Journal of Steroid Biochemistry & Molecular Biology 161 (2016) 84–91

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Original article

Estrogen receptors modulate striatal metabotropic receptor type 5 in intact and MPTP male mice model of Parkinson's disease

S. Al-Sweidi[a,b], M. Morissette[a], T. Di Paolo[a,b,*]

[a] Neuroscience Research Unit, Centre Hospitalier Universitaire de Québec, CHUL, Quebec City, Quebec G1V 4G2, Canada
[b] Faculty of Pharmacy, Laval University, Quebec City, Quebec G1K 7P4, Canada

ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 23 December 2015
Accepted 7 February 2016
Available online 9 February 2016

Keywords:
Estrogen receptor alpha
Estrogen receptor beta
Knock out
Parkinson
Striatum
Metabotropic glutamate receptor 5
BDNF

ABSTRACT

Glutamate is the most important brain excitatory neurotransmitter and glutamate overactivity is well documented in Parkinson's disease (PD). Metabotropic glutamate (mGlu) receptors are reported to interact with membrane estrogen receptors (ERs) and more specifically the mGlu5 receptor subtype. 17β-estradiol and mGlu5 antagonists have neuroprotective effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. We previously reported that ERα and ERβ are involved in neuroprotection following MPTP toxicity. The present study investigated the implication of ERs on the mGlu5 receptor adaptive response to MPTP toxicity in the brain of wild type (WT), ER knockout (ERKO)α and ERKOβ male mice. Autoradiography of [^{3}H]ABP688 specific binding to striatal mGlu5 receptors showed a dorsal/ventral gradient similar for WT, ERKOα and ERKOβ mice with higher values ventrally. The lateral septum had highest [^{3}H]ABP688 specific binding that remained unchanged in all experimental groups. ERKOα and ERKOβ mice had similarly lower striatal [^{3}H]ABP688 specific binding than WT mice as measured also by Western blots. MPTP dose-dependently decreased striatal [^{3}H]ABP688 specific binding in WT but not in ERKOα and ERKOβ mice; this correlated positively with striatal dopamine concentrations. A 17β-estradiol treatment for 10 days left unchanged striatal [^{3}H]ABP688 specific binding of unlesioned mice of the three genotypes. 17β-estradiol treatment for 5 days before MPTP and for 5 days after partially prevented the mGlu5 receptor decrease only in WT MPTP mice and this was associated with higher BDNF striatal contents. These results thus show that in male mice ERs affect striatal mGlu5 receptor levels and their response to MPTP.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Glutamate is the most prominent neurotransmitter, being present in over 50% of nervous tissue [1]. There are two types of glutamate receptors, ionotropic and metabotropic (mGlu) receptors [2], both of which play a role in chronic brain disorders and neurodegenerative diseases such as Parkinson's disease (PD) [3]. mGlu receptors are G protein-coupled receptors which are categorized into three families according to their sequence homology, signal transduction mechanisms and pharmacological properties [3]. Group I receptors includes mGlu1 along with mGlu5 receptors. Group II receptors include mGlu2 and mGlu3 receptors, while Group III includes mGlu4 and mGlu6-8 receptors [3].

The depletion of striatal dopamine (DA) is a main feature of the neurodegenerative process of PD [4]. 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 [5,6]. Nevertheless for the majority of PD patients, these therapies eventually loose effectiveness and are associated with side-effects [7]. 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.

17β-estradiol is neuroprotective in 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 treatment with 17β-estradiol before MPTP prevents the loss of striatal DA [8], DA transporter (DAT), and

Abbreviations: A, adenosine; DA, dopamine; CNS, central nervous system; CREB, cAMP response element-binding protein; DAT, dopamine transporter; ERK, extracellular signal-regulated kinase; ER, estrogen receptor; ERKO, estrogen receptor knock out; L-DOPA, levodopa; MAPK, mitogen-activated protein kinase; mGlu, metabotropic glutamate; MPEP, [2-methyl-6-(phenylethynyl)pyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl] pyridine; PD, Parkinson's disease; RIPA, radioimmunoprecipitation lysis buffer; VMAT2, vesicular monoamine transporter 2; WT, wild-type.

* Corresponding author at: Neuroscience Research Unit, Centre Hospitalier Universitaire de Québec, 2705 Laurier Boulevard, Quebec, Quebec G1V 4G2, Canada. Fax: +1 418 654 2298.
E-mail address: therese.dipaolo@crchul.ulaval.ca (T. Di Paolo).

http://dx.doi.org/10.1016/j.jsbmb.2016.02.004
0960-0760/© 2016 Elsevier Ltd. All rights reserved.

vesicular monoamine transporter 2 (VMAT2) [9]. Using specific agonists for ERα and ERβ we have previously shown that ERα agonists protect against MPTP toxicity in male mice [10]. In estrogen receptor (ER) knock out (ERKOα and ERKOβ) male mice, we previously showed a more extensive MPTP-induced DA depletion 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 [11]. 17β-estradiol partially prevented the MPTP-induced decrease in striatal DA levels only in WT mice [11].

Activation of mGlu5 receptors contributes to nigro-striatal damage induced by MPTP in mice whereas inhibition with its subtype-selective antagonists [2-methyl-6-(phenylethynyl) pyridine (MPEP) is neuroprotective [12]. The highly specific metabotropic glutamate receptor 5 antagonist, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl] pyridine (MTEP) was also shown to protect dopaminergic and noradrenergic neurons from degeneration in MPTP-treated monkeys [13].

Colocalization and immunoprecipitation reveal that ERs directly interact with mGlu receptors [14]. In the striatum, ERα but not ERβ is reported to regulate mitogen-activated protein kinase (MAPK)-dependent cAMP response element-binding protein (CREB) phosphorylation through mGlu5 receptor signaling [15]. Hence, the present study thus investigated the implication of ERα and ERβ on the mGlu5 receptor adaptive response to MPTP toxicity in the striatum of wild type (WT), ERKOα and ERKOβ male mice.

2. Material 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 to eight 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 [11]. The MPTP doses (7, 9 and 11 mg/kg) that specifically affected striatal DA while sparing serotonin concentrations in WT mice [11] 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.

Then, 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 to investigate 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 of 17β-estradiol such as we used previously [10,11]. On day 5, mice received four injections of MPTP (9 mg/kg, per intraperitoneal injection) at a 2-hour interval, while the control groups received saline solution. Treatments with 17β-estradiol or vehicle were continued until day 10. The next day, mice were killed with an air/isoflurane mixture and decapitated; brains were quickly removed and frozen in a mixture of isopentane/dry ice and then stored at −80 °C.

2.2. Preparation of brain tissue

Frozen brains were cut on a cryostat in 12 μm thick slices at striatal region. Coronal sections of the striatum (bregma 0.62 mm) were done according to the mouse brain atlas by Franklin and Paxinos [16].

2.3. [^{3}H]ABP688 autoradiography

mGlu5 receptor specific binding was evaluated with [^{3}H] ABP688 binding (81.6 Ci/mmol, gift from Novartis, Switzerland), a high-affinity and selective mGlu5 receptor antagonist [17], according to our published conditions [18,19] using 5 nM [^{3}H] ABP688 and 10 μM MPEP to estimate non-specific binding. Slide-mounted tissue sections were exposed to BioMax MR films (Kodak; along with Amersham standard [^{3}H]-microscales, USA) for 14 days at room temperature. For all autoradiographies presented in this study, we used 6 brain slices for each animal.

2.4. Western blot

Small frozen striatal pieces taken from coronal brain sections were homogenized in radioimmunoprecipitation assay (RIPA) lysis buffer supplemented with protease and phosphatase inhibitors purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Homogenates were allowed to solubilize for 30 min on ice and then centrifuged at 16,000 × *g* for 15 min. Protein content of supernatants was measured with a modified Lowry assay (Micro BCA protein assay kit, Thermo Scientific, Rockford, IL). An equal amount of protein (14 μg) from each sample was loaded on the gel along with a prestained protein ladder for the determination of molecular weights. Proteins samples, previously heated at 95 °C for 15 min, were resolved using 6% or 12% SDS-polyacrylamide gel electrophoresis for mGlu5 receptor and BDNF respectively with a Triple wide mini-vertical gel system (C.B.S. Scientific Company, Del Mar, CA) and transferred by electrophoresis to polyvinylidine difluoride (PVDF) membranes. The membranes were blocked with 5% bovine serum albumin (BSA) diluted in 0.1% Tween 20/ Phosphate-buffered saline and incubated overnight with the primary antibodies. Antibody against mGlu5 receptor (diluted 1:5000) was obtained from Abcam (Cambridge, MA, USA). The antibody against BDNF (diluted 1:500) was from Santa Cruz Biotechnology (Santa Cruz, CA) while antibody against βIII-tubulin was purchased from EDM Millipore Corporation (Temecula, CA) and was used as a loading control. After incubation with the primary antibody, membranes were washed and incubated with horseradish peroxidase-coupled secondary antibodies (Cell Signaling Technology, Pickering, On: diluted 1:5000). Immunoreactive bands were detected using an enhanced chemiluminescence system (Clarity Western ECL substrate, Bio-Rad, Mississauga, ON). Densitometric analysis of bands was performed using AlphaView Image Analysis Systems (FluorChem Q Alpha Innotech). Experiments were repeated two to three times.

2.5. Statistical analysis

Autoradiograms of specific binding were analyzed using Scion Image 1.63 software. The striatum was subdivided into four sub regions including dorsolateral (DL), dorsomedial (DM), ventrolateral (VL), and ventromedial (VM) (Fig. 1). The statistical analyses were done using GraphPad Prism software (v.6.0b) for Macintosh Computer. A one-way ANOVA was used to compare MPTP and/or

Download English Version:

https://daneshyari.com/en/article/1991245

Download Persian Version:

https://daneshyari.com/article/1991245

Daneshyari.com