Glutamate *N*-methyl-D-aspartate Receptor Antagonists Rapidly Reverse Behavioral and Synaptic Deficits Caused by Chronic Stress Exposure

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Background: Despite widely reported clinical and preclinical studies of rapid antidepressant actions of glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonists, there has been very little work examining the effects of these drugs in stress models of depression that require chronic administration of antidepressants or the molecular mechanisms that could account for the rapid responses.

Methods: We used a rat 21-day chronic unpredictable stress (CUS) model to test the rapid actions of NMDA receptor antagonists on depressant-like behavior, neurochemistry, and spine density and synaptic function of prefrontal cortex neurons.

Results: The results demonstrate that acute treatment with the noncompetitive NMDA channel blocker ketamine or the selective NMDA receptor 2B antagonist Ro 25-6981 rapidly ameliorates CUS-induced anhedonic and anxiogenic behaviors. We also found that CUS exposure decreases the expression levels of synaptic proteins and spine number and the frequency/amplitude of synaptic currents (excitatory postsynaptic currents) in layer V pyramidal neurons in the prefrontal cortex and that these deficits are rapidly reversed by ketamine. Blockade of the mammalian target of rapamycin protein synthesis cascade abolishes both the behavioral and biochemical effects of ketamine.

Conclusions: The results indicate that the structural and functional deficits resulting from long-term stress exposure, which could contribute to the pathophysiology of depression, are rapidly reversed by NMDA receptor antagonists in a mammalian target of rapamycin dependent manner.

Key Words: Antidepressant, depression, ketamine, rapamycin, spines, synaptogenesis

Major depressive disorder (MDD) is a debilitating and recurring psychiatric disorder that affects up to 17% of the American population (1). Despite a wide range of antidepressants available, only one third of the patients show significant mood improvement in response to an initial antidepressant treatment (2). Moreover, there is a time lag of weeks to months with currently available medications, further highlighting a major unmet need for novel rapid-acting and more efficacious antidepressant agents. Recent studies with ketamine, a noncompetitive glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonist, may address this critical need. Ketamine produces rapid antidepressant responses (within hours) in treatment-resistant MDD patients (3–5). However, the widespread use of ketamine is limited by the potential for toxicity and abuse, and studies are being conducted in animal models to elucidate the mechanisms underlying the actions of ketamine and to develop safe, rapid-acting agents.

Preclinical studies have demonstrated antidepressant actions of ketamine in rodent behavioral despair models, including the forced swim test (FST) and learned helplessness (LH) paradigm (6–10). However, FST and LH are responsive to acute or subchronic antidepressant treatments and do not provide a rigorous test of the rapid actions of ketamine (11). In the current study, we used a chronic unpredictable stress (CUS) paradigm, which results in anhedonia, a core symptom of depression, that is responsive to chronic (3 weeks) but not acute or short-term antidepressant treatment (12). In addition, chronic stress exposure also causes atrophy of neurons in rodent prefrontal cortex and hippocampus (13–20), effects that could contribute to decreased volume of these regions reported in brain imaging studies of MDD patients (14,21–24). Another aim of the current study is to determine if ketamine can rapidly reverse the neuronal atrophy and functional deficits caused by CUS exposure.

We have recently reported that NMDA antagonists rapidly increase the density and function of spine synapses in the prefrontal cortex (PFC) and that these effects, as well as the behavioral responses in the FST and LH, are mediated by activation of the mammalian target of rapamycin (mTOR) (25). The mTOR pathway has been implicated in activity-dependent synaptic plasticity and is localized in neuronal dendrites and spines, where it controls the synthesis of proteins that are required for new synapse formation (26). *N*-methyl-D-aspartate antagonist stimulation of mTOR-mediated synaptogenesis provides a mechanism for rapid reversal of stress-mediated and/or depression-mediated deficits (25).

In the present study, we report the ability of a single dose of NMDA antagonists to rapidly reverse the behavioral and synaptic deficits caused by long-term CUS exposure in an mTOR-dependent manner. These results highlight mTOR and upstream signaling pathways as pivotal targets for development of novel rapid-acting and efficacious antidepressant agents.

Methods and Materials

Animals

Male Sprague-Dawley rats weighing 175 to 250 g were pair-housed and maintained in standard conditions with a 12-hour

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light/dark cycle and ad libitum access to food and water. Animal use and procedures were in accordance with the National Institutes of Health guidelines and approved by the Yale University Animal Care and Use Committees.

CUS Procedure

Animals were exposed to a variable sequence of mild and unpredictable stressors for 21 days, a procedure that we have found produces depressive-like behavioral changes (27,28). A total of 10 different stressors were used (2 stressors per day, see Figure 1A). The stressors included rotation on a shaker, placement in a 4°C ambient, lights off for 3 hours (10:00 AM–1:00 PM), lights on overnight, strobe light overnight, aversive odor, 45° tilted cages, food and water deprivation, crowded housing, and isolation housing.

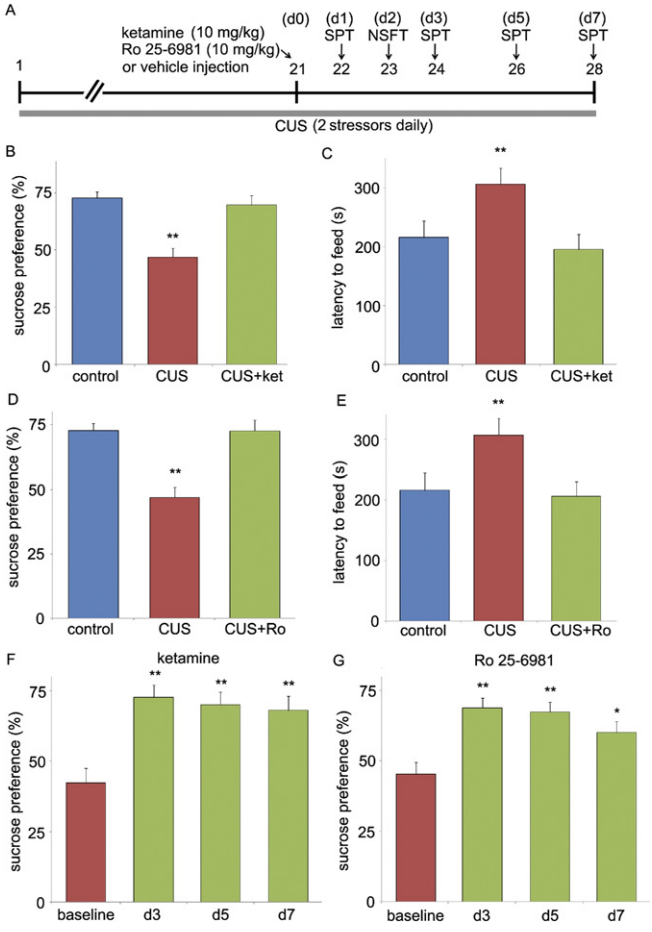

Figure 1. *N*-methyl-D-aspartate receptor antagonists produce rapid antidepressant responses in a chronic unpredictable stress (CUS) paradigm. **(A)** Schematic demonstrating the time line for CUS exposure, drug administration, and behavioral testing. Numbers in parentheses represent days after drug administration. Rats were exposed to CUS and administered ketamine or Ro 25-6981 (both at 10 mg/kg intraperitoneal) on day 21. The sucrose preference test was conducted 1 day later **(B, D)** and novelty-suppressed feeding test 2 days after drug treatment **(C, E)**. Ketamine and Ro 25-6981 administration in CUS rats reversed the decreased sucrose preference and increased latency to feed to the level of nonstressed control rats. The sucrose preference test was also conducted at 3, 5, and 7 days after ketamine or Ro 25-6981 **(F, G)**. Baseline was measured on day 21 before drug injections. Values represent mean ± SEM (n = 6 per group. $^{**}p < .01$, $^{*}p < .05$, analysis of variance). ket, ketamine; NSFT, novelty-suppressed feeding test; Ro, Ro 25-6981; SPT, sucrose preference test.

Drug Administration and Surgical Procedure

Animals received a single acute intraperitoneal (IP) injection of vehicle, ketamine, or Ro 25-6981 on day 21 of CUS treatment. Based on previous studies (25), the dose used for both drugs was 10 mg/kg. Tissue was collected for molecular assays or animals were tested in behavioral paradigms as described below. For experiments involving central administration of inhibitors, rats were implanted with guide cannulae (22 ga) into the lateral ventricles (coordinates from bregma: −.9 mm anterior/posterior, −1.5 mm medial/lateral, −3.3 mm dorsal/ventral from dura). The surgical procedures were carried out under Nembutal anesthesia (IP 55 mg/kg; Butler Schein, Chicago, Illinois). Postoperative care consisted of perisurgical administration of carprofen (5 mg/kg; Butler Schein) and topical triple antibiotic ointment. During recovery, animals carried a dummy cannula. After a 7-day recovery period, rapamycin (.2 nmol in 2 μL; Sigma, St. Louis, Missouri) or a vehicle (dimethyl sulfoxide, DMSO; Sigma) was delivered 30 minutes before drug injections at the rate of .25 μL/min, with an injection cannula (26 ga) protruding .5 mm beyond the guide cannula. These doses were chosen based on previous reports demonstrating effective and selective inhibition of the respective targets (25,29). The injection cannula stayed in the guide cannula for 1 minute after infusions.

Behavioral Tests

Sucrose Preference Test. For the sucrose preference test (SPT), rats were exposed to a palatable sucrose solution (1%; Sigma) for 48 hours, followed by 4 hours of water deprivation and a 1 hour exposure to two identical bottles, one filled with sucrose solution and the other with water. This procedure was adapted from previous studies and has been used previously in our laboratory (30,31). Sucrose and water consumption were determined by measuring the change in the volume of fluid consumed. Sucrose preference was defined as the ratio of the volume of sucrose versus total volume of sucrose and water consumed during the 1-hour test.

Novelty-Suppressed Feeding Test. The novelty-suppressed feeding test (NSFT) was performed as previously described (31). Before testing, rats were food-deprived overnight. Rats were placed in an open field (76.5 cm × 76.5 cm × 40 cm, Plexiglas) with a small amount of food in the center. Animals were allowed to explore the open field for 8 minutes. The latency to feed, specifically the time it took for the animal to approach and take the first bite of the food, was recorded by a stopwatch. Home cage food intake was measured right after the test as a control value.

Immunoblotting

Prefrontal cortex synaptoneurosomes were prepared as previously reported (25) and sonicated in protein lysis buffer. Protein concentration was determined by bicinchoninic acid protein assay. For western blotting, equal amounts of protein (10–20 μg) for each sample were loaded into 10% to 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Polyvinylidene difluoride membranes (Bio-Rad, Hercules, California) with transferred proteins were blocked in phosphate buffered saline + .1% Tween 20 (PBST; Sigma) for 1 hour and kept with primary antibodies overnight at 4°C. The following primary antibodies were used: synapsin I (BD Biosciences, San Jose, California), postsynaptic density protein 95 (PSD95) (Invitrogen, Carlsbad, California), and glutamate receptor 1 (GluR1) (Abcam, Cambridge, Massachusetts). The next day, blots were washed three times in PBST and incubated with horseradish peroxidase conjugated anti-mouse or anti-rabbit secondary antibody (1:5000 to 1:10000; Vector Laboratories, Burlingame, California) for 1 hour. After three final washes with PBST, bands were