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Original article

# Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex

W. Zhou [1], N. Wang [1], C. Yang, X.-M. Li, Z.-Q. Zhou **, J.-J. Yang *

*Department of Anesthesiology, School of Medicine, Jinling Hospital, Nanjing University, No. 305, East Zhongshan Road, Nanjing 210002, China*

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ABSTRACT

Ketamine exerts fast acting, robust, and lasting antidepressant effects in a sub-anesthetic dose, however, the underlying mechanisms are still not fully elucidated. Recent studies have suggested that ketamine's antidepressant effects are probably attributed to the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. The present study aimed to observe the effects of AMPA receptor modulators on mammalian target of rapamycin (mTOR) and brain-derived neurotrophic factor (BDNF) expression during the procedure of ketamine exerting antidepressant effects. Therefore, we pretreated rats with NBQX, an AMPA receptor antagonist, or CX546, an AMPA receptor agonist, and subsequently observed the immobility time during the forced swimming test (FST) and the hippocampal and prefrontal cortical levels of mTOR and BDNF. The results showed ketamine decreased the immobility time of rats during the FST and increased the hippocampal and prefrontal cortical mTOR and BDNF. NBQX pretreatment significantly increased the immobility time and decreased the levels of mTOR and BDNF when compared with vehicle 1 (DMSO) pretreatment. CX546 pretreatment significantly decreased the immobility time and increased the levels of mTOR and BDNF when compared with vehicle 2 (DMSO + ethanol) pretreatment. Our results suggest ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex.



## 1. Introduction

Major depressive disorder (MDD) afflicts about 16% of the population and becomes one of the leading causes of total disability and economic burden [12]. Serotonin and/or norepinephrine reuptake inhibitors are widely used to alleviate the symptoms of depression in clinical practice. Unfortunately, the delayed onset time and the low remission rate of these conventional antidepressants are still major challenges [16,10]. Therefore, there is an urgent need to look for a fast-acting and effective antidepressant in the near future.

Ketamine, an antagonist of *N*-methyl-D-aspartate (NMDA) receptors, is often clinically used as an anesthetic agent especially for pediatric operation. Recently, mounting studies have shown that a sub-anesthetic dose of ketamine exerts rapid, robust, and lasting antidepressant effects in depressed animals and patients [7,14,20,23,26]. Furthermore, it is suggested that the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors is involved in the antidepressant effects of ketamine; however, the precise mechanisms remain ambiguous [2,15,17,18].

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that regulates the initiation of protein translation, and is expressed in dendritic development that controls new protein synthesis [8]. Li et al. [14] have reported that the activation of mTOR in prefrontal cortex underlies the antidepressant effects of ketamine in rats. Some case studies have also reported that mTOR is activated in depressed patients' peripheral blood after acute ketamine administration [5,27].

Brain-derived neurotrophic factor (BDNF) is a secreted protein, which acts on certain neurons in the nervous system, supports the survival of neurons, and activates the growth and differentiation of new neurons and synapses [1,11]. Garcia et al and Autry at al [3,9] have demonstrated that the upregulation of hippocampal BDNF contributes to the antidepressant effects of ketamine in depression models of rodents.

Considering that mTOR and BDNF are the two important biomarkers in the process of ketamine exerting antidepressant effects, we aimed to evaluate the changes in mTOR and BDNF in rat

* Corresponding author. Tel.: +86 25 52323834; fax: +86 25 84806839.
** Co-corresponding author.
*E-mail addresses:* zq_zhou@163.com (Z.-Q. Zhou), yjyangjj@126.com (J.-J. Yang).
[1] The two authors contributed equally to this work.

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brain after pretreatment with AMPA receptor antagonist or agonist during the antidepressant process of ketamine. As the alternations of hippocampus and prefrontal cortex contribute to the pathogenesis of mood disorders [19,25], we chose rat hippocampus and prefrontal cortex for the determination of mTOR and BDNF in the present study.

## 2. Materials and methods

### 2.1. Rats and groupings

Eighty adult male Wistar rats (200–300 g body weight) were purchased from the Shanghai Animal Center, Shanghai, China. The animals were housed 5 per cage with food and water available *ad libitum*, and were maintained on a 12 h light/dark cycle (lights on at 7:00 am). Rats in the present study were randomly divided into eight groups ($n = 10$ each): vehicle 1 + saline, vehicle 1 + 10 mg/kg ketamine, 5 mg/kg NBQX + 10 mg/kg ketamine, 10 mg/kg NBQX + 10 mg/kg ketamine, vehicle 2 + saline, 1 mg/kg CX546 + saline, vehicle 2 + 10 mg/kg ketamine, and 1 mg/kg CX546 + 10 mg/kg ketamine. Animals involved in this experiment were treated in accordance with the Guide for Care and Use of Laboratory Animals of National Institutes of Health.

### 2.2. Drugs and interventions

Ketamine was obtained from Gutian Pharmaceutical Company (Fujian, China). NBQX (an AMPA receptor antagonist) and CX546 (an AMPA receptor agonist) were purchased from Tocris company (UK). Animals submitted to the forced swimming test (FST) were intraperitoneally pretreated with vehicle 1 (DMSO), vehicle 2 (DMSO + ethanol), or drug (NBQX or CX546), respectively. Thirty minutes later, rats were intraperitoneally injected with ketamine 10 mg/kg or the same volume of saline, respectively, and were subjected to the behavioral test at 0.5 h after administration. Immediately after the FST, the rats were sacrificed and then the hippocampus and the prefrontal cortex were dissected and stored at −80 °C for later biochemical analysis.

### 2.3. FST

The FST was applied according to the previous documents [6,24]. Rats were exposed to a cylindrical tank with water in which rats cannot touch the bottom of the tank. The tank is 60 cm in tall, 30 cm in diameter, and was filled with water (22–23 °C) in a depth of 30 cm. Water in the tank was changed for each rat. All the procedures were conducted during 9:00–15:00. Rats were first placed in the water for 15 min (pretest session). Twenty-four hours later, rats were placed in the water for a 6-min session (test session). The immobility time during the last 5 min of the 6-min test was recorded in seconds by two expert observers who were blinded to the grouping.

### 2.4. Western blotting

Phosphorylated mTOR (p-mTOR) levels in hippocampus and prefrontal cortex were evaluated by Western blotting. Equal amount of proteins (10–20 μg) for each sample were loaded into 10–15% SDS PAGE gel for electrophoresis. Polyvinylidene difluoride (PVDF) membranes with transferred proteins were blocked with 2% BSA in PBST (PBS + 0.1% Tween-20) for 1 hr and kept with primary antibodies overnight at 4 °C. The following primary antibody was used: phosphor-mTOR (ser2448)(1:2000, Cell Signaling). The next day, blots were washed three times in PBST and incubated with horseradish peroxidase conjugated anti-mouse or anti-rabbit secondary antibody (1:5000 to 1:10000) for 1 hr. Bands were detected using enhanced chemiluminescence (ECL) and blots then were incubated in the stripping buffer for 30 min at 50–55 °C. The stripped blots were kept blocking solution for 1 hr and incubated with the primary antibody directed against total levels of the β-actin for loading control. Densitometric analysis of immunoreactivity for each protein was conducted using Image J software.

### 2.5. Enzyme-linked immunosorbent assay (ELISA)

BDNF levels in hippocampus and prefrontal cortex were measured by anti-BDNF sandwich-ELISA, according to the manufacturer instructions (Chemicon, USA). Briefly, rat hippocampus and prefrontal cortex was homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ethylene glycol tetraacetic acid (EGTA), respectively. Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in diluent and standard curve of BDNF ranged from 7.8 to 500 pg/mL. Beyond these limits, BDNF concentrations could not be accurately extrapolated from the standard curve. The amount of BDNF was determined by absorbance in 450 nm. The standard curve demonstrates a direct relationship between Optical Density (OD) and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

### 2.6. Statistical analysis

Data are expressed as mean ± S.E.M. and analyzed by the Statistical Product for Social Sciences (SPSS version 16.0, IL, USA). Comparisons were made by one-way analysis of variance followed by Bonferroni tests for post-hoc comparisons. Difference was considered to be significant at $P < 0.05$.

## 3. Results

Fig. 1 showed that compared with the vehicle 1 + saline group, the immobility time of rats decreased in the other three groups ($P < 0.05$). Pretreatment with NBQX 5 mg/kg and 10 mg/kg significantly attenuated the ketamine-induced decrease in the immobility time in the FST ($P < 0.05$).

Fig. 2 showed that compared with the vehicle 2 + saline group, the immobility time of rats decreased in the vehicle 2 + ketamine and CX546 + ketamine groups ($P < 0.05$), but did not in the CX546 + saline group ($P > 0.05$). Moreover, the immobility time

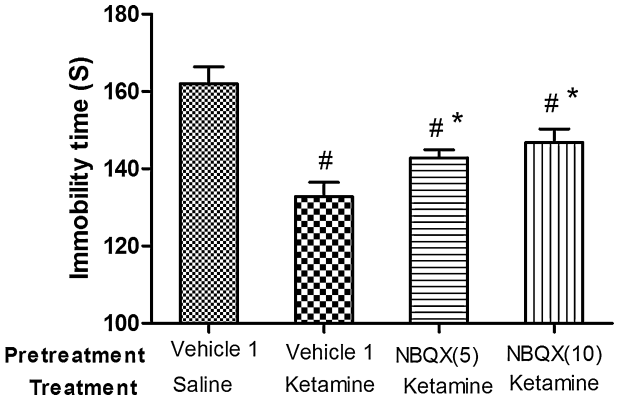


**Fig. 1.** Immobility time (mean ± S.E.M.) of rats during the FST after NBQX pretreatment. Each group had ten subjects. $^{\#}P < 0.05$, compared with vehicle 1 + saline; $^{*}P < 0.05$, compared with vehicle 1 + ketamine.