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ORIGINAL INVESTIGATION

Serotonin-1A receptor stimulation mediates effects of a metabotropic glutamate 2/3 receptor antagonist, 2S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3-(xanth-9-yl) propanoic acid (LY341495), and an *N*-methyl-D-aspartate receptor antagonist, ketamine, in the novelty-suppressed feeding test

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Abstract
Rationale α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor stimulation has been proposed to be a common neural mechanism of metabotropic glutamate 2/3 (mGlu2/3) receptor antagonists and an *N*-methyl-D-aspartate receptor antagonist, ketamine, exerting antidepressant effects in animal models. AMPA receptor stimulation has also been shown to mediate an increase in the extracellular level of serotonin (5-HT) in the medial prefrontal cortex by an mGlu2/3 receptor antagonist in rats. However, involvement of the serotonergic system in the actions of mGlu2/3 receptor antagonists and ketamine is not well understood.
Objectives We investigated involvement of the serotonergic system in the effects of an mGlu2/3 receptor antagonist, 2S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid (LY341495), and ketamine in a novelty-suppressed feeding (NSF) test in mice.
Results The intraperitoneal administration of LY341495 or ketamine at 30 min prior to the test significantly shortened latency to feed, which was attenuated by an AMPA receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydr obenzo[*f*]-quinoxaline-7-sulfonamide (NBQX). The effects of LY341495 and ketamine were no longer observed in mice pretreated with a tryptophan hydroxylase inhibitor, *para*-chlorophenylalanine (PCPA). Moreover, the effects of LY341495 and ketamine were blocked by a 5-HT1A receptor antagonist, *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridynyl) cyclohexane-carboxamide (WAY100635), but not by a 5-HT2A/2C receptor antagonist, ritanserin. Likewise, an AMPA receptor potentiator, 2,3-dihydro-1,4-benzodioxin-7-yl-(1-piperidyl)methanone (CX546), shortened latency to feed in the NSF test, which was prevented by depletion of 5-HT and blockade of 5-HT1A receptor.
Conclusions These results suggest that AMPA receptor-dependent 5-HT release and subsequent 5-HT1A receptor stimulation may be involved in the actions of an mGlu2/3 receptor antagonist and ketamine in the NSF test.

Keywords AMPA receptor · Depression · Ketamine · Metabotropic glutamate 2/3 receptor antagonist · Novelty-suppressed feeding test · Serotonin · 5-HT1A receptor

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Introduction

Accumulated evidence has shown that modulating the glutamatergic system may be a useful approach for treating depressive symptoms. Indeed, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, ketamine, has been reported to exert both rapid and sustained antidepressant effects in patients with major depressive disorder (MDD), treatment-resistant depression (TRD) (Berman et al. 2000; Zarate et al. 2006; Mathew et al. 2010; Ibrahim et al. 2011), and bipolar

disorder (Diazgranados et al. 2010). Thus, elucidating the mechanisms underlying antidepressant effects of agents acting on glutamatergic systems, such as ketamine, may lead to novel therapies for MDD. Recently, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor stimulation has been reported to play a pivotal role in the antidepressant effects of ketamine (Koike et al. 2011a; Maeng et al. 2008), and the subsequent stimulation of tropomyosin-related kinase B (TrkB) (Autry et al. 2011; Koike et al. 2013) and the mammalian target of rapamycin (mTOR) signaling pathway may lead to an increase in the synthesis of synaptic proteins (Li et al. 2010).

Manipulating metabotropic glutamate (mGlu) receptors has also been reported to exert antidepressant effects in several animal models of depression. We previously reported that mGlu2/3 receptor antagonists exerted antidepressant effects in several animal models (Chaki et al. 2004; Karasawa et al. 2005; Pałucha-Poniewiera et al. 2010; Yoshimizu et al. 2006) and that the effects of mGlu2/3 receptor antagonists were blocked by pretreatment with an AMPA receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydr obenzo[*f*]quinoxaline-7-sulfonamide (NBQX) (Karasawa et al. 2005; Pałucha-Poniewiera et al. 2010), an mTOR antagonist, rapamycin (Dwyer et al. 2012; Koike et al. 2011b), and a TrkB tyrosine kinase inhibitor, K252a (Koike et al. 2013). Thus, mGlu2/3 receptor antagonists and ketamine may share similar neuronal mechanisms that may be triggered by AMPA receptor stimulation to exert antidepressant effects.

Moreover, AMPA receptor stimulation mediates an increase in the extracellular level of serotonin (5-HT) in the medial prefrontal cortex (mPFC) by an mGlu2/3 receptor antagonist, as well as an NMDA receptor antagonist, in rats (Karasawa et al. 2005; López-Gil et al. 2007). Given the fact that the interaction between serotonergic and glutamatergic systems is implicated in psychiatric disorders, such as psychosis and anxiety (Marek et al. 2000; Kłodzinska et al. 2002; Stachowicz et al. 2007), the serotonergic system may contribute to the antidepressant effect of mGlu2/3 receptor antagonists and ketamine. However, involvement of the serotonergic system in actions of mGlu2/3 receptor antagonists and ketamine is not well understood. In this study, we first confirmed the involvement of AMPA receptor stimulation in the antidepressant effects of an mGlu2/3 receptor antagonist, 2S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid (LY341495), and ketamine in the novelty-suppressed feeding (NSF) test, which is useful for evaluating mechanisms responsible for actions of antidepressants. Moreover, to investigate the involvement of the serotonergic system in antidepressant effects of LY341495 and ketamine, we used the NSF test to examine the effects of LY341495 and ketamine following pretreatment with a tryptophan hydroxylase inhibitor, *para*-chlorophenylalanine (PCPA); a 5-HT1A receptor antagonist, *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridynyl) cyclohexane-carboxamide (WAY100635); and a 5-HT2A/2C receptor antagonist, ritanserin.

Materials and methods

Animals and housing

Nine-week-old male C57BL/6 J mice (Charles River Laboratories, Yokohama, Japan) were used for all experiments. The animals were maintained under a controlled temperature (23± 3 °C) and humidity (50±20 %) with a 12-h light/dark cycle (lights on at 7:00 a.m.). Food and water were provided ad libitum, except for food deprivation for 24 h prior to the NSF test. All studies were performed according to the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments (1987).

Drug administration

LY341495 (Tocris Cookson Ltd., Bristol, UK) was dissolved in 1/15 M phosphate buffer (pH8.0). Ketamine (Veterinary Ketalar® 50; Sankyo Yell Pharmaceutical Co., Ltd., Tokyo, Japan) was diluted with saline. CX546 (Tocris Cookson) was dissolved in 10 % hydroxypropyl-beta-cyclodextrin. NBQX (Tocris Cookson) was suspended in saline. WAY100635 (Sigma-Aldrich Co., St. Louis, MO, USA) was dissolved in saline. (±)-8-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT) (Research Biochemicals Inc., Natick, MA, USA) was dissolved in saline. PCPA (Sigma-Aldrich) and ritanserin (Sigma-Aldrich) were suspended in 0.5 % methylcellulose (MC). *Para*-chloroamphetamine (PCA) (Sigma-Aldrich) was dissolved in distilled water. LY341495 (1 mg/kg) and ketamine (30 mg/kg) were administered intraperitoneally (i.p.) 30 min prior to the test. CX546 (10 mg/kg) was administered subcutaneously (s.c.) 30 min prior to the test. NBQX (10 mg/kg) and WAY100635 (0.3, 1, 3 mg/kg) were administered s.c. 35 min and 60 min prior to the test, respectively. Ritanserin (0.5 mg/kg) and PCA (10 mg/kg) were administered i.p. 60 min and 10 min prior to the test, respectively. 8-OH-DPAT (0.1-3 mg/kg) was administered s.c. 30 min prior to the test. A tryptophan hydroxylase inhibitor, PCPA (300 mg/kg) was administered i.p. twice daily (at 7:00–11:00 and 16:00–19:00) for 3 consecutive days, and the tests were conducted 18 h after the final administration. All drugs were injected at a volume of 10 ml/kg body weight. Doses for systemic administration of LY341495, ketamine, and NBQX were selected based on previous studies (Koike et al. 2011b, 2013). Doses for systemic administration of WAY100635 and ritanserin were selected according to previous studies (Dursun and Handley 1996; Iijima et al. 2007). The dose for systemic

administration of PCPA was selected according to a previous study (O'Leary et al. 2007).

PCA-induced head-twitch response

A PCA-induced head-twitch response was performed according to a previously reported method (Balsara et al. 1986). Mice were weighed and individually placed in open-topped plastic cages and removed only for injections. To observe the head-twitch response, the mice were placed in open-topped plastic cages immediately after treatment with PCA (10 mg/kg i.p.). The number of head twitches was counted for 2 min at 18-min intervals between 10 and for up to 52 min. The 2-min scores were accumulated to give a total number of head twitches per mouse, and the total number for each mouse in the group was used to compute the mean value for the group.

Novelty-suppressed feeding test in mice

The NSF test was performed during a 5-min period, as described previously (Iijima et al. 2012). Of note, we previously reported that fluvoxamine showed effect following treatment for 28 days, whereas both ketamine and LY341495 showed effect after a single treatment under the same conditions (Iijima et al. 2012; Koike et al. 2013). Mice were weighed, and all food was removed from their cages. Water continued to be provided ad libitum. Twenty-four hours after food removal, mice were transferred to the testing room, placed in a clean holding cage, and allowed to habituate for 30 min. The testing apparatus consisted of a Plexiglas box (45×45×20 cm) in an illuminated (approximately 1,000 lux), soundproofed box. The floor of the box was covered with 1 cm of wooden bedding. A small piece of mouse chow was placed in the center of the arena on a white circular filter paper (11 cm in diameter). Each mouse was placed in the corner of the testing arena, and the time until the first feeding episode was recorded. Immediately after the mouse began to eat the chow; the tested animal was placed alone in its home cage with a weighed piece of chow for 5 min. At the end of this period, the amount of food consumed was determined by weighing the piece of chow. After all the mice from a single cage were tested, they were returned to their home cage, with food and water provided ad libitum. No treatment affected the amount of food consumed at the respective doses tested (data not shown).

Serotonin content in the prefrontal cortex

The 5-HT content was measured according to a previously reported method (van den Buuse 2013). Mice were sacrificed at 18 h after the final PCPA administration, and the brain was rapidly removed and the frontal cortex dissected on ice. Brain tissues were stored at −80 °C until use, when they were homogenized with ice-cold 0.1 M perchloric acid and centrifuged at 17,360×g at 4 °C. The 5-HT content in the supernatant was measured using an enzyme-linked immunosorbent assay (ELISA; Labor Diagnostika Nord, Nordhorn, Germany).

Statistical analysis

Results are expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined using a one- or two-way analysis of variance (ANOVA), followed by Dunnett's test and least significant difference (LSD) post hoc test for comparing the treated group with a control group and multigroup comparisons, respectively. Statistical differences between two sets of groups were determined using the Student's *t* test. A value of $p<0.05$ was considered statistically significant.

Results

Effect of an AMPA receptor antagonist on the action of LY341495, ketamine, or CX546 in the NSF test

Decrease in latency to feed induced by LY341495 (1 mg/kg i.p.) was blocked by pretreatment with an AMPA receptor antagonist, NBQX (10 mg/kg s.c.) [LY341495, $F(1,42)=3.42$, $p=0.07$; NBQX, $F(1,42)=1.92$, $p=0.17$; interaction, $F(1,42)=6.4$, $p<0.05$] (Fig. 1a). Decrease in latency to feed induced by ketamine (30 mg/kg i.p.) was blocked by pretreatment with NBQX (10 mg/kg s.c.) [ketamine, $F(1,43)=3.40$, $p=0.07$; NBQX, $F(1,43)=7.58$, $p<0.01$; interaction, $F(1,43)=4.94$, $p<0.05$] (Fig. 1b). Decrease in latency to feed induced by an AMPA receptor potentiator, CX546 (10 mg/kg s.c.), was blocked by pretreatment with NBQX (10 mg/kg s.c.) [CX546, $F(1,38)=1.61$, $p=0.21$; NBQX, $F(1,38)=1.72$, $p=0.20$; interaction, $F(1,38)=4.45$, $p<0.05$] (Fig. 1c). In contrast, NBQX (10 mg/kg s.c.) per se did not affect latency to feed in the NSF test (Fig. 1a–c).

Verification of 5-HT depletion after PCPA pretreatment

PCPA (300 mg/kg i.p. twice daily for 3 days) significantly reduced the 5-HT content in the frontal cortex by 74.8 % compared with 0.5 %MC-treated group ($p<0.01$) (Table 1).

Effect of PCPA on PCA-induced head-twitch response

The head-twitch response induced by treatment with a 5-HT release-promoting agent, PCA (10 mg/kg i.p.), was markedly reduced in mice pretreated with PCPA (300 mg/kg i.p. twice daily for 3 days) [PCA, $F(1,36)=110.74$, $p<0.001$; PCPA, $F(1,36)=90.05$, $p<0.001$; interaction, $F(1,36)=84.53$, $p<0.001$] (Fig. 2).

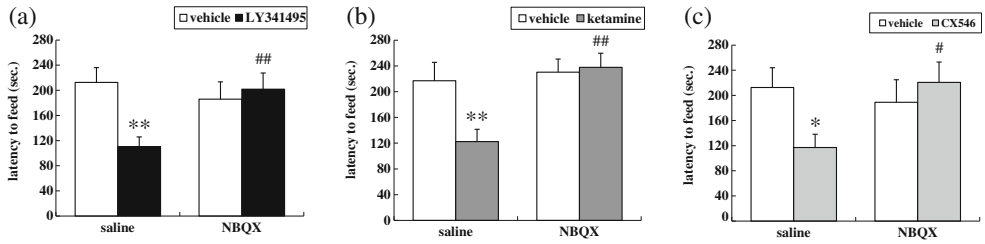

Fig. 1 Effect of an AMPA receptor antagonist on the action of LY341495, ketamine, or CX546 treatment in the NSF test in mice. **a** LY341495 (1 mg/kg i.p.) was administered 30 min prior to the test; **b** ketamine (30 mg/kg i.p.) was administered 30 min prior to the test; **c** CX546 (10 mg/kg s.c.) was administered 30 min prior to the test; NBQX (10 mg/kg s.c.) was administered 35 min prior to the test. Values indicate mean ± S.E.M.: **a** n=11–12, **b** n=11–12, **c** n=9–12). *p<0.05, **p<0.01 compared with saline-treated vehicle, #p<0.05, ##p<0.01 compared with saline-treated agents: **a** LY341495, **b** ketamine, **c** CX546 (LSDpost hoc test)

Effect of 5-HT depletion on the action of LY341495, ketamine, or CX546 in the NSF test

Decrease in latency to feed induced by LY341495 (1 mg/kg i.p.) was blocked by pretreatment with PCPA (300 mg/kg i.p. twice daily for 3 days) [LY341495, $F(1,44)=2.13$, $p=0.15$; PCPA, $F(1,44)=2.51$, $p=0.12$; interaction, $F(1,44)=4.12$, $p<0.05$] (Fig. 3a). Decrease in latency to feed induced by ketamine (30 mg/kg i.p.) was blocked by pretreatment with PCPA (300 mg/kg i.p. twice daily for 3 days) [ketamine, $F(1,41)=5.06$, $p<0.05$; PCPA, $F(1,41)=5.45$, $p<0.05$; interaction, $F(1,41)=4.15$, $p<0.05$] (Fig. 3b). Decrease in latency to feed induced by CX546 (10 mg/kg s.c.) was blocked by pretreatment with PCPA (300 mg/kg i.p. twice daily for 3 days) [CX546, $F(1,43)=4.86$, $p<0.05$; PCPA, $F(1,43)=4.32$; $p<0.05$; interaction, $F(1,43)=5.34$, $p<0.05$] (Fig. 3c). In contrast, PCPA (300 mg/kg i.p. twice daily for 3 days) per se did not affect latency to feed in the NSF test (Fig. 3a, b, c).

Effect of a 5-HT1A receptor antagonist on the action of LY341495, ketamine, or CX546 in the NSF test

Decrease in latency to feed induced by LY341495 (1 mg/kg i.p.) was blocked by pretreatment with a 5-HT1A receptor antagonist, WAY100635 (1, 3 mg/kg s.c.) [$F(3,44)=4.85$, $p<0.05$] (Fig. 4a). Decrease in latency to feed induced by ketamine (30 mg/kg i.p.) was blocked by pretreatment with WAY100635 (3 mg/kg s.c.) [$F(3,43)=4.47$, $p<0.05$] (Fig. 4b). Decrease in latency to feed induced by CX546 (10 mg/kg s.c.) was blocked by pretreatment with WAY100635 (3 mg/kg s.c.) [CX546, $F(1,44)=4.65$, $p<0.05$; WAY100635, $F(1,44)=3.34$, $p=0.07$; interaction, $F(1,44)=6.18$, $p<0.05$] (Fig. 4c). In contrast, WAY100635 (3 mg/kg s.c.) per se did not affect latency to feed in the NSF test (Fig. 4c).

Table 1 Effect of PCPA treatment on 5-HT content in the frontal cortex in mice

	5-HT content (ng/mg tissue)	Percent charge
0.5 % MC	4.33±0.80	–
PCPA	1.09±0.24**	−74.8

Values are mean ± S.E.M. (n=4)

MC methylcellulose

*p<0.01 compared with 0.5 % MC (Student's t test)

Effect of a 5-HT2A/2C receptor antagonist on the action of LY341495, ketamine, or CX546 in the NSF test

Decrease in latency to feed induced by LY341495 (1 mg/kg i.p.) was not blocked by pretreatment with a 5-HT2A/2C receptor antagonist, ritanserin (0.5 mg/kg i.p.) [LY341495, $F(1,43)=15.07$, $p<0.001$; ritanserin, $F(1,43)=0.01$, $p=0.91$; interaction, $F(1,43)=0.34$, $p=0.56$] (Fig. 5a). Decrease in latency to feed

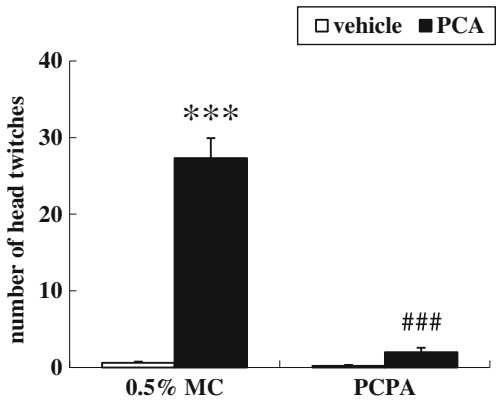

Fig. 2 Effect of 5-HT depletion on PCA-induced head-twitch response in mice. Mice were treated with -PCPA (300 mg/kg i.p.) or 0.5 % MC twice daily for 3 consecutive days until the day before the test, and head-twitch responses induced by injection of PCA (10 mg/kg i.p.) were counted. Values indicate mean ± S.E.M. (n=10). ***p<0.001 compared with 0.5 % MC-treated vehicle, ###p<0.001 compared with 0.5 % MC-treated PCA (LSD post hoc test)

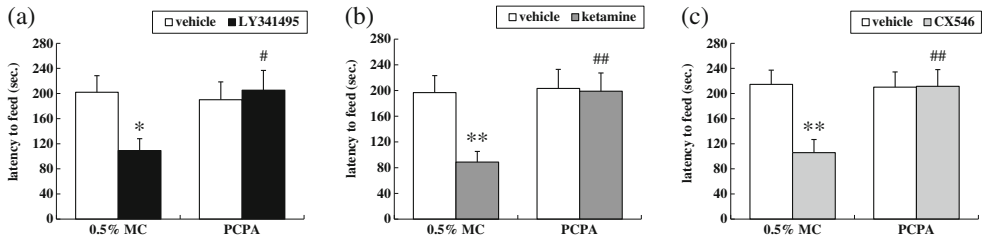

Fig. 3 Effect of 5-HT depletion on the action of LY341495, ketamine, or CX546 in the NSF test in mice. **a** LY341495 (1 mg/kg i.p.) was administered 30 min prior to the test; **b** ketamine (30 mg/kg i.p.) was administered 30 min prior to the test; **c** CX546 (10 mg/kg s.c.) was administered 30 min prior to the test; PCPA (300 mg/kg i.p.) was administered twice daily for 3 consecutive days until the day before the test. Values indicate mean ± S.E.M.: **a** n=12, **b** n=11–12, **c** n=11–12. *p<0.05, **p<0.01 compared with 0.5 % MC-treated vehicle, #p<0.05, ##p<0.01 compared with 0.5 % MC-treated agents: **a** LY341495, **b** ketamine, **c** CX546 (LSD post hoc test)

induced by ketamine (30 mg/kg i.p.) was not blocked by pretreatment with ritanserin (0.5 mg/kg i.p.) [ketamine, $F(1,41)$=16.02, p<0.001; ritanserin, $F(1,41)$=0.36, p=0.55; interaction, $F(1,41)$=0.03, p=0.87] (Fig. 5b). Decrease in latency to feed induced by CX546 (10 mg/kg s.c.) was not blocked by pretreatment with ritanserin (0.5 mg/kg i.p.) [CX546, $F(1,44)$=17.05, p<0.001; ritanserin, $F(1,44)$=0.75, p=0.39; interaction, $F(1,44)$=0.00, p=0.95] (Fig. 5c). In contrast, ritanserin (0.5 mg/kg i.p.) per se did not affect latency to feed in the NSF test (Fig. 5a, b, c).

Effect of 8-OH-DPAT in the NSF test

8-OH-DPAT (0.1-3 mg/kg s.c.) did not affect latency to feed in the NSF test [$F(4,35)$=0.24, p=0.92] (Fig. 6). Subcutaneous administration of 8-OH-DPAT at the doses used in this study has been reported to exert pharmacological effects in rodent models, including the NSF test (Luscombe et al. 1993; Zhang et al. 2010). Thus, the dosages used in our study should be relevant.

Discussion

Previous reports indicate that AMPA receptor stimulation mediates the antidepressant effects of mGlu2/3 receptor antagonists and ketamine in animal models of depression (Karasawa et al. 2005; Koike et al. 2011b; Maeng et al. 2008). First, we confirmed that AMPA receptor blockade cancels the effects of an mGlu2/3 receptor antagonist, LY341495, and ketamine in the NSF test, as observed in other models. Our study indicates that an AMPA receptor antagonist, NBQX, blocked the effects of LY341495 and ketamine in the NSF test. Therefore, AMPA receptor stimulation may contribute to the effects of LY341495 and ketamine in the NSF test. This finding is in line with our results that show an AMPA receptor potentiator, CX546, also exerted the effect in the NSF test, which was also blocked by NBQX.

The primary aim of the study was to investigate the involvement of the serotonergic system in the effects of LY341495 and ketamine in the NSF test. The effects of LY341495 and ketamine were blocked by pretreatment with a tryptophan hydroxylase inhibitor, PCPA, and a 5-HT1A

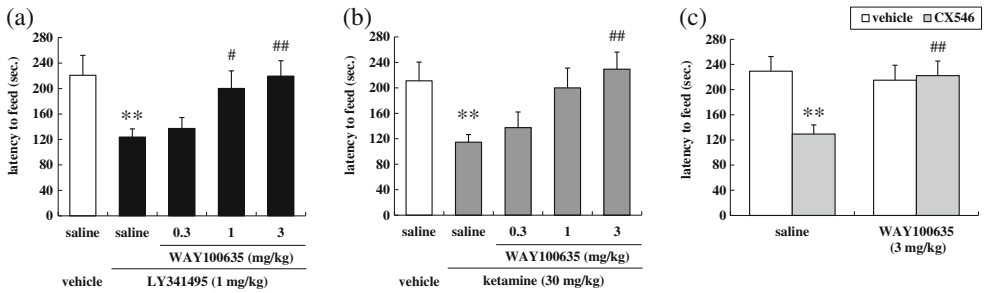

Fig. 4 Effect of a serotonin 5-HT1A receptor antagonist on the action of LY341495, ketamine, or CX546 in the NSF test in mice. **a** LY341495 (1 mg/kg i.p.) was administered 30 min prior to the test; **b** ketamine (30 mg/kg i.p.) was administered 30 min prior to the test; **c** CX546 (10 mg/kg s.c.) was administered 30 min prior to the test; WAY100635 (0.3, 1, 3 mg/kg s.c.) was administered 60 min prior to the test. Values indicate mean ± S.E.M.: **a** n=12, **b** n=11–12, **c** n=12. **p<0.01 compared with saline-treated vehicle (Student's t test), #p<0.05, ##p<0.01 compared with saline-treated agents: **a** LY341495; **b** ketamine (Dunnett's test). **p<0.01, ***p<0.001 compared with saline-treated vehicle, ##p<0.01, ###p<0.001 compared with saline-treated agents; **c** CX546 (LSD post hoc test)

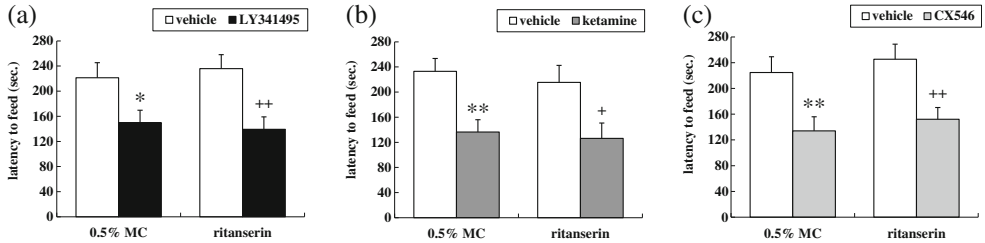

Fig. 5 Effect of a 5-HT2A/2C receptor antagonist, ritanserin, on the action of LY341495, ketamine, or CX546 in the NSF test in mice. **a** LY341495 (1 mg/kg i.p.) was administered 30 min prior to the test; **b** ketamine (30 mg/kg i.p.) was administered 30 min prior to the test; **c** CX546 (10 mg/kg s.c.) was administered 30 min prior to the test; ritanserin (0.5 mg/kg i.p.) was administered 60 min prior to the test. Values indicate mean ± S.E.M.: **a** n=11–12; **b** n=10–12; **c** n=12. *p<0.05, **p<0.01 compared with 0.5 % MC-treated vehicle. + p<0.05, ++p<0.01 compared with ritanserin-treated vehicle (LSD post hoc test)

receptor antagonist, WAY100635, in the NSF test. These results are the first to suggest that the serotonergic system may play a key role in the effects of the mGlu2/3 receptor antagonist and ketamine in the NSF test.

We confirmed that treatment with PCPA (300 mg/kg twice daily for 3 days) caused a 74.8 % reduction in 5-HT content in the frontal cortex compared with the 0.5 % MC-treated group and abolished the head-twitch response induced by a 5-HT-release-promoting agent, PCA. These results indicate that treatment with PCPA is sufficient for pharmacological depletion of 5-HT in the brain. Pretreatment with PCPA blocked the effects of LY341495 and ketamine in the NSF test, suggesting that the effects of LY341495 and ketamine may be mediated by 5-HT release in the NSF test. Moreover, similar to LY341495 and ketamine, the effect of CX546 was blocked by pretreatment with PCPA. Given that the effects of LY341495 and ketamine were blocked by an AMPA receptor antagonist, 5-HT release through AMPA receptor stimulation may play an important role in the effects of LY341495 and ketamine in the NSF test.

Next, we tested 5-HT receptor antagonists to identify the postsynaptic 5-HT receptor subtype responsible for the effects of LY341495 and ketamine and found that these effects were blocked by a 5-HT1A receptor antagonist, WAY100635, but not by a 5-HT2A/2C receptor antagonist, ritanserin, suggesting that among the 5-HT receptor subtypes, 5-HT1A receptor stimulation may mediate the effects of LY341495 and ketamine in the NSF test. Of note, the dose of WAY100635 used in the study has been reported to block postsynaptic 5-HT1A receptor (Ago et al. 2003; Sakaue et al. 2000). We also investigated whether AMPA receptor stimulation exerts the effect via stimulation of postsynaptic 5-HT1A receptor, as observed in the effects of LY341495 and ketamine. The effect of CX546 was also blocked by WAY100635 but not by ritanserin, suggesting that AMPA receptor stimulation exerts its effects by indirectly activating the postsynaptic 5-HT1A receptor. Taken together, 5-HT release through AMPA receptor stimulation by LY341495 and ketamine activates the postsynaptic 5-HT1A receptor, which may lead to the effects of LY341495 and ketamine in the NSF test.

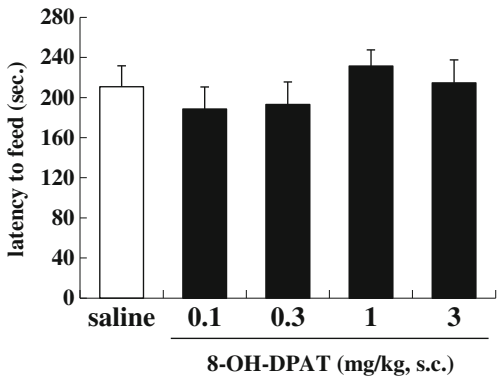

Fig. 6 Effect of 8-OH-DPAT in the NSF test in mice. 8-OH-DPAT (0.1-3 mg/kg s.c.) was administered 30 min prior to the test. Values indicate mean ± S.E.M. (n=8)

The pharmacological significance of findings from this study regarding actions of an mGlu2/3 receptor antagonist and ketamine remains to be elucidated. Warden et al. (2012) reported that the selective stimulation of a subset of dorsal raphe neurons by mPFC projections using an optogenetic technique caused profound antidepressant effects in rodents, whereas activation of the entire dorsal raphe nucleus additionally caused an increase in locomotor activity. Thus, serotonergic transmission, which is selectively stimulated by mPFC-dorsal raphe projection, may play a pivotal role in antidepressant effects. This assumption is underpinned by the finding that deep brain stimulation of the mPFC exerts the antidepressant effect that was prevented by 5-HT depletion (Hamani et al. 2010). Moreover, deep brain stimulation of the mPFC also exerts an acute effect in the NSF test (Hamani et al. 2010), indicating that the mPFC- dorsal raphe pathway might also play an important role in the effects observed in the NSF test. Previously, we reported that mGlu2/3 receptor antagonists activate dorsal raphe 5-HT neurons, and activation of mPFC pyramidal neuron projections to the dorsal raphe nucleus

through AMPA receptor activation may be involved in this effect (Karasawa et al. 2005; Kawashima et al. 2005). In addition, the increase in 5-HT release caused by an NMDA receptor antagonist is reportedly attenuated by intra-mPFC injection of NBQX, and it is postulated that an enhanced glutamatergic output from mPFC neurons, including those projecting to the dorsal raphe nucleus, may increase 5-HT cell firing, leading to cortical 5-HT efflux (López-Gil et al. 2007). Taken together, these findings suggest that activation of a subset of dorsal raphe neurons regulated by mPFC projections, presumably through AMPA receptor stimulation, may be involved in the actions of LY341495 and ketamine in the NSF test. Of note, given that deep brain stimulation of the mPFC reportedly improves symptoms of patients with TRD (Lozano et al. 2008), this mechanism might be involved, at least in part, in the efficacy of ketamine for TRD treatment.

In this study, we found that the 5-HT1A receptor agonist, 8-OH-DPAT, had no effect, wehereas the effects of both LY341495 and ketamine were attenuated by a 5-HT1A receptor antagonist in the NSF test. This discrepancy can be explained by the involvement of selective serotonergic pathways in antidepressant effects, where the 5-HT1A receptor in discrete brain regions stimulated by subset 5-HT neurons (presumably stimulated by mPFC projection) is important for exerting the effects. The roles of dorsal raphe 5-HT neurons regulated by mPFC projections in the antidepressant effects of mGlu2/3 receptor antagonists and ketamine need to be clarified in future studies.

Contrary to our findings, an mGlu2/3 receptor antagonist has been reported to exert an antidepressant effect independent of the serotonergic system in the tail-suspension test (Pałucha-Poniewiera et al. 2010). The antidepressant effect of the acute effect of ketamine has also been reported to be independent of the serotonergic system in the forced swimming test (Gigliucci et al. 2013). Although these differences might arise from differences in paradigms and conditions used in the studies, results cannot be clearly explained at present. Further studies are required to elucidate involvement of the serotonergic system in the effects of mGlu2/3 receptor antagonists and ketamine in several models of depression.

In conclusion, we provide here the first evidence that, in addition to AMPA receptor blockade, actions of both an mGlu2/3 receptor antagonist and ketamine were attenuated by 5-HT depletion and 5-HT1A receptor blockade in the NSF test. These results suggest that AMPA receptor-dependent 5-HT release and the subsequent 5-HT1A receptor stimulation may be involved in actions of an mGlu2/3 receptor antagonist and ketamine in the NSF test. Understanding the mechanisms underlying the activities of agents on glutamatergic systems should lead to novel therapies for MDD.

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