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Rapid Communication

RAPID COMMUNICATION

Lack of Antidepressant Effects of Low-Voltage-Sensitive T-Type Calcium Channel Blocker Ethosuximide in a Chronic Social Defeat Stress Model: Comparison with (R)-Ketamine

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Abstract

Background: A recent study demonstrated that low-voltage-sensitive T-type calcium channel blocker ethosuximide shows rapid antidepressant actions. This study was conducted to compare the antidepressant actions of ethosuximide and (*R*)-ketamine in a chronic social defeat stress model.
Methods: Ethosuximide (100, 200, or 400 mg/kg), (*R*)-ketamine (10 mg/kg), or saline was administered i.p. to chronic social defeat stress-susceptible mice. Subsequently, locomotion test, tail suspension test, forced swimming test, and 1% sucrose preference test were performed.
Results: (*R*)-ketamine showed rapid and long-lasting antidepressant actions in chronic social defeat stress-susceptible mice. In contrast, ethosuximide did not attenuate the increased immobility time of tail suspension test and forced swimming test in chronic social defeat stress-susceptible mice. In the sucrose preference test, ethosuximide did not improve decreased sucrose preference in chronic social defeat stress-susceptible mice.
Conclusions: Unlike (*R*)-ketamine, ethosuximide did not show rapid and sustained antidepressant effects in a chronic social defeat stress model. Therefore, it is unlikely that low-voltage-sensitive T-type calcium channel inhibitors may have ketamine-like robust antidepressant actions.

Keywords: antidepressant, ethosuximide, (*R*)-ketamine, T-VSCC

Introduction

(*R*,*S*)-ketamine, the *N*-methyl-D-aspartate receptor (NMDAR) antagonist, exhibits rapid and sustained antidepressant effects in treatment-resistant patients with major depressive disorder or bipolar disorder (Newport et al., 2015; Kishimoto et al., 2016). Although (*R*,*S*)-ketamine is the most attractive antidepressant for the treatment of depression (Monteggia and Zarate, 2015; Duman et al., 2016; Hashimoto, 2016a, 2016b), the precise mechanisms underlying its antidepressant actions remain elusive.

(*R*,*S*)-ketamine is a racemic mixture containing equal parts of (*R*)-ketamine (or arketamine) and (*S*)-ketamine (or esketamine). Previously, we reported that (*R*)-ketamine shows greater potency and longer-lasting antidepressant effects than (*S*)-ketamine in several animal models of depression (Zhang et al., 2014; Yang et al., 2015, 2017a, 2017b, 2018a; Fukumoto et al., 2017). Unlike (*S*)-ketamine, (*R*)-ketamine does not induce psychotomimetic side effects or exhibit abuse potential in rodents (Yang et al., 2015, 2016).

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Significance Statement

The discovery of rapid and long-lasting antidepressant effects of ketamine in patients with treatment-resistant depression is the most important advance in the field of depression research in the past half-century. A recent study shows that low-voltage-sensitive T-type calcium channel (T-VSCC) blocker ethosuximide has ketamine-like rapid antidepressant effects in rodents. Here, we report that, unlike (*R*)-ketamine, ethosuximide did not have rapid and sustained antidepressant effects in a chronic social defeat stress model. It is therefore unlikely that T-VSCC inhibitors have ketamine-like robust antidepressant actions.

A study using positron emission tomography demonstrated a marked reduction of dopamine $D_{2/3}$ receptor binding in conscious monkey striatum after a single infusion of (*S*)-ketamine but not that of (*R*)-ketamine (Hashimoto et al., 2017), suggesting that (*S*)-ketamine-induced dopamine release might be associated with acute psychotomimetic and dissociative side effects in humans (Hashimoto et al., 2017). Therefore, (*R*)-ketamine could be a safer antidepressant in humans than (*S*)-ketamine (Hashimoto, 2016a, 2016b).

Recently, Yang et al. (2018b) reported that blockade of NMDAR-dependent bursting activity in the lateral habenula (LHb) by (*R*,*S*)-ketamine mediates the rapid antidepressant actions of (*R*,*S*)-ketamine in rodent models of depression. Furthermore, local blockade of NMDAR or low-voltage-sensitive T-type calcium channels (T-VSCCs) in the LHb is sufficient to induce rapid antidepressant effects in rodents (Yang et al., 2018b). Interestingly, i.p. administration of T-VSCC blocker ethosuximide (200 mg/kg) in chronic restraint stress (CRS) mice caused rapid antidepressant effects in both the forced swimming test (FST) and the sucrose preference test (SPT) (Yang et al., 2018b). However, the antidepressant effects of ethosuximide in a chronic social defeat stress (CSDS) model have not been reported. In addition, direct comparison with (*R*,*S*)-ketamine [or (*R*)-ketamine] in the same model was not reported.

The present study was, therefore, undertaken to compare the rapid and long-lasting antidepressant effects of ethosuximide and (*R*)-ketamine in a CSDS model.

Methods and Materials

Animals

Male adult C57BL/6 mice (n = 70), aged 8 weeks (body weight 20-25 g, Japan SLC, Inc., Hamamatsu, Japan) and male adult CD1 (ICR) mice (n = 20), aged 13 to 15 weeks (body weight >40 g, Japan SLC, Inc.) were used. Animals were housed under controlled temperatures and 12-hour-light/-dark cycles (lights on between 7:00 AM and 7:00 PM), with ad libitum food (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee (permission no. 30–357). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animals were deeply anaesthetized with isoflurane before being killed by cervical dislocation. All efforts were made to minimize suffering.

Materials

(*R*)-ketamine hydrochloride was prepared by recrystallization of (*R*,*S*)-ketamine (Ketalar, ketamine hydrochloride, Daiichi Sankyo Pharmaceutical Ltd., Tokyo, Japan) and D-(-)-tartaric acid, as described previously (Zhang et al., 2014). The dose (10 mg/kg as hydrochloride) of (*R*)-ketamine dissolved in the physiological saline was used as previously reported (Zhang et al., 2014; Yang et al., 2015, 2017a, 2017b, 2018a). Three doses of ethosuximide (100, 200, or 400 mg/kg: Tokyo Chemical Industry Co., Ltd, Tokyo, Japan) were used since the dose (200 mg/kg) was used in a previous study (Yang et al., 2018b). Ethosuximide was dissolved in the physiological saline. Other reagents were purchased commercially.

CSDS Model

The procedure of CSDS was performed as previously reported (Yang et al., 2015, 2017a, 2017b, 2018a; Zhang et al., 2015; Dong et al., 2017). The C57BL/6 mice were exposed to a different CD1 aggressor mouse for 10 min/d for consecutive 10 days. When the social defeat session ended, the resident CD1 mouse and the intruder mouse were housed in one-half of the cage separated by a perforated Plexiglas divider to allow visual, olfactory, and auditory contact for the remainder of the 24-hour period. At 24 hours after the last session, all mice were housed individually. On day 11, a social interaction test was performed to identify subgroups of mice that were susceptible and unsusceptible to social defeat stress. This was accomplished by placing mice in an interaction test box (42 × 42 cm) with an empty wire-mesh cage (10 × 4.5 cm) located at one end. The movement of the mice was tracked for 2.5 minutes, followed by 2.5 minutes in the presence of an unfamiliar aggressor confined in the wire-mesh cage. The duration of the subject's presence in the "interaction zone" (defined as the 8-cm-wide area surrounding the wiremesh cage) was recorded by a stopwatch. The interaction ratio was calculated as time spent in an interaction zone with an aggressor/time spent in an interaction zone without an aggressor. An interaction ratio of 1 was set as the cutoff: mice with scores <1 were defined as "susceptible" to social defeat stress and those with scores ≥1 were defined as "resilient". Approximately 70% to 80% of mice were susceptible after CSDS. Susceptible mice were randomly divided in the subsequent experiments. Control C57BL/6 mice without CSDS were housed in the cage before the behavioral tests.

Treatment and Behavioral Tests

The CSDS-susceptible mice were divided into 5 groups. Subsequently, saline (10 mL/kg), (*R*)-ketamine (10 mg/kg), or ethosuximide (100, 200, or 400 mg/kg) was administered i.p. into mice (Figure 1A). Behavioral tests, including locomotion test (LMT), tail suspension test (TST), FST, and 1% SPT, were performed as reported previously (Yang et al., 2015, 2017a, 2017b, 2018a; Dong et al., 2017). LMT and TST were performed 2 and 4 hours after a single injection, respectively. SPT was performed 2, 4, and 7 days after a single injection. In contrast, the previous study (Yang et al., 2018b) performed FST and SPT in CRS mice 1 hour after a single injection of ethosuximide (200 mg/kg).

Locomotion

The locomotor activity was measured by an animal movement analysis system SCANETMV-40 (MELQUEST Co., Ltd., Toyama, Japan). The mice were placed in experimental cages (length × width × height: 560 × 560 × 330 mm). The cumulative

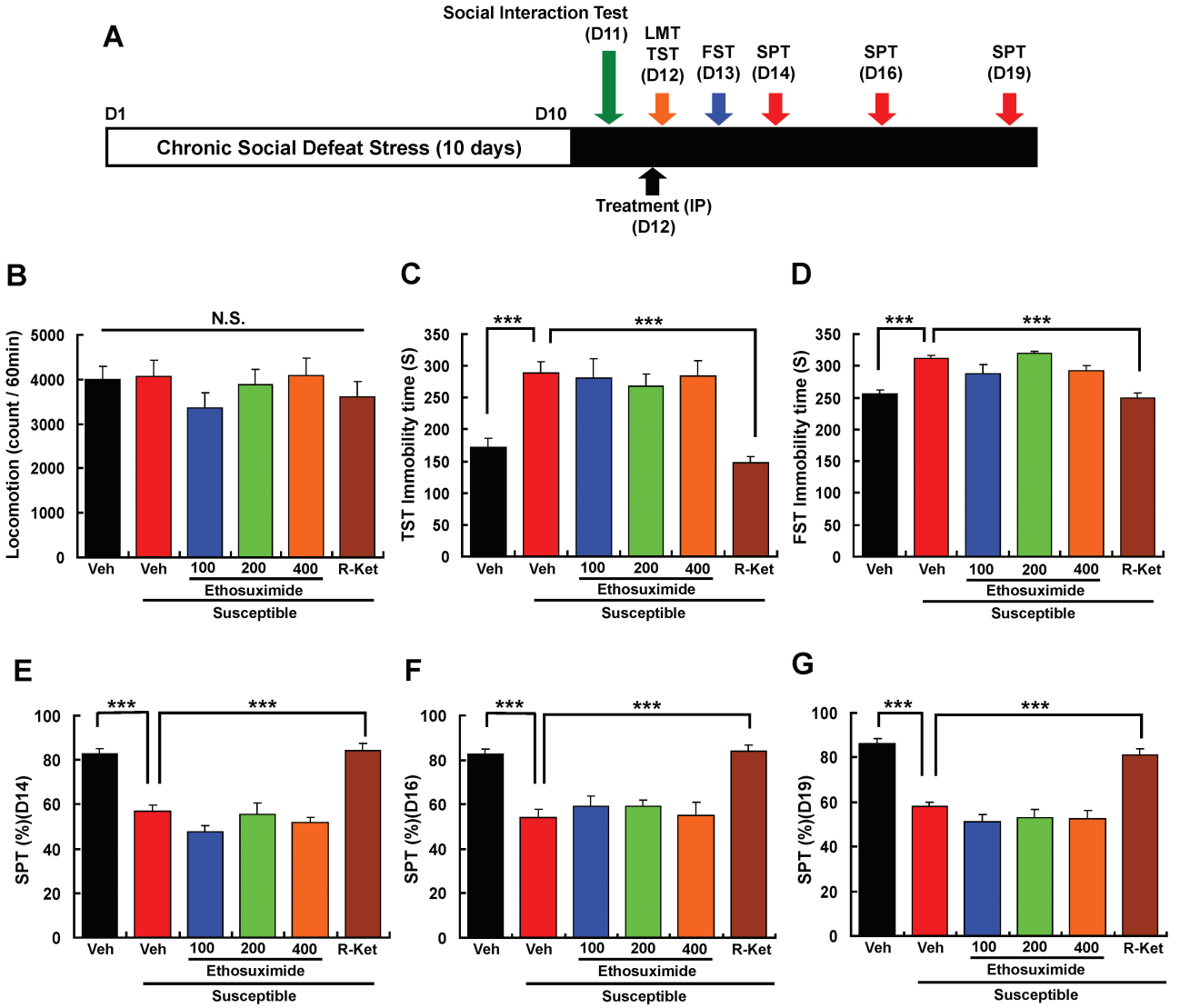

Figure 1. Schedule of a chronic social defeat stress (CSDS) model, treatment, and behavioral tests. (A) CSDS was performed from day 1 to day 10, and the social interaction test (SIT) was performed on day 11. Saline (10 mL/kg), (R)-ketamine (10 mg/kg), or ethosuximide (100, 200, or 400 mg/kg) was administered i.p. in the susceptible mice on day 12. Locomotion test (LMT) and tail suspension test (TST) were performed 2 and 4 hours after a single injection, respectively. Sucrose preference test (SPT) was performed 2, 4, and 7 days after a single injection. (B) LMT (day 12). (C) TST (day 12). (D) FST (day 13). (E) SPT (day 14). (F) SPT (day 16). (G) SPT (day 19). The values represent the mean ± SEM (n = 9). **P < .01, ***P < .001 compared with vehicle-treated susceptible mice. N.S., not significant.

locomotor activity counts were recorded for 60 minutes. Cages were cleaned between testing session.

TST

A small piece of adhesive tape placed approximately 2 cm from the tip of the tail for mouse. A single hole was punched in the tape and mice were hung individually on a hook. The immobility time was recorded for 10 minutes. Mice were considered immobile only when they hung passively and completely motionless.

FST

The FST was conducted using an automated forced-swim apparatus (SCANET MV-40; MELQUEST Co., Ltd.). Mice were placed individually in a cylinder (diameter: 23 cm; height: 31 cm) containing 15 cm of water maintained at a temperature of 23°C ± 1°C. The immobility time was calculated using the activity time as (total) – (active) time by the apparatus analysis software. The immobility time of each mouse was recorded for a period of 6 minutes.

SPT

Mice were exposed to water and 1% sucrose solution for 48 hours, followed by 4 hours of water and food deprivation and a 1-hour exposure to 2 identical bottles (water and 1% sucrose solution). The bottles containing water and sucrose were weighed before and at the end of this period. The sucrose preference was calculated as a percentage of sucrose solution consumption to the total liquid consumption.

Statistical Analysis

The data show as the mean ± SEM. Analysis was performed using PASW Statistics 20 (formerly SPSS Statistics; SPSS, Tokyo, Japan). The behavioral data were analyzed using 1-way ANOVA, followed

by posthoc Fisher's Least Significant Difference test. $P < .05$ was considered statistically significant.

Results

Effects of (*R*)-Ketamine and Ethosuximide in a CSDS Model

Locomotion showed no difference ($F_{5,53}=0.728$, $P=.606$) among the 6 groups (Figure 1B). One-way ANOVA of TST data showed statistical significance ($F_{5,53}=9.725$, $P<.001$) among the 6 groups (Figure 1C). Posthoc tests showed that (*R*)-ketamine (10 mg/kg) significantly attenuated the increased immobility times of TST in susceptible mice after CSDS (Figure 1C). However, ethosuximide (100, 200, or 400 mg/kg) did not attenuate the increased immobility times of TST in susceptible mice after CSDS (Figure 1C). One-way ANOVA of FST data showed statistical significance ($F_{5,53}=11.695$, $P<.001$) among the 6 groups (Figure 1D). Posthoc tests showed that (*R*)-ketamine (10 mg/kg) significantly attenuated the increased immobility times of FST in susceptible mice after CSDS (Figure 1D). However, ethosuximide (100, 200, or 400 mg/kg) did not attenuate the increased immobility times of FST in susceptible mice after CSDS (Figure 1D). One-way ANOVA of SPT data showed statistical significances (2 days after a single injection: $F_{5,53}=22.796$, $P<.001$; 4 days after a single injection: $F_{5,53}=13.157$, $P<.001$; 7 days after a single injection: $F_{5,53}=26.211$, $P<.001$) among the 6 groups (Figure 1E–G). Posthoc tests showed that sucrose preference of (*R*)-ketamine-treated group was significantly higher from saline-treated group. However, sucrose preference of ethosuximide-treated group was not different from saline-treated group (Figure 1E–G).

Next, we examined the effects of ethosuximide in the control (no CSDS) mice. A single dose of ethosuximide (200 mg/kg) did not show antidepressant-like effects in the control nice (Figure 2).

These results suggest that, unlike (*R*)-ketamine, ethosuximide does not have rapid and sustained antidepressant effects in mice with depression-like phenotype in a CSDS model.

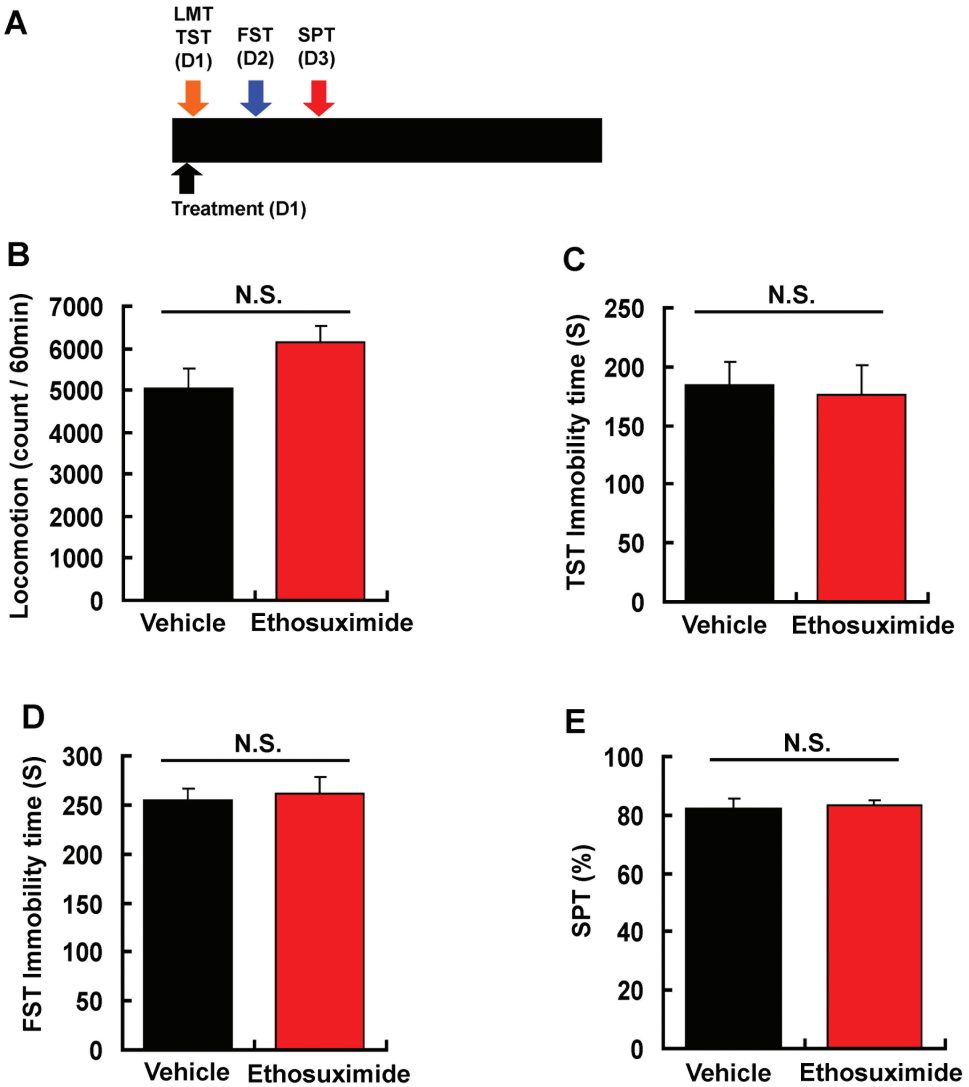

Figure 2. Lack of antidepressant-like effects of ethosuximide in control mice. (A) Saline (10 mL/kg) or ethosuximide (200 mg/kg) was administered i.p. in the control (no chronic social defeat stress [CSDS]) mice. Locomotion test (LMT) and tail suspension test (TST) were performed 2 and 4 hours after a single injection, respectively. Forced swimming test (FST) was performed 1 day after a single injection. Sucrose preference test (SPT) was performed 2 days after a single injection. (B) LMT (day 1). (C) TST (day 1). (D) FST (day 2). (E) SPT (day 3). The values represent the mean ± SEM (n = 9). N.S., not significant.

Discussion

In the present study, we demonstrated that ethosuximide did not show rapid and sustained antidepressant effects in CSDS-susceptible mice, although (*R*)-ketamine had rapid and long-lasting antidepressant effects in the same model. In this study, we used the 3 doses (100, 200, or 400 mg/kg) of ethosuximide in a CSDS model, although a previous study used the dose 200 mg/kg (Yang et al., 2018b). This study suggests that ethosuximide does not have ketamine-like antidepressant actions in a CSDS model.

It is reported that (*R,S*)-ketamine blocks burst of neurons in the LHb, rapidly relieving depression-like behaviors in rodents (Yang et al., 2018b). Local infusion of (*R,S*)-ketamine (25 μg/each side) into the LHb of congenitally learned helplessness rats significantly improved depression-like behaviors without affecting locomotor activity (Yang et al., 2018b). However, the dose (25 μg/each side) used in the study (Yang et al., 2018b) was higher than that of previous reports [10 or 30 ng/each side in the infralimbic region of medial prefrontal cortex of control (no stress) rats (Fuchikami et al., 2015); 2 μg/each side in the infralimbic region of medial prefrontal cortex of LH rats (Shirayama and Hashimoto, 2017)]. Furthermore, (*R,S*)-ketamine (25 mg/kg for rats and 10 mg/kg for mice) reduced the number of bursting LHb neurons and relieved depression-like behaviors in the congenitally learned helplessness rats and CRS mice (Yang et al., 2018b). They performed the behavioral tests (FST and SPT) 1 hour after a single injection, and, unfortunately, they did not examine sustained (24 hours) and long-lasting (7 days) antidepressant effects. The study suggests that, by blocking NMDAR-dependent LHb bursts, (*R,S*)-ketamine can release this inhibition brake onto the reward centers to elicit its rapid antidepressant effects (Yang et al., 2018b). Although LHb may play, in part, a role in the rapid antidepressant effects of (*R,S*)-ketamine, further detailed study on LHb theory of ketamine's antidepressant actions is needed.

Interestingly, Yang et al. (2018b) demonstrated that the LHb burst was dependent on T-VSCC and that T-VSCC blocker ethosuximide abolished burst firing and relieved depression-like behaviors in CRS mice. In the study, they used the only dose (200 mg/kg) of ethosuximide in the CRS model, and they did not include control (no CRS) mice (Figure 4c–d in Yang et al., 2018b). Furthermore, they did not examine the sustained (24 hours after injection) and long-lasting (7 days after injection) antidepressant effects of ethosuximide, although (*R,S*)-ketamine or (*R*)-ketamine shows sustained and longer-lasting antidepressant effects in a CSDS model (Yang et al., 2015, 2017a, 2017b, 2018a; Zhang et al., 2015). Although the reasons underlying the discrepancy (our study vs Yang et al., 2018b) are currently unclear, the use of models (CSDS model vs CRS model) and the breeding and housing for animals (Japan vs China) may contribute to the discrepancy. To assess ketamine-like antidepressant effects of T-VSCC inhibitors, further detailed study using other T-VSCC blockers is needed. Although ethosuximide did not show ketamine-like antidepressant effects in a CSDS model, the mechanisms by which (*R*)-ketamine results in rapid and long-lasting antidepressant effects remain to be determined.

In conclusion, this study suggests that, unlike (*R*)-ketamine, T-VSCC blocker ethosuximide did not show the rapid and long-lasting antidepressant effects in a CSDS model. Therefore, it is unlikely that T-VSCC blockers have ketamine-like robust antidepressant effects, although further study is needed.

Acknowledgments

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Statement of Interest

Dr Hashimoto is an inventor on a filed patent application on "The use of (*R*)-ketamine in the treatment of psychiatric diseases" by Chiba University. Dr. Hashimoto has received research support from Dainippon-Sumitomo, Otsuka, and Taisho. Other authors declare no conflict of interest.

References

Dong C, Zhang JC, Yao W, Ren Q, Ma M, Yang C, Chaki S, Hashimoto K (2017) Rapid and sustained antidepressant action of the mGlu2/3 receptor antagonist MGS0039 in the social defeat stress model: comparison with ketamine. Int J Neuropsychopharmacol 20:228–236.

Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016) Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat Med 22:238–249.

Fuchikami M, Thomas A, Liu R, Wohleb ES, Land BB, DiLeone RJ, Aghajanian GK, Duman RS (2015) Optogenetic stimulation of infralimbic PFC reproduces ketamine's rapid and sustained antidepressant actions. Proc Natl Acad Sci U S A 112:8106–8111.

Fukumoto K, Toki H, Iijima M, Hashihayata T, Yamaguchi JI, Hashimoto K, Chaki S (2017) Antidepressant potential of (*R*)-ketamine in rodent models: comparison with (*S*)-ketamine. J Pharmacol Exp Ther 361:9–16.

Hashimoto K (2016a) R-ketamine: a rapid-onset and sustained antidepressant without risk of brain toxicity. Psychol Med 46:2449–2451.

Hashimoto K (2016b) Ketamine's antidepressant action: beyond NMDA receptor inhibition. Expert Opin Ther Targets 20:1389–1392.

Hashimoto K, Kakiuchi T, Ohba H, Nishiyama S, Tsukada H (2017) Reduction of dopamine D2/3 receptor binding in the striatum after a single administration of esketamine, but not R-ketamine: a PET study in conscious monkeys. Eur Arch Psychiatry Clin Neurosci 267:173–176.

Kishimoto T, Chawla JM, Hagi K, Zarate CA, Kane JM, Bauer M, Correll CU (2016) Single-dose infusion ketamine and non-ketamine N-methyl-d-aspartate receptor antagonists for unipolar and bipolar depression: a meta-analysis of efficacy, safety and time trajectories. Psychol Med 46:1459–1472.

Monteggia LM, Zarate C Jr (2015) Antidepressant actions of ketamine: from molecular mechanisms to clinical practice. Curr Opin Neurobiol 30:139–143.

Newport DJ, Carpenter LL, McDonald WM, Potash JB, Tohen M, Nemeroff CB, APA Council of Research Task Force on Novel Biomarkers and Treatments (2015) Ketamine and other NMDA antagonists: early clinical trials and possible mechanisms in depression. Am J Psychiatry 172:950–966.

Shirayama Y, Hashimoto K (2017) Effects of a single bilateral infusion of R-ketamine in the rat brain regions of a learned helplessness model of depression. Eur Arch Psychiatry Clin Neurosci 267:177–182.

Yang C, Shirayama Y, Zhang JC, Ren Q, Yao W, Ma M, Dong C, Hashimoto K (2015) R-ketamine: a rapid-onset and sustained antidepressant without psychotomimetic side effects. Transl Psychiatry 5:e632.

Yang C, Han M, Zhang JC, Ren Q, Hashimoto K (2016) Loss of parvalbumin-immunoreactivity in mouse brain regions after repeated intermittent administration of esketamine, but not R-ketamine. Psychiatry Res 239:281–283.

Yang C, Qu Y, Abe M, Nozawa D, Chaki S, Hashimoto K (2017a) (*R*)-ketamine shows greater potency and longer

lasting antidepressant effects than its metabolite (2*R*,6*R*)-hydroxynorketamine. Biol Psychiatry 82:e43–e44.

Yang C, Qu Y, Fujita Y, Ren Q, Ma M, Dong C, Hashimoto K (2017b) Possible role of the gut microbiota-brain axis in the antidepressant effects of (*R*)-ketamine in a social defeat stress model. Transl Psychiatry 7:1294.

Yang C, Ren Q, Qu Y, Zhang JC, Ma M, Dong C, Hashimoto K (2018a) Mechanistic target of rapamycin-independent antidepressant effects of (*R*)-ketamine in a social defeat stress model. Biol Psychiatry 83:18–28.

Yang Y, Cui Y, Sang K, Dong Y, Ni Z, Ma S, Hu H (2018b) Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. Nature 554:317–322.

Zhang JC, Li SX, Hashimoto K (2014) R (-)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. Pharmacol Biochem Behav 116:137–141.

Zhang JC, Yao W, Dong C, Yang C, Ren Q, Ma M, Han M, Hashimoto K (2015) Comparison of ketamine, 7,8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. Psychopharmacology (Berl) 232:4325–4335.