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ORIGINAL ARTICLE

The involvement of AMPA–ERK1/2–BDNF pathway in the mechanism of new antidepressant action of prokinetic meranzin hydrate

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Abstract It was recently discovered that ketamine can relieve depression in a matter of hours through an action on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. This is much more rapid than the several weeks required for the available antidepressants to show therapeutic efficacy. However, ketamine has negative side effects. The aim of this study was to determine whether the natural prokinetic drug meranzin hydrate (MH) has a fast-acting antidepressant effect mediated by AMPA receptors. By means of in vivo and in vitro experiments, we found that (1) treatment of rats with MH at 9 mg/kg decreased immobility time in a forced swimming test (FST), as did the popular antidepressant fluoxetine and the AMPA receptor positive modulator aniracetam. Pretreatment of rats with NBQX (10 mg/kg), an antagonist of AMPA receptors, blocked this effect of MH. (2) MH increased number of crossings of forced swimming rats in the open field test. (3) FST enhanced hippocampal ERK1/2, p-ERK1/2 and BDNF expression levels. MH (9 mg/kg) treatment further up-regulated hippocampal p-ERK1/2 and BDNF expression levels, and this effect was prevented by NBQX. (4) MH-increased BDNF expression corresponded with MH-decreased immobility time in the FST. (5) In vitro experiments, we found that incubation of rats hippocampus slices with MH (10, 20 μM respectively) increased concentrations of BDNF and p-ERK1/2. This effect of MH (20 μM) were prevented by NBQX. In conclusion, in animals subjected to acute stress, the natural prokinetic drug MH produced a rapid effect mediated by AMPA receptors and involving BDNF modulation through the ERK1/2 pathway.

Keywords MH · Forced swimming test · BDNF · Glutamate · AMPA · ERK1/2

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Introduction

Recently, it was discovered that, in some patients, depression could be relieved by ketamine in a matter of hours (Zarate et al. 2006). However, many of the available monoaminergic-based antidepressant drugs (serotonin, noradrenaline and dopamine) often required several weeks to achieve a therapeutic effect (Fumagalli et al. 2009; Quan et al. 2011; Porsolt et al. 1977). Further study showed that the AMPA subtype of glutamate receptor was a crucial

target for ketamine's antidepressant effects (Maeng et al. 2008). In fact, ketamine no longer prevented depressive behavior in an animal model after AMPA receptors were blocked (Maeng et al. 2008). These findings suggested that compounds that trigger AMPA receptors may eliminate depressive behavior as rapidly as ketamine. Despite this progress, ketamine (which is used as a club and anesthetic drug and known as "special K") is unlikely to be used widely given its multiple side effects including cognitive and gastrointestinal disorders (aan het Rot et al. 2010).

The effects and side effects of ketamine highlight the urgent need for an alternative rapid antidepressant drug, possibly a natural compound targeting AMPA receptors. Meranzin hydrate (MH) is a natural compound isolated from the fruit Fructus Aurantii (Huang et al. 2011). A patent application regarding this compound was filed by us in 2008 (Patent number: CN101940566). Our previous study in rats showed that MH induced prokinetic effects similar to Fructus Aurantii through activation of H_1 histamine receptors (Huang et al. 2011). In patients, we performed a pharmacokinetic study of the prokinetic compound MH following oral administration of the classic antidepressant Chaihu-Shugan-San (CSS) (Qiu et al. 2011), which contains both MH and Fructus Aurantii (Li et al. 2009; Kim et al. 2005). The antidepressant effects of CSS and its constituents are clearly described in our previous report (Kim et al. 2005). Our recent work further shows that CSS and its component ferulic acid have anti-depressive effects resulting from polypharmacological mechanisms (Zhang et al. 2011).

Therefore, we hypothesized that MH accounted for part of the antidepressive effects of CSS and that its mechanism of action involved AMPA receptors, a target of rapid antidepressants.

To test this hypothesis, we examined first whether the mechanisms underlying the rapid antidepressant effects of MH were related with AMPA receptors in animals subjected to an acute stress, one of the causal factors for development of depression (Brown 2011). Secondly, we examined the pathway mediated by AMPA subtype of glutamate receptor in the treatment of MH. It has been reported that glutamate mediates anti-depressive effects through release of brain-derived neurotrophic factor (BDNF) (Alexander et al. 2010; Mitsukawa et al. 2006). BDNF, as a marker of neuroadaptive changes, is popularly selected as a causal index of depression and anti-depression (Alexander et al. 2010; Martinowich et al. 2007; Vaishnav Krishnan et al. 2008). The ERK1/2 pathway has been widely shown to link glutamate and BDNF (Mayer et al. 2006; Hayashi et al. 1999). Therefore, we examined whether the AMPA receptor-mediated anti-depressive effect of MH involved the AMPA–ERK1/2–BDNF pathway.

Materials and methods

Animals

Male Sprague–Dawley (SD) rats (Changsha, China) weighing 150–180 g were housed under standardized environmental conditions [22 ± 2°C, a 12 h light/dark cycle (6.30 a.m. to 6.30 p.m.) and 50 ± 10 % relative humidity] and with free access to food and water. All experimental procedures were conducted in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (1988) and approved by the Animal Experimental Center for Central South University (Changsha, China).

Forced swimming test (FST)

The FST was applied for 5 min as a type of stress (Mayer et al. 2006). Animals were exposed to acute forced swim stress (FSS) for 5 min. During the single 5-min stress period, scoring was done by two independent observers blind to the treatment conditions. The behavior was scored every 5 s based on the criteria listed below. Each rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water (Porsolt et al. 1977, 1978). Animals were sacrificed 15 min or 60 min after the forced swimming tests.

Open-field test (OFT)

Analysis of rat spontaneous activity was carried out in an open field apparatus according to the publication (Ramos and Mormede 1997). Rat was placed in the center of the open-field apparatus, and the locomotor activity was assessed immediately before the forced swim test. The open-field apparatus was a field, 70 cm in diameter, which was demarcated into 18 approximately equal areas. Hand-operated counters were used to score locomotion (number of line crossings within 5 min). A separate researcher, who was blind to the treatment group, scored the behavior in the open field. Experiments were performed in a dark room, and the apparatus was illuminated by a 60 W bulb positioned 1 m above the center of the circle.

Western blot analysis

Primary antibodies for ERK1/2 (rabbit monoclonal), P-ERK1/2 (rabbit monoclonal), BDNF (rabbit monoclonal), and β-actin (rabbit monoclonal), as well as horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody were obtained from Bamstead International. All

other reagents were obtained from Wako Pure Chemicals, unless otherwise specified.

Rats were decapitated after behavioral tests. The brains were quickly removed and the hippocampus was dissected out on dry ice (Qi et al. 2008). All hippocampus samples were stored at −80 °C until required for further experiments. Western blot analysis was performed as described by Qi et al. (2008). Levels of ERK1/2, PiERK1/2, BDNF and β-actin were determined in a different NC using the same procedures.

In vitro experiments

Preparation and incubation of slices

Fifteen minutes after the forced swimming test, rats were decapitated, and hippocampal brain slices were quickly prepared using a Vibroslice at 4 °C. Then, slices were preincubated in artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 2.4 $CaCl_2$, 26 $NaHCO_3$, ten glucose (pH 7.4, constantly gassed with 95 % O_2/5 % CO_2) for 45 min at 37 °C (Dell'Anno et al. 2012; Oliveira et al. 2002).

Slices treatment

After the preincubation period, hippocampal slices from the vehicle group ($n = 8$) were incubated in ACSF (pH 7.4) for 30 min. Slices from the other groups were incubated in ACSF containing MH (10 μM and 20 μM, $n = 16$) or the AMPA receptor antagonist NBQX + MH (NBQX 20 μM; MH 20 μM; $n = 8$) for 30 min.

After the 30-min incubation periods, the mediums of all groups were replaced with fresh incubation solution and slices were incubated for 3 h at 37 °C (equilibrated with 95 % O_2/5 % CO_2, "reperfusion" phase).

ELISA assays

BDNF and p-ERK1/2 concentrations in the incubation solution obtained at the end of the experiments were determined using commercially available BDNF and p-ERK1/2 enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol.

Experimental design

Experiment 1: Rats received administration of MH (4.5 or 9 mg/kg), fluoxetine (10 mg/kg), aniracetam (10 mg/kg) or vehicle and, 30 min later, were subjected to the FST for 5 min. The blank group of rats received vehicle and was not subjected to the swim stress. Thirty minutes after the forced swim test, the above groups all submitted to the behavioral tests.

Experiment 2: Rats received MH (9 mg/kg) or vehicle and were subjected to the forced swim test for 5 min. The blank (control) group of rats was treated with vehicle and was not subjected to the swim stress. Rats in the antagonism group received NBQX (10 mg/kg) followed, 30 min later, by MH. A further 30 min later they were exposed to the forced swim test for 5 min. Fifteen minutes after the swim stress, all rats anesthetized and sacrificed, the hippocampus was removed and processed for western blot analysis for ERK1/2, Pi-ERK1/2 and BDNF contents.

Experiment 3: Rats received MH (9 mg/ kg) or vehicle and were submitted to the forced swim test for 5 min. Sixty minutes after the swim stress, all rats were anesthetized and killed, and the hippocampus was removed and processed for western blot analysis of BDNF content.

Experiment 4: In vitro ($n = 8$ rats/group) experiments investigating the effects of MH on the AMPA–ERK1/2–BDNF pathway were performed as described above.

Data analysis

Data are expressed as mean ± SEM. Comparisons of data were performed using one-way or two-way ANOVA followed by Duncan's post hoc test. A value of $P < 0.05$ was considered to represent statistical significance.

Drugs

Meranzin hydrate (Huaxi Medical University Medicine Factory, Chengdu, China): 4.5 or 9 mg/kg (Fig. 1) (Huang et al. 2011; Qiu et al. 2011); Fluoxetine (supplied by Eli Lilly and Company Limited, USA): 10 mg/kg (a positive antidepressive control) (Qi et al. 2008); aniracetam (supplied by Nanjing Shenghe Pharmaceutical Industry Limited, Nanjing, China): 10 mg/kg (an AMPA receptor positive modulator) (Okuyama and Aihara 1988); NBQX (Sigma, St. Louis, MO, USA), an antagonist to AMPA subtype of glutamate receptors (Pitt et al. 2000), 10 mg/kg (Szewczyk et al. 2010). NBQX was dissolved in 1 M

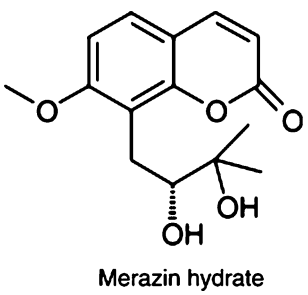

Fig. 1 The structure of meranzin hydrate

sodium hydroxide before administration. Aniracetam was dissolved in DMSO. All other drugs were dissolved in distilled water and given by gavage.

Results

Effects of MH, fluoxetine or aniracetam treatment in the forced swimming test

There was a significant treatment effect in the forced swimming test for 5 min [F (5, 42) = 26.856, $P < 0.01$] with MH (9 mg/kg), aniracetam (10 mg/kg) and fluoxetine (10 mg/kg) significantly reducing the immobility time compared with vehicle ($n = 8$, Duncan $P < 0.01$). However, there was no difference in immobility time between FST rats and FST-MH (4.5 mg/kg) rats (see Fig. 2).

In the open field test, MH at the dose of 9 mg/kg significantly increased the number of crossing compared with vehicle group [F (2, 35) = 35.72, $P < 0.01$]. But there was no difference between FST rats and FST-aniracetam rats (Duncan, $P > 0.05$) (see Fig. 3).

Effects of MH alone or in combination with NBQX (10 mg/kg) in the forced swimming test

Compared with vehicle, treatment with MH alone significantly decreased immobility time in the forced swim test [F (5, 42) = 1.324, $P < 0.01$]. The effects of MH were prevented by pretreatment with NBQX (Duncan, $P < 0.01$). However, NBQX + vehicle treatment did not

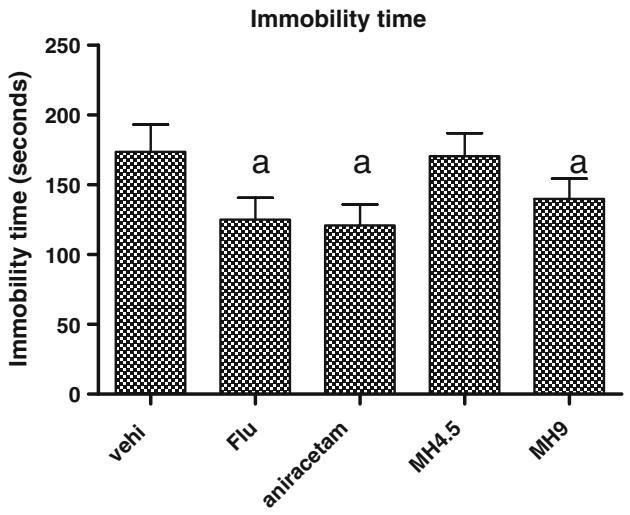

Fig. 2 MH (9 mg/kg), aniracetam and Fluoxetine reduced immobility time of rats (8 per group) in the forced swimming test (FST). Vehi, FST rats pretreated with 1.5 ml saline; Flu, FST rats pretreated with fluoxetine (10 mg/kg); Aniracetam, FST rats pretreated with aniracetam (10 mg/kg); MH4.5, FST rats pretreated with MH at the dose of 4.5 mg/kg; MH9, FST rats with MH at the dose of 9 mg/kg. Values are mean ± SEM. "*a*" indicates $P < 0.01$ compared with vehicle group

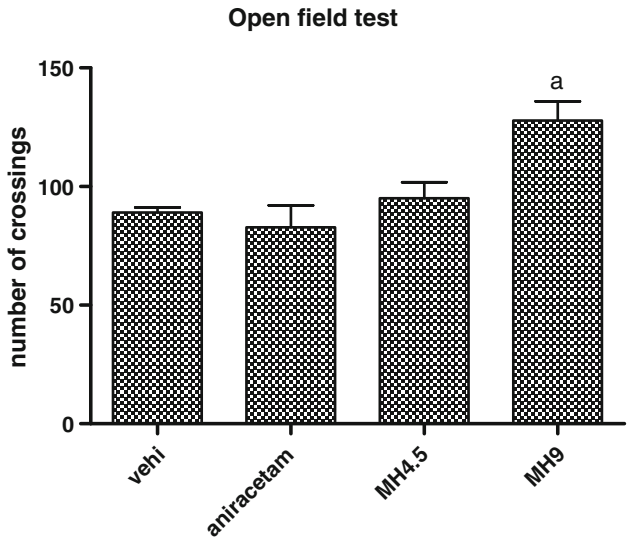

Fig. 3 MH (9 mg/kg) increased the number of crossing of rats (8 per group) in the open field test 30 min after forced swimming test (FST). Vehi, FST rats pretreated with 1.5 ml saline; Aniracetam, FST rats pretreated with aniracetam (10 mg/kg); MH4.5, FST rats pretreated with MH at the dose of 4.5 mg/kg; MH9, FST rats with MH at the dose of 9 mg/kg. Values are mean ± SEM. "*a*" indicates $P < 0.01$ compared with vehicle group

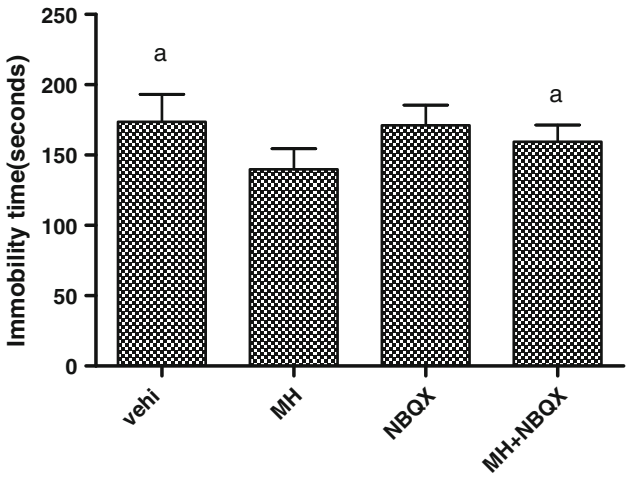

Fig. 4 Pretreatment with NBQX (10 mg/kg) prevented MH (9 mg/kg) effects in the forced swimming test (8 rats per group). Vehi, FST rats pretreated with 1.5 ml saline; MH, FST rats with MH at the dose of 9 mg/kg; NBQX, FST rats pretreated with NBQX (10 mg/kg); MH + NBQX, FST rats pretreated with MH (9 mg/kg) and NBQX (10 mg/kg). Values are mean ± SEM. "*a*" indicates $P < 0.01$ compared with MH group

induce significant changes in immobility time compared with vehicle treatment (Duncan, $P > 0.05$) (see Fig. 4).

Effects of MH, alone or in combination with NBQX, on expression of ERK1/2 and pERK1/2 in the hippocampus

pERK1/2 and ERK1/2 levels in the hippocampus were significantly increased in FST rats compared with the blank group [F (3, 28) = 13.37 and F (3, 28) = 41.40, respectively, $P < 0.01$]. The FST-MH group showed further increased hippocampus levels of pERK1/2 compared with the vehicle group [F (3, 28) = 13.37, $P < 0.01$]. NBQX prevented the increased pERK1/2 levels by MH [F (3, 28) = 13.37, $P < 0.01$]. However, there was no significant difference in the hippocampal levels of ERK1/2 between the FST-MH group and the vehicle group [F (3, 28) = 41.40, $P > 0.05$] (see Figs. 5 and 6).

Effects of MH, alone or in combination with NBQX, on expression of BDNF in the hippocampus

The levels of hippocampal BDNF in FST rats were significantly increased compared with the levels in the blank group [F (7, 56) = 67.25, $P < 0.01$]. Treatment with MH induced further up-regulation of BDNF levels compared with the levels in the vehicle group (Duncan, $P < 0.01$). NBQX prevented the effect of MH on BDNF levels (Duncan, $P < 0.01$) (see Figs. 7 and 6).

Moreover, MH was associated with BDNF levels with an increase in time-dependent manner in hippocampus. BDNF levels in MH-treated rats were higher at 60 min after the forced swimming stress than at 15 min after the test [F (7, 56) = 67.25, $P < 0.05$]. Interestingly, there was negative correlation between immobility time and hippocampal BDNF levels 60 min after stress (Pearson correlation, $r = -0.583$, $n = 16$, $P < 0.05$). However, there was no correlation between immobility time and hippocampal

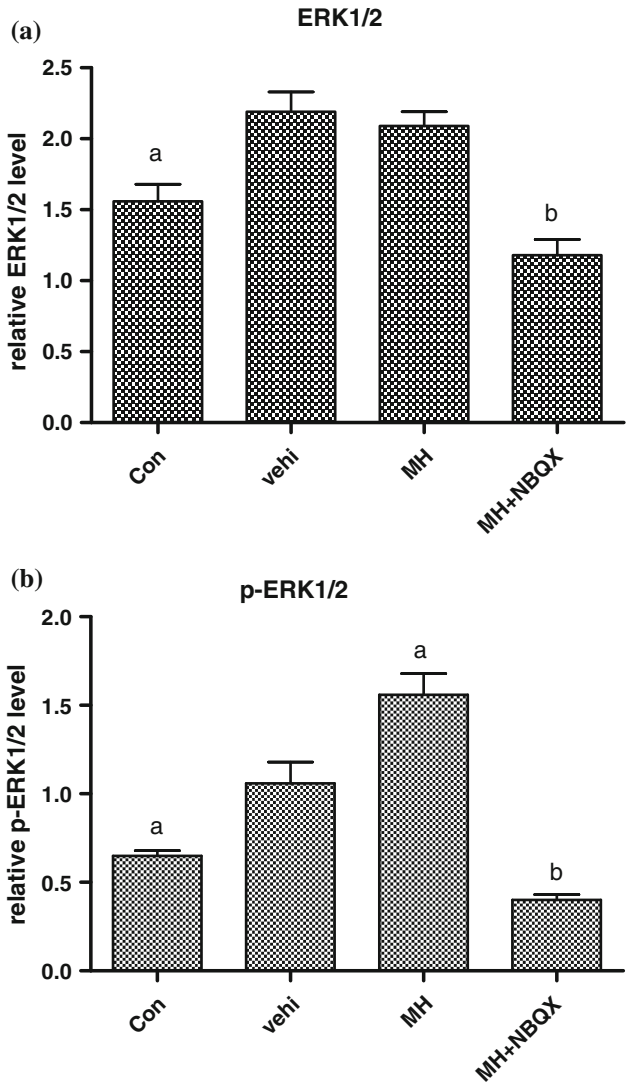

Fig. 5 MH modulated the activities of hippocampus P-ERK1/2, but not hippocampus ERK1/2 in the forced swimming test (FST, 8 rats per group). Con, non-FST rats pretreated with 1.5 ml saline; Vehi, FST rats pretreated with 1.5 ml saline; MH, FST rats with MH at the dose of 9 mg/kg; MH + NBQX, FST rats pretreated with MH (9 mg/kg) and NBQX (10 mg/kg). Values are mean ± SEM. "*a*" indicates $P < 0.01$ compared with vehicle group. "*b*" indicates $P < 0.01$ compared with MH group

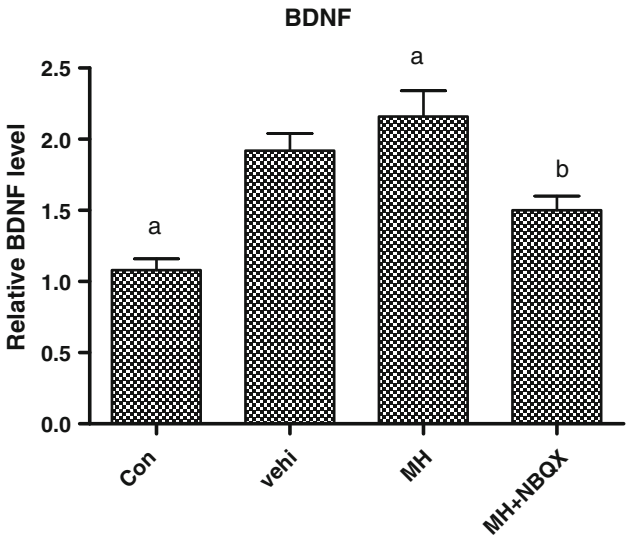

Fig. 6 MH modulated the activities of hippocampus BDNF in rats (8 per group) 15 min after forced swimming test (FST). *Con*, non-FST rats pretreated with 1.5 ml saline; Vehi, FST rats pretreated with 1.5 ml salinel; MH, FST rats with MH at the dose of 9 mg/kg; MH + NBQX, FST rats pretreated with MH (9 mg/kg) and NBQX (10 mg/kg). Values are mean ± SEM. "*a*" indicates $P < 0.01$ compared with vehicle group. "*b*" indicates $P < 0.01$ compared with MH group

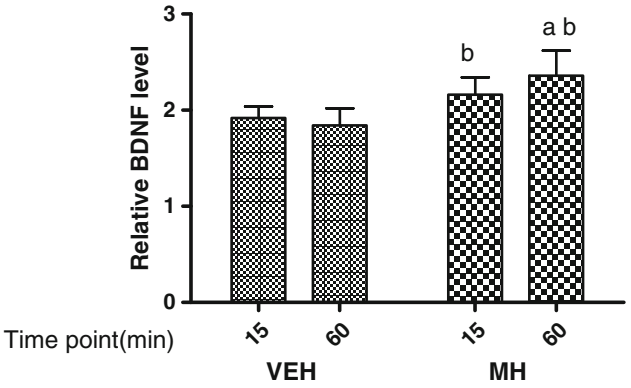

Fig. 7 MH affected the timing of forced swimming stress-induced modification of BDNF (8 rats per group). VEH, FST rats with 1.5 ml saline; MH, FST rats pretreated with MH (9 mg/kg). BDNF levels were measured using Western blot analysis 15 or 60 min after FST. In this graph, "*a*" versus 15 min after FST in the MH group ($P < 0.05$); "*b*" versus the VEH group at corresponding time point ($P < 0.01$)

BDNF levels at 15 min after stress (Pearson correlation, $r = -0.297$, $n = 16$, $P > 0.05$) (see Figs. 8 and 9).

Effects of incubation with MH and the AMPA receptor antagonist NBQX on the p-ERK1/2 in vitro ELISA assays

Increases in p-ERK1/2 levels were observed in the incubation medium with 10 or 20 μM MH, compared with vehicle group [F (3, 32) = 52.22, $P < 0.01$ both].

When compared with 10 μM MH, exposure to 20 μM MH caused a significant increase in p-ERK1/2 concentration in the incubation medium [F (3, 32) = 52.22, $P < 0.01$]. Co-incubation of MH (20 μM) with the AMPA receptor antagonist NBQX (20 μM) abolished the 20 μM MH-induced increases in p-ERK1/2 concentration [F (3, 32) = 52.22, $P < 0.01$] (see Fig. 10).

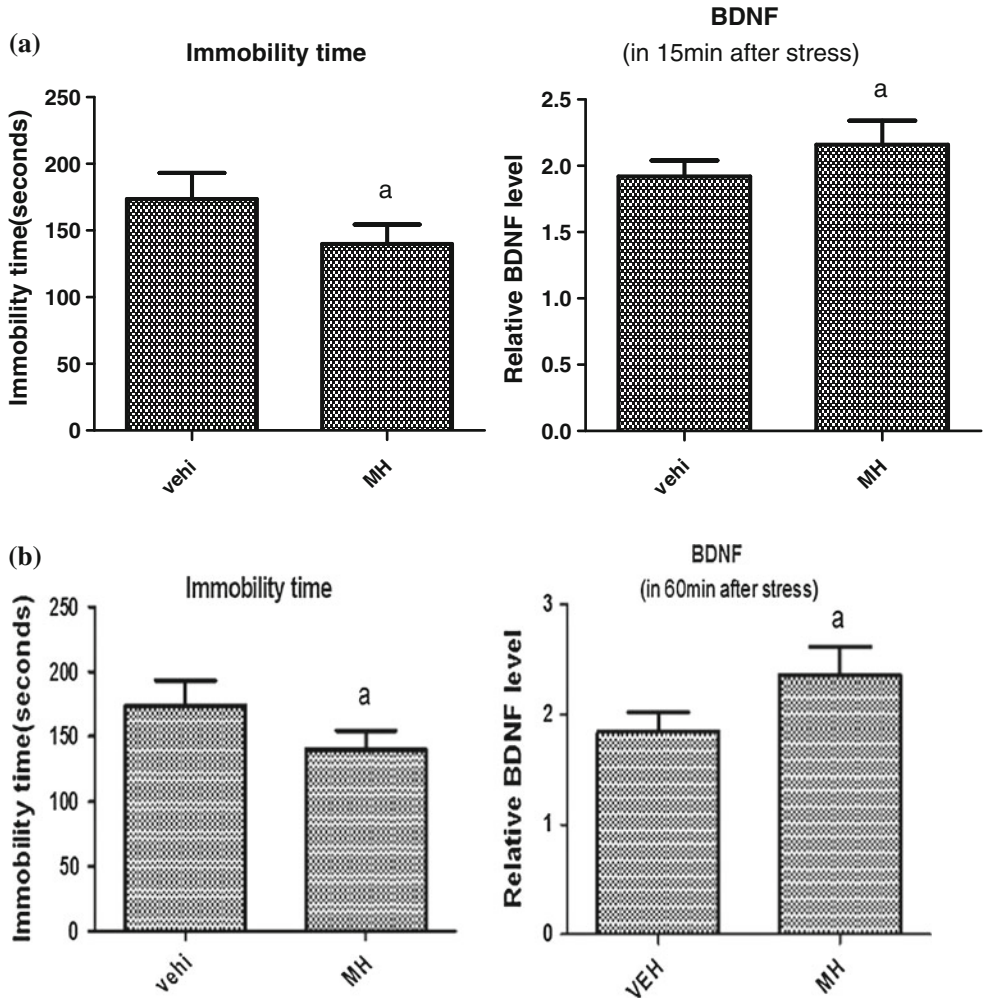

Fig. 8 MH-induced antidepressant-like effects were associated with activities of hippocampal BDNF in 60 min after the forced swim stress (**b** graph), but were not associated with activities of hippocampal BDNF in 15 min after the forced swim stress (**a** graph). Vehi, FST rats with 1.5 ml saline; MH, FST rats pretreated with MH (9 mg/kg). Values are mean ± SEM. "*a*" indicates $P < 0.01$ compared with vehicle group

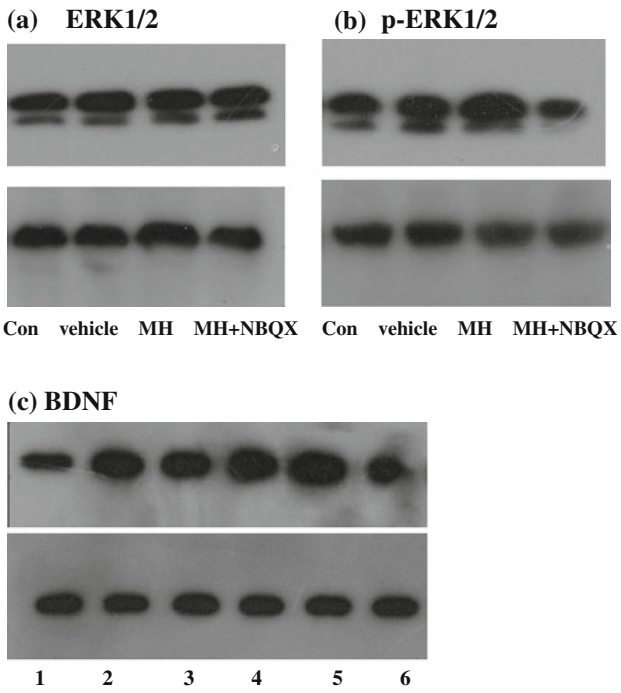

Fig. 9 The levels of ERK1/2 (**a**), P-ERK1/2 (**b**) and BDNF (**c**) were analyzed by Western blotting using specific antibodies for each [in graph **c**, *1* Con group, *2* vehicle group (15 min after FST), *3* vehicle group (60 min after FST), *4* MH group (15 min after FST), *5* MH group (60 min after FST), *6* MH + NBQX group (15 min after FST)]. Con, non-FST rats pretreated with 1.5 ml saline; Vehicle, FST rats pretreated with 1.5 ml saline; MH, FST rats with MH at the dose of 9 mg/kg; MH + NBQX, FST rats pretreated with MH (9 mg/kg) and NBQX (10 mg/kg). The images shown are representative bands from one animal of each group

Effects of incubation of MH and AMPA receptor antagonist NBQX on the BDNF in vitro ELISA assays

Increases in BDNF levels were observed in the incubation medium with 10 μM or 20 μM MH, compared with vehicle group [F (3, 32)= 72.29, $P < 0.01$ both]. BDNF concentration was also increased in exposure to 20 μM MH compared with 10 μM MH [F (3, 32) = 72.29, $P < 0.05$]. The 20 μM MH-induced increases in BDNF concentration were abolished by co-incubation of MH (20 μM) with 20 μM NBQX [F (3, 32) = 72.29, $P < 0.01$] (see Fig. 11).

Discussion

The present results show that MH at 9 mg/kg (Huang et al. 2011; Qiu et al. 2011) reduced immobility time analogous to the popular antidepressant fluoxetine and the AMPA receptor positive modulator aniracetam in the forced swimming test. It is known that stress is an important factor of depression (Brown 2011). This work is the first to suggest that MH has a potential antidepressant effect. However, other popular prokinetic drugs (Gao 2007) do not have anti-depressive effects (Mayer et al. 2006); indeed, among these, metoclopramide inversely produces depression-like symptoms (Cozza et al. 2003; Phillips et al. 2011). In addition, the present report indicated that MH (9 mg/kg) increased the number of crossing of FST rats in open field test (OFT). Thus, the rapid antidepressant-like effect of MH may be also ascribed to an alteration in the locomotor activity.

Accumulating research suggests that depressed patients experience malfunction of glutamate and gamma-amino butyric acid (GABA) systems (Bhagwagar et al. 2007; Choudary et al. 2005). AMPA receptor is an often demonstrated glutamate receptor. One future avenue for rapid treatment of depression may link with glutamate system and target AMPA receptor to enhance synaptic signaling. However, there have been no reports on the effects of natural prokinetic drugs based upon these data (Szewczyk et al. 2010; Sen and Sanacora 2008). For the first time, we propose that the antidepressant action of the prokinetic drug MH is mediated by AMPA receptors. Our results show that the antidepressant-like effect of MH is inhibited by pre-treatment with NBQX, an AMPA subtype glutamate receptor antagonist, at a dose that did not induce any effect by itself. This suggests that the effects of MH in the forced swimming test depend on activation of AMPA receptors.

Regarding the AMPA receptor-mediated mechanisms of MH, our study also showed that hippocampal BDNF expression was up-regulated after MH treatment and that this up-regulation was prevented by pre-treatment with the AMPA antagonist NBQX. The present results are consistent with those of previous reports. Other positive modulators of AMPA receptors are effective in models of depression and produce neuroprotective effects via BDNF (Bai et al. 2001; Li et al. 2001). For example, Ampakines, positive AMPA-type glutamate receptor modulators, can increase the protein levels of BDNF in mice and facilitate synaptic plasticity in both animals and humans (Rex et al. 2006). Stress-induced transcription of BDNF isoforms was modulated by the AMPA receptor potentiator Org 26576 in the rat hippocampus (Fumagalli et al. 2012). In light of these findings, our study supports the idea that AMPA receptors exert neuroprotective effects through an increase in BDNF expression during the acute action of MH.

In addition, our results show that MH induces BDNF mRNA expression in a time-dependent manner, while other similar analyses were only carried out at a single time-point (Calabtese et al. 2009; Fumgalli et al. 2011; Legutko et al. 2001). Interestingly, the present study is the first to show that the effects of MH on stress-coping in the FST occur in parallel with increased BDNF expression, suggesting that changes in BDNF expression may, at least in part, underlie the therapeutic effect of MH; this is different from the

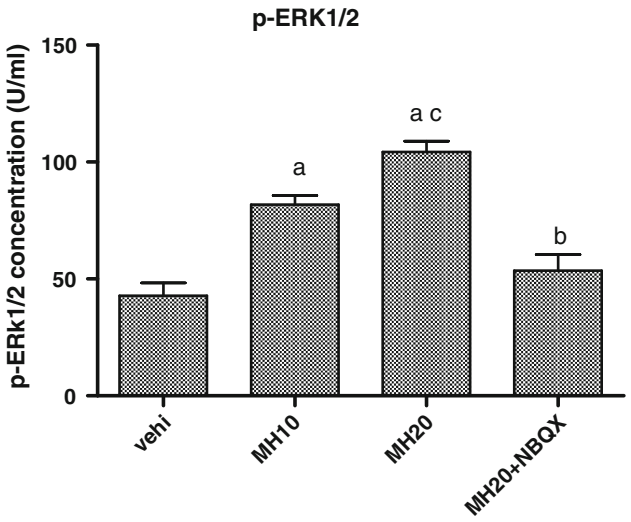

Fig. 10 Effects of incubation of MH and AMPA receptor antagonist NBQX on p-ERK1/2 concentration in vitro brain slice experiment using ELISA kits. Vehi, slices of FST rats incubated in artificial cerebrospinal fluid (ACSF). MH10, slices of FST rats incubated in ACSF and MH (10 μM). MH20, slices of FST rats incubated in ACSF and MH (20 μM). MH20 + NBQX, slices of FST rats incubated in ACSF, MH (20 μM) and NBQX (20 μM). "*a*" indicates $P < 0.01$ compared with vehicle group. "*b*" indicates $P < 0.01$ compared with MH20 group. "*c*" indicates $P < 0.01$ compared with MH10 group

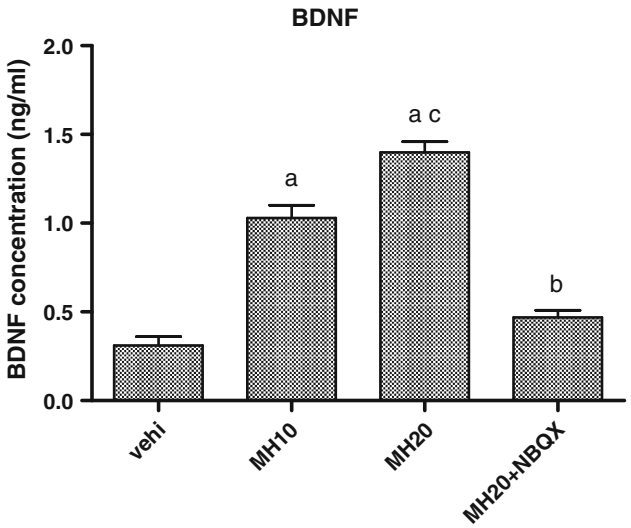

Fig. 11 Effects of incubation of MH and AMPA receptor antagonist NBQX on BDNF concentration in vitro brain slice experiment using ELISA kits. Vehi, slices of FST rats incubated in artificial cerebrospinal fluid (ACSF); MH10, slices of FST rats incubated in ACSF and MH (10 μM); MH20, slices of FST rats incubated in ACSF and MH (20 μM); MH20 + NBQX, slices of FST rats incubated in ACSF, MH (20 μM) and NBQX (20 μM). "*a*" indicates $p < 0.01$ compared with vehicle group. "*b*" indicates $p < 0.01$ compared with MH20 group. "*c*" indicates $p < 0.05$ compared with MH10 group

effects of monoamines, including 5-hydroxide tryptamine (5-HT) (Homberg et al. 2010; Meyer et al. 2001). Selective serotonin reuptake inhibitors (SSRIs) often produce an antidepressant effect for several weeks after changes in 5-HT levels (Meyer et al. 2001). Interestingly, the correlation between immobility time and BDNF expression only occurred 60 min after stress, but not 15 min after stress, which suggested that the correlation is also dependent on time.

Furthermore, BDNF is reported to exert its biological effects through activating intracellular signaling molecules, including extracellular signal-regulated kinase (ERK) 1/2, a component of the mitogen-activated protein kinase (MAPK) signaling pathway (Martinowich et al. 2007; Kawashima et al. 2010). In the present study, intriguingly, hippocampal pERK1/2 levels in stress rats were up-regulated by MH treatment and this up-regulation was prevented by pre-treatment with the AMPA receptor antagonist NBQX, in accordance with the change in BDNF expression. As described above, in addition to MH-decreased immobility time, the MH-induced increases in BDNF and Pi-ERK1/2 expression levels were both abolished by pre-treatment with the AMPA antagonist NBQX.

Importantly, through in vitro brain slice experiments, the present work showed that MH produced a neuroprotective effect in forced swimming rats and that this effect was mediated by AMPA receptors. The AMPA receptor-mediated mechanism of action of MH was also closely related to Pi-ERK1/2 and BDNF levels in vitro. Thus, based on our in vivo and in vitro experiments, we inferred that the antidepressant action induced by MH is mediated by AMPA receptors acting via the MAPK–ERK1/2 pathway to enhance BDNF levels.

Of note, Both AMPA receptor and BDNF are involved in neuroplastic changes (Qi et al. 2008; Sen and Sanacora 2008; Fumgalli et al. 2011; Wager-Smith 2011; Xuan et al. 2004). Hippocampal synaptic plasticity not only plays a key role in the pathogenesis of depression and antidepression, but also mediates hippocampus-dependent memory (Szewczyk et al. 2010; Fumgalli et al. 2011; Kawashima et al. 2010; Derkach et al. 2007). In further study, we shall investigate the memory function of prokinetic MH at a deeper level.

In conclusion, these results suggest that AMPA receptors play an important role in mediating the effects of MH in animals subjected to acute stress. We also show for the first time that prokinetic MH, acting via AMPA receptors, the ERK1/2 pathway and subsequent modulation of BDNF levels, has potential antidepressant properties that could be of benefit for the treatment of depression.

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Conflict of interest The authors declare no conflicts of interest.

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