DOI 10.1007/s00702-003-0045-3
J Neural Transm (2003) 110: 1215–1224

Journal of Neural Transmission

Acute effects of estrogen upon methamphetamine induced neurotoxicity of the nigrostriatal dopaminergic system

T. M. Gajjar, L. I. Anderson, and **D. E. Dluzen**

Department of Anatomy, Northeastern Ohio Universities College of Medicine (NEOUCOM), Rootstown, OH, U.S.A.

Received January 13, 2003; accepted July 20, 2003

Summary. Estrogen acts as a neuroprotectant of the nigrostriatal dopaminergic system when given chronically to female mice prior to Methamphetamine (MA) insult. In this report, we tested the acute effects of Estradiol Benzoate (EB-10 μg in Oil) in ovariectomized CD-1 mice to function as a neuroprotectant when administered prior to (Experiment 1) or after (Experiment 2) MA treatment. Striatal dopamine (DA) concentrations and DOPAC/DA ratios were measured to assess the neuroprotective effects of EB. In Experiment 1, we observed that EB exerted a neuroprotective effect upon striatal dopamine concentrations when administered at 24 and 12, but not at 0.5, hours prior to MA injection and upon DOPAC/DA ratios when administered at 24, 12 and 0.5 hours prior to MA. In Experiment 2, no evidence for estrogen to protect the striatum from MA insult was obtained when EB was administered at 15, 30, 60 or 120 minutes after MA. These results show that EB can act as a modulator of MA-induced nigrostriatal dopaminergic neurotoxicity suggestive of a neuroprotectant, when administered within 0.5 hour of MA insult as assessed by measures of DOPAC/DA, but fails to prevent depletion of DA when given after MA insult. The data suggest that estrogen may exert this rapid neuroprotective effect through a non-genomic mechanism.

Keywords: Neurodegeneration, corpus striatum, Parkinson's disease, neuroprotection.

Introduction

Various epidemiological studies show that Parkinson's disease is more common in males as compared to females (Diamond et al., 1990; Dluzen et al., 1998; Baldereschi et al., 2000; Schrag et al., 2001; Swerdlow et al., 2001). This male preponderance has in part been attributed to estrogen, which can act as a neuroprotectant in females. Estrogen is widely accepted as a neuroprotective agent against stroke (Toung et al., 1998; Fukuda et al., 2000; Liao et al., 2001; Wise et al., 2001) and neurodegenerative disorders like Alzheimer's disease

(Granholm et al., 2000; Miller et al., 2001; Owens et al., 2002). Further, laboratory experiments have shown that striatal dopamine (DA) depletion by neurotoxins like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Dluzen et al., 1996a, b; Miller et al., 1998; Granbois et al., 2000; Callier et al., 2001), methamphetamine (MA) (Dluzen and McDermott, 2000, 2002; Yu and Liao, 2000a; Gao and Dluzen, 2001; Yu et al., 2002) and 6-hydroxydopamine (Dluzen, 1997) were decreased when rodents were pretreated with estrogen. Moreover, striatal DA output evoked by neurotoxins which target the nigrostriatal dopaminergic (NSDA) system is significantly decreased in the presence of estrogen (Disshon and Dluzen, 1997, 2000; Arvin et al., 2000; Myers et al., 2003). Taken together, these data demonstrate the capacity for estrogen to function as a neuroprotectant of the NSDA system (Reviewed in: Dluzen et al., 1998; Miller et al., 1998; Dluzen, 2000; Dluzen and Horstink, 2003; Horstink et al., 2003) Interestingly, in contrast to these data which indicate neuroprotection, estrogen does not have any neurorestorative effect when administered one week after MA (Gao and Dluzen, 2001; Dluzen and McDermott, 2002).

In these previous reports, this protective effect of estrogen was produced when the estrogen was administered as long term release pellets of 17-β estradiol at 7 days before injection of MA (Dluzen and McDermott, 2002) or through a series of daily estrogen treatments (Yu and Liao, 2000a). It is not known what effects estrogen treatment would have if given within shorter time periods (minutes to hours) either before or after MA injection. The purpose of this report is to assess the potential for acute neuroprotective or neurorestorative effects of estrogen upon MA induced neurotoxicity. To address this issue, in Experiment 1, mice were injected with estrogen at 24, 12 or 0.5 hours before an injection of MA. The second major goal of this report was to determine whether estrogen can act as a neurorestorative agent when given immediately after MA injection as has been demonstrated for the ischemic injury model of nervous system (Yang et al., 2000). Accordingly, in Experiment 2, estrogen was administered at 0.25, 0.5, 1 or 2 hours after MA injection. For both experiments, DA and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations were measured at 1 week post-MA to determine whether any neuroprotective effects were observed as indicated by striatal DA concentrations and DOPAC/DA ratios. Measurement of the DOPAC/DA ratios was included since these may provide a more sensitive indicator of NSDA functioning with which to complement the DA concentration measurements.

Materials and methods

Animals

Female CD-1 mice (retired breeders – 6–9 months of age) were used for this experiment (N = 28 – Exp 1 and N = 32 – Exp 2). Mice were housed individually in plastic cages with free access to food and water. They were maintained at a room temperature of ~22°C with a humidity controlled atmosphere and a 12 hour light-dark cycle (lights on at 0600 hr). All

conditions were maintained according to NIH regulations and approved by the IACUC at NEOUCOM.

Ovariectomy

All mice were ovariectomized prior to use in the experiment. Ovariectomies were performed to remove the primary source of endogenous estrogen from the mice and thereby permit greater control with the estrogen treatments administered. The mice were anesthetized with Ketaset (Ketamine HCl + Acepromazine Maleate in a 10:2 proportion at 100 mg/kg) through an intramuscular injection. Incisions were made on the posterolateral sides of the abdomen and the ovaries were exposed by dissection. The ovaries were ligated and removed. The abdominal muscular layers sutured and skin apposed with wound clips. At least one week was allowed for healing and depletion of endogenous circulating estrogen before the mice were used in the experiments.

Treatments

Estradiol Benzoate (EB): The EB (17-β Estradiol 3-Benzoate – Sigma) was made up in Sesame oil (Sigma) at a final concentration of 10 μg/0.1 ml. The dosage for injection was 10 μg for each mouse. The EB was injected subcutaneously within the dorsal neck region.

Methamphetamine (MA): The MA was made in Normal Saline (Baxter) and prepared fresh just before use in the experiment. Each mouse received a single intraperitoneal injection at a dose of 40 mg/kg body weight.

Procedure

Experiment 1: The purpose of Experiment 1 was to: (1) determine whether a single acute administration of EB could produce neuroprotection of the NSDA system and (2) establish the temporal relationship between this EB administration and the demonstration of a neuroprotective effect. The mice were divided into 5 groups; 4 groups were given EB at 24 (n = 7), 12 (n = 7) or 0.5 (n = 6) hours prior to MA while the fourth group (n = 4) was given EB at 24 hours prior to treatment with the vehicle for MA (saline). The fifth group (n = 4) received only MA.

Experiment 2: Like that of Experiment 1, the purpose of Experiment 2 was to determine whether a single, acute administration of EB could function as a neuroprotectant, however, now we examined the effect of EB when administered after MA treatment. In addition, the temporal relationship of this effect was examined. The mice were divided into 5 groups; 4 groups received MA followed by the EB at 15 (n = 6), 30 (n = 7), 60 (n = 6) and 120 (n = 7) minutes post-MA. The fifth group (n = 6) received only MA.

Catecholamine assay

For both experiments, the mice were euthanized by rapid decapitation at one week post-MA and their striatum dissected, weighed, and put in 0.1 N perchloric acid at 4°C. While maintaining a temperature of 4°C, the tissue was sonicated (Heat Systems Ultrasonics) for 20 seconds and then centrifuged for 15 minutes. The supernatant fluid was separated and DA and DOPAC concentrations were determined by injection into a 20 microliter loop on an ESA High Pressure Liquid Chromatography system with a coulochem II detector set at E1:400 mV, R1:2 μA, E2: – 350 mV, R2: – 100 nA. Biogenic amines were separated on a Biophase C-18, 3 μm sphere column (Bioanalytical Systems Inc.). The Phase 2 Buffer was prepared from 50 mM sodium acetate, 27.4 mM citric acid, 10 mM sodium hydroxide, 0.1 mM sodium octyl sulfate, 0.1 mM EDTA in distilled water. Final pH was adjusted to 4.5 and the buffer was filtered before running the samples. The sensitivity of this assay was <12.5 pg/20 ml as determined by the reliable determinations of peaks within the standards. DA and DOPAC concentrations were expressed as pg/mg of brain tissue.

Analysis

For both experiments, comparisons among the groups were performed using separate one-way ANOVAs. The Fisher's protected LSD post-hoc tests was applied to identify individual pair-wise differences if the overall difference among the groups was statistically significant. A $p < 0.05$ was required for the results to be considered statistically significant.

Results

Experiment 1

Dopamine concentration. A summary of the DA concentrations from the five groups is presented in Fig. 1A. Analyses of the data indicated that the overall difference among the groups was statistically significant ($F_{4,23} = 6.63$, $p = 0.011$). Post hoc comparisons revealed that the DA concentrations were significantly increased in mice treated with EB at 24 hours and receiving the vehicle for MA and mice treated with EB at 24 and 12 hours prior to MA as compared to the MA only group. Though the DA levels in the 0.5 hour group were higher than controls, they were not statistically significant. In addition, striatal DA concentrations of mice receiving EB at 24 hours followed by the vehicle for MA were significantly increased compared with mice receiving EB at 12 and 0.5 hours prior to MA.

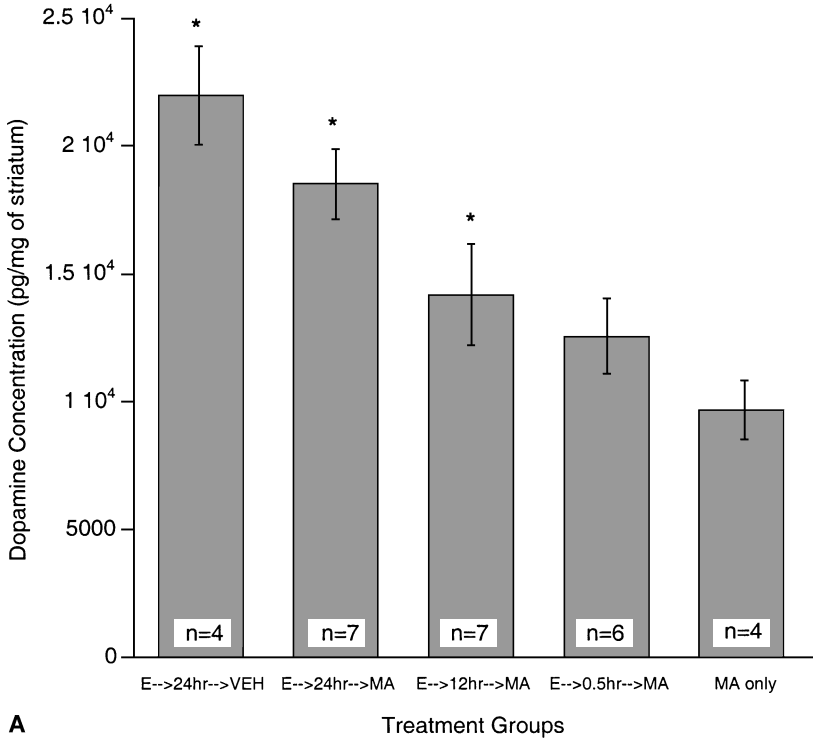
DOPAC/DA ratio. The DOPAC/DA ratios are shown in Fig. 1B. Analyses of the data reveal that the difference among the groups was statistically significant ($F_{4,23} = 5.49$, $p = 0.003$). Post-hoc comparisons revealed that all animals treated with EB showed significantly lower ratios compared to MA only controls.

Experiment 2

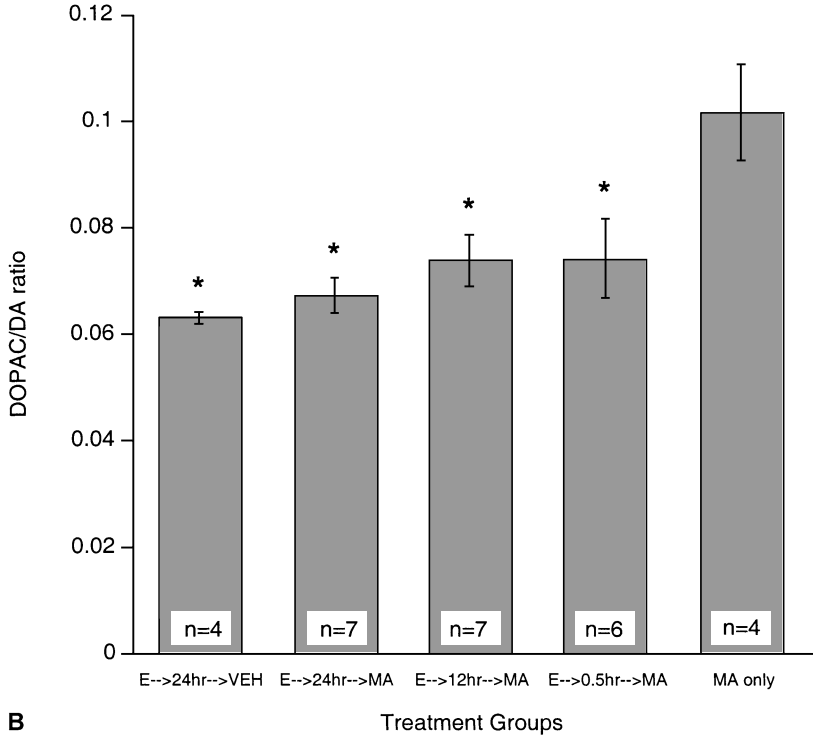
Dopamine concentration. Striatal DA concentrations obtained from mice of Experiment 2 are summarized in Fig. 2A. Analyses of the data indicate that there was no overall statistically significant difference in the DA concentrations among the different groups ($F_{4,27} = 1.68$, $p = 0.16$).

DOPAC/DA ratio. The ratios of DOPAC/DA from the groups of Experiment 2 are shown in Fig. 2B. Analyses of these data show that no overall

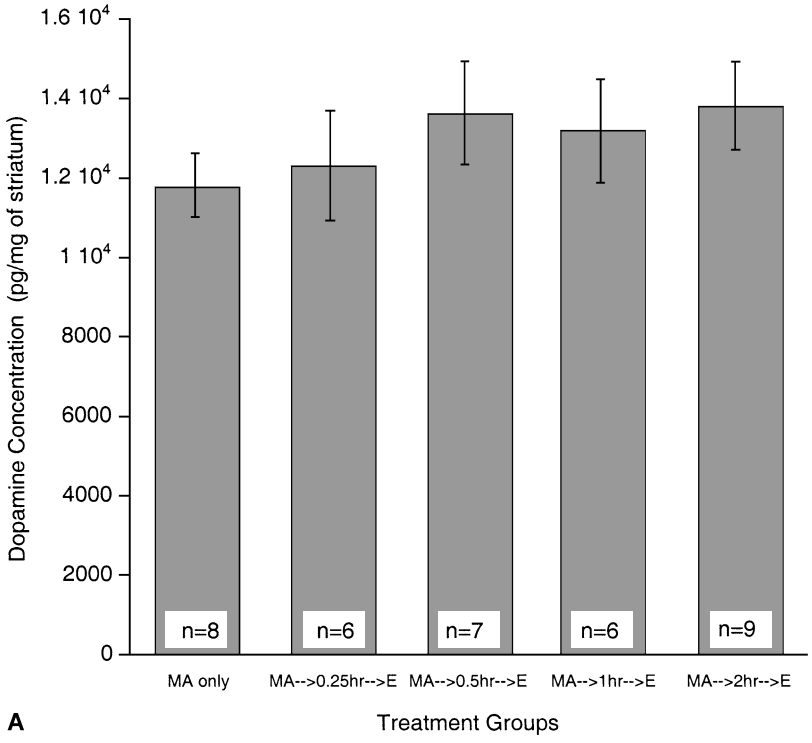
Fig. 1. A Dopamine (DA) concentrations (Mean ± SEM – in pg/mg) from Experiment 1. Ovariectomized CD-1 female mice were treated with 10 µg Estradiol Benzoate (E) at 24, 12 or 0.5 hours prior to a single injection of 40 mg/kg methamphetamine (MA). Control groups consisted of ovariectomized mice treated with E at 24 hours prior to an injection of the vehicle for MA and non-E treated mice receiving MA only. DA concentrations of mice receiving E at 24 and 12 hours prior to MA, as well as mice treated with E at 24 hours prior to an injection of the vehicle for MA, were significantly greater than that of the MA only group (as indicated by the *). The numbers contained within each bar indicate the number of animals within each treatment group. **B** DOPAC/DA ratios (Mean ± SEM) from the mice of Experiment 1 as described in caption of Fig. 1A. The DOPAC/DA ratios of all mice treated with estradiol benzoate were significantly different from that of the MA only group


2.5 10^4
2 10^4
1.5 10^4
1 10^4
5000
0
Dopamine Concentration (pg/mg of striatum)
*
*
*
n=4
n=7
n=7
n=6
n=4
E-->24hr-->VEH
E-->24hr-->MA
E-->12hr-->MA
E-->0.5hr-->MA
MA only
Treatment Groups

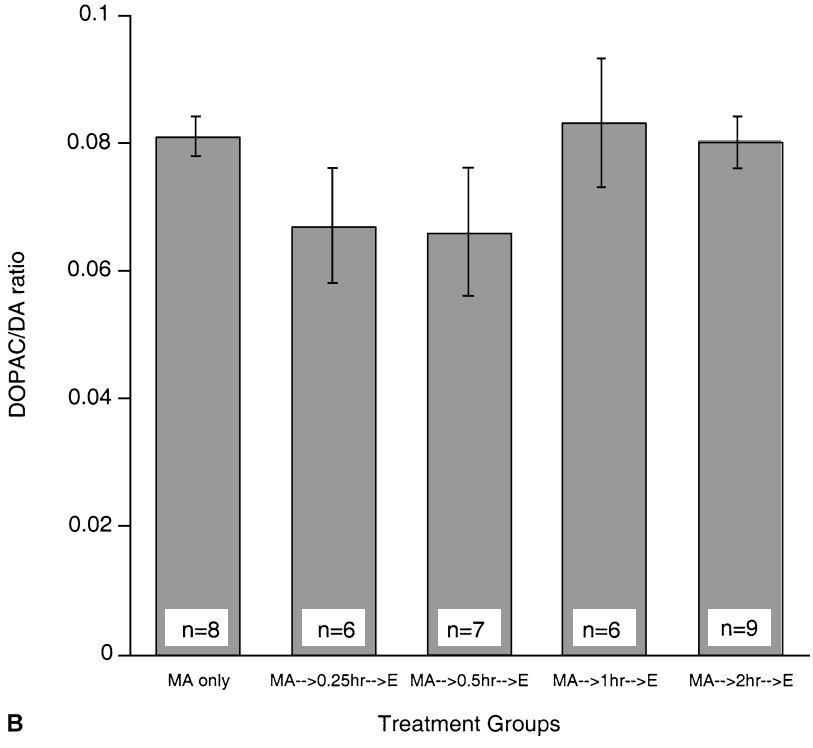
A


0.12
0.1
0.08
0.06
0.04
0.02
0
DOPAC/DA ratio
*
*
*
*
n=4
n=7
n=7
n=6
n=4
E-->24hr-->VEH
E-->24hr-->MA
E-->12hr-->MA
E-->0.5hr-->MA
MA only
Treatment Groups

B


1.6 10^4
1.4 10^4
1.2 10^4
1 10^4
8000
6000
4000
2000
0
Dopamine Concentration (pg/mg of striatum)
n=8
n=6
n=7
n=6
n=9
MA only
MA-->0.25hr-->E
MA-->0.5hr-->E
MA-->1hr-->E
MA-->2hr-->E
Treatment Groups

A


0.1
0.08
0.06
0.04
0.02
0
DOPAC/DA ratio
n=8
n=6
n=7
n=6
n=9
MA only
MA-->0.25hr-->E
MA-->0.5hr-->E
MA-->1hr-->E
MA-->2hr-->E
Treatment Groups

B

statistically significant difference was present among the groups ($F_{4,27} = 1.05$, $p = 0.403$).

Discussion

The results of Experiment 1 indicate that EB can significantly diminish the amount of striatal DA depletion induced by MA when administered $\geq$12 hours before MA. The 0.5 hour period shows a decrease in DA depletion that was not statistically different from the MA only group. With the measure of DOPAC/DA ratios, a significant difference was observed for all the EB treatment groups as compared to the MA only group. Based upon the DOPAC/DA ratios our data indicate that the EB treatment as early as 0.5 hour prior to MA can exert a modulatory action upon MA-induced neurodegeneration of the NSDA system suggestive of a neuroprotective effect. The present findings that EB administered prior to MA can function as a neuroprotectant support related findings which show that estrogen administration at several days to one week prior to MA can produce a preservation of striatal DA concentrations (Yu and Liao, 2000a; Dluzen and McDermott, 2002). However, the results of Experiment 1 reveal several important extensions with regard to this capacity for estrogen to function as a neuroprotectant. First, it provides a time frame in which estrogen is effective as a neuroprotectant. The data show that under the conditions tested (EB in oil), estrogen was clearly effective as a neuroprotectant (indicated by DOPAC/DA ratios) as early as 0.5 hours prior to MA. Second, this neuroprotection can result from a single acute injection of EB in oil as compared the more chronic estrogen exposure resulting from treatment with an estrogen pellet or repeated EB injections. Third, the neuroprotection can result from an estrogen administration that would be considered within a physiological range, like that obtained following treatment with estrogen pellets (Gao and Dluzen, 2001). Related data in the rat indicate that a 10 μg dose of EB in oil produces serum estradiol levels considered to be physiological (Vongher and Frye, 1999). In addition, the fact that MA-induced striatal DA depletion varies as a function of the estrous cycle within the mouse (Yu and Liao, 2000b) provides further support for the notion that this neuroprotection can occur under conditions of physiological changes in gonadal steroid hormones. Fourth, the data suggest that EB administration at 24 hours prior to MA may *prevent* striatal DA depletion produced by this regimen of MA since DA concentrations of this group did not differ statistically from the mice receiving the EB at 24 hours prior to treatment with the MA vehicle. Finally, the results showed an

Fig. 2. A Dopamine (DA) concentrations (Mean ± SEM – in pg/mg) from Experiment 2. Ovariectomized CD-1 female mice received a single injection of 40 mg/kg methamphetamine (MA) followed by 10 μg Estradiol Benzoate (E) at 0.25, 0.5, 1 or 2 hours post-MA. A separate group of ovariectomized, non-E treated mice receiving MA only served as a control. No statistically significant differences in DA concentrations were obtained among the five treatment groups of Experiment 2. The numbers contained within each bar indicate the number of animals within each treatment group. **B** DOPAC/DA ratios (Mean ± SEM) from the mice of Experiment 2 as described in caption of Fig. 2A. No statistically significant differences in DOPAC/DA ratios were obtained among the five treatment groups of Experiment 2

interesting divergence with regard to the two parameters measured in that DA concentrations of the EB at 0.5 hour group failed to differ statistically from the MA only group while the DOPAC/DA ratios of this group was statistically different from the MA only group. Therefore, with a parameter that provides a potentially more dynamic and sensitive assessment of NSDA functioning (DOPAC/DA), it appears that EB is exerting a modulatory, neuroprotective effect upon MA-induced neurotoxicity when administered as early as 0.5 hours prior to MA.

The fact that EB was effective in altering the DOPAC/DA ratios at all time periods tested shows that estrogen can exert very rapid effects upon modulating MA induced effects upon NSDA functioning. Rapid responses to EB upon stimulated DA release have also been reported in rats (Becker and Rudick, 1999). These findings, which show such rapid effects, suggest a non-genomic action of estrogen. In general, a basic criterion for non-genomic actions of gonadal steroid hormones includes a relatively short period of time to exert an effect (McEwen et al., 1990), although a temporal criterion alone may not necessarily provide for a distinguishing dichotomy between genomic and non-genomic actions.

The results of Experiment 2 show that the different experimental groups receiving EB at 0.25–2 hours post-MA were not statistically different from the MA-only controls for both DA concentrations and DOPAC/DA ratios. These findings demonstrate that estrogen does not have any protective or preventive effect when administered immediately after MA insult. These results support previous findings which show an absence of any neuroprotective effect of estrogen when administered at 7 days post-MA insult (Gao and Dluzen, 2001; Dluzen and McDermott, 2002). Combining the findings from the present and past results indicate that neither an acute nor long-term perturbation of the NSDA system as induced by MA can be altered subsequently by estrogen. Thus, estrogen does not appear to exert any neurorestorative effects on MA-induced nigrostriatal degeneration. In this way, although estrogen can serve as a neuroprotectant of the NSDA system, the potential for estrogen as a therapeutic agent after MA toxicity or its potential in Parkinsonism-related degeneration of nigrostriatal pathway where the pathology involves depletion of DA seems unlikely as based upon this animal model.

The lack of any estrogen protection when administered after MA can be contrasted with the findings in the ischemia model of brain lesion where estrogen does have neuroprotective effects after the ischemic insult (Yang et al., 2000). Such a difference can be attributed to the dissimilarities in pathogenesis for stroke compared to Parkinson's disease. In addition, the experiments showing neuroprotective effects of estrogen after ischemic insult are obtained with the use of supraphysiological doses of estrogen. In the present report, we attempted to limit our estrogen treatments to levels which would be considered within a physiological range.

In summary, the data presented in this report demonstrate that estrogen can act as a neuroprotective agent upon DOPAC/DA ratios of the NSDA system when given as early as 0.5 hours prior to MA. The presence of beneficial effects of EB at the 0.5 hour time period suggests that this neuroprotection by estrogen

involves a non-genomic action. When EB was administered acutely, within 0.25–2 hours after MA, we failed to obtain any prevention of the MA induced neurotoxicity. Taken together, these data show that estrogen can exert a clear neuroprotective (as administered pre-MA), but no neuropreventative (as administered post-MA), effect upon the NSDA system.

References

Arvin M, Fedorkova L, Disshon KA, Dluzen DE, Leipheimer RE (2000) Estrogen modulates responses of striatal dopamine neurons to MPP+: evaluations using in vitro and in vivo techniques. Brain Res 872: 160–171

Baldereschi M, DiCarlo A, Rocca A, Vanni P, Maggi S, Perissinotto E, Grigoletto F, Amaducci L, Inzitari D, for the ILSA Working Group (2000) Parkinson's disease and parkinsonism in a longitudinal study. Two fold higher incidence in men. Neurology 55: 1358–1363

Becker JB, Rudick CN (1999) Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: a microdialysis study. Pharmacol Biochem Behav 64: 53–57

Callier S, Morissette M, Grandbois M, Pelaprat D, Di Paolo T (2001) Neuroprotective properties of 17beta-estradiol, progesterone, and raloxifene in MPTP C57BI/6 mice. Synapse 41: 131–138

Diamond SG, Markham CH, Hoehn MM, McDowell FH, Muenter MD (1990) An examination of male–female differences in progression and mortality of Parkinson's disease. Neurology 40: 763–766

Disshon KA, Dluzen DE (1997) Estrogen as a modulator of MPTP-induced neurotoxicity: effects upon striatal dopamine release. Brain Res 764: 9–16

Disshon KA, Dluzen DE (2000) Estrogen reduces acute striatal dopamine responses in vivo to the neurotoxin MPP+ in female, but not male rats. Brain Res 868: 95–104

Dluzen DE (1997) Estrogen decreases corpus striatal neurotoxicity in response to 6-hydroxydopamine. Brain Res 767: 340–344

Dluzen DE (2000) Neuroprotective effects of estrogen upon the nigrostriatal dopaminergic system. J Neurocytol 29: 387–399

Dluzen DE, McDermott JL (2000) Neuroprotective role of estrogen upon methamphetamine and related neurotoxins within the nigrostriatal dopaminergic system. Ann NY Acad Sci 914: 112–126

Dluzen DE, McDermott JL (2002) Estrogen, anti-estrogen and gender differences in methamphetamine neurotoxicity. Ann NY Acad Sci 965: 136–156

Dluzen DE, Horstink MWIM (2003) Estrogen as a neuroprotectant of the nigrostriatal dopaminergic system: laboratory and clinical considerations. Endocrine 21: 67–75

Dluzen DE, McDermott JL, Liu B (1996a) Estrogen alters MPTP-induced neurotoxicity in female mice: effects on striatal dopamine concentrations and release. J Neurochem 66: 658–666

Dluzen DE, McDermott JL, Liu B (1996b) Estrogen as a neuroprotectant against MPTP-induced neurotoxicity in C57/B1 mice. Neurotoxicol Teratol 18: 603–606

Dluzen DE, Disshon KA, McDermott JL (1998) Estrogen as a modulator of striatal dopaminergic neurotoxicity. In: Marwah J, Teitelbaum H (eds) Recent advances in neurodegenerative disorders. Prominent Press, Scottsdale, AZ, pp 149–192

Fukuda K, Yao H, Ibayashi S, Nakahara T, Uchimura H, Fujishima M, Hall ED (2000) Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats. Stroke 31: 155–160

Gao X, Dluzen DE (2001) Tamoxifen abolishes estrogen's neuroprotective effect upon methamphetamine neurotoxicity of the nigrostriatal dopaminergic system. Neuroscience 103: 385–394

Grandbois M, Morissette M, Callier S, Di Paolo T (1999) Ovarian steroids and raloxifene prevent MPTP-induced dopamine depletion in mice. Neuroreport 11: 343–346

Granholm AC (2000) Oestrogen and nerve growth factor – neuroprotection and repair in Alzheimer's disease. Expert Opin Invest Drugs 9: 685–694

Horstink MWIM, Strijks E, Dluzen DE (2003) Estrogen and Parkinson's disease. Adv Neurol 91: 107–114

Liao S, Chen W, Kuo J, Chen C (2001) Association of serum estrogen level and ischemic neuroprotection in female rats. Neurosci Lett 297: 159–166

McEwen BS, Corini H, Schumacher M (1990) Steroid effects on neuronal activity: when is genome involved? Ciba Foundation Symposium 153: 3–21 (Steroids and neuronal activity)

Miller DB, Ali SF, O'Callaghan JP, Laws SC (1998) The impact of gender and estrogen on striatal dopamine neurotoxicity. Ann NY Acad Sci 844: 153–165

Miller MM, Monjan AA, Buckholtz NS (2001) Estrogen replacement therapy for the potential treatment or prevention of Alzheimer's disease. Ann NY Acad Sci 949: 223–234

Myers RE, Anderson LI, Dluzen DE (2003) Estrogen, but not testosterone, attenuates methamphetamine-evoked dopamine output from superfused striatal tissue of female and male mice. Neuropharmacology 44: 624–632

Owens CT (2002) Estrogen replacement therapy for Alzheimer disease in postmenopausal women. Ann Pharmacother 37: 1273–1281

Schrag A, Ben-Shlomo Y, Quinn NP (2001) Cross sectional prevalence survey of idiopathic Parkinson's disease and parkinsonism in London. Br J Med 321: 21–22

Swerdlow RH, Parker WD, Currie LJ, Bennet JP, Harrison MB, Trugman JM, Wooten GF (2001) Gender ratio differences between Parkinson's disease patient and their affected relatives. Parkinsonism Relat Disord 7: 129–133.26

Toung TJ, Traystman RJ, Hurn PD (1998) Estrogen-mediated neuroprotection after experimental stroke in male rats. Stroke 29: 1666–1670

Vongher JM, Frye CA (1999) Progesterone in conjunction with estradiol has neuroprotective effects in an animal model of neurodegeneration. Pharmacol Biochem Behav 64: 777–785

Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M, Rosewell KL (2001) Estradiol is a protective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies. Brain Res Rev 37: 313–319

Yang SH, Shi J, Day AL, Simpkins JW (2000) Estradiol exerts neuroprotective effects when administered after ischemic insult. Stroke 31: 745–755

Yu L, Liao P-C (2000a) Estrogen and progesterone distinctively modulate methamphetamine-induced dopamine and serotonin depletions in C57BL/6J mice. J Neural Transm 107: 1139–1147

Yu L, Liao P-C (2000b) Sexual differences and estrous cycle in methamphetamine-induced dopamine and serotonin depletions in the striatum of mice. J Neural Transm 107: 419–427

Yu L, Kuo Y, Cherng CG, Chen H-H, Hsu C-H (2002) Ovarian hormones do not attenuate methamphetamine-induced dopaminergic neurotoxicity in mice gonadectomized at 4 weeks postpartum. Neuroendocrinology 75: 282–287

Authors' address: Dr. D. Dluzen, Department of Anatomy, NEOUCOM, 4209 State Route 44, PO Box 95, Rootstown, OH 44272-0095, U.S.A., e-mail: ded@neoucom.edu