Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Effect of acute NR2B antagonist treatment on long-term potentiation in the rat hippocampus

John D Graef[a,*], Kimberly Newberry[a], Amy Newton[a], Rick Pieschl[a], Eric Shields[b], Fu-ni Luan[b], Jean Simmermacher[b], David Luchetti[c], Eric Schaeffer[c], Yu-Wen Li[a], Laszlo Kiss[a], Linda J Bristow[a]

[a]Neuroscience Biology, Bristol-Myers Squibb Company, Wallingford, CT, USA
[b]Pharmaceutical Candidate Optimization, Bristol-Myers Squibb Company, Wallingford, CT, USA
[c]Neuroscience Clinical Biomarkers, Bristol-Myers Squibb Company, Wallingford, CT, USA

ARTICLE INFO

Article history:
Accepted 10 March 2015
Available online 19 March 2015

Keywords:
Treatment-resistant depression
Plasticity
Hippocampus
NMDA receptors
NR2B subunit
Long-term potentiation
Ex vivo

ABSTRACT

The long lasting antidepressant response seen following acute, i.v. ketamine administration in patients with treatment-resistant depression (TRD) is thought to result from enhanced synaptic plasticity in cortical and hippocampal circuits. Using extracellular field recordings in rat hippocampal slices, we show that a single dose of the non-selective NMDA receptor antagonist ketamine or CP-101,606, a selective antagonist of the NR2B subunit of the NMDA receptor, enhances hippocampal synaptic plasticity induced with high frequency stimulation (HFS) 24 h after dosing – a time at which plasma concentrations of the drug are no longer detectable in the animal. These results indicate that acute inhibition of NMDA receptors containing the NR2B subunit can lead to long-lasting changes in hippocampal plasticity.

1. Introduction

Current evidence from both humans and animals supports the idea that depression may be related to an impairment of neuronal plasticity. For example, studies in humans have demonstrated that depression is associated with decreased volumes of the hippocampus, a highly plastic brain structure important for learning and memory (Sheline et al., 1996). Moreover, functional studies using EEG have shown that depressed patients exhibit impaired cortical plasticity, and that chronic administration of the antidepressant sertraline increases plasticity in control subjects (Normann et al., 2007).

The role of NMDA receptors in synaptic plasticity has been extensively studied (Bliss and Collingridge, 2013) and is supportive of recent clinical studies demonstrating the therapeutic potential of NMDA antagonists in treatment-resistant depression (TRD) (Berman et al., 2000; Preskorn et al., 2008; Zarate et al., 2006). These investigations have shown that a

Abbreviations: aCSF, Artificial cerebrospinal fluid; BDNF, Brain derived neurotrophic factor; EEG, Electroencephalography; fEPSP, Field excitatory postsynaptic potential; i.p., Intraperitoneal; i.v., Intravenous; HFS, High frequency stimulation; NMDAR, N-Methyl-D-Aspartate Receptor; NR2B, NMDA receptor 2B subtype; s.c., Subcutaneous; TRD, Treatment resistant depression

*Correspondence to: Genetically-defined Diseases, Bristol-Myers Squibb, 5 Research Parkway, Wallingford, CT 06492.
E-mail address: john.graef@bms.com (J. Graef).

http://dx.doi.org/10.1016/j.brainres.2015.03.019
0006-8993/

single i.v. infusion of the non-selective NMDA receptor blocker ketamine produces rapid and long-lasting antidepressant effects in patients that begin to manifest after the drug can no longer be detected in the plasma. However, ketamine causes psychotomimetic and dissociative side effects, limiting its therapeutic potential. The selective inhibition of NMDA receptors containing the NR2B subunit has been proposed as an alternate approach to non-selective NMDA receptor modulation in order to produce antidepressant efficacy without ketamine-like side effects (Ibrahim et al., 2012). Therefore, one potential mechanism by which NMDA receptor blockers may produce antidepressant-like effects is through enhanced plasticity.

Studies investigating the mechanism of action underlying ketamine's rapid and sustained antidepressant effects have demonstrated that pathways involved in increasing the levels of brain-derived neurotrophic factor (BDNF) are required for efficacy in animal models (Autry et al., 2011; Zhou et al., 2013), and increased expression of BDNF has been shown to induce and maintain plasticity both in vitro (Ying et al., 2002) and in vivo (Messaoudi et al., 2002).

Long-term potentiation (LTP) is widely-believed to be a cellular mechanism underlying learning and memory and can be used as a measure of plasticity within discrete neuronal circuits. Therefore we interrogated the hypothesis that the therapeutic effect of NMDA receptor antagonists in TRD patients is dependent upon enhanced plasticity by measuring the effects of acute treatment of either ketamine or the selective NR2B antagonist, CP-101,606 (Kawai et al., 2007) on ex vivo rat hippocampal LTP.

2. Results

2.1. *Ketamine, but not CP-101,606 acutely impairs LTP in the CA1 region*

We first examined the acute effects of the non-selective NMDAR channel blocker ketamine and the NR2B-selective antagonist CP-101,606 on both baseline fEPSPs responses and the induction of LTP in the CA1 region of the hippocampus. After a stable 20 min baseline period, either ketamine or CP-101,606 was bath-applied to hippocampal slices for a 20 min period prior to LTP induction and during high-frequency stimulation (HFS). As can be seen in Fig. 1A, application of 10 μM ketamine had no effect on baseline responses (control 2.0±1.5% change from baseline, $n=8$ slices; ketamine −0.1±1.4% change from baseline, $n=8$ slices), but significantly impaired LTP following HFS conditioning as compared to control slices (control 165.7±11.6% of baseline, $n=8$ slices; ketamine 117.0±5.0% of baseline, $n=8$ slices; $p<0.01$, unpaired t test). Fig. 1B shows that application of 10 μM CP-101,606 had no effect on either the slope of the evoked fEPSPs (control 0.01±2.97% change from baseline, $n=6$ slices; CP-101,606 3.9±2.4% change from baseline, $n=7$ slices) or induction of LTP (control 175.3±25.3% of baseline, $n=6$ slices; CP-101,606 173.6±10.7% of baseline, $n=7$ slices).

2.2. *i.p. Ketamine and s.c. CP-101,606 enhance LTP 24 h post-treatment, but not at 72 h*

We next tested the effects of either i.p. administration of the non-selective NMDAR channel blocker ketamine or s.c. administration of the NR2B-selective antagonist CP-101,606 on the magnitude of LTP 24 and 72 h after injection. As demonstrated in Fig. 2A, 24 h following a 30 mg/kg i.p. injection of ketamine produced a significant enhancement of LTP as compared to vehicle-treated rats (vehicle-treated: 198.0±20.5% of baseline, $n=5$ rats; ketamine-treated: 277.8±35.9% of baseline, $n=5$ rats; $p<0.05$, paired t test). However, this effect on LTP was no longer apparent 3 days post-injection (Fig. 2B; vehicle-treated: 227.3±17.4% of baseline, $n=5$ rats; ketamine-treated: 208.3±16.2% of baseline, $n=5$ rats). Similar results were seen following treatment with CP-101,606. LTP was enhanced 24 h after a single s.c. injection of 30 mg/kg CP-101,606 as compared to vehicle controls (Fig. 2C; vehicle-treated: 167.0±9.6% of baseline, $n=5$ rats; CP-101,606-treated: 209.9±11.0% of baseline, $n=5$ rats; $p<0.05$, paired t test), but not at 3 days post-injection (Fig. 2D; vehicle-treated: 216.4±25.0% of baseline, $n=5$ rats; CP-101,606-treated: 214.6±6.2% of baseline, $n=5$ rats). These results demonstrate that a single systemic exposure of either 30 mg/kg ketamine (i.p.) or 30 mg/kg CP-101,606 (s.c.) can significantly enhance hippocampal LTP ex vivo 24 h after treatment, but that this effect is lost at 72 h post-dose.

2.3. *i.v. Ketamine enhances LTP 24 and 72 h post-treatment, whereas i.v. CP-101,606 only enhances LTP 24 post-exposure*

In order to mimic the route of delivery that is used in the clinic to produce ketamine's anti-depressant effects, we next tested the effects of a 3 mg/kg i.v. dose of either ketamine or CP-101,606 on LTP. As can be seen in Fig. 3, both ketamine and CP-101,606 significantly enhanced ex vivo LTP 24 h after a 3 mg/kg i.v. treatment (Fig. 3A; vehicle-treated: 182.9±7.7% of baseline, $n=4$ rats; ketamine-treated: 231.6±7.7% of baseline, $n=4$ rats, $p<0.05$ paired t test; Fig. 3C; vehicle-treated: 177.8±15.6% of baseline, $n=6$ rats; CP-101,606-treated: 260.2±40.0% of baseline, $n=6$ rats, $p<0.05$ paired t test). In addition, ketamine produced a sustained enhancement of LTP as demonstrated by a significantly greater magnitude of LTP 72 post-treatment (Fig. 3B; vehicle-treated: 218.3±16.3% of baseline, $n=5$ rats; ketamine-treated: 314.3±20.1% of baseline, $n=6$ rats, $p<0.05$ paired t test). This is in contrast to CP-101,606, where the magnitude of LTP elicited in hippocampal slices from rats treated with 3 mg/kg i.v. 72 h prior was not statistically different from vehicle-treated controls (Fig. 3D; vehicle-treated: 230.0±46.2% of baseline, $n=6$ rats; CP-101,606-treated: 237.0±20.7% of baseline, $n=6$ rats). These data indicate that i.v. administration of both ketamine and CP-101,606 significantly enhanced ex vivo hippocampal LTP 24 h post-dose, and that ketamine, but not CP-101,606 exhibits a sustained effect that can be detected 72 h post-treatment. A summary of LTP effects for all conditions tested is shown in Fig. 5.

2.4. *NR2B occupancy associated with enhanced LTP*

After establishing that a 3 mg/kg i.v. dose of CP-101,606 could enhance ex vivo hippocampal LTP 24 h post-exposure, we