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Overexpression of parkin in the rat nigrostriatal dopamine system protects against methamphetamine neurotoxicity

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ABSTRACT

Methamphetamine (METH) is a central nervous system psychostimulant with a high potential for abuse. At high doses, METH causes a selective degeneration of dopaminergic terminals in the striatum, sparing other striatal terminals and cell bodies. We previously detected a deficit in parkin after binge METH in rat striatal synaptosomes. Parkin is an ubiquitin-protein E3 ligase capable of protecting dopamine neurons from diverse cellular insults. Whether the deficit in parkin mediates the toxicity of METH and whether parkin can protect from toxicity of the drug is unknown. The present study investigated whether overexpression of parkin attenuates degeneration of striatal dopaminergic terminals exposed to binge METH. Parkin overexpression in rat nigrostriatal dopamine system was achieved by microinjection of adeno-associated viral transfer vector 2/6 encoding rat parkin (AAV2/6-parkin) into the substantia nigra *pars compacta*. The microinjections of AAV2/6-parkin dose-dependently increased parkin levels in both the substantia nigra *pars compacta* and striatum. The levels of dopamine synthesizing enzyme, tyrosine hydroxylase, remained at the control levels; therefore, tyrosine hydroxylase immunoreactivity was used as an index of dopaminergic terminal integrity. In METH-exposed rats, the increase in parkin levels attenuated METH-induced decreases in striatal tyrosine hydroxylase immunoreactivity in a dose-dependent manner, indicating that parkin can protect striatal dopaminergic terminals against METH neurotoxicity.

Introduction

Methamphetamine (METH) is a psychostimulant drug, which is widely abused in the United States and worldwide. When taken at high doses, METH has toxic effects on the dopaminergic (DAergic) system in the brain in experimental animals and humans. In rats, administration of binge high-dose METH causes persistent deficits in DAergic markers in striatal DAergic terminals but has little effect on other DAergic nerve endings, such as those terminating in the nucleus accumbens (Broening et al., 1997; Cass, 1997; Haughey et al., 1999; Morgan and Gibb, 1980), and on DA cell bodies in the substantia nigra *pars compacta* (SNc) from which striatal DAergic terminals originate (Harvey et al., 2009; Hotchkiss and Gibb, 1980; Ricaurte et al., 1982). Chronic METH users do not display classic Parkinsonian motor impairments; however, they are at higher risk for developing Parkinson's disease (PD) than non-users (Callaghan et al., 2010, 2011). Abuse of high doses of METH can cause impairment of cognitive skills, agitation, violent behavior, anxiety, confusion, and paranoia. Despite years of active research in the area of METH abuse and its related neurotoxicity, to date, there are no specific medications that counteract the damaging effects of METH on the brain. Thus, there is a compelling need to discover new molecular drug targets in order to develop novel pharmaceuticals that can protect the CNS from the toxic effects of acute METH overdose.

Parkin is an ubiquitin-protein E3 ligase; its primary function is to add polyubiquitin chains to proteins destined for degradation by the 26S proteasome (Moore, 2006; Shimura et al., 2000; Zhang et al., 2000). A decrease in parkin function leads to toxic accumulation of unwanted proteins and has been implicated in the etiology of various neurodegenerative disorders, including PD (Buneeva and Medvedev, 2006; Lim, 2007; Olzmann et al., 2008). Conversely, overexpression of parkin protects DA neurons against a variety of cellular insults *in vitro* and *in vivo*, most importantly against those involved in mediating METH neurotoxicity, such as DA-induced oxidative stress, inhibition of mitochondrial function, and impairment of the proteasome (Darios et al., 2003; Jiang et al., 2004, 2012; Kirik and Bjorklund, 2005; Lo Bianco et al., 2004; Oluwatosin-Chigbu et al., 2003; Petrucelli

Abbreviations: AAV, adeno-associated virus; AMC, amido-4-methylcoumarin; AP, anterior–posterior; CNS, central nervous system; DA, dopamine; DAB, diaminobenzidine; METH, methamphetamine; ML, medio-lateral; SAL, saline; SNc, substantia nigra *pars compacta*; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Suc-LLVY-AMC, Suc-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin; TH, tyrosine hydroxylase; TU, transducing unit; V, ventral; V_{max}, maximal velocity.

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et al., 2002; Yang et al., 2005, 2011). These findings suggest the importance of parkin in the functioning and maintenance of DA neurons.

Our previous *in vivo* study demonstrated that binge METH was followed by a rapid decrease in parkin levels in rat striatal synaptosomes, which persisted for a minimum of 24 h after METH administration (Moszczynska and Yamamoto, 2011). Whether the deficit in parkin mediates the toxicity of METH is not known and no studies, to date, have examined whether parkin could protect DAergic terminals against neurotoxicity of the drug. Thus, the major aim of the present study was to determine whether overexpression of parkin in the nigrostriatal DA system protects DAergic terminals in the striatum against binge high-dose METH. In order to gain further insight into the role of parkin in METH neurotoxicity and potential mechanisms of parkin neuroprotection, we also investigated the effects of parkin overexpression on the levels of tyrosine hydroxylase (TH) (a rate-limiting enzyme in DA synthesis), the activity of 20S proteasome, and METH-induced hyperthermia. We are the first to report that the overexpression of parkin in the nigrostriatal DA system attenuates METH toxicity to striatal DAergic terminals in a dose-dependent manner. These results, together with our previous findings, suggest that parkin deficit mediates, in part, toxicity of METH to striatal DAergic terminals *in vivo*.

Materials and methods

Animals

Adult male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing 175–220 g at the time of the arrival were housed two per cage under a 12 h light/dark cycle in a temperature-controlled room (21–23 °C). Food and water were available *ad libitum*. Temperature of the rats was measured *via* a rectal probe digital thermometer (Thremalert TH-5; Physitemp Instruments, Clifton, NJ, USA). All animal procedures were conducted in strict accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Wayne State University (# A 06-03-10). All surgery was performed under anesthesia, and all efforts were made to minimize suffering of animals.

Adeno-associated viral transfer vectors

The AAV2/6 gene transfer vectors, non-coding AAV2/6 (with a DNA segment cloned upstream of the pgk promoter to adapt the size of vector genome to AAV packaging capacity) and rat parkin-encoding AAV2/6 (AAV2/6 and AAV2/6-parkin), were a kind gift from Dr. Patrick Aebischer at the Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland. The cDNA encoding rat parkin was cloned into the pAAV-pgk-MCS transfer vector, and serotype 6 adeno-associated viral particles (AAV2/6) were produced and titered as described previously (Dusonchet et al., 2009). The virus titers for AAV2/6-parkin and non-coding AAV2/6 were 4.7×10^{10} TUs/ml (transducing units/ml) and 4.4×10^{10} TUs/ml, respectively. Virus-containing suspensions of 1×10^{7} or 2×10^{7} TUs in 2 μl volume were microinjected into the brain.

Experimental design

Following 1 week of acclimation, rats were stereotaxically injected with non-coding (1×10^{7} or 2×10^{7} TUs) or parkin-coding (1×10^{7} or 2×10^{7} TUs) transfer vector suspensions into the left SNc. After 3 weeks, the rats were sacrificed by decapitation without treatment or treated with binge METH or saline and sacrificed by perfusion a week later. Dissected or sliced brains were stored at −80 °C until assayed. The untreated rats were used for validation and evaluation of parkin overexpression in the nigrostriatal system and for evaluation of its effects on activity of the 20S proteasome. Saline- and METH-treated rats were used to assess the effects of parkin overexpression on the extent of METH toxicity and for evaluation of METH-induced hyperthermia. TH levels were assessed at 3 weeks after microinjections (western blotting) and at 4 weeks after microinjections (immunohistochemistry). The experimental design is illustrated in Fig. 1.

Stereotaxic surgery

Rats were anesthetized with an intraperitoneally (i.p.) administered mixture of xylazine (20 mg/ml) and ketamine hydrochloride (100 mg/ml). When necessary, supplementary doses of ketamine were administered to maintain surgical levels of anesthesia. AAV2/6 vectors (AAV2/6 or AAV2/6-parkin) were unilaterally injected into the left SNc with the following coordinates: −5.6 mm (AP) from Bregma, −2 mm (ML) from Bregma, −7.6 mm (V) from the dura according to the Paxinos and Watson's rat brain atlas. Viral suspensions were injected with a 5 μl Hamilton syringe at a rate of 0.15 μl/min using a syringe pump (Harvard Apparatus, Holliston, MA, USA). The syringe was left in place for 5 min at −7.6 mm than withdrawn to −6.6 mm and −5.5 mm, staying in each location for 5 min, then raised slowly out of the brain over 2 min.

Methamphetamine administration

Three weeks after AAV2/6 or AAV2/6-parkin microinjection, rats were randomly divided into two groups and injected (i.p.) with either

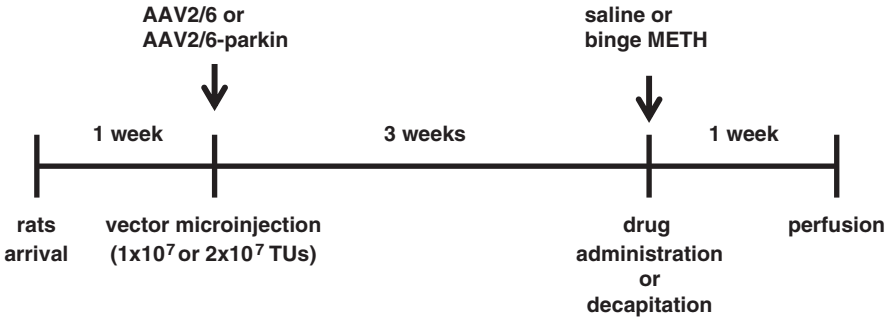

Fig. 1. Experimental design. Following 1 week of acclimation, adult male Sprague–Dawley rats were stereotaxically injected with non-coding or parkin-coding adeno-associated transfer vectors (AAV2/6 and AAV2/6-parkin) into the left substantia nigra *pars compacta* (SNc). After 3 weeks, the rats were sacrificed without treatment or treated with binge saline (1 ml/kg×4., every 2 h, i.p.) or binge METH (7.5 mg/kg×4, every 2 h, i.p.). Untreated rats were used for evaluation and validation of parkin overexpression. To assess the effects of parkin overexpression on the extent of METH toxicity, saline and METH-treated rats were sacrificed by perfusion 1 week after the day of METH and saline treatment. The arrows indicate stereotaxic microinjection (AAV2/6 vectors) and drug injection (METH or saline) times. Abbreviations: AAV, adeno-associated virus; TUs, transducing units; METH, methamphetamine.