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Effects of estrogens on striatal damage after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in male and female mice

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ABSTRACT

Emerging evidence shows a beneficial effect of estrogens for Parkinson's disease, yet the exact potency of these compounds implicated remain obscured. In this study, we investigated the neuroprotective effect of 17β-estradiol and estrone against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced striatal toxicity in mice. The neuroprotective effects of both compounds were evaluated by HPLC and Western blot analyses 5 days after the last of 4 consecutive injections of MPTP at 1-h intervals to mice. Subacute treatment (10 days) with estrone or 17β-estradiol at low doses (0.05 and 0.2 mg/kg) showed no significant changes against MPTP-induced damage of striatal dopamine terminals in mice. Furthermore, acute treatment with estrone at high doses (0.5 and 2.0 mg/kg) showed no significant alterations against MPTP-induced damage of striatal dopamine terminals in mice. In contrast, acute treatment with 17β-estradiol at high doses exhibited a neuroprotective effect against the damage of striatal dopamine terminals in both male and female mice after MPTP treatments. The results demonstrate that estrogen therapy with high doses may have a neuroprotective effect on the damage of striatal dopamine terminals in the MPTP-induced mice. These findings may lead to be development of estrogen therapy for the prevention and treatment of Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease commonly characterized by the progressive loss of dopaminergic neurons in the substantia nigra (McGeer et al., 1988). The loss of dopaminergic neurons afferents to the striatum and putamen results in motor dysfunction, including tremor, rigidity and bardykinesia (Olanov et al., 2003). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 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 dyskinesias (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). Furthermore, estrogen improves motor disability in parkinsonian postmenopausal woman with motor fluctuations and decreases the risk of PD in postmenopausal woman (Tsang et al., 2000; Benedetti et al., 2001). Symptoms of PD and L-dopa-induced dyskinesias are also shown to be modulated by estrogens (Di Paolo, 1994; Giladi and Honigman, 1995), while an anti-dopaminergic effect of estrogens on parkisonian symptoms is reported (Session et al., 1994). These findings suggest that estrogen may play a protective role in PD. In experimental animals, numerous studies have been reported that estrogen can protect against MPTP- and 6-hydroxydopamine (6-OHDA)-induced depletion of striatal dopamine levels and its metabolites and prevent the loss of tyrosine hydroxylase-immunoreactive nigral neurons (Murray et al., 2003; Ramirez et al., 2003; D'Astous et al., 2004; Quesada and Micevych, 2004; Tripanichkul et al., 2006). However, in a previous experimental study, long-term estrogen replacement treatment of ovariectomized rats did not protect the dopaminergic neurons from an insult with 6-OHDA (Ferraz et al., 2003). In humans, estrogen therapy is reported to be beneficial to women with early PD prior to

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initiation of L-dopa (Saunders-Pullman et al., 1999), but not at late stages of the disease (Strijks et al., 1999). Therefore, it still remains to ascertain whether estrogen can exert a neuroprotective effect in an animal model of PD.

In the present study, therefore, we investigated the neuroprotective effect of estrogens (17β-estradiol and estrone) against MPTP-induced striatal toxicity in mice with two schedules of estrogen treatment.

2. Materials and methods

2.1. Experimental animals and treatments

Male and female C57BL/6 mice (Nihon SLC Co., Shizuoka, Japan), 7–12 weeks of age, were used in this study. The animals were housed in a controlled environment (23 ± 1 °C, 50 ± 5% humidity) and were allowed food and tap water *ad libitum*. The room lights were on between 8:00 and 20:00.

2.2. Effect of subacute treatment with estrone or 17β-estradiol (male mice)

Male animals were divided into 10 groups: (1) vehicle (0.1% Tween 80)-treated group; (2) estrone (0.2 mg/kg)-treated group; (3) MPTP- and vehicle-treated group; (4) MPTP- and estrone (0.05 mg/kg)-treated group; (5) MPTP- and estrone (0.2 mg/kg)-treated group; (6) vehicle (0.1% Tween 80)-treated group; (7) 17β-estradiol (0.2 mg/kg)-treated group; (8) MPTP- and vehicle-treated group; (9) MPTP- and 17β-estradiol (0.05 mg/kg)-treated group; (10) MPTP- and 17β-estradiol (0.2 mg/kg)-treated group. Each group received a pre-treatment of estrone, 17β-estradiol or vehicle 6 days prior to the MPTP treatments. The intraperitoneal (i.p.) pre-treatment consisted of two daily injections of estrone, *17β-estradiol* or vehicle. On day 6, the mice received four injections of MPTP (10 mg/kg, i.p.) at 1-h intervals, while vehicle-treatment group received saline solution. The treatments with estrone, 17β-estradiol or vehicle were continued until day 10. On day 11 (5 days after MPTP treatments), the mice were killed by cervical dislocation, the striatum quickly removed and kept at −80 °C.

2.3. Effect of acute treatment with estrone or 17β-estradiol (male mice)

Male animals were divided into 10 groups: (1) vehicle (0.1% Tween 80)-treated group; (2) estrone (2.0 mg/kg)-treated group; (3) MPTP- and vehicle-treated group; (4) MPTP- and estrone (0.5 mg/kg)-treated group; (5) MPTP- and estrone (2.0 mg/kg)-treated group; (6) vehicle (0.1% Tween 80)-treated group; (7) 17β-estradiol (2.0 mg/kg)-treated group; (8) MPTP- and vehicle-treated group; (9) MPTP- and 17β-estradiol (0.5 mg/kg)-treated group; (10) MPTP- and 17β-estradiol (2.0 mg/kg)-treated group. The mice were injected four times with MPTP (10 mg/kg, i.p.) at 1-h intervals. The animals were injected i.p. with estrone, *17β-estradiol* or vehicle before 30 min and 90 min after the first administration of MPTP. For groups (1), (2), (6) and (7), the vehicle-treated, estrone (2.0 mg/kg)-treated and 17β-estradiol (2.0 mg/kg)-treated animals were injected with the same manner instead of MPTP. Five days after MPTP treatments, the mice were killed by cervical dislocation, the striatum quickly removed and kept at −80 °C.

2.4. Effect of acute treatment with 17β-estradiol (female mice)

Female animals were divided into five groups: (1) vehicle (0.1% Tween 80)-treated group; (2) 17β-estradiol (2.0 mg/kg)-treated group; (3) MPTP- and vehicle-treated group; (4) MPTP- and 17β-estradiol (0.5 mg/kg)-treated group; (5) MPTP- and 17β-estradiol (2.0 mg/kg)-treated group. The mice were injected four times with MPTP (10 mg/kg, i.p.) at 1-h intervals. The animals were injected i.p. with 17β-estradiol or vehicle before 30 and 90 min after the first administration of MPTP. For groups (1) and (2), the vehicle-treated and 17β-estradiol (2.0 mg/kg)-treated animals were injected with the same manner instead of MPTP. Five days after MPTP treatments, the mice were killed by cervical dislocation, the striatum quickly removed and kept at −80 °C.

2.5. Measurement of dopamine, DOPAC and HVA levels

The mice were killed by cervical dislocation 5 days after MPTP treatments, as described previously (Yokoyama et al., 2008). 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, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) 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). Each group consisted of five mice. In addition, the dissection procedure was performed in less than 2 min at 10–12 a.m.

2.6. Western blot analysis

The mice were killed by cervical dislocation 5 days after MPTP treatments, as described previously (Yokoyama et al., 2008). The striatal 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 fluroride, 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; 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. TH protein levels ($n = 4$ mice), DAT protein levels ($n = 3$ mice) and GFAP protein levels ($n = 3$–4 mice). Each group consisted of 3–4 mice. In addition, the dissection procedure was performed in less than 2 min at 10–12 a.m.

2.7. Statistical analysis

All values were expressed as the means ± S.E. and statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by 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 MPTP-treated group (Fisher's protected LSD multiple comparison test). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with vehicle-treated or MPTP-treated group (Student's *t*-test).

3. Results

3.1. Measurement of dopamine, DOPAC and HVA levels

3.1.1. Effects of subacute treatment with estrone or 17β-estradiol on the striatal dopamine, DOPAC and HVA contents in male mice 5 days after MPTP treatments

Subacute treatment with estrone or 17β-estradiol showed no significant changes in dopamine, DOPAC and HVA levels in the striatum after MPTP treatments. Furthermore, both compounds showed no significant alterations in the dopamine, DOPAC and HVA contents in the striatum, as compared to vehicle-treated group (Fig. 1).

3.1.2. Effects of acute treatment with estrone on the striatal dopamine, DOPAC and HVA contents in male mice 5 days after MPTP treatments

Acute treatment with estrone showed no significant changes in dopamine and HVA levels in the striatum after MPTP treatments. However, estrone exhibited a significant increase in the striatal DOPAC levels after MPTP treatment. Also, this compound alone showed no significant alterations in the dopamine, DOPAC and HVA levels in the striatum, as compared to vehicle-treated group (Fig. 2).