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RESEARCH ARTICLE

Tramadol reinforces antidepressant effects of ketamine with increased levels of brain-derived neurotrophic factor and tropomyosin-related kinase B in rat hippocampus

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Abstract **Ketamine exerts rapid and robust antidepressant properties in both animal models and depressed patients and tramadol possesses potential antidepressant effects. Brain-derived neurotrophic factor (BDNF) is an important biomarker for mood disorders and tropomyosin-related kinase B (TrkB) is a high affinity catalytic receptor for BDNF. We hypothesized that tramadol pretreatment might reinforce ketamine-elicited antidepressant effects with significant changes in hippocampal BDNF and TrkB levels in rats. Immobility time of rats receiving different treatment in the forced swimming test (FST) was observed. Levels of BDNF and TrkB in hippocampus were measured by enzyme linked immunosorbent assay. Results showed that tramadol (5 mg/kg) administrated alone neither elicited antidepressant effects nor altered BDNF or TrkB level. However, pretreatment with tramadol (5 mg/kg) enhanced the ketamine (10 mg/kg) -elicited antidepressant effects and upregulated the BDNF and TrkB levels in hippocampus. In conclusion, tramadol pretreatment reinforces the ketamine-elicited antidepressant effects, which is associated with the increased levels of BDNF and TrkB in rat hippocampus.**

Keywords tramadol; ketamine; antidepressant; brain-derived neurotrophic factor; tropomyosin-related kinase B

Introduction

Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist for glutamate and has been shown to exert antidepressant effects in animals and humans [1,2]. Tramadol is a moderate analgesic with dual mechanisms of action: exciting the mu-opioid receptors and inhibiting the reuptake of noradrenaline (NE) and serotonin (5HT) [3,4]. Commonly used antidepressants mirtazapine, duloxetine, and venlafaxine, also inhibit NE and 5HT reuptake and this shared monoaminergic action leads to the research of tramadol as a potential treatment for depression [3,4].

Brain-derived neurotrophic factor (BDNF), a kind of neurotrophin, is known as an important biomarker in the process of the antidepressant actions. Depressed patients usually have a lower level of BDNF in both central nervous system and peripheral blood [5]. It has been reported that the forced swimming test (FST) decreases the hippocampal BDNF mRNA level, whereas the antidepressant treatment increases the hippocampal BDNF mRNA level [6]. Moreover, the intracerebral infusion of BDNF also presents antidepressant effects in two classical animal models of depression including the learned helplessness and the FST[7]. Previous research suggests that the antidepressant effects of ketamine are mainly attributed to the increase of BDNF in rat hippocampus [8]. Therefore, the increased BDNF in hippocampus would be the underlying mechanism of ketamine exerting antidepressant effects.

Tropomyosin-related kinase B (TrkB) is a high affinity catalytic receptor for BDNF, and mediates the multiple effects of BDNF. Previous literature indicates that BDNF-TrkB receptor signaling is necessary for the actions of antidepressant drugs [9]. Similarly, several lines of evidence have demonstrated that antidepressants activate BDNF-TrkB receptor signaling within an hour after drug administration via different mechanisms [10,11].

Until now, little information is available about the action of tramadol pretreatment on the ketamine-induced antidepressant effects. Therefore, the main purpose of the present study was to evaluate the action of tramadol pretreatment on the

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ketamine-induced antidepressant effects in rats receiving FST, and the minor purpose was to determine the associated changes of BDNF and TrkB levels in rat hippocampus.

Materials and methods

Animals

Forty male Wistar rats (200–300 g body weight) were purchased from Shanghai Animal Center, Shanghai, China. The animals were housed 5 per cage with food and water available *ad libitum* and exposed to a 12 h : 12 h (7 : 00–19 : 00) light/dark cycle. The experimental procedures were approved by the Institutional Animal Ethics Committee of Jinling Hospital, Nanjing University.

Forced swimming test (FST)

FST was applied according to previous reports [12,13] to evaluate the effects of antidepressants mainly. The test included two separate exposures to a cylindrical tank (30-cm diameter, 60-cm height) filled with water (22–23°C) to a 30 cm level in which rats cannot touch the bottom of the tank. Water in the tank was changed after every rat. All the procedures were conducted during 9 : 00–15 : 00. Rats were placed in the water for 15 min before drug pretreatment (pretest session). Twenty-four hours later, rats were placed in the water again for 5 min (test session), and the immobility time were recorded in seconds. Immobility was defined as that the rat remains floating in the water without struggling and makes only those movements necessary to keep its head above water.

Groupings and drug interventions

Ketamine was obtained from Gutian Pharmaceutical Company (Fujian, China). Tramadol was purchased from Grunenthal GmbH (Germany). Rats were equally randomized into 4 groups. One day before drug intervention, rats were insulted in the FST for 15 min as pretest session. Twenty four hours later, rats were intraperitoneally injected with saline + saline ($n = 10$), tramadol (5 mg/kg) + saline ($n = 10$), saline + ketamine (10 mg/kg) ($n = 10$), and tramadol (5 mg/kg) + ketamine (10 mg/kg) ($n = 10$) as drug pretreatment and drug administration, respectively. There was a 30-min-interval between the drug pretreatment and the drug administration. All the saline and drugs were given in a same volume of 1 ml. Thirty minutes after the drug administration, the FST was conducted for 5 min as test session and the immobility time was recorded. After the behavioral tests, rat hippocampus was harvested and stored at −80°C for the determination of the levels of BDNF and TrkB. The dose of tramadol and ketamine was determined based on previous studies of Jesse *et al.* [14]and Beurel *et al.* [15].

Enzyme linked immunosorbent assay (ELISA)

BDNF and TrkB levels in hippocampus were measured by anti-BDNF and anti-TrkB sandwich-ELISA respectively, according to the manufacturer instructions (Chemicon, USA). Briefly, rat hippocampus was homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ethylene glycol tetraacetic acid (EGTA). Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1 : 2 in sample diluent and standard curve ranged from 7.8 to 500 pg/ml of BDNF (or TrkB). The plates were then washed four times with sample diluent and a monoclonal anti-BDNF (or anti-TrkB) rabbit antibody diluted 1 : 1 000 in sample diluent was added to each well and incubated for 3 h at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1 : 1 000) was added to each well and incubated at room temperature for 1 h. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF and TrkB were determined by absorbance in 450 nm respectively. The standard curve demonstrates a direct relationship between optical density (OD) and BDNF (or TrkB) concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

Statistical analysis

Data are presented as mean ± SD. Statistical analyses were made by one-way analysis of variance and *post hoc* comparisons were performed by least significant difference tests. These statistical analyses were conducted by Statistical Product for Social Sciences (SPSS version 17.0). Differences were considered to be significant at $P < 0.05$.

Results

Rats in the saline + ketamine group had shorter immobility time than in the saline + saline group and rats in the tramadol + ketamine group had shorter immobility time than in the saline + ketamine group ($F_{(3-36)} = 51.813$; $P < 0.001$; Fig. 1).

Rats in the saline + ketamine group had higher BDNF level ($F_{(3-36)} = 8.113$; $P = 0.039$; Fig. 2) and TrkB level ($F_{(3-36)} = 13.927$; $P = 0.001$; Fig. 3) in hippocampus than in the saline + saline group. Rats in the tramadol + ketamine group had higher BDNF level ($F_{(3-36)} = 8.113$; $P = 0.039$; Fig. 2) and TrkB level ($F_{(3-36)} = 13.927$; $P = 0.001$; Fig. 3) in hippocampus than in the saline + ketamine group.

Discussion

Previous studies have shown that ketamine at the dose of 10 mg/kg may produce rapid and significant antidepressant

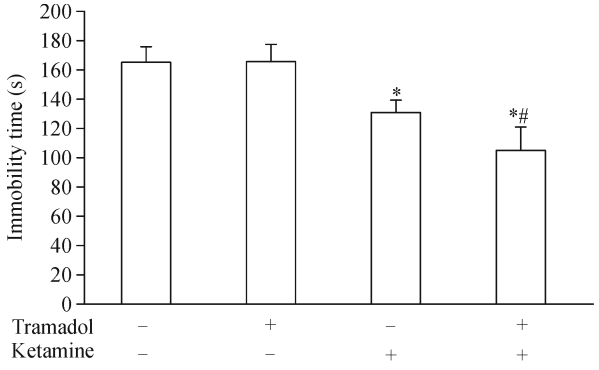

Fig. 1 Effects of different interventions (saline + saline, tramadol [5 mg/kg]+ saline, saline + ketamine [10 mg/kg], and tramadol [5 mg/kg]+ ketamine [10 mg/kg], i.p.) on the immobility time of rats in the FST. Bars represent mean ± SD. $*P < 0.05$, the other three groups vs. the saline + saline group; $\#P < 0.05$, the tramadol + ketamine group vs. the saline + ketamine group.

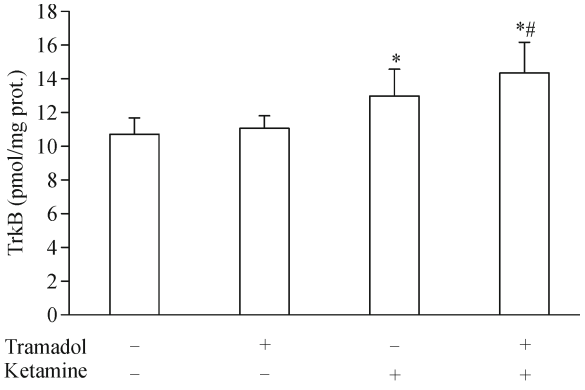

Fig. 3 Effects of different interventions (saline + saline, tramadol [5 mg/kg]+ saline, saline + ketamine [10 mg/kg], and tramadol [5 mg/kg]+ ketamine [10 mg/kg], i.p.) on the expression of hippocampal TrkB of rats in the FST. Bars represent mean ± SD. $*P < 0.05$, the other three groups vs. the saline + saline group; $\#P < 0.05$, the tramadol + ketamine group vs. the saline + ketamine group.

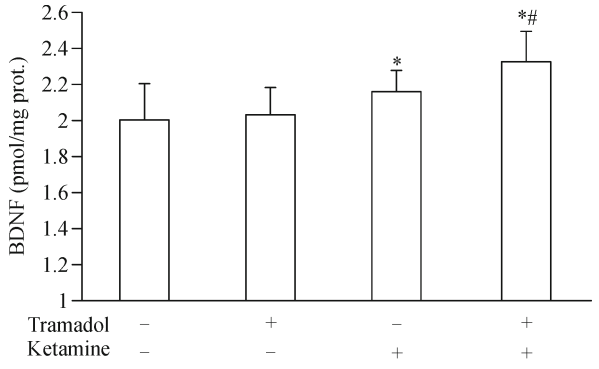

Fig. 2 Effects of different interventions (saline + saline, tramadol [5 mg/kg]+ saline, saline + ketamine [10 mg/kg], and tramadol [5 mg/kg]+ ketamine [10 mg/kg], i.p.) on the expression of hippocampal BDNF of rats in the FST. Bars represent mean ± SD. $*P < 0.05$, the other three groups vs. the saline + saline group; $\#P < 0.05$, the tramadol + ketamine group vs. the saline + ketamine group.

effects in the FST and other rat depression models[15,16]. Our preliminary experiment showed that the intraperitoneal administration of tramadol (10 mg/kg) alone presented antidepressant-like effects, while 5 mg/kg could not elicit any antidepressant potency in rats in the FST. Therefore, we chose ketamine at the dose of 10 mg/kg to elicit ketamine's antidepressant effects and administrated tramadol at a dose of 5 mg/kg to avoid tramadol's antidepressant effects in the present study.

The primary result of the present study was that tramadol pretreatment further reduced the ketamine-shortened immobility time. The secondary result was that the enhanced antidepressant effects of ketamine was associated with the increased levels of BDNF and TrkB in rat hippocampus.

Previous data suggest that tramadol and ketamine have potential to be novel antidepressants because they target monoaminergic transmitter and brain glutamate, respectively [17]. A preclinical study has shown that ketamine suppresses the serotonin transporter and norepinephrine transporter in cultured human embryonic kidney 293 cell [18]. Recently, Zarate *et al.* [1] have demonstrated that ketamine exerts rapid and robust antidepressant properties through upregulating the monoaminergic transmitter. Some studies have also found that tramadol has the ability to inhibit the reuptake of noradrenaline and serotonin in the central nervous system [4,17], which is also the main mechanism of the conventional antidepressants [19]. Moreover, Yalcin *et al.* [4] have observed that the increase of serotonin and norepinephrine neurotransmitter is the core mechanism of tramadol exerting its antidepressant effects. So, we suggested that tramadol elicited potential antidepressant effects via increasing monoamines in synaptic cleft and has a synergic effect with ketamine at this point [20].

BDNF plays an important role in the treatment of major depressive disorder. Increasing evidences suggest that common antidepressants have been implicated in the increased expression of BDNF and TrkB signaling in a ligand-dependent manner [21,22]. It has been reported that antidepressant agents significantly increase TrkB mRNA expression in the rodent brain [22]. Furthermore, several lines of evidence have revealed that the upregulation of BDNF in rat hippocampus is associated with ketamine-induced antidepressant effects [8,23]. However, Faron-Górecka and coworkers [24] indicate that the antidepressant potency of tramadol is related to dopamine and alpha 1-adrenoceptor

function, rather than upregulating the levels of mRNA encoding BDNF and TrkB. Acute and chronic stress decreases the neurotrophin levels in central nervous system, which was reversed by the antidepressants administration [5]. In our study, tramadol (5 mg/kg) administrated alone neither elicited significant antidepressant effects in FST, nor increased the levels of BDNF and TrkB. Interestingly, it showed that tramadol pretreatment enhanced the ketamine-induced antidepressant effects, which was associated with the increased expression of BDNF and TrkB in hippocampus.

In the present study, we showed a synergic effect of tramadol and ketamine in rats receiving FST. The upregulated levels of BDNF and TrkB would be one of the potential mechanisms for tramadol enhancing the ketamine-induced antidepressant effects. We concluded that tramadol pretreatment enhances ketamine-elicited antidepressant effects associated with the increased levels of BDNF and TrkB.

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