Neuroscience 236 (2013) 99–109

ESTROGEN RECEPTORS AND LESION-INDUCED RESPONSE OF STRIATAL DOPAMINE RECEPTORS

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Abstract—Neuroprotection by 17β-estradiol and an estrogen receptor (ER) agonist against 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP) lesion were shown to implicate protein kinase B (Akt) signaling in mice. In order to evaluate the associated mechanisms, this study compared estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) intact or knockout (KO) and wild-type (WT) C57Bl/6 male mice following MPTP treatment of 7, 9, 11 mg/kg and/or 17β-estradiol. Striatal D1 and D2 dopamine (DA) receptors were measured by autoradiography with the specific ligands [^{3}H]-SCH 23390 and [^{3}H]-raclopride, respectively and signaling by Western blot for Akt, glycogen synthase kinase 3β (GSK3β) and extracellular-regulated signal kinases (ERK1 and ERK2). Control ERKOβ mice had lower striatal [^{3}H]-SCH 23390 specific binding than WT and ERKOα mice; both KO mice had lower [^{3}H]-raclopride specific binding. Striatal D1 receptors decreased with increasing doses of MPTP in correlation with striatal DA concentrations in ERKOα mice and remained unchanged in WT and ERKOβ mice. Striatal D2 receptors decreased with increasing doses of MPTP in correlation with striatal DA concentrations in WT and ERKOα mice and increased in ERKOβ mice. In MPTP-lesioned mice, 17β-estradiol treatment increased D1 receptors in ERKOα and ERKOβ mice and D2 receptors in WT and ERKOβ mice. MPTP did not affect striatal pAkt/Akt and pGSK3β/GSK3β levels in WT and ERKOα mice, while in vehicle-treated ERKOβ mice these levels were higher and increased with MPTP lesioning. Striatal pERK1/ERK1 and pERK2/ERK2 levels showed to a lesser extent a similar pattern. In conclusion, ERs affected the response of striatal DA receptors to a MPTP lesion and post receptor signaling.

Key words: MPTP, 17β-estradiol, Akt, GSK3, ERK, estrogen receptor.

INTRODUCTION

Dopamine (DA) is an important brain neurotransmitter playing a major role in locomotion, motivation and cognitive processes (Bjorklund and Dunnett, 2007a,b). Brain DA signaling is mediated through a family of receptors coupled to G proteins. These receptors are grouped into two classes on the basis of the G-proteins to which they couple: D1 and D5 receptors are called D1-like DA receptors which stimulate adenylyl cylase, while the D2-like DA receptors including D2, D3 and D4 receptors are negatively coupled to adenylyl cylase (Missale et al., 1998). The striatum is known as the brain region with the highest density of D1 and D2 receptors (Missale et al., 1998). In rodents, these two receptors are segregated in the striatal output pathways with D1 receptors in the direct and D2 receptors in the indirect pathway (Gerfen and Surmeier, 2011). Striatal D1 receptors are localized on post synaptic GABAergic spiny projection neurons, whereas D2 receptors are expressed both presynaptically on nigrostriatal terminals and postsynaptically on GABAergic spiny projection neurons (Roth, 1979).

The striatum is implicated in a wide variety of psychomotor disorders such as Parkinson's disease (PD), schizophrenia and drug abuse; altered DA neurotransmission is reported to play an important role in these diseases (Gerfen and Surmeier, 2011). Drug treatments of these psychiatric and neurodegenerative diseases involve the regulation of dopaminergic neurotransmission and DA receptors are an important target. PD principally involves the death of DA neurons in the substantia nigra (SN) projecting to the striatum and the gold standard treatment is to replace the lost DA by the precursor L-DOPA (Gerfen and Surmeier, 2011). By contrast, in schizophrenia overactive DA neurotransmission is treated with antipsychotics that block D2 receptors (Shin et al., 2011). The long-term adaptation of DA systems to the lack of DA or alternatively to its overactivity is important to understand since D2 receptors are a target of numerous therapeutic agents.

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Abbreviations: 17β-E_2, 17β-estradiol; Akt, protein kinase B; BSA, bovine serum albumin; D1, D1 dopamine receptor; D2, D2 dopamine receptor; DA, dopamine; EEDQ, N-ehtoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; ERK, extracellular signal-regulated kinase; ERKO, estrogen receptor knock out; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; GSK3, glycogen synthase kinase 3; MAPK, mitogen-activated protein kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PBS, phosphate-buffered saline; PD, Parkinson's disease; SDS, sodium dodecyl sulfate; SN, substantia nigra; WT, wild type.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.12.058

D1 and D2 receptors are reported to change in parkinsonian rodents and monkeys as well as humans suffering from PD, with an increase for striatal D2 receptors measured in numerous studies, whereas for D1 receptors increases, decreases and no change are documented (Laihinen et al., 1994; Morissette et al., 1996; Antonini et al., 1997; Goulet et al., 1997; Surmeier et al., 2010).

Estrogen receptors (ER) alpha (ERα) and beta (ERβ) are present in the nigrostriatal pathway (Kuppers and Beyer, 1999; Kuppers et al., 2000; Shughrue et al., 2000; Mitra et al., 2003). It is well documented that 17β-estradiol modulates both D1 and D2 receptors in the striatum (Bourque et al., 2009; Sanchez et al., 2010); this was not correlated with changes of mRNA levels of these receptors (Le Saux et al., 2006) but was associated with reduced receptor degradation as evaluated with these DA receptor kinetics after irreversible inhibition with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (Morissette et al., 1992).

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered as a contamination of a meperidine analog taken by drug users and produced similar motor symptoms as idiopathic PD (Langston et al., 1983). MPTP was since then documented as a selective neurotoxin for nigrostriatal dopaminergic neurons and is used extensively to model PD. MPTP provides specific neurotoxicity in animals such as mice, monkeys, cats and pigs, whereas rats are resistant to it (Kopin and Markey, 1988; Przedborski et al., 2001). The MPTP mouse model of PD has been extensively used in neuroprotection experiments and relevant to the present study in hormone neuroprotection (Bourque et al., 2009).

Substantial evidence shows that 17β-estradiol protects nigrostriatal DA against MPTP toxicity in male mice, whereas androgens are not protective (Bourque et al., 2009). 17β-Estradiol is also shown to be protective in the 6-hydroxydopamine (6-OHDA) model of PD on behavioral and biochemical DA markers (Baraka et al., 2011). The latter study used ovariectomized 6-OHDA rats and reported neuroprotection with 17β-estradiol, the ERα agonist PPT and the selective estrogen receptor modulator, raloxifene; whereas the ERβ agonist DPN and tamoxifen were inactive as seen by their lack of neuroprotective activity in the male MPTP mouse model (Bourque et al., 2009). Hence, estrogenic drugs protected rodents of both sexes against toxins affecting nigrostriatal DA neurons. The present experiments were therefore performed in the male MPTP mouse model that has been extensively used in our laboratory. Moreover, male mice were used in order to consider the epidemiologic results demonstrating that more men than women develop PD (Wooten et al., 2004; Shulman, 2007).

Both D1 and D2 receptors are reported to stimulate mitogen-activated protein kinase (MAPK)/extracellular-regulated kinases (ERK) signaling (Cai et al., 2000), whereas the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt)/glycogen synthase kinase 3β (GSK3β) signaling pathway is shown to be affected by a D2 but not D1 receptor agonist and antagonist (Sutton and Rushlow, 2011). MPTP lesion and dopaminergic treatments affect these signaling pathways (Bychkov et al., 2007; Morissette et al., 2010). Hence, ERs modulate neuroprotection and 17β-estradiol could combat MPTP toxicity through mechanisms involving the regulation of receptors and Akt/GSK3β and ERK1/2 signaling.

The aims of the present study were to seek the response of striatal D1 and D2 receptors to a MPTP lesion and the implication of ERα and ERβ in this response using knockout (KO) mice for these ERs. Receptor specific binding to striatal D1 and D2 receptors were measured and compared to DA levels and to ERK1/2 and Akt/GSK3β signaling.

EXPERIMENTAL PROCEDURES

Animals and treatments

Adult male C57Bl/6 wild-type (WT), estrogen receptor alpha knockout (ERKOα) and estrogen receptor beta knockout (ERKOβ) mice (7–12 weeks, 18–28 g) were purchased from Taconic Laboratories (Hudson, NY, USA). MPTP and 17β-estradiol were purchased from Sigma Chemical (St-Louis, MO, USA). Mice were housed 3–4 per cage under a 12:12-h light/dark cycle at 22–23 °C. Animals received mouse chow and water *ad libitum* and were equally distributed for age and weight in experimental groups of 6–8 animals. All efforts were made to minimize animal suffering and to reduce the number of mice used. The Laval University Animal Care Committee approved all the animal studies.

We previously reported striatal biogenic amine concentrations of WT C57Bl/6 male mice following an extended MPTP dose–response study up to 20 mg/kg (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, were thus used for comparison of MPTP dose–responses of ERKOα, ERKOβ and WT mice (Morissette et al., 2007). Mice (6 per group) received a total of four (0.1 ml) intraperitoneal injections of the vehicle (0.9% saline with 0.3% gelatin) or a saline solution of MPTP (each containing respectively 7, 9 or 11 mg/kg), one every 2 h during the light cycle (9:00 am, 11:00 am, 1:00 pm and 3:00 pm) and were killed 5 days after treatment with MPTP.

Second, the effect of 17β-estradiol and MPTP in WT, ERKOα and ERKOβ were compared. Four groups comprised of 8 mice per group of both ERKOα and ERKOβ mice were compared to WT mice. An intermediate dose of 9 mg/kg MPTP was selected and the effect of 17β-estradiol treatment was investigated 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, 2 μg per day as we previously used (Callier et al., 2000; Morissette et al., 2007), while control mice received vehicle injections. On day 5, mice received a total of four separate 0.1-ml intraperitoneal injections of the vehicle treatment or a saline solution of MPTP (9 mg/kg of MPTP per injection) at 2-h intervals during the light cycle (9:00 am, 11:00 am, 1:00 pm and 3:00 pm). Treatments with 17β-estradiol or vehicle were continued until day 10. Mice were killed 5 days after the MPTP lesion, or 24-h after the last 17β-estradiol treatment, with an air/halothane mixture and decapitated; brains were quickly removed and frozen in a mixture of isopentane/dry ice and then stored at −80 °C.