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Neuronal injury, but not microglia activation, is associated with ketamine-induced experimental schizophrenic model in mice

Yue Hou, Hongli Zhang, Guanbo Xie, Xinyue Cao, YaNan Zhao, Yang Liu, Zhihao Mao, Jingyu Yang *, Chunfu Wu *

Department of Pharmacology, Shenyang Pharmaceutical University, 110016 Shenyang, PR China

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ABSTRACT

Schizophrenia is a chronic debilitating psychiatric disorder affecting as many as 1% of the population worldwide. Unfortunately, its etiology and pathophysiology are poorly defined. Previous studies have shown that neuronal injury and microglia activation were observed in the schizophrenic patients. The present study aims to evaluate the role of neurons and microglia in ketamine-induced experimental schizophrenic model to further understand its pathophysiology. Firstly, ketamine was used to simulate the behavior abnormalities associated with schizophrenia. The effects of ketamine on mouse locomotor activity, Y-maze task, novel object recognition, and forced swimming test were studied. The results showed that ketamine (25, 50, and 100 mg/kg i.p.) administered acutely or repeatedly (for 7 days) can increase the locomotor number significantly. In Y-maze task, ketamine (25, 50, and 100 mg/kg) impaired spontaneous alternation after both acute and repeated treatments. In novel object recognition test, acute or chronic ketamine treatment showed no significant effect on mouse exploratory preference behavior. In forced swimming test, repeated treatment of ketamine (100 mg/kg) enhanced the immobility duration. Secondly, immunohistochemical method was used to study the changes of neurons and microglia. The results showed that acute treatment of ketamine (100 mg/kg) had no effect on neurons in the prefrontal cortex or hippocampus (1, 3, 5, and 7 days after the treatment). In contrast, repeated treatment of ketamine caused neuronal impairment in mouse hippocampus (3rd day, 5th day and 7th day after the final administration). The results of immunohistochemistry demonstrated that microglia in the prefrontal cortex and hippocampus were not affected after acute or repeated administration of ketamine. Finally, the neuronal impairment caused by repeated administration of ketamine was further investigated from the oxidative stress aspects. The results showed that repeated administration of ketamine increased nitric oxide (NO) and nitric oxide synthase (NOS) in prefrontal cortex, hippocampus and serum, while decreased SOD in hippocampus and serum. In summary, chronic ketamine treatment to mice successfully mimics the core behavioral deficits in schizophrenia. It is demonstrated for the first time that neuronal injury was associated with the chronic ketamine-induced experimental schizophrenic model, while microglial cells may play a little role in this model. Oxidative stress may contribute to the significant neuronal injury in mouse brain induced by chronic ketamine treatment.

1. Introduction

Schizophrenia is a chronic debilitating psychiatric disorder affecting as many as 1% of the population worldwide (Reus, 2008). Typical symptoms of schizophrenia can be separated into positive symptoms (e.g., hallucinations, delusions, and thought disorder), negative symptoms (e.g., deficits in social interaction, emotional expression, and motivation), and cognitive dysfunction (e.g., impaired attention/information processing, problem-solving, processing speed, verbal and visual learning, and memory and working memory) (Nuechterlein et al., 2004; Pearlson, 2000).

At present, the pathophysiology of schizophrenia is only partially understood and the investigation of it relies on suitable animal models, which simulate core behavioral aspects of human psychosis associated with schizophrenia. For example, amphetamine, used extensively in modeling psychosis, was used quite successfully in bringing forth the concept of dopamine as one of the prominent players in the pathophysiology of schizophrenia (Carlsson et al., 1997). However, it does not induce the negative symptoms of schizophrenia (Sams-Dodd, 1998). In contrast, N-methyl-D-aspartate (NMDA) receptor antagonists, such as ketamine, phencyclidine (PCP), and MK-801 were reported to induce a wider spectrum of behavioral responses that encompass positive, negative, and cognitive schizophrenia-like symptoms in healthy human

* Corresponding authors at: Department of Pharmacology, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, PR China. Tel./fax: +86 24 23843567.
E-mail addresses: yangjingyu2006@gmail.com (J. Yang), wucf@syphu.edu.cn (C. Wu).

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volunteers (Javitt and Zukin, 1991) and rodents (Chatterjee et al., 2011). Thus, NMDA receptor antagonist-induced animal models have become an important tool of choice to study the pathophysiology of schizophrenia.

As one of the non-competitive NMDA receptor antagonists, ketamine is known for its strong psychotomimetic effects in humans and rodents (Krystal et al., 1994). Controlled administration of ketamine in healthy volunteers leads to positive, negative, and cognitive symptoms similar to those observed in schizophrenic patients (Krystal et al., 2003). Subanesthetic ketamine administration also induces behavioral alterations in animals. Acute administration of ketamine impairs attentional set-shifting in rodents (Kos et al., 2011; Nikiforuk et al., 2010). Both acute and chronic treatments of ketamine can induce hyperlocomotor response and reduce the transfer-latency time in passive avoidance test (Chatterjee et al., 2011). In a recent study, an increased stereotyped activity and grooming with a decreased rearing as compared to controls were observed in mice treated subchronically with subanesthetic doses of ketamine (Rao et al., 2012).

A variety of lines of converging evidence implicates that neuronal injury is associated with schizophrenia. For example, a decrease in basilar dendrites of pyramidal cells has been found in schizophrenic medial prefrontal cortex (Broadbelt et al., 2002). It was reported that the schizophrenia subjects had 40% fewer total ring intersections per neuron than comparison subjects and a smaller basilar dendritic field size was evident in proximal and distal branches, which indicated that abnormal dendritic outgrowth or maintenance contributes to reduced neuropil and prefrontal connectivity in schizophrenia (Black et al., 2004). Glantz and Lewis have reported a decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia (Glantz and Lewis, 2000).

In recent years, microglial cells have shown to be involved in many CNS illnesses. Monji et al. have proposed the microglia hypothesis of schizophrenia (Monji et al., 2009). It was found that the number of activated microglia was higher in schizophrenic brains than in control brains (Wierzba-Bobrowicz et al., 2005). A quantitative positron emission tomography study has shown microglia activation in recent-onset schizophrenia (van Berckel et al., 2008). Postmortem study reveals the increased microglia densities in schizophrenic patients who had committed suicide (Steiner et al., 2006). However, inconsistent role of microglia in schizophrenia has been also observed. A postmortem study has revealed that microglia show no statistically significant differences between the patients with schizophrenia and the control patients (Arnold et al., 1998).

Although ketamine has been used to mimic the schizophrenia-like symptoms in rodents, the characteristic of this model is still not well-defined. Moreover, whether or not the neurons or microglia is involved in ketamine-induced schizophrenia-like symptoms is not clear. Therefore, in the present study, we attempted to evaluate the role of neurons and microglia in ketamine-induced experimental schizophrenic model.

2. Materials and methods

2.1. Animals

Male Swiss–Kunming mice with body weight of 25–30 g were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. The animals were housed under standard conditions with a 12–12 h light–dark cycle (lights on 7 h) and free access to food and water. The mice were used for the behavioral experiments after they had been adapted to laboratory conditions for at least 5 days. All animal use procedures were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University.

2.2. Drugs

Ketamine hydrochloride (Fujian Gutian Medicine Co., Ltd.) was dissolved in 0.9% saline and i.p. in a volume of 0.1 ml/10 g body weight. Controls received 0.9% saline alone.

2.3. Experimental protocol

The animals were randomly divided into control group and ketamine groups (25, 50, 100 mg/kg, i.p.). In the acute experiment, mice in the control or ketamine groups were treated with 0.9% saline or ketamine only once. In the chronic experiment, mice in the control or ketamine groups were treated with 0.9% saline or ketamine once daily for 7 days, respectively.

2.4. Behavioral assessment

2.4.1. Locomotor activity

The locomotor activity was tested as described earlier (Hou et al., 2006). Mice were randomly assigned to each group. After the initial 10-min habituation process, the mice were treated with 0.9% saline or ketamine and then the locomotor activity of the mice was measured in a locomotor monitoring cage (25 × 25 × 25 cm, Shanghai Mobiledatum Information Technology Co., Ltd., China) for 60 min. The experimenter was blind to the medication status. The animal's movement was recorded and analyzed using a computerized video-tracking system (Ethovision@ 8.0, Noldus Information Technology, Wageningen, Netherlands).

2.4.2. Forced swimming test

The forced swimming test was performed as previously reported (Noda et al., 1997, 2000). In brief, mice were placed individually in glass cylinders (20 cm height, 12 cm diameter) containing 10 cm depth of water at 25 °C. After 5 min, the animals were removed from water, dried and returned back to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured for 3 min. Mice which were floating motionless were considered to be immobile. The experimenter was blind to the medication status. The animal's movement was recorded and analyzed using a computerized video-tracking system (Ethovision@ 8.0, Noldus Information Technology, Wageningen, Netherlands).

2.4.3. Y-maze task

Spontaneous alternation was assessed in the Y-maze task (Bild et al., 2013; Józwiak et al., 2006). Each arm of the maze was 38 cm long, 12 cm high and 5 cm wide, and converged to an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The total number of arm entries and alternation (defined as consecutive entries into all three arms without repetitions) was scored. Total number of arm entries was collected cumulatively over 8 min. The percent alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries − 2) × 100. In the acute experiment, mice were injected once with 0.9% saline or ketamine 30 min before the test session in the Y-maze. In the chronic experiment, the Y-maze task was done 30 min after the final injection of 0.9% saline or ketamine. The experimenter was blind to the medication status.

2.4.4. Novel object recognition (NOR) test

NOR test was performed as previously reported (Hashimoto et al., 2005; Kunitachi et al., 2009). The apparatus for this task consisted of a black open field box (50 × 50 × 50 cm). The procedure for the novel object recognition test consisted of different sessions: habituation, training and retention. Before the test, mice were habituated in the box for 3 days. During a training session, two objects (various objects differing in shape and color but similar in size) were placed in the box

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