Behavioural Brain Research 316 (2017) 1–10

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Antidepressant-like effects of long-term sarcosine treatment in rats with or without chronic unpredictable stress

Kuang-Ti Chen[a,1], Ching-Hsiang Wu[b], Mang-Hung Tsai[c,1], Ya-Chieh Wu[d], Ming-Jia Jou[e], Chih-Chia Huang[f,g,h,**], I-Hua Wei[c,*]

[a] Institute of Basic Medical Science, China Medical University, Taichung, Taiwan
[b] Department of Anatomy, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Department of Anatomy, China Medical University, Taichung, Taiwan
[d] Department of Nursing, Ching-Kuo Institute of Management and Health, Keelung, Taiwan
[e] School of Chinese medicine for post baccalaureate, I Shou University, Kaohsiung, Taiwan
[f] Department of Psychiatry, China Medical University Hospital, Taichung, Taiwan
[g] Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan
[h] Department of Psychiatry, China Medical University, Taichung, Taiwan

HIGHLIGHTS

- Long-term sarcosine treatment exerts antidepressant-like effects in the forced swim test in chronic unpredictable stress (CUS)-exposed rats but not in naive rats.
- Long-term sarcosine treatment increases the expression of the mTOR signaling-related proteins and increases AMPAR membrane insertion in the hippocampus in both naive rats and CUS-exposed rats.
- The distinct sensitivity to long-term sarcosine treatment in rats with or without CUS is found after long-term sarcosine treatment, which is not associated with the activated mTOR signaling pathway or increased AMPAR membrane insertion.

ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form 31 May 2016
Accepted 2 June 2016
Available online 21 August 2016

Keywords:
Sarcosine
Long-term treatment
mTOR
AMPA
Depression

ABSTRACT

Sarcosine, an *N*-methyl-D-aspartate receptor enhancer, can improve depression-like behavior in rodent models and depression in humans. We found that a single dose of sarcosine exerted antidepressant-like effects with rapid concomitant increases in the mammalian target of rapamycin (mTOR) signaling pathway activation and enhancement of α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor (AMPAR) membrane insertion. Sarcosine may play a crucial role in developing novel therapy for depression. For a detailed understanding of sarcosine, this study examined the effects of long-term sarcosine treatment on the forced swim test (FST), mTOR signaling, and AMPAR membrane insertion in rats. The effects of long-term sarcosine treatment were examined in naive rats and rats exposed to chronic unpredictable stress (CUS). Long-term sarcosine treatment (560 mg/kg/d for 21 d) significantly ameliorated the increased immobility induced by CUS in the FST, reaffirming the potential role of sarcosine as an antidepressant for depressed patients. The same long-term treatment exhibited no such effect in naive rats despite increased mTOR activation and AMPAR membrane insertion in both groups. Our findings clearly show CUS-exposed rats are sensitive to long-term sarcosine treatment in FST and the response at the same dose is absent in naïve rats.

* Corresponding author at: Department of Anatomy, China Medical University, No. 91 Hsueh-Shih Road, Taichung, Taiwan.
** Corresponding author at: Department of Psychiatry, China Medical University Hospital, Taichung, Taiwan, No. 2, Yuh-Der Road, Taichung, Taiwan.
E-mail addresses: chihchiahuang@yahoo.com.tw (C.-C. Huang), ihwei@mail.cmu.edu.tw (I.-H. Wei).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.bbr.2016.06.004

Nevertheless, the distinct sensitivity to long-term sarcosine treatment in rats with or without CUS is not associated with the activated mTOR signaling pathway or increased AMPAR membrane insertion. Additionally, understanding the behavioral and molecular basis of distinct responses is vital important for developing personalized treatment programs to increase the probability of success when treating depression.

1. Introduction

Recently, several glutamatergic system modulators acting at *N*-methyl-D-aspartate receptors (NMDARs), including NMDAR antagonists, NMDAR partial agonists, an NMDAR coagonist, and reversible glycine transporter inhibitors, have been shown to be efficacious in patients with major depressive disorder and in preclinical studies [1–12]. Sarcosine, an endogenous amino acid, is a competitive inhibitor of the type I glycine transporter (GlyT1) [13] and an NMDAR coagonist [14]. Because of these 2 properties, sarcosine can enhance NMDAR function. We previously conducted both rodent behavior tests and a trial of sarcosine treatment in patients with major depression and demonstrated that sarcosine treatment elicits similar antidepressant-like effects in both acute and chronic stress models of depression and achieves a much higher remission rate than that achieved by standard selective serotonin reuptake inhibitor (SSRI) treatment for major depression [15]. Subsequently, we found that a single dose of sarcosine rapidly exerted antidepressant-like effects with a concomitant increase in the mammalian target of rapamycin (mTOR) signaling pathway activation and enhancement of α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor (AMPAR) membrane insertion in the hippocampus [16]. The involvement of the mTOR signaling pathway or AMPAR membrane insertion may be a convergent change induced by antidepressant drugs [2,17–23]. Moreover, the mechanism of the antidepressant sarcosine is similar to that of several glutamatergic system modulators with rapid antidepressant potential [2,17,19,20]; thus, sarcosine may be an attractive option for next-generation antidepressants.

To date, the effect of sarcosine has not been extensively studied. The effects of long-term sarcosine treatment on mTOR signaling activation or AMPAR membrane insertion and the relationship between antidepressant-like effects have not been investigated. Long-term treatment may be more similar to that in clinical practice, where long-term administration may be required for producing and maintaining clinical therapeutic effects; therefore, more compelling evidence of the antidepressant-like effect of long-term treatment is required. Thus, to determine its potential as an antidepressant in humans, we investigated the antidepressant-like activity of long-term sarcosine administration in the forced swim test (FST) in naive rats and rats exposed to the chronic unpredictable stress (CUS) paradigm and studied its effect on the mTOR signaling pathway and AMPAR membrane insertion by measuring the expression of phospho-mTOR (pmTOR), phospho-extracellular signal-regulated protein kinase (pERK), phospho-Akt (pAkt), and phospho-AMPA GluR1 serine845 (pGluR1ser845) in the hippocampus.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 250–350 g, were used for the experiments. Five rats were housed per cage, with food and water available ad libitum in the laboratory animal center, and were maintained on a 12-h light–dark cycle (light, 07:00–19:00) at 23 °C ± 1 °C in a 60% humidity-controlled environment. After at least a 7-day acclimation period in the center, the rats were transferred to the testing room and were immediately used for subsequent experiments. The study protocol was approved by the Institutional Animal Care and Use Committee of China Medical University, Taiwan.

2.2. Drugs

Sarcosine (Merck Millipore, #807666) at a dose of 560 mg/kg or saline was injected intraperitoneally (i.p.) once daily for 21 days at a dose of 0.01 mL/g of body weight.

2.3. Experimental procedure

In this study, the antidepressant-like effects of 21-day sarcosine treatment was examined using the FST. To evaluate the general locomotor activity for the possibility of false-positive result in FST, elevated plus maze (EPM) test was conducted 24 h before the FST [24,25]. Immediately after EPM, rats had a 15-min conditioning swim. This study consisted of 2 experiments. In Experiment 1, the effect of long-term sarcosine treatment was evaluated in naive rats. In Experiment 2, this effect was examined in rats exposed to CUS.

In Experiment 1, the rats were subdivided into saline and sarcosine groups (Fig. 1A). Each experimental group comprised 8 rats. The 2 groups of rats were injected i.p. once daily for 21 days with saline or sarcosine at 560 mg/kg. The EPM was conducted on the 22nd day 20–22 h after the last injection. The pretest for the FST was then performed for 15 min. The FST was performed on the 23rd day (Fig. 1A).

In Experiment 2, the effect of long-term sarcosine treatment was examined in CUS-exposed rats. The rats were subdivided into 3 groups: control, saline with CUS, and sarcosine with CUS (Fig. 1B). Each experimental group comprised 8 or 9 rats. The rats were handled daily (home cage control) or subjected to the CUS procedure (2 stressors daily) for 35 days. On the 15th day of the CUS procedure, the rats exposed to CUS were administered saline or sarcosine (560 mg/kg) i.p. daily for the last 21 days of the CUS procedure. The subsequent EPM test, pretest for the FST, and FST were then conducted as they were in Experiment 1 (Fig. 1B).

Moreover, immediately after the FST, 4 rats in each group were sacrificed through an intramuscular injection of combinations of zoletil (30 mg/kg) and xylazine (10 mg/kg), and these rats were then rapidly decapitated. The hippocampus was removed and stored at −80 °C for biochemical analysis.

2.4. Behavioral assays

2.4.1. FST

The FST was performed in an acrylic cylinder (diameter, 20 cm; height, 40 cm) filled to a height of 30 cm with water at 25 °C. Rats first had a 15-min conditioning swim before being placed in the swimming apparatus again 24 h later. Their behavior was monitored for 5 min as described in our previous reports [15,16].