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Rapid Communication

RAPID COMMUNICATION

Cognitive Impairment That Is Induced by (*R*)-Ketamine Is Abolished in NMDA GluN2D Receptor Subunit Knockout Mice

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Abstract

Although the *N*-methyl-D-aspartate receptor antagonist ketamine has attracted attention because of its rapid and sustained antidepressant effects in depressed patients, its side effects have raised some concerns. Ketamine is a racemic mixture of equal amounts of the enantiomers (*R*)-ketamine and (*S*)-ketamine. The neural mechanisms that underlie the differential effects of these enantiomers remain unclear. We investigated cognitive impairment that was induced by ketamine and its enantiomers in *N*-methyl-D-aspartate GluN2D receptor subunit knockout (GluN2D-KO) mice. In the novel object recognition test, (*RS*)-ketamine and (*S*)-ketamine caused cognitive impairment in both wild-type and GluN2D-KO mice, whereas (*R*)-ketamine induced such cognitive impairment only in wild-type mice. The present results suggest that the GluN2D subunit plays an important role in cognitive impairment that is induced by (*R*)-ketamine, whereas this subunit does not appear to be involved in cognitive impairment that is induced by (*RS*)-ketamine or (*S*)-ketamine.

Keywords: antidepressant, cognitive impairment, enantiomer, GluN2D, ketamine

Ketamine is a phencyclidine (PCP) derivative and noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist that has been used as an anesthetic agent. Recent studies have revealed that a subanesthetic dose of ketamine exerts rapid and sustained antidepressant effects, including improvements in suicidal ideation, in depressed patients (Krystal et al., 2013). However, ketamine also has notable side effects, such as psychotomimetic symptoms, abuse potential, and neurotoxicity. Ketamine is a racemic mixture of equal amounts of the enantiomers (*R*)-ketamine and (*S*)-ketamine. (*S*)-Ketamine has been thought to be an active isomer because of its higher affinity for the NMDA receptor and greater anesthetic potency (Domino, 2010). A recent clinical trial reported the rapid onset of robust antidepressant effects of (*S*)-ketamine in patients with treatment-resistant depression (Singh et al., 2016). However, (*R*)-ketamine has also been reported to exert more potent and sustained antidepressant effects than (*S*)-ketamine, without causing such adverse effects as psychotomimetic behaviors, neurotoxicity, and abuse potential in animal models (Zhang et al., 2014; Yang et al., 2015; Fukumoto et al., 2017). The neural mechanisms that underlie these differential effects of ketamine and its enantiomers remain unclear.

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The NMDA receptor subunit family is composed of GluN1, GluN2A-D, and GluN3A-B subunits. We previously reported that PCP significantly increased locomotor activity, caused motor impairment, and increased extracellular dopamine levels in wild-type but not GluN2D-KO mice (Hagino et al., 2010; Yamamoto et al., 2013). We also reported that GluN2D-KO mice did not develop ketamine-induced locomotor sensitization (Yamamoto et al., 2016). Furthermore, we found that the GluN2D subunit plays an important role in the sustained but not rapid antidepressant effects of (*R*)-ketamine, whereas this subunit does not appear to be involved in the antidepressant effects of (*RS*)-ketamine or (*S*)-ketamine (Ide et al., 2017; Ide and Ikeda, 2018). However, the role of the GluN2D subunit in other side effects of ketamine and its enantiomers is still unknown. The present study investigated the role of GluN2D subunits in cognitive impairment that was induced by ketamine and its enantiomers.

Wild-type and homozygous GluN2D-KO mouse littermates (20–32 g, >18 weeks old) on a C57BL/6 genetic background (Ikeda et al., 1995) were used. All of the experiments were performed with approval from the Animal Use and Care Committee of the Tokyo Metropolitan Institute of Medical Science. The mice were housed 4 to 6 per cage with free access to food and water. (*R*)-Ketamine hydrochloride and (*S*)-ketamine hydrochloride were prepared by the recrystallization of (*RS*)-ketamine (Ketalar, ketamine hydrochloride, Daiichi Sankyo Pharmaceutical Ltd., Tokyo, Japan) (Zhang et al., 2014) and dissolved in saline.

Cognitive impairment was evaluated using the novel object recognition (NOR) test, which was performed according to Botton et al. (Botton et al., 2010) with modifications. In the habituation phase (day 1), each mouse was allowed to freely explore the square open-field arena (600 mm × 600 mm) in the absence of objects for 10 minutes in a sound-isolated room with constant illumination (~500 lux) at the level of the test apparatus. The mouse was then removed from the arena and placed in its home cage. During the familiarization phase (day 2), individual mice were placed in the open-field arena that contained 2 different sample objects (A + B) for 10 minutes. Immediately after the familiarization phase, (*RS*)-ketamine, (*R*)-ketamine, (*S*)-ketamine (10 or 20 mg/kg), or saline was injected i.p. After 24 hours, in the test phase (day 3), the animals were returned to the open-field arena with 2 objects: 1 was identical to the sample and the other was novel (A + C). During both the familiarization and test phases, the objects were located in opposite corners of the arena, and the location of the novel object relative to the familiar object was counterbalanced between animals. All of the objects that were used in this study had different shapes but identical sizes (45-mm height). Object exploration time was automatically defined as the length of time that the animal directed its nose within 30 mm of the object. The data are expressed as the recognition index: RI = time exploring object C / (total time spent exploring objects A + C) × 100 (%). Each session was recorded using video cameras that were located above the arena and analyzed using the DVTrack video tracking system (Muromachi Kikai, Tokyo, Japan). The results are expressed as mean ± SEM and were analyzed using 1-way ANOVA) folloed by the Sidak multiple-comparison post hoc test or Student's *t* test. One-sample *t* tests were used to determine whether the RI in each experimental group was different from the 50% chance level. Values of $P < .05$ were considered statistically significant.

In wild-type mice (Figure 1A), ketamine and its enantiomers significantly affected the RI in the NOR test (1-way ANOVA: $F_{6,60} = 3.33$, $P = .0068$). The post hoc tests revealed that the higher dose (20 mg/kg) of (RS)-ketamine ($P = .0063$), (R)-ketamine ($P = .043$), and (S)-ketamine ($P = .0027$) significantly reduced the RI compared with the saline-treated group (Figure 1A). The post hoc tests also showed that only (S)-ketamine ($P = .024$) and not (*RS*)-ketamine ($P = .268$) or (R)-ketamine ($P = .605$) significantly reduced the RI at the lower dose (10 mg/kg) compared with the saline-treated group. One-sample *t* tests showed that the saline-treated group significantly discriminated objects ($P = .002$). The groups that received the lower dose of (RS)-ketamine ($P = .093$) and (R)-ketamine ($P = .086$) but not the lower dose of (S)-ketamine ($P = .826$) or higher dose of (RS)-ketamine ($P = .826$), (R)-ketamine ($P = .460$), or (S)-ketamine ($P = .495$) tended to significantly discriminate objects. These results suggest that ketamine and its enantiomers cause cognitive impairment and that this effect of (S)-ketamine is more potent than (*RS*)-ketamine and (*R*)-ketamine in wild-type mice.

In GluN2D-KO mice (Figure 1B), ketamine and its enantiomers significantly affected the RI in the NOR test (1-way ANOVA: $F_{6,60} = 3.54$, $P = .0045$). The post hoc tests revealed that the higher dose (20 mg/kg) of (RS)-ketamine ($P = .049$) and (S)-ketamine ($P = .0029$) but not (R)-ketamine ($P = .876$) significantly reduced the RI compared with the saline-treated group (Figure 1B). The post hoc tests also showed that only (S)-ketamine ($P = .038$) and not (RS)-ketamine ($P = .905$) or (R)-ketamine ($P = .985$) significantly

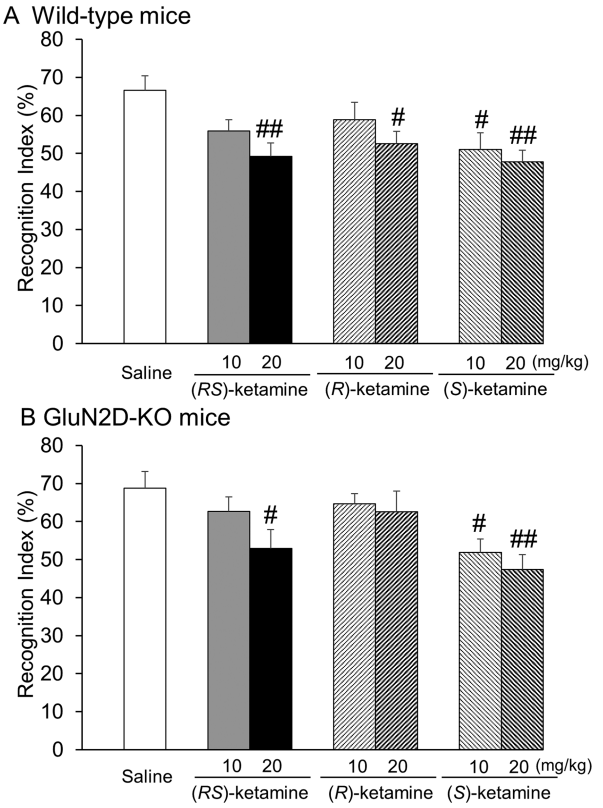

Figure 1. Cognitive impairment that is induced by ketamine and its enantiomers in the novel object recognition (NOR) test. (A–B) Recognition index (RI) in wild-type mice (A) (saline: [n = 11, 66.6% ± 3.9%]; [RS]-ketamine: 10 mg/kg [n = 8, 55.9% ± 3.0%], 20 mg/kg [n = 10, 49.2% ± 3.6%]; [R]-ketamine: 10 mg/kg [n = 9, 58.9% ± 4.5%], 20 mg/kg [n = 10, 52.5% ± 3.3%]; [S]-ketamine: 10 mg/kg [n = 9, 51.0% ± 4.4%], 20 mg/kg [n = 10, 47.8% ± 3.1%]) and GluN2D-KO mice (B) (saline [n = 11, 68.7% ± 4.4%]; [RS]-ketamine: 10 mg/kg [n = 8, 62.6% ± 3.9%], 20 mg/kg [n = 10, 53.0% ± 4.9%]; [R]-ketamine: 10 mg/kg [n = 9, 64.7% ± 2.6%], 20 mg/kg [n = 10, 62.6% ± 5.4%]; [S]-ketamine, 10 mg/kg [n = 9, 51.9% ± 3.5%], 20 mg/kg [n = 10, 47.4% ± 3.9%]) in the NOR test. The data are expressed as mean ± SEM. #$P < .05$, ##$P < .01$, compared with saline-injected mice (Sidak multiple-comparison post hoc test).

reduced the RI at the lower dose (10 mg/kg) compared with the saline-treated group. Although GluN2D-KO mice exhibited a decrease in the total distance travelled during the familiarization phase (day 2) compared with wild-type mice (wild-type mice: 23.0 ± 1.8 m; GluN2D-KO mice: 15.7 ± 0.8 m; Student's *t* test, $P < .001$), 1 sample *t* tests showed that saline-treated GluN2D-KO mice significantly discriminated objects ($P = .002$). Furthermore, 1 sample *t* tests showed that the groups that received the lower dose of (*RS*)-ketamine ($P = .014$) and (*R*)-ketamine ($P = .001$) and higher dose of (*R*)-ketamine ($P = .045$) but not the lower dose of (*S*)-ketamine ($P = .595$) or higher dose of (*RS*)-ketamine ($P = .563$) or (*S*)-ketamine ($P = .519$) significantly discriminated objects. These results indicate that (*RS*)-ketamine and (*S*)-ketamine but not (*R*)-ketamine induced cognitive impairment in GluN2D-KO mice.

Ketamine and other NMDA receptor antagonists, such as MK-801 and PCP, have been used to produce cognitive impairment in an animal model of schizophrenia and have psychotomimetic effects in humans (Newcomer et al., 1999; Cadinu et al., 2018). Subanesthetic doses of ketamine impaired recognition memory in the NOR test (Pitsikas et al., 2008). A recent clinical trial reported that intranasal (*S*)-ketamine induced transient cognitive impairment (Morrison et al., 2018). Differences in cognitive impairment that are produced by enantiomers of ketamine have not been previously studied to our knowledge. The present results showed that the higher, subanesthetic dose (20 mg/kg) of (*RS*)-ketamine, (*R*)-ketamine, and (*S*)-ketamine significantly impaired recognition memory in the NOR test. However, only (*S*)-ketamine and not (*RS*)-ketamine or (*R*)-ketamine significantly impaired recognition memory at the lower dose (10 mg/kg) compared with the saline-treated group. The present results are consistent with a previous study that showed that (*S*)-ketamine has higher affinity for the NMDA receptor (Domino, 2010). Previous studies reported more potent and sustained antidepressant effects of (*R*)-ketamine (Zhang et al., 2014; Yang et al., 2015; Fukumoto et al., 2017). We also previously reported that the lower dose of ketamine and its enantiomers is sufficient to exert antidepressant actions in wild-type mice (Ide et al., 2017; Ide and Ikeda, 2018). Although further human clinical studies are needed for validation, (*R*)-ketamine appears to be a safer antidepressant than (*S*)-ketamine with regard to cognitive impairment. Furthermore, (*R*)-ketamine induced cognitive impairments only in wild-type mice and not in GluN2D-KO mice in the present study. These results indicate that a weak but evident inhibitory effect of (*R*)-ketamine on recognition is mediated by NMDA receptors that contain the GluN2D subunit. Clarification of the mechanism of cognitive impairment that is mediated by NMDA receptors that contain the GluN2D subunit will contribute to optimizing the clinical use of (*R*)-ketamine and (*RS*)-ketamine as antidepressants and developing better novel antidepressants.

The inhibition of NMDA receptors on γ-aminobutyric acid (GABA)ergic interneurons could be one mechanism of action of subanesthetic doses of ketamine (Zanos and Gould, 2018). GABAergic interneurons are also known to regulate numerous cognitive functions. Furthermore, chronic ketamine treatment in rodents caused cognitive impairment and reduced the number of parvalbumin (Parv)-expressing neurons, which are a major population of GABAergic interneurons in the prefrontal cortex (PFC) and hippocampus (Sabbagh et al., 2013; Hauser et al., 2017). Yang et al. (2016) recently showed that repeated intermittent (*S*)-ketamine administration (10 mg/kg, once weekly for 8 weeks) but not (*R*)-ketamine administration caused the loss of Parv immunoreactivity in the medial PFC and hippocampus in mice. Thus, treatment with (*RS*)-ketamine, especially (*S*)-ketamine, appears to reduce the number of Parv-positive GABAergic interneurons in the PFC and hippocampus, resulting in cognitive impairment. Although the expression of the GluN2D subunit has been found in presynaptic axons of many GABAergic neurons, GluN2D mRNA expression is mainly restricted to diencephalic, mesencephalic, and brainstem structures (Watanabe et al., 1992). In the present study, (*R*)-ketamine induced cognitive impairment only in wild-typemice and not in GluN2D-KO mice, whereas (*S*)-ketamine induced cognitive impairment in both wild-type and GluN2D-KO mice. Thus, (*R*)-ketamine and (*S*)-ketamine appear to produce cognitive impairment through different mechanisms in different brain regions. We previously showed that PCP increased the number of Fos-positive neurons in the striatum and PFC in wild-type but not GluN2D-KO mice (Yamamoto et al., 2013). Therefore, PCP and ketamine may inhibit presynaptic GluN2D-containing NMDA receptors on GABAergic neurons that project to the PFC, thus decreasing GABA release and activating the PFC–dorsal striatum pathway. This activation may underlie acute PCP- and (*R*)-ketamine-induced psychiatric effects. The involvement of GluN2D subunits in these signaling pathways deserves further investigation.

NMDA receptors have been suggested to be required for the formation of NOR memory (Rampon et al., 2000; de Lima et al., 2005; Winters and Bussey, 2005). The present results suggest a pivotal role for the GluN2D subunit in cognitive impairment that is induced by (*R*)-ketamine but not (*RS*)-ketamine or (*S*)-ketamine. We previously found that GluN2D-KO mice did not develop ketamine-induced hyperlocomotion (Yamamoto et al., 2016). Our previous study that used the tail-suspension test and a mouse model of restraint stress-induced depression indicated that the GluN2D subunit plays an important role in the sustained but not rapid antidepressant effects of (*R*)-ketamine, but this subunit does not appear to be involved in the antidepressant effects of (*RS*)-ketamine or (*S*)-ketamine (Ide et al., 2017; Ide and Ikeda, 2018). Altogether, ketamine and its enantiomers appear to induce cognitive impairment, locomotor sensitization, and rapid and sustained antidepressant effects through different mechanisms, including a mechanism that involves NMDA receptors that contain the GluN2D subunit. Future studies of enantiomers are required to determine the specific role of the GluN2D subunit in the effects of ketamine.

The present results suggest that the GluN2D subunit plays an important role in cognitive impairment that is induced by (*R*)-ketamine, whereas this subunit does not appear to be involved in such effects of (*RS*)-ketamine or (*S*)-ketamine.

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