Neuropharmacology 62 (2012) 2363–2372

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

# Sex and temporally-dependent effects of methamphetamine toxicity on dopamine markers and signaling pathways

Mélanie Bourque [a,b], Dean E. Dluzen [c], Thérèse Di Paolo [a,b,*]

[a] Molecular Endocrinology and Genomic Research Center, Centre de recherche du CHUQ (CHUL), Quebec City, Quebec, Canada G1V 4G2
[b] Faculty of Pharmacy, Laval University, Quebec City, Quebec, Canada G1K 7P4
[c] Department of Anatomy and Neurobiology, Northeast Ohio Medical University (NEOMED), Rootstown, OH 44272, USA

ARTICLE INFO

*Article history:*
Received 18 July 2011
Received in revised form
10 February 2012
Accepted 13 February 2012

*Keywords:*
Methamphetamine
Dopamine
Sex difference
Akt
Neurotoxicity
Striatum

ABSTRACT

Methamphetamine induces a greater neurodegenerative effect in male versus female mice. In order to investigate this sex difference we studied the involvement of Akt and extracellular signal-regulated kinase (ERK1/2) in methamphetamine toxicity as a function of time post-treatment (30 min, 1 and 3 days). Methamphetamine-induced decreases in dopamine concentrations and dopamine transporter (DAT) specific binding in the medial striatum were similar in female and male mice when evaluated 1 day post-methamphetamine (40 mg/kg). At 3 days post-methamphetamine, striatal dopamine concentration and DAT specific binding continued to decline in males, whereas females showed a recovery with increases in dopamine content and DAT specific binding in medial striatum at day 3 versus day 1 post-methamphetamine. The reduction in striatal vesicular monoamine transporter 2 specific binding observed at 1 and 3 days post-methamphetamine showed neither a sex- nor temporal-dependant effect. Under the present experimental conditions, methamphetamine treatments had modest effects on dopamine markers measured in the substantia nigra. Proteins assessed by Western blots showed similar reductions in both female and male mice for DAT proteins at 1 and 3 days post-methamphetamine. An increase in the phosphorylation of striatal Akt (after 1 day), glycogen synthase kinase 3β (at 1 and 3 days) and ERK1/2 (30 min post-methamphetamine) was only observed in females. Striatal glial fibrillary acidic protein levels were augmented in both females and males at 3 days post-methamphetamine. These results reveal some of the sex- and temporally-dependent effects of methamphetamine toxicity on dopaminergic markers and suggest some of the signaling pathways associated with these responses.
© 2012 Elsevier Ltd. All rights reserved.

## 1. Introduction

Methamphetamine (MA) abuse can induce long-term effects resulting in neurotoxicity in humans. Post-mortem analyses of brain in chronic MA users have found reduced levels of striatal dopamine (DA), tyrosine hydroxylase and dopamine transporter (DAT) (Wilson et al., 1996). Positron emission tomography studies have also revealed a decrease in DAT and vesicular monoamine transporter 2 (VMAT2) densities, D2 receptors, serotonin transporter as well as the activation of microglia in striatum of MA users (Johanson et al., 2006; Sekine et al., 2008, 2006; Volkow et al., 2001a; Volkow et al., 2001b). Moreover, MA users are reported to have a higher incidence for developing neurodegenerative disorders such as Parkinson's disease (Callaghan et al., 2010).

Gender differences are present with regard to use and response to MA. Women appear more addicted to MA and show a lower level of DA evoked by amphetamine than men (Liu and Dluzen, 2007; Munro et al., 2006). Studies performed with rodents have also shown sex differences in response to MA treatment. Greater neurotoxic effects of MA have been reported in male mice, as shown by a higher reduction in striatal DA concentration and DAT specific binding than comparably treated female mice (Bourque et al., 2011).

Difference in DA systems between males and females could contribute to this sex difference in MA toxicity. DA transporters seem to be important in the toxicity induced by MA, since knockout of the DAT does not induce striatal DA neurotoxicity in male mice

*Abbreviations:* DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; ERK, extracellular signal-regulated kinase; GFAP, glial fibrillary acidic protein; GPER1, G protein-coupled estrogen receptor 1; GSK3β, glycogen synthase kinase 3β; MA, methamphetamine; pAkt, phosphorylated Akt at serine 473; pERK1/2, phosphorylated ERK1/2; pGSK3β, phosphorylated GSK3β at serine 9; PI3K, phosphatidylinositol-3 kinase; VMAT2, vesicular monoamine transporter 2; [$^{125}$I]-RTI-121, 3β-(4-[$^{125}$I]iodophenyl)tropane-2β-carboxylic acid isopropyl ester; [$^{3}$H]-TBZ-OH, [$^{3}$H]dihydrotetrabenazine.

* Corresponding author. Molecular Endocrinology and Genomic Research Center, Centre de recherche du CHUQ (CHUL), 2705 Laurier Blvd, Quebec City, Quebec, Canada G1V 4G2. Tel.: +1 418 654 2296; fax: +1 418 654 2761.
*E-mail address:* therese.dipaolo@crchul.ulaval.ca (T. Di Paolo).

0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2012.02.009

treated with MA (Fumagalli et al., 1998). And, a reduction of DA vesicular storage as achieved by a heterozygous VMAT2 knockout in male mice or by using a mutant strain of male and female mice only expressing a 5–10% of the normal amount of VMAT2 resulted in an increased toxicity in response to MA treatment (Fumagalli et al., 1999; Guillot et al., 2008). Interestingly, in female rodents, where decreased MA-induced toxicity is observed, greater function of DAT and VMAT2 activities is present (Bhatt and Dluzen, 2005; Dluzen et al., 2008; Ji et al., 2007; Morissette and Di Paolo, 1993; Walker et al., 2000). Thus, a more efficient DA uptake and vesicular storage could decrease the degree of oxidative stress and favor a more efficient protection of DA terminals. Therefore, one goal of this report was to investigate how specific binding to DA transporters was modulated as a function of sampling at some critical times after MA treatment. In this way, we can provide an overview of the modulation of MA upon DA transporters as a function of these sex differences and time post-MA administration.

The phosphatidylinositol-3 kinase (PI3K)/Akt and extracellular signal-regulated kinase (ERK1/2) pathways are important regulators of cell functions (Manning and Cantley, 2007; Pearson et al., 2001) and these two pathways are activated in response to MA and amphetamine treatment (Garcia et al., 2005; Hebert and O'Callaghan, 2000; Shi and McGinty, 2006; Wei et al., 2007). These two pathways converge downstream to glycogen synthase kinase 3β (GSK3β), a constitutive active kinase which can be inhibited by phosphorylation on its serine 9 by Akt and ERK1/2 (Hetman et al., 2002; Manning and Cantley, 2007). Activation of GSK3β is associated with neuronal apoptosis (Enguita et al., 2005) and is shown to mediate toxin-induced striatal neuron death (Chen et al., 2004) whereas inhibition of this kinase favors cell survival (Hetman et al., 2000). We have recently shown that Akt and GSK3β are altered with MA treatment, in a manner more pronounced in male mice as shown at 7 days post-MA (Bourque et al., 2011). Moreover, the decrease observed in DA concentration and DAT specific binding was correlated with the reduction of phosphorylated Akt. A previous report demonstrated that inhibition of Akt by amphetamine reduces DAT cell surface expression (Garcia et al., 2005) and ERK1/2 could also regulate DAT cell surface expression (Moron et al., 2003). In our preceding experiment, we have not observed a long-term effect of MA on ERK1/2 when sampled at 1 week post-treatment in female and male mice (Bourque et al., 2011). However, previous studies have reported activation of ERK1/2 occurs shortly after MA and amphetamine administration (Hebert and O'Callaghan, 2000; Shi and McGinty, 2006). Therefore, a thorough investigation into the involvement of Akt and ERK pathways in these sex differences to MA toxicity requires a time-dependent evaluation.

Accordingly, in the present experiment, we investigated the potential sex differences in the toxicity induced by MA on dopaminergic markers at various critical time points after MA treatment (30 min, 1 and 3 days). These time periods were selected since significant sex differences in MA-induced DA depletion are present at 3 days, but not at 30 min post-MA (Dluzen et al., 2010). The measures of dopaminergic markers at different time points after MA administration could provide important information related to sex difference in MA toxicity and enable us to track the pathways and markers associated with these sex differences.

## 2. Materials and methods

### 2.1. Animals and treatments

Intact female and male CD-1 mice (2–3 months old) were purchased from Charles River Laboratories (Wilmington, MA, USA). Mice were housed individually in plastic cages, to avoid the potential for stress-induced fighting. Mice were allowed free access to food and water, while maintained at room temperature of approximately 22 °C under a 12 h light–dark cycle (lights on at 06:00 h). All conditions were according to NIH regulations and approved by the Institutional Animal Care and Use Committee (IACUC) at Northeast Ohio Medical University (NEOMED). All efforts were made to minimize the number of animals used and their suffering.

Mice were treated with a single intraperitoneal injections of 40 mg/kg MA (Sigma, St Louis, MO) in saline ($n = 4$ per group). A control group ($n = 7$ per group) of mice received a saline solution. This schedule of MA administration was chosen based on our previous data with this dose in male and female mice (Dluzen et al., 2011, 2010) and an earlier study of a dose/regime dependent MA response experiment on striatal DA (Liu and Dluzen, 2006). Moreover, MA at 40 mg/kg has been used by many investigators (Barrett et al., 2001; Jayanthi et al., 2004; Xie et al., 2002; Zhu et al., 2005) and found to have a good survival rate (Jayanthi et al., 2004; Liu and Dluzen, 2006; Zhu et al., 2006).

### 2.2. Brain preparation

Mice were killed by rapid decapitation 30 min, 1 day or 3 days post-MA or saline. Brains were removed, bisected and a unilateral striatum was used to assay DA and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations. The contralateral hemisphere was frozen in liquid nitrogen. The striatum (bregma 1.54 at 0.38 mm) and the substantia nigra (bregma −2.80 at −3.88 mm) of the contralateral hemisphere were cut on a cryostat in 12 μm slices for autoradiography and *in situ* hybridization and striatal dissection was performed with 50 μm slices for Western blots. Slices were kept at −80 °C until assayed.

### 2.3. Striatal dopamine assay

The unilateral striatum used for determination of DA and DOPAC concentrations was dissected, weighed and placed in 0.1N $HClO_4$ at 4 °C. The samples were sonicated and centrifuged. The supernatants were used to measure DA and DOPAC concentrations by high-performance liquid chromatography with electrochemical detection, as previously described (Gao and Dluzen, 2001). Concentrations of DA and DOPAC were expressed as pg per mg of tissue weight. In addition, the DOPAC/DA ratio was calculated from these concentrations.

### 2.4. DAT and VMAT2 autoradiography

DAT autoradiography in the striatum and substantia nigra was performed as previously described (Callier et al., 2001). DAT specific binding used 20 pmol of the ligand 3β-(4-[$^{125}$I]iodophenyl)tropane-2β-carboxylic acid isopropyl ester ([$^{125}$I]-RTI-121) (2200 Ci/mmol, PerkinElmer, Boston, MA, USA). Non-specific binding was evaluated with binding in the presence of 100 nM of Mazindol (Sandoz Pharmaceuticals, Dorval, Quebec). Brain slices were apposed to Kodak films (Biomax), 31 h for the striatum and 72 h for the substantia nigra. VMAT2 autoradiography in the striatum and the substantia nigra was performed using the specific ligand [$^{3}$H] dihydrotetrabenazine ([$^{3}$H]-TBZ-OH, American Radiolabeled Chemicals, St. Louis, MO, USA) (Kilbourn and Frey, 1996). Specific binding was evaluated using 20 nM of [$^{3}$H]-TBZ-OH (20 Ci/mmol) and 1 μM of cold TBZ-OH for the non-specific binding. Slices were exposed to Kodak films (Biomax), 4 weeks for the striatum and 6 weeks for the substantia nigra. Films were analyzed using the software NIH Image 1.63.

### 2.5. DAT in situ hybridization

DAT mRNA in the substantia nigra was measured by *in situ* hybridization using a cDNA probe labeled with [$^{35}$S]-UTP, as previously described (Jourdain et al., 2005). Slices were exposed to Kodak films (Biomax) for 3 days and analyzed using the software NIH Image 1.63.

### 2.6. Western blots

Western blots were performed as previously described (Bourque et al., 2011). Striatum were homogenized in lysis buffer (0.5% SDS, 8 M urea, 2% β-mercaptoethanol, 1% phenylmethylsulfonyl 100 mM) supplemented with protease and phosphatase inhibitors (inhibitor cocktails from Sigma, Oakville, ON). Homogenates were kept on ice for 30 min to allow solubilization and then sonicated. Proteins content was measured with a Bradford protein assay (Bio-Rad, Mississauga, ON). Proteins were resolved using 12% SDS-polyacrylamide gel electrophoresis with a triple wide mini-vertical gel system (C.B.S. Scientific Company, Del Mar, CA) and electrophoretically transferred to polyvinylidine difluoride (PVDF) membrane. The membranes were blocked with 5% bovine albumin serum (BSA) diluted in 0.1% Tween 20/phosphate-buffered saline and incubated overnight with the primary antibodies. The antibodies against phosphorylated Akt at serine 473 (pAkt, diluted 1:1000), phosphorylated GSK3β at serine 9 (pGSK3β, diluted 1:1000), Akt (diluted 1:2000), GSK3β (diluted 1:2000), phosphorylated ERK1/2 (pERK1/2, diluted 1:1000), ERK1 (diluted 1:1000), ERK2 (diluted 1:1000) and glial fibrillary acidic protein (GFAP) (diluted 1:1000) were obtained from Cell Signaling Technology (Pickering, ON). G protein-coupled estrogen receptor 1 (GPER1)/GPR30 antibody (diluted 1:5000) was obtained from Abcam (Cambridge, MA). βIII-tubulin antibody (diluted 1:10,000) was from Chemicon International (Hornby, ON). Anti-DAT monoclonal antibody (diluted

Download English Version:

https://daneshyari.com/en/article/2493527

Download Persian Version:

https://daneshyari.com/article/2493527

Daneshyari.com