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PCN FRONTIER REVIEW

Rapid-acting antidepressant ketamine, its metabolites and other candidates: A historical overview and future perspective

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Major depressive disorder (MDD) is one of the most disabling psychiatric disorders. Approximately one-third of the patients with MDD are treatment resistant to the current antidepressants. There is also a significant therapeutic time lag of weeks to months. Furthermore, depression in patients with bipolar disorder (BD) is typically poorly responsive to antidepressants. Therefore, there exists an unmet medical need for rapidly acting antidepressants with beneficial effects in treatment-resistant patients with MDD or BD. Accumulating evidence suggests that the *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine produces rapid and sustained antidepressant effects in treatment-resistant patients with MDD or BD. Ketamine is a racemic mixture comprising equal parts of (*R*)-ketamine (or arketamine) and (*S*)-ketamine (or esketamine). Because (*S*)-ketamine has higher affinity for NMDAR than (*R*)-ketamine, esketamine was developed as an antidepressant. On 5 March 2019, esketamine nasal spray was approved by the US Food and Drug Administration. However, preclinical data suggest that (*R*)-ketamine exerts greater potency and longer-lasting antidepressant effects than (*S*)-ketamine in animal models of depression and that (*R*)-ketamine has less detrimental side-effects than (*R,S*)-ketamine or (*S*)-ketamine. In this article, the author reviews the historical overview of the antidepressant actions of enantiomers of ketamine and its major metabolites norketamine and hydroxynorketamine. Furthermore, the author discusses the other potential rapid-acting antidepressant candidates (i.e., NMDAR antagonists and modulators, low-voltage-sensitive T-type calcium channel inhibitor, potassium channel Kir4.1 inhibitor, negative modulators of γ-aminobutyric acid, and type A [$GABA_A$] receptors) to compare them with ketamine. Moreover, the molecular and cellular mechanisms of ketamine's antidepressant effects are discussed.

Keywords: *gut microbiota, (R)-ketamine (or arketamine), (S)-ketamine (or esketamine), (S)-norketamine.*

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Major depressive disorder (MDD) is a chronic and debilitating mood disorder that affects approximately 320 million people worldwide. Its incidence increased by >18% between 2005 and 2015.[1] In addition, approximately 800 000 individuals take their own life annually, which indicates that suicide is also a serious problem for public health. The currently available antidepressants have certain limitations (e.g., long time lags and low response rates). Approximately 30% of MDD patients are refractory to the current antidepressants.[2] Thus, there exists an unmet medical need for rapidly acting novel antidepressants that are also effective in treatment-resistant patients and in patients with suicide ideation.

Bipolar disorder (BD) is a major psychiatric disorder that exhibits extreme mood swings (i.e., mania or hypomania and depression). Most patients with BD spend a significant amount of their time experiencing either subsyndromal depression or major depression.[3,4] Bipolar depression is typically poorly responsive to antidepressants, with a risk of switch to mania with antidepressant treatment being extremely high.[5] A cohort study suggests that the treatment failure rates of bipolar depression are higher than those of MDD.[6] Therefore, there is also an unmet medical need for treatment options for refractory patients with bipolar depression, indicating one of the most important issues faced by psychiatrists and researchers.[4]

Accumulating evidence has revealed the robust antidepressant effects and anti-suicidal effects of ketamine, the *N*-methyl-D-aspartate receptor (NMDAR) antagonist, in treatment-resistant patients with MDD or BD.[7–10] The discovery of the robust antidepressant actions of ketamine is regarded as the greatest advancement in mood disorder research in the past 60 years.[11] In this article, the author reviews the historical overview and the molecular and cellular mechanisms of ketamine's antidepressant actions. Furthermore, the antidepressant actions of ketamine and its major metabolites are discussed. Moreover, the other potential rapid-acting antidepressant candidates (i.e., other NMDAR antagonists, NMDAR modulators, low-voltage-sensitive T-type calcium channel inhibitor, potassium channel Kir4.1 inhibitor, negative modulators of γ-aminobutyric acid type A [$GABA_A$] receptors) are also discussed. Finally, the author discusses the future perspective of ketamine and its metabolites.

Brief history of phencyclidine and ketamine

Phencyclidine (PCP; formerly known as 'CI-395' and 'Sernyl'; Ki = 0.06 μM for NMDAR; Fig. 1 and Table 1)[12] was synthesized in 1956 at the pharmaceutical company Park Davis. From 1957 to 1958, PCP was administered as an anesthetic compound in humans undergoing surgery. It was considered that PCP was a safe compound in humans; however, some patients treated with PCP exhibited severe

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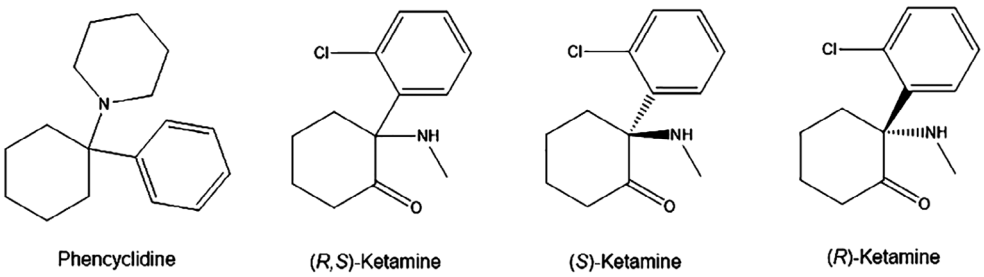

Fig.1 Chemical structure of phencyclidine, (*R,S*)-ketamine, and enantiomers of ketamine.

and prolonged post-surgery delirium. Subsequently, Luby *et al.*[14] investigated the effects of PCP in healthy control subjects and in schizophrenia patients. They found that PCP was an excellent drug model of schizophrenia as PCP could produce schizophrenia-like symptoms in healthy subjects.[14] Currently, the PCP model of schizophrenia has been accepted by several scientists.[15–17] With more clinical experience, it became clear that PCP was not useful as an anesthetic agent in humans.

Subsequently, scientists at Park Davis synthesized a series of short-acting derivatives of PCP. The compound CI-581 (known as 'ketamine': Ki = 0.53 μM[12] or Ki = 1.06 μM[13] for NMDAR; Fig. 1 and Table 1) was synthesized in 1962. In 1964, Dr Edward F. Domino and colleagues (University of Michigan) conducted the first clinical study of ketamine in humans.[18] In 1970, ketamine was introduced as a short-acting anesthetic drug for humans;[17–19] however, ketamine produced certain side-effects, such as psychotomimetic effects and dissociation in humans.[20,21] Several ketamine users showed derealization, including visual hallucinations, a sense of unity with external environmental factors, and out-of-body experiences.[22] Ketamine has been widely used as a recreational drug worldwide,[17,18,23,24] although it is also suggested to be useful for substance use disorders.[25] Importantly, ulcerative cystitis in ketamine users is one of most serious concerns.[26,27]

Ketamine is a racemic mixture of equal amounts of the two enantiomers: (*R*)-enantiomer and (*S*)-enantiomer. (*S*)-ketamine (Ki = 0.30 μM[12] or Ki = 0.69 μM[13] for NMDAR) has a three- to fourfold greater affinity for the NMDAR than (*R*)-ketamine (Ki = 1.4 μM[12] or Ki = 2.57 μM[13] for NMDAR; Fig. 1 and Table 1). Furthermore, the IC_{50} values for (*S*)-ketamine were similar among the four subtypes (GluN2A, GluN2B, GluN2C, GluN2D) of NMDAR, whereas the IC_{50} for (*R*)-ketamine varied slightly among these subtypes.[28] In humans, (*S*)-ketamine as an anesthetic is twofold more potent than (*R,S*)-ketamine and approximately threefold more potent than (*R*)-ketamine.[29] Therefore, (*S*)-ketamine has been widely used in some countries as an anesthetic drug.

Table 1. Binding affinities of (+)-MK-801, phencyclidine, enantiomers of ketamine, and its metabolites to the NMDA receptor

Compounds	Ki (μM): Ebert *et al.*[12]	Ki (μM): Moaddel *et al.*[13]
(+)-MK-801	0.0019	0.0047
Phencyclidine	0.06	ND
(*R,S*)-Ketamine	0.53	1.06
(*S*)-Ketamine	0.3	0.69
(*R*)-Ketamine	1.4	2.57
(*S*)-Norketamine	1.7	2.25
(*R*)-Norketamine	13	26.46
(2*S*,6*S*)-Hydroxynorketamine	ND	21.19
(2*R*,6*R*)-Hydroxynorketamine	ND	>100

ND, not determined.

Ketamine's antidepressant effects in humans

MDD

In a review article, Domino described his experience of a patient, an abuser of PCP and ketamine, in the late 1970s or early '80s: 'She stated that ketamine and PCP worked quickly and were much better antidepressants but they did not last as long so she took them again and again.[18]

In 2000, Berman and colleagues (Yale University) reported the rapidly acting antidepressant effects of ketamine in MDD patients. This was the first double-blinded, placebo-controlled trial showing the rapidly acting antidepressant effects of ketamine in patients with MDD.[30] Subsequent studies replicated the robust antidepressant actions of ketamine in refractory MDD patients.[31–34] The seminal study of Krystal *et al.*[21] reported dissociative and psychotomimetic effects of ketamine at the dose of 0.5 mg/kg. These side-effects gradually disappeared within 60–120 min after a single infusion. Several previous studies have used placebo control for randomized, double-blinded, clinical trials. However, Murrough *et al.*[35] used the other anesthetic agent midazolam as an active control. The ketamine-treated patients showed greater improvement in depressive symptoms than the midazolam-treated patients. Furthermore, the score for suicide ideation in the ketamine-treated patients was lower than in the midazolam-treated patients, indicating that ketamine could be useful for suicide ideation.[36] In addition, unlike midazolam, adjunctive treatment with ketamine significantly reduces suicidal ideation in patients with depression.[37]

Response rates of ketamine ranged from 25% to 85% at 24 h post-injection and from 14% to 70% at 72 h post-injection.[38] Murrough *et al.*[39] gave six ketamine infusions over a 12-day period in treatment-resistant MDD patients, and 70.8% of these patients were responders to ketamine. Moreover, the repeated infusions were tolerated, without serious side-effects.[39] Singh *et al.*[40] demonstrated that twice- and thrice-a-week infusions of ketamine maintained the antidepressant efficacy in treatment-resistant MDD patients.

In addition to intravenous infusions, other multiple routes of ketamine administration, such as intramuscular, nasal, oral, rectal, subcutaneous, and sublingual administrations, have been used.[41,42] The bioavailability of ketamine administered via intramuscular injection was slightly lower (93%) than intravenous infusion (100%, by definition; Table 2).[41] Due to the first-pass metabolism, oral ketamine's bioavailability was reportedly low (17%–29%) and that via intranasal, rectal, and sublingual administrations was 8%–45%, 11%–25%, and 24%–30%, respectively (Table 2).[41,42] Thus, it seems that low bioavailability of intranasal administration causes lower efficacy compared to intravenous and intramuscular administrations.

An open-label study of oral ketamine (0.5 mg/kg/day, 28 days) improved the depressive symptoms in depressed patients receiving hospice care; however, significant antidepressant effects were not shown until Day 14 of administration.[43] Furthermore, the oral ketamine (150 mg/day for 6 weeks) group had significantly lower

Table 2. Pharmacokinetic profiles of (*R*,*S*)-ketamine in humans

Route	Dose	Bioavailability	Tmax (min)
Intravenous†	1–4.5 mg/kg	100%	3
Intramuscular†	6.5–13 mg/kg	93%	5–10
Intranasal†	0.5–1.0 mg/kg	8%–45%	10–20
Oral†	0.25–0.5 mg/kg	17%–29%	30
Rectal†	9–10 mg/kg	11%–25%	30–45
Sublingual‡	25 mg	24%–30%	30–45

†From Li and Vlisides.[41]
‡From Peltoniemi *et al.*[42]

depressive scores compared to the diclofenac group.[44] Moreover, oral ketamine (150 mg/day for 6 weeks) decreased the time lag for antidepressant actions of sertraline in MDD patients.[45] Thus, low oral bioavailability of ketamine might contribute to its slow-acting antidepressant effects in depressed patients. Collectively, it seems that oral administration of ketamine produces lower efficacy than intravenous and intramuscular administrations.

Bipolar depression

In 2010, Diazgranados and colleagues (US National Institutes of Health [NIH]) reported that ketamine showed rapid antidepressant actions in refractory BD patients.[46] Subsequently, Zarate *et al.*[47] replicated the robust antidepressant effects of ketamine in refractory patients with BD. In addition, six injections of ketamine produced antidepressant and anti-suicidal effects in Chinese patients with MDD and BD.[48]

The switch to mania from depression is an important side-effect of using antidepressants to treat bipolar depression.[49,50] Although the use of ketamine might produce adverse reactions (i.e., dissociative symptoms) in BD patients, the risk of switch from depression to mania remains to be reported.[51,52] However, two case reports showed ketamine-induced switch from depression to mania in treatment-resistant patients.[53,54] Nonetheless, it is noteworthy that ketamine has robust antidepressant effects for bipolar depression.

Comparison of ketamine enantiomers in healthy control subjects

Mathisen *et al.*[55] reported that both (*S*)-ketamine (0.45 mg/kg) and (*R*)-ketamine (1.8 mg/kg) caused side-effects, such as blurred vision, altered hearing, dizziness, proprioceptive disturbances, and illusions, in patients with oral pain. The incidence of psychotomimetic side-effects of the (*S*)-ketamine group was higher than that of the (*R*)-ketamine group, although the dose of (*S*)-ketamine (0.45 mg/kg) was lower than that of (*R*)-ketamine (1.8 mg/kg).[55] Furthermore, it is well known that experiencing illusion and alterations in hearing, vision, and proprioception is attributable to (*S*)-ketamine's actions.[56] Vollenweider *et al.*[57] administered 15 mg of (*S*)- or (*R*)-ketamine by intravenous injection and measured its effect on psychological state and cerebral glucose metabolism. Whereas (*S*)-ketamine caused psychotic reactions, including depersonalization and hallucinations, the same dose of (*R*)-ketamine did not produce any psychotic symptoms in the same subjects and most of them experienced a state of relaxation.[57] Positron emission tomography showed that (*S*)-ketamine markedly increased the glucose utilization in the frontal cortex and thalamus whereas (*R*)-ketamine significantly suppressed glucose metabolic rate in several brain areas.[57] These findings suggest that (*S*)-ketamine contributes to the acute side-effects of ketamine, whereas (*R*)-ketamine may not be associated with these side-effects.[58]

(*S*)-ketamine

As NMDAR inhibition was believed to play a crucial role in ketamine's robust antidepressant effects in MDD patients, the Janssen Pharmaceutical Company selected the (*S*)-enantiomer of ketamine as an antidepressant candidate (Table 3). An intravenous infusion of (*S*)-ketamine is reported to elicit rapid-acting and sustained antidepressant actions in refractory patients with MDD.[60] The most common adverse events (i.e., headache, nausea, dissociation) of (*S*)-ketamine were transient.[60]

The intranasal single injection of (*S*)-ketamine produced a decline of cognitive performance in healthy control subjects 40 min after a single dose (84 mg).[61] There was no difference in the driving performance in healthy control subjects at 8 h after the intranasal single injection of (*S*)-ketamine (84 mg).[62] The Janssen Pharmaceutical Company reported the results of a phase 2 study of (*S*)-ketamine. The intranasal injections of (*S*)-ketamine (28, 56, or 84 mg twice a week) showed good efficacy for the rapid reduction in the depressive symptoms in treatment-resistant MDD patients. Changes in depressive scores in all three (*S*)-ketamine groups were superior to those in the placebo group.[63] Subsequently, Canuso *et al.*[64] reported the beneficial effects of intranasal (*S*)-ketamine infusions in depressed patients with imminent risks for suicide. (*S*)-ketamine significantly decreased the depressive symptoms and suicidality at both 4 and 24 h after the initial injection, although these beneficial effects were not shown after 4 weeks of treatment.[64]

The Janssen Pharmaceutical Company presented the five phase 3 trials of intranasal administration of (*S*)-ketamine in treatment-resistant patients. Only two of the five phase 3 trials demonstrated positive results. On 12 February 2019, the majority of panelists of the US Food and Drug Administration (FDA) voted to recommend to approve an (*S*)-ketamine nasal spray, though two voted against it because of its insufficient efficacy and potential for abuse.[65] On 5 March 2019, the US FDA approved (*S*)-ketamine nasal spray for treatment-resistant patients. Because of the risk of serious adverse effects, it is only available through a restricted distribution system, under the Risk Evaluation and Mitigation Strategy.[66] At present, the clinical trial of (*R*)-ketamine in depressed patients with MDD or BD remains to be reported.

Predictable biomarkers of ketamine's antidepressant effects in MDD patients

Ketamine can produce antidepressant effects in approximately two-thirds of refractory MDD patients, with effects lasting for >7 days.[38] However, the detrimental side-effects (i.e., psychotomimetic effects and dissociation) were evident after a single injection. Therefore, predictable biomarkers that distinguish responders or non-responders to ketamine are necessary.[10]

The Val/Val carrier (rs6265) in the brain-derived neurotrophic factor (*BDNF*) gene in MDD patients shows a higher ketamine response than the Met carriers,[67] suggesting that the Val66Met polymorphism in the *BDNF* gene might be a potential genetic biomarker for ketamine's responder. The Met allele in Asian populations occurs at a frequency of 40%–50%, whereas it occurs at a frequency of 20%–30% in Caucasian populations.[68] However, this finding was not replicated from the samples in a Taiwanese population.[34]

In 2015, we reported that baseline interleukin-6 (IL-6) could be a predictive blood biomarker for the rapid antidepressant effects of ketamine.[69] Furthermore, there is a positive correlation between the decrease in levels of tumor necrosis factor-α 40 min post-injection of ketamine and ketamine's antidepressant actions.[70] Thus, the rapid anti-inflammatory actions of ketamine might contribute to its rapid antidepressant effects. Conversely, changes in the IL-6 levels after ketamine infusion were not associated with the antidepressant response.[71] Furthermore, several pro-inflammatory cytokines exhibited significant changes in the blood of treatment-resistant MDD patients after ketamine injection.[72] Moreover, the low baseline levels of fibroblast growth factor 2 might be related to ketamine's

Table 3. List of ketamine enantiomers, ketamine metabolites, and NMDAR-related compounds as novel antidepressants

Drug	Company or institute	Mechanisms	Status
(*S*)-Ketamine (Esketamine)	Janssen/J&J	NMDAR antagonist	Approved
(*R*)-Ketamine (Arketamine, PCN 101)	Perception Neuroscience	NMDAR antagonist	Phase 1 in 2019
(2*R*,6*R*)-Hydroxynorketamine	NIMH, USA	AMPAR activator	Phase 1 in 2019
Rapastinel	Allergan	NMDAR modulator	Phase 3 (negative of three phase 3 trials)
AV-101 (L-4-chlorokynurenine)	VistaGen Therapeutics	NMDAR antagonist	Phase 2 (negative Phase 2 trials)
NRX-101 (D-cycloserine plus lurasidone)	NeuroRx Pharma	NMDAR modulator plus 5-HT_{2A} receptor antagonist	Phase 2
AGN-241751	Allergan	NMDAR modulator	Phase 1
AXS-05 (dextromethorphan plus bupropion)	Axsome Therapeutics	NMDAR antagonist plus norepinephrine and dopamine reuptake inhibitor	Phase 1

From Reardon[59] with a slight modification.
AMPAR, AMPA receptor; NIMH, National Institute of Mental Health; NMDAR, NMDA receptor.

antidepressant response.[72] Nonetheless, future studies using a large sample size are necessary to confirm the potential of these predictable biomarkers for ketamine responders.[73]

In 2018, Kadriu *et al.*[74] reported increased blood levels of osteoprotegerin (OPG)/receptor activator of nuclear factor kB Ligand (RANKL) ratio and osteopontin after ketamine infusion, suggesting that the OPG/RANK/RANKL system might be implicated in ketamine's antidepressant effects. Unlike (*S*)-ketamine and (2*R*,6*R*)-hydroxynorketamine (HNK), (*R*)-ketamine attenuated the higher blood levels of RANKL in a chronic social-defeat stress (CSDS) model.[75,76] There was also a positive correlation between ketamine-induced behavioral outcome (i.e., anhedonia) and OPG/RANKL ratio. In addition, (*R*)-ketamine, but not (2*R*,6*R*)-HNK, significantly improved the decreased bone mineral density of CSDS-susceptible mice.[76] Collectively, it is likely that the bone turnover markers might be involved in the antidepressant effects of (*R*)-ketamine.[75,76] Furthermore, (*R*)-ketamine would be a potential therapeutic drug for abnormalities in bone metabolism in depressed patients.[76]

A metabolomics analysis showed that the ketamine responder group showed a decrease in blood levels of kynurenine and an increase in the bioavailability of arginine compared to the non-responder group.[77] Further studies focusing on the kynurenine and arginine pathways for ketamine's antidepressant actions are thus deemed necessary.

Shaw *et al.*[78] reported that ketamine (0.5 mg/kg) increased β amplitudes, decreased the peak γ frequency in the visual cortex, and that ketamine could amplify γ band amplitudes in the visual cortex of healthy control subjects. Subsequently, Nugent *et al.*[79] reported the effects of ketamine on γ power in healthy control subjects and MDD patients. Both groups showed the increased resting γ power after a single infusion of ketamine. The γ power at baseline in MDD patients might influence the relationship between post-ketamine γ power and ketamine's antidepressant effects. Interestingly, higher γ power after ketamine injection was related to better antidepressant response in MDD patients with lower baseline γ. These findings were replicated by the same group.[80] Collectively, it is likely that baseline γ power could be a predictable biomarker for ketamine's beneficial actions in MDD patients, although further study is needed.

Enantiomers of ketamine and its metabolites

Antidepressant effects of (*R*)-ketamine and (*S*)-ketamine in rodents

Based on an assumption that the effect of ketamine is mediated by the NMDAR antagonisms, several drugs inhibiting NMDAR have been developed as new antidepressants (Table 3). However, the antidepressant actions of ketamine in MDD patients are more potent than those of non-ketamine NMDAR antagonists,[7,8] which suggests that NMDAR inhibition does not play a major role in ketamine's antidepressant effects in humans. In early 2010, we hypothesized that (*R*)-ketamine would produce more potent antidepressant actions compared to (*S*)-ketamine. To confirm this hypothesis, we purified two enantiomers from (*R,S*)-ketamine using the recrystallization method because two enantiomers of ketamine were not available. In 2014, we first demonstrated that (*R*)-ketamine produced longer-lasting antidepressant actions than (*S*)-ketamine in mice after neonatal dexamethasone exposure.[81–83] In 2015, we confirmed more potent antidepressant actions of (*R*)-ketamine in the other rodent models, such as mouse CSDS and rat learned helplessness (LH) models, compared to (*S*)-ketamine.[84] In 2016, Zanos and colleagues (University of Maryland and NIH, USA) confirmed our findings on (*R*)-ketamine versus (*S*)-ketamine in rodent models.[85] Subsequently, Fukumoto and colleagues reported that, unlike (*S*)-ketamine, (*R*)-ketamine and (*R,S*)-ketamine (24 h after a single injection) significantly reversed depression-like behavior in rats after repeated corticosterone treatments.[86] Importantly, the levels of both the enantiomers in the brain and blood after a single injection were the same, which suggests that the differences in the potency of two enantiomers are not correlated to the differences in their pharmacokinetic profiles.[85,86] The potent NMDAR antagonist (+)-MK-801 caused rapid antidepressant effects in CSDS-susceptible mice, although (+)-MK-801 did not elicit a long-lasting antidepressant effect,[87] supporting the previous reports.[88,89] Cumulatively, it is unlikely that the potent NMDAR inhibition might be involved in the long-lasting antidepressant actions of ketamine.

Brain regions for the antidepressant actions of ketamine

Ketamine could attenuate the decreased levels of BDNF and PSD-95 (postsynaptic density protein 95) in the prefrontal cortex (PFC), dentate gyrus (DG), and CA3 of the hippocampus from CSDS-susceptible mice.[90,91] Conversely, ketamine exhibited no effect against the increased levels of BDNF, and PSD-95 in the nucleus accumbens (NAc) from CSDS-susceptible mice.[90,91] Interestingly, (*R*)-ketamine induces more potent therapeutic effects on reduced synaptogenesis (i.e., dendritic spine density, PSD-95) and BDNF–tropomyosin receptor kinase B (TrkB) cascade in the PFC and hippocampus from CSDS-susceptible mice in comparison with (*S*)-ketamine.[84]

Fuchikami and colleagues reported that ketamine infusion into the infralimbic region of the medial PFC (mPFC) reproduced the antidepressant-like actions of systemic ketamine administration, and that optogenetic stimulation of the infralimbic region of mPFC showed ketamine-like antidepressant-like effects in control-naive rats.[92] Subsequently, Shirayama *et al.*[93] demonstrated that direct injection of (*R*)-ketamine into the infralimbic region of the mPFC or hippocampus (i.e., CA3 and DG) of LH rats produced antidepressant effects. Conversely, direct injection of (*R*)-ketamine into other brain regions (i.e., the prelimbic cortex of mPFC, the shell and core of the NAc, central nucleus of the amygdala, and basolateral amygdala) had no effect.[93] In addition, we also showed that increased BDNF–TrkB signaling in the NAc might contribute to depression-like phenotypes after withdrawal from repeated methamphetamine administration and that ketamine did not improve depression-like phenotypes in these mice.[94] Taken together, it seems that the NAc might not play a key role in the therapeutic effects of ketamine[93–95] although further study is needed.

In contrast, ketamine restored a decreased dopamine neuron population activity and synaptic plasticity in the NAc and hippocampus pathway in Wistar-Kyoto rats exposed to inescapable, uncontrollable footshocks.[96] Furthermore, Yao *et al.*[97] reported that long-term potentiation (LTP) in the NAc of control naive mice was disrupted 24 h after ketamine injection, and that ketamine-induced LTP loss in the NAc was maintained for 7 days. It is currently unclear whether ketamine-induced LTP loss in the NAc is associated with its antidepressant actions. A pilot clinical study using magnetic resonance imaging showed that ketamine may normalize larger left NAc volume in MDD patients.[98] It is also unclear whether ketamine-induced normalization of left NAc volume in MDD patients is related to its antidepressant actions. Further detailed study using a large sample size is necessary to assess the role of NAc in the antidepressant actions of ketamine.

In 2018, Yang *et al.*[99] demonstrated that ketamine-induced blockade of NMDAR-dependent bursting activity in the lateral habenula (LHb) might mediate its rapid-acting antidepressant effects in rodents with depression-like phenotype, suggesting that rapid-acting antidepressant effects of ketamine are dependent on ketamine's action in the LHb, with a primary site of action.[100] In contrast, the dose (25 μg/each side) of ketamine[99] was higher than that of the previous report (2 μg/each side of (*R*)-ketamine in the mPFC of LH rats[93]). It may be, therefore, of interest to investigate whether direct injection of (*R*)-ketamine in the LHb from LH rats shows antidepressant effects.

Side-effects of ketamine

Although the psychotomimetic effects and dissociative symptoms in humans after ketamine infusion are well known, off-label ketamine treatment for depression has become popular in the USA.[101–103] In 2018, an estimated 300 clinics were providing off-label ketamine treatment to patients with depression, although the US FDA had not approved ketamine for depression.[59] This situation is expected to change due to the recent approval of esketamine nasal spray by the US FDA.

(*S*)-ketamine-induced locomotor activity in mice was reported to be higher than that of (*R*)-ketamine,[84,104] although one study showed that these two enantiomers exerted equipotent hyperlocomotion at the same dose in rats and mice.[86] Antidepressant dose (10 mg/kg) of (*R*)-ketamine did not increase locomotor activity of rats and mice.[84,86] (*S*)-ketamine, but not (*R*)-ketamine, caused prepulse inhibition (PPI) deficits in mice.[84] Furthermore, the ED_{50} of (*R*)-ketamine (6.33 mg/kg) for PPI deficits in rats was higher than that of (*S*)-ketamine (2.86 mg/kg),[105] indicating that (*R*)-ketamine disrupts PPI with 2.5-fold lower potency than (*S*)-ketamine. In a conditioned place preference (CPP) test, (*S*)-ketamine increased the CPP score in mice.[84] However, (*R*)-ketamine did not alter the CPP score in mice.[84] Therefore, an antidepressant dose of (*R*)-ketamine may not produce side-effects (i.e., psychotomimetic and dissociative effects and abuse liability) in humans.[84] In collaboration with Dr Hideo Tsukada (Hamamatsu Photonics Co., Ltd., Japan), we demonstrated a significant reduction in the dopamine $D_{2/3}$ receptor binding potential in the monkey striatum after a single intravenous injection of (*S*)-ketamine (0.5 mg/kg for 40-min), but not (*R*)-ketamine (0.5 mg/kg for 40-min).[106] This positron emission tomography study suggests that (*S*)-ketamine-induced marked dopamine release from the presynaptic terminal may contribute to acute side-effects (i.e., psychotomimetic and dissociation) in humans.

In 1989, Olney and colleagues (Washington University) reported that the NMDAR antagonists (i.e., PCP, ketamine, and (+)-MK-801) caused neuropathological changes in the retrosplenial cortex of rat brains.[107] These neuropathological changes are well known as 'Olney's lesion.' The order of potency of NMDAR antagonist-induced neuropathological changes is known to be correlated with the potency of these compounds for NMDAR.[107] Furthermore, it is reported that these NMDAR antagonists induce heat shock protein HSP-70 protein (a marker of reversible neuronal injury) in the retrosplenial cortex.[108–110] (*R,S*)-ketamine, (*S*)-ketamine, and (+)-MK-801 produced HSP-70 protein in the rat retrosplenial cortex whereas (*R*)-ketamine did not produce HSP-70 protein in this brain region.[111]

The reduction of parvalbumin (PV)-immunoreactivity in the PFC is associated with psychosis.[112] A single dose of (*S*)-ketamine produced the reduction of PV-immunoreactivity in the mPFC.[84] In contrast, a single dose of (*R*)-ketamine did not cause the reduction of PV-immunoreactivity in the same brain region.[84] Furthermore, repeated injections with (*S*)-ketamine, but not with (*R*)-ketamine, caused a significant reduction in PV-immunoreactivity in the mPFC.[113] Thus, it seems that reduction of PV-immunoreactivity in the PFC after injection of (*S*)-ketamine might be involved in the risk of psychosis.

Very recently, we compared antidepressant effects and side-effects in mice after intranasal administration of (*R,S*)-ketamine and its two enantiomers.[114] The order of potency of antidepressant effects was (*R*)-ketamine > (*R,S*)-ketamine > (*S*)-ketamine. In contrast, the order of side-effects was (*S*)-ketamine > (*R,S*)-ketamine > (*R*)-ketamine. Thus, intranasal administration of (*R*)-ketamine might be a safer antidepressant than (*R,S*)-ketamine and (*S*)-ketamine.

Taken all together, these preclinical and clinical studies suggest a possibility that (*R*)-ketamine may be a safer antidepressant than (*S*)-ketamine and (*R,S*)-ketamine.[114–119]

(2*R*,6*R*)-hydroxynorketamine

Ketamine undergoes extensive metabolism via *N*-demethylation to norketamine by the cytochrome P450 (CYP) enzymes in the liver initially. Following this *N*-demethylation, norketamine is further metabolized to HNK (Fig. 2) and dehydronorketamine.[58] Several metabolites of HNK, including (2*R*,6*R*;2*S*,6*S*)-HNK and (2*S*,6*R*; 2*R*,6*S*)-HNK, were detected in human plasma following ketamine infusion.[120,121]

In 2016, Zanos and colleagues demonstrated that the generation of (2*R*,6*R*)-HNK (Ki >10 μM for NMDAR; Table 1[13]) in the body was essential for (*R,S*)-ketamine's antidepressant actions in rodents.[85] Other researchers have also supported this finding: Chou *et al.*[122] showed reversal of inescapable shock-induced depression-like behaviors following intra-vlPAG (ventrolateral periaqueductal gray) infusion of (2*R*,6*R*)-HNK in the modified rat LH paradigm. Pham *et al.*[123] reported that intracortical administration of (2*R*,6*R*)-HNK exerted antidepressant-like effects in control naive mice. Fukumoto *et al.*[124] also reported that (2*R*,6*R*)-HNK displayed sustained antidepressant-like activity in socially isolated mice. These findings are consistent with the initial report by Zanos and colleagues.[85]

It is reported that (2*R*,6*R*)-HNK inhibits LTP in the NAc[97] similarly to ketamine, and (2*R*,6*R*)-HNK could enhance structural plasticity in mouse mesencephalic and human iPSC-derived dopaminergic neurons via AMPA receptor (AMPAR)-dependent BDNF.[125] Furthermore, (2*R*,6*R*)-HNK promoted dendrite outgrowth in human

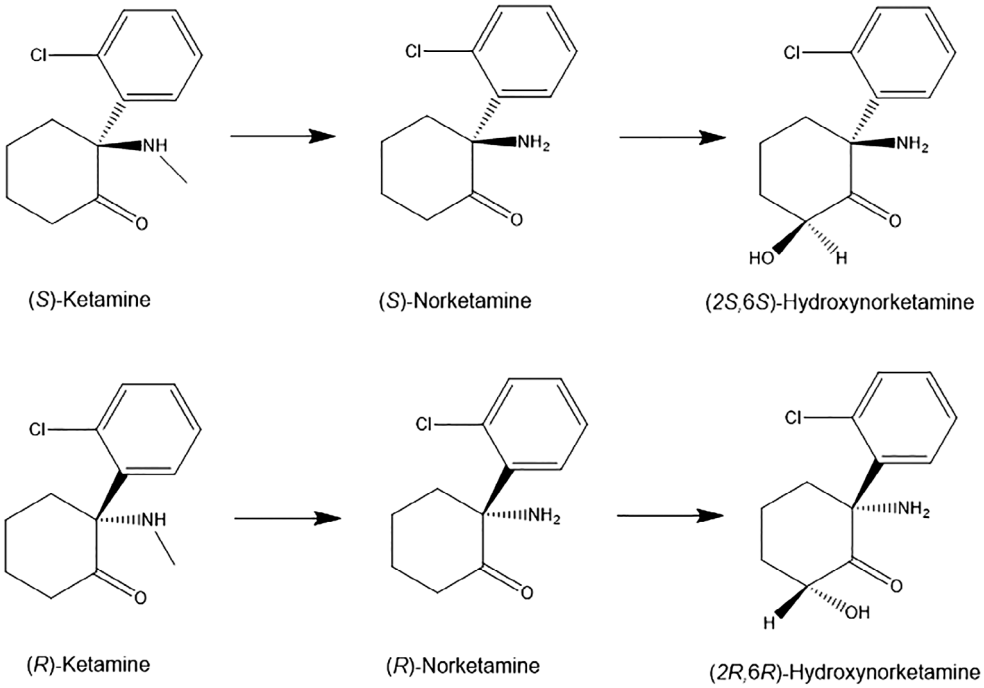

Fig.2 Major metabolites norketamine and hydroxynorketamine of (*S*)-ketamine and (*R*)-ketamine.

iPSC-derived neurons through AMPAR.[126] Although (2*R*,6*R*)-HNK can affect synaptic plasticity in the NAc and dopaminergic neurons, it is unknown whether these actions of (2*R*,6*R*)-HNK are associated with its antidepressant actions. Further study is necessary to assess the role of dopaminergic system in the antidepressant actions of ketamine and (2*R*,6*R*)-HNK. Furthermore, Wray *et al.*[127] reported that ketamine and (2*R*,6*R*)-HNK may mediate antidepressant-like actions through the translocation of GαS from lipid rafts.

On the other hand, however, we reported that (2*R*,6*R*)-HNK did not produce antidepressant effects in CSDS and lipopolysaccharide (LPS)-induced models although (*R*)-ketamine elicited rapid and long-lasting antidepressant effects in the same models.[128] Furthermore, neither (*R*)-norketamine nor (2*R*,6*R*)-HNK, which are the major metabolites of (*R*)-ketamine, showed any antidepressant effect in a rat LH model, although its parent compound (*R*)-ketamine demonstrated potent antidepressant actions in the same model.[129]

Intracerebroventricular (i.c.v.) injection of (*R*)-ketamine revealed rapid and long-lasting antidepressant actions in a CSDS model. Conversely, the i.c.v. injection of (2*R*,6*R*)-HNK did not produce antidepressant actions in the same model, although the brain level of (2*R*,6*R*)-HNK after i.c.v. injection of (2*R*,6*R*)-HNK was higher than that after i.c.v. injection of (*R*)-ketamine.[130] In addition, direct injection of (*R*)-ketamine into the brain regions (i.e., the infralimbic region of the mPFC, CA3, and the DG of the hippocampus) demonstrated long-lasting antidepressant actions in a rat LH model.[93] Together, it seems that (*R,S*)-ketamine (or (*R*)-ketamine) itself in the brain could exert antidepressant actions in rodents.

Zanos *et al.*[85] also reported the deuterium isotope effects of metabolism and the antidepressant effects of (*R,S*)-ketamine. Using (*R*)-d_2-ketamine, we replicated the deuterium isotope effects of metabolism of (*R*)-d_2-ketamine to (2*R*,6*R*)-d_1-HNK. In contrast, both (*R*)-ketamine and (*R*)-d_2-ketamine produced similar rapid and long-lasting antidepressant effects in CSDS-susceptible mice.[131] These findings suggest no deuterium isotope effect in the antidepressant actions of (*R*)-ketamine.

Ketamine is reportedly metabolized mainly by CYP enzymes in the liver.[58,85] To prevent the metabolism of (*R*)-ketamine to (2*R*,6*R*)-HNK, we used the CYP inhibitors (e.g., ticlopidine hydrochloride and 1-aminobenzotriazole). Treatment with these two CYP inhibitors before (*R*)-ketamine (3 mg/kg) injection increased the blood (*R*)-ketamine levels, whereas (2*R*,6*R*)-HNK was not detected in the blood. In the presence of CYP inhibitors, (*R*)-ketamine (3 mg/kg) produced antidepressant actions although the dose did not show antidepressant effects in the absence of CYP inhibitors. These results suggest that the production to (2*R*,6*R*)-HNK from (*R*)-ketamine is not necessary for its antidepressant effects.[132] Furthermore, we did not detect any sex difference in the pharmacokinetic profile and the antidepressant actions of (*R*)-ketamine in an LPS-induced mouse model of depression.[133]

These findings suggest that production of (2*R*,6*R*)-HNK from (*R,S*)-ketamine is not essential for the antidepressant effects of (*R,S*)-ketamine (or (*R*)-ketamine). A recent study by Zanos *et al.*[134] demonstrated that (*R*)-ketamine may exert antidepressant actions partly via conversion to (2*R*,6*R*)-HNK. Thus, the word *essential* was changed to the word *partly* in their conclusion.[85,134]

Although the reasons underlying these discrepancies remain unclear, variations in the strain, species, or experimental conditions may contribute to these discrepancies.[124] More interestingly, healthy control subjects showed significant increases in depressive symptom for up to 1 day after a single ketamine infusion,[80] suggesting that ketamine does not produce antidepressant effects in healthy control subjects. Collectively, the use of rodents with or without depression-like phenotypes may contribute to these discrepancies.[135]

(2*R*,6*R*)-HNK is a final metabolite of (*R*)-ketamine; however, the antidepressant action of (2*R*,6*R*)-HNK as a compound independent from (*R,S*)-ketamine (or (*R*)-ketamine) needs to be studied.[136–138] Furthermore, the intravenous (or intranasal) injections of (*S*)-ketamine can produce rapid and sustained antidepressant effects in treatment-resistant MDD patients.[60,63,64] It is also possible that (2*R*,6*R*)-HNK is not necessary for (*R,S*)-ketamine's antidepressant effects as (2*R*,6*R*)-HNK is not generated from (*S*)-ketamine in humans. Furthermore, a recent study showed that (2*R*,6*R*)-HNK has favorable oral bioavailability in three species (mouse, rat, dog).[139] In 2019, a clinical trial of (2*R*,6*R*)-HNK in humans will be performed at NIH, USA (Table 3). Therefore, it is of great interest to compare the antidepressant actions of (*R*)-ketamine and (2*R*,6*R*)-HNK in MDD patients.

(*S*)-norketamine

Ketamine-induced side-effects are suggested to be associated with NMDAR inhibition.[59,102,103,140] (*S*)-ketamine is metabolized to (*S*)-

norketamine (Ki = 1.70 μM[12] or Ki = 2.25 μM[13] for NMDAR) by the CYP enzymes (Table 1 and Fig. 2). (*S*)-norketamine shows rapid and sustained antidepressant actions in CSDS and LPS models of depression. Potency of antidepressant actions of (*S*)-norketamine is similar to its parent compound (*S*)-ketamine although (*S*)-norketamine's antidepressant actions are less potent than (*R*)-ketamine. Interestingly, unlike (*S*)-ketamine, (*S*)-norketamine did not produce behavioral and biochemical abnormalities (i.e., rewarding effects, PPI deficits, increases in baseline γ-oscillations, and reduction of PV-immunoreactivity in the mPFC) in mice.[141] Thus, it is possible that side-effects of (*S*)-norketamine in humans are significantly lower than (*S*)-ketamine (Table 1). Taken all together, (*S*)-norketamine and its prodrugs would be safer antidepressants than (*S*)-ketamine in humans.[10,141,142] Notably, unlike (*S*)-ketamine, it should be noted that (*S*)-norketamine is not a schedule compound.

Other NMDAR antagonists and modulators

Inspired by the rapid antidepressant effects of ketamine, application of other NMDAR antagonists for treatment of depression has also been studied. Memantine (Ki = approximately 1.0 μM for NMDAR) is a low-to-moderate-affinity NMDAR antagonist that is currently used in the treatment of Alzheimer's disease. Unlike ketamine, memantine does not cause psychotomimetic effects at therapeutic doses.[143] In a double-blind, placebo-controlled study, memantine (5–20 mg/day for 8 weeks) was not effective in the treatment of MDD.[144] Although the precise mechanism(s) underlying the differences are currently unknown, the differential effects of ketamine and memantine at intracellular signaling coupled to NMDAR at rest may contribute to the difference.[145]

Lanicemine (AZD6765; Ki = 0.56–1.5 μM for NMDAR) is also a low-trapping NMDAR antagonist that shares several pharmacological profiles of ketamine at the NMDAR.[146–148] A phase 2b trial using a large sample size showed that lanicemine (50 or 100 mg/day, three infusions a week for 12 weeks) did not show antidepressant actions in treatment-resistant MDD patients.[148] Furthermore, ketamine significantly increased the average PFC global brain connectivity with global signal regression (GBCr) during infusion and at 24 h post-treatment, whereas lanicemine showed no significant effects on GBCr compared with the placebo,[149] suggesting differences between ketamine and lanicemine. Moreover, (*R*)-ketamine demonstrated antidepressant actions in a CSDS model, whereas lanicemine did not produce any antidepressant effect.[150] Hence, the development of lanicemine was discontinued.

Rapastinel (formerly GLYX-13; Table 3) had antidepressant-like actions in rodents, and this compound does not have ketamine-like side-effects.[151,152] Furthermore, the 5-hydroxytryptamine subtype-2 receptor-mediated synaptic and behavioral actions of rapastinel may be associated with the lack of psychotomimetic side-effects of this compound.[153] A recent study suggests that the positive modulation of NMDAR by rapastinel might show rapid and sustained antidepressant effects.[154] Furthermore, (*R*)-ketamine elicited longer-lasting antidepressant actions than rapastinel in a CSDS model.[155] Intravenous administration of rapastinel (5 or 10 mg/kg) showed antidepressant actions in depressed patients who did not respond to another antidepressant.[156] However, on 6 March 2019, Allergan announced that three acute pivotal phase 3 studies of rapastinel in MDD patients did not meet their primary endpoint (Table 3).[157] AGN-241751 (Table 3), an orally bioavailable positive modulator of NMDAR, is being developed as a rapid and sustained antidepressant.[158]

A clinical study of AV-101 (L-4-chlorokynurenine: a potent antagonist at the glycine site of NMDAR), NRX-101 (D-cycloserine plus lurasidone), and AXS-05 (dextromethorphan plus bupropion) is also underway (Table 3).[59,159] On 3 May 2019, the company VistaGen stated that AV-101 did not show antidepressant actions in depressed patients (Table 3).

Low-voltage-sensitive T-type calcium channel inhibitor and potassium channel Kir4.1 inhibitor

In 2018, Yang and colleagues (Zhejiang University, China) demonstrated that the blockade of NMDAR-dependent bursting activity by ketamine in the LHb promotes its rapid-acting antidepressant effects in rodents. Furthermore, LHb bursting requires both NMDAR and low-voltage-sensitive T-type calcium channels (T-VSCC). Interestingly, the T-VSCC inhibitor ethosuximide (200 mg/kg) could show rapid antidepressant actions in rodents[99]; however, the data are lacking in the control (no stress) group, analysis of dose dependency, and positive control (i.e., ketamine) group. In contrast, unlike (*R*)-ketamine, ethosuximide (100, 200, 400 mg/kg) did not produce any antidepressant action in CSDS-susceptible mice.[160] Therefore, T-VSCC inhibitors might not exert ketamine-like robust antidepressant actions. Nonetheless, it would be interesting to investigate whether ethosuximide can produce ketamine-like rapid antidepressant actions in patients with depression since ethosuximide is an anticonvulsant commonly used for absence seizures in adults and children. A clinical study of ethosuximide in treatment-resistant patients with MDD is underway at Zhejiang University (NCT03887624).

The inwardly rectifying potassium channel Kir4.1 in astroglia is reportedly responsible for potassium buffering.[161] The same group demonstrated upregulation of astroglial Kir4.1 in the LHb of rats with depression-like phenotype.[162] In addition, astrocyte-specific gain and loss function of Kir4.1 in the LHb could regulate neuronal bursting and depression-like phenotypes, suggesting that Kir4.1 in the LHb may be a therapeutic target for depression.[162] We reported that the levels of Kir4.1 and $GABA_B$ (γ-aminobutyric acid, type B) receptor subunit 1 in the parietal cortex were higher in the MDD group than in the control group.[163] Interestingly, there was a positive correlation between Kir4.1 protein and $GABA_B$ receptor subunit 1 in the parietal cortex in the control group, but not in the MDD group.[163] Thus, increased expression of Kir4.1 might be involved in the pathophysiology of MDD. However, unlike (*R*)-ketamine, non-specific Kir4.1 inhibitors (i.e., quinacrine and sertraline) did not produce any antidepressant action in CSDS-susceptible mice. Therefore, Kir4.1 channel inhibitors might not produce ketamine-like antidepressant effects; however, future studies using potent and selective Kir4.1 channel inhibitors are warranted.[164]

Negative modulators of α5 $GABA_A$ receptors

The $GABA_A$ (γ-aminobutyric acid, type A) receptors play an important role in several psychiatric disorders, such as MDD, as the regulation of $GABA_A$ receptors reportedly influences glutamate neurotransmission.[165,166] Two negative allosteric modulators (NAM; MRK-016 and L-655,708) of α5 $GABA_A$ receptors showed rapid-acting antidepressant actions in chronic-restraint and chronic unpredictable mild stress (CUMS) models.[167] Furthermore, MRK-016 produced ketamine-like rapid-acting antidepressant actions, through AMPAR-dependent increase coherent neuronal activity.[168] Furthermore, L-655,708 and another $GABA_B$ receptor, MAM RY-080, had antidepressant-like effects in rodents.[169,170] However, unlike (*R*)-ketamine, MRK-016 did not produce an antidepressant effect 7 days after a single dose in a CSDS model although MRK-016 elicited rapid-acting antidepressant effects in the same model. Conversely, L-655,708 did not produce any antidepressant effect in the same model.[171]

Molecular and cellular mechanisms of ketamine's antidepressant actions

The molecular and cellular mechanisms underlying ketamine's antidepressant effects have been proposed in several preclinical studies.[11,172–178]

AMPAR activation

In 1997, Moghaddam and colleagues (Yale University) reported that low doses (10–30 mg/kg) of ketamine can increase extracellular levels of glutamate in the rat PFC, suggesting that ketamine might stimulate glutamatergic neurotransmission in the PFC.[179] It is reported that the activation of AMPAR is required for ketamine's antidepressant effects as the pretreatment of AMPAR antagonist blocked the ketamine's

antidepressant effects in rodents.[88] Subsequently, the role of AMPAR activation in the antidepressant effects of ketamine and its two enantiomers has been replicated.[89,180–184]

Although the molecular and cellular mechanisms underlying robust antidepressant actions of ketamine remain unclear, the rapid antidepressant actions of ketamine are believed to occur via the blockade of NMDAR located at inhibitory interneurons, which causes the disinhibition of the pyramidal cells, thereby resulting in a burst of glutamatergic transmission. Collectively, AMPAR activation plays a key role in the antidepressant actions of ketamine and its enantiomers.[11,84,174] Conversely, AMPAR activation may not be necessary for the antidepressant effects of (*S*)-norketamine in a CSDS model.[141] A clinical study using AMPAR antagonist perampanel, an FDA-approved drug, is underway at Yale University to assess the role of AMPAR in the antidepressant action of ketamine (NCT03367533).

Mammalian target of the rapamycin complex 1 signaling

The mammalian target of the rapamycin complex 1 (mTORC1) is a ubiquitous protein kinase involved in protein synthesis and synaptic plasticity. In 2010, Li and colleagues (Yale University) reported that the mTORC1 signaling plays a key role in ketamine's antidepressant effects as the pretreatment (i.c.v. or direct injection into mPFC) of rapamycin (an mTOR inhibitor) blocked the antidepressant effects of ketamine in rats.[180] Ketamine could increase the numbers of synaptic proteins and increase the numbers and functions of new spine synapses in the rat PFC through activation of the mTORC1 signaling.[180,185] Subsequent preclinical studies have supported the role of mTORC1 signaling in the antidepressant actions of ketamine.[186,187] Conversely, Autry and colleagues (University of Texas Southwestern Medical Center) reported that systemic administration of rapamycin (1.0 mg/kg, intraperitoneal [i.p.]) did not block the antidepressant actions of ketamine and that the phosphorylation of mTOR in the mPFC was not detected by ketamine injection.[89] Thus, the role of mTORC1 in the antidepressant actions of ketamine remains controversial. One reason underlying the discrepancy is due to the different route of treatment (i.e., i.c.v. or direct injection into the mPFC vs i.p.). However, it is reported that rapamycin (10 mg/kg, i.p.) improved social interaction deficits in mouse models of tuberous sclerosis complex,[188] indicating that the dose (10 mg/kg) is effective in the brain disorders. In addition, the role of mTORC1 in the antidepressant actions of (*R*)-ketamine and (*S*)-ketamine remains unclear. Recently, we showed that mTORC1 signaling in the PFC plays a role in the antidepressant actions of (*S*)-ketamine, but not of (*R*)-ketamine, and that extracellular signal-regulated kinase (ERK) signaling might be involved in the antidepressant effects of (*R*)-ketamine, but not of (*S*)-ketamine (Fig. 3).[189]

Interestingly, Denk *et al.*[190] reported the first evidence of increased phosphorylated mTOR protein in the blood from an MDD patient after a single dose of (*S*)-ketamine. Subsequently, Yang *et al.*[191] also demonstrated that ketamine showed rapid antidepressant actions with acute increases in the mTOR expression in the blood from MDD patients. It is, therefore, interesting to investigate whether (*R*)-ketamine can influence the ERK (or mTOR) and their phosphorylation in the blood from patients with MDD or BD.

Very recently, Abdallah and colleagues (Yale University) demonstrated that pretreatment with rapamycin did not block the rapid and sustained antidepressant effects of ketamine in treatment-resistant MDD patients. Unexpectedly, rapamycin prolonged the sustained antidepressant actions of ketamine in these patients. There was a significantly higher response rate in the rapamycin plus ketamine group (41%) than that in the placebo plus ketamine group (13%).[192] These data do not support the previous preclinical report[180] from the same university; however, the treatment route of these two studies was different (i.e., oral administration for human study vs i.c.v. or direct injection into mPFC of rodents). Therefore, further detailed studies on the role of mTORC1 signaling in ketamine's antidepressant actions are warranted.

BDNF–TrkB signaling

Multiple lines of evidence suggest that BDNF and its receptor TrkB play a crucial role in depression and in the mechanisms of antidepressants.[95,193–196] In 2011, it was reported that ketamine's rapid-acting antidepressants depend on the rapid synthesis of BDNF as ketamine does not elicit antidepressant-like effects in inducible BDNF knock-out mice.[89] Subsequent studies have supported the key role of the BDNF–TrkB cascade in the antidepressant actions of ketamine.[183,197] ANA-12 (a TrkB inhibitor) significantly blocked the rapid and long-lasting antidepressant actions of both (*R*)-ketamine and (*S*)-ketamine in CSDS-susceptible mice.[84] (*R*)-ketamine produced more potent beneficial effects on reduced synaptogenesis and BDNF–TrkB cascade in the PFC and hippocampus (i.e., CA3 and DG) from CSDS-susceptible mice than (*S*)-ketamine.[84] Furthermore, it is reported that the regulation of glutamate transporter 1 on astrocytes through TrkB activation is involved in the beneficial effects of ketamine on behavioral abnormalities and morphological changes in the hippocampus of CUMS-exposed rats.[198] Collectively, the long-lasting activation of BDNF–TrkB cascade in the PFC and hippocampus might be implicated in the long-lasting antidepressant effects of ketamine and its enantiomers.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) plays a role in stress and stress-related disorders, including depression.[199,200] Viral-mediated hippocampal knockdown of VEGF produced depressive-like behaviors and decrease in hippocampal neurogenesis in rats, which partially recovered after injection of ketamine. This study suggests that ketamine-induced VEGF expression might partially contribute to neurogenesis in the hippocampus and the antidepressant-like effects of ketamine.[201]

In 2019, Deyama and colleagues showed that antidepressant-like effects of ketamine in control naive rats were blocked by forebrain excitatory neuron-specific deletion of either VEGF or its receptor Flk-1 or by intra-mPFC injection of a VEGF neutralizing antibody.[202] Furthermore, intra-mPFC infusions of VEGF are sufficient to produce rapid ketamine-like behavioral actions, and these effects are blocked by neuron-specific Flk-1 deletion. Moreover, inhibition of neuronal VEGF signaling blocks the neurotrophic and synaptogenic effects of ketamine.[202] This study suggests that neuronal VEGF-Flk-1 cascade in the mPFC might be implicated in ketamine's antidepressant actions although further study using rodents with depression-like phenotype is needed.

In addition, co-infusion of a VEGF neutralizing antibody blocked the rapid and sustained antidepressant-like actions of intra-mPFC BDNF, and neuron-specific mPFC deletion of VEGF blocked the antidepressant-like actions of BDNF. This study suggests that antidepressant-like and neurotrophic effects of BDNF may require VEGF signaling.[203]

Hyperpolarization-activated cyclic nucleotide-gated channel

The inhibition or deletion of the hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1) is reported to occlude completely the synaptic and behavioral actions of ketamine,[204] suggesting that the reduced activity of presynaptic HCN1 channels by ketamine-induced blockade of NMDAR may be involved in the antidepressant actions of ketamine. Furthermore, the lentiviral-mediated knockdown of HCN1 protein prevented depression-like behaviors after CUMS.[205] The systemic administration of HCN channel inhibitor cilobradine (DK-AH269) produced rapid and sustained antidepressant actions in a CSDS model.[206] Therefore, HCN1 inhibitors may prove to be novel potential antidepressants; however, further studies on their direct comparison with ketamine are needed.

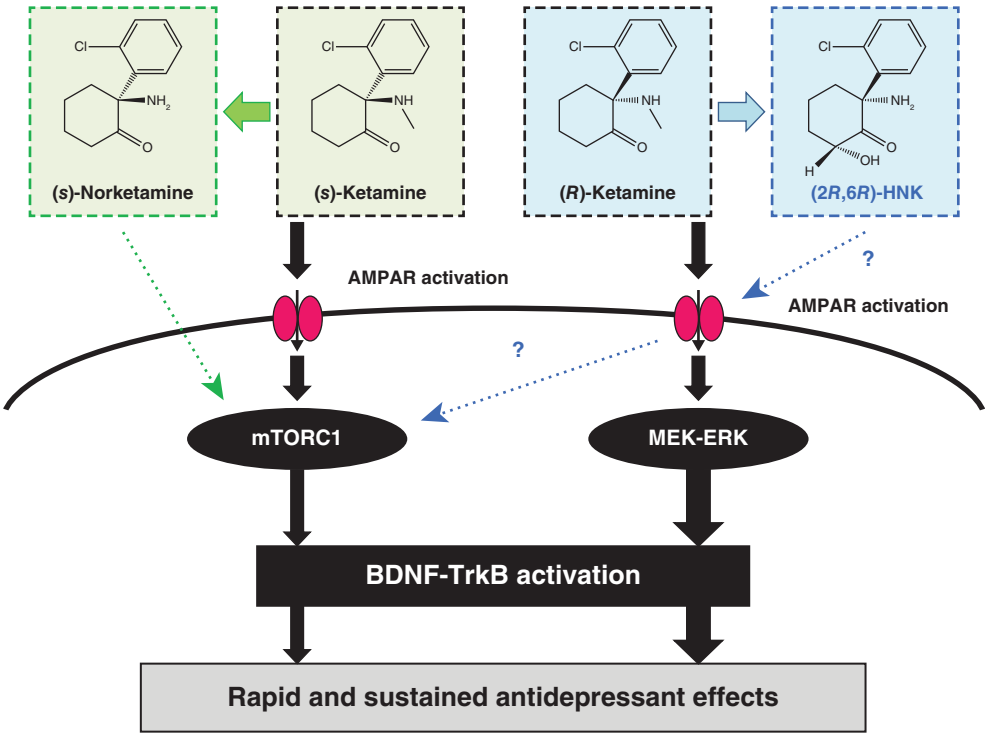

Fig.3 Proposed cellular mechanisms of (*S*)-ketamine, (*R*)-ketamine, (*S*)-norketamine, and (2*R*,6*R*)-hydroxynorketamine (HNK) for antidepressant effects. Both (*S*)-ketamine and (*R*)-ketamine activate AMPA receptors (AMPAR). Subsequently, (*S*)-ketamine and (*R*)-ketamine activate mammalian target of the rapamycin complex 1 (mTORC1) signaling and MAPK/ERK kinase (MEK)–extracellular signal-regulated kinase (ERK) signaling, respectively, and then activate brain-derived neurotrophic factor (BDNF)–tropomyosin receptor kinase B (TrkB) signaling, resulting in antidepressant effects. Antidepressant effects of (*R*)-ketamine are more potent than (*S*)-ketamine, although the precise mechanisms underlying the different efficacies of two enantiomers are currently unknown.[189] In contrast, (*S*)-norketamine, a major metabolite of (*S*)-ketamine, may not activate AMPAR. (*S*)-norketamine activates mTORC1 signaling and then activates BDNF–TrkB signaling, resulting in antidepressant effects.[141] Zanos *et al*.[85] demonstrated that metabolism of (2*R*,6*R*)-HNK from (*R*,*S*)-ketamine is essential for ketamine's antidepressant actions, and that AMPAR activation and mTORC1 signaling may play a role in the antidepressant effects of (2*R*,6*R*)-HNK. However, our data do not support the conclusion of Zanos *et al*.[85] In addition our data suggest that, unlike (*R*)-ketamine, (2*R*,6*R*)-HNK does not have robust antidepressant actions in rodents with depression-like phenotype.

Glycogen synthase kinase-3

Ketamine is reported to robustly increase the serine phosphorylation of both glycogen synthase kinase-3 (GSK-3)α and GSK-3β in the cerebral cortex and hippocampus. The ketamine's antidepressant actions in the LH paradigm were completely absent in GSK3 knock-in mice with seine-to-alanine mutations, suggesting that the phosphorylation of GSK3 by ketamine is essential for its antidepressant action.[207] Furthermore, the synaptogenic and antidepressant-like actions of ketamine in rats were potentiated when administered together with lithium (a nonselective GSK-3 inhibitor) or a GSK-3β inhibitor SB216763,[208,209] suggesting the role of the GSK-3 pathway in the antidepressant actions of ketamine. However, unlike ketamine, SB216763 did not produce any rapid and long-lasting antidepressant effect in a CUMS model.[210] Therefore, future studies elucidating the role of GSK-3β in ketamine's antidepressant effects are necessary.

P11

The protein p11 (also known as 'S100A10') is widely expressed in several brain regions that are involved in depression.[211,212] Ketamine could improve the decreased expressions of BDNF and p11 in the hippocampus of CUMS rats.[213] Interestingly, ketamine did not produce sustained antidepressant effects in rats after the knockdown of hippocampal p11.[213] Collectively, hippocampal p11 may be involved in the sustained antidepressant effects of ketamine.

Opioid receptor system

Previous reports have demonstrated interactions between ketamine and opioid receptors. The order of affinity for opioid receptor subtypes is mu > kappa > delta. (*S*)-ketamine is known to bind approximately two- to fourfold stronger to mu and kappa receptors than does (*R*)-ketamine.[214,215] In 2018, Williams and colleagues (Stanford University) demonstrated that pretreatment with naltrexone (the opioid receptor antagonist) markedly diminished the ketamine-induced antidepressant effects in treatment-resistant MDD patients.[216] The lack of impact of naltrexone on dissociative experiences suggests that opiate receptors are not central to ketamine's dissociative effects; this report concludes that the opioid system activation is necessary to produce rapid-acting antidepressant effects of ketamine.[216] However, there has been much debate about this paper.[217–221] In contrast, pilot data from Yale University demonstrated that pretreatment with naltrexone did not affect the antidepressant actions of ketamine in patients with depression and alcohol use disorder.[222–224] Further clinical trials using a large sample size are needed to better understand whether opioid receptor activation is necessary for ketamine's antidepressant actions in patients with depression.

Recently, we reported that naltrexone did not block the rapid-acting and sustained antidepressant effects of ketamine in a CSDS model or LPS-induced inflammation model.[225] If opioid receptors are implicated in the antidepressant actions of ketamine, (*S*)-ketamine must be more potent than (*R*)-ketamine in animal models of depression. Therefore, the opioid receptor system might not play a role in ketamine's antidepressant actions.[225]

microRNA

The microRNA (miRNA) are a class of non-coding RNA (approximately 22 nts) that regulate the translation of mRNA by binding to the 3′ untranslated region of mRNA in a sequence-specific manner. Repeated electroconvulsive shock therapy and ketamine possessed miRNA-598-5p as a common target.[226] Yang *et al*.[227] reported that

miR-206 is a crucial gene for BDNF expression in the hippocampus of control naive rats after ketamine injection. Furthermore, the treatment with an antagonist to miR-448-3p could diminish the antidepressant effects of ketamine in the LH paradigm, suggesting that the upregulation of miR448-3p could have an antidepressant action.[228] A new study has demonstrated that miR-29b-3p in the PFC plays a role in the antidepressant effects of ketamine in a CUMS model.[229] Collectively, miRNA may play a role in the antidepressant effects; however, further detailed studies are needed to confirm this role.

Role of gut microbiota

Multiple lines of evidence suggest that gut microbiota might be involved in the pathophysiology of depression and in the antidepressant-like effects of certain potential candidates.[230–238] Recently, we investigated the possible role of gut microbiota in the antidepressant effects of (*R*)-ketamine in CSDS-susceptible mice. At the class level, (*R*)-ketamine, but not (*S*)-ketamine, could attenuate the reduced levels of Mollicutes in the susceptible mice. At the genus level, (*R*)-ketamine was more potent than (*S*)-ketamine in reducing the levels of *Butyricimonas* in the susceptible mice.[239] Furthermore, (*R*)-ketamine, unlike lanicemine, could attenuate the altered levels of *Bacteroidales*, *Clostridiales*, and *Ruminococcaceae* in the susceptible mice.[150] Moreover, the phylum *Actinobacteria* and the class *Coriobacteriia* might be associated with ketamine's antidepressant effects in the LPS model.[240] At the genus level, ketamine could amplify *Lactobacillus*, *Turicibacter*, and *Sarcina* by 3.3-, 26-, and 42-fold, respectively. In contrast, ketamine reduced the opportunistic pathogens *Mucispirillum* and *Ruminococcus*.[241] Cumulatively, the antidepressant actions of (*R*)-ketamine (or ketamine) might be, in part, mediated through the gut–brain axis, although further studies on the role of the gut–brain axis are essential.

Concluding remarks and future directions

The development of rapid-acting antidepressants for treatment-resistant patients with MDD or BD is an unmet medical need. Multiple lines of evidence have demonstrated the robust antidepressant and anti-suicidal effects of ketamine in treatment-resistant patients with MDD or BD. Thus, the discovery of ketamine's antidepressant effects in these patients is serendipity in the field of mood disorders.[242] On 5 March 2019, the US FDA approved (*S*)-ketamine nasal spray (Spravato) for treatment-resistant depression (Table 3). A phase 1 study of (*R*)-ketamine and (2*R,*6*R*)-HNK was initiated in early 2019 (Table 3). Therefore, it will be very interesting to perform a direct comparison of (*R*)-ketamine, (*S*)-ketamine, and (2*R,*6*R*)-HNK in patients with depression.

Considering the high attrition rates, substantial costs, and the slow pace of development of novel drugs, old drug repurposing is increasingly becoming an attractive approach.[243] (*R*)-ketamine was discarded for a long period when (*S*)-ketamine was prepared from (*R,S*)-ketamine to provide (*S*)-ketamine as an anesthetic drug in several countries. If (*R*)-ketamine could elicit rapid and sustained antidepressant effects without side-effects in depressed patients with MDD or BD, it will exemplify drug repurposing from a racemic mixture in the field of psychiatric disorders. Finally, the identification of novel molecular and cellular targets responsible for the rapid and sustained antidepressant effects of ketamine and its enantiomers will thus be useful for the development of novel antidepressants without the detrimental side-effects of ketamine.

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Disclosure statement

Dr Hashimoto is the inventor of filed patent applications on 'The use of *R*-ketamine in the treatment of psychiatric diseases' and '(*S*)-norketamine and salt thereof as pharmaceutical' by Chiba University. Dr Hashimoto also declares that he has received research support and consultant fees from Dainippon Sumitomo, Otsuka, and Taisho.

Author contributions

K.H. wrote the article.

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