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BRAIN RESEARCH

Research Report

Estrogen down-regulates glial activation in male mice following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxication

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ABSTRACT

Emerging evidence suggests beneficial effect of estrogen for Parkinson's disease (PD), yet the exact mechanisms implicated remain obscured. Activated glia observed in MPTP mouse model and in PD may participate in the cascade of deleterious events that ultimately leads to dopaminergic nigral neuronal death. In vitro studies demonstrate that estrogen can modify the microglial and astroglial expression of inflammatory mediator, such as cytokines and chemokines implicated in neuroinflammation and neurodegeneration. To determine whether estrogen-elicited neuroprotection in PD is mediated through glia, adult male C57Bl/6 mice were treated with 17β-estradiol (E2) for a total of 11 days. Following 5 days of pretreatment with E2, they were injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on the sixth day. The brains were collected on day 11. Immunohistochemistry and quantitative study were used to assess the number of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the substantia nigra pars compacta (SNpc) and that of activated astrocytes and activated microglia in the SNpc and the striatum. Pretreatment with E2 decreased the loss of TH-IR nigral neurons and diminished the deficit of TH-IR striatal fibers triggered by MPTP. The neuroprotective effect of E2 was coincident with an attenuation of a glial response within the nigra and the striatum. These findings suggest that the neuroprotective effects of E2 evidenced in MPTP mouse model might mediate through an inhibition of reactive glia. However, direct neuroprotective effects of E2 upon TH-IR neurons cannot be excluded.

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Abbreviations:
DAB, 3,3′-diaminobenzidine tetrahydrochloride
E2, 17β-estradiol
ER, estrogen receptors
GFAP, glial fibrillary acidic protein
GSI-B_4, *Griffonia simplicifolia* isolectin B_4
iNOS, inducible nitric oxide synthase
IFN-γ, interferon-gamma
-IR, immunoreactive
IL-1, interleukin-1
IL-6, interleukin-6
MPP^+, 1-methyl-4-phenylpyridinium
MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PBS, phosphate buffered saline
PD, Parkinson's disease
SEM, standard error
SNpc, substantia nigra pars compacta
TH, tyrosine hydroxylase
TNF-α, tumor necrosis factor-alpha
6-OHDA, 6-hydroxydopamine

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease commonly characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and their projections in the striatum (McGeer et al., 1988a,b). Epidemiological studies have shown a greater prevalence of PD in men compared to women (Diamond et al., 1990; Kurtzke and Goldberg, 1988). Furthermore, estrogen improves motor disability in parkinsonian postmenopausal women with motor fluctuations (Tsang et al., 2000) and reduces the risk of PD in postmenopausal women (Benedetti et al., 2001). Altogether, these data indicate a beneficial effect of this ovarian hormone in the progression of PD. The evidence of a neuroprotective role of estrogen in PD is provided by studies in PD animal models. These studies have reported that estrogen decreases the striatal dopamine depletion in MPTP-intoxicated mice (Callier et al., 2001; Dluzen et al., 1996, 2001; Grandbois et al., 2000; Ramirez et al., 2003) and in 6-hydroxydopamine (6-OHDA)-lesioned rats (Datla et al., 2003; Dluzen, 1997; Murray et al., 2003). Besides, in vitro studies have revealed that estrogen can protect against 1-methyl-4-phenylpyridinium (MPP^+) induced dopaminergic neuronal death (Sawada et al., 2002). Nevertheless, the effect of E2 on protecting against loss of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the SNpc of PD animal model remains controversial (Ferraz et al., 2003; Murray et al., 2003; Quesada and Micevych, 2004).

Although data suggest that estrogen confers benefits in PD, the mechanisms by which the hormone exerts its neuroprotection remains poorly understood. Glial reaction observed in PD and in PD animal model may contribute to the degeneration of dopaminergic neurons (Kohutnicka et al., 1998; Liberatore et al., 1999; McGeer et al., 1988a,b; Wu et al., 2002). Indeed, activated astrocytes and microglia express broad array of neurotoxic molecules, including pro-inflammatory cytokines, pro-inflammatory prostaglandins, reactive oxygen species and reactive nitrogen species (Knott et al., 2000; Liberatore et al., 1999; Norenberg, 1996; Qin et al., 2002). Previous studies have reported that glia have estrogen receptors and are targets for estrogen actions (Azcoitia et al., 1999; Bruce-Keller et al., 2000; Garcia-Ovejero et al., 2002; Hosli et al., 2001; Langub and Watson, 1992; Lei et al., 2003; Mor et al., 1999; Platania et al., 2003; Santagati et al., 1994; Vegeto et al., 2001). Additionally, glia have been implicated in the regenerative and neuroprotective effects of estrogen (Garcia-Estrada et al., 1999; Garcia-Segura et al., 1999a,b; Jones et al., 1999; Rozovsky et al., 2002; Sortino et al., 2004).

In view of the wide-ranging effect of estrogen on glia, it is of interest whether neuroprotection of estrogen in PD animal model is mediated through glia. The present study investigated the effect of 17β-estradiol (E2) on glial reaction in the SNpc and the striatum of adult male mice following acute MPTP intoxication. Further, we also examined if E2 was able to protect against MPTP-induced loss of TH-IR nigral neurons and their projections to the striatum.

2. Results

2.1. *Estrogen attenuates MPTP-mediated loss of TH-IR neurons in the SNpc and TH-IR fibers in the striatum*

In MPTP-treated animals, only 34% of the TH-IR nigral neurons survived MPTP injection ($P < 0.05$ vs. control animals). In contrast, about 1.7 times as many TH-IR nigral neurons in MPTP/E2-treated animals survived MPTP intoxication ($P < 0.05$ vs. MPTP-treated animals) (Figs. 1 and