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Estrogen receptors and gonadal steroids in vulnerability and protection of dopamine neurons in a mouse model of Parkinson's disease

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ABSTRACT

17β-estradiol is well known to have neuroprotective effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. We investigated the neuroprotective contribution of estrogen receptors (ERα and ERβ) against MPTP toxicity by examining the membrane dopamine (DA) transporter (DAT), the vesicular monoamine transporter 2 (VMAT2) and tyrosine hydroxylase (TH) in ER knock out (ERKO) C57Bl/6 male mice compared to their plasma steroid levels. A dose–response to MPTP comparing wild-type (WT) to ERKO mice was studied. WT mice were also compared to ERKO mice pretreated with 17β-estradiol alone and with MPTP. Specific radioligand binding autoradiography and *in situ* hybridization for DAT, VMAT2 and TH were assayed in the striatum and the substantia nigra (SN). Intact ERKOβ mice had both striatal transporters levels lower than WT and ERKOα mice. MPTP caused a dose-dependant loss of both striatal transporters that correlated with striatal DA concentrations. Compared to WT and ERKOβ mice, ERKOα mice DAT, VMAT2 and TH were affected at lower MPTP doses. In the striatum and SN, ERKOα mice were more vulnerable and 17β-estradiol protected against MPTP toxicity only in WT mice. ERKOα mice blood plasma had higher levels of testosterone, dihydrotestosterone and 3β-diol compared to the plasma of WT and ERKOβ mice. 17β-estradiol treatment increased estradiol plasma levels in all genotypes. Striatal DA concentrations and SN TH mRNA correlated inversely with plasma testosterone and 3β-diol levels. Hence, in male mice the lack of ERα or ERβ altered their basal plasma steroid levels and both striatal DA transporters as well as their susceptibility to MPTP toxicity.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer and is likely to increase due to the aging population (review: Siderowf and Stern, 2003). Motor impairment in PD results from the loss of striatal dopamine (DA), due to the death of DA neurons in the substantia nigra (SN). There is no cure for PD but the motor symptoms are alleviated by replacement of DA by its precursor levodopa (L-DOPA) or by treatment with direct DA receptor agonists (Hornykiewicz, 2002; Olanow et al., 2009). Nevertheless for the majority of PD patients, these therapies eventually loose effectiveness and are associated with side-effects (Katzenschlager and Lees, 2002). Thus, there is a need for therapies to prevent the loss of DA neurons and/or halt disease progression. Estrogenic drugs could bring such disease modifying therapies for PD.

There are two families of transporters responsible for controlling extracellular DA concentrations; these are the DA transporter (DAT) and the vesicular monoamine transporter 2 (VMAT2) (Guillot and Miller, 2009). The striatum has dense and heterogeneous DAT distribution, the transporter is found on plasma membranes of axon terminals and immunocytochemistry shows that DAT is colocalized with tyrosine hydroxylase (TH) and the D2 DA receptor (Ciliax et al., 1999). DAT allows the uptake of DA into the cytoplasm from the extracellular space, while VMAT2 is responsible for storing DA in synaptic vesicles and reduction of its levels in the nigrostriatal system is seen in animal models of PD and in PD patients

Abbreviations: CNS, central nervous system; DA, dopamine; DAT, membrane dopamine transporter; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; 3β-diol, 5α-androstan 3-β, 17β-diol; DOPAC, 3,4-Dihydroxyphenylacetic acid; 17β-E_2, 17β-estradiol; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; ERKO, estrogen receptor knock out; E1, estrone; ERK, extracellular-signal-regulated kinase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; P450scc, cytochrome P450 sidechain cleavage; P450c17, cytochrome P450 17α-hydroxysteroid/C17 20-lyase; PPT, 1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole; 5α-R, 5α-reductase; [^{125}I]-RTI-121, 3β-(4-^{125}I-iodophenyl)trophane-2β-carboxylic acid; SN, substantia nigra; TH, tyrosine hydroxylase; [^{3}H]-TBZ-OH, [^{3}H]-dihydrotetrabenazine; VMAT2, vesicular monoamine transporter type 2; WT, wild-type.

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(Guillot and Miller, 2009; Le Saux and Di Paolo, 2006). The actions of these transporters are regulated by presynaptic receptors and protein kinases (Guillot and Miller, 2009). Hence, the amount of free DA depends on DAT levels on the plasma membrane and the presence of VMAT2 on synaptic vesicles.

Epidemiological and clinical studies support a beneficial effect of estrogens against the development and progression of PD. A greater prevalence and incidence of PD is described in men than in women (Shulman and Bhat, 2006; Wooten et al., 2004). Men with PD show symptoms requiring medical attention during earlier stages of the disorder than women suggesting that the disease progresses more rapidly in men, thus supporting that estrogen can provide neuroprotective effects (Saunders-Pullman, 2003). Gender differences in symptoms were also seen in outcome studies after stereotactic surgery for PD (Shulman and Bhat, 2006). Also, an inverse association between factors reducing estrogen stimulation during life and PD is found, supporting the hypothesis that endogenous estrogens play a role in its development (review: Bourque et al., 2009). Therapy with 17β-estradiol was reported to be beneficial at an early stage of PD, before initiation of L-DOPA (review: Bourque et al., 2009).

17β-estradiol is neuroprotective in both male and female mice against a variety of central nervous system (CNS) insults such as protection of DA neurons against the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), where pre-treatment with 17β-estradiol before MPTP prevents the loss of striatal DA and its metabolites (review: Bourque et al., 2009), DAT, and VMAT2 (D'Astous et al., 2003). Dluzen and colleagues suggest that estrogen protects by decreasing DAT's binding affinity, thus not allowing entry of neurotoxic compounds, like the MPTP ion 1-methyl-4-phenylpyridinium (MPP+), into dopaminergic nerve terminals (Dluzen, 2000). Furthermore, the neuroprotective effect of 17β-estradiol appears to be mediated through interaction with estrogen receptors (ERs). In male mice, 17β-estradiol which binds to and activates ERs, is neuroprotective against striatal MPTP toxicity; whereas 17α-estradiol, the isomer with low ER affinity, lacks neuroprotective activity (Callier et al., 2000) and estriol and estrone, weak agonists on ERs, show poor or no activity to protect against MPTP toxicity (Jourdain et al., 2005). Thus, the potencies of the above compounds to protect against MPTP toxicity parallel their activity on ERs. There are two main ER subtypes, ERα and ERβ (Green et al., 1986; Kuiper and Gustafsson, 1997). ERα is widely expressed throughout the body and mediates most of the feminizing effects of estradiol (Mitra et al., 2003). By contrast, ERβ has a much more restricted distribution, of which expression in the brain is notable (Kuiper and Gustafsson, 1997). Both ERs have been detected in the mouse striatum and SN (Mitra et al., 2003). Moreover, no sex difference was observed for ERα and ERβ levels in mouse striatum during development and in adulthood (Kuppers and Beyer, 1999). Using specific agonists for ERα and ERβ we have previously shown that ERα agonists protect against MPTP toxicity in male mice (D'Astous et al., 2004).

The intact male mouse MPTP animal model of PD is representative of PD pathology and, to unravel the neuroprotective effects of ERs, striatal catecholamine concentrations of ER knock out (ERKOα and ERKOβ) male mice were previously published (Morissette et al., 2007). The degree of MPTP-induced DA and DOPAC depletion was greater in ERKOα than in wild-type (WT) male mice, whereas ERKOβ mice exhibited no change in MPTP sensitivity but they showed a lower DA turnover than WT and ERKOα mice. 17β-estradiol partially prevented the MPTP-induced decrease in striatal DA and 3,4-dihydroxyphenylacetic acid (DOPAC) levels only in the WT mice (Morissette et al., 2007). Therefore, we hypothesize that sparing of striatal DA concentrations from MPTP toxicity in these WT mice is due to neuroprotection of DA neurons by endogenous steroids and administered 17β-estradiol acting on ERs. Hence, in the present study we investigated the effect of ER genotype on blood steroid levels and various DA markers in these mice. We explored the contribution of the striatal and SN DAT, VMAT2 and TH in MPTP toxicity and neuroprotection by 17β-estradiol in WT mice compared to ERKOα and ERKOβ male mice.

2. Materials and methods

2.1. Animals and treatments

Adult WT, ERKOα and ERKOβ male C57Bl/6 mice (7–12 weeks, 18–28 g, WT and ERKO mice) were purchased from Taconic Laboratories (Hudson, NY, USA). MPTP and 17β-estradiol were purchased from Sigma Chemical (St-Louis, MO, USA). In order to minimize the possible variability of the response to MPTP treatment, WT and ERKO mice were of C57Bl/6 background and were equally distributed for age and weight in experimental groups of six animals. The Laval University Animal Care Committee approved all the animal studies. All efforts were made to minimize animal suffering and to reduce the number of mice used.

An extended MPTP dose–response up to 20 mg/kg was performed in WT male C57Bl/6 mice and striatal biogenic amine concentrations of these mice was previously reported (Morissette et al., 2007). The MPTP doses (7, 9 and 11 mg/kg) that specifically affected striatal DA while sparing serotonin concentrations in WT mice (Morissette et al., 2007) were used for comparison of MPTP dose–responses of ERKOα, ERKOβ and WT mice. Mice received four 0.1 ml intraperitoneal injections with saline or a saline solution of MPTP at a two-hour interval and were killed 5 days after treatment with MPTP.

The effect of 17β-estradiol and MPTP toxicity in ERKOα and ERKOβ was compared to WT mice. Four groups of both ERKOα and ERKOβ mice were compared to WT mice. An intermediate dose of 9 mg/kg MPTP was selected and we investigated the effect of 17β-estradiol treatment in intact and MPTP mice. Each group received a 5-day pre-treatment of 17β-estradiol or vehicle prior to MPTP injections. The pre-treatment consisted of two daily subcutaneous injections (in the dorsal part of the neck) of 17β-estradiol, while control mice received injections of vehicle (0.9% saline with 0.3% gelatin). Concentrations used were 2 μg per day for 17β-estradiol such as we used previously (D'Astous et al., 2004; Morissette et al., 2007). On day 5, mice received four injections of MPTP (9 mg/kg, per intraperitoneal injection) at a 2-h interval, while the control group received saline solution. Treatments with 17β-estradiol or vehicle were continued until day 10. The next day, mice were killed with an air/halothane mixture and decapitated; trunk blood was collected and brains were quickly removed and frozen in a mixture of isopentane/dry ice and then stored at −80 °C. Gas chromatography and negative chemical ionization mass spectrometry was used to assay steroid plasma levels as described (Labrie et al., 2007).

2.2. Preparation of brain tissue

Frozen brains were cut on a cryostat in 12 μm thick slices at striatal and SN *pars compacta* regions. Coronal sections for the anterior striatum (bregmas between 1.54 and 1.18 mm), middle striatum (bregmas between 0.50 and 0.14 mm), posterior striatum (bregmas between −0.34 and −0.70 mm) and the SN (bregmas −2.70 mm to −3.28 mm) were done according to the mouse brain atlas by Franklin and Paxinos (Franklin and Paxinos, 1997).

2.3. DAT and VMAT2 autoradiography

Autoradiography using isopropylester 3β-(4-^{125}I-iodophenyl)trophane-2β-carboxylic acid ([^{125}I]-RTI-121) to the DAT was done on the striatum and the SN as previously described (Callier et al., 2001). DAT specific binding was measured using 20 pM [^{125}I]-RTI-121 (2200 Ci/mmol, Perkin–Elmer, Woodbridge, ON, Canada) in the presence of 100 nM of mazindol to estimate nonspecific binding. The striatal slices were exposed to Kodak BIOMAX film for two days and the SN slices for four days.

Autoradiography using [^{3}H]-dihydrotetrabenazine ([^{3}H]-TBZ-OH) for VMAT2 binding was done on the striatum and the SN using 20 nM of [^{3}H]-TBZ-OH (20 Ci/mmol, ARC, Saint Louis, MO USA) in the presence of 1 μM of cold TBZ-OH to estimate nonspecific binding as described (Kilbourn and Frey, 1996). Striatal slices were exposed to sensitive Kodak BIOMAX film for four weeks and the SN slices for six weeks.

2.4. DAT, VMAT2 and TH in situ hybridization

DAT mRNA levels in the SN were measured by *in situ* hybridization using a sequence encoding the entire rat DAT (Callier et al., 2000). The percentage of homologies between the rat and the mouse cDNA sequences used to generate the radioactive probe for the DAT transporter is 95% (GenBank accession no. NM_012694 and NM_010020.3). The whole rat DAT sequence was subcloned into the *Eco*RI site of pBlueScript and used in the preparation of sense and antisense strands of cRNA