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Sub-anesthetic concentrations of (R,S)-ketamine metabolites inhibit acetylcholine-evoked currents in α7 nicotinic acetylcholine receptors

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Abstract

The effect of the (R,S)-ketamine metabolites (R,S)-norketamine, (R,S)-dehydronorketamine, (2S, 6S)-hydroxynorketamine and (2R,6R)- hydroxynorketamine on the activity of α7 and α3β4 neuronal nicotinic acetylcholine receptors was investigated using patch-clamp techniques. The data indicated that (R,S)-dehydronorketamine inhibited acetylcholine-evoked currents in α7-nicotinic acetylcholine receptor, IC_{50} = 55 ± 6 nM, and that (2S,6S)-hydroxynorketamine, (2R, 6R)-hydroxynorketamine and (R,S)-norketamine also inhibited α7-nicotinic acetylcholine receptor function at concentrations ≤1μM, while (R,S)-ketamine was inactive at these concentrations. The inhibitory effect of (R,S)-dehydronorketamine was voltage-independent and the compound did not competitively displace selective α7-nicotinic acetylcholine receptor ligands [^{125}I]-α-bungarotoxin and [^{3}H]-epibatidine indicating that (R,S)-dehydronorketamine is a negative allosteric modulator of the α7-nicotinic acetylcholine receptor. (R,S)-Ketamine and (R,S)-norketamine inhibited (S)-nicotine-induced whole-cell currents in cells expressing α3β4-nicotinic acetylcholine receptor, IC_{50} 3.1 and 9.1μM, respectively, while (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine were weak inhibitors, IC_{50} >100μM. The binding affinities of (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine at the NMDA receptor were also

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Disclosure

Dr. Zarate (CAZ) is listed as a co-inventor on a patent application for the use of ketamine and its metabolites in major depression. CAZ has assigned his rights to the US government but will share a percentage of any royalties that may be received by the government. CAZ, IWW, and RM have submitted a patent, assigned to the US government, for use of ketamine metabolites in treatment of bipolar disorder and major depression.

determined using rat brain membranes and the selective NMDA receptor antagonist [^{3}H]-MK-801. The calculated K_i values were 38.95 μM for (S)-dehydronorketamine, 21.19 μM for (2S,6S)-hydroxynorketamine and > 100 μM for (2R,6R)-hydroxynorketamine. The results suggest that the inhibitory activity of ketamine metabolites at the α7-nicotinic acetylcholine receptor may contribute to the clinical effect of the drug.

Keywords

Dehydronorketamine; hydroxynorketamine; norketamine; depression; pain; negative allosteric modifiers; neuronal nicotinic acetylcholine receptors

1. Introduction

(R,S)-Ketamine (Fig. 1) is a chiral phencyclidine derivative that was developed as an anesthetic agent (Hirota and Lambert, 2011). (R,S)-Ketamine is extensively transformed by microsomal enzymes into a number of metabolites including (R,S)-norketamine, (R,S)-dehydronorketamine and a series of hydroxynorketamines including (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine (Fig. 1) (Trevor, et al., 1983), and the P450 isoforms associated with these transformation have been recently identified (Desta, et al., 2012). Initial studies utilizing (R,S)-ketamine, (R,S)-norketamine and (2S,6S;2R,6R)-hydroxynorketamine determined that the CNS activities associated with general anesthesia and recovery were produced by (R,S)-ketamine and (R,S)-norketamine but not (2S,6S;2R, 6R)-hydroxynorketamine (Leung and Baillie, 1986). Thus, (R,S)-ketamine was identified as an anesthetic agent, (R,S)-norketamine as an “active” metabolite and (2S,6S;2R,6R)-hydroxynorketamine was considered inactive. When subsequent studies indicated that (R,S)-ketamine and (R,S)-norketamine inhibited the NMDA receptor, this activity became the accepted explanation of the pharmacological effects of (R,S)-ketamine and (R,S)-norketamine (Hirota and Lambert, 2011).

Since the operating hypothesis was centered upon the activity of (R,S)-ketamine and (R,S)-norketamine, most metabolic, pharmacokinetic, pharmacological and clinical studies have concentrated on these compounds. However, while this approach may be valid for anesthetic dosing of (R,S)-ketamine, it does not appear to be applicable to sub-anesthetic dosing of the compound, which is currently being investigated for treatment of neuropathic and acute pain and depression (Hirota and Lambert, 2011). For example, a population-pharmacokinetic analysis of patients receiving low-dose (R,S)-ketamine for the treatment of bipolar depression demonstrated that following the distribution of (R,S)-ketamine and (R,S)-norketamine may not accurately reflect the pharmacodynamics and that (2S,6S)-hydroxynorketamine, (2R,6R)- hydroxynorketamine and (R,S)-dehydronorketamine were major plasma components (Zhao, et al., 2012). In addition, a study of clinical response in 67 patients treated with low-dose (R,S)-ketamine for major depressive disorder and bipolar depression indicated plasma concentrations of (R,S)-dehydronorketamine, (2S,5S;2R,5R)-hydroxynorketamine, and (2S,5R;2R,5S)- hydroxynorketamine were associated with lower psychotomimetic or dissociative side effects (Zarate, et al., 2012). Thus, the observed clinical responses produced by low dose (R,S)-ketamine may be due to unexplored pharmacological activities of (R,S)-ketamine metabolites.

Recent studies have demonstrated that mecamylamine has therapeutic efficacy in the treatment of depression and that this effect may be related to its activity as an open-channel non-competitive inhibitor of neuronal nicotinic acetylcholine receptors (Philip, et al., 2010). (R,S)-Ketamine is also an open-channel non-competitive inhibitor of homomeric (α7) and heteromeric (α4β2, α3β4) nicotinic acetylcholine receptor subtypes (Yamakura, et al.,

2000; Coates, et al., 2001), but little is known about the activity of (R,S)-ketamine metabolites at the nicotinic acetylcholine receptor. This study was designed to assess the effect of (R,S)-norketamine, (2S,6S)-hydroxynorketamine, (2R,6R)- hydroxynorketamine and (R,S)-dehydronorketamine on agonist-induced whole-cell current in cells expressing α3β4-nicotinic acetylcholine receptor and α7-nicotinic acetylcholine receptor and their binding affinities to the NMDA receptor. In addition, the binding affinities of (R)- and (S)-dehydronorketamine at the α7-nicotinic acetylcholine receptor were determined.

2. Material and methods

2.1. Materials

(R,S)-Ketamine, (R)-ketamine, (S)-ketamine, (R,S)-norketamine, (R)-norketamine, (S)-norketamine, (R,S)-dehydronorketamine, (R)-dehydronorketamine, (S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine were prepared as previously described (Moaddel, et al., 2010). Minimum Essential Medium with Earles Salts and L-glutamine (MEM), fetal bovine serum (FBS), penicillin, streptomycin, geneticin were purchased from Gibco (Carlsbad, CA, USA). (±)-Epibatidine dihydrochloride, (S)-nicotine tartrate, acetylcholine chloride, and the other chemicals used in the study were purchased from Sigma (St. Louis, MO, USA). Mecamylamine was purchased from Ascent Scientific (Princeton, NJ, USA), and [^{125}I]-α-bungarotoxin, [^{3}H]-MK-801 and [^{3}H]-epibatidine dihydrochloride were purchased from PerkinElmer Life Sciences (Boston, MA, USA). Methyllycaconitine was generously provided by Research Triangle Institute through the National Institute on Drug Abuse.

2.2. Cell lines and culture

The KXα3β4R2 (expressing rat α3β4-nicotinic acetylcholine receptor) and KXα7R1 (expressing rat α7-nicotinic acetylcholine receptor) cell lines have been previously described (Xaio, et al., 1998; Xaio, et al., 2009). The cell lines were maintained in MEM supplemented with 10% FBS, 100 units/ml penicillin G, 100 μg/ml streptomycin and 0.7 mg/ml geneticin at 37°C with 5% CO_2 in a humidified incubator.

2.3. Whole-cell current recording in KXα7R1 cells

The KXα7R1 cell line was studied in the whole-cell configuration of the patch-clamp technique using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). The borosilicate glass capillary electrodes (Sutter Instrument Company, Novato, CA, USA), had a resistance of 2.5–3.5 MΩ when filled with 110mM trisphosphate dibasic, 28mM Tris base, 11mM EGTA, 2mM $MgCl_2$, 0.1mM $CaCl_2$ and 4mM Mg-ATP (pH 7.3). In some cells ~85% of electrode resistance was compensated electronically, so that the effective series resistance in the whole-cell configuration was accepted when less than 20 MΩ. Generation of voltage-clamp protocols and acquisition of the data were carried out using pCLAMP 9.0 software (Molecular Devices). Sampling frequency was 5 kHz and current signals were filtered at 5 or 10 kHz before digitization and storage. All experiments were performed at room temperature (22–25°C).

KXα7R1 cells were studied 2–3 days after plating on 15-mm round plastic cover slips (Thermanox, Nalge Nunc, Napierville, IL, USA) in an experimental chamber mounted on the stage of an Olympus IX50 inverted microscope (Olympus Corporation, Tokyo, Japan). The experimental chamber was constantly perfused (1–2 ml/min) with 140mM NaCl, 3mM KCl, 2mM $MgCl_2$, 25mM D-glucose, 10mM HEPES and 2mM $CaCl_2$ (pH 7.4). The high speed solution exchange system, HSSE-2 (ALA Scientific Instruments, Westbury, NY, USA) was used to deliver control and test solutions. The delay in switching between

solutions was ~10ms. Data presented herein were obtained through subtraction from the leak current.

The cells expressing α7-nicotinic acetylcholine receptors were initially exposed to 280μM acetylcholine (~EC_{50}), followed by pre-exposure (1–3 min) to test compounds at various concentrations prior to co-application with 280μM acetylcholine. The acetylcholine-evoked currents in the presence of the test compounds were measured at −80 mV and normalized to the amplitude of the current elicited by acetylcholine alone. The IC_{50} and nH values for (R,S)-dehydronorketamine were evaluated with the Origin 5.0 program (Microcal, North Hampton, MA, USA). The acetylcholine-evoked currents in the presence of (R,S)-dehydronorketamine were measured at −80 mV and normalized to the amplitude of the current elicited by acetylcholine alone. Values were plotted against the concentrations of (R,S)-dehydronorketamine on a logarithm scale and fitted with the equation:

$$I=I_{max}/1+(IC_{50}/[\text{antagonist}])^{nH}$$

Where: I is the current amplitude at the antagonist concentration [antagonist]; I_{max} is the maximum current; nH is the Hill coefficient. Results are presented as the mean ± S.E.M for the number of cells (*n*), where n = 4 per concentration.

2.4. Whole-Cell current recording α3β4-nicotinic acetylcholine receptor

Whole cell current was measured using EPC 10 HEKA Instruments amplifier. All drugs were applied by fast perfusion system (DynaFlow, Celletricon AB, Mölndal, Sweden) with 16 channels chip. Cells were clamped at -60mV holding potential. KXα3β4R2 cells were collected at 70% confluence and suspended in HEPES [10mM, pH 7.4] containing 120mM NaCl, 3.1mM KCl, 2mM $CaCl_2$, 5mM glucose at room temperature. The glass pipettes with 5–7M resistance were filled with HEPES [10 mM, pH 7.2], containing 145mM CsCl, 1mM $MgCl_2$, 2mM ATP, 1mM EGTA. All experiments were performed under visual control using an Olympus CKX 41 inverted microscope (Olympus Corporation). Test compounds were applied at concentrations of 0.1nM, 1nM, 10nM, 100nM, 1μM, 10μM and 100μM in the absence of (S)-nicotine for stimulation or in the presence of 100μM (S)-nicotine for inhibition measurements. For inhibition studies, the decrease in current relative to 100μM (S)-nicotine (100%) was determined. Raw data was collected using PatchMaster Version 2.32 software (Celletricon AB) and analysed using GraphPad Prism Version 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA).

2.5 KXα7R1 membrane binding studies

Binding of [^{125}I]-α-bungarotoxin and of [^{3}H]-epibatidine to membranes prepared from the KXα7R1 cell line was measured as described previously (Xaio, et al., 2009). Briefly, membrane preparations were incubated with ~ 1.7 nM [^{125}I]-α-bungarotoxin or ~ 2.1 nM [^{3}H]-epibatidine in Tris.HCl [50 mM, pH7.4] containing 0.2 mg/ml BSA for 2h at 24°C in a final volume of 0.1 ml. Total binding and nonspecific binding were determined in the absence and presence of 1,000 μM (S)-nicotine. Ten concentrations of each test compound were incubated to afford a binding inhibition curve. Bound and free ligands were separated by vacuum filtration through Whatman GF/C filters (Thermo Fisher Scientific, Waltham, MA, USA) treated with 0.5% polyethylenimine and 0.5 mg/ml BSA. The filter-retained radioactivity was measured by liquid scintillation counting. Specific binding was defined as the difference between total binding and nonspecific binding. Data from competition binding assays were analyzed using GraphPad Prism version 5.0 (GraphPad Software).

2.6 NMDA receptor binding studies

The binding affinities of the test compounds to the NMDA receptor were determined using rat brain membranes and the high affinity and selective antagonist [^{3}H]MK-801 using previously described procedures (Wong, et al., 1986). In brief, membranes were prepared from previously frozen rat brains by homogenization in Tris buffer [5 mM, pH 7.7], centrifugation, re-homogenization and a second centrifugation. The final membrane pellet was suspended in 5 mM Tris buffer at a concentration of 1.0 g original wet weight per 50 ml. Binding was conducted in a final volume of 1.0 ml, using 1.65 nM [^{3}H]MK-801 as the radioligand and unlabeled MK-801 (10 μM) to define non-specific binding. The reaction was incubated for 1 h at 25°C and filtered over glass fiber filters soaked in 0.05% polyethyleneimine (PEI, EMD Millipore Corp, Belleria, MA, USA).

3. Results

3.1 The effect on α7-nicotinic acetylcholine receptor activity

The effect of the test compounds on the function of α7-nicotinic acetylcholine receptors was evaluated using patch-clamp technique in the whole-cell configuration. Initial studies examined their ability to induce current activation at concentrations equal to 10, 50 and 200 nM. No measurable effect was observed at any tested concentration (n = 4), as illustrated for (R,S)-dehydronorketamine (Fig. 2).

The ability of the test compounds to affect acetylcholine-induced current was assessed using 280 μM acetylcholine, corresponding to previously determined EC_{50} concentration for rat α7-nicotinic acetylcholine receptor (Xiao, et al., 2009) and 100 nM or 1 μM of the test compounds. (R,S)-Ketamine had no observable effect, data not shown, which is consistent with previous finding that (R,S)-ketamine is a non-competitive inhibitor of α7-nicotinic acetylcholine receptor with an IC_{50} ~20μM with no significant inhibitory activity at concentrations ≤1 μM (Coates and Flood, 2001). At 100 nM concentrations, (R,S)-ketamine metabolites inhibited acetylcholine-induced current in a range from ~60%, (R,S)-dehydronorketamine (Fig. 3A), to ~45%, (R,S)-norketamine (Table 1). Acetylcholine-activated current recovered upon washout of (R,S)-dehydronorketamine (Fig. 3A). The inhibitory potency of (R,S)-dehydronorketamine was assessed at a range of concentrations from 0.0001 to 10 μM, and the IC_{50} was 55 ± 6 nM (nH = 0.66 ± 0.05; n = 3–5 for each tested concentration) (Fig. 3B). The effect of voltage on the (R,S)-dehydronorketamine mediated inhibition of acetylcholine-induced current was examined using a 30 nM concentration of (R,S)-dehydronorketamine and by varying the holding potential between −100 and + 80 mV, (Fig. 4A,B). The inhibitory effect of (R,S)-dehydronorketamine on α7-nicotinic acetylcholine receptors was voltage-independent (n=3) (Fig. 4B), suggesting that (R,S)-dehydronorketamine does not act as an ion channel blocker on this nicotinic acetylcholine receptor subtype.

In order to determine whether the inhibitory effect of (R,S)-dehydronorketamine in α7-nicotinic acetylcholine receptor is a function of competitive binding to the agonist binding site, the binding affinities, K_i values, of (R)-dehydronorketamine and (S)-dehydronorketamine were determined using cellular membranes from the KXα7R1 cell line. Competitive displacement studies were carried out using the selective α7-nicotinic acetylcholine receptor ligands [^{125}I]-α-bungarotoxin and [^{3}H]-epibatidine with (S)-nicotine and methyllycaconitine as positive controls. In previous studies utilizing KXα7R1 cellular membranes (Xiao, et al., 2009), [^{125}I]- α-bungarotoxin, K_d = 0.38 nM, was displaced by methyllycaconitine, K_i = 9.1 nM, and by (S)-nicotine, K_i = 570 nM. Under the same experimental conditions, (S)-dehydronorketamine and (R)-dehydronorketamine did not displace [^{125}I]-α-bungarotoxin at concentrations up to 1,000 μM (Fig. 5A). These results were confirmed using [^{3}H]-epibatidine, K_d = 1.8 nM (Xiao, et al., 2009), which was fully

displaced by methyllycaconitine and (S)-nicotine, but was not displaced by (S)-dehydronorketamine or (R)-dehydronorketamine at concentrations up to 1,000 μM (Fig 5B). While non-specific binding at the higher concentrations of epibatidine produced a large scatter in the data, the results demonstrate that (S)-dehydronorketamine and (R)-dehydronorketamine have low binding affinities for the binding site probed by epibatidine and are consistent with the results from the binding studies using [^{125}I]-α-bungarotoxin.

3.2 The effect on α3β4-nicotinic acetylcholine receptor activity

The effect of the test compounds on the function of the α3β4-nicotinic acetylcholine receptors was evaluated using patch-clamp technique in the whole-cell configuration. The compounds were first examined for their ability to induce current activation at the range of concentrations 0.1 nM-100 μM. No measurable response was observed at any tested concentration, data not shown. The ability of the test compounds to affect (S)-nicotine-induced current was assessed using 100 μM (S)-nicotine and appropriate concentration range of (R,S)-ketamine and metabolites (Fig. 6). (R,S)-Ketamine and (R,S)-norketamine produced a significant suppression of the currents with IC_{50} values of 3.1μM and 9.1μM, respectively. (R,S)-Ketamine has been previously reported as a non-competitive inhibitor of the α3β4 nicotinic acetylcholine receptor, IC_{50} = 9.5μM (Yamakura, et al., 2000). The application of (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine had no significant effect on the induced current, IC_{50} > than 200μM (Fig 5) indicating that these compounds had little effect on $\alpha_3\beta_4$-nicotinic acetylcholine receptor activity.

3.3 Binding affinities to the NMDA receptor

The binding affinities of the compounds used in this study to the NMDA receptor were investigated using displacement binding studies (Table 2). The marker ligand was MK-801, a non-competitive inhibitor of the NMDA receptor, which binds to phencyclidine binding sites within the ion pore of the receptor.

The calculated K_i values for (S)-ketamine (0.69 μM), (R)-ketamine (2.57 μM), (S)-norketamine (2.25 μM) and (R)-norketamine (24.46 μM) were consistent with previously reported data as were the enantioselectivities with the (S)-enantiomer having a greater relative binding affinity the (R)-enantiomer (Ebert, et al., 1997). The binding affinities of (S)-dehydronorketamine (38.95 μM) and (2S,6S)-hydroxynorketamine (21.19 μM) were weaker than the corresponding (S)-enantiomers of ketamine and norketamine, but stronger than the corresponding (R)-dehydronorketamine (74.55 μM) and (2R,6R)-hydroxynorketamine (> 100 μM) enantiomers (Table 2).

4. Discussion

Numerous *in vitro* studies have determined that (R,S)-ketamine is extensively metabolized by microsomal enzymes producing (R,S)-norketamine (Trevor *et al.*, 1983; Kharasch and Labroo, 1992; Desta, et al., 2012), (R,S)- dehydronorketamine (Bolze and Boulieu, 1998; Desta, et al., 2012) and a series of diastereomeric hydroxynorketamine metabolites (Adams *et al.*, 1981; Trevor *et al.*, 1983; Woolf and Adams, 1987). The *in vitro* data were confirmed in studies in healthy volunteers (Turfus, *et al.*, 2009) and patients receiving the drug in the treatment of bipolar and major depression (Zhao, et al., 2012; Zarate, et al., 2012) and complex regional pain syndrome (Moaddel, et al., 2010). However, while the extensive metabolism of (R,S)-ketamine has been recognized, little is known about the pharmacological activity of its metabolites other than (R,S)-norketamine. This study reports the initial examination of the pharmacological activity of (2S,6S)-hydroxynorketamine, (2R,

6R)-hydroxynorketamine, (R)-dehydronorketamine and (S)-dehydronorketamine at the α7 nicotinic acetylcholine receptor, α3β4-nicotinic acetylcholine receptor and NMDA receptor.

In this study, patch-clamp techniques were utilized to determine the pharmacological effect of (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine on the activity of the α7 nicotinic acetylcholine receptor and α3β4-nicotinic acetylcholine receptor. The data from the patch-clamp studies utilizing KXα7R1 cells indicate that 100 nM concentrations of (R,S)-norKetamine, (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine inhibited acetylcholine-induced current, while (R,S)-ketamine had no activity at this concentration (Table 1). (R,S)-dehydronorketamine appeared to be the most potent inhibitor of the tested metabolites, IC_{50} = 55 ± 6 nM, acting as a negative allosteric modulator of the α7-nicotinic acetylcholine receptor. The allosteric modulation of nicotinic acetylcholine receptor by (R,S)-dehydronorketamine is consistent with recent studies that have characterized allosteric binding sites at the protein lipid interface of the nicotinic acetylcholine receptor, to which general anesthetics bind and potentially modulate different transitions of the receptor (Nury, et al., 2010). The magnitude of the inhibitory effects of (R,S)-norketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine were similar (Table 1) indicating that inhibition of the α7-nicotinic acetylcholine receptor by these metabolites may play a role in the therapeutic effects of (R,S)-ketamine.

The effect of (R,S)-ketamine, (R,S)-norketamine, (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine on the α3β4-nicotinic acetylcholine receptor was also investigated. The data indicate that both (R,S)-ketamine and (R,S)-norketamine effectively inhibited (S)-nicotine-induced current in KXα3β4R2 cells with IC_{50} values of 3.1 μM and 9.1 μM, respectively (Fig. 6). Under the same conditions (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2S,6R)-hydroxynorketamine were essentially inactive with IC_{50} values >200 μM.

(R,S)-Ketamine and (R,S)-norketamine have been previously characterized as NMDA receptor antagonists and the clinical effects of (R,S)-ketamine are attributed to this pharmacological effect (Hirota and Lambert, 2011). Therefore, we determined the ability of (2S,6S)-hydroxynorketamine, (2R,6R)-hydroxynorketamine, (R)-dehydronorketamine and (S)-dehydronorketamine to displace the NMDA receptor marker ligand [^{3}H]-MK801 in rat brain tissue preparations. The results indicate that the metabolites interact weakly with the phencyclidine-binding site of the NMDA receptor as the calculated K_i values ranged from 21 μM (2S,6S)-hydroxynorketamine to >100 μM (2R,6R)-hydroxynorketamine (Table 2). The observed affinities were lower than those obtained using (S)-ketamine (0.69 μM), (R)-ketamine (2.57 μM) and (S)-norketamine (2.25 μM) as the displacers, while the K_i of (2S, 6S)-hydroxynorketamine was equivalent to that of (R)-norketamine (26.46 μM) (Table 2). These results are consistent with the data from an earlier study of (R,S)-ketamine and (R,S)-norketamine at the NMDA receptor in which (R,S)-ketamine had the highest binding affinity to the receptor (K_i = 0.53 μM) (Ebert, et al., 1997).

The relative binding affinities of the tested compounds demonstrated that an S-configuration at the 2-position of the phencyclidine ring was associated with a higher affinity than the corresponding compounds with an R-configuration at that site. These results are consistent with previous NMDA receptor binding data obtained with ketamine and norketamine stereoisomers (Ebert, et al., 1997), and with the observations that (R,S)-ketamine is a more potent anesthetic agent than (R,S)-norketamine and that (S)-ketamine and (S)-norketamine are more potent than the corresponding (R)-enantiomers (Hirota and Lambert, 2011). It is of interest to consider that the large difference in the K_i values between the enantiomeric (2S, 6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine may explain the initial

observation that the hydroxynorketamine metabolite is pharmacologically inactive since the (2S,6S;2R,6R)-hydroxynorketamine racemate was used in these studies (Leung and Baillie, 1986). However, even though the calculated K_i values for (2S,6S)-hydroxynorketamine and (S)-dehydronorketamine suggest that these compounds may act as inhibitors of the NMDA receptor, the clinical effect should be weak and on the order of the inhibition produced by (R)-norketamine.

The results of this study indicate that (R,S)-dehydronorketamine is a potent and selective inhibitor of the α7-nicotinic acetylcholine receptor with little effect at the α3β4-nicotinic acetylcholine receptor or NMDA receptor. Preliminary studies have also indicated that this compound had no effect on acetylcholine-evoked currents in α4β2-nicotinic acetylcholine receptor (data not shown). The data also demonstrate that (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine are inhibitors of the α7-nicotinic acetylcholine receptor with little effect at the α3β4-nicotinic acetylcholine receptor and NMDA receptor.

The potential clinical relevance of the inhibition of α7-nicotinic acetylcholine receptor activity by (R,S)-ketamine metabolites is suggested by the plasma concentrations of (R,S)-dehydronorketamine in patients with bipolar depression treated with a 40-min infusion of 0.5 mg/kg (R,S)-ketamine. In these patients the average plasma concentrations of (R,S)-dehydronorketamine were above the IC_{50} value for the inhibition of α7-nicotinic acetylcholine receptor activity at each measured time point, including 24 h after the completion of the infusion (Zarate, et al., 2012). It is important to note that 41% of the bipolar depression patients treated with (R,S)-ketamine reported a positive response 24 h after treatment and the response rate was 30% at 72 h, and that the plasma concentrations of (R,S)-dehydronorketamine, (2S,5S;2R,5R)-hydroxynorketamine, and (2S,5R;2R,5S)-hydroxynorketamine were associated with lower psychotomimetic or dissociative side effects (Zarate, et al., 2012). The contribution of an α7-nicotinic acetylcholine receptor antagonist such as (R,S)-dehydronorketamine to a positive response in the treatment of depression is consistent with the emerging clinical use of these agents (Mineur and Picciotto, 2010; Philip, et al., 2010). This is exemplified by the use of mecamylamine in the treatment of depression, which like ketamine is an open-channel non-competitive inhibitor of the α7-nicotinic acetylcholine receptor and α3β4-nicotinic acetylcholine receptor (Papke, et al., 2001).

The results of this study indicate that a further examination of the pharmacological effects of (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine is warranted and studies involving cellular and animal models have been initiated. The results will be presented elsewhere.

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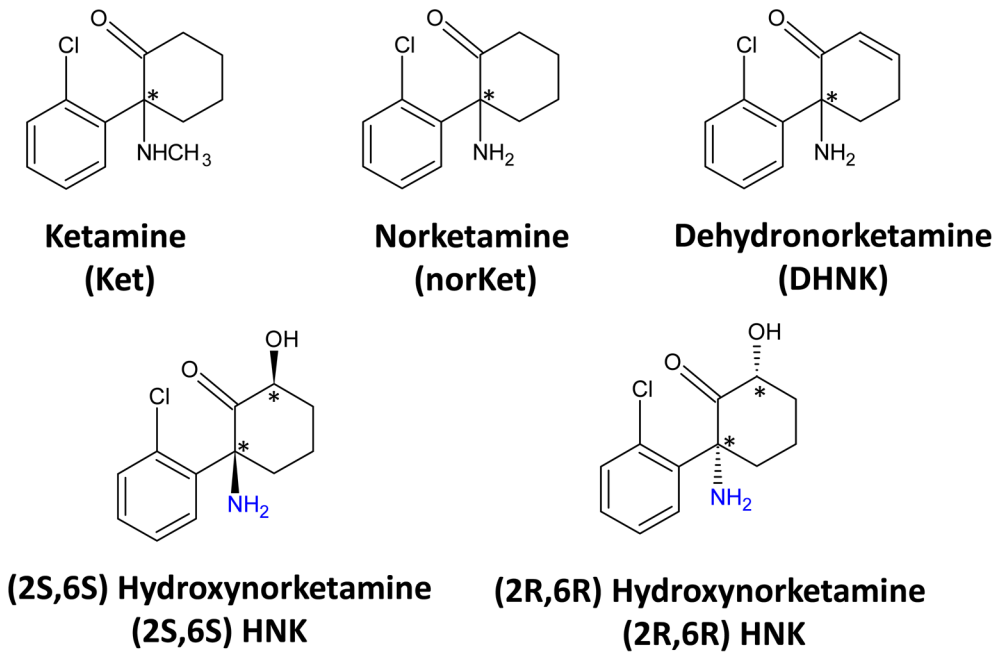

Figure 1.
The structures of the compounds used in this study

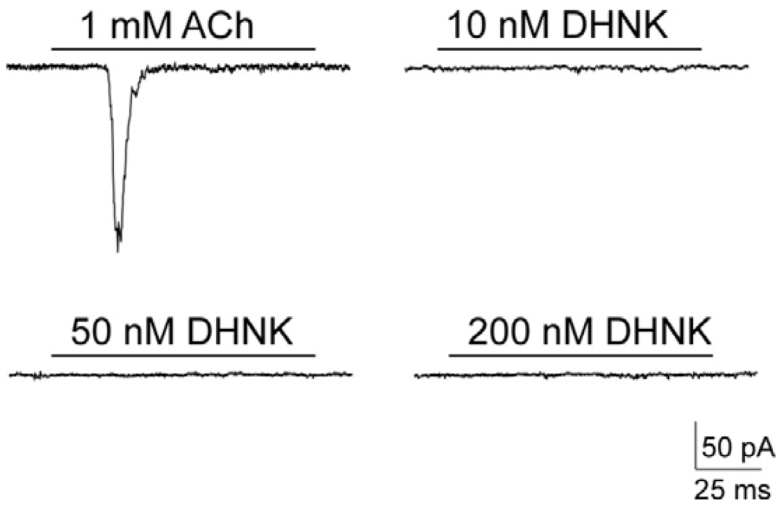

Figure 2.
The effect of 10, 50 and 200nM (R,S)-dehydronorketamine {(R,S)-DHNK} on the current traces recorded using KXα7R1 cells in the whole-cell configuration of patch-clamp technique in the presence of 1 mM ACh with a **h**olding potential of −80mV.

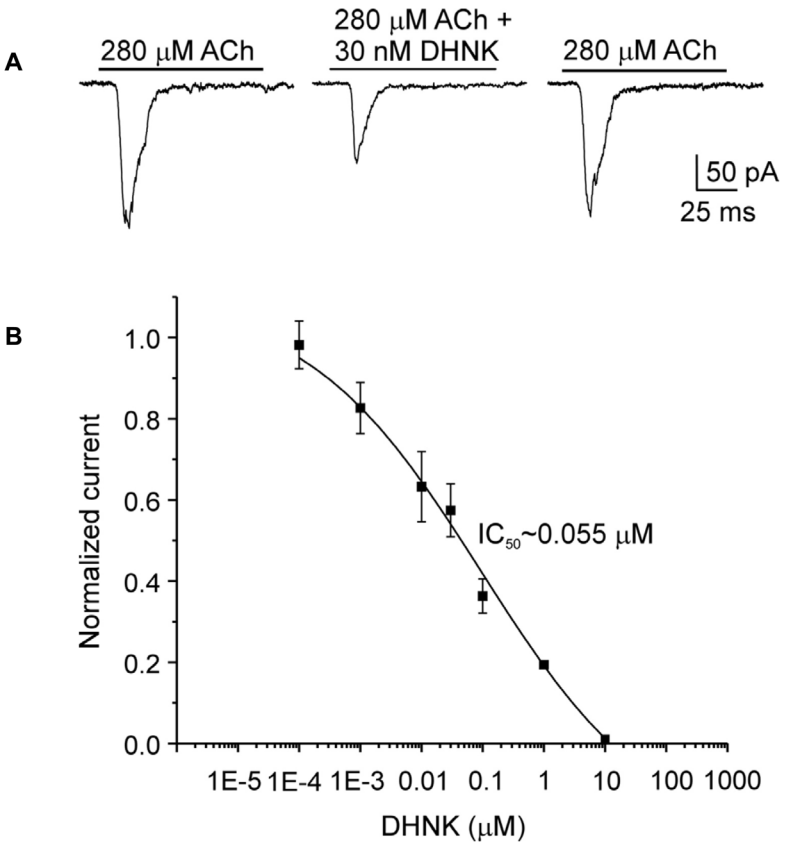

Figure 3.

A. The effect of 30 nM (R,S)-DHNK on the ACh-induced current in KXα7R1 cells illustrates a reversible pronounced (45%) inhibition of the whole-cell current. Holding potential was −80 mV.

B. Dose-dependent effect of (R,S)-DHNK on the ACh-induced current in KXα7R1 cells. The curve was fitted to Hill equation. Symbols and bars represent the mean ± S.E.M.

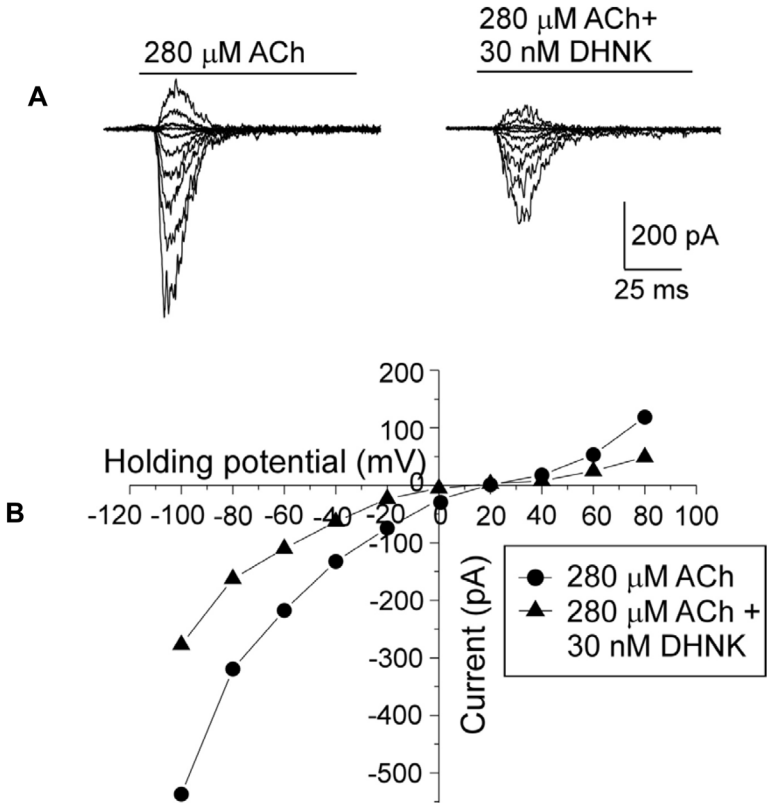

Figure 4.

A. Superimposed traces of the ACh-induced currents in the absence and presence of DHNK in KXα7R1 cells.

B. Voltage-dependence of inhibitory effect of (R,S)-DHNK on the ACh-induced current in KXα7R1 evoked at various holding potentials from −100 to +80 mV, quantified at its maximal amplitude in the absence (circles) and presence (triangles) of 30 nM DHNK and plotted versus the corresponding holding potential.

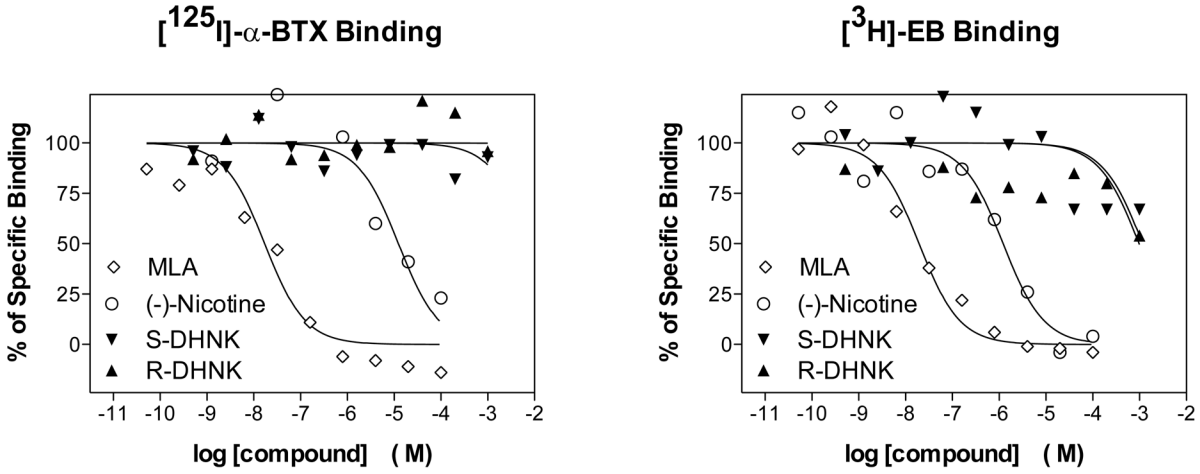

Figure 5.
Competitive displacement of [^{125}I]-α-BTX and [^{3}H]-EB from cellular membranes obtained from the KXα7R1 cell line by (R)-DHNK, (S)-DHNK, MLA and (S)-nicotine.

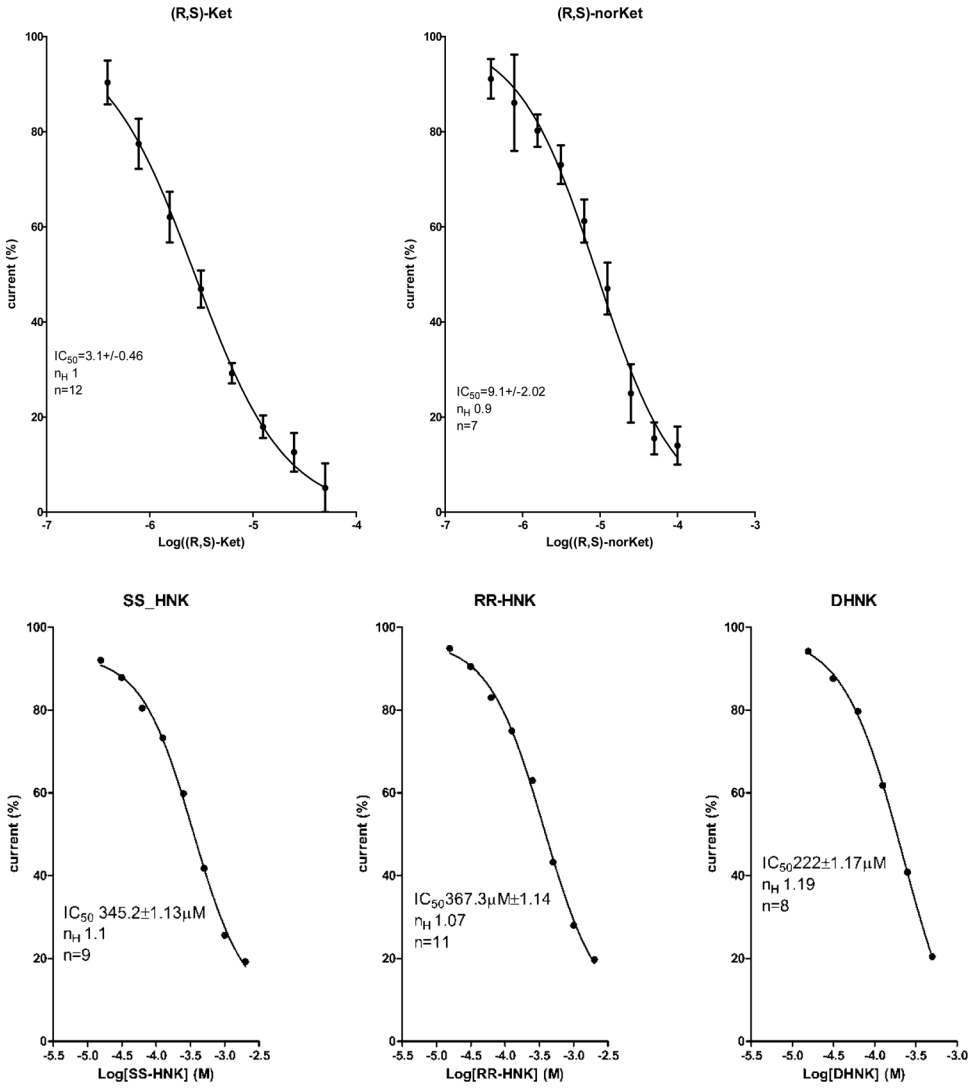

Figure 6.

The effect of the concentration of (R,S)-ketamine {(R,S)-Ket}, (R,S)-norketamine {(R,S)-norKet}, (2S,6S)-hydroxynorketamine {SS-HNK}, (2R,6R)-hydroxynorketamine {RR-HNK} and (R,S)-dehydronorketamine {(R,S)-DHNK} on the (S)-nicotine-induced current in KXα3β4R2 cells determined using whole-cell configuration of the patch-clamp technique. The calculated IC_{50} values are included in the figure.

Table 1

The effect of (R,S)-norketamine, (R,S)-dehydronorketamine, (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine on the currents evoked by 280 μM acetylcholine in the KXα7R1 cell line.

Compound	Concentration	Mean	SE
(R,S)-norKetamine	100 nM	0.548	0.099
	1000 nM	0.287	0.075
(R,S)-dehydronorketamine	100 nM	0.407	0.053
	1000 nM	0.205	0.013
(2S,6S)-hydroxynorketamine	100 nM	0.461	0.06
	1000 nM	0.361	0.07
(2R,6R)-hydroxynorketamine	100 nM	0.489	0.077
	1000 nM	0.354	0.084

The data was obtained at −80 mV and normalized to the amplitude of the current elicited by acetylcholine alone, n = 4.

Table 2

The binding affinities (K_i values) of (R)- and (S)-ketamine, (R)- and (S)-norketamine, (R)- and (S)-dehydronorketamine and (2S,6S)- and (2R,6R)-hydroxynorketamine to the NMDA receptor determined using displacement binding studies with [^{3}H]-MK801as the marker ligand, where n = 4 for MK801 and 2 for all other measurements.

Compound	K_i (μM)
MK801	0.0047 ± 0.0004
(R,S)-Ketamine	1.06 ± 0.14
(S)-Ketamine	0.69 ± 0.09
(R)-Ketamine	2.57 ± 0.28
(S)-norKetamine	2.25 ± 0.22
(R)-norKetamine	26.46
(S)-Dehydronorketamine	38.95
(R)-Dedhydronorketamine	74.55
(2S,6S)-Hydroxynorketamine	21.19
(2R,6R)-Hydroxynorketamine	>100