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Research article

Sub-anesthetic doses of ketamine exert antidepressant-like effects and upregulate the expression of glutamate transporters in the hippocampus of rats

Xianlin Zhu [a], Gang Ye [a], Zaiping Wang [a,*], Jie Luo [b], Xuechao Hao [b]

[a] Department of Anesthesiology, The Central Hospital of Enshi, Autonomous Prefecture, Enshi, Hubei, 445000, China
[b] Department of Anesthesiology, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

HIGHLIGHTS

- Excitatory amino acid transporters (EAATs)-mediated glutamate reuptake dysfunction involved in the pathogenesis of depression.
- Sub-anesthetic doses of ketamine induced antidepressant-like effects in rats.
- Ketamine up-regulated the expression of EAATs and decreased the concentration of extracellular glutamate in the hippocampus of depressive-like rats.

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ABSTRACT

Clinical studies on the role of the glutamatergic system in the pathogenesis of depression found that ketamine induces an antidepressant response, but the molecular mechanisms remain unclear. The present study investigated the effects of sub-anesthetic doses of ketamine on the glutamate reuptake function in the rat hippocampus. Chronic unpredictable mild stress (CUMS) was applied to construct animal models of depression. Sixty adult male Sprague-Dawley rats were randomly assigned to 5 groups and received a different regimen of CUMS and ketamine (10, 25, and 50 mg/kg) treatment. The sucrose preference test and open-field test were used to assess behavioral changes. The expression levels of excitatory amino acid transporters (EAATs) were measured by western blot. Microdialysis and high-performance liquid chromatography (HPLC) were used to detect hippocampal glutamate concentrations. We found that the expression of EAAT2 and EAAT3 were obviously downregulated, and extracellular concentrations of glutamate were significantly increased in the hippocampi of depressive-like rats. Ketamine (10, 25, and 50 mg/kg) upregulated the expression of EAAT2 and EAAT3, decreased the hippocampal concentration of extracellular glutamate, and alleviated the rats' depressive-like behavior. The antidepressant effect of ketamine may be linked to the regulation of EAAT expression and the enhancement of glutamate uptake in the hippocampus of depressive-like rats.

1. Introduction

Depression is a prevalent and disabling psychiatric illness that affects millions of individuals worldwide, resulting in enormous personal suffering and public health costs [1]. Traditional antidepressants such as monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs) usually take weeks to months to produce a therapeutic response, and more than 30% of patients with depression exhibit refractory or intolerant responses to current available antidepressant medications [2]. In contrast, recent clinical studies have demonstrated that the *N*-methyl-D-aspartate (NMDA) antagonist ketamine induces a rapid (within h) antidepressant response [3,4], and is effective in patients with major depressive disorder who are treatment-resistant to traditional antidepressants [5]. However, the molecular mechanisms underlying this process remain unclear.

Multiple lines of evidence have supported a critical role for the glutamatergic system in the pathophysiology of depression, and it is believed to be a key target in mood regulation [6,7]. Glutamate

* Corresponding author.
E-mail addresses: zhuxianlin8@sohu.com (X. Zhu), 343205560@qq.com (Z. Wang).

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is a critical excitatory neurotransmitter in the mammalian brain, and its reuptake is essential for normal synaptic transmission. High levels of extracellular glutamate can mediate excitotoxicity and is implicated in the pathogenesis of many brain diseases [8]. However, the clearance of released glutamate is not assumed by its synaptic degradation. Excitatory amino acid transporters (EAATs), also named glutamate transporters, transport glutamate from the extracellular to the intracellular spaces, thereby efficiently controlling the extracellular concentration of glutamate [9]. Currently, five distinct EAATs (EAAT1-5) that transport glutamate have been cloned. EAAT1 and EAAT2 are predominantly localized on astrocytes and abundant in the hippocampus and cerebral cortex. In contrast, EAAT3 is a neuronal transporter, which is expressed in the pre-and postsynaptic regions of neurons, while EAAT4 and EAAT5 appear mainly restricted to expression on the cerebellum and the retina, respectively [10]. Our previous study found that EAAT2 expression was markedly downregulated in the hippocampus of depressive-like rats [11]. However, whether the antidepressant effect of ketamine is related to regulating glutamate reuptake functions requires further study.

The aim of this study was to investigate the effects of ketamine on depressive behaviors in rats and the potential roles of EAAT-mediated glutamate reuptake function in this process.

2. Materials and methods

2.1. Animals

Healthy adult male Sprague-Dawley rats, weighing 200–250 g, aged 2–3 months, were obtained from the Laboratory Animal Center of Chongqing Medical University. The rats were housed and maintained in standard laboratory conditions (22 ± 2 °C and a 12:12-h light-dark cycle) with free access to feed and water for 1 week before further experiments. All the procedures were approved by the Ethics Committee of Chongqing Medical University and carried out according to the animal care guidelines of the National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Animal models of depressive-like behavior

Chronic unpredictable mild stress (CUMS) was applied to construct animal models of depressive-like behavior as previously described [12]. The rats were housed in individual cages and randomly exposed to one of the following stressors per day for 28 consecutive days: cold water swimming at 4 °C for 5 min; hot stress in an oven at 45 °C for 5 min; pinching the tail for 1 min; food deprivation for 24 h; water deprivation for 24 h; caged in a soiled cage for 24 h; shaking for 20 min (once per second); social crowding (25 rats per cage); cage being tilted to 30° from the horizontal for 24 h; and continuous lighting for 24 h. After the CUMS procedure, 48 rats with depressive-like behavior were obtained.

2.3. Experimental groups and treatments

A group of 12 healthy male rats (with same age and weight) were set as the control group (group C). Forty-eight depressive-like behavior rats were randomly assigned to four groups (n = 12): group D, group DK1, group DK2, and group DK3. Group C did not receive any treatment; rats in group D were treated with normal saline (10 ml/kg, i.p.); rats in group DK1 were treated with i.p. injection of 10 mg/kg ketamine (concentration at 1 mg/ml, No. KH091201, Jiangsu Hengrui Medicine, China); rats in group DK2 were treated with i.p. injection of 25 mg/kg ketamine; rats in group DK3 were treated with i.p. injection of 50 mg/kg ketamine. The aforementioned treatments were given once per day for 5 consecutive days.

2.4. Behavior test

2.4.1. Sucrose preference test

The sucrose preference test was performed as previously described to evaluate the anhedonia in rats (the core symptom of depression) [13]. In the first 24 h, rats were exposed to two bottles of 1% (w/v) sucrose solution to habituate them to consumption of a sucrose solution. In the next 24 h, one bottle of sucrose solution was replaced with a bottle of sterile water. After 23 h of water and food deprivation, each rat was exposed to two identical bottles with one containing 1% sucrose and the other one containing sterile water. All rats were allowed to drink water freely for 1 h. Sucrose preference percentage (SPP) was calculated according to the following formula: SPP (%) = [sucrose solution intake (ml)/(sucrose solution intake (ml)+sterile water intake (ml)] × 100.

2.4.2. Open-field test (OFT)

To evaluate spontaneous locomotor and exploratory activities of rats in a novel environment, the OFT was performed as described previously [14]. The open-field apparatus consisted of a black wooden square arena (100 × 100 × 50 cm) in a quiet room with dim illumination. The floor of the box was marked with a grid dividing it into 25 equal-size squares. Each animal was tested individually and only once in the apparatus. It was placed in the central square and observed for 5 min. Parameters assessed were horizontal ambulation (the number of squares crossed, indicating general locomotors) and the times of rearing (when a rat stood completely erect on its hind legs, indicating exploratory behavior). The OFT was performed and scored by trained and experienced observers who were blind to the diagnoses of the animals. The SPT and OFT was performed twice, once in the 24 h after CUMS treatments was completed, and the other in the 24 h after last time ketamine treatment.

2.5. Microdialysis and tissue preparation

After completion of the behavioral experiments, 6 rats were randomly selected from each group for use in the microdialysis study [15]. Briefly, the rats were anesthetized with 2% pentobarbital sodium (40 mg/kg, i.p.) and then fixed in a stereotaxic frame (Kopf Instruments, California). A microdialysis probe (MAB 4.15.2 Cu, Microbiotech, Sweden) was inserted unilaterally into the dorsal hippocampus. (A/P, −3.6 mm; L, 2.0 mm; D/V, −4.0 mm). The microdialysis pipeline was filled with artificial cerebrospinal fluid (NaCl 147 mmol/L, KCl 2.7 mmol/L, $CaCl_2$ 1.2 mmol/L, $MgCl_2$ 0.85 mmol/L) and continuously perfused at a flow rate of 2.5 μl/min by a microinfusion pump. After allowing the system to equilibrate for 1 h, samples (25 ml) were collected in tubes containing 2 ml of acetic acid and frozen (−20 °C) immediately for further analysis. After the completion of the microdialysis, all rats were killed under anesthesia with 2% pentobarbital sodium (50 mg/kg, i.p.). The bilateral hippocampi were quickly removed and immediately cooled in liquid nitrogen and stored in a refrigerator at −80 °C.

2.6. Western blotting analysis

Frozen hippocampi were weighed and homogenized in protein buffer consisting of 3 ml of radioimmunoprecipitation assay (RIPA) lyses buffer (US Biological, USA) and 30 μl complete cocktail protease inhibitor (Roche Molecular Biochemicals, Germany) per gram of tissue. After centrifugation with 12000 rpm at 4 °C for 10 min, the supernatant was collected and stored at −20 °C until used. Following a bovine serum albumin (BSA) micro assay (Pierce, Rockford, IL) and spectrophotometry to assess protein levels, every