Common Neurotransmission Recruited in (R,S)-Ketamine and (2R,6R)-Hydroxynorketamine–Induced Sustained Antidepressant-like Effects

To the Editor:

Racemic (R,S)-ketamine, an N-methyl-D-aspartate receptor (NMDAR) antagonist, exhibits a rapid and persistent antidepressant activity at subanesthetic doses in treatment-resistant depressed patients and in preclinical studies in rodents (1). (R,S)-ketamine also induces stress resilience (2). Molecular and cellular mechanisms mediating these activities are unknown. However, (R,S)-ketamine unlikely exerts its antidepressant-like activity solely via NMDAR blockade. We previously reported that (R,S)-ketamine-induced increases in presynaptic serotonin (5-hydroxytryptamine [5-HT]) release in the medial prefrontal cortex (mPFC) is correlated with its antidepressant-like activity in mice (3). The control exerted by the mPFC is important in regulating stress processing and in mediating antidepressant-like activity of both selective serotonin reuptake inhibitors and ketamine. However, regulation of synaptic excitatory/inhibitory balance and concomitant changes in glutamate (Glu)/gamma-aminobutyric acid (GABA) neurotransmission induced by (R,S)-ketamine in rodent mPFC are unclear (4).

Recently, Zanos et al. (5) reported that the metabolism of (R,S)-ketamine to (2R,6R)-hydroxynorketamine (HNK) is essential for the antidepressant-like activity and involves early activation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA) in the mPFC and hippocampus. Conversely, Yang et al. (6) suggested that (R)-ketamine displays greater potency and longer-lasting antidepressant effects than (2R,6R)-HNK. However, brain regions and neurotransmitters supporting (2R,6R)-HNK effects are unknown. Here, we compared the sustained antidepressant-like activity and neurotransmitters’ changes of (R,S)-ketamine and (2R,6R)-HNK in BALB/cJ mice (Janvier Labs, Le Genest-Saint-Isle, France) using the forced swim test (FST), a preclinical test to screen antidepressant-like activity of drugs (7), coupled to microdialysis. Cortical extracellular levels of 5-HT, GABA, Glu (Gluext), and glutamine (Glnext) were measured by high-pressure liquid chromatography coupled to either an electrochemical detector or mass spectrometry (3).

Male adult BALB/cJ mice, 9 to 12 weeks of age (body weight = 20 to 25 g; Janvier Labs, Le Genest-Saint-Isle, France) were used. This study was approved by the Institutional Animal Care and Use Committee in France (permission #92-196 to AMG). (R,S)-ketamine hydrochloride (Sigma-Aldrich, Saint-Quentin-Fallavier, France) and (2R,6R)–HNK hydrochloride (synthesized by the Organic Chemistry Collaborative Center, New York) were dissolved in vehicle before use. The data (mean ± SEM) were analyzed using one-way analysis of variance and Fisher’s protected least-squares difference post hoc test.

(R,S)-ketamine, (2R,6R)-HNK (acute 10 mg/kg, intraperitoneal), or vehicle (NaCl, 0.9%) was administered 24 hours prior testing. On the next day (t24h), the FST was performed for 6 minutes during the 120 minutes of dialysate collection (Figure 1A). Similar to (R,S)-ketamine–elicited antidepressant-like effects at t24h as already reported (3), systemic (2R,6R)-HNK increased swimming duration (a serotonergic parameter) and extracellular levels of 5-HT in the mPFC (Figure 1B–D). Thirty minutes after its systemic administration, (2R,6R)-HNK plasma levels were five times higher than those of (R,S)-ketamine, but both drugs were no longer detected at t24h (Figure 1E), while they display a sustained antidepressant-like activity (8). These data agree with their pharmacokinetic parameters (9) and underline the presence of brain adaptive mechanisms.

To know whether the effects of (R,S)-ketamine and (2R,6R)-HNK in the mPFC were due to increases in Glu release and/or to changes in its reuptake, we performed the zero-net-flux method of quantitative microdialysis. The slope of zero-net-flux regression line is an in vivo estimate of Glu cerebral uptake (10,11). (2R,6R)-HNK significantly increased basal Glu release (Figure 1F), while (R,S)-ketamine only trended to increase it (p = .09 vs. control mice). Glu uptake was not reduced by these two drugs because cortical slope/extracellular fraction of the probe did not change (Figure 1G and insert).

In a second cohort of mice, 1 nmol/side of (R,S)-ketamine or (2R,6R)-HNK or vehicle (cerebrospinal fluid) was perfused locally at 0.2 μL/min into the mPFC (bilateral) for 2 minutes. At t24h, both (R,S)-ketamine and (2R,6R)-HNK increased swimming duration and cortical extracellular levels of 5-HT (Figure 1H–J) and decreased Glnext/Gluext ratio in the mPFC (Figure 1K). (R,S)-ketamine, but not (2R,6R)-HNK, increased cortical extracellular levels of GABA (Figure 1L, M), while (2R,6R)-HNK increased Gluext (Figure 1N–O) versus vehicle-treated mice. (R,S)-ketamine only trended to increase area under the curve Gluext values (p = .13 vs. control mice).

Using ex vivo 1H-[13C]–nuclear magnetic resonance spectroscopy, changes in mPFC Glu/GABA/Gln cycling in rat brain tissues occurred 30 minutes, but not 24 hours post-injection of (R,S)-ketamine (from 1 to 80 mg/kg, intraperitoneal) (12). The nuclear magnetic resonance technique measures the Glu/GABA/Gln cycling, used as marker of glial and neuronal metabolism, whereas in vivo microdialysis can measure a Glnext/Gluext ratio following their neuronal/glial release and/or reuptake.

Our data suggest that (2R,6R)-HNK contributes to the neurochemical and behavioral effects of (R,S)-ketamine. (2R,6R)-HNK is the major active metabolite found in the plasma and brain of mice after (R,S)-ketamine administration (5). Brain tissue concentration of (2R,6R)-HNK was about 25% of that of (R,S)-ketamine (9). Unlike (R,S)-ketamine, (2R,6R)-HNK does not bind to NMDAR at antidepressant-relevant concentrations (13), but rather increases mPFC glutamatergic AMPAR activity. Here, activation of the mPFC results in an excitatory effect and increased Glu release by pyramidal cells (Figure 1F, N, O), without affecting its reuptake (Figure 1G): various 5-HT receptor subtypes on pyramidal neurons and GABA interneurons are known to modulate mPFC neuronal activity (14,15). This mechanism can, at least partially, support...
Swimming Duration (sec)

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<tr>
<th>Time (min)</th>
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<th>(R,S)-HNK 10 mg/kg</th>
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Extracellular 5-HT Levels

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<th>(R,S)-HNK 10 mg/kg</th>
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Figure 1. (2R,6R)-hydroxynorketamine (HNK) and (R,S)-ketamine (Ket) display sustained antidepressant-like activity. (A) Experimental protocol: the surgery and drugs administration (either intraperitoneal (i.p.) or locally into the medial prefrontal cortex (intra-mPFC)) were carried out 24 hours before testing. On the next day (t24h), the microdialysis was performed in the same mice. Samples were collected from the right and left cortex in each mouse for 120 minutes, i.e., before, during, and after the FST. Swimming duration was scored for the last 4 minutes of the 6-minute testing period. Systemic administration of vehicle (Veh), (R,S)-Ket, or (2R,6R)-HNK. (B) Similar to (R,S)-Ket, (2R,6R)-HNK increased the swimming duration in the FST (F_{2,22} = 11.72, p < .0002; n = 10 mice per group). (C) Time course of drug effects on mPFC extracellular levels of 5-hydroxytryptamine (5-HT_{exc}) (R,S)-Ket and (2R,6R)-HNK increased cortical 5-HT_{exc} (in pmol/sample) vs. Veh-treated mice (F_{2,22} = 22.64, p < .0001). The gray area indicates the duration of the FST. (D) (R,S)-Ket and (2R,6R)-HNK increased cortical 5-HT_{exc} (area under the curve [AUC] values as percentages of Veh group) (F_{2,22} = 8.39, p = .0007). (E) Plasma levels of (R,S)-Ket and (2R,6R)-HNK were measured at two different time points, 30 minutes and 24 h after their systemic acute administration. (R,S)-Ket was quickly
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(2R,6R)-HNK antidepressant-like activity. It should result in potentiation of excitatory neurotransmission in the mPFC, but (2R,6R)-HNK decreased Gluext and increased Glnext, thus leading to a decrease in Glnext/Gluext ratio. In agreement with these data, an increased cerebrospinal fluid Glu/Gln ratio was found in depressed patients, suggesting abnormalities in the glia-neuron communication in the brains of these subjects (16).

Consistent with the effects of AMPAR antagonists (3), our data suggest that AMPAR activation is required for ketamine’s effects. We used the BALB/cJ strain of mice for its baseline anxiety phenotype (7), while Yang et al. (6) used a C57 strain. Behavioral effects may be mouse strain dependent because of regional differences in neurotransmitter metabolism across strains that have been previously described (17). (2R,6R)-HNK effects on mPFC Glu release by pyramidal neurons together with those of (R,S)-ketamine on cortical GABA release by interneurons led to a sustained antidepressant-like activity. Chronic stress yielding a depressed phenotype decreased brain tissue GABA levels, and a subanesthetic dose of (R,S)-ketamine normalized these changes (18). Furthermore, major depression is associated with low plasma and cerebrospinal fluid GABA concentrations and an increase in cortical GABA concentrations after selective serotonin reuptake inhibitor treatment of depressed patients (19).

In conclusion, this analysis of excitatory/inhibitory neurotransmitters’ response to (R,S)-ketamine and its metabolites paves the way for further studies to decipher the molecular and cellular mechanisms underlying their sustained antidepressant-like activity, as new data suggest that (2R,6R)-HNK is an open-channel blocker of NMDAR (20).

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RAB and CAD are named on a nonprovisional patent application for the prophylactic use of ketamine against stress-related psychiatric disorders. All other authors report no biomedical financial interests or potential conflicts of interest.

Article Information

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