

The effect of ketamine on microglia and pro-inflammatory cytokines in the hippocampus of depression-like rat

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ABSTRACT

Objectives

Mounting evidence suggests that activation of microglia and inflammatory response play an important role in the pathogenesis of depression. A single or repeated sub-anesthetic dose of ketamine administration induced fast and potent antidepressant effect. We investigated the effect of ketamine on microglia activation and pro-inflammatory cytokines levels in hippocampus of depression-like rats.

Methods

Forty-eight rats were equally randomized into four groups Control+saline group, Control+ketamine group, Chronic unpredictable mild stress (CUMS)+saline group and CUMS+ketamine group. 0.9% saline or 10 mg/kg ketamine was given once daily for 7 consecutive days. The sucrose preference test (SPT) open field test (OFT) and forced swimming test (FST) were performed before and day 7 after drug treatment. The hippocampus was subsequently harvested for the detection of levels of tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6 and levels of CD11b and Iba1. The CD11b-positive cells in the CA3 and DG region of the hippocampus were also stained by immunohistochemistry.

Results

CUMS induced the decrease of sucrose solution intake volume, the increase of the immobility time, the up-regulation of TNF- α , IL-1 β , IL-6, CD11b and Iba1 levels of the hippocampus and the increase of CD11b-positive microglia in the CA3 and DG region of the hippocampus. Ketamine administration could reverse this effect.

Conclusion

Ketamine's antidepressant effect on depression-like rats is accompanied by the inhibition of microglia activation and pro-inflammatory cytokines levels in the hippocampus.

Keywords

Depression, Ketamine, Microglia, Pro-inflammatory cytokines

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Introduction

Depression is a common clinical mental disorder [1], it is estimated that depression will be the second global burden of disease after ischemic heart disease by 2020 [2]. Traditional antidepressants need several weeks to produce antidepressant effect, however, risk of suicide increases during this time [3]. Therefore, there is an emergency need to exploit novel antidepressants with fast-acting and high remission rate.

Recent studies have shown the antidepressant effect of ketamine, acting as a nonselective N-methyl-D-aspartate (NMDA) receptor antagonist [4]. A single sub-anesthetic dose of ketamine administration displayed rapid and obvious antidepressant properties, but the antidepressant effect of ketamine just maintains a short time [4,5]. It has been demonstrated that repeated ketamine administration can maintain ketamine's antidepressant effects [6]. Ketamine can inhibit microglia activation stimulated by lipopolysaccharide (LPS) and pulmonary inflammatory responses induced by sepsis [7,8]. However, whether the microglia deactivation and anti-inflammatory cytokines mediate ketamine's antidepressant effect is unclear. We hypothesize that the microglia deactivation and down-regulation of pro-inflammatory cytokines may be one of the possible mechanisms underlying ketamine's antidepressant effects. Thus, we try to investigate the effect of ketamine on the inhibition of microglia activation and pro-inflammatory cytokines in the CUMS rats.

Materials and methods

■ Animals

Forty-eight male adult Sprague-Dawley rats (age of 50 days, weight of 200-250 g) were purchased from the Southwest Medical University Animal Centre. They were housed three per cage with food and water available ad libitum. The rats were maintained on a 12-h light/dark cycle (lights on at 7.00 a.m.) at a temperature of 23°C ± 1°C. They were left to acclimate for 7 days before subjected to CUMS. All experimental procedures were approved by the Animal Ethics Committee of Southwest Medical University and were performed in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health, USA.

■ CUMS and drug administration

The CUMS procedure was carried out according to previous reports [9]. The rats in CUMS group were exposed to 9 stressors with 1 time per day. The CUMS procedure sustained 6 weeks and the same stressor was not applied consecutively over two days to prevent rats from tolerance to the stressors. The stressors included: (1) cage tilting 45°C for 24 h, (2) wet bedding for 24 h (200 ml water to wet bed), (3) hot swimming at 45°C for 5 min, (4) cold swimming at 4°C for 5 min, (5) fasting for 24 h, (6) water deprivation for 24 h, (7) rotation for 60 min (at 30 revolutions per minute), (8) tail nip for 1 min (1 cm from the end of the tail), and (9) inversion of light/dark cycle for 24 h. The rats in control group were fed in a quiet room for 6 weeks except necessary procedures, such as routine cage cleaning.

After 6 weeks, 24 Control rats were equally randomized into Control+saline group and Control+ketamine group, 24 CUMS rats were equally randomized into CUMS+saline group and CUMS+ketamine group. 0.9% saline or 10 mg/kg ketamine was intraperitoneally injected once daily for 7 consecutive days [10].

■ Behavioral tests and tissue preparation

The behavioral tests were conducted after CUMS model and 7 days after drug administration.

Sucrose preference test

The anhedonia is the core symptom of depression, and it is reflected by the percentage of sucrose solution consumption. The rats were habituated two bottles of 1% sucrose solution for 24 h. The one bottle of sucrose solution was changed into water at the second day. The rats were allowed to drink 1% sucrose solution (100 ml) and the same volume of water for 3 h after fasting and water deprivation 24 h. Sucrose preference was defined as the percentage of sucrose solution consumption of the total fluid volume during 3 h.

Open-field test

The open field consists of a blue arena of 100 cm × 100 cm square floor surrounded by 50 cm high walls. The floor of the apparatus was divided into 25 equal squares (20 cm × 20 cm each) by white lines. Rats were gently placed into the center of floor, and the number of crossing and rearing activity performed by each rat was recorded by an expert observer for 5 minute.

Forced swimming test (FST)

The forced swimming test was conducted in a cylinder with 65 cm tall, 30 cm in diameter and filled with 40 cm-depth of water (22-23°). The FST was performed for 6 min, and the immobility time (in seconds) was recorded during the final 5min. The immobility time was defined as the time during which the rat stood still without struggling or used only essential movements to keep the head above water or contact the bottom more than 1 second.

After the final behavioral test, the rats were anesthetized with sodium pentobarbital and sacrificed. The skulls were removed and hippocampus was dissected after perfused with 4% paraformaldehyde and then stored in 70% alcohol until embedded with paraffin.

■ Enzyme-linked immunosorbent assay (ELISA)

TNF- α , IL-1 β and IL-6 levels in hippocampus were measured using ELISA-kit (Shanghai MLBIO Biotechnology, China). Briefly, rat hippocampus tissue was homogenized in iced RIPA buffer with PMSF, and protein concentration was measured by BCA protein assay (Bioworld Technology, USA), then according to the manufacturer's instructions operation, concentrations of TNF- α , IL-1 β and IL-6 were calculated by referring to a standard curve.

■ Western blotting

CD11b and Iba1 levels in hippocampus were measured by western blot analysis. The hippocampus tissue was homogenized in iced RIPA buffer with PMSF, and then protein concentration was measured by BCA protein assay (Bioworld Technology, USA). Protein samples were separated on 10% or 15% SDS-PAGE and transferred to polyvinylidene fluoride membrane (PVDF). Membranes were blocked with 5% non-fat milk for 1h and incubated with primary antibodies [CD11b (1:200, Santa Cruz, Iba1 (1:1000, ProteinTech Group, Chicago, USA, GAPDH (1:3000; ProteinTech Group, Chicago, USA)] at 4°C overnight. The membranes were washed with TBST buffer and incubated with secondary antibody (1:5000) at room temperature for 1 h. The intensity of the protein bands was quantified using Image J software.

Immunohistochemistry (IHC)

CD11b-positive cells in the CA3 and DG region of the hippocampus were measured by

immunohistochemistry. The rat hippocampus was embedded in paraffin, and the paraffin blocks were sectioned at 5 μ m. The sections were deparaffinized, ethanol hydrated, and submitted to heat-induced antigen retrieval. 3% hydrogen peroxide was used to quench endogenous peroxidases, and the sections were rinsed with PBS, and blocked with 10 % norm goat serum, and subsequently incubated with the primary antibodies of CD11b (Santa Cruz, USA), and then incubated with the corresponding goat anti-mouse secondary antibody (Santa Cruz, USA). 3,3-diaminobenzidine (DAB) was used for the chromogenic reaction. Finally, the sections were dehydrated, cleared, and coverslipped. Images were captured with OLYMPUS digital imaging system (40 \times). The immunopositive cells were counted within 5-image (1360 \times 1024 pixels) of the hippocampal CA3 and DG region.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 16.0 and GraphPad Prism 5.0 were used for statistical analysis. All data were presented as mean \pm SEM. Differences between two groups were determined by Student's *t*-test and multigroup were determined by one-way ANOVA followed by Tukey's post hoc test. *p* values less than 0.05 were considered as statistically significant differences.

Results

■ Effects of CUMS exposure and ketamine treatment on the behavioral indexes

As depicted in **Figure 1**, compared with the control, rats in CUMS group displayed the significant decrease of 1% sucrose solution intake volume ($t=5.624$, $P<0.001$, **Figure 1a**) and the increase of immobility time in FST ($t=5.597$, $P<0.001$, **Figure 1b**). Compared with the CUMS+Saline group, rats in CUMS +Ketamine group displayed the significant increase of 1% sucrose solution intake volume ($F=6.522$, $P=0.0014$, **Figure 1a**) and the decrease of immobility time in FST ($F=5.624$, $P=0.01$, **Figure 1b**). However, CUMS exposure and ketamine administration did not affect the number of crossings ($t=0.7908$, $P=0.4346$; $F=0.6773$, $P=0.5114$; **Figure 1c**) and rearings ($t=0.6700$, $P=0.5030$; $F=0.4896$, $P=0.6920$; **Figure 1d**) in OFT.

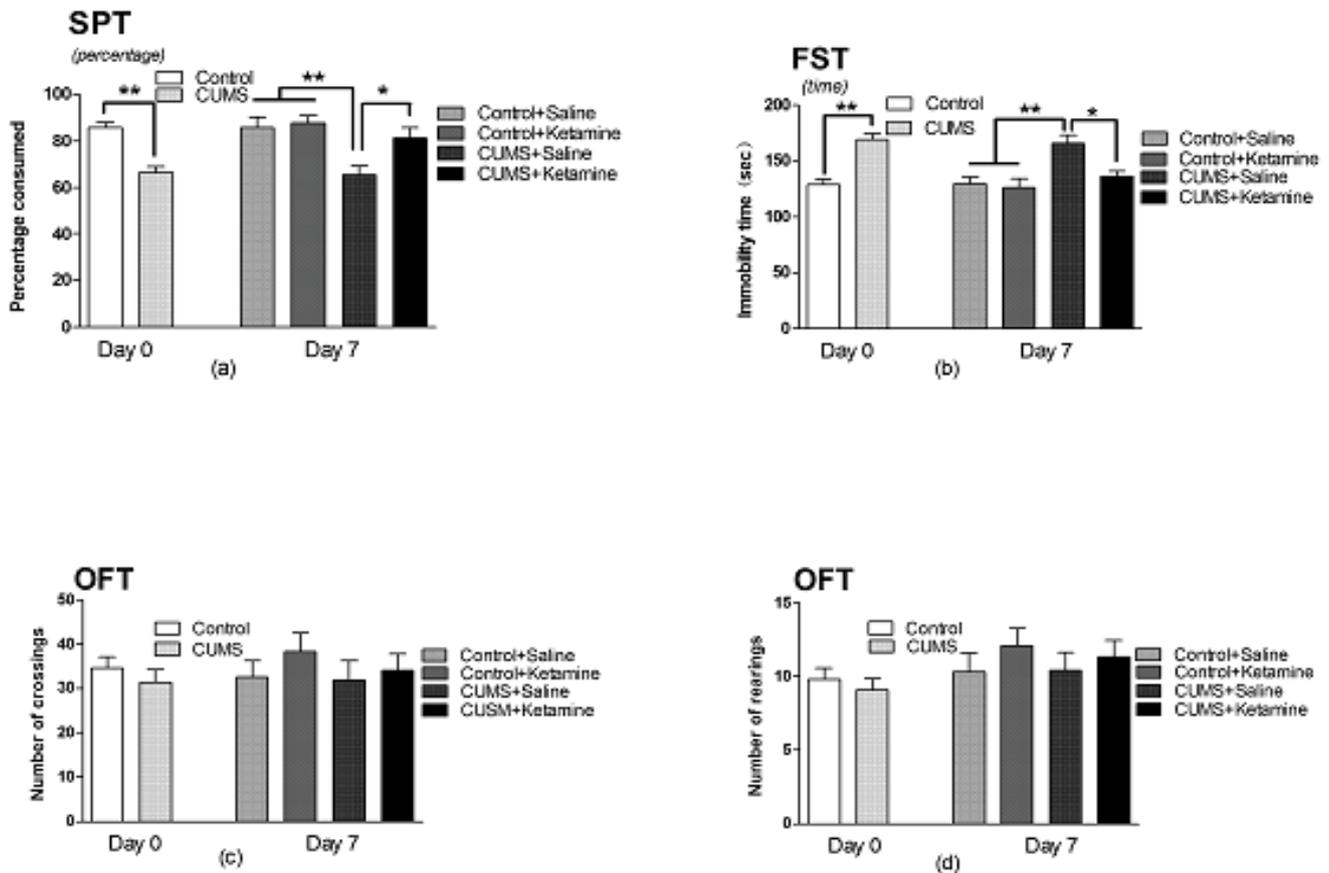


Figure 1: Effects of CUMS exposure (Day 0=after six weeks of CUMS) and chronic administration of Ketamine (Day 7=after 7 days ketamine administration) on the percentage of sucrose solution consumption (a), immobility time (b), number of crossings (c) and rearings (d). Data are presented as the mean \pm SEM of 12 rats per group. * $p < 0.05$, ** $p < 0.01$.

■ **Effects of ketamine on the levels of TNF- α , IL-1 β and IL-6 in the hippocampus**

As depicted in **Figure 2**, compared with the control group, the levels of TNF- α , IL-1 β and IL-6 were significantly increased in the CUMS rat hippocampus ($F=5.330$, $P=0.0073$, **Figure 2a**; $F=4.736$, $P=0.0118$, **Figure 2b**; $F=6.725$, $P=0.0026$, **Figure 2c**). Interestingly, repeated ketamine treatment significantly attenuated the increase of TNF- α , IL-1 β , and IL-6 levels in the hippocampus of CUMS rats (**Figure 2a**, **Figure 2b** and **Figure 2c**).

■ **Effects of ketamine on the levels of CD11b and Iba1 in the hippocampus**

As depicted in **Figure 3**, compared with the control group, the levels of CD11b ($F=7.411$, $P=0.0016$, **Figure 3b**) and Iba1 ($F=4.735$, $P=0.0118$, **Figure 3b**) were significantly increased in the hippocampus of CUMS rat. Moreover, repeated ketamine treatment reversed the increase of CD11b and Iba1

levels in the hippocampus of CUMS rats (**Figure 3b**).

■ **Effects of ketamine on CD11b-positive cells in the CA3 and DG region of the hippocampus**

As depicted in **Figure 4**, compared with the control, the CD11b-positive cells were significantly increased in the CA3 ($F=5.213$, $P=0.0080$, **Figure 4b**) and DG ($F=4.914$, $P=0.0102$, **Figure 4b**) regions of the hippocampus in the CUMS rat. The increase of CD11b-positive cells in CA3 and DG regions of the hippocampus were reversed by repeated ketamine treatment (**Figure 4b**).

Discussion

Recently, preclinical and clinical studies have demonstrated that ketamine have fast and obvious antidepressant effects [11], but the mechanisms are still unclear. Relative studies reveal that neuroinflammation may contribute

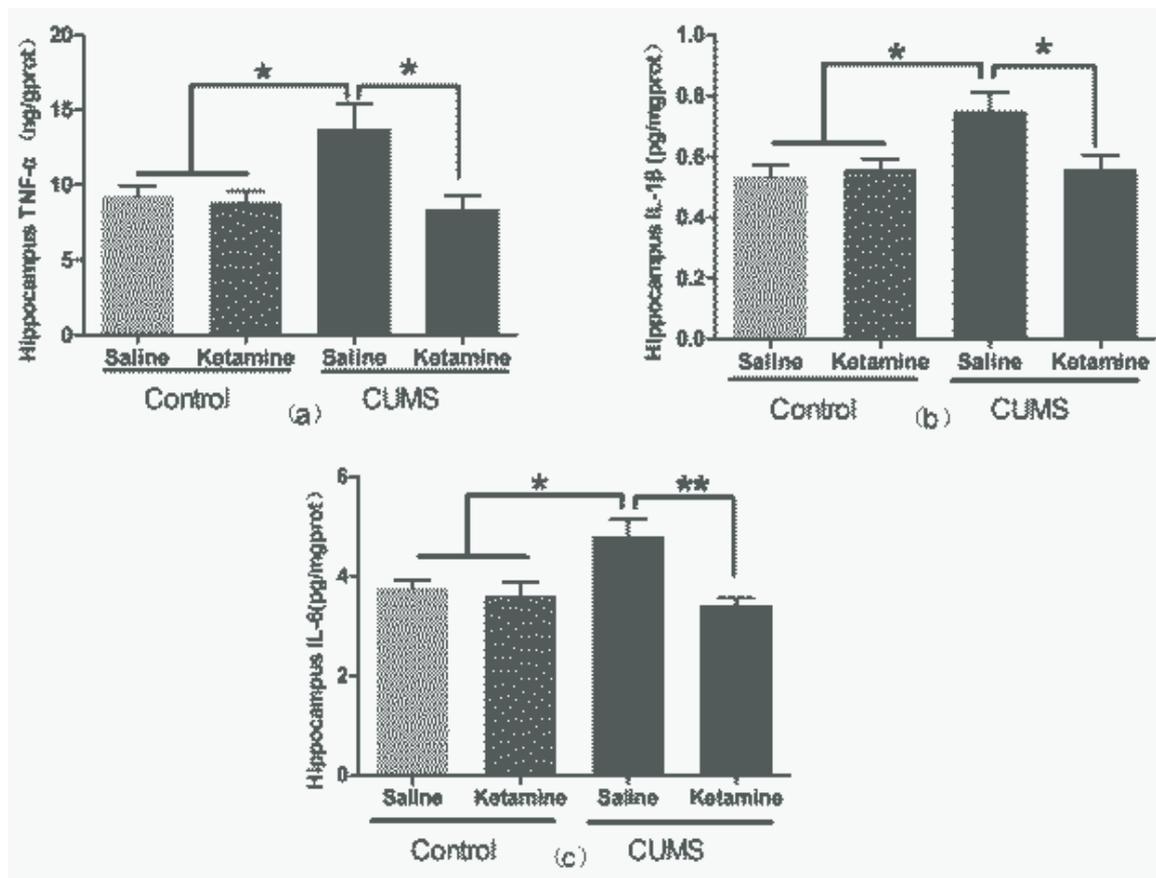


Figure 1: Effects of ketamine on the levels of TNF- α (a), IL-1 β (b), and IL-6(c) in the hippocampus. Data are presented as the mean \pm SEM of 6 rats per group. *p<0.05, **p<0.01.

to depression etiology [12]. As is well-known, the microglia plays a key role in inflammation response of CNS [13]. And several researches have reported that ketamine could inhibit inflammatory cytokine release in systemic inflammatory response syndrome [14,15]. Thus, we hypothesized that ketamine's antidepressant effects may be related to its anti-inflammation action in CNS. We investigated the effect of ketamine on microglia activation and pro-inflammatory cytokines release in CNS on depression-like animal model.

There are limitations in methodology and ethics on the human central nervous system. Therefore, the establishment of animal model about CNS disorders is widely used for further research [16,17]. CUMS model is widely used in depression research [18]. So in the present study, we chose CUMS procedure to establish the depression-like rat model. Clinical stress-related depressive patients often have obvious depressive mood. Many behavior tests, such as

the percentage of sugar solution consumption (which reflects the emotional activities about interest or anhedonia) and immobility time in FST (which reflects desperate state) were used to evaluate the depression-like state of CUMS rats. There was no difference between control and CUMS at the baseline of behavioral test before the CUMS exposure, but after the CUMS, compared with Control, rats in CUMS significantly displayed the decrease of sucrose solution intake volume and the increase of immobility time. Our results also showed that the CUMS model was successfully established.

Several researches have shown that inflammatory processes were involved in the pathogenesis of major depression [19,20]. Interestingly, the pharmacological antidepressant treatment was associated with the reduction of inflammatory cytokines levels and depressive symptoms [21]. Dowlati *et al.* found the pro-inflammatory cytokines of TNF- α and IL-6 in

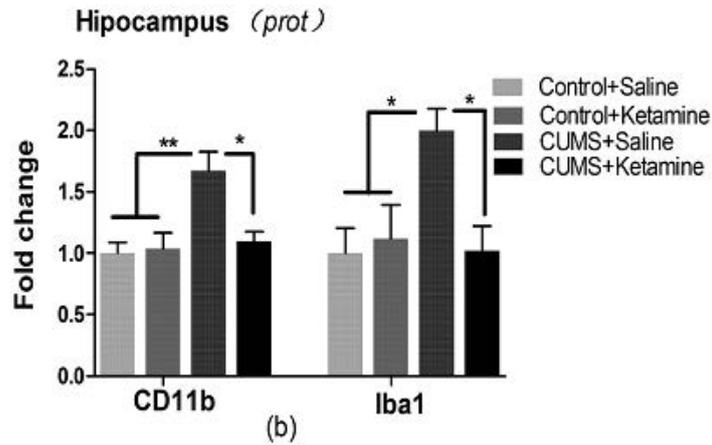
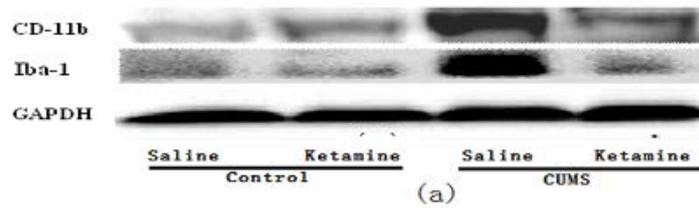


Figure 3: Effects of ketamine on the levels of CD11b and Iba1 in the hippocampus. (a) The expression of CD11b and Iba1 in the hippocampus of the four groups. (b) Quantitative results of CD11b and Iba1 immunoblots. Data are presented as the mean \pm SEM of 6 rats per group. * p <0.05, ** p <0.01.

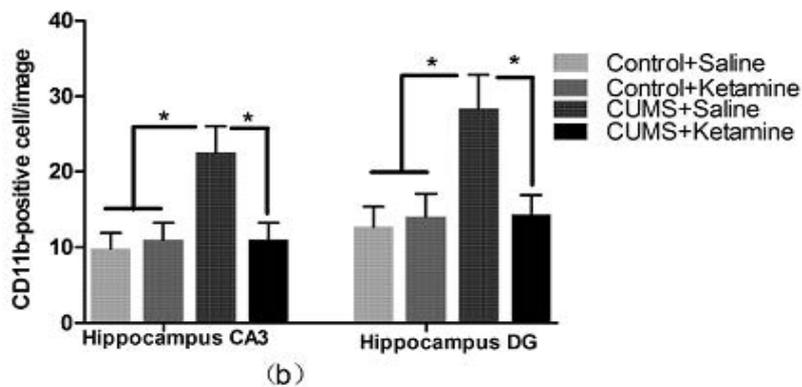
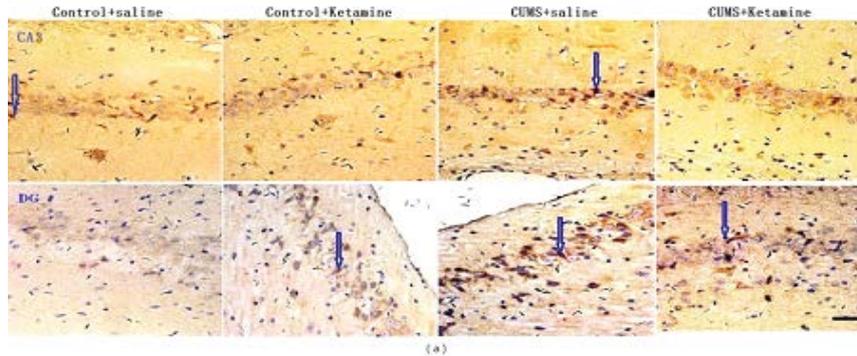


Figure 4: Effects of ketamine on CD11b-positive cells in the CA3 and DG regions of the hippocampus. (a) Appearance of CD11b-positive cells in the CA3 and DG regions of the hippocampus. Scale bar: 50 μ m. (b) The results are the mean \pm SEM of 5 images in the CA3 and DG regions of the hippocampus randomly selected from 6 rats in each group. * p <0.05.

depressive patients were significantly higher than that in normal subjects [22]. Maes *et al.* have reported that psychological stress increased the levels of TNF- α , IL-6, IL-1 receptor antagonist (IL-1Ra), interferon gamma (IFN-gamma) and IL-10 in subjects [23]. Animal researches also supported that stress could increase the levels of inflammation cytokines such as TNF- α and IL-6 in periphery or the central nervous system [24,25]. In the present study, we also demonstrated that CUMS exposure increased the levels of TNF- α , IL-6 and IL-1 β in hippocampus.

Previous studies have shown that active microglia played an important role in the neuroinflammation response because the active microglia may lead to the up-regulation of CD11b and Iba1, and produce neurotoxicity and pro-inflammatory cytokines [26,27]. Postmortem study results provided the evidences that microglia was activated in the anterior cingulate cortex, frontal cortex and hippocampus of patients with depression [28,29]. Therefore, active microglia and levels of pro-inflammatory cytokines may be used as predictors to evaluate antidepressant treatment [30,31]. Chronic stress was associated with microglia activation and morphological change [32,33]. Our study also showed that the CUMS exposure induced microglia activation in hippocampus (up-regulation of protein CD11b and Iba1).

A single sub-anesthetic dose of ketamine administration showed rapid and obvious antidepressant properties, but just maintained a short time [4,5]. Graeme *et al.* have reported sub-anesthetic dose of ketamine administration for 5 consecutive days relieved patients' depressive symptoms quickly and maintained several weeks [34]. Garcia *et al.* have demonstrated that repeated ketamine administration also produced antidepressant effect [35]. In our study, sub-anesthetic dose of ketamine administration for 7 consecutive days showed remarkable relief of behavioral changes induced by CUMS exposure, and was associated with the inhibition of microglia and pro-inflammatory cytokines in hippocampus, and it has been reported that ketamine may decrease the levels of inflammation cytokines in sepsis patients

[36], inhibit the activation of microglia and the levels of inflammation cytokines in cells induced by lipopolysaccharide (LPS) [7,8]. The mechanism may be that the pro-inflammation cytokines induced indoleamine 2, 3-dioxygenase (IDO) activation (the limited enzyme in kynurenine pathway), further activated kynurenine pathway to produce neuroactive metabolites and disturb the homeostasis between monoamine and glutamate neurotransmitter, which plays a key role in the pathogenesis of depression [37-39]. In addition, the pro-inflammation cytokines also promoted release of adrenocortical hormone to activate the hypothalamic pituitary gonadal axis (HPA), hyperactive HPA may induce hypercortisolemia and elicit symptoms of depression [40,41]. Therefore, these results showed that ketamine's antidepressant effects were associated with the inhibition of microglia activation and pro-inflammatory cytokines in the CUMS rats.

In conclusion, CUMS exposure elicited the depression-like symptoms and was associated with the microglia activation and the increase of pro-inflammatory cytokines levels in the hippocampus. The repeated ketamine administration relieved the depression-like symptoms and was associated with the inhibition of microglia activation and pro-inflammatory cytokines levels in the hippocampus. The anti-inflammatory effect of ketamine in CNS may be one of the possible mechanisms underlying ketamine's antidepressant effects.

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Conflict of interest

The authors have no potential conflicts of interest to disclose.

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