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Research report

Differential regulation of GluA1 expression by ketamine and memantine

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HIGHLIGHTS

- Memantine potentiates Schaffer collateral-CA1 excitatory synaptic transmission in hippocampal area CA1.
- Administration of memantine or ketamine enhances GluA1 S845 phosphorylation, but only ketamine elevates the expression of GluA1.
- Memantine-induced potentiation of SC-CA1 synaptic phosphorylation and GluA1 phosphorylation are not occluded by GAGA receptor blockade.
- Ketamine but not memantine enhances the phosphorylation of mTOR, in a time course parallel to the elevation of GluA1 expression.
- Neither ketamine or memantine reduces the phosphorylation of eEF2; instead, ketamine enhances eEF2 phosphorylation 30 min following administration.

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ABSTRACT

Evidence from preclinical and clinical studies shows that ketamine, a noncompetitive NMDA receptor antagonist, exerts rapid and sustained antidepressant responses. However, ketamine's psychotomimetic side effects and abuse liability limit the clinical use of the compound. Interestingly, memantine, another NMDA receptor channel blocker, processes no defined antidepressant property but is much safer and clinical tolerated. Understanding why ketamine but not memantine exhibits rapid antidepressant responses is important to elucidate the cellular signaling underlying the fast antidepressant actions of ketamine and to design a new safer generation of fast-acting antidepressants. Here we show that ketamine but memantine caused a rapid and sustained antidepressant-like responses in forced swim test (FST). Both drugs enhanced GluA1 S845 phosphorylation and potentiated Schaffer collateral-CA1 synaptic transmission. However, ketamine but not memantine elevated the expression of GluA1. Incubating acutely prepared hippocampal slices with ketamine but not memantine enhanced mTOR phosphorylation in a time course parallel to the time course of GluA1 elevation. Our results suggest that distinct properties in regulation of mTOR phosphorylation and synaptic protein expression may underlie the differential effectiveness of ketamine and memantine in their antidepressant responses.

1. Introduction

Major depressive disorder (MDD) is a serious public health problem with a lifetime prevalence about 16% worldwide [4,13,34]. Existing treatments for MDD usually take weeks to months to achieve their antidepressant effects, and a significant number of patients do not have adequate improvement even after months of treatment. In addition, increased risk of suicide attempts is a concern during the first month of standard antidepressant therapy [23]. Thus, improved therapeutics that show rapid and uniform efficacy and better safety are urgently needed.

Emerging evidence from clinic trails demonstrate that a single dose of ketamine, a noncompetitive ionotropic glutamatergic NMDA receptor antagonist, produced rapid antidepressant responses in patients suffering from MDD [25]. Depressed,

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treatment-resistant patients reported alleviation of core symptoms of major depression within hours of a single dose of intravenously infused ketamine, with effects lasting up to 1 week or even longer [39,15,17]. Subsequent studies reported that ketamine significantly reduced suicidal ideality in individuals with treatment-resistant depression [1]. The discovery of ketamine's rapid-acting effect opens up a potentially promising new approach to treating depression. However, the psychotomimetic properties and abuse potential limit ketamine to be promoted as a general treatment for depression [5]. Understanding the underlying mechanism responsible for ketamine's beneficial behavioral effects may be the key to developing novel, safe, and fast-acting antidepressants.

Memantine, another noncompetitive NMDAR antagonist, has memory-improvement action and is a widely prescribed treatment for Alzheimer's disease. Memantine is a well-tolerated drug and lacks the psychotomimetic side effect at therapeutic doses. However, the antidepressant action of memantine is elusive and controversial [31,24]. In clinical trials, chronic memantine administration did not elicit consistent antidepressant response in depressed patients compared with patients received placebo [22,20]. Understanding why ketamine, but not memantine, generates fast antidepressant responses may help us identify the critical molecular events that mediate the antidepressant actions of ketamine and design new generation of fast and safe antidepressant.

2. Materials and methods

2.1. Animals and drug treatments

Sprague-Dawley (SD) rats were purchased from Harlan Laboratories (Indianapolis, IN, USA). Rats were kept on a 12/12-h light/dark cycle and allowed ad libitum access to food and water. Both male and female rats used for tissue preparation or behavioral tests in this study were eight- to fourteen-week old. Memantine hydrochloride (Sigma) and ketamine hydrochloride (Henry Schein) were dissolved (or diluted) in saline. Rats received ketamine or memantine injection (i.p.) at the time indicated in the text before forced swim test or euthanasia to assess molecular signaling. Force swim tests were conducted by an observer blinded to drug treatment. All experimental procedures involving animals were performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Southern Illinois University, Carbondale.

2.2. Hippocampal slice preparation

This method was described in detail in our previous publications [7,6]. Briefly, rats were killed by decapitation after sedation with Euthasol solution (containing 390 mg/ml sodium pentobarbital and 50 mg/ml phenytoin sodium; 0.5 ml/kg, i.p.; Henry Schein, Melville, NY). Brains were rapidly removed and hippocampal dissection was done in ice-cold artificial cerebrospinal fluid (ACSF; 124 mM NaCl, 3 mM KCl, 1.25 mM $NaH2PO_4$, 1.0 mM $MgCl_2$, 2.5 mM $CaCl_2$, 26 mM $NaHCO_3$ and 10 mM glucose) bubbled with 95% O_2/5% CO_2. Isolated hippocampi were sectioned into 400 μM slices using a vibratome (VT 1000S, Leica) and kept in a holding chamber at room temperature (20–22 °C) at the interface of ACSF and humidified in 95% O_2/5% CO_2 for minimally 1 h.

2.3. Extracellular field potential recordings

After incubation, hippocampal slices were transferred to a submersion-type recording chamber and perfused at room temperature with ACSF (flow rate = 1–2 ml/min). Concentric bipolar tungsten electrodes were placed in stratum radiatum of area CA1 to stimulate Schaffer collateral afferents. Extracellular recording pipettes (1–2 MΩ) were filled with ACSF and placed 100–150 μm from the stimulating electrodes in stratum radiatum. Stimuli (100 μs duration) were delivered at 0.05 Hz. The stimulus intensity was set at 150% of threshold intensity, resulting in a field EPSPs (fEPSPs) of 0.1–0.2 mV. All compounds were applied by perfusion. Field EPSPs were recorded using Axoclamp 2B or Axopatch 200B amplifiers (Molecular Devices), and signals were amplified 100× and filtered at 10 kHz before digitization using a Digidata 1440A A/D converter (Molecular Devices).

2.4. Forced swim test

In the pretest session rat was individually placed into a water-filled Plexiglas cylinder (10,000 ml cylinder filled with 6000 ml water of temperature 20–22 °C) for 15 min. The animal was then removed, dried with a towel and returned to home cage. The water in the cylinders was changed between subjects. A test session was performed 24 h after pretest. The test session, also 15 min, was recorded by a video camera positioned on the side of the cylinder to evaluate locomotor activity. The first 5 min of the test session was analyzed and scored by an observer blind to group assignment. The time that the test animal spent in the first 5 min of test trial without making any movements (no obvious movement of any limb) beyond those required to keep its head above water was measured as immobility time. A decrease in immobility time (i.e., more swimming/struggling while in the water) is suggestive of an antidepressant-like response.

2.5. Isolation of synpatosomes

Hippocampal slices, prepared as described above, were maintained at room temperature for at least 1 h. Area CA1 wedges were dissected from the slices. Eight to twelve hippocampal CA1 wedges were pooled and homogenized in 800 μl Syn-PER™ synaptic protein extraction reagent (ThermoFisher Scientific). Homogenates (total fractions) were collected and centrifuged at 1200 × *g* for 10 min to remove cell debris, and the supernatant was centrifuged at 15,000 × *g* for 20 min at 4 °C. The pellets, containing synaptosomes, were gently re-suspended in the modified RIPA buffer (Fisher Scientific, 100 μl for each sample, containing 150 mM NaCl, 20 mM Hepes, 1% Triton X-100, 0.5% SDS, 2 mM EDTA, and supplemented with a cocktail of protease and phosphatase inhibitors). Total protein concentration in the re-suspended synaptosomes was measured by bicinchoninic acid assay (BCA assay), and was normalized by adding the modified RIPA buffer.

2.6. Western blot analysis

After added with equal volume 2 X loading buffer, synaptosome solution was heated at 95 °C for 5 min and loaded into 6–10% Bis-Tris gels (Bio-Rad, Hercules, CA, USA). After running in 1 × NuPAGE MOPS SDS buffer (Fisher Scientific), the gel was transferred onto polyvinylidene difluoride membranes in 1 × NuPAGEge transfer buffer (in 20% methanol, vol/vol). The membrane was blocked with 5% nonfat dry milk (wt/vol) in buffer containing 1 M Tris-buffered saline and 0.1% Tween-20 (vol/vol), and incubated with primary antibodies against S845-phosphorylated GluA1 (1: 1000, Cell Signaling Technology, Danvers, MA, USA, #13185), phospho-mTOR (Ser2448) (1:1000, Cell Signaling Technology, #2971), and phspho-eEF2 (Thr56) (1:1000, Cell Signaling Technology, #2331), respectively, overnight at 4 °C. After three rinses in TBS-Tween, the membrane was incubated for 1 h at room temperature in horseradish peroxidase-conjugated goat anti-rabbit IgG (1: 1000, Fisher Scientific, #31462). The immunoblot was developed with enhanced chemiluminescence (Fisher Scientific). Membranes were