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Inhibition of p38 pathway-dependent MPTP-induced dopaminergic neurodegeneration in estrogen receptor alpha knockout mice

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ABSTRACT

Approximately, 7–10 million people in the world suffer from Parkinson's disease (PD). Recently, increasing evidence has suggested the protective effect of estrogens against nigrostriatal dopaminergic damage in PD. In this study, we investigated whether estrogen affects 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced behavioral impairment in estrogen receptor alpha (ERα)-deficient mice. MPTP (15 mg/kg, four times with 1.5-h interval)-induced dopaminergic neurodegeneration was evaluated in ERα wild-type (WT) and knockout (KO) mice. Larger dopamine depletion, behavioral impairments (Rotarod test, Pole test, and Gait test), activation of microglia and astrocytes, and neuroinflammation after MPTP injection were observed in ERα KO mice compared to those in WT mice. Immunostaining for tyrosine hydroxylase (TH) after MPTP injection showed fewer TH-positive neurons in ERα KO mice than WT mice. Levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC, metabolite of dopamine) were also lowered in ERα KO mice after MPTP injection. Interestingly, a higher immunoreactivity for monoamine oxidase (MAO) B was found in the substantia nigra and striatum of ERα KO mice after MPTP injection. We also found an increased activation of p38 kinase (which positively regulates MAO B expression) in ERα KO mice. In vitro estrogen treatment inhibited neuroinflammation in 1-methyl-4-phenyl pyridium (MPP+)-treated cultured astrocyte cells; however, these inhibitory effects were removed by p38 inhibitor. These results indicate that ERα might be important for dopaminergic neuronal survival through inhibition of p38 pathway.

Introduction

Parkinson's disease (PD) is pathologically characterized by dopaminergic neurodegeneration in the substantia nigra and consequent depletion of dopamine in the striatum (Wu et al., 2009). A significant loss of these neurons and subsequent striatal dopamine (DA) (Okuda et al., 2006) takes place before symptoms occur. This helps in the clinical diagnosis of the disorder. The main symptoms of PD are bradykinesia (slowness of movement), muscle rigidity, postural instability, and resting tremor.

Epidemiological studies conducted in different countries have revealed that men are at a higher risk for developing PD at all ages (Baldereschi et al., 2000; Swerdlow et al., 2001; Taylor et al., 2007; Van Den Eeden et al., 2003). Furthermore, meta-analysis studies indicate that twice as many men than women suffer from PD (Elbaz et al., 2002).

Studies on human DA release kinetics have shown that women have higher levels of DA release than men do (Riccardi et al., 2006). Similarly, in rodent studies, female Sprague–Dawley rats were shown to have higher levels of DA release than male counterparts by fast-scan cyclic voltammetry on brain slices (Walker et al., 2000).

The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model has been extensively used, as the system recapitulates several cardinal features of PD such as dopaminergic neuronal loss in

Abbreviations: AD, Alzheimer's disease; CNS, central nervous system; DA, dopamine acid; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; GFAP, glial fibrillary acidic protein; KO, knockout; MAO, monoamine oxidase; MAPK, mitogen-activated protein kinase.; MPP+, 1-methyl-4-phenyl pyridium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PBS, phosphate-buffered saline; PD, Parkinson's disease; TH, tyrosine hydroxylase; WT, wild type.

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the substantia nigra and depletion of dopamine level in the striatum (Forno et al., 1993; German et al., 1996; Onofrj and Ghilardi, 1990). In astrocytes, monoamine oxidase (MAO) B produces 1-methyl-4-phenyl pyridium (MPP+), an active neurotoxic metabolite of MPTP, which causes the MPTP-mediated dopaminergic neurotoxicity. Once MPP+ is released from astrocytes, it is delivered to dopaminergic neurons through the dopamine transporter (DAT) (Youdim et al., 2006). MAO B plays a key role in MPTP neurotoxicity. Several studies demonstrated that a selective MAO B inhibitor (e.g., deprenyl) prevents MPTP-induced dopaminergic neurotoxicity (Delogu et al., 2014; Yasar et al., 2006). A recent meta-analysis shows that activation of MAO B was associated with PD risk in 20 case–control studies with a total of 2846 cases (Liu et al., 2014). In addition, overexpression of MAO B in astrocytes causes dopaminergic neurodegeneration (Mallajosyula et al., 2008). These findings suggest that an increase of MAO B activity can affect pathogenesis of neurodegenerative diseases.

Estrogen is important in the maintenance of normal brain function. Increasing evidence reveals a neuroprotective role of estrogen in neurodegenerative diseases such as Alzheimer's disease (AD), PD, and stroke (Galanopoulou et al., 2003; Hurn and Macrae, 2000; Wise, 2002). The neuroprotective effect of estrogen in PD has been demonstrated by various animal studies showing the protective effect of high levels of estrogen against MPTP-induced dopaminergic neurodegeneration (Disshon and Dluzen, 1997; Dluzen, 2000; Dluzen et al., 1996; Grandbois et al., 2000). Estrogen is effective in lowering the level of MAO B, which is a causal factor for the degeneration of dopaminergic neurons (Holschneider et al., 1998).

The p38 mitogen-activated protein kinase (MAPK) has been activated in PD models and is implicated in neuronal cell death (Mathiasen et al., 2004; Silva et al., 2005). Indeed, phosphorylation of p38 induces the expression of the proapoptotic protein Bax in dopaminergic neurons of the midbrain in mice (Chipuk and Green, 2008). Rotenone induces apoptosis in dopaminergic SH-SY5Y cells through the activation of p38 MAPKs (Newhouse et al., 2004), and its toxicity is prevented by activation of ERK1/2 signaling pathways (Hsuan et al., 2006). MAO B expression could be regulated by MAPK. In activated microglia and astrocytes, the activation of MAO B was prevented by inhibition of the p38 MAPK pathway (Wong et al., 2002). Thus, the MAPK pathway could be significant in the neuroprotective mechanism of estrogen in the development of PD. Therefore, in this study, we investigated whether estrogen exhibits a protective effect against MPTP-induced dopaminergic neurodegeneration, and possible mechanisms of such an effect.

Materials and methods

Animals and MPTP treatment

Estrogen receptor alpha (ERα) knockout (KO) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All the experimental procedures carried out in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Chungbuk National University (approval number: CBNUA-144-1001-01). Four experimental groups of 10-month-old male mice (n = 8) weighing 28–30 g were studied: the C57BL/6 WT (control) group1/2 (n = 16) and the ERα KO group (n = 16). ERα KO mice grow and reproduce normally with no obvious phenotypic difference from the wild type. All mice were housed in a room at a temperature of 21–25 °C and relative humidity of 45–65% with a controlled light–dark cycle.

The mice were administered intraperitoneal injections of MPTP (15 mg/kg, Sigma-Aldrich, St Louis, MO, USA) or saline four times at 1.5-h intervals. The MPTP dose was used to differentiate MPTP-induced dopaminergic neurotoxicity between ERα WT and KO mice. The mice were sacrificed 7 days after injections for experimental analyses.

Behavioral tests

We performed behavioral tests 6 days after MPTP injection to examine whether there is any difference in the neurotoxicant-caused behavioral deficit between ERα WT and ERα KO mice. Rotarod, pole, and gait tests were the behavioral tests used.

Rotarod test

The rotarod test was conducted as described previously (Choi et al., 2012). The mice were trained for 2 consecutive days before MPTP injections in acceleration mode (2–20 rpm) over 5 min. The training was repeated with a constant speed (16 rpm) until the mice were able to stay on the rod for at least 300 s.

Pole test

The pole test was carried out as described by Choi et al. (2012). The test was conducted in triplicate and the average values were used for each animal.

Gait test

The gait test was also performed in accordance with previous description (Choi et al., 2012). The mice were subjected to two training trials to get adapted to the environment. A single test trial was performed, and stride length was measured as the distance between successive paw prints. Data were presented as the average of five strides for each animal.

Collection and preservation of brain tissues

After the behavior tests, the mice were euthanized with diethyl ether and then perfused with phosphate-buffered saline (PBS). The brains were immediately removed from the skull, and the tissues were stored at −80 °C until biochemical analysis.

High-performance liquid chromatography analysis of dopamine and its metabolite

Dopamine and its metabolites in the striatum were measured by high-performance liquid chromatography (HPLC). Briefly, tissues were sonicated in chilled 0.1 M perchloric acid containing dihydroxybenzylamine as an internal standard. After centrifugation (15,000 ×*g*, 30 min, 4 °C), the supernatant was diluted with mobile phase (75-mM NaH2PO4, 1.7-mM octane sulfonic acid, 10% methanol; pH 3.0), and 10 μl of sample was isocratically eluted through a 80 × 4.6 mm C18 column (Waters Associates, Milford, MA, USA) with a flow rate of 1.5 ml/min. Neurochemicals such as dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were detected by a two-channel electrochemical detector (Waters Associates) at a potential of 1.5 mV, and their concentrations were normalized by wet tissue weight.

Measurement of dopamine

Lysates of whole brain tissue were obtained through protein extraction buffer containing protease inhibitor. Dopamine levels were also determined using each specific enzyme-linked immunosorbent assay (ELISA) kit (Wuhan Huamei Biotech Co. Ltd., Hubei, China). In brief, 100 μl of sample was placed in the precoated plate and incubated for overnight at 4 °C. After washing each well of the precoated plate with buffer, 100 μl of labeled antibody solution was added, and the mixture was incubated at 4 °C for 1 h in dark. After washing, chromogen was added, and the mixture was incubated at room temperature for 30 min in dark. Finally, the resulting color was assayed at 450 nm