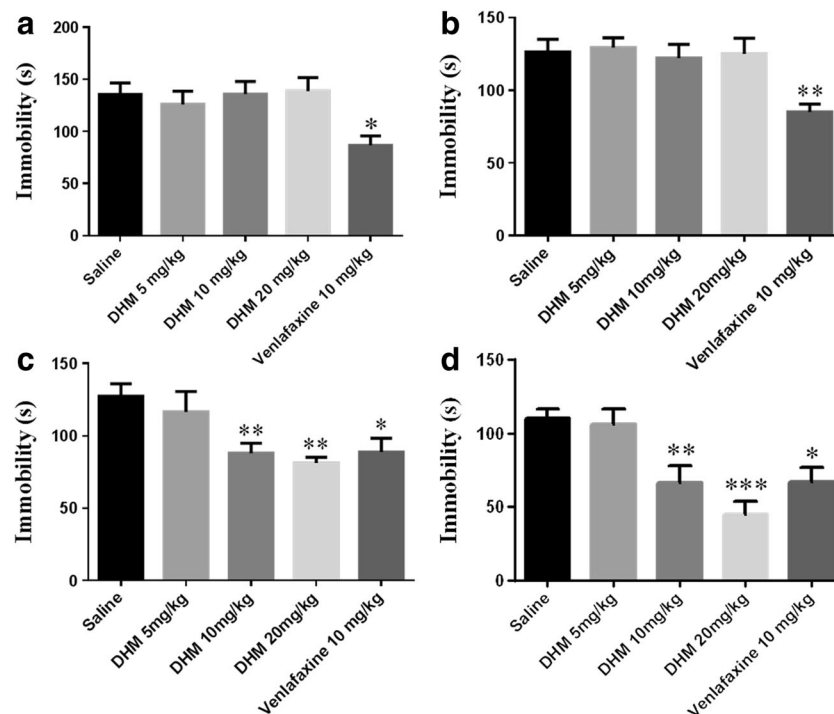
Total RNA was extracted from cultured hippocampal neurons using RNAiso plus reagent (TaKaRa Biotechnology, Dalian, China), and 1  $\mu$ g RNA was reverse-transcribed into cDNA using moloney murine leukemia virus reverse transcriptase, deoxynucleotide triphosphate, recombinant RNase inhibitor, and oligo (dT) according to the manufacturer's instructions. cDNAs were amplified using the following specific primers: GAPDH, forward primer: 5'-TGTGTCCGTCGTGGATCTGA-3', reverse primer: 5'-TTGCTGTTGAAGTCGCAGGAG-3'; TNF- $\alpha$ , forward primer: 5'-CAGGAGGGAGAACAGAACTCCA-3', reverse primer: 5'-CCTGGTTGGCTGCTTGCTT-3'; and IL-6, forward primer: 5'-TTCCATCCAGTTGCCTTCTT-3', reverse primer: 5'-CAGAATTGCCATTGCACAAC-3'. Quantitative tests of mRNA expression were performed by SYBR Premix II (TaKaRa, Dalian, China) according to the manufacturer's instructions. Expression of target genes was normalized to GAPDH and quantified relative to expression in the respective control. Results were analyzed and expressed as previously described (Ni et al. 2015; Wu et al. 2015).

### Enzyme-linked immunosorbent assay

To measure TNF- $\alpha$  and IL-6, hippocampal tissue was collected from mice following the LPS-induced model of depression and controls. Samples were homogenized ultrasonically in RIPA buffer with protease inhibitor (Roche, Mannheim, Germany). Supernatants were collected after centrifugation (12,000 rpm, 20 min, 4 °C) and protein concentration measured by BCA protein kit (Tiangen, Beijing, China). TNF- $\alpha$  and IL-6 commercial ELISA kits were purchased from Boster Biosciences Co., Wuhan, China, and protein expression measured according to the manufacturer's instructions. OD values were assessed by Microplate Reader (Infinite M200 PRO, Tecan, Switzerland) (Ni et al. 2015).

### Statistical analysis

Data were analyzed by GraphPad Prism® (version 6.0) software and presented as means  $\pm$  SEM. One-way ANOVAs were followed by Dunnett's test for comparisons with vehicle control groups or Newman-Keul's test for multiple-group comparisons. For data on weight gain during the CUMS procedure, repeated measures ANOVA was employed. A probability value of  $p < 0.05$  was considered statistically significant.



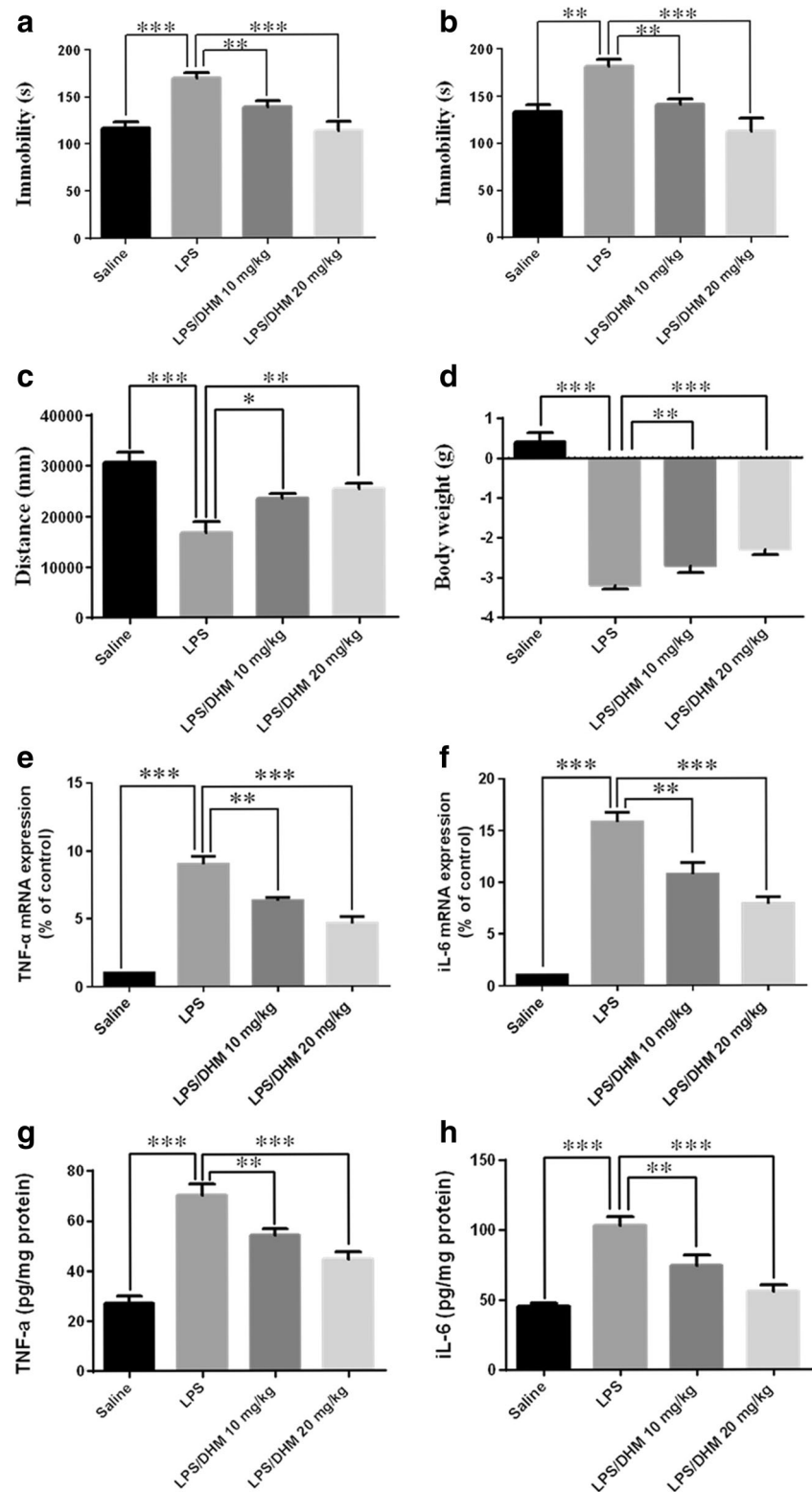
**Fig. 1** Effects of treatment with DHM or venlafaxine on immobility time in the tail suspension test (TST) and forced swimming test (FST) in naive mice. Male C57BL/6 mice were divided into five groups: saline; DHM 5.0, 10.0, and 20.0 mg/kg; and venlafaxine 10.0 mg/kg. Mice received intraperitoneal (i.p.) injections of DHM or venlafaxine 2 h before behavioral tests: **a** TST and **b** FST immobility times after a single

administration of DHM or venlafaxine and **c** TST and **d** FST immobility times after repeated administration of DHM or venlafaxine once daily for three consecutive days. At least 12 mice were used in each group. Values are presented as means  $\pm$  SEM. Statistical analyses were performed by one-way ANOVA followed by Dunnett's test: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs saline

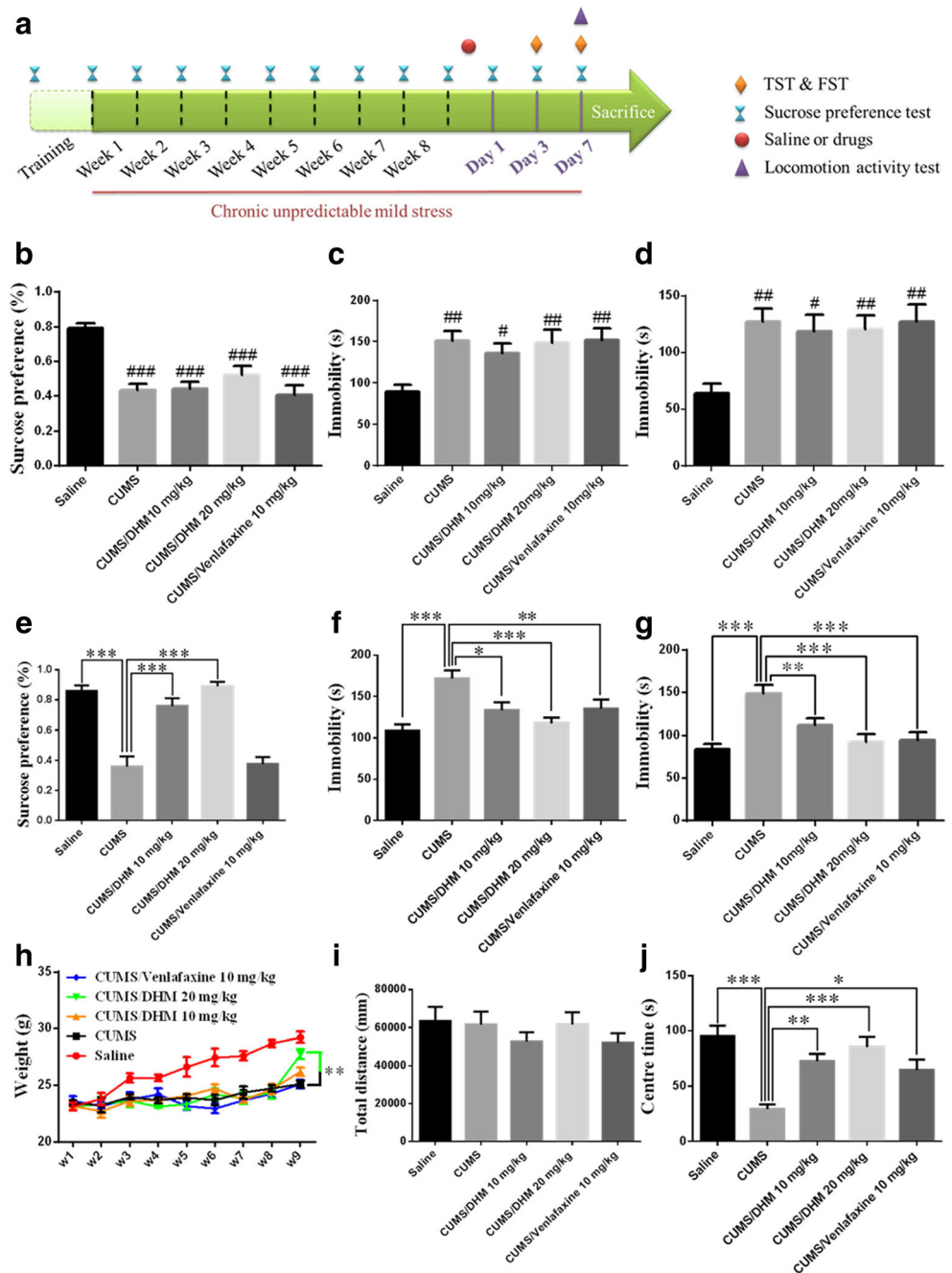


**Fig. 2** Effects of DHM on LPS-induced changes in the tail suspension test (TST) and forced swimming test (FST) and on locomotor activity, body weight, TNF- $\alpha$ , and IL-6. Male C57BL/6 mice were divided into four groups: saline, LPS 0.83 mg/kg, LPS 0.83 mg/kg + DHM 10.0 mg/kg, and LPS 0.83 mg/kg + DHM 20.0 mg/kg. Mice received intraperitoneal (i.p.) injections of DHM once daily for three consecutive days. On the last day, LPS was injected i.p. 30 min after DHM treatment. Immobility times in **a** TST and **b** FST, and **c** locomotor activity, as total distance traveled, were evaluated 24 h after LPS injection. **d** Body weight (g) was recorded before and 24 h after LPS administration and the difference calculated. At least 12 mice were used in each group. Following the behavioral studies, mice were sacrificed and hippocampal tissue collected for assay of **e** TNF- $\alpha$  mRNA, **f** IL-6 mRNA, **g** TNF- $\alpha$  protein, and **h** IL-6 protein after 3 days of treatment with DHM treatment, 10.0 and 20.0 mg/kg. At least 12 mice were used in each group. Values are presented as means  $\pm$  SEM. Statistical analyses for **a–h** were performed by one-way ANOVA followed by the Newman-Keuls test.

\* $p < 0.05$ , \*\* $p < 0.01$ ,  
\*\*\* $p < 0.001$



**Fig. 3** Effects of treatment with DHM or venlafaxine in the chronic unpredictable mild stress (CUMS) model. **a** Experimental design. Mice were subjected to CUMS over 8 weeks and then divided randomly into four treatment groups: CUMS, CUMS + DHM 10.0 mg/kg i.p., CUMS + DHM 20.0 mg/kg i.p., or venlafaxine 10.0 mg/kg i.p. once daily for 7 days; naïve mice received saline as controls. Sucrose preference test (SPT), tail suspension test (TST), and forced swimming test (FST) were carried out after three (b–d) and seven (e–g) days of drug treatment. **h** Body weight was recorded weekly. Total distance traveled (i) and time spend in the center area (j) were recorded after 7 days of drug treatment. At least 12 mice were used in each group. Values are presented as means  $\pm$  SEM. Statistical analyses for b–d were performed by one-way ANOVA followed by Dunnett's test; statistical analysis for e–j were performed by one-way ANOVA (repeated measures ANOVA for h) followed by the Newman-Keuls test; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  vs saline



## Results

### DHM reduces immobility time in FST and TST

We first examined the antidepressant-like effect of DHM using the FST and TST in naïve mice. As shown in Fig. 1a, b, a 2-h pretreatment with DHM (10.0 and 20.0 mg/kg) did not alter immobility time in either FST or TST, while the typical antidepressant venlafaxine (10.0 mg/kg) significantly decreased immobility time. However, pretreatment with DHM (10.0 and 20.0 mg/kg) once per day for 3 days resulted in a

marked reduction in immobility time in dose-dependent manner (Fig. 1c, d). In contrast, pretreatment with DHM for 1 or 2 days did not elicit similar antidepressant effects. These results indicated that the antidepressant effect of DHM requires repeated administration over 3 days.

### DHM attenuates LPS-induced depression-related behaviors and inflammatory markers

We next employed an inflammation-induced, depression-related model in mice, induced by acute injection of LPS.

As expected, acute LPS injection (0.83 mg/kg) induced depression-like behavior in mice in terms of increases in immobility times in FST and TST and reduced locomotion (Fig. 2a, b) (Guan et al. 2015; Kurosawa et al. 2016; Wohleb 2016). DHM (10.0 and 20.0 mg/kg) reversed the LPS-induced increase in immobility time in both TST and FST (Fig. 2a, b) and markedly attenuated LPS-induced inhibition of locomotion in terms of total distance traveled (Fig. 2c). DHM (10.0 and 20.0 mg/kg) attenuated LPS-induced weight loss 24 h after LPS injection (Fig. 2d). DHM (10.0 and 20.0 mg/kg) also attenuated the LPS-induced increase in hippocampal TNF- $\alpha$  and IL-6 mRNA (Fig. 2e, f) and protein levels (Fig. 2g, h).

### DHM attenuates CUMS-induced depression-related and anxiety-related behaviors and disruption to the ERK1/2-CREB-BDNF pathway

The experimental procedure for the CUMS model is shown in Fig. 3a. There were no differences in sucrose preference between the five groups at the beginning of the experiment. At 3 and 7 days following CUMS for 8 weeks, mice showed the expected marked reduction in sucrose preference (Fig. 3b, e) and increase in immobility time in both FST and TST (Fig. 3c, d, f, g), together with a decrease in time exploring the center area of the activity monitor but without alteration in distance traveled (Fig. 3i, j). Three days of DHM treatment (10.0 and 20.0 mg/kg) did not alter CUMS-induced decrease in sucrose preference (Fig. 3b) or increase in FST and TST time (Fig. 3c, d). However, 7 days of DHM treatment reversed CUMS-induced decrease in sucrose preference (Fig. 3e) and increase in FST and TST time (Fig. 3f, g), together with reversal of CUMS-induced decrease in time exploring the center area of the activity monitor but without alteration in distance traveled (Fig. 3i, j); 7 days of DHM treatment also reversed CUMS-induced weight loss (Fig. 3h). In contrast, 7 days of treatment with venlafaxine (10.0 mg/kg) did not influence any of these CUMS-induced effects (Fig. 3b–j).

Phosphorylation of ERK1/2, CREB, and GSK-3 $\beta$  (ser-9) were decreased, and the expression of BDNF was downregulated in the hippocampus of mice exposed to CUMS (Fig. 4a, b). DHM treatment (10.0 and 20.0 mg/kg daily for 7 days) reversed these CUMS-induced alterations in ERK1/2, CREB, GSK-3 $\beta$ , and BDNF (Figs. 4a, b). To further confirm these observations, we studied the effects of DHM in naïve mice and primary cultured hippocampal neurons. DHM (5.0, 10.0, and 20.0 mg/kg in hippocampus tissue; 5, 25, and 50  $\mu$ M in primary cultured hippocampal neurons) increased phosphorylation of ERK1/2, CREB, and GSK-3 $\beta$  at ser-9 (Fig. 4c, d).

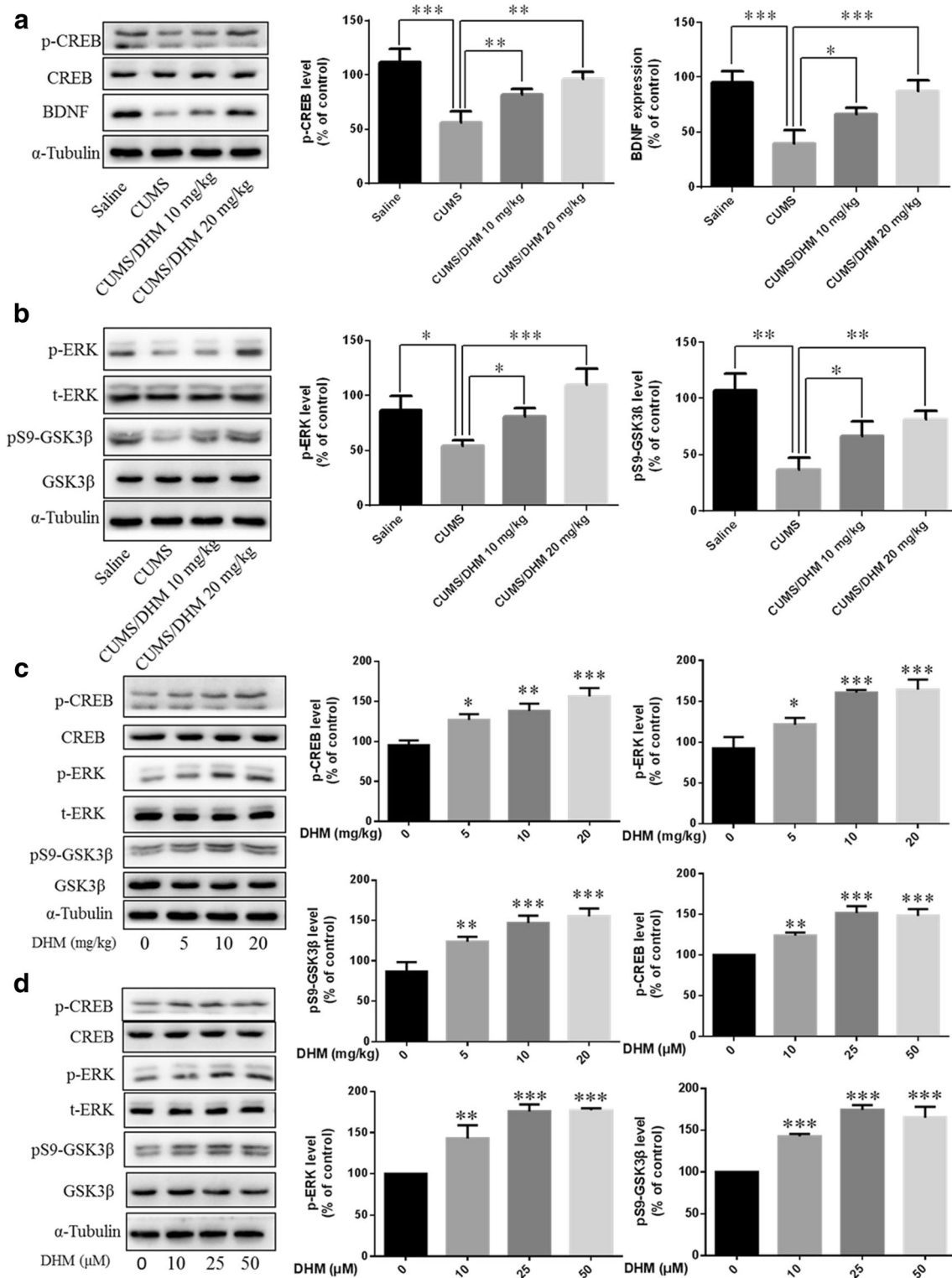
Since a 7-day treatment with DHM elevated hippocampal BDNF expression in mice exposed to CUMS (Fig. 4a), we further investigated DHM-stimulated expression of BDNF in vivo and in primary cultured hippocampal neurons.

Administration of DHM (20.0 mg/kg) to naïve mice increased expression of hippocampal BDNF in a time-dependent manner, with this effect evident by day 3 and continuing to day 7; in contrast, venlafaxine (10.0 mg/kg) was without effect (Fig. 5a, b). Primary cultured hippocampal neurons treated with DHM (10–50  $\mu$ M) for 48 h also exhibited increased expression of BDNF (Fig. 5c). In agreement with in vivo observations, expression of BDNF in primary cultured neurons was enhanced in a time-dependent manner following treatment with 25  $\mu$ M DHM, with this effect evident by day 2 and continuing to day 3 (Fig. 5d). To confirm the signaling pathways for DHM-stimulated BDNF expression, we employed U0126, a ERK1/2 inhibitor, and LY294002, a selective inhibitor of Akt, which activates GSK-3 $\beta$  indirectly. In primary cultured hippocampal neurons, stimulation of BDNF expression by 25  $\mu$ M DHM was inhibited by each of 10  $\mu$ M LY294002 and 300 nM U0126 (Fig. 6a), indicating that stimulation of BDNF expression by DHM is dependent on ERK1/2 activation and GSK-3 $\beta$  inhibition.

## Discussion

Currently available antidepressant drugs require administration for a number of weeks to attain full clinical efficacy. In addition, these drugs can also cause a number of serious adverse effects. Thus, there is an urgent need to develop novel antidepressant drugs. The present study employed depression-related phenotypes in experimental mouse models and demonstrated that DHM, a natural occurring flavonoid purified from *Ampelopsis grossedentata*, elicited antidepressant-like effects. In the inflammation (LPS)-induced mouse model of depression, DHM reduced immobility time in both the TST and FST and inhibited inflammatory cytokines. We also observed that DHM attenuated depression-related behaviors in the CUMS mouse model in a time-dependent manner. This antidepressant-like effect was evident following 7 days of DHM treatment. Relative to the serotonin and norepinephrine reuptake inhibitor venlafaxine, our results indicate that 7 days of treatment with DHM elicited an antidepressant-like effect on CUMS-induced depression-related behaviors without central stimulatory effects (Supplementary figure S1). We further demonstrated that DHM promoted hippocampal BDNF expression both in vivo and in primary cultured hippocampal neurons in a time-dependent manner. Additionally, we demonstrated that activation of ERK1/2 and CREB and inhibition of GSK-3 $\beta$  contribute to DHM-stimulated BDNF expression. The present data indicate that DHM may be a promising antidepressant candidate.

Neuroinflammation is increasingly recognized as an important factor in the development of depression and/or as a potential index of therapeutic response in depressive illness. Acute LPS is a reliable model for inducing inflammation-

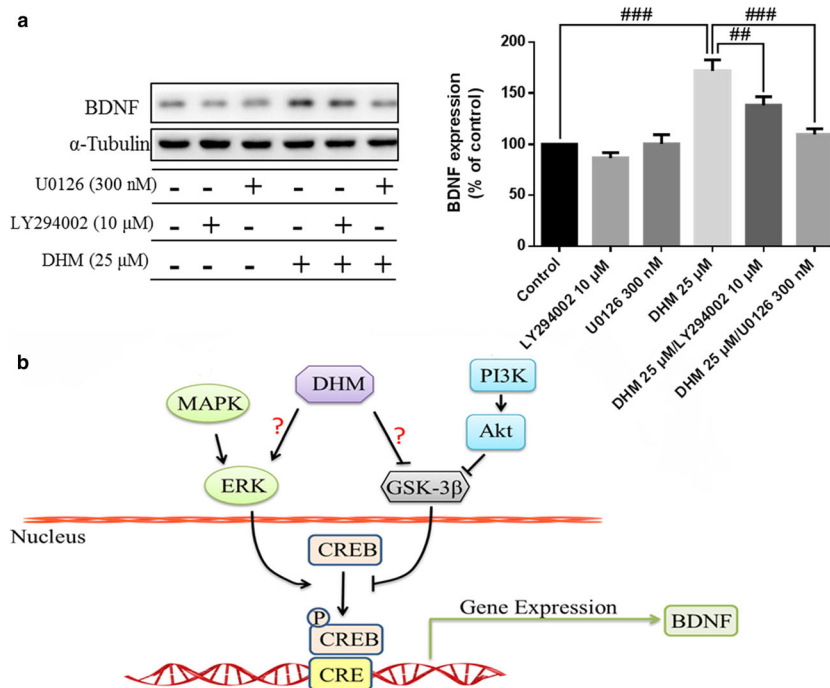
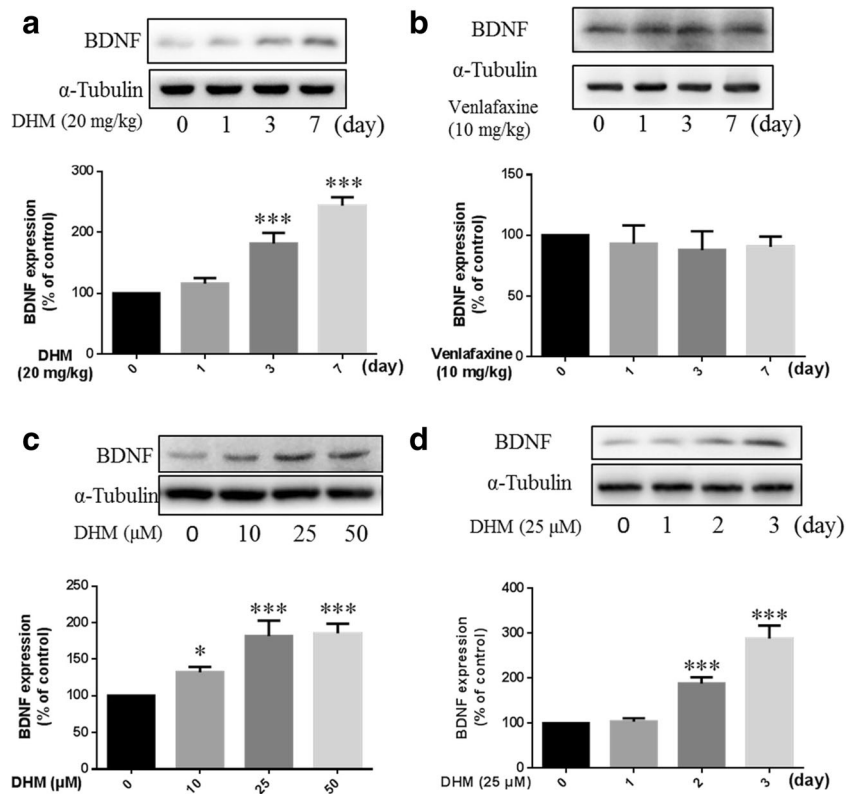


**Fig. 4** Effects of DHM on ERK1/2, CREB, and GSK-3 $\beta$  pathways and stimulated BDNF expression in vivo and in vitro. Following the behavioral studies of Fig. 4, mice were sacrificed and hippocampal tissue dissected and processed for assay. **a, b** Western blots with respective antibodies. **c** Naïve mice were treated i.p. with DHM 5.0, 10.0, and 20.0 mg/kg, sacrificed after 2 h and hippocampal tissue dissected for immunoblot assays. **d** Primary cultured hippocampal neurons were treated with DHM 10.0, 25.0, and 50.0  $\mu$ M for 1 h and

cells collected for immunoblot assays. For each of **a, b, c,** and **d,** representative images for immunoblots are shown in the left panel and quantitative data shown in the right panels. Values are presented as means  $\pm$  SEM from at least three independent experiments. Statistical analyses for **a** and **b** were performed by one-way ANOVA followed by the Newman-Keuls test. Statistical analyses for **c** and **d** were performed by one-way ANOVA followed by Dunnett's test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs control



**Fig. 5** Effects of **a** DHM and **b** venlafaxine on BDNF expression in hippocampus. Mice were treated i.p. with 20.0 mg/kg DHM or 10.0 mg/kg venlafaxine once daily for 1, 3, and 7 days and sacrificed at these different times. BDNF expression in hippocampal tissue was measured by immunoblot. Primary cultured hippocampal neurons were **c** treated with 10.0, 25.0, and 50.0  $\mu$ M DHM for 48 h, or **d** treated with 25.0  $\mu$ M DHM for 1, 2, or 3 days, before collection for immunoblot assays to determine BDNF expression. For each of **a**, **b**, **c**, and **d**, representative images for immunoblots are shown in the upper panels and quantitative data shown in the lower panels. Values are presented as means  $\pm$  SEM from at least three independent experiments. Statistical analyses were performed by one-way ANOVA followed by Dunnett’s test: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs control



**Fig. 6** Effects of the selective Akt inhibitor LY294002 and the selective MEK1/2 inhibitor U0126 on DHM-stimulated BDNF expression. **a** Primary cultured hippocampal neurons were pretreated in the presence or absence of 10  $\mu$ M LY294002 or 300 nM U0126 prior to and during DHM treatment for an additional period of 48 h. For **a**, representative images for immunoblots are shown in the left panel and quantitative data shown in the right panel. Values are presented as means  $\pm$  SEM. Statistical

analyses were performed by one-way ANOVA followed by the Newman-Keuls test: # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$ . **b** Potential signaling mechanisms for DHM-mediated antidepressant-like activity: DHM increases phosphorylation of ERK1/2 to activate CREB and enhance expression of BDNF; DHM also inhibits GSK-3 $\beta$ , via phosphorylating GSK-3 $\beta$  at ser-9, to enhance BDNF expression. However, how DHM regulates ERK1/2 and GSK-3 $\beta$  remains unclear

related depression-like phenotypes; it also elicits the expression and secretion of inflammatory cytokines such as TNF- $\alpha$  and IL-6, which appear critical in the pathological processes of inflammation-related depression (Kurosawa et al. 2016; O'Connor et al. 2009; Park et al. 2011; Sulakhiya et al. 2016; Wang et al. 2010). We demonstrated that LPS-induced increases in immobility time and weight loss were prevented by a 3-day pretreatment with DHM. In addition, DHM inhibited LPS-stimulated expression and secretion of TNF- $\alpha$  and IL-6 in mice. These results indicated that 3 days of pretreatment with DHM prevented LPS-induced depression-related behavior and attenuated associated LPS-induced increases in hippocampal inflammatory processes. Thus, inhibition of inflammation may also contribute to the antidepressant-like action of DHM.

It is interesting to note that antidepressant-like activity on LPS-induced depression-related behavior in mice was observed after 3 days of DHM treatment; acute administration of DHM for 1 or 2 days did not elicit antidepressant-like activity. This may be associated with BDNF expression (BoJiang et al. 2016), as increased expression of BDNF in the hippocampus was evident after 3 days of DHM treatment and elevated BDNF expression in primary cultured hippocampal neurons was evident only after 2–3 days of DHM treatment. DHM activates ERK1/2 and GSK-3 $\beta$ , and both ERK1/2 and GSK-3 $\beta$  are known to be involved in the regulation of BDNF expression (Beurel and Jope 2010; Bui et al. 2012; Gimenez-Cassina et al. 2012; Omata et al. 2011; Sreaton et al. 2004; Tsai et al. 2008; Xue et al. 2016; Zunszain et al. 2013). Such DHM-induced activation of the ERK1/2-CREB-BDNF pathway may contribute to its antidepressant-like activity, and there is growing evidence that BDNF plays an important role in many neuropsychiatric diseases, including MDD (Duman and Voleti 2012; Mai et al. 2002; Sun et al. 2016; Xu et al. 2016; Xue et al. 2016).

Potential signaling mechanisms for DHM-mediated antidepressant-like activity are indicated in Fig. 6b, but the question of how DHM upregulates the phosphorylation level of ERK1/2 and GSK-3 $\beta$  needs further research. Furthermore, the mechanism(s) underlying the time-dependent induction of BDNF expression by DHM remain to be determined. While our studies focus on BDNF expression in the hippocampus, future studies should also include investigation of pro-BDNF and any role(s) for the prefrontal cortex and nucleus accumbens in these processes.

In summary, the present work demonstrates that (a) DHM elicits a more rapid antidepressant-like effect than does the typical antidepressant venlafaxine and that (b) this effect may involve stimulation of BDNF expression, possibly via activation of the ERK1/2-CREB pathway and inhibition of GSK-3 $\beta$ , and inhibition of neuroinflammation. These findings provide the first evidence that DHM may be a promising candidate for the treatment of MDD.

**Acknowledgements** This work was supported by funds from the National Science Foundation of China (81130023, 30825042). Support provided from the Priority Academic Program Development of Jiangsu Higher Education Institutes (PAPD) and the Jiangsu key laboratory grant (BM2013003) is also appreciated.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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