



Inhibition of sigma-1 receptor reduces N-methyl-D-aspartate induced neuronal injury in methamphetamine-exposed and -naïve hippocampi

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ABSTRACT

Acute and prolonged methamphetamine (METH) exposure has been reported to moderate the function of N-methyl-D-aspartate type glutamate receptors (NMDAr) in the hippocampus. These effects have been found to be associated with enhanced NMDAr-dependent release of Ca^{2+} from IP_3 -sensitive intracellular stores. The present studies were designed to extend these findings and examine the role of the endoplasmic membrane (ER) bound orphan receptor, the sigma-1 receptor, in NMDA-induced neuronal injury and METH withdrawal-potentiated NMDA-induced neuronal injury. Organotypic hippocampal slice cultures were exposed to METH (0 or 100 μM) for 6 days and withdrawn for 7 days, then exposed to NMDA (0 or 5 μM) for 24 h. Additional cultures were also exposed to this regimen and were co-incubated with BD1047 (100 μM), a specific inhibitor of ER-bound sigma-1 receptors, for the 24 h NMDA exposure. Cytotoxicity was assessed by analysis of propidium iodide uptake. These studies demonstrated that protracted METH exposure and withdrawal significantly potentiated the neuronal injury produced by NMDA exposure. Further, co-exposure to BD1047 with NMDA markedly attenuated neuronal injury in METH-naïve and METH-withdrawn organotypic cultures. As a whole, these data demonstrate that prolonged METH exposure, even at non-toxic concentrations, significantly alters glutamate receptor signaling. Inhibition of sigma-1 receptor-dependent Ca^{2+} release from the ER entirely prevented NMDA-induced toxicity in METH-naïve cultures and markedly reduced METH-potentiated toxicity. These findings demonstrate the importance of Ca^{2+} -induced intracellular Ca^{2+} release in excitotoxic insult and suggest that blockade of glutamatergic overactivity may represent a therapeutic target in the treatment of METH withdrawal.

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Methamphetamine (METH) abuse has increased substantially in the United States in the last decade. Chronic METH use has been associated with decreases in dopaminergic transporters (DAT), D2 receptors, and hippocampal volume as well as cognitive deficits, including attention/psychomotor speed, learning and memory, and fluency [1,12,38]. While DAT measures displayed improvement after protracted abstinence (~9 months) from METH, this does not appear to correlate with improvements on behavioral deficits (time gaiting and delayed recall tests) [40].

Mechanisms identified in METH neurotoxicity primarily involve the oxidation and depletion of monoamines [3,27]. However, several studies have suggested that METH may interact with glutamatergic systems, particularly the N-methyl-D-aspartate (NMDA) receptor system [13,23,31,32,41,42]. Previously our lab has reported that METH may act as a functional NMDA receptor (NMDAr) antagonist, and possibly as a competitive NMDAr

antagonist [31]. These findings suggest that chronic antagonism of the NMDAr by METH may result in multiple adaptive responses that could increase the likelihood of neuronal excitability during withdrawal. Specifically, Moriguchi et al. [23] reported increased NMDAr mediated EPSP amplitudes even upon hyperpolarization of the cell in neostriatal slices following long-term withdrawal (7 days) from METH. Furthermore, previous studies have suggested that long-term withdrawal from chronic METH results in a potentiated neurotoxic response to NMDA in CA1 pyramidal cells, which requires the co-activation of NMDAr and inositol triphosphate receptors (IP_3), since antagonism of either receptor alleviates the potentiated response [32]. Currently, it is unknown how METH may interact with the IP_3 receptor to produce the observed potentiated neurotoxic response. However, previous studies have suggested that the IP_3 receptor is linked to the sigma-1 receptor and that METH may exert its effects on IP_3 receptor mediated Ca^{2+} release via interactions with the sigma-1 receptor [5,36,40].

Two subtypes of sigma receptors have been identified [4,14]. An endogenous ligand has yet to be determined for these receptors, however, a variety of endogenous and pharmacological compounds have displayed binding affinity for the sigma-1 receptor, including psychomotor stimulants [7,8,10,26,34,35]. In fact,

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previous studies have reported that cocaine and METH preferentially bind the sigma-1 receptor (METH: $\sigma 1=2.16 \mu\text{M} \pm 0.25$; $\sigma 2=46.67 \mu\text{M} \pm 10.34$) rather than the sigma-2 receptor [11,25,30]. Furthermore, sigma-1 receptor antagonists have been shown to attenuate behavioral sensitization and locomotor effects of cocaine and METH as well as modulating cocaine state dependent learning [16,18,19,25,28,29,37]. These results suggest that sigma-1 receptors uniquely contribute to psychomotor stimulant behavioral profiles.

Chronic exposure to METH may also affect expression and function of sigma-1 receptors. Following chronic self-administration of METH (5 days per week, for 5 weeks; 0.1 mg/kg), a significant upregulation of sigma-1 receptor mRNA in the frontal cortex and hippocampus was observed [33]. Furthermore, both METH self-administering animals and yoked controls have displayed a significant upregulation of the sigma-1 receptor in the ventral tegmental area and substantia nigra after chronic exposure (5 days per week, for 5 weeks; 0.1 mg/kg) [9]. Changes in sigma-1 receptor expression following METH exposure may play a role in IP₃ receptor expression and IP₃ receptor mediated Ca²⁺ release, since sigma-1 receptors are linked to IP₃ receptors via the cytoskeleton protein, ankyrin [6]. Following activation by agonists, including cocaine, the sigma-1 and ankyrin complex dissociates from the IP₃ receptor, resulting in increased IP₃ receptor mediated Ca²⁺ release [5,6]. Given the similar sigma-1 receptor binding and behavioral profiles of METH and cocaine as well as the potential interactions with IP₃ receptor mediated Ca²⁺ release, the current studies examined the effects of sigma-1 receptor antagonism following chronic METH exposure on NMDA-induced neurotoxicity in the hippocampus.

Eight-day-old, male and female, Sprague–Dawley rat pups (Harlan Laboratories, Indianapolis, ID, USA) were sacrificed, and their brains were aseptically removed. After extraction, brains were transferred to ice-cold dissecting medium (Minimum Essential Medium (MEM: Gibco BRL, Gaithersburg, MD), 25 mM HEPES, 20 mM glutamine, and 50 μM streptomycin/penicillin). Bilateral hippocampi were then removed and cleaned of extra tissue. Upon completion of cleaning, hippocampi were transferred to ice-cold culture medium (dissecting media with the addition of 36 mM glucose, 25% Hanks' balanced salt solution (HBSS: Gibco BRL, Gaithersburg, MD), and 25% heat inactivated horse serum (HIHS: Sigma, St. Louis, MO)). Afterwards unilateral hippocampi were sectioned coronally at 200 μm using a McIlwain Tissue Chopper (Campden Instruments Ltd, Lafayette, ID, USA). Once sectioned three intact hippocampi slices were plated onto Millicell-CM biopore membrane inserts, with 1 ml of pre-incubated culture medium added to the bottom of each well. Plates were then placed in an incubator, at 37 °C (gas composition 5% CO₂, 21% oxygen, and 74% nitrogen) for five days to allow hippocampi to affix to the silicon membrane. Care of all animals was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23).

METH-naïve cultures were aged to 20 days in vitro (DIV), and medium was changed every 5 days to mimic treatment of METH-exposed and -withdrawn tissue described below. At 19 DIV, METH-naïve slice cultures were exposed to cell culture medium with the addition of propidium iodide (PI; 3.74 μM : Molecular Probes, Eugene, OR, USA), PI-medium with the addition of NMDA (5 μM), PI-medium with the addition of the sigma receptor antagonist BD1047 (100 μM) or PI-medium with the addition of NMDA and BD1047 for 24 h. Following PI exposure, all cultures were imaged and cytotoxicity was assessed as described below. All drugs (NMDA: Sigma, St. Louis, MO, USA; BD1047: TOCRIS, Bristol, UK) were dissolved in cell culture medium and applied to the bottom of culture plate well in 1 ml increments.

At 6 DIV, slices were exposed to METH (100 μM ; Sigma) dissolved in cell culture medium. Slices were exposed to METH for a

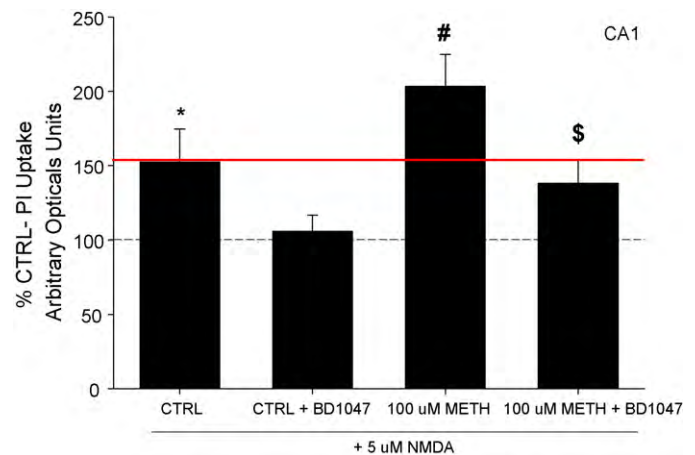


Fig. 1. PI uptake following long-term withdrawal from METH, and NMDA exposure. Data is presented as percent control, 100% line represents CTRL tissue (naïve to METH, NMDA, and BD1047). Significant PI uptake following NMDA exposure was observed in all tissue. METH w/d tissue displayed a potentiated response to NMDA exposure, beyond CTRL tissue exposed to NMDA (above the red line). Co-exposure to BD1047 reduced NMDA toxicity, regardless of treatment. * $p < 0.01$ vs. CTRL; # $p < 0.01$ vs. CTRL + NMDA; \$ $p < 0.01$ vs. 100 μM METH + NMDA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

total of 6 days with METH cell culture medium replenished at day 3 of exposure. Following 6 days of METH exposure (13 DIV), slices were withdrawn from METH for 7 days. At that time, slice cultures were treated with PI, PI and NMDA, PI and BD1047, or PI, NMDA, and BD1047 as stated above.

PI is a polar stain that only penetrates cell membranes of damaged or potentially dying cells. Once in the cell PI binds to nucleic acids to produce a bright red fluorescence at 630 nm [43]. Hippocampi were visualized with SPOT Advanced version 4.0.2 software for Windows (W. Nuhsbaum Inc., McHenry, IL, USA) at a 5 \times objective with a Leica DMIRB microscope (W. Nuhsbaum Inc.: McHenry, IL, USA) that is fitted for fluorescent detection (mercury-arc lamp). The emission wavelength of PI is 620 nm in the visual range, and has a peak excitation wavelength of 536 nm. PI was excited using a band-pass filter exciting wavelengths between 510 and 560 nm. Intensity of PI fluorescence was analyzed by densitometry using NIH Image J software in the pyramidal cell layers of the CA1 and CA3 as well as the granule cell layer of the DG of the hippocampus proper. To minimize variability in PI uptake between replications, each replication was converted to percent control before analysis.

Two-way ANOVAs were initially employed analyzing the effect of treatment [CTRL, CTRL + NMDA, CTRL + BD1047, CTRL + NMDA + BD1047, METH w/d, METH w/d + NMDA, METH w/d + BD1047, METH w/d + NMDA + BD1047] and gender in each subregion of the hippocampus proper. However, there were no significant differences observed in regards to gender, so all further statistics collapse gender across treatment groups. One-way ANOVAs were employed examining the effects of treatment in each subregion of the hippocampus proper, since previous studies have demonstrated that the pyramidal cell layer of the CA1 selectively displays NMDA-induced neurotoxicity, particularly following METH withdrawal [32]. Statistical outliers were discovered utilizing Grubb's test for outliers. Post hoc comparisons were made using the Fisher's LSD method. All significance levels were set at $p < 0.05$.

At 19 DIV METH-naïve tissue was exposed to NMDA (5 μM) and PI for 24 h. NMDA exposure produced a significant increase in PI uptake in the pyramidal cell layer of the CA1 in METH-naïve tissue. NMDA treatment increased PI uptake 125% compared to NMDA-

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