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What is hydroxynorketamine and what can it bring to neurotherapeutics?

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Abstract

(*R,S*)-Ketamine was initially developed as an anesthetic agent and its pharmacological properties were determined on the basis of this clinical use. However, pharmacological studies in rat led to the development of the 'Ketamine Paradigm', whereby (*R,S*)-ketamine and its N-demethylated metabolite (*R,S*)-norketamine were deemed the active compounds whereas the other ketamine metabolites were considered inactive. Recent *in vivo* and *in vitro* studies with (*2S,6S*)-hydroxynorketamine, a previously identified 'inactive' metabolite, have demonstrated that this compound is an active and selective inhibitor of the α7 subtype of the nicotinic acetylcholine receptor and that this activity contributes to the pharmacological responses associated with the antidepressant activity of (*R,S*)-ketamine. Thus, it appears that it is necessary to reassess the 'Ketamine Paradigm' in regards to the use of sub-anesthetic doses of (*R,S*)-ketamine in the treatment of treatment-resistant depression.

Keywords

depression; D-serine; nicotinic acetylcholine receptors; NMDA receptor; serine racemase

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(*R,S*)-Ketamine (Ket) produces rapid and short-lived anesthesia and this effect has been associated with inhibition of the *N*-methyl-D-aspartate receptor (NMDA) receptor [1–3]. While Ket has a wide margin of clinical safety, it also produces dissociative and hallucinogenic effects and has been classified as a drug of abuse [2,4]. Ket's high potential for abuse resulted in limited clinical interest in the drug until the 1990's when Trullas and Skolnick demonstrated that NMDA receptor antagonists displayed antidepressant effects [5] that led to the initiation of a search for glutamate-based antidepressants [6]. In 2000, Berman and coworkers reported that Ket had antidepressant effects [7], which was followed in 2006 by the confirmatory studies of Correll and Futter [8] and Zarate, *et al.*[9]. The study by Zarate, *et al.* demonstrated for the first time in patients with treatment-resistant major depressive disorder that a single sub-anesthetic dose of Ket produced rapid antidepressant responses [9]. Since then, there has been a dramatic surge in laboratory and clinical studies of Ket as reflected by a keyword literature survey conducted using 'ketamine' and 'antidepressant' [10]. The search identified 432 papers between 1974 and 2014, with the majority appearing after 2010. It should be noted that sub-anesthetic dosing of Ket has been used in the treatment of neuropathic pain such as Complex Regional Pain Syndrome, c.f. [11] and in pediatric sedation, c.f. [12]. This review only focuses on the antidepressant effects of the drug and its metabolites.

To date, most of the studies of Ket in antidepressant therapy have been designed, conducted and analyzed using a commonly accepted view that Ket and its N-demethylated metabolite (*R,S*)-norketamine (norKet) are the active agents, that the clinical effect is due to inhibition of the NMDA receptor, and that other Ket metabolites are inactive, c.f. [2,3]. We define this operating hypothesis as the 'Ketamine Paradigm'.

The 'Ketamine Paradigm' is based upon the results of an early pharmacological study in the rat of the anesthetic properties of Ket, norKet and an additional metabolite (*2S,6S;2R,6R*)-hydroxynorketamine [(*2S,6S;2R,6R*)-HNK] [13]. The data demonstrated that Ket and norKet produced the CNS activities associated with general anesthesia and increased spontaneous locomotor activity during the post-anesthetic recovery phase. The (*2S,6S;2R,6R*)-HNK metabolite was inactive in both of these tests and was labeled as an 'inactive' metabolite. Ket is extensively and stereoselectively transformed by multiple hepatic cytochrome P450 isoforms into a broad array of metabolites, including diastereomeric hydroxyketamines, diastereomeric HNKs and (*R,S*)-dehydronorketamine [14,15]. On the basis of the 'Ketamine Paradigm' all of these metabolites were assumed to be 'inactive' and their pharmacological activities were, therefore, not investigated.

While there is little doubt that the 'Ketamine Paradigm' describes the anesthetic effects of Ket and norKet, data from our laboratory suggested that this hypothesis did not explain the antidepressant response in patients with treatment-resistant depression treated with a 40-min infusion of Ket (0.5 mg/kg) [16]. Instead, the data indicated that there were potential associations between antidepressant response and the plasma concentrations of diastereomeric HNK and some of the HNK metabolites and that these metabolites were major circulating metabolites in patients with bipolar depression and treatment-resistant depression at 230-min postinfusion [16]. The results prompted us to examine the

pharmacological activities of (*2S,6S*)-HNK as this isomer proved to be more active in preliminary *in vitro* studies as compared to (*2R,6R*)-HNK and the racemic mixture, (*2S,6S; 2R,6R*)-HNK [Singh, Wainer 2014, unpublished data].

Our *in vitro* pharmacological studies of (*2S,6S*)-HNK demonstrated that the compound did not significantly reduce the binding of [^{3}H]-MK-801 at the NMDA receptor, indicating that (*2S,6S*)-HNK is not a potent inhibitor of NMDA receptor activity as compared to Ket and norKet [17], which is consistent with the earlier findings that (*2S,6S;2R,6R*)-HNK did not produce anesthesia in the rat [13]. The results also demonstrated that (*2S,6S*)-HNK was a potent (IC_{50} <100 nM) and selective inhibitor of the α7 subtype of the nicotinic acetylcholine receptor (α7-nAChR), a ligand-gated ion channel whose activation contributes to increased intracellular Ca^{2+} flux. The α7-nAChRs are expressed in the presynaptic terminal suggesting that selective antagonists of these receptors may be useful in the treatment of CNS pathologies.

One potential consequence of α7-nAChR inhibition is the attenuation of the activity of serine racemase (SR). SR is a pyridoxal-5′-phosphate-dependent enzyme whose activation is dependent upon the binding of divalent cations, such as Mg^{2+} and Ca^{2+} [18]. SR is the source of endogenous D-serine (D-Ser), and intracellular Ca^{2+} concentrations have been shown to affect the production of D-Ser [18,19]. D-Ser is a key NMDA receptor co-agonist that plays a critical role in long-term potentiation and NMDA-induced neurotoxicity [18,20,21]. Variations in endogenous D-Ser levels have been correlated with a number of CNS diseases and pathological states, as increased levels of D-Ser in the CNS have been linked to amyotrophic lateral sclerosis and Alzheimer's disease while decreases in CNS concentrations of D-Ser have been associated with schizophrenia [18]. Thus, modulation of SR activity is an area of pharmacological and clinical interest [18,22].

We have recently demonstrated that intracellular D-Ser concentrations in PC-12 cells can be attenuated by the inhibition of cellular receptors and transporters [23–25]. Incubation with (*2S,6S*)-HNK decreased the intracellular concentration of D-Ser [23,25], which was attributed to (*2S,6S*)-HNK-mediated inhibition of the basal activity of α7-nAChR. This inhibition lowers intracellular Ca^{2+}, which, in turn, reduces the magnitude of Ca^{2+}-activated SR and consequently the intracellular D-Ser concentrations. A similar reduction in intracellular D-Ser concentration was observed after incubation with gabapentin and (*S*)-pregabalin, which reduce intracellular Ca^{2+} concentrations via inhibition of the α_2-δ subunit of voltage-gated calcium channels [24]. Based upon these observations and the recent report identifying a relationship between reduced D-Ser concentrations and attenuated NMDA receptor activity in rat and mouse models [22], it is reasonable to assume that a (*2S,6S*)-HNK-associated reduction in D-Ser production should also result in reduced NMDA receptor activation and associated neurotoxicity, synaptic death and depression.

The incubation of PC-12 cells with (2*S*,6*S*)-HNK also led to *de novo* protein synthesis of the monomeric form of SR [23,25]. The effect was associated with the mammalian target of rapamycin (mTOR) signaling pathway and involved phosphorylation-dependent modulation of multiple intracellular proteins, including extracellular signal-regulated kinases (ERK1/2), protein kinase B (Akt), eukaryotic initiation factor 4E binding protein (4E-BP1) and p70S6

kinase (p70S6K). The unique aspect of the effect of (2*S*,6*S*)-HNK on α7-nAChR activity is that it leads to increased monomeric form of SR expression but not increased D-Ser production as the concurrent decrease in intracellular Ca^{2+} concentration prevents activation of the newly synthesized enzyme.

The results from a recent study in the Wistar rat suggest that the rapid antidepressant effect produced by Ket is due to the activation of the mTOR pathway in the prefrontal cortex of the animal [26,27]. The administration of Ket produced a rapid increase in the relative concentration of the phosphorylated forms of ERK1/2 (pERK1/2), AKT (pAkt), 4E-BP1 (p4E-BP1) and p70S6 kinase (pp70S6K) and the number and function of new spine synapses in the prefrontal cortex [26,27]. Previous studies in the rat have demonstrated that Ket is rapidly converted to norKet and (*2S,6S*;*2R,6R*)-HNK, as these metabolites are detected in plasma and brain tissues within 2 min following intravenous administration of Ket [13]. The rapid appearance of (*2S,6S*;*2R,6R*)-HNK in brain tissue and the *in vitro* effects of (2*S*,6*S*)-HNK in PC-12 cells suggested that this Ket metabolite may contribute to the synaptogenesis and anti-depressive effects observed in the rat after the administration of Ket. We examined this possibility through the administration of (2*S*,6*S*)-HNK to Wistar rats and assessment of the compound's brain tissue concentrations and the corresponding changes in the mTOR pathway [25]. The results indicated that pharmacologically relevant concentrations of (2*S*,6*S*)-HNK are rapidly (within 10 min) attained in the CNS, and that the compound stimulates the activating phosphorylation of mTOR and its downstream targets in rat pre-frontal cortex tissue. Although the effect of (2*S*,6*S*)-HNK on synaptogenesis was not examined, our results indicate that the activation of the mTOR signaling pathway by (2*S*, 6*S*)-HNK results in an increase in the *de novo* synthesis of m-SR in PC-12 cells. Thus, the signaling process initiated by this compound was successfully translated into increased protein expression.

The data from *in vitro* and *in vivo* studies of (2*S*,6*S*)-HNK demonstrate that the compound has potent pharmacological activities and contributes to the effects produced by subanesthetic doses of Ket. These results suggest that it is time to reassess the use of the 'Ketamine Paradigm' when studying the antidepressant effects produced by Ket and, perhaps, to create a new clinical paradigm based upon (2*S*,6*S*)-HNK and the other understudied metabolites of Ket. In addition, the link between increased endogenous D-Ser plasma levels and CNS diseases and pathological states, such as Alzheimer's and Parkinson's diseases [18,22], suggests a broader clinical use for (2*S*,6*S*)-HNK. Because the administration of (2*S*,6*S*)-HNK produces decreased D-Ser production and improved neurogenesis, and the compound is orally bioavailable [Moaddel, Wainer 2014, unpublished data], (2*S*,6*S*)-HNK should be a clinically useful addition to the treatment of these diseases as well as providing a template for new drugs and a new 'Ketamine Metabolite Paradigm'.

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Zarate have assigned their rights in the patent to the US government, but will receive a percentage of any royalties that may be received by the government.

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