Abolished ketamine effects on the spontaneous excitatory postsynaptic current of medial prefrontal cortex neurons in GluN2D knockout mice

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Abstract
Ketamine, a non-competitive antagonist of the N-methyl-D-aspartate receptor (NMDAR), generates a rapidly-acting antidepressant effect. It exerts psychomimetic effects, yet demands a further investigation of its mechanism. Previous research showed that ketamine did no longer promote hyperlocomotion in GluN2D knockout (KO) mice, which is a subunit of NMDAR. In the present study, we tested whether GluN2D-containing NMDARs participate in the physiological changes in the medial prefrontal cortex (mPFC) triggered by ketamine. Sub-anesthetic dose of ketamine (25 mg/kg) elevated the frequency of spontaneous excitatory postsynaptic currents (sEPSC) in wild-type (WT) mice, but not in GluN2D KO mice, 1 h after the injection. The amplitude of sEPSC and paired-pulse ratio (PPR) were unaltered by ketamine in both WT and GluN2D KO mice. These findings suggest that GluN2D-containing NMDARs might play a role in the ketamine-mediated changes in glutamatergic neurons in mPFC and, presumably, in ketamine-induced hyperlocomotion.

Keywords: Ketamine, Medial prefrontal cortex, N-methyl-D-aspartate receptor (NMDAR), Spontaneous excitatory postsynaptic current (sEPSC), GluN2D

Ketamine is an N-methyl-D-aspartate receptor (NMDAR) antagonist and has been widely used as an anesthetic drug over the past two decades. Due to its rapid antidepressant effect, ketamine became a breakthrough in the clinical research of depression. Ketamine exerts a rapid and long-lasting antidepressant effect in a dose-dependent manner [1], where a sub-anesthetic dose of ketamine (0.3–1 mg/kg in humans [2] and 5–10 mg/kg in animals [3], respectively) has been reported to be effective in alleviating depressive symptoms [2]. However, ketamine also has notable side effects, such as psychotomimetic symptoms, abuse potential, and neurotoxicity. For instance, a higher dose of ketamine (25–50 mg/kg) triggered dissociation [4] and hyperlocomotion in mice [5].

The NMDAR subunit family is composed of GluN1, GluN2A-D, and GluN3A-B subunits. The GluN2D-containing NMDARs reach maximal expression at the first postnatal week and become restricted in a few cell types including interneurons of the hippocampus and the prefrontal cortex [6, 7]. Previous research has implicated GluN2D-containing NMDARs in the sustained antidepressant effect [8] and the cognitive impairment effect [9] of (R)-ketamine, an enantiomer of racemic ketamine.
Furthermore, GluN2D knockout (KO) mice did not develop ketamine-induced locomotor sensitization [5]. However, the physiological mechanism through which GluN2D-containing NMDARs contribute to ketamine-induced hyperlocomotion remains largely unknown. To address this question, we measured spontaneous excitatory postsynaptic currents (sEPSC) and paired-pulse ratio in the medial prefrontal cortex (mPFC) layer 5 pyramidal neurons of wild-type (WT) and GluN2D KO mice 1 h after the injection of a sub-anesthetic dose of ketamine, a dose known to trigger hyperlocomotion in rodents (Fig. 1A).

WT and homozygous GluN2D KO mice of both sexes were used for experiments, aged 6–16 weeks at the time of recording. Saline or ketamine (25 mg/kg) ((R,S)-ketamine hydrochloride, Yuhan Corporation, Seoul, Korea) was intraperitoneally administered 1 h before the decapitation. Mice were anesthetized with isoflurane and sacrificed by decapitation in accordance with the regulation and policy approved by Institutional Animal Care and Use Committee in Seoul National University. Coronal slices with 350 μm thickness were obtained as previously described [10].

One or two coronal slices were selected according to their coordinates from Bregma (AP: +1.70) and transferred to a submerged chamber for whole-cell recording, continuously perfused with artificial cerebrospinal fluid (ACSF) that contained (in mM): 124 NaCl, 3 KCl, 26 NaHCO3, 1.25 NaH2PO4, 2 MgSO4, 15 d-glucose and 2 CaCl2 (carbonated with 95% O2 and 5% CO2). Pyramidal neurons in layer 5 of the mPFC were recognized by their perpendicular distance from the midline. Recordings were made primarily within the infralimbic cortex, leading to the disinhibition of pyramidal neurons predominantly inhibiting presynaptic GABAergic interneurons, resulting in increased number of excitatory synapses of layer 5 pyramidal neurons made by inputs from layer 2/3 neurons. The stimulation electrode was placed in layer 2/3 perpendicularly aligned with the patch pipette in layer 5. Two successive electronic simulations were delivered with varying interpulse intervals of 50–200 ms. The paired-pulse ratio was calculated by dividing the peak amplitude of the second EPSC by that of the first EPSC and 4 sweeps were averaged.

Interestingly, no significant differences were observed in the paired-pulse ratio (Fig. 1E–G). Even though we could not exclude the possibility that changes in release probability in other synapses or excitability of presynaptic neurons caused the increase in the sEPSC frequency, PPR data are consistent with the hypothesis that the observed elevation in sEPSC frequency is a result of an increased number of excitatory synapses.

WT mice displayed increased sEPSC frequency of mPFC pyramidal neurons when ketamine was injected (Fig. 1C). Previous research has shown that ketamine predominantly inhibits presynaptic GABAergic interneurons, leading to the disinhibition of pyramidal neurons in the mPFC [3, 11]. However, we consider it unlikely

(See figure on next page.)

**Fig. 1** Effects of ketamine on the sEPSC of mPFC neurons. (A) Experimental scheme and the recording site in the mPFC. (B) Representative traces of sEPSCs. (C) WT mice displayed elevated sEPSC frequency compared to their saline-injected counterparts, whereas GluN2D KO mice did not (n = 18–21 cells, 5–6 mice/group; ketamine effect, F1, 78 = 8.473, p = 0.0047; genotype, F1, 78 = 5.067, p = 0.0272; interaction, F1, 78 = 15.79, p = 0.0002; WT ketamine vs saline, p < 0.0001; GluN2D KO ketamine vs saline, p = 0.7099). (D) No significant differences were observed in the amplitude of sEPSC. (E) Representative traces of paired-pulse ratio (PPR) at 150 ms inter stimulus interval (F, G). No significant differences were observed in PPR at any of the inter-stimulus intervals examined (50–200 ms) (n = 13–14 cells, 4–5 mice/group). Electrophysiology data are represented as mean ± SEM. sEPSC, PPR: 2-way ANOVA with Post hoc Sidak’s multiple-comparisons test. **** p < 0.0001. ns, nonsignificant.
Fig. 1 (See legend on previous page.)
that the elevated sEPSC frequency in the current study was a direct reflection of the disinhibition of excitatory neurons, as ketamine would have been washed out during the preparation of the brain slice. Rather, we speculate that it was a consequence of the synaptic-activity dependent synaptogenesis [12, 13] that might have been induced and stabilized by disinhibition of pyramidal neurons. The resultant increase in the glutamatergic transmission in mPFC likely has augmented dopamine release in both mPFC and striatum resulting in hyperlocomotion, as previously suggested [14, 15].

In contrast, GluN2D KO mice did not show increased sEPSC frequency in response to the ketamine injection. Moreover, they did not develop ketamine-induced hyperlocomotion [5] and the increase of extracellular dopamine level in mPFC in response to phencyclidine, another non-competitive NMDAR antagonist [16]. Given that GluN2D-containing NMDARs are mainly expressed in mPFC interneurons [7], our data suggest a possibility that ketamine disinhibits pyramidal neurons partially by blocking GluN2D-containing NMDARs in interneurons leading to the hyperlocomotion.

In conclusion, the present study adds evidence to the view that GluN2D-containing NMDARs may participate in the process through which ketamine increases glutamatergic synapses of pyramidal neurons in the mPFC, and thereby provides a potential mechanism of ketamine-triggered hyperlocomotion.

Abbreviations
NMDAR: N-methyl-D-aspartate receptor; mPFC: Medial prefrontal cortex; sEPSC: Spontaneous excitatory postsynaptic currents; WT: Wild-type; KO: Knockout; PPR: Paired-pulse ratio; ACSF: Artificial cerebrospinal fluid.

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Authors’ contributions
DHH, IH, SJ, PP, MM, KI, JYB, HP and BKK designed the study. DHH, IH, and JEC performed the experiments. DHH, IH, JEC, and BKK analyzed the data. DHH, IH, JEC, KI, and BKK wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data in the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All procedures were conducted in accordance with the animal care standards of the Institutional Animal Use and Care Committee.

Competing interests
The authors declare that they have no competing interests.

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