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Amphetamine-induced locomotor activity is reduced in mice following MPTP treatment but not following selegiline/MPTP treatment

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Abstract

MPTP treatment has been used in mice to cause dopaminergic neuronal cell loss and subsequent behavioral abnormalities. As such, this animal model is often used as a method for the characterization of putative novel therapeutics for disease states characterized by dopamine loss, such as Parkinson's disease. Previous reports of behavioral abnormalities in mice following MPTP intoxication, however, have been conflicting. For example, open field spontaneous activity has been reported to increase, decrease or not change in MPTP treated mice. Accordingly, a more robust and direct functional measure of MPTP-induced central dopamine depletion is needed. In the present manuscript, we report on the characterization of amphetamine-induced locomotor activity as a sensitive functional endpoint for dopamine loss following MPTP treatment. We found that the amphetamine-induced locomotor activity of C57BL/6 mice was reduced in a dose-dependent manner following treatment with MPTP. This reduction of activity was associated with decreases in central dopamine levels. Further, the potential for use of this endpoint to evaluate putative therapeutics is exemplified by the amelioration of these effects following pre-treatment with the MAO-B inhibitor selegiline.

Keywords: Amphetamine; MPTP; Locomotor activity; Parkinson's disease; Striatum; Dopamine

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a selective loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). The resulting loss of dopaminergic input to the striatum causes a variety of motor deficits, including rigidity, tremor, akinesia, bradykinesia, and postural instability (Lang and Lozano, 1998a; Dauer and Przedborski, 2003).

One common preclinical manipulation that causes loss of dopaminergic innervation of the striatum and, as such, is considered an animal model of Parkinsonism involves treating mice with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Sundstrom et al., 1990; Dauer and Przedborski, 2003). In C57BL/6 mice, acute administration of MPTP (20 mg/kg, 4×2 h) has been shown to cause a loss of approximately 90% of terminal DA in the striatum (Itzhak et al., 1999). Despite the substantial loss of striatal DA, consistent changes in functional locomotor-based endpoints have not been forthcoming. In fact, a wide variety of seemingly conflicting behavioral outcomes have been reported (Sedelis et al., 2001). These include hyperactivity (Chia et al., 1996), hypoactivity (Fredriksson and Archer, 1994; Fredriksson et al., 1999), and no change in activity (Tomac et al., 1995). Notably, more subtle changes in motor activity, such as those assessed by the grid test (Tillerson et al., 2002; Tillerson and Miller, 2003), are affected in a manner consistent with central DA depletion following MPTP treatment. Nonetheless, additional behavioral tests that measure functional outputs simply, consistently and reliably are needed in order to evaluate the efficacy of therapeutic interventions for PD.

DA agonists clinically attenuate the motor symptoms of Parkinsonism, an effect mediated by increasing DA receptor stimulation in the basal ganglia (Lang and Lozano, 1998b; Schapira, 2005). D-amphetamine (AMPH) is an indirect DA agonist that causes a transient elevation of synaptic DA levels in the striatum and nucleus accumbens, resulting in time-dependent increases in locomotor activity in normal mice (Glick and Masanico, 1974; Helmeste and Seeman, 1982). Accordingly, we reasoned that amphetamine-induced locomotor activity may provide a

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quantitative measure of DA integrity following MPTP treatment. Thus, MPTP treated mice should demonstrate a compromised response to AMPH compared to saline-treated controls relative to their level of MPTP-induced striatal denervation.

The purpose of the present study was to assess the use of amphetamine-induced locomotor activity as a functional endpoint in the MPTP-mouse model of Parkinson's disease. We hypothesized that increasing doses of MPTP would cause greater central DA loss in terminal fields. The lower levels of available DA would then result in an attenuated response to amphetamine challenge in the locomotor activity assay. We report that increasing doses of MPTP did indeed lead to a reduction in levels of striatal DA. Lower levels of striatal DA corresponded with a muted response to amphetamine-induced locomotor activity. Finally, we report that pretreatment with the irreversible MAO-B inhibitor, selegiline, inhibits MPTP-induced neurotoxicity resulting in a favorable functional outcome.

2. Materials and methods

2.1. Animals

Adult male C57BL/6 mice (Taconic Farms, Germantown, NY) weighing 18–20 g upon arrival were housed in groups of five and maintained on a 12/12 h light dark cycle with food and water available ad libitum. After arrival to the facility all animals were allowed to acclimate for 3–5 days before testing. Animals were housed and tested in an Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited facility in strict compliance with all applicable regulations.

2.2. MPTP dosing and behavioral testing

Mice were dosed 4 times every 2 h with 10, 15 or 20 mg/kg MPTP (total dose=40, 60 or 80 mg/kg, respectively) by subcutaneous (SC) administration (Sigma, St. Louis, MO) or saline. In studies where the neuroprotective effects of selegiline were assessed, selegiline (1 mg/kg; (*R*)-(−)-*N*,α-Dimethyl-*N*- (2-propynyl)phenethylamine hydrochloride; Sigma) was administered intraperitoneally (IP) 30 min prior to each dose of MPTP. On day 5 following MPTP dosing, mice were tested in clear Plexiglas locomotor boxes (Med Associates, Georgia, VT) measuring 27 cm×27 cm. Photocells were located horizontally on the sides of the boxes, 1.7 and 4.2 cm above floor level. Infrared beam breaks were recorded by software and total distance traveled (in centimeters) was calculated for 3 h in 5-min intervals.

Mice were placed individually in the boxes and were allowed to habituate for 1 h. At 1 h, the recording software was paused and the mice were administered 3 mg/kg D-amphetamine (Sigma, St. Louis, MO) by IP injection. Following injection, the mice were placed back in the locomotor arena and recording was resumed for 2 h. Using similar procedures we have typically noted a lack of enhanced locomotor activity following vehicle injections in control animals (see, e.g., Kinney et al., 2003).

Data are expressed as the baseline normalized mean±S.E.M. distance traveled recorded at 5 min intervals over the test period beginning at the time of amphetamine injection. The data were analyzed using repeated measures analysis of variance followed by post-hoc testing using a one-tailed Dunnett test, where appropriate. A difference was considered significant when $p \leq 0.05$. For all repeated measures analyses, the Huynh–Feldt corrected *p*-values are reported.

2.3. Striatal dissections

One week following behavioral testing, 6 mice from each treatment group were randomly selected for striatal DA analysis. Brains were removed and the striata were dissected. The striata were placed in microcentrifuge tubes, weighed, and immediately placed on dry ice. These samples were stored at −70 °C until analyzed for DA levels.

2.4. Striatal DA analysis

Mouse striata were homogenized in ice-cold 0.2 M perchloric acid, 1 mM EDTA and centrifuged at 10,000×*g* for 10 min. The supernatants were filtered through a 0.2 μm filter and DA was determined by HPLC using an Antec Leyden EC detector set at +0.7 V and a 5 μm, 150×4.6 mm Beckman Ultrasphere ODS

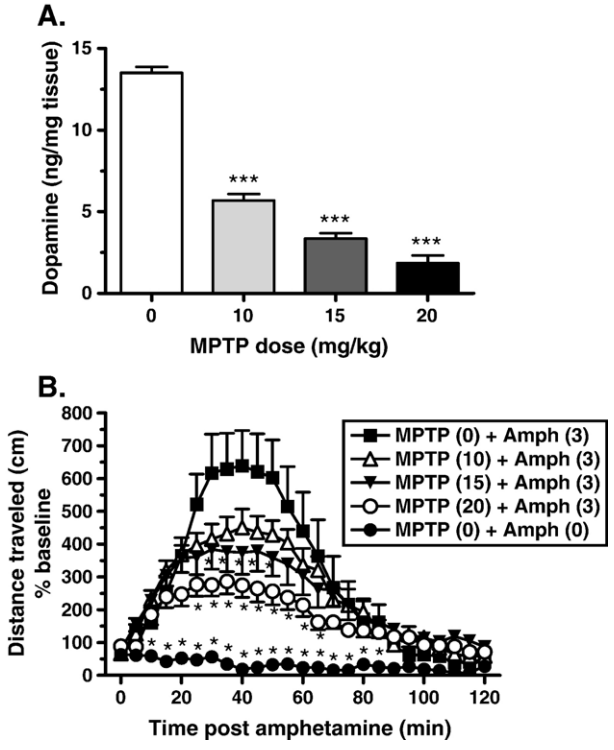

Fig. 1. (A) The differential effect of increasing concentrations of MPTP (4×10–20 mg/kg SC) on striatal dopamine levels (N=6/group). Asterisks represent a significant difference from the saline control group (0 mg/kg), ***$p<0.001$. Error bars represent the S.E.M. (B) The effect of various treatment conditions on amphetamine-induced locomotor activity in mice (N=8/group). MPTP was administered 5 days prior to behavioral testing. Following a 1 h acclimation period, amphetamine was delivered at t=0. Values in parentheses represent dose (mg/kg). Asterisks represent a significant difference from the MPTP(0)+Amph (3) (i.e., amphetamine-alone) group, *$p<0.05$. Error bars represent the S.E.M. MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Amph, D-amphetamine.

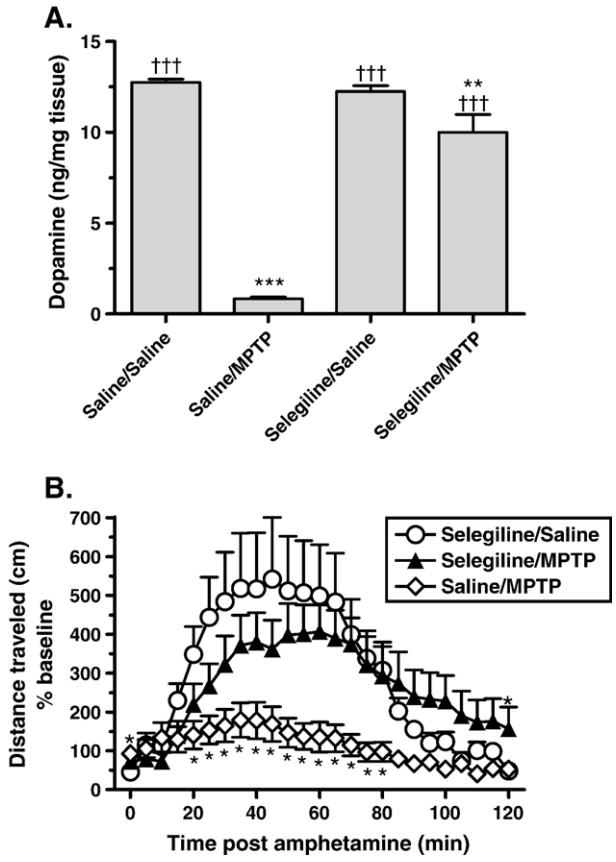

Fig. 2. (A) The effect of MPTP (4×20 mg/kg), selegiline (4×1 mg/kg) or selegiline+MPTP on striatal dopamine levels (N=6/group). Asterisks represent a significant difference from the saline/saline group, **p<0.01, ***p<0.001. Daggers represent a significant difference from the saline/MPTP treated group, †††p<0.001. Error bars represent the S.E.M. (B) The effect of MPTP (4×20 mg/kg), selegiline (4×1 mg/kg) or selegiline+MPTP on amphetamine induced locomotor activity in mice (N=7–8/group). MPTP was administered 5 days prior to behavioral testing. Following a 1 h acclimation period, amphetamine was delivered at t=0. Asterisks represent a significant difference from the selegiline/saline (i.e., no MPTP) group, *p<0.05. Error bars represent the S.E.M. MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

column. The mobile phase consisted of 50 mM sodium phosphate, 50 mM citric acid, 0.14 mM EDTA, 0.28 mM OSA, 5% acetonitrile, pH 4.5, delivered at 1 ml/min. The detector signal was processed using EZChrom Elite software and DA levels were quantitated using an external standard.

DA levels are expressed in ng/mg of wet tissue±S.E.M. The data were analyzed by analysis of variance followed by post-hoc testing using a one-tailed Dunnett test for the MPTP dose-effect study (Fig. 1). Since more than one comparator was used for post-hoc evaluation of the selegiline neuroprotection study (Fig. 2), the Tukey HSD test was used for post-hoc evaluation in this study. A difference was considered significant when $p \leq 0.05$.

3. Results

3.1. MPTP dose response

As shown in Fig. 1A, striatal DA levels decreased in a dose-dependent manner with increasing doses of MPTP. Analyses revealed a significant treatment related effect [F(3,20)=170.6, p<0.001], with a >85% reduction of striatal dopamine observed following treatment with the highest dose of MPTP tested (4×20 mg/kg). Post-hoc testing further confirmed a significant reduction in DA levels for all MPTP treated groups when compared to saline treated controls (p<0.001 for all doses).

As shown in Fig. 1B, MPTP treatment inhibited amphetamine-induced locomotor activity in a manner that was consistent with the decreased striatal DA levels observed in Fig. 1A. Consistent with this observation, repeated measures analysis of variance confirmed a significant effect of MPTP treatment on locomotor activity [F(4,34)=16.25, p<0.001] and a significant treatment by time interaction [F(96,816)=7.95, p<0.001]. Post-hoc testing further confirmed significantly less activity in all MPTP treated groups that received amphetamine relative to the control group that received saline and amphetamine only. This was particularly evident at the 15 and 20 mg/kg dose of MPTP.

3.2. Selegiline neuroprotection

As shown in Fig. 2A, pretreatment of mice with 4×1 mg/kg selegiline significantly inhibited the loss of striatal DA following treatment with 4×20 mg/kg MPTP. This effect was confirmed by analysis of variance, which demonstrated a significant main effect of treatment [F(3,20)=111.8, p<0.001]. Post-hoc analysis confirmed that mice administered selegiline and MPTP had significantly higher striatal DA levels relative to mice that received saline and MPTP (p<0.001). Independent of MPTP, selegiline alone (i.e., selegiline/saline) had no effect on striatal DA levels relative to saline/saline treated mice (p>0.05). While selegiline did significantly abate MPTP induced decreases in DA levels, a significant reduction in striatal DA levels was observed relative to saline/saline treated mice (p<0.01).

Amphetamine-induced locomotor changes were observed in a manner that was predicted by striatal DA levels depicted in Fig. 2A. Since the saline/saline group and the selegiline/saline groups did not differ in this task (treatment and treatment by time interaction, p's>0.05), the latter group was used as the relevant comparator in Fig. 2B for clarity of presentation. As shown in Fig. 2B, MPTP treatment inhibited amphetamine-induced locomotor activity relative to selegiline treated mice that had received vehicle (selegiline/saline). By contrast, mice that received selegiline/MPTP did not differ significantly from selegiline/saline treated mice. Repeated measures analysis of variance confirmed a significant effect of treatment [F(3,26)=8.97, p<0.001] and a significant treatment by time interaction [F(69,598)=4.36, p<0.002]. Post-hoc testing further confirmed significantly less activity in the saline/MPTP group relative to the selegiline/saline group and a further lack of difference in locomotor response in selegiline/MPTP mice relative to the selegiline/saline group.

4. Discussion

The present study was designed to test the hypothesis that murine locomotor activity in response to an amphetamine

challenge would be attenuated by prior MPTP treatment in a manner consistent with a loss of central DA. Consistent with this hypothesis, the present results show that increasing doses of MPTP lead to a dose-dependent loss in striatal DA and an associated attenuation of locomotor response to amphetamine challenge. Further, the present results suggest that this functional outcome measure is also sensitive to neuroprotective intervention. Thus, pretreatment of mice with selegiline resulted in a sparing of the MPTP effects on amphetamine-induced locomotor activity. Selegiline is an irreversible monoamine oxidase-B (MAO-B) inhibitor that prevents the metabolism of MPTP to its toxic metabolite, MPP^+, in the brain (Cohen et al., 1984; Heikkila et al., 1984b) and has been shown to inhibit MPTP toxicity in mice (Heikkila et al., 1984b; Castagnoli et al., 1999).

The neurotoxicity of MPTP to nigrostriatal DA neurons in mice is well established (Heikkila et al., 1984a; Fuller and Hemrick-Luecke, 1985; Sundstrom et al., 1987; Willis and Donnan, 1987). MPTP induces oxidative stress in nigrostriatal dopaminergic neurons (Adams and Odunze, 1991; Chiueh et al., 1993; Mohanakumar et al., 2002). Using amphetamine-induced locomotor activity, we show that this MPTP neurotoxicity can be expressed in a functional endpoint in a dose-dependent manner. This test therefore represents a reliable locomotor based assessment of MPTP treated mice and augments well-described changes in grid performance, which is also correlated with MPTP induced central DA depletion (Tillerson et al., 2002; Tillerson and Miller, 2003).

In summary, the present study describes an easily quantified behavioral assay that provides a reliable functional endpoint for striatal dopamine loss in the MPTP mouse model of Parkinson's disease. Mice treated with MPTP show significantly reduced locomotor response to amphetamine challenge. This reduced response is dependent on the dose of MPTP and is inhibited following treatment with the neuroprotective agent, selegiline. As such, this study provides the first comprehensive evaluation of amphetamine-induced locomotor activity in the MPTP mouse as a behavioral endpoint that correlates with the extent of the striatal DA loss and confirms an earlier report that evaluated the neuroprotective effect of the calpain inhibitor MDL-28170 in a similar paradigm (Crocker et al., 2003). Accordingly, the present data provide a novel method to allow for a reliable and easily quantified functional impairment in the MPTP mouse model. Such a method is of value for the evaluation of novel neuroprotective therapeutic interventions that are expected to result in functional improvement for human disease states characterized by a loss of striatal DA (e.g., Parkinson's disease).

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