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Characterization of binge-dosed methamphetamine-induced neurotoxicity and neuroinflammation

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ABSTRACT

Methamphetamine (MA) is a potent, highly addictive psychostimulant abused by millions of people worldwide. MA induces neurotoxicity, damaging striatal dopaminergic terminals, and neuroinflammation, with striatal glial activation leading to pro-inflammatory cytokine and reactive oxygen species production. It is unclear whether MA-induced neuroinflammation contributes to MA-induced neurotoxicity. In the current study, we examined the linkage between the time course and dose response of MA-induced neurotoxicity and neuroinflammation. Adult male mice underwent a binge dosing regimen of four injections given every 2 h with doses of 2, 4, 6, or 8 mg/kg MA per injection, and were sacrificed after 1, 3, 7, or 14 days. Binge MA treatment dose-dependently caused hyperthermia and induced hypoactivity after one day, though activity returned to control levels within one week. Striatal dopamine (DA) was diminished one day after treatment with at least 4 mg/kg MA, while DA turnover rates peaked after seven days. Although striatal tyrosine hydroxylase and DA transporter levels were also decreased one day after treatment with at least 4 mg/kg MA, they trended toward recovery by day 14. All doses of MA activated striatal glia within one day. While astrocyte activation persisted, microglial activation was attenuated over the two weeks of the study. These findings help clarify the relationship between MA-induced neuroinflammation and neurotoxicity, particularly regarding their temporal and dose-specific dynamics.

1. Introduction

Methamphetamine (MA) is a potent and highly addictive psychostimulant, abused by millions of people worldwide (UNODC World Drug Report, 2010). People abuse MA to induce euphoria, and increase energy, attention, and libido. However, long-term MA abuse is associated with a host of systemic and neurological maladies. Systemic afflictions include cardiovascular pathology and liver, kidney, and respiratory failure (Schep et al., 2010). Neurologically, MA abusers exhibit cognitive and psychomotor impairment, and have increased risk for psychosis, strokes, and seizures, with greater drug exposure correlating with greater risk and severity of complications (Simon et al., 2000; Volkow et al., 2001; Darke et al., 2008).

These impairments are accompanied by neurological damage, particularly in the striatum. Chronic MA abusers have markedly depleted levels of dopamine (DA), tyrosine hydroxylase (TH), and dopamine transporter (DAT) in the striatum (Wilson et al., 1996; Volkow et al., 2001), indicating damage to dopaminergic axon terminals. Similarly, rodents and non-human primates also exhibit striatal dopaminergic neurotoxicity following MA treatment, characterized by depleted DA, decreased TH and DAT (Wagner et al., 1980; Deng et al., 1999; Harvey et al., 2000), and striatal nerve terminal damage revealed by silver stains (Bowyer et al., 1994; O'Callaghan and Miller, 1994). Interestingly, while the dopaminergic terminals in the striatum are damaged, the cell bodies from which they project in the substantia nigra pars compacta are not destroyed (Harvey et al., 2000): a fact which may account for the capacity for recovery, as well as the rarity of parkinsonian symptoms in MA abusers.

MA induces neurotoxicity via multiple mechanisms, including excitotoxicity, oxidative stress, apoptosis induction, and hyperthermia (Krasnova and Cadet, 2009). Recently, neuroinflammation has been implicated as an additional mechanism. Neuroinflammation has been linked with several neurological disorders, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, and stroke (Czlonkowska and Kurkowska-Jastrzebska, 2011). It is well

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established that MA exposure activates microglia and astrocytes in culture as well as in animal and human studies (Sheng et al., 1994; Sekine et al., 2008; Krasnova and Cadet, 2009; Yue et al., 2012; Clark et al., 2013). Such gliosis may contribute to MA-induced neurotoxicity, as activated microglia and astrocytes produce reactive oxygen species that are harmful to neurons, and proinflammatory cytokines to propagate the neuroinflammatory cascade and recruit additional immune cells from the periphery, potentially amplifying the oxidative damage to neurons (Clark et al., 2013). However, microglia and astrocytes also serve neuroprotective roles, producing neurotrophic factors, and promoting healing (Czeh et al., 2011; Singh et al., 2011). Furthermore, attempts to prevent MA-induced neurotoxicity by blocking the activation of microglia or astrocytes have yielded contradictory results (Thomas and Kuhn, 2005; Kawasaki et al., 2006; Sriram et al., 2006). Thus, it becomes difficult to define the relationship between the glial activation central to neuroinflammation and the neurotoxicity resulting from MA exposure.

Previous studies have focused on individual doses or time points following MA treatment; however, no comprehensive characterization of the dose–response and time-course of MA-induced neurotoxicity has yet been published. In this study, we used a binge-dosing paradigm rather than a single injection for MA administration, to more accurately replicate patterns of MA use by human abusers. By administering a wide range of doses (2–8 mg/kg MA x4 injections) and examining several time points (1, 3, 7, and 14 days post-MA), we have characterized the dose–response and time-course of MA-induced striatal dopaminergic terminal damage and glial activation, as well as changes in locomotor behavior, and acute effects on core temperature, to pursue links between MA-induced neuroinflammation and neurotoxicity.

2. Materials and methods

2.1. Animals

Adult male C57BL/6 mice ordered from Taconic (Germantown, NY), aged 12–14 weeks, were used in this study. Only males were used in order to avoid the confounding effects of different sex hormone profiles between genders and the different phases of the estrous cycle in females, since these differences in sex hormone levels alter susceptibility to methamphetamine-induced neurotoxicity (Yu and Liao, 2000). Mice were maintained on a 12 h light:12 h dark cycle, with ad libitum access to food and water. Data regarding group sample sizes and body mass at sacrifice are shown in Supplementary Table 1. All procedures were approved by the University Committee on Animal Resources of the University of Rochester Medical Center.

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neuro.2015.08.006.

2.2. MA administration and animal handling

MA was prepared by the University of Rochester Pharmacy Department. To mimic patterns of MA abuse in humans, we chose to use a binge dosing paradigm, in which mice received a total of four intraperitoneal (i.p.) injections of saline ($n = 56$) or MA at doses of 2 mg/kg ($n = 60$), 4 mg/kg ($n = 60$), 6 mg/kg ($n = 72$), or 8 mg/kg ($n = 72$) per injection, administered every 2 h. Core body temperatures were recorded prior to the first injection and 1 h after each injection using a TH-5 Monitoring Thermometer (Physitemp Instruments, Clifton, NJ). Mice from each treatment group were sacrificed 1 day, 3 days, 7 days, and 14 days following MA treatment via either cervical dislocation for neurotransmitter analysis or transcardial perfusion for immunohistochemical analysis. Following cervical dislocation, the striatum and cortex were dissected, quickly frozen on dry ice, and stored at −80 °C. To control for potential inter-hemispheric differences, an equal number of right and left hemispheres from mice in each dose group on each day was used. Transcardial perfusions were performed on mice anesthetized with sodium pentobarbital (Lundbeck Inc., Deerfield, IL), perfusing first with 0.1 M phosphate buffer with 0.25% sodium nitrite and 0.1% sodium heparin (Sigma Aldrich, St. Louis, MS), then with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were postfixed overnight, then stored in 30% sucrose at 4 °C. One mouse from the saline group, one mouse from the 2 mg/kg dose group, two mice from the 4 mg/kg dose group, three mice from the 6 mg/kg dose group, and nine mice from the 8 mg/kg dose group died before their sacrifice dates and therefore were not used for neurotransmitter or immunohistochemical analyses.

2.3. Locomotor activity

Mice in the 14 day group underwent locomotor activity testing using the Opto-Varimex-Minor (Columbus Instruments, Columbus, OH), which detects horizontal, ambulatory, and vertical movement when a mouse crosses photobeams within the chamber. Horizontal activity was defined as any nonambulatory movement in the *X–Y* plane, which can include stereotyped movement; ambulatory activity required three successive photobeam breaks in the *X–Y* plane; and vertical activity was defined as photobeam breaks of the *Z* plane, such as rearing-related behaviors. Mice were habituated to the testing chamber with three 5-min sessions on each of three consecutive days prior to the start of MA injections. Activity levels from the third day of habituation were used to determine baseline activity for each mouse. The animals' activity levels were assessed one 1, 3 days, 7 days, and 13 days following MA treatment with 45-min sessions on each testing day.

2.4. High-performance liquid chromatography

Striatal and cortical levels of dopamine and its metabolites metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (HIAA), and norepinephrine (NE) were assessed using high-performance liquid chromatography (HPLC; Waters 2695) with electrochemical detection. Tissue samples were placed in perchloric acid (0.1 N), sonicated, and centrifuged at 10,000 × *g* for 20 min before storing supernatants at −80 °C. Separation was achieved using the MD-150 Analytical Column (ThermoScientific, 3.2 mm × 15 cm, 3 μm; part no.70-0636) with commercially available MDTM mobile phase (ThermoScientific; part no. 70-1332) containing 10% acetonitrile, 89% water, and 1% sodium phosphate monobasic monohydrate. Detection occurred with a Water 2465 Electrochemical detector and in situ silver/silver chloride (ISSAC) electrode maintained at a potential of 0.8 V. Tissue pellets were dissolved in 1 mL of 0.5 N NaOH and total protein content was determined using the Bio-Rad BA protein assay (Hercules, CA). In order to conserve animals and avoid a confounding effect of behavior on neurochemical endpoints, animals used for behavioral testing were omitted from HPLC analysis.

2.5. Immunohistochemistry and image analysis

Brains from perfused mice were sectioned at 30 μm on a microtome. The free-floating sections were stored in cryoprotectant (30% sucrose (Sigma–Aldrich), 30% ethylene glycol (Sigma–Aldrich) in 0.1 M phosphate buffer (PB)) at −20 °C until use.