Molecular and Cellular Endocrinology 311 (2009) 62–68

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Gender differences on MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxicity in C57BL/6 mice

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ARTICLE INFO

Article history:
Received 25 February 2009
Received in revised form 13 July 2009
Accepted 15 July 2009

Keywords:
Gender
Parkinson's disease
Western blot analysis
Dopamine transporter
Dopamine system
Mice

ABSTRACT

The aim of this study was to investigate the impact of gender difference in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated animal model of Parkinson's disease (PD). In the present study, we investigated the time-dependent alterations of dopamine and its metabolites, striatal tyrosine hydroxylase (TH) protein, dopamine transporter (DAT) protein, glial fibrillary acidic protein (GFAP) protein and midbrain TH protein and motor function in male and female mice 5 h and 1, 3 and 7 days after four administrations of MPTP (20 mg/kg) at 2-h intervals. The present study showed that the decrease of dopamine, DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanillic acid) content in female mice was more pronounced than that in male animals 1, 3 and 7 days after MPTP treatment. Our Western blot analysis study also demonstrated that the decrease of both striatal and midbrain TH protein levels in female mice was more pronounced than that in male animals from 1 to 7 days after MPTP treatment. As compared to male mice, in contrast, the increase of striatal GFAP protein levels in female mice was observed from 5 h to 7 days after MPTP treatment. Furthermore, the present study showed that motor deficits were found in both male and female mice 1 and 7 days after MPTP treatment. In the present study, moreover, the decrease of striatal DAT protein levels in female mice was more pronounced than that in male animals 1, 3 and 7 days after MPTP treatment. These results demonstrate that our administrations of MPTP at 2-h intervals can cause more severe damage in female mice as compared with male animals. The gender difference may be due to the decrease of DAT expression caused by MPTP. Thus our findings provide further valuable information for the pathogenesis of PD.

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1. Introduction

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP) provides one of the most valuable approaches to analyze critical aspects of Parkinson's disease (PD) in animal model. When administrated systemically to primates or rodents is a neurotoxin that selectively destroys dopaminergic neurons of the substantia nigra in both humans and animals (Langston et al., 1984; Heikkila et al., 1984). It was discovered as a side-product of an illicit meperidine drug synthesis; humans that took MPTP developed Parkinson-like symptoms of idiopathic PD and responded to antiparkinsonian medication (Langston et al., 1983, 1984). MPTP-treated mice and MPTP-treated monkeys are presently the best animals to model PD in neuroprotection experiments and investigate antiparkinsonian drugs and their motor side-effect such as dyskinesia (Jakowec and Petzinger, 2004; Smeyne and Jakson-Lewis, 2005).

Epidemiological studies have shown a prevalence of PD in men compared to women (Diamond et al., 1990). In fact, PD occurs 1.5 times more frequently in men than in women (Haaxma et al., 2007; Rodriguez-Navarro et al., 2008). In women, the age at onset of PD correlates with the end of the fertile life (Rocca et al., 2008).

Furthermore, female hormone estrogen improves motor ability in parkinsonian postmenopausal women with motor fluctuations as well as to reduce the risk of PD in postmenopausal women (Tsang et al., 2000; Benedetti et al., 2001). Symptoms of PD and L-dopa-induced dyskinesia are also shown to be modulated by estrogens (Di Paolo, 1994; Giladi and Honigman, 1995), while an anti-dopaminergic effect of estrogens on parkinsonian symptoms is reported (Session et al., 1994). In experimental animals, numerous studies have been reported that estrogen can protect against MPTP- and 6-OHDA (6-hydroxydopamine)-induced depletion of striatal dopamine levels and its metabolites and prevent the loss of tyrosine hydroxylase (TH)-immunoreactive nigral neurons (Murray et

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doi:10.1016/j.mce.2009.07.011

al., 2003; Ramirez et al., 2003; D'Astous et al., 2004; Quesada and Micevych, 2004; Tripanichkul et al., 2006). These findings suggest that female hormone may play a protective role in PD.

Interestingly, a gender effect on dopamine transporter (DAT) heterogeneity has been reported in human, with females having a higher heterogeneity in the striatum than males (Kuikka et al., 1999). Furthermore, a gender effect on DAT density has been reported in animals (Rivest et al., 1995). Moreover, DAT binding site density is higher in females than males in both animals and human studies (Morissette and Di Paolo, 1993; Lavalaye et al., 2000). It is known that DAT is a primary determinant of the lifetime of extracellular dopamine (Jones et al., 1998; Borland and Michael, 2004) as well as an important therapeutic target for neurostimulants commonly prescribed for a number of disorders (Whyte et al., 2004). From these observations, we speculate that gender-based differences in susceptibility to MPTP neurotoxicity may be explained by differences in DAT expression between males and females. However, the exact mechanisms for the gender differences in susceptibility to MPTP neurotoxicity in C57BL/6 mice are not fully understood.

In the present study, therefore, we investigated exactly the gender differences in susceptibility to MPTP neurotoxicity.

2. Materials and methods

2.1. Experimental animals and treatments

Male and female C57BL/6 mice (Nihon SLC Co., Shizuoka, Japan), 8 weeks of age, were used in this study. The animals were housed in a controlled environment ($23 \pm 1\,^{\circ}C$, $50 \pm 5\%$ humidity) and were allowed food and tap water *ad libitum*. The room lights were on between 8:00 and 20:00. All experiments were performed in accordance with the Guidelines for Animal Experiments of the Tokushima University School of Medicine. The male and female mice were injected intraperitoneally (i.p.) with four administrations of MPTP (20 mg/kg) at 2-h intervals, the total dose per mouse being 80 mg/kg. The vehicle animals were injected i.p. in the same manner with saline treatment instead of MPTP.

2.2. Measurement of dopamine, DOPAC and HVA levels

The mice were killed by cervical dislocation 5 h and 1, 3 and 7 days after MPTP treatments. After cervical dislocation, the striatum were rapidly dissected out and sonicated in ice-cold 0.2 M perchloric acid containing 100 ng/ml isoproterenol as an internal standard. Dopamine, DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanillic acid) were quantified by HPLC with an electrochemical detector (ECD) (Eicom, Kyoto, Japan). Concentrations of dopamine and its metabolites were expressed as μg/g tissue weight, as described previously (Araki et al., 2001; Kurosaki et al., 2005; Yokoyama et al., 2008). Each group consisted of five mice. In addition, the dissection procedure was performed in less than 2 min.

2.3. Western blot analysis

The mice were killed by cervical dislocation 5 h and 1, 3 and 7 days after MPTP treatments. The striatal and midbrain tissues were homogenized in HEPES-buffered sucrose (0.32 M sucrose containing 4 μg/ml pepstatin, 5 μg/ml aprotinin, 20 μg/ml trypsin inhibitor, 4 μg/ml leupeptin, 0.2 mM phenylmethanesulfonyl fluoride, 2 mM EDTA, 2 mM EGTA, and 20 mM HEPES, pH 7.2) using a microtube homogenizer. Protein concentrations were determined using a BCA kit (PIERCE, IL, USA). The homogenates were solubilized in Laemmli's sample buffer. Ten micrograms of protein from each sample were separated on 5–20% SDS-PAGE gel using constant current. Separated proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (ATTO, Tokyo, Japan) for 1 h with semi-dry blotting system. The PVDF membranes were incubated for 1 h at room temperature with phosphate-buffered saline containing 0.1% Tween 20 (PBST) and 0.5% skim milk, followed by overnight incubation at room temperature with desired antibodies. The anti-tyrosine hydroxylase (TH) antibody (1:5000, Chemicon International Inc., USA) and anti-dopamine transporter (DAT) antibody (1:2000, Chemicon International Inc., USA) as a marker of dopaminergic neurons and anti-glial fibrillary acidic protein (GFAP) antibody (1:2000, Sigma, USA) as a marker of reactive astrocytes were diluted in PBST containing 0.5% skim milk. Membranes were washed three times for 10 min at room temperature and incubated with horseradish peroxidase-conjugated secondary antibody in PBST containing 0.5% Skim milk for 1 h. Immunoreactive bands were visualized by enhanced chemiluminescent autoradiography (ECL Kit, Amersham, USA), according to manufacturer's instructions. Actin antibody (Sigma, USA) and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) antibody (Santa Cruse Biotechnology Inc., USA) were used as a house keeping protein to confirm that equal amounts of protein were loaded in each line. Optical densities were determined using a computerized image analysis system (Dolphin-DOC, Kurabo, Osaka, Japan) as described previously (Takagi et al., 2007; Ookubo et al., 2008; Watanabe et al., 2008). TH protein levels were expressed as % of vehicle using ratios to actin protein levels. DAT and GFAP protein levels were expressed as % of vehicle using ratios to GAPDH protein levels. Each group consisted of 3–4 mice. In addition, the dissection procedure was performed in less than 2 min.

2.4. Behavioral testing

2.4.1. Rota rod test

The Rota rod treadmill (Constant Speed Model, Ugo Basile, Varese, Italy) consists of a plastic rod, 6 cm in diameter and 36 cm long, with a non-slippery surface 20 cm above the base (trip plate). This rod is divided into five equal sections by six discs (25 cm in diameter), which enables five mice to walk on the rod at the same time. In the present study, rotor mode was used (28 rpm for 10 min). The time from when the animal was placed on the rod to when it fell off was recorded as the performance time. Each group consisted of 4–5 mice.

2.4.2. Pole test

The mouse was placed head downward near the top of a vertical rough-surfaced pole (diameter 8 mm, height 50 cm). The time until the mouse reached the floor (locomotion activity time; TLA) were recorded with the cuff-off limit of 30 s. Each group consisted of 4–5 mice.

2.5. Statistical analysis

All values were expressed as the means ± SE and statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by *post hoc* Fisher's protected LSD multiple comparison test or Student's *t*-test (Stat View version 5.0, SAS Institute Inc., USA). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with each vehicle-treated group (Fisher's protected LSD multiple comparison test). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with each vehicle-treated male group or MPTP-treated male group (Student's *t*-test).

3. Results

3.1. Measurement of dopamine, DOPAC and HVA levels

As shown in Fig. 1, four administrations of MPTP at 2-h intervals to male and female mice produced marked depletion of dopamine, DOPAC and HVA content in the striatum after 5 h and 1, 3 and 7 days. The decrease of dopamine, DOPAC and HVA content in MPTP-treated female mice was more pronounced than that in MPTP-treated male animals. In contrast, the increase of turnover (DOPAC + HVA/dopamine) in MPTP-treated female mice was more pronounced than that in MPTP-treated male animals.

3.2. Western blot analysis of TH, DAT and GFAP protein

As shown in Figs. 2 and 3, four administrations of MPTP at 2-h intervals to male and female mice produced marked depletion of striatal TH and DAT protein levels after 5 h and 1, 3 and 7 days. The decrease of striatal TH and DAT protein levels in MPTP-treated female mice was more pronounced than that in MPTP-treated male animals. Furthermore, in vehicle-treated animals, a significant decrease of the DAT protein levels in male mice was observed compared with that in female animals. As shown in Fig. 4, a significant decrease of midbrain TH levels was also observed in male and female mice 5 h and 1, 3 and 7 days after MPTP treatment. The decrease of midbrain TH levels in MPTP-treated female mice was more pronounced than that in MPTP-treated male animals after 1, 3 and 7 days. In contrast, as shown in Fig. 5, a significant increase of striatal GFAP levels was found in MPTP-treated female mice after 3 and 7 days. A significant increase of striatal GFAP levels was also observed in MPTP-treated male mice after 7 days. The increase of striatal GFAP levels in MPTP-treated female mice was more pronounced than that in MPTP-treated male animals.

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