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REGULAR RESEARCH ARTICLE

(*R*)-Ketamine Induces a Greater Increase in Prefrontal 5-HT Release Than (*S*)-Ketamine and Ketamine Metabolites via an AMPA Receptor-Independent Mechanism

Yukio Ago, Wataru Tanabe, Momoko Higuchi, Shinji Tsukada, Tatsunori Tanaka, Takumi Yamaguchi, Hisato Igarashi, Rei Yokoyama, Kaoru Seiriki, Atsushi Kasai, Takanobu Nakazawa, Shinsaku Nakagawa, Kenji Hashimoto, Hitoshi Hashimoto

Laboratory of Molecular Neuropharmacology (Dr Ago, Mr Tanabe, Ms Higuchi, Mr Tsukada, Dr Tanaka, Mr Igarashi, Mr Yokoyama, Drs Seiriki, Kasai, Nakazawa, H. Hashimoto), and Laboratory of Biopharmaceutics (Dr Ago, Mr Yamaguchi, Dr Nakagawa), Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan; Interdisciplinary Program for Biomedical Sciences, Institute for Transdisciplinary Graduate Degree Programs (Dr Seiriki), and Department of Pharmacology, Graduate School of Dentistry (Dr Nakazawa), Osaka University, Suita, Osaka, Japan; Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan (Dr K. Hashimoto); Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University and University of Fukui, Suita, Osaka, Japan (Dr H. Hashimoto); Division of Bioscience, Institute for Datability Science (Dr H. Hashimoto), and Transdimensional Life Imaging Division, Institute for Open and Transdisciplinary Research Initiatives (Dr H. Hashimoto), Osaka University, Suita, Osaka, Japan.

Y.A. and W.T contributed equally to this work.

Correspondence: Yukio Ago, PhD, Associate Professor; Laboratory of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Osaka University; 1–6 Yamada-oka, Suita, Osaka 565–0871, Japan (ago@phs.osaka-u.ac.jp).

Abstract

Background: Although recent studies provide insight into the molecular mechanisms of the effects of ketamine, the antidepressant mechanism of ketamine enantiomers and their metabolites is not fully understood. In view of the involvement of mechanisms other than the *N*-methyl-D-aspartate receptor in ketamine's action, we investigated the effects of (*R*)-ketamine, (*S*)-ketamine, (*R*)-norketamine [(*R*)-NK], (*S*)-NK, (2*R*,6*R*)-hydroxynorketamine [(2*R*,6*R*)-HNK], and (2*S*,6*S*)-HNK on monoaminergic neurotransmission in the prefrontal cortex of mice.
Methods: The extracellular monoamine levels in the prefrontal cortex were measured by in vivo microdialysis.
Results: (*R*)-Ketamine and (*S*)-ketamine acutely increased serotonin release in a dose-dependent manner, and the effect of (*R*)-ketamine was greater than that of (*S*)-ketamine. In contrast, (*S*)-ketamine caused a robust increase in dopamine release

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Significance Statement

Several molecular mechanisms underlying the antidepressant-like effects of (*R*,*S*)-ketamine have been proposed, which focus on the glutamatergic system. However, (*R*)-ketamine has been reported to possess greater potency and longer-lasting antidepressant effects than (*S*)-ketamine in several animal models of depression despite (*R*)-ketamine having lower affinity for the *N*-methyl-D-aspartate receptor than (*S*)-ketamine. The present study demonstrates the differences between ketamine enantiomers in inducing prefrontal serotonin (5-HT) and dopamine but not noradrenaline release. (*R*)-Ketamine induced a greater increase in 5-HT release than (*S*)-ketamine, but this effect was AMPA receptor independent. (*S*)-Ketamine caused a robust increase in dopamine release compared with (*R*)-ketamine via an AMPA receptor-dependent mechanism. A ketamine metabolite (2*R*,6*R*)-hydroxynorketamine induced a slight increase in 5-HT and noradrenaline release and (*S*)-norketamine increased dopamine and noradrenaline release. These findings provide a neurochemical basis for understanding the pharmacological differences and mechanisms of (*R*)-ketamine, (*S*)-ketamine, and their metabolites.

compared with (*R*)-ketamine. Both ketamine enantiomers increased noradrenaline release, but these effects did not differ. (2*R*,6*R*)-HNK caused a slight but significant increase in serotonin and noradrenaline but not dopamine release. (*S*)-NK increased dopamine and noradrenaline but not serotonin release. Differential effects between (*R*)-ketamine and (*S*)-ketamine were also observed in a lipopolysaccharide-induced model of depression. An α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4- tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX), attenuated (*S*)-ketamine-induced, but not (*R*)-ketamine-induced serotonin release, whereas NBQX blocked dopamine release induced by both enantiomers. Local application of (*R*)-ketamine into the prefrontal cortex caused a greater increase in prefrontal serotonin release than that of (*S*)-ketamine.

Conclusions: (*R*)-Ketamine strongly activates the prefrontal serotonergic system through an AMPA receptor-independent mechanism. (*S*)-Ketamine-induced serotonin and dopamine release was AMPA receptor-dependent. These findings provide a neurochemical basis for the underlying pharmacological differences between ketamine enantiomers and their metabolites.

Keywords: (*R*)-ketamine, (*S*)-ketamine, monoamine, prefrontal cortex, AMPA receptors

Introduction

Accumulating evidence has indicated that the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine (racemic ketamine; (*R*,*S*)-ketamine) has rapid and potent antidepressant effects in major depressive disorder including treatment-resistant depression (Berman et al., 2000; Zarate et al., 2006a; Murrough et al., 2013; Newport et al., 2015; Su et al., 2017). (*R*,*S*)-Ketamine also produces antisuicidal effects in treatment-resistant depression (Price et al., 2009; Murrough et al., 2015; Grunebaum et al., 2018; Wilkinson et al., 2018). Some clinical trials have demonstrated that intranasal administration of esketamine [(*S*)-ketamine] showed rapid and sustained (>2 months) antidepressant effects in treatment-resistant depression (Daly et al., 2018) and resulted in rapid improvement in depressive symptoms and suicidality in patients at imminent risk for suicide (Canuso et al., 2018). On March 5, 2019, an (*S*)-ketamine nasal spray was approved as a new antidepressant for treatment-resistant depression by the US Food and Drug Administration (FDA News Release, 2019). Several molecular mechanisms underlying the antidepressant-like effects of ketamine have been proposed, especially focusing on the glutamatergic system such as synaptic or GluN2B-selective extra-synaptic NMDA receptor inhibition, inhibition of NMDA receptors localized on GABAergic interneurons, and the role of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor activation (Maeng et al., 2008; Chaki, 2017; Pałucha-Poniewiera, 2018; Zanos and Gould, 2018; Duman et al., 2019). However, other NMDA antagonists, including memantine and lanicemine, which bind to the receptor at the same site as ketamine, do not exhibit consistent evidence for clinical antidepressant efficacy (Zarate et al., 2006b, 2013; Smith et al., 2013; Newport et al., 2015; Sanacora et al., 2017). Moreover, several animal studies have demonstrated that (*R*)-ketamine has greater potency and longer lasting antidepressant effects than (*S*)-ketamine (Zhang et al., 2014a; Yang et al., 2015, 2017a, 2017b, 2018b; Zanos et al., 2016; Fukumoto et al., 2017), while (*S*)-ketamine (Ki = 0.30 μM) has a higher affinity for the NMDA receptor than (*R*)-ketamine (Ki = 1.4 μM) (Ebert et al., 1997). Therefore, mechanisms other than the NMDA receptor also play an important role in mediating the antidepressant effects of ketamine.

In addition to the glutamatergic system, recent preclinical studies indicate the potential involvement of the monoaminergic system in the antidepressant actions of ketamine (du Jardin et al., 2016b). Furthermore, the monoaminergic system has been implicated in the antidepressant effects of numerous currently used drugs. Regarding the antidepressant-like effects of ketamine, a serotonin (5-HT) synthesis inhibitor, *p*-chlorophenylalanine (PCPA), attenuated the acute (Fukumoto et al., 2016) and sustained (Pham et al., 2017) antidepressant-like effects of (*R*,*S*)-ketamine. The sustained antidepressant effects were also attenuated by intra-medial prefrontal cortex (PFC) injection of a 5-HT_{1A} receptor antagonist, WAY100635 (Fukumoto et al., 2018). Additionally, a recent study shows that activation of *Drd1* (the dopamine [DA]-D_1 receptor)-expressing pyramidal cells in the medial PFC produces rapid and long-lasting antidepressant responses, and the disruption of *Drd1* activity blocked the rapid antidepressant effects of (*R*,*S*)-ketamine (Hare et al., 2019). These findings suggest that the monoaminergic system is involved at least partly in the acute and sustained antidepressant-like effects of ketamine. Previous microdialysis studies have shown that acute administration of (*R*,*S*)-ketamine in the dose range showing antidepressant activity increased the extracellular levels

of 5-HT and DA in the PFC (Lorrain et al., 2003; Pham et al., 2017; Kinoshita et al., 2018). Witkin et al. (2016) reported that (*S*)-ketamine (10 mg/kg, s.c.) increased the extracellular levels of 5-HT, DA, noradrenaline (NA), histamine, and acetylcholine but not glutamate or γ-aminobutyric acid in rat PFC. However, the neurochemical effects of (*R*)-ketamine are not fully understood, and there is no comparative study to our knowledge of ketamine enantiomers. In this study, we aimed to clarify the effects of (*R*)-ketamine and (*S*)-ketamine on the in vivo release of monoamines in the PFC of both normal mice and a lipopolysaccharide (LPS)-induced mouse model of depression (Zhang et al., 2014b). Since the AMPA receptor is suggested to be involved in the antidepressant-like effects of ketamine (Maeng et al., 2008; Chaki, 2017; Pałucha-Poniewiera, 2018; Zanos and Gould, 2018; Duman et al., 2019), we subsequently examined the effects of 2,3-dioxo-6-nitro-1,2,3,4- tetrahydrobenzo[f] quinoxaline-7-sulfonamide (NBQX), an AMPA receptor antagonist, on (*R*)-ketamine- and (*S*)-ketamine-induced changes in monoamine release. Some metabolites of ketamine, such as (*S*)-norketamine [(*S*)-NK] and (2*R*,6*R*)-hydroxynorketamine [(2*R*,6*R*)-HNK] have been shown to exert antidepressant-like effects (Zanos et al., 2016; Chou et al., 2018; Pham et al., 2018; Yang et al., 2018a; Fukumoto et al., 2019). Thus, this study also investigated the effects of metabolites of ketamine enantiomers such as (*R*)-NK, (*S*)-NK, (2*R*,6*R*)-HNK, and (2*S*,6*S*)-HNK on central monoaminergic transmission in mice.

Materials and Methods

Animals and Drugs

All animal studies were approved by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences, Osaka University. All experimental procedures were conducted in accordance with the guidelines of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). Every effort was made to minimize animal suffering and to reduce the number of animals used. Eight-week-old male C57BL/6J mice were obtained from SHIMIZU Laboratory Supplies Co., Ltd. (Kyoto, Japan) and housed in cages (28 cm × 17 cm × 12 cm) in groups of 5 or 6 animals under controlled environmental conditions (22 ± 1°C; 50 ± 10% relative humidity; a 12-hour light-dark cycle, lights on at 8:00 AM; food and water ad libitum) for at least 1 week before use in the experiments. (*R*)-Ketamine hydrochloride and (*S*)-ketamine hydrochloride were prepared by recrystallization of (*R*,*S*)-ketamine (Ketalar, ketamine hydrochloride, Daiichi Sankyo Pharmaceutical Ltd., Tokyo, Japan) and D-(-)-tartaric acid and L-(+)-tartaric acid, respectively (Zhang et al., 2014a). (*R*)-NK, (*S*)-NK, (2*R*,6*R*)-HNK, and (2*S*,6*S*)-HNK were purchased from Tocris Bioscience (Bristol, UK). NBQX disodium salt and LPS (serotype O111:B4) were purchased from Abcam (Cambridge, UK) and Sigma-Aldrich (St. Louis, MO), respectively. All drugs were dissolved in saline (0.9% [w/v] solution of NaCl). All drugs except NBQX were administered i.p. at a volume of 10 mL/kg body weight. NBQX was s.c. injected at a volume of 10 mL/kg body weight. The doses of (*R*)-ketamine, (*S*)-ketamine, and their metabolites used here were selected according to previous studies (Zhang et al., 2014a; Yang et al., 2015, 2018a; 2017a, 2017b, 2018b; Zanos et al., 2016; Fukumoto et al., 2017, 2019; Pham et al., 2017, 2018; Chou et al., 2018). To induce depression-like models, mice were i.p. injected with LPS (0.5 mg/kg) 24 hours before the microdialysis experiment, as previously described (Zhang et al., 2014b).

In Vivo Microdialysis

Microdialysis experiments were performed as previously reported (Ago et al., 2013; Hara et al., 2016; Tanaka et al., 2017). Briefly, each mouse was anesthetized with a mixture of medetomidine (0.3 mg/kg, i.p.), midazolam (4 mg/kg, i.p.), and butorphanol (5 mg/kg, i.p.) and stereotaxically implanted unilaterally and counterbalanced left or right with a guide-cannula for a dialysis probe (Eicom Corp., Kyoto, Japan) positioned in the PFC (*A* +1.9 mm, *L* ±0.5 mm, *V* –0.8 mm, from the bregma and skull) (Franklin and Paxinos, 1997). The cannula was cemented in place with dental acrylic, and the animal was kept warm and allowed to recover from anesthesia. Postoperative analgesia was performed with a single injection of buprenorphine (0.1 mg/kg, i.p.). The active probe membranes were 3 mm long. Two days after surgery, the probe was perfused with Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, and 2.2 mM $CaCl_2$; Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) at a constant flow rate of 1 μL/min. A stabilization period of 3 hours was established before the onset of the experiment. Microdialysis samples (20 μL) were collected every 20 minutes and injected immediately onto a high-performance liquid chromatography column for simultaneous assay of 5-HT, DA, and NA (Hara et al., 2016; Tanaka et al., 2017). The concentrations of 5-HT, DA, and NA in brain microdialysates were determined using high-performance liquid chromatography with an electrochemical detector (HTEC-500; Eicom Corp.). After the experiments, Evans Blue dye was microinjected through the cannula to histologically verify the position of the probe, and only data from animals with correct probe placement were used in the analysis.

Statistics

All results are presented as the mean ± SEM. Data from microdialysis were calculated as the percentage of change from dialysate baseline concentrations, with 100% defined as the average of 3 fractions before the drug administration. Data were analyzed using 2- or 3-way ANOVA for treatment or dosage as the inter-subject factor and repeated measures with time as the intra-subject factor, followed by the Tukey-Kramer post hoc test when the interaction was significant. Statistical analyses were performed using the Statview 5.0J software package for Apple Macintosh (SAS Institute Inc., Cary, NC). A value of $P < .05$ was considered statistically significant.

Results

Effects of (*R*)-Ketamine, (*S*)-Ketamine, and Their Metabolites on Extracellular 5-HT, DA, and NA Levels in the PFC of Normal Mice

Baseline levels (mean ± SEM) of extracellular 5-HT, DA, and NA in the PFC (not corrected for in vitro probe recovery) were 1.03 ± 0.07, 0.87 ± 0.06, and 1.02 ± 0.05 pg/fraction (20 μL), respectively (n = 95, calculated from Figures 1, 3, and 4). For 5-HT release, a single administration of (*R*)-ketamine at doses of 10 mg/kg ($F_{8,64} = 3.722$, $P = .0013$) and 20 mg/kg ($F_{8,64} = 17.055$, $P < .0001$) and (*S*)-ketamine at doses of 10 mg/kg ($F_{8,64} = 2.100$, $P = .0485$) and 20 mg/kg ($F_{8,64} = 5.517$, $P < .0001$) increased extracellular 5-HT levels in a dose-dependent manner (Figure 1A). The increase in 5-HT release by (*R*)-ketamine was significantly greater than (*S*)-ketamine (3-way ANOVA with repeated measures: $F_{16,192} = 2.362$, $P = .0032$). (2*R*,6*R*)-HNK (20 mg/kg) caused a slight but significant increase in 5-HT release in the PFC ($F_{8,64} = 2.676$, $P = .0133$),

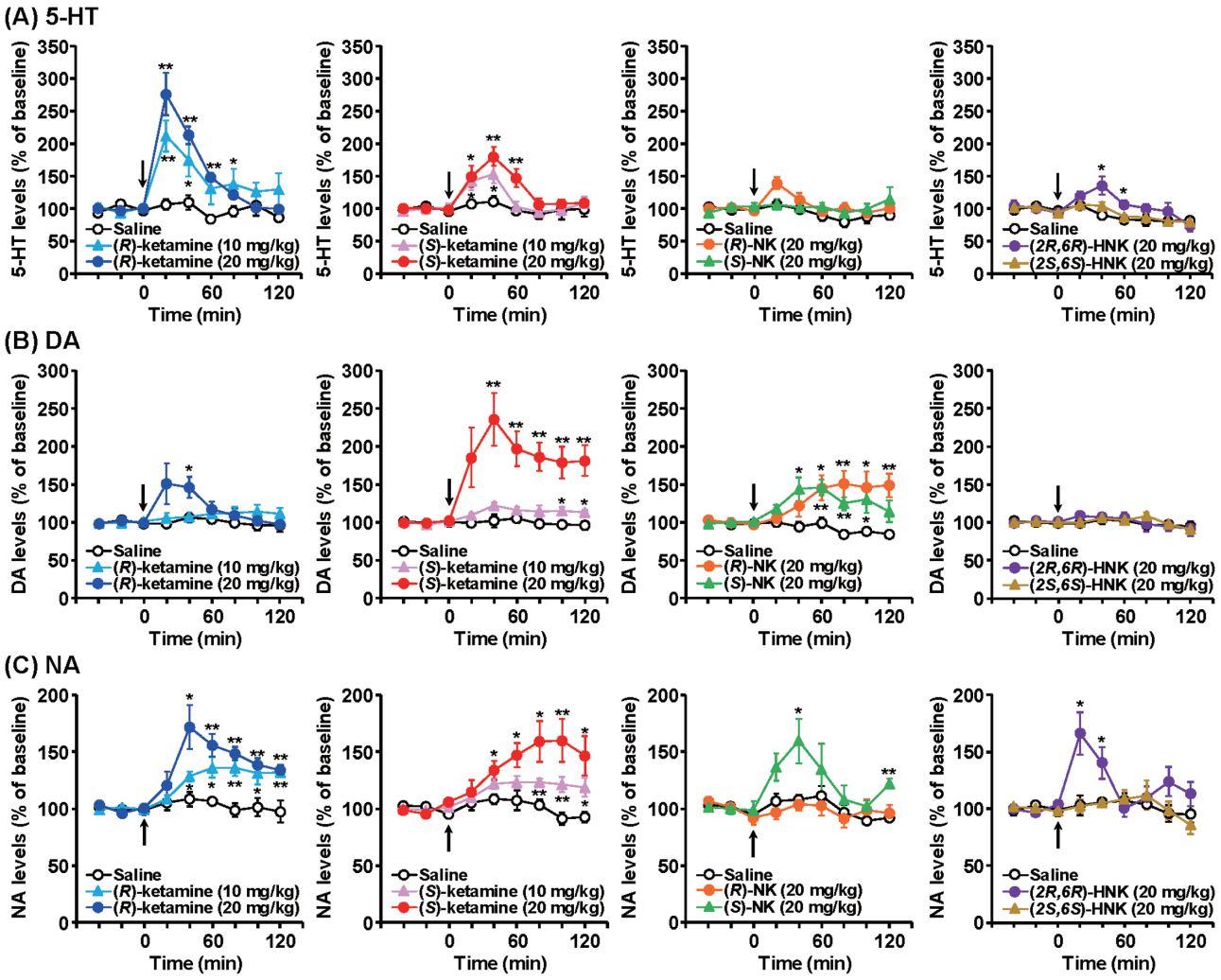

Figure 1. The effects of (*R*)-ketamine, (*S*)-ketamine, and their metabolites on extracellular serotonin (5-HT) (A), dopamine (DA) (B), and noradrenaline (NA) (C) levels in the prefrontal cortex (PFC) of mice. (*R*)-ketamine (10, 20 mg/kg), (*S*)-ketamine (10, 20 mg/kg), (*R*)-norketamine [(*R*)-NK] (20 mg/kg), (*S*)-NK (20 mg/kg), (2*R*,6*R*)-hydroxynorketamine [(2*R*,6*R*)-HNK] (20 mg/kg), (2*S*,6*S*)-HNK (20 mg/kg), or saline was i.p. injected at 0 minutes (arrow). Results are expressed as the mean ± SEM of 5 mice per group. *P < .05, **P < .01, compared with the saline-treated mice at each time point.

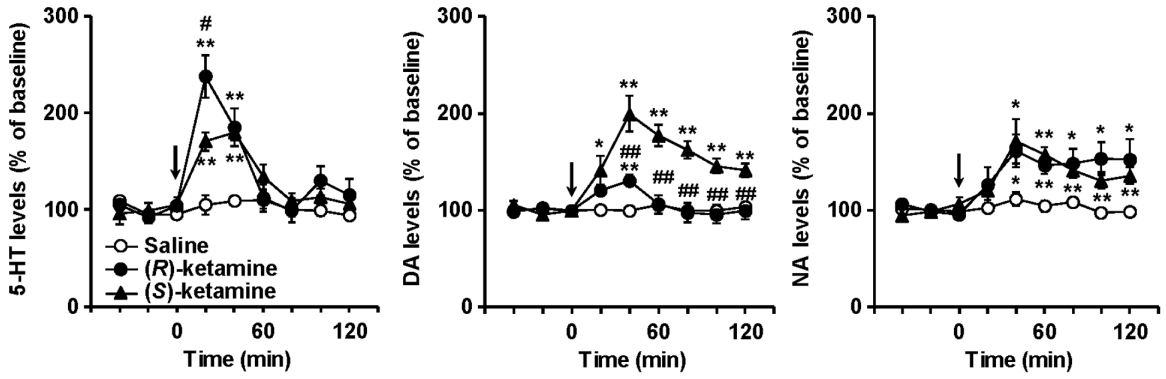

Figure 2. The effects of (*R*)-ketamine and (*S*)-ketamine on extracellular monoamine levels in the prefrontal cortex (PFC) of lipopolysaccharide (LPS)-treated mice. Mice were i.p. injected with LPS (0.5 mg/kg) 24 hours before the experiment. (*R*)-Ketamine (20 mg/kg), (*S*)-ketamine (20 mg/kg), or saline was i.p. injected at 0 minutes (arrow). Results are expressed as the mean ± SEM of 5 mice per group. *P < .05, **P < .01, compared with saline-treated mice at each time point. #P < .05, ##P < .01, compared with (*S*)-ketamine-treated mice at each time point.

whereas (*R*)-NK (20 mg/kg, $F_{8,64}$ = 1.582, *P* = .1480), (*S*)-NK (20 mg/kg, $F_{8,64}$ = 0.960, *P* = .4749), or (2*S*,6*S*)-HNK (20 mg/kg, $F_{8,64}$ = 0.559, *P* = .8072) did not affect prefrontal 5-HT release.

For DA release, a single administration of (*R*)-ketamine at a dose of 20 mg/kg ($F_{8,64}$ = 2.776, *P* = .0106), but not 10 mg/kg ($F_{8,64}$ = 1.067, *P* = .3974), and (*S*)-ketamine at doses of 10 mg/

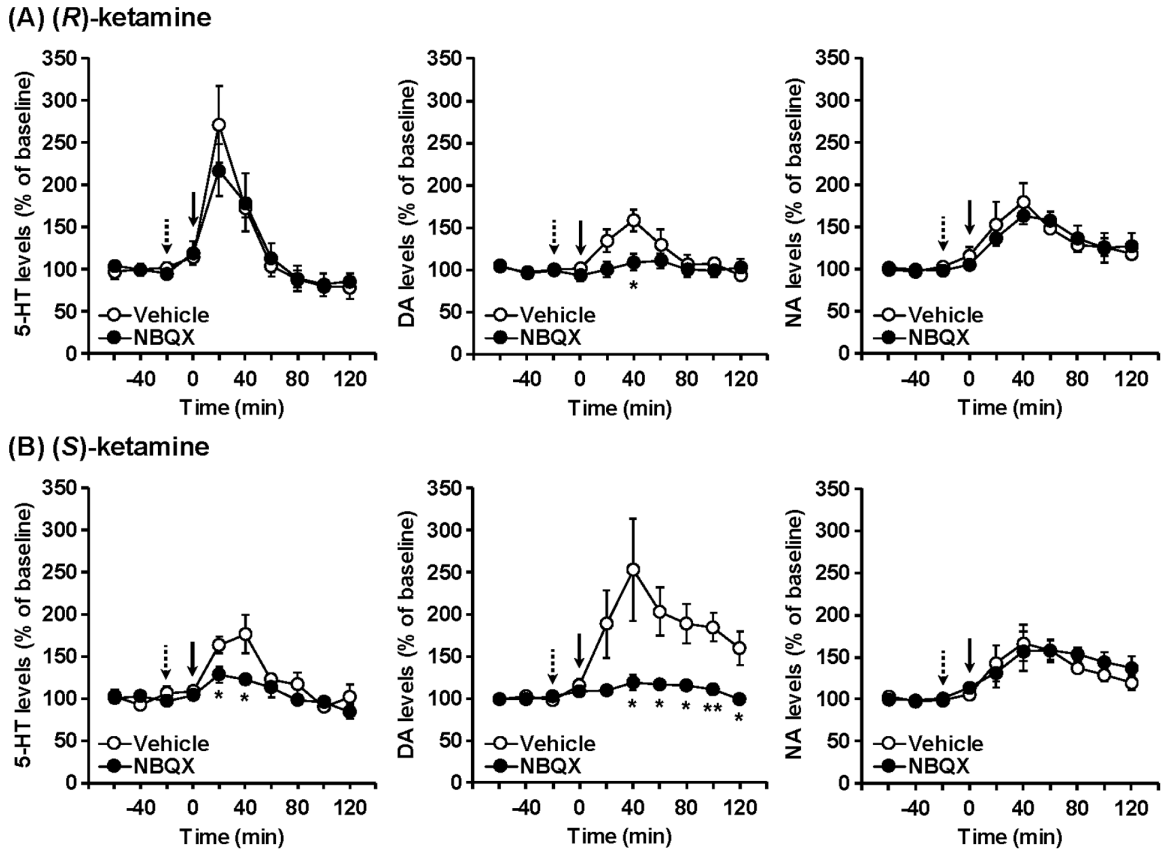

Figure 3. The effects of NBQX on (R)-ketamine- and (S)-ketamine-induced monoamine release in the prefrontal cortex (PFC) of mice. (R)-Ketamine (20 mg/kg) (A) or (S)-ketamine (20 mg/kg) (B) was i.p. injected at 0 minutes (solid arrow). NBQX (10 mg/kg) or vehicle was s.c. injected 20 minutes before ketamine treatment (dotted arrow). Results are expressed as the mean ± SEM of 5 mice per group. *P < .05, **P < .01, compared with vehicle-pretreated mice at each time point.

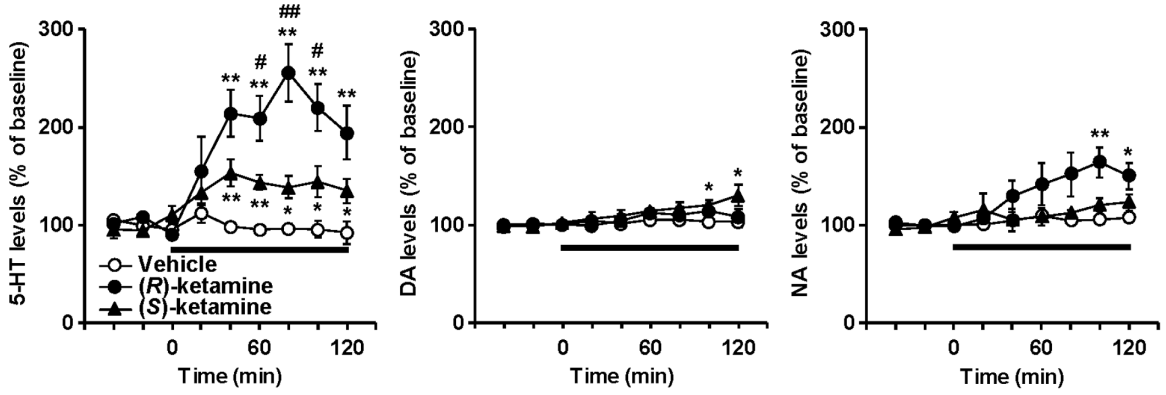

Figure 4. The effects of local application of (R)-ketamine and (S)-ketamine on extracellular monoamine levels in the prefrontal cortex (PFC) of mice. (R)-Ketamine (50 μM), (S)-ketamine (50 μM), or vehicle was perfused into the PFC via the dialysis probe for the time indicated by the horizontal bar. Results are expressed as the mean ± SEM of 5 mice per group. *P < .05, **P < .01, compared with the vehicle-treated mice at each time point. #P < .05, ##P < .01, compared with the (S)-ketamine-treated mice at each time point.

kg ($F_{8,64}$ = 2.412, P = .0242) and 20 mg/kg ($F_{8,64}$ = 5.197, P < .0001) increased extracellular DA levels in the PFC (Figure 1B). The increase in DA release by (S)-ketamine was significantly greater than (R)-ketamine (3-way ANOVA with repeated measures: $F_{16,192}$ = 2.366, P = .0031). (R)-NK (20 mg/kg, $F_{8,64}$ = 7.253, P < .0001) and (S)-NK (20 mg/kg, $F_{8,64}$ = 4.364, P = .0003) caused increases in DA release in the PFC, whereas (2R,6R)-HNK (20 mg/kg, $F_{8,64}$ = 0.301, P = .9628) or (2S,6S)-HNK (20 mg/kg, $F_{8,64}$ = 0.651, P = .7316) did not affect prefrontal DA release.

For NA release, a single administration of (R)-ketamine at doses of 10 mg/kg ($F_{8,64}$ = 5.179, P < .0001) and 20 mg/kg ($F_{8,64}$ = 7.632, P < .0001) and (S)-ketamine at doses of 10 mg/kg ($F_{8,64}$ = 3.754, P = .0012) and 20 mg/kg ($F_{8,64}$ = 5.019, P < .0001) increased extracellular NA levels in a dose-dependent manner (Figure 1C). There

was no significant difference between the effects of (*R*)-ketamine and (*S*)-ketamine (3-way ANOVA with repeated measures: $F_{16,192}=1.470$, $P=.1142$). (*S*)-NK (20 mg/kg, $F_{8,64}=2.506$, $P=.0196$) and (2*R*,6*R*)-NK (20 mg/kg, $F_{8,64}=4.155$, $P=.0005$) caused increases in NA release in the PFC, whereas (*R*)-NK (20 mg/kg, $F_{8,64}=0.640$, $P=.7411$) or (2*S*,6*S*)-HNK (20 mg/kg, $F_{8,64}=0.413$, $P=.9090$) did not affect prefrontal NA release.

Effects of (*R*)-Ketamine and (*S*)-Ketamine on Prefrontal Monoamine Release in an LPS-Induced Mouse Model of Depression

Baseline levels of extracellular 5-HT, DA, and NA in the PFC of LPS-treated mice were 0.73±0.08, 0.67±0.13, and 0.76±0.09 pg/fraction (20 µL), respectively (n=15, calculated from Figure 2). (*R*)-Ketamine (20 mg/kg, $F_{8,64}=10.460$, $P<.0001$) and (*S*)-ketamine (20 mg/kg, $F_{8,64}=7.205$, $P<.0001$) increased prefrontal 5-HT release in LPS-treated mice, and the increase by (*R*)-ketamine in 5-HT release was significantly greater than (*S*)-ketamine ($F_{8,64}=2.400$, $P=.0249$) (Figure 2). Conversely, (*R*)-ketamine (20 mg/kg, $F_{8,64}=3.672$, $P=.0014$) and (*S*)-ketamine (20 mg/kg, $F_{8,64}=13.957$, $P<.0001$) increased prefrontal DA release in LPS-treated mice, and the increase by (*S*)-ketamine in DA release was significantly greater than (*R*)-ketamine ($F_{8,64}=7.909$, $P<.0001$). (*R*)-Ketamine (20 mg/kg, $F_{8,64}=3.121$, $P=.0049$) and (*S*)-ketamine (20 mg/kg, $F_{8,64}=4.696$, $P=.0001$) increased prefrontal NA release in LPS-treated mice, and there was no significant difference between the effects of (*R*)-ketamine and (*S*)-ketamine ($F_{8,64}=0.581$, $P=.7898$).

Involvement of the AMPA Receptor in the (*R*)-Ketamine- and (*S*)-Ketamine-Induced Increases in Prefrontal Monoamine Release

Pretreatment with the AMPA receptor antagonist NBQX (10 mg/kg) attenuated (*S*)-ketamine-induced prefrontal 5-HT release ($F_{9,72}=2.152$, $P=.0356$), whereas it did not affect (*R*)-ketamine-induced 5-HT release ($F_{9,72}=0.700$, $P=.7066$) (Figure 3). NBQX blocked both (*R*)-ketamine-induced ($F_{9,72}=3.074$, $P=.0036$) and (*S*)-ketamine-induced ($F_{9,72}=4.355$, $P<.001$) DA release. NBQX did not affect either (*R*)-ketamine-induced ($F_{9,72}=0.451$, $P=.9019$) or (*S*)-ketamine-induced ($F_{9,72}=0.552$, $P=.8310$) NA release.

Effects of the Local PFC Application of (*R*)-Ketamine and (*S*)-Ketamine on the Extracellular Monoamine Levels in the PFC

Local application of (*R*)-ketamine and (*S*)-ketamine at a dose of 10 µM had minimal effects on monoamine release in the PFC (data not shown). Local application of (*R*)-ketamine ($F_{8,64}=9.785$, $P<.0001$) and (*S*)-ketamine ($F_{8,64}=2.979$, $P=.0067$) at a dose of 50 µM increased extracellular 5-HT levels in the PFC, and the increase by (*R*)-ketamine in 5-HT release was significantly greater than (*S*)-ketamine ($F_{8,64}=3.469$, $P=.0022$) (Figure 4). Local application of (*S*)-ketamine ($F_{8,64}=2.512$, $P=.0193$), but not of (*R*)-ketamine ($F_{8,64}=0.950$, $P=.4826$), caused a slight increase in DA release. Conversely, the local application of (*R*)-ketamine ($F_{8,64}=4.647$, $P=.0002$), but not (*S*)-ketamine ($F_{8,64}=0.742$, $P=.6539$), increased NA release.

Discussion

In this study, we identified differences between (*R*)-ketamine and (*S*)-ketamine in their abilities to induce prefrontal 5-HT and DA but not NA release. Both (*R*)-ketamine and (*S*)-ketamine caused an increase in 5-HT release, and the effect of (*R*)-ketamine was significantly greater than that of (*S*)-ketamine. In contrast, (*S*)-ketamine caused a robust increase in DA release compared with (*R*)-ketamine. Both (*R*)-ketamine and (*S*)-ketamine increased NA release, but these have similar effects. Although it is unclear exactly how these differences would contribute to the pharmacological differences between (*R*)-ketamine and (*S*)-ketamine, several reports show differences in the effects of ketamine enantiomers on antidepressant-like activity and psychosis- or addiction-related behaviors. (*R*)-Ketamine exhibits more potent and longer acting antidepressant-like effects than (*S*)-ketamine (Zhang et al., 2014a; Yang et al., 2015, 2017a). Pham et al. (2017) previously reported that (*R*,*S*)-ketamine-induced increases in 5-HT release in the medial PFC were positively correlated with its antidepressant-like activity in BALB/cJ mice. Additionally, local injection of (*R*,*S*)-ketamine into the PFC induces sustained antidepressant-like effects (Pham et al., 2017; Fukumoto et al., 2018), and this effect was mediated by the local activation of 5-HT_{1A} receptors in the PFC (Fukumoto et al., 2018). These findings suggest that enhanced prefrontal serotonergic activity by (*R*)-ketamine could contribute to its potent and sustained antidepressant-like effect. The ketamine metabolites (*R*)-NK, (*S*)-NK, and (2*S*,6*S*)-HNK did not affect 5-HT release in the PFC, although (2*R*,6*R*)-HNK caused a slight increase in 5-HT release. Thus, the serotonergic system might not be involved mainly in the antidepressant-like effects of ketamine metabolites, except for (2*R*,6*R*)-HNK. Interestingly, like (*R*,*S*)-ketamine, both systemic and local injection of (2*R*,6*R*)-HNK caused an increase in baseline 5-HT release in the PFC 24 hours after injection (Pham et al., 2018), although (2*R*,6*R*)-HNK does not bind to NMDA receptors at antidepressant-relevant concentrations (Gould et al., 2017). In this study, (*R*)-ketamine, (*S*)-ketamine, (*S*)-NK, and (2*R*,6*R*)-HNK increased prefrontal NA release. (*S*)-Ketamine, (*S*)-NK, and (*R*)-NK, but not (2*R*,6*R*)-HNK, increased prefrontal DA release. (*R*)-NK at 20 mg/kg, but not at lower doses (5 and 10 mg/kg), significantly attenuates increased immobility time in the forced swim test in LPS-treated mice, although (*S*)-NK is more potent than (*R*)-NK (Yang et al., 2018a). These findings raise the possibility that increases in NA and DA release in the PFC might contribute at least partly to the antidepressant-like effects of ketamine and its metabolites. Of note, (*R*,*S*)-ketamine-induced antidepressant-like effects are blocked by the disruption of DA-D_1 receptor activity in the PFC (Hare et al., 2019). The prefrontal DA system has been implicated in playing a pivotal role in depression and antidepressant actions (Ago et al., 2005, 2017; Furuyashiki, 2012; Rogóż, 2013; Watt et al., 2014). Therefore, the enhanced activity of the prefrontal dopaminergic system by (*S*)-ketamine would contribute to its antidepressant-like activity, whereas DA-D_1 receptors might not play a major role in the antidepressant actions of (*R*)-ketamine (Chang et al., 2019). Conversely, (*S*)-ketamine might have a higher potential for inducing psychotomimetic effects such as hyperactivity, pre-pulse inhibition deficits, and rewarding effects compared with (*R*)-ketamine (Yang et al., 2015), probably due to the affinity for the NMDA receptor. In addition, a positron emission tomography study showed that a single infusion of (*S*)-ketamine, but not (*R*)-ketamine, causes a reduction in the binding availability of $\text{DA-D}_{2/3}$ receptors in the striatum of conscious monkeys, suggesting that (*S*)-ketamine induces DA release (Hashimoto et al., 2017). Similar to this observation, in this study, (*S*)-ketamine caused a robust increase in DA release in the PFC, while (*R*)-ketamine had a small effect. Thus, the exact role of DA release induced by ketamine enantiomers and their metabolites remains unclear. Further studies investigating the

effects of ketamine and its metabolites in other brain regions such as the striatum, nucleus accumbens, and hippocampus are required for full elucidation.

Previous studies have suggested the involvement of AMPA receptor activation in the antidepressant-like action of (*R*)-ketamine, (*S*)-ketamine, and (*R*,*S*)-ketamine (Maeng et al., 2008; Koike et al., 2011; Walker et al., 2013; Koike and Chaki, 2014; Zhou et al., 2014; Yang et al., 2015; Zhang et al., 2016; Fukumoto et al., 2017; Kinoshita et al., 2018). Microdialysis studies also showed that raphe AMPA receptors mediate at least in part (*R*,*S*)-ketamine-induced 5-HT release in the rat PFC (Nishitani et al., 2014; Pham et al., 2017). Moreover, AMPA itself induces 5-HT and DA release in the PFC (Araki et al., 2014). These observations suggest that AMPA receptors might be involved in the behavioral and neurochemical effects of ketamine. In the present study, the AMPA receptor antagonist NBQX blocked (*R*)-ketamine- and (*S*)-ketamine-induced DA, but not NA release in the PFC, suggesting the involvement of AMPA receptors in regulating the dopaminergic system by both ketamine enantiomers. Interestingly, NBQX partially blocked (*S*)-ketamine-induced prefrontal 5-HT release, while it did not affect (*R*)-ketamine-induced prefrontal 5-HT release. This finding suggests that AMPA receptor activation is not involved in (*R*)-ketamine-induced 5-HT release. A positron emission tomography study showed that subanesthetic doses of ketamine transiently decrease 5-HT transporter (SERT) binding in conscious monkeys, and ketamine infusion transiently increased 5-HT but not DA levels in the extracellular fluid of the PFC of conscious monkeys (Yamamoto et al., 2013). These findings suggest that subanesthetic ketamine might enhance serotonergic transmission by the inhibition of SERT activity. In vitro studies have reported that ketamine at concentrations $>10^{-6}$ M inhibited the uptake of [^{3}H]5-HT by the SERT transfected into human embryonic kidney 293 cells in a dose-dependent manner (Nishimura et al., 1998; Zhao and Sun, 2008). We also observed that local application of (*R*)-ketamine and (*S*)-ketamine into the PFC induced increases in prefrontal 5-HT release, and the effect of (*R*)-ketamine was significantly greater than that of (*S*)-ketamine. These phenomena might be related to a greater increase in 5-HT release by systemic administration of (*R*)-ketamine than (*S*)-ketamine, although (*R*)-ketamine (*Ki* = 148 μM) and (*S*)-ketamine (*Ki* = 156 μM) have similar affinities for the SERT (Nishimura and Sato, 1999). Therefore, the mechanism of (*R*)-ketamine-induced prefrontal 5-HT release remains unknown.

Previous studies showed that the depletion of 5-HT by PCPA abolished the acute antidepressant effects of (*R*,*S*)-ketamine (30 mg/kg, 30 minutes prior to the forced swim test) in control C57BL/6J mice (Fukumoto et al., 2016), and the sustained antidepressant effects of (*R*,*S*)-ketamine (10 mg/kg, 24 hours prior to the forced swim test) in highly anxious BALB/cJ mice (Pham et al., 2017). Additionally, the acute (1 hour) and sustained (24 hours) antidepressant effects of (*S*)-ketamine (15 mg/kg) in Flinders Sensitive Line rats (a genetic model of depression) were abolished by 5-HT depletion, suggesting that the acute and sustained antidepressant-like effects of (*S*)-ketamine in Flinders Sensitive Line rats depend on the endogenous 5-HT concentration (du Jardin et al., 2016a). In contrast, depletion of 5-HT by PCPA did not abolish the acute antidepressant effects of (*R*,*S*)-ketamine (25 mg/kg, 1 hour prior to the forced swim test) in control Sprague–Dawley rats (Gigliucci et al., 2013). Furthermore, PCPA did not abolish the acute (4 hours prior to the tail-suspension test) and long-lasting (2 or 5 days after a single dose in the sucrose preference test) antidepressant effects of (*R*)-ketamine (10 mg/kg) in a chronic social defeat stress model in male C57BL/6 mice (Zhang et al., 2018). Although the reasons for these discrepancies are currently unknown, several factors such as different animal models used (normal vs stress-induced or genetic models of depression), behavioral tests, and different doses and isomers of ketamine could account for them. Thus, the role of 5-HT in the acute and sustained antidepressant effects of (*R*,*S*)-ketamine and its enantiomers might differ depending on the experimental conditions. In this regard, it might be important to see whether the differential effects of (*R*)-ketamine and (*S*)-ketamine on prefrontal monoaminergic transmission in normal animals are also observed in depression-like models. LPS is known to cause depression-like behaviors in the forced swim and tail-suspension tests (Zhang et al., 2014b). Both (*R*)-ketamine and (*S*)-ketamine show antidepressant-like effects in an LPS-induced depression model, but the potency of (*R*)-ketamine is higher than that of (*S*)-ketamine (Yang et al., 2017a; Yamaguchi et al., 2018). In this study, we found that (*R*)-ketamine and (*S*)-ketamine enhanced serotonergic and dopaminergic neurotransmission, respectively, in LPS-treated mice as seen in normal mice. This finding implies that (*R*)-ketamine would produce pronounced 5-HT release, leading to antidepressant effects under some conditions of depression.

In conclusion, our study showed that (*R*)-ketamine and (*S*)-ketamine differentially affect serotonergic and dopaminergic neurotransmission in the PFC in particular. (*R*)-Ketamine caused a greater increase in 5-HT release than (*S*)-ketamine, but this effect was AMPA receptor-independent. Ketamine-induced DA but not NA release was AMPA receptor-dependent. (2*R*,6*R*)-HNK acutely induced a slight increase in 5-HT release. (*S*)-NK, which potentially has antidepressant activity, enhances DA and NA, but not 5-HT release. These findings provide a neurochemical basis for understanding the pharmacological differences and the mechanisms of action of (*R*)-ketamine, (*S*)-ketamine, and their metabolites.

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Statement of Interest

Dr. Kenji 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. Kenji Hashimoto has received research support from Sumitomo Dainippon Pharma Co., Ltd., Otsuka Pharmaceutical Co., Ltd., and Taisho Pharmaceutical Co., Ltd. The other authors declare no conflicts of interest.

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