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THE LONG-LASTING ANTIDEPRESSANT EFFECTS OF RAPASTINEL (GLYX-13) ARE ASSOCIATED WITH A METAPLASTICITY PROCESS IN THE MEDIAL PREFRONTAL CORTEX AND HIPPOCAMPUS

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Abstract—Rapastinel (GLYX-13) is an N-methyl-D-aspartate receptor (NMDAR) modulator that has characteristics of a glycine site partial agonist. Rapastinel is a robust cognitive enhancer and facilitates hippocampal long-term potentiation (LTP) of synaptic transmission in slices. In human clinical trials, rapastinel has been shown to produce marked antidepressant properties that last for at least one week following a single dose. The long-lasting antidepressant effect of a single dose of rapastinel (3 mg/kg IV) was assessed in rats using the Porsolt, open field and ultrasonic vocalization assays. Cognitive enhancement was examined using the Morris water maze, positive emotional learning, and contextual fear extinction tests. LTP was assessed in hippocampal slices. Dendritic spine morphology was measured in the dentate gyrus and the medial prefrontal cortex. Significant antidepressant-like or cognitive enhancing effects were observed that lasted for at least one week in each model. Rapastinel facilitated LTP 1 day–2 weeks but not 4 weeks post-dosing. Biweekly dosing with rapastinel sustained this effect for at least 8 weeks. A single dose of rapastinel increased the proportion of whole-cell NMDAR current contributed by NR2B-containing NMDARs in the hippocampus 1 week post-dosing, that returned to baseline by 4 weeks post-dosing. The NMDAR antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) blocked the antidepressant-like effect of rapastinel 1 week post dosing. A single injection of rapastinel also increased mature spine density in both brain regions 24 h post-dosing. These data demonstrate that rapastinel produces its long-lasting antidepressant effects via triggering NMDAR-dependent processes that lead to increased sensitivity to LTP that persist for up to two weeks. These data also suggest that these processes led to the alterations in dendritic spine morphologies associated with the maintenance of long-term changes in synaptic plasticity associated with learning and memory.

Key words: NMDA receptor, GLYX-13, depression, LTP, medial prefrontal cortex, hippocampus.

INTRODUCTION

NMDA receptors have recently become targets for drug development for the treatment of depression (Danysz and Parsons, 1998; Machado-Vieira et al., 2009; Skolnick et al., 2009; Krystal et al., 2013; Martinowich et al., 2013). *In vivo* imaging studies show decreased glutamate levels in the prefrontal cortex/anterior cortex of depressed patients (Hasler et al., 2007; Luykx et al., 2012) and postmortem data suggest that N-methyl-D-aspartate receptor (NMDAR) protein levels are altered in the prefrontal cortex of depressed patients (Feyissa et al., 2009). Ketamine, a potent NMDA channel blocker, has been found to reduce depression scores in subjects with major depressive disorder (MDD) following a single dose (Berman et al., 2000; Zarate et al., 2006; aan het Rot et al., 2010; Martinowich et al., 2013), with efficacy apparent within a few hours and lasting approximately 1–2 weeks following a single dose (Berman et al., 2000; Zarate et al., 2006; aan het Rot et al., 2010). Similarly, CP-101,606, an NR2B subunit-selective NMDAR antagonist, reduced depression scores in another study, but was also associated with ketamine-like psychotomimetic side effects, though these were reduced when the dose level of CP-101,606 was reduced (Preskorn et al., 2008). Another NR2B-selective antagonist, MK-0657, also reduced secondary efficacy measures of depression by

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Abbreviations: ACSF, artificial cerebrospinal fluid; (±)-CPP, (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; fEPSPs, field excitatory postsynaptic potentials; LTD, long-term depression; LTP, long-term potentiation; MDD, major depressive disorder; MPFC, medial prefrontal cortex; MWM, Morris water maze; NIH, novelty-induced hypophagia; NMDAR, N-methyl-D-aspartate receptor; PEL, positive emotional learning; USVs, ultrasonic vocalizations.

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day 5 of oral administration in five subjects, reportedly without causing psychotomimetic side effects (Ibrahim et al., 2012). Lanicemine, a low-trapping NMDA channel blocker produces sustained antidepressant effects but with psychotomimetic side effects (Sanacora et al., 2014).

Rapastinel, an NMDA receptor modulator with glycine-site partial agonist properties is currently in a phase II clinical development program as an adjunctive therapy for MDD (clinicaltrials.gov identifier NCT01684163). A single dose of rapastinel produces a robust antidepressant response in humans that lasts at least 1 week post-dosing (Moskal et al., 2014), and repeat weekly or bi-weekly dosing with rapastinel maintains this effect and produces further gains without evidence of tachyphylaxis.

Rapastinel was derived from cloning a hypervariable region of a monoclonal antibody that was shown to be a NMDAR-specific glycine site partial agonist (Moskal et al., 2005). In rat hippocampal slices, rapastinel has been shown to preferentially enhance conductance of NR2B-containing NMDARs at rat Schaffer collateral-CA1 synapses *in vitro* (Zhang et al., 2008), and enhance the magnitude of long-term potentiation (LTP) of synaptic transmission while simultaneously reducing that of long-term depression (LTD), which differentiates rapastinel from other NMDAR modulators such as D-cycloserine (Zhang et al., 2008; Burgdorf et al., 2011c). In animal studies, rapastinel has been shown to enhance performance in a variety of hippocampal-dependent learning tasks, including trace eyeblink conditioning and the Morris water maze (MWM), in both young adult and learning-impaired aged rats (Burgdorf et al., 2011c) and produces an antidepressant-like effect in Porsolt, learned helplessness, and novelty-induced hypophagia (NIH) tests in rats, without ketamine-like dissociative, addictive or sedative side effects (Burgdorf et al., 2013). Rapastinel facilitates positive emotional learning (PEL) and produces antidepressant-like effects when injected directly into the infralimbic or prelimbic medial prefrontal cortex (MPFC), but not into dorsal-lateral control sites (primary/secondary motor cortex; see (Burgdorf et al., 2011a, 2013).

The studies reported here were undertaken to determine if there is an association between metaplasticity and the long-lasting antidepressant effects in humans. The findings reported here showed that metaplasticity mechanisms associated with LTP-like processes do play a key role in these processes and may be relevant for the long-lasting antidepressant effects observed with other NMDA receptor modulators as well (Burgdorf et al., 2013).

EXPERIMENTAL PROCEDURES

Animals

Adult male (2–3-month-old) Sprague–Dawley (SD) rats were purchased from Harlan (USA) for behavioral and dendritic spine morphology studies, or Charles River (USA) for electrophysiological studies. Rats were housed in lucite cages with aspen wood chip bedding, maintained on a 12:12 light:dark cycle (lights on at 5 AM), and given *ad libitum* access to Purina lab chow (USA) and tap water throughout the study. All experiments were approved by the Northwestern University or New York Medical College Animal Care and Use Committees.

Drugs

Rapastinel was synthesized in free base form by Sai Life Sciences (Hyderabad, India), and was administered in 1 ml/kg 0.9% sterile saline vehicle. The dose of 3 mg/kg IV for rapastinel was chosen because it was the optimal antidepressant dose in Porsolt testing based on a previous dose–response (1–56 mg/kg IV) study (Burgdorf et al., 2013). The NMDA receptor antagonist (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) was purchased from Sigma (St. Louis, MO, USA), and was also administered in 1 ml/kg 0.9% sterile saline vehicle. The dose of CPP (10 mg/kg IP) was chosen based on a previous report that this dose could block the antidepressant-like effects of a putative NMDAR modulator in the Porsolt test without exhibiting behavioral effects on its own in (Zhang et al., 2013).

Porsolt test

The Porsolt forced swim test adapted for use in rats was performed as previously described (Page et al., 1999; Burgdorf et al., 2009). Animals were placed in a 46 cm tall × 20 cm in diameter clear glass tube filled to 30 cm with tap water (23 ± 1 °C) for 15 min on the first day (habituation) and 5 min on the subsequent test day. Water was changed after every other animal. Animals were videotaped, and floating time as defined as the minimal amount of effort required to keep the animals head above water was scored offline by a blinded experimenter with high inter-rater reliability (Pearson's $r > .9$).

Animals were tested 1 week post-dosing with rapastinel (3 mg/kg IV) or 0.9% sterile saline (1 ml/kg) vehicle (Fig. 1A), or received a dose of CPP (10 mg/kg IP) 1 h before the 1 week test point (Fig. 4B). Alternatively, animals received pre-treatment with CPP (10 mg/kg IP) 1 h before rapastinel administration and were tested 1 h after rapastinel administration (Results Section). The broad spectrum NMDAR glutamate site antagonist CPP was chosen for these studies because it does not produce an antidepressant response in the Porsolt test (Zhang et al., 2013) unlike the NMDAR channel blockers like ketamine, MK-801 or the NR2B-specific antagonist Ro25-6981 (Maeng et al., 2008; Burgdorf et al., 2013).

Open field test

Open field testing was performed as previously described (Burgdorf et al., 2009). Time spent in the open compartment has been shown to be increased by some classes of anxiolytic/antidepressant compounds (Prut and Belzung, 2003). Testing consisted of placing an animal in a 40 cm × 40 cm × 20 cm high opaque plexiglas open field cage divided into nine equally sized 13.3 cm × 13.3 cm sections under red lighting for 10 min. Between animals, boli and urine were removed from the