Ketamine Enhances GluR1 Ser845 Phosphorylation in Prefrontal Cortex

Mingfa Huang1, Ke Zhang2, and Xiang Cai*1

1The Institute of Neuroscience, The Second Affiliated Hospital of Guangzhou Medical University, Guangzhou, P.R. China
2Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL, USA

Abstract
The mechanisms underlying the rapid-acting antidepressant effects of ketamine have not been fully identified. In current study, we observed that a single subanesthesia dose of ketamine strongly decreased the immobility time of Sprague–Dawley rats in forced swim test. Ketamine injection significantly increased GluR1 expression and GluR1 Ser845 phosphorylation in prefrontal cortex. Intracerebroventricular injection of PKA inhibitor H89 completely eliminated the fast antidepressant-like responses of ketamine in force swim test. Our results suggest that activation of PKA and phosphorylation of GluR1 Ser845 are critical for fast antidepressant action of ketamine.

Introduction
Major depressive disorder (MDD) is a serious public health problem with a lifetime prevalence of 17% in the United States [1]. Despite the high incidence of MDD and its socioeconomic impact, the etiology of MDD remains largely unknown. Existing treatments for MDD usually take weeks to months to achieve their antidepressant effects, and a significant number of patients do not have adequate improvement even after months of treatment. In addition, increased risk of suicide is a major public health concern during the first month of standard antidepressant therapy. Thus, improved therapeutics that exert antidepressant effects within hours or several days of administration are urgently needed.

Recent clinical trials show that a single subanesthesia dose (0.5- 20 mg/kg) of ketamine, a noncompetitive ionotropic glutamatergic NMDA receptor antagonist, produces rapid antidepressant responses in patients suffering from MDD [2]. Treatment-resistant, depressed patients reported alleviation of core symptoms of major depression within hours of a single dose of intravenously infused ketamine, with effects lasting up to 2 weeks [3]. Subsequent studies reported significant efficacy of ketamine in reducing suicidal ideation in individuals exhibiting treatment-resistant depression [4].

The discovery of ketamine’s rapid-acting antidepressant effect has been exciting. However, the psychotomimetic properties and abuse potential of ketamine necessitate caution in promoting this compound as a general treatment for depression. Understanding the underlying mechanism of action of ketamine linked to behavioral improvement is of significant importance for developing novel, safe and fast-acting antidepressants.

Materials and methods
Animals
Male and female Sprague–Dawley rats weighing 200–350 g were used for all behavioral experiments (purchased from Guangdong Animal Experiment Center, Guangzhou, Guangdong, China). Animals were maintained on a 12 h light/dark cycle in a temperature- and humidity-controlled facility with ad libitum access to food and water. All experiments were in accordance with US National Institutes of Health guidelines and approved by the Guangzhou Medical University Institutional Animal Care and Use Committee.

Forced swim test
In the pretest session, rats were placed into a Plexiglas cylinder (65-cm-height, 30-cm-diameter) filled to a height of 45 cm water (22°C to 24 °C) for 15 min. Water was changed between subjects and rats were dried by paper towel after swim. After ketamine or saline injection for different period of time, the test session were performed twenty four hours later. The test session was 15 min for each subject and was recorded by a video camera positioned on the side of the cylinder. However, only the first 5 min of test session was analyzed and scored by an observer blind to group assignment. A decrease in immobility time is suggestive of an antidepressant-like response.

Acute prefrontal brain slices preparation
These methods are described in detail in our previous publications [5,6]. Rats were killed by decapitation after sedation with. Prefrontal cortices (PFC) were isolated and sectioned into 400-μm-thick slices in ice-cold ACSF ( in mM: 124 NaCl, 3 KCl, 1.25 NaH2PO4, 1.5 MgCl2, 2.5 CaCl2, 26 NaHCO3, and 10 glucose, bubbled with 95% O2/5% CO2) using a Leica VT 1200S vibratome (Leica Microsystems Inc., Bannockburn, IL, USA). The slices were maintained at room
temperature for over 1 hour in an interface holding chamber in a humidified atmosphere saturated with 95% O2/5% CO2. The slices were then transferred to submission-type bottles containing 10 ml ASCF and bubbled with 95% O2/5% CO2.

Intracerebroventricular injection

Rats were deeply anesthetized with isoflurane and heads were mounted in a stereotaxic instrument. Double cannulae (26 gauge; Plastics One, Roanoke, VA, USA) were inserted bilaterally into left and right lateral ventricles (coordinates relative to bregma: −0.9 mm anterior/posterior (AP), ±1.5 mm medial/lateral (ML), −3.3 mm dorsal/ventral (DV) from dura). Postoperative care consisted in administration of carprofen (5 mg/kg) and topical triple antibiotic for 3 days. After a 7-day recovery period, rats were injected with 50 nM H89 (in 10 µl ACSF) or same amount of DMSO (in 10 µl ACSF) into the lateral ventricles at the rate of 0.25 µl/min with an injection cannula (26GA) protruding 0.5 mm beyond the guide cannula 1 hour before ketamine injections (i.p.). The injection cannula stayed in the guide cannula for 5 min after infusions.

Western blotting

After drug treatment, prefrontal homogenates were lysed using buffer containing 50 mM Tris-Cl, 150 mM NaCl, 0.02% NaN3, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate, 5 µg/ml leupeptin and 1 µg/ml aprotinin. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked at room temperature (22°C to 24°C) for 1 h in a solution containing 130 mM NaCl, 2.5 mM KCl, 10 mM Na2HPO4, 1.5 mM KH2PO4, 0.1% Tween 20 and 5% BSA (pH 7.4). The membranes were treated with the primary antibodies against Ser845-phosphorylated GluR1 (1:1000; Millipore AB5849). After rinses in TBS-Tween, the membrane was incubated for 1 hr at room temperature in horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000, Cell Signaling Technology #7074). The immunoblot was developed with enhanced chemiluminescence (Amersham). Membranes were then stripped, blocked, and reprobed with antibodies against GluR1 (0.5 µg/ml; Thermo Scientific PA1-37776) or β-actin (1:2000; Cell Signaling Technology #4967). Levels of phosphorylation, expressed as the ratio of phospho-specific intensity divided by total protein intensity and computed with ImageJ, were used for statistical analysis. For display purposes, blots were cropped and brightness and contrast were adjusted globally using Photoshop.

Statistical analysis

Data are presented as mean ± SE. Significance of the difference between two groups was assessed with unpaired two-tailed t-test or Bonferroni test after one-way repeated ANOVA test. A p value less than 0.05 is considered statistically significant.

Results

Ketamine reduced immobility of animal in forced swim test

We examine the antidepressant effect of ketamine and detected ketamine’s acute effect in Sprague–Dawley rats for significant behavioral responses in forced swim test, an antidepressant (AD)-predictive task. Compared to saline injection group, 1 h, 3 h and 6 h ketamine injection (10 mg/kg, i.p.) significantly reduced immobility time of animals during forced swim test (Figure 1).

Ketamine enhanced GluR1 Ser845 phosphorylation in PFC

Previous studies show that AMPA receptor activation is necessary for ketamine's antidepressant-like effect [7,8]. While, AMPA receptor phosphorylation is critical for the activation of the receptor. Among the multiple phosphorylation sites of AMPA receptor, the phosphorylation of GluR1 Ser845 site is important for AMPA receptor translocation from cytosol onto postsynaptic membrane. We therefore examine the impact of ketamine on GluR1 Ser845 phosphorylation in prefrontal cortex, a brain region that is heavily involved in the etiology of depression. After ketamine injection, prefrontal cortical brain slices were prepared as described in the Methods. Using the acutely prepared prefrontal cortical slices, western blot experiments detected that ketamine application time-dependently increased GluR1 Ser845 phosphorylation and total GluR1 (Figure 2 A–C).

The fast acting antidepressant response of ketamine.

GluR1 Ser845 is phosphorylated by PKA. To test whether GluR1 Ser845 phosphorylation is necessary for ketamine’s antidepressant effect, H89 (50 nM, in 10 µl ACSF), a PKA inhibitor was infused intracerebroventricularly (i.c.v.) 1 hour before ketamine injection (i.p.). H89 administration alone increased immobility time of animal in forced swim test. However, H89 totally blocked ketamine-induced reduction of immobility in forced swim test (Figure 3).

Discussion

Previous studies show that AMPA receptor activation and synaptic protein synthesis is necessary for ketamine-induced antidepressant-like responses [7,8]. It has been speculated that inhibition of tonically-active GABAergic interneurons and activation of voltage-gated calcium channels underlie ketamine-induced synaptogenesis [7,9]. However, blocking GABAergic transmission by picrotoxin, a GABA receptor antagonist, does not affect depressive behaviors [8], indicating that blocking GABAergic transmission-induced disinhibition of principle neurons is not sufficient to generate the rapid antidepressant response of ketamine. Monteggia et al. [10,11] demonstrated that ketamine deactivates eukaryotic elongation factor 2 (eEF2) kinase (also called CaMKII), resulting in reduced eEF2 phosphorylation.
and de-suppression of BDNF translation as well as increases surface expression of AMPA receptors. However, other researchers observed that relative to CaMKII, CaMKIII exhibits approximately two orders of magnitude greater affinity for calmodulin and is sensitive to >5-fold lower calcium concentrations. They also demonstrated that CaMKIII activity and eEF2 phosphorylation are suppressed by NMDA antagonist only in the condition when synaptic transmission is blocked by TTX [12]. Furthermore, a recent clinical research study observed that the expression of eEF2 (p-eEF2) increases in the blood plasma of depressed patients treated with ketamine [13]. These results suggest that suppression of eEF2 phosphorylation probably could not fully account for ketamine-induced increase of BDNF release and AMPA receptor expression, especially in vivo where TTX is absent.

Phosphorylation of AMPA receptors has been shown to play an important role in expression of synaptic plasticity at excitatory synapses. There are at least ten phosphorylation sites that have been identified on the C-terminal domains of AMPA receptor GluR1-GluR4 subunits [14–16]. Among these phosphorylation sites, GluR1 Ser845 is phosphorylated by PKA [17–19]. Phosphorylation of GluR1 Ser845 increases the open-channel probability of AMPA receptors and therefore enhances the function of AMPA receptor [19, 20]. Furthermore, GluR1 Ser845 phosphorylation is suggested to be important for rapid synaptic insertion of GluR1 subunit-containing receptors [21]. Our result show that ketamine strongly enhanced GluR1 Ser845 phosphorylation, suggesting that ketamine may enhance the function of AMPA receptor through phosphorylation of the receptor, which may underlie the rapid antidepressant effect of ketamine. Indeed, H89 totally inhibited the antidepressant-like effect of ketamine in forced swim test, indicating that PKA activation and GluR1 Ser845 phosphorylation are necessary for the rapid antidepressant action of ketamine.

Competing interests

The authors declare no competing interests

Acknowledgments

This study was supported by NIMH R01 (NIMHMH088628), SIU School of Medicine startup funds (501871), the National Natural Science Foundation of China (81271488), the Pearl River Scholar Program by Guangdong Province, China, the National Natural Science Foundation of China (81271488), the Science and Technology Program by Guangzhou, China (2014Y2-00096), the Foundation for High-level Talents in Higher Education of Guangdong, China (310109-012), the Research Project for Colleges and Universities in Guangzhou (12A002S).

References

1. Kessler D, Sharp D, Lewis G (2005) Screening for depression in primary care. Br J Gen Pract 55: 605–610. [Crossref]
2. Mathew SJ, Shah A, Lapidus K, Clark C, Jarun N, et al. (2012) Ketamine for treatment-
resistant unipolar depression: current evidence. *CNS Drugs* 26: 189-204. [Crossref]

3. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, et al. (2000) Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* 47: 351-354. [Crossref]

4. aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, et al. (2010) Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol Psychiatry* 67: 139-145. [Crossref]

5. Cai X, Kallarackal AJ, Kvarta MD, Goluskin S, Gayler K, et al. (2013) Local potentiation of excitatory synapses by serotonin and its alteration in rodent models of depression. *Nat Neurosci* 16: 464-472. [Crossref]

6. Cai X, Liang CW, Muralidharan S, Kao JP, Tang CM, et al. (2004) Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* 44: 351-364. [Crossref]

7. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, et al. (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* 329: 959-964. [Crossref]

8. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, et al. (2011) NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* 475: 91-95. [Crossref]

9. Duman RS, Aghajanian GK (2012) Synaptic dysfunction in depression: potential therapeutic targets. *Science* 338: 68-72. [Crossref]

10. Kavalali ET, Monteggia LM (2012) Synaptic mechanisms underlying rapid antidepressant action of ketamine. *Am J Psychiatry* 169: 1150-1156. [Crossref]

11. Monteggia LM, Gideons E, Kavalali ET (2013) The role of eukaryotic elongation factor 2 kinase in rapid antidepressant action of ketamine. *Biol Psychiatry* 73: 1199-1203. [Crossref]

12. Sutton MA, Taylor AM, Ito HT, Pham A, Schuman EM (2007) Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron* 55: 648-66. [Crossref]

13. Yang C, Zhou ZQ, Guo ZQ, Shi JY, Yang JJ (2013) Acute increases in plasma mammalian target of rapamycin, glycogen synthase kinase-3beta, and eukaryotic elongation factor 2 phosphorylation after ketamine treatment in three depressed patients. *Biol Psych* 73: e35-36. [Crossref]

14. Carvalho AL, Kameyama K, Huganir RL (1999) Characterization of phosphorylation sites on the glutamate receptor 4 subunit of the AMPA receptors. *J Neurosci* 19: 4748-4754. [Crossref]

15. Chung IH, Xia J, Scannev RH, Zhang X, Huganir RL (2000) Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *J Neurosci* 20: 7258-7267. [Crossref]

16. Lee HK, Barbarese M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405: 955-959. [Crossref]

17. Barria A, Derkach V, Soderling T (1997) Identification of the Ca2+/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate-type glutamate receptor. *J Biol Chem* 272: 32727-32730. [Crossref]

18. Mammen AL, Kameyama K, Roche KW, Huganir RL(1997) Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent protein kinase II. *J Biol Chem* 272: 32528-32533. [Crossref]

19. Roche KW, O’Brien RJ, Mammen AL, Bernhardt J, Huganir RL (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* 16: 1179-1188. [Crossref]

20. Banke TG, Bowie D, Lee H, Huganir RL, Schousboe A, et al. (2000) Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J Neurosci* 20: 89-102. [Crossref]

21. Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, et al. (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nat Neurosci* 6: 136-143. [Crossref]

Copyright: ©2016 Huang M. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.