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Tamoxifen protects male mice nigrostriatal dopamine against methamphetamine-induced toxicity

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ABSTRACT

The selective estrogen receptor modulator tamoxifen and estradiol were shown to protect nigrostriatal dopamine concentration loss by methamphetamine in female mice whereas male mice were protected only by tamoxifen. The present study examined the protective properties of tamoxifen in male mice on several nigrostriatal dopaminergic markers and body temperature. Intact male mice were administered 12.5 or 50 μg tamoxifen 24 h before methamphetamine treatment. Basal body temperatures of male mice remained unchanged by the tamoxifen treatment. Methamphetamine reduced striatal dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid concentrations, striatal and substantia nigra dopamine and vesicular monoamine transporter specific binding as well substantia nigra dopamine and vesicular monoamine transporter mRNA levels and increased striatal preproenkephalin mRNA levels. These methamphetamine effects were not altered by 12.5 μg tamoxifen except for increased striatal dopamine metabolites and turnover. Tamoxifen at 50 μg reduced the methamphetamine effect on striatal dopamine concentration, dopamine transporter specific binding and prevented the increase in preproenkephalin mRNA levels; in the substantia nigra tamoxifen prevented the decrease of dopamine transporter mRNA levels. The present results show a tamoxifen dose-dependent prevention of loss of various dopaminergic markers against methamphetamine-induced toxicity in male mice. Since this is the only known hormonal protection of male mice against methamphetamine toxicity, these findings provide important new information on specific parameters of nigrostriatal dopaminergic function preserved by tamoxifen.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder mainly characterized by a progressive and selective depletion of DA neurons in the substantia nigra (SN) [1]. A greater prevalence and incidence of PD is described in men than in women [2,3], suggesting the involvement of estrogen in this difference. Metamphetamine

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Abbreviations: DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; MA, methamphetamine; MAO, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PPE, preproenkephalin; SN, substantia nigra; TMX, tamoxifen; VMAT2, vesicular monoamine transporter2; [^{125}I]-RTI-121, 3β-(4-[^{125}I]iodophenyl)tropane-2β-carboxylic acid isopropyl ester; [^{3}H]-TBZ-OH, [^{3}H]dihydrotetrabenazine.

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(MA) is a potent and addictive psychostimulant shown to cause toxicity of striatal nerve terminals [4,5]. Studies in MA treated rodents, to model neuronal terminal loss as in PD, have shown that male mice are more sensitive to the effect of the toxin than females and show a more severe depletion in striatal dopamine (DA) concentration [6,7]. Estrogens are shown to protect against DA loss in animal models of PD, such as in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydoxydopamine and MA-treated mice [6–11]. Treatment with estrogen in ovariectomized female mice before MA injection results in neuroprotective effects on the nigrostriatal dopaminergic system [10,11]. Although estrogen shows neuroprotective properties in female mice, no such effects of estrogen upon MA neurotoxicity were observed in male mice [12,13].

Tamoxifen (TMX), a non-steroidal anti-estrogen, presents agonist and antagonist estrogenic action in many organs, including the central nervous system. TMX is shown to modulate the nigrostriatal dopaminergic system [14] and to exert neuroprotective effects on striatal DA depletion induced by MA in both male and female mice [7,11,15]. In addition, TMX protects against DA transporter (DAT) and vesicular monoamine transporter-2 (VMAT2) MA-induced loss in ovariectomized female mice [11]. Accordingly, while both estrogen and TMX can serve as neuroprotectants against MA-induced DA depletion in female mice, only TMX displays this capacity in male mice [7,15]. Hence, TMX shows interesting modulatory effects in the nigrostriatal dopaminergic system of male mice that warrants further investigation.

In the present study, we investigated the modulation of the MA responses by TMX upon several nigrostriatal dopaminergic markers as well as body temperature within intact male mice. Measures of body temperature were included since this parameter represents an important component of MA-induced DA neurotoxicity [16–18]. This neurotoxin at moderate doses causes injury to striatal neuron terminals, decreases DA and metabolite concentrations as well as DAT and VMAT2 specific binding and increases preproenkephalin (PPE) mRNA levels [11,19,20]. The dopaminergic markers investigated provide information on early stages of neuronal dopaminergic degeneration [7,10,11,21] and were used in the present investigation to evaluate the effects of a MA lesion and treatment by TMX in striatum and substantia nigra pars compacta of male mice.

2. Materials and methods

2.1. Animals and treatments

Intact male CD-1 mice (2–3 months) were purchased from Charles River Laboratories (Wilmington, MA, USA). Mice were housed individually, to avoid the potential for stress-induced fighting, in plastic cages with free access to food and water, while maintained at room temperature of approximately 22 °C under a 12 h light:12 h 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 Northeastern Ohio Universities College of Medicine (NEOUCOM). All efforts were made to minimize the number of animals used and their suffering.

Mice were treated with TMX (Sigma, St. Louis, MO, USA) in sesame oil at a final concentration of 12.5 ($n = 8$) or 50 ($n = 8$) μg per animal. TMX or sesame oil was injected subcutaneous in the dorsal neck region, 24 h before MA injections (four injections of 10 mg/kg in saline at 2 h intervals, intraperitoneal). A MA alone group of mice ($n = 16$) received sesame oil and a control group of mice ($n = 9$) received sesame oil and saline solution.

2.2. Body temperature measures

In order to assess whether TMX would alter body temperatures, this parameter was measured in a separate group of TMX-treated mice. Intact male mice (as described above) were treated with 0 (sesame oil controls), 12.5 or 50 μg TMX ($N = 4$/group) and body temperatures were sampled at 24 and 27 h post-TMX/oil treatment using a rectal probe Traceable Digital Thermometer (VWR Scientific). These two time periods were selected to determine whether the TMX treatment may alter body temperatures at the time when MA was administered to the mice and thereby affect these MA-induced toxicity responses.

2.3. Brain preparation

Mice were euthanized by rapid decapitation 1 week post-MA or saline. Brains were removed, bisected and a unilateral striatum was used to assay DA and metabolite concentrations. The contralateral hemisphere was frozen in liquid nitrogen. The striatum (bregma 1.54 at −0.82 mm) and the substantia nigra (bregma −2.80 at −3.88 mm) [22] of the contralateral hemisphere were cut in a cryostat in 12 μm slices. Slices were kept at −80 °C until assayed.

2.4. Striatal dopamine assay

The unilateral striatum used for determination of DA and metabolite 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, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations by high-performance liquid chromatography with electrochemical detection, as previously described [13]. Concentrations of DA, DOPAC and HVA were expressed as pg per mg of tissue weight. In addition, the DOPAC/DA and HVA/DA ratios were also calculated from these concentrations.

2.5. DAT and VMAT2 autoradiography

DAT autoradiography in the striatum and the substantia nigra (SN) was performed as previously described [9]. 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, Perkin-Elmer, Boston, MA, USA). Non-specific binding was evaluated using 100 nM of mazindol. Slices were apposed to Kodak films (Biomax), 29 h for the SN and 15 h for the striatum. VMAT2 autoradiography in the striatum and the SN was performed using the specific ligand [^{3}H]dihydrotetrabenazine ([^{3}H]-TBZ-OH, American Radiolabeled Chemicals, St. Louis, MO, USA) [23]. 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 SN and 5 weeks for the striatum. Films were analyzed using the software NIH Image 1.68. The striatum was quantified into four equal quadrants: dorso-medial (DM), ventro-medial (VM), dorso-lateral (DL) and ventro-lateral (VL).

2.6. DAT, VMAT2 and PPE in situ hybridization

DAT mRNA in the SN was measured by *in situ* hybridization using a cDNA probe labeled with [^{35}S]-UTP, as previously described [21]. VMAT2 mRNA was measured by *in situ* hybridization using a complementary oligonucleotide [^{35}S]-d-ATP labeled, as described [21]. PPE mRNA in the striatum was measured by *in situ* hybridization using a complementary oligonucleotide [^{35}S]-d-ATP labeled, as described [24]. Slices were exposed to Kodak films (Biomax), for 3 days (DAT mRNA), 4 weeks (VMAT2 mRNA) and 5 days (PPE mRNA) and analyzed using the software NIH Image 1.68.

2.7. Statistical analysis

Statistical comparisons of the data were performed with a one-way analysis of variance (ANOVA) using Stat View 4.51 for Macintosh Computer software, followed by a post hoc analysis with a Fisher probability of least significant difference test. A simple regression model was used to determine the coefficient of correlation and the significance of the degree of linear relationship between variables. A $p < 0.05$ was required for the results to be considered statistically significant.

3. Results

3.1. Body temperature measures

Body temperature remained unchanged by treatment with TMX with no statistically significant differences obtained among the groups at either time period sampled. The mean ± S.E.M. body temperature (°C) at 24 h post-treatment of control mice was 37.58 ± 0.22, 37.61 ± 0.20 for TMX 12.5 μg treated mice and 37.74 ± 0.24 for TMX 50 μg treated mice ($p = 0.874$). Body temperatures as measured at 27 h post-treatment were 36.06 ± 1.09 for control mice, 37.63 ± 0.21 for TMX 12.5 μg treated mice and 36.92 ± 0.27 for TMX 50 μg treated mice ($p = 0.295$).

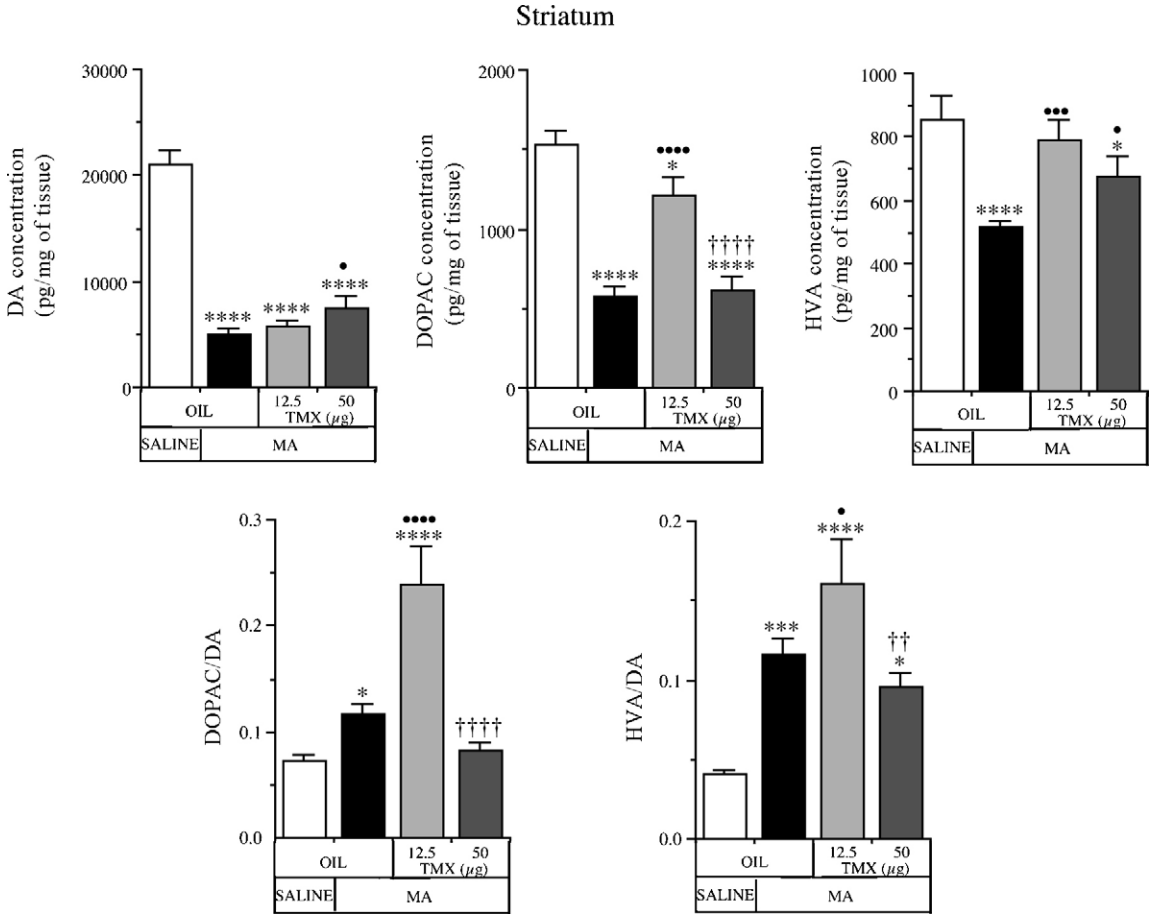

Fig. 1 – Effects of MA and TMX on striatal DA, DOPAC and HVA concentrations, as well as DOPAC/DA and HVA/DA ratios in male mice as compared with saline-treated mice (control). $^{*}p < 0.05$, $^{***}p < 0.005$ and $^{****}p < 0.0001$ vs. control; $^{\bullet}p < 0.05$, $^{\bullet\bullet\bullet}p < 0.005$ and $^{\bullet\bullet\bullet\bullet}p < 0.0001$ vs. MA; $^{\dagger\dagger}p < 0.01$ and $^{\dagger\dagger\dagger\dagger}p < 0.0001$ vs. TMX (12.5 μg). Values are the means (pg/mg of tissue) ± S.E.M. of 8–16 mice per group.

3.2. Striatal dopamine/metabolite concentrations and ratios

MA treatment led to a decrease in the striatum of DA concentrations of 76%, DOPAC of 62% and HVA of 40% as compared to control values (Fig. 1). Administration of TMX at 50 μg, but not at 12.5 μg, prevented partially the MA-induced DA depletion, as indicated by significantly higher striatal DA when compared with mice receiving the oil vehicle and MA. Mice that received 12.5 μg of TMX had significantly increased striatal DOPAC and HVA concentrations than MA treated mice receiving the oil vehicle; DOPAC levels were significantly lower and HVA levels were at control values in these mice. At 50 μg of TMX striatal DOPAC concentrations of MA-treated mice were as oil-treated MA mice but HVA concentrations were significantly increased; both metabolites were significantly decreased as compared with control values and DOPAC concentrations were significantly reduced as compared with mice that received 12.5 μg TMX ($p < 0.0001$). The striatal DOPAC/DA and HVA/DA ratios, used as an index of DA turnover, showed a statistically significant increase in the 12.5 μg TMX-treated group as compared to all other groups. The striatal HVA/DA ratio was significantly elevated in the oil + MA-treated mice and mice that received 50 μg TMX as compared with controls; the increase was significantly greater in mice that received the 12.5 versus 50 μg TMX (DOPAC/DA $p < 0.0001$; HVA/DA $p = 0.0068$).

3.3. DAT [^{125}I]-RTI-121 and VMAT2 [^{3}H]-TBZ-OH specific binding in striatum and substantia nigra pars compacta

High [^{125}I]-RTI-121 and [^{3}H]-TBZ-OH specific binding was observed in the striatum with a lateral/medial gradient (Fig. 2). More specifically, the dorso-medial striatum had the lowest DAT and VMAT2 specific binding compared to the ventro-lateral part; the ventro-medial and dorso-lateral striatum showed intermediate binding to these transporters (Fig. 3). The ventro-lateral striatum indicated higher DAT (33% $p < 0.0001$) and VMAT2 (213%, $p < 0.0001$) specific binding compared to the dorso-medial striatum of the control mice.

In both the dorso- and ventro-medial striatum, administration of MA significantly decreased to 37 and 45% of controls, respectively, [^{125}I]-RTI-121 specific binding; this was partially prevented with the 50 μg dose of TMX as levels in this group were significantly increased as compared with the oil + MA group (Fig. 3). The dorso- and ventro- lateral striatum were more affected by MA than the medial region ($p < 0.0001$) with a significant decrease to 17 and 15% of controls, respectively, of [^{125}I]-RTI-121 specific binding that was not prevented by either doses of TMX. MA-induced the greatest decrease of [^{3}H]-TBZ-OH specific binding to 36% of controls in the dorso-medial striatum compared to the ventro-medial (60%, $p = 0.0003$), dorso-lateral (55%, $p = 0.0031$) and ventro-lateral (60%, $p = 0.0003$) striatum. These decreases in [^{3}H]-TBZ-OH specific binding were not prevented by TMX.

In the SN high [^{125}I]-RTI-121 and [^{3}H]-TBZ-OH specific binding were measured (Fig. 4) and MA administration led to a significant decrease of 18% for DAT and 45% for VMAT2 specific binding, respectively (Fig. 5). For both transporters, no effect of the pretreatment with TMX was observed on the MA insult.

3.4. In situ hybridization of DAT, VMAT2 and PPE mRNA

MA administration significantly decreased DAT and VMAT2 mRNA levels by 44 and 20%, respectively (Figs. 4 and 6). The decrease in DAT mRNA induced by MA was prevented by

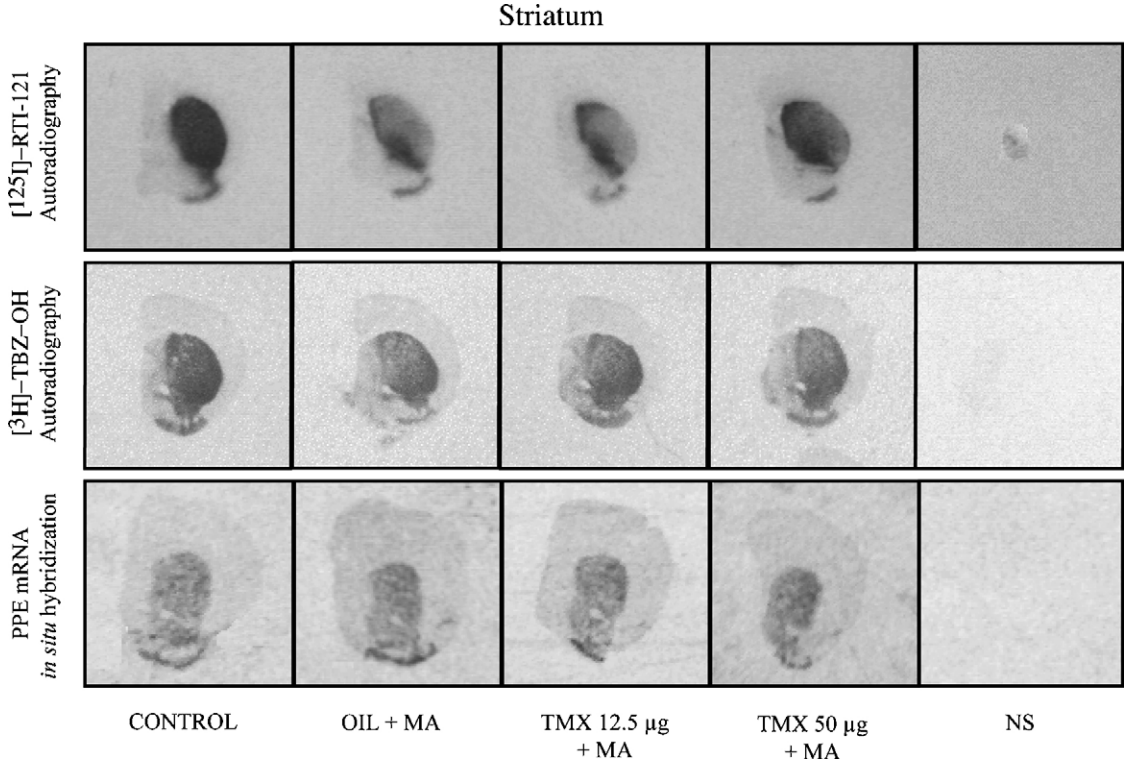

Fig. 2 – Examples of autoradiograms of [^{125}I]-RTI-121 and [^{3}H]-TBZ-OH binding as well as PPE mRNA in striatum of male mice treated with MA and TMX as compared to saline-treated mice (control). NS = non-specific binding.

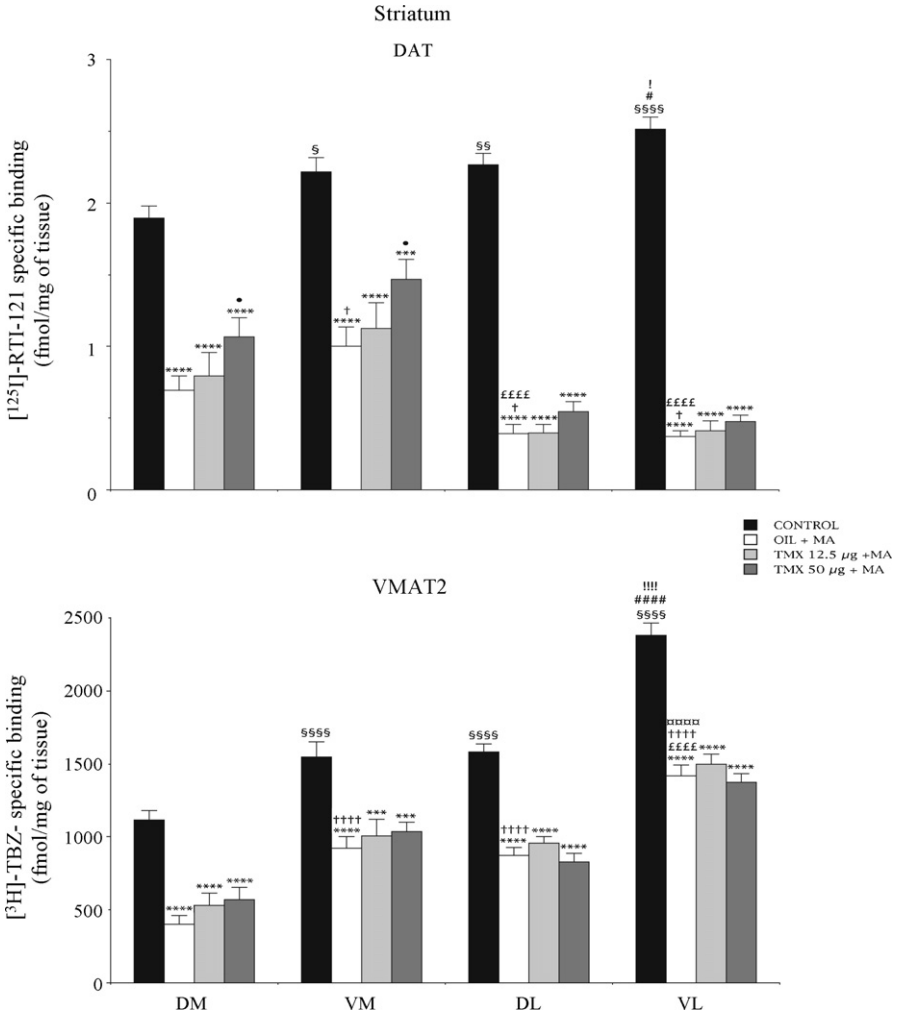

Fig. 3 – Effects of MA and TMX on DAT and VMAT2 specific binding in sub-regions of the striatum (DM, dorso-medial; VM, ventro-medial; DL, dorso-lateral; VL, ventro-lateral) of male mice as compared with saline-treated mice (control). $^{***}p < 0.005$ and $^{****}p < 0.0001$ vs. the corresponding sub-region in the control group; $^{\bullet}p < 0.05$ vs. the corresponding sub-region in the MA group; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ and $^{\S\S\S\S}p < 0.0001$ vs. the DM striatum of controls; $^{\#}p < 0.05$ and $^{\#\#\#\#}p < 0.0001$ vs. the VM striatum of controls; $^{!}p < 0.05$ and $^{!!!!}p < 0.0001$ vs. the DL striatum of controls; $^{\dagger}p < 0.05$ and $^{\dagger\dagger\dagger\dagger}p < 0.0001$ vs. the DM striatum of MA-treated mice; $^{£££££}p < 0.0001$ vs. the VM striatum of MA-treated mice; $^{\square\square\square\square}p < 0.0001$ vs. DL striatum of MA-treated mice. Values are the means (fmol/mg of tissue) ± S.E.M. of 8–16 mice per group.

treatment with 50, but not 12.5 μg TMX (significant difference between 12.5 and 50 μg TMX, $p = 0.0064$). Treatment with TMX (12.5 or 50 μg) did not prevent the decrease in VMAT2 mRNA levels induced by MA. MA treatment significantly increased PPE mRNA levels in both medial and lateral striatum by 15 and 19%, respectively (Figs. 2 and 7). In both regions of the striatum, this increase was only prevented by the 50 μg dose of TMX. A significant difference between 12.5 and 50 μg TMX was observed in the medial striatum ($p = 0.0153$).

3.5. *Correlation of dopamine markers*

A significant positive correlation was observed between DA concentration and striatal/SN DAT specific binding as well as between DA concentration and striatal/SN VMAT2 specific binding; to be more concise, striatal transporters specific binding were grouped in their medial–lateral parts (Table 1). A significant positive correlation was observed between DAT versus VMAT2 specific binding in both the medial and lateral striatum. Moreover, for both transporters, specific binding in striatum showed a significant positive correlation when correlated with their respective transporter specific binding and mRNA in the SN. A significant negative correlation was observed between DA and PPE mRNA as well as both transporters with PPE mRNA. In the SN, DAT correlated with VMAT2 specific binding and mRNA levels, respectively. The DAT and VMAT2 specific binding in the SN correlated with their respective mRNA levels.

4. Discussion

The present results show a partial protective effect of TMX (50 μg) in male mice against MA-induced toxicity for striatal

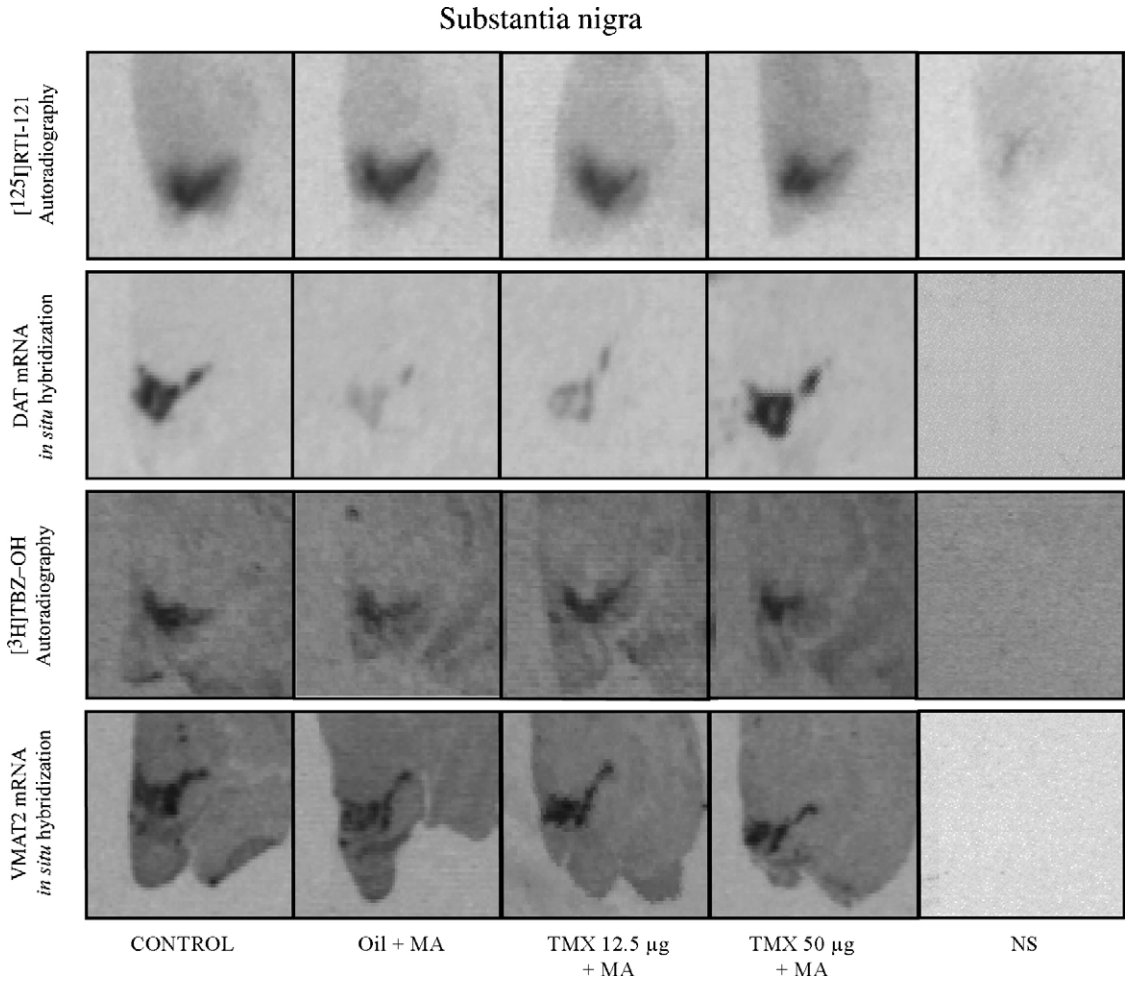

Fig. 4 – Examples of autoradiograms of [^{125}I]-RTI-121 and [^{3}H]-TBZ-OH binding as well as both transporters in the substantia nigra of male mice treated with MA and TMX as compared to saline-treated mice (control). NS = non-specific binding.

DA and HVA concentrations and DAT specific binding and a complete neuroprotection for SN DAT and PPE mRNA levels. The lower dose of TMX (12.5 μg) did not protect striatal DA concentrations, DAT and VMAT2 specific binding and mRNA levels but increased extensively striatal DOPAC and HVA concentrations. These protective effects to the 50 μg TMX treatment are in accordance with previous reports for striatal DA concentrations [7,15], but now provide an important extension of these previous findings as revealed by the dose dependency and effects exerted upon other markers of nigrostriatal dopaminergic function.

The substantially increased levels of striatal DOPAC concentrations and DOPAC/DA ratios in the 12.5 μg TMX-treated group are intriguing as this dose was ineffective in modifying any of the other parameters measured as part of this report. Therefore, while this dose apparently fails to offer any ''neuroprotection'', it remains effective as a modulator of MA-induced effects upon nigrostriatal dopaminergic function. One possible explanation is that this dose of TMX may have been effective in inducing a compensatory increase in dopaminergic activity. It is known that estrogen can alter DA synthesis, increase striatal DA turnover and modulate monoamine oxidase (MAO) activity [25–27]. Since TMX can exert estrogenic effects on DA synthesis, and these effects appear to be dose dependent, the increased DOPAC and DOPAC/DA ratios observed to the 12.5 μg TMX dose may represent agonist properties of this selective estrogen receptor modulator. In this way, the MA-induced decrease in striatal DA content, depletion in level of striatal tyrosine hydroxylase [19,28] and inhibition of MAO activity [20,29,30] may have been modulated by this low dose of TMX treatment. The marked increase in DOPAC levels may also result from a decrease in the vesicular sequestering of DA as SN mRNA of VMAT2 is significantly decreased in the 12.5 μg TMX group. HVA and HVA/DA ratio in 12.5 μg TMX MA-treated group compared to MA-treated mice were also elevated. Thus, increases of both DA metabolites, DOPAC and HVA as well as DA turnover (indicated with DOPAC/DA and HVA/DA ratios) support that 12.5 μg TMX induces an overall compensatory increase of DA activity. In MA + 50 μg TMX-treated mice striatal DA and HVA but not DOPAC levels were higher than mice that received MA alone and the DOPAC/DA returned to control values and HVA/DA were lower than in the MA + 12.5 μg TMX. The effect of 50 μg TMX on striatal dopamine and metabolites likely involves additional targets than observed for the lower dose of 12.5 TMX as we observed for the DAT.

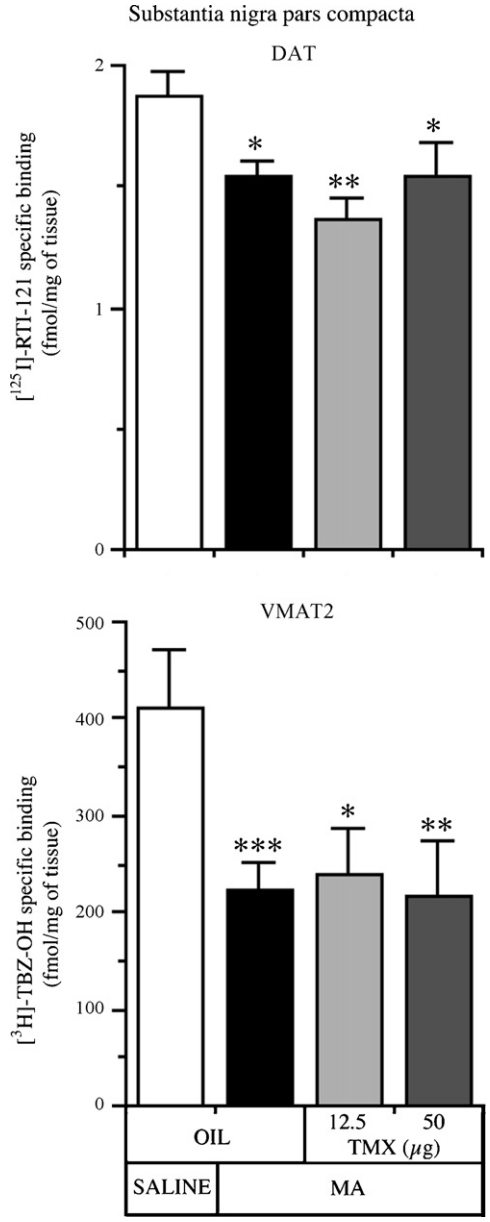

Fig. 5 – Effects of MA and TMX on DAT and VMAT2 specific binding in substantia nigra of male mice as compared with saline-treated mice (control). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.005$ vs. saline-treated mice (control). Values are the means (fmol/mg of tissue) ± S.E.M. of 8–16 mice per group.

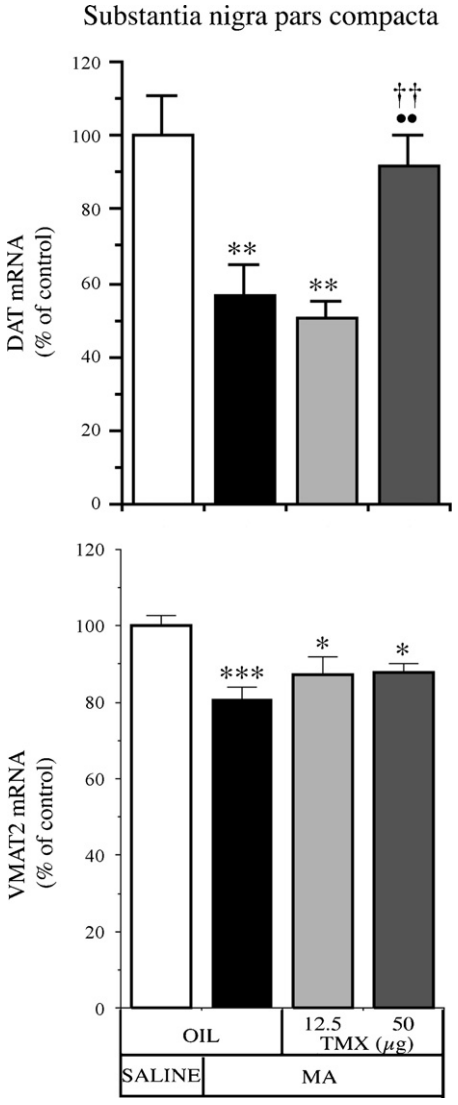

Fig. 6 – Effects of MA and TMX on DAT and VMAT2 mRNA levels in the substantia nigra of male mice as compared with saline-treated mice. $^{**}p < 0.01$ and $^{***}p < 0.005$ vs. saline-treated mice (control); $^{••}p < 0.01$ vs. MA; $^{††}p < 0.01$ vs. TMX (12.5 μg). Values are the means (expressed as percentage of relative optical density) ± S.E.M. of 8–16 mice per group.

The present results show moderate protection of TMX (50 μg) on DAT in the medial striatum, a region less affected by MA and no protection of striatal VMAT2. Similarly, this dose of TMX effectively prevented MA toxicity on mRNA of the DAT in the SN while no effect was observed for SN VMAT2 mRNA. The maintenance of DAT mRNA levels is not likely to be a compensatory mechanism in response to MA insult since mRNA levels were decreased in the MA group. This complete protection of DAT transporter mRNA levels by TMX (50 μg) is not reflected at the protein level in the striatum and SN likely because of differences in the kinetics of response to the lesion.

In intact control mice, the higher DAT and VMAT2 specific binding in lateral than the medial striatum observed in the present study was as reported by other investigators for the DAT [19]. More specifically, we observed the lowest DAT and VMAT2 specific binding in the dorso-medial quadrant and the highest in the ventro-lateral quadrant of the striatum. The lesion induced by MA upon the DAT was greater in the lateral than the medial striatum. This regional difference in MA-induced toxicity was previously observed [11,19]. By contrast, the greatest MA-induced decrease of VMAT2 specific binding was in the dorso-medial striatum compared to the other sub-regions of the striatum that were equally depleted of this transporter. Lower doses of MA, like that of other dopaminergic toxins, are more effective in altering presynaptic

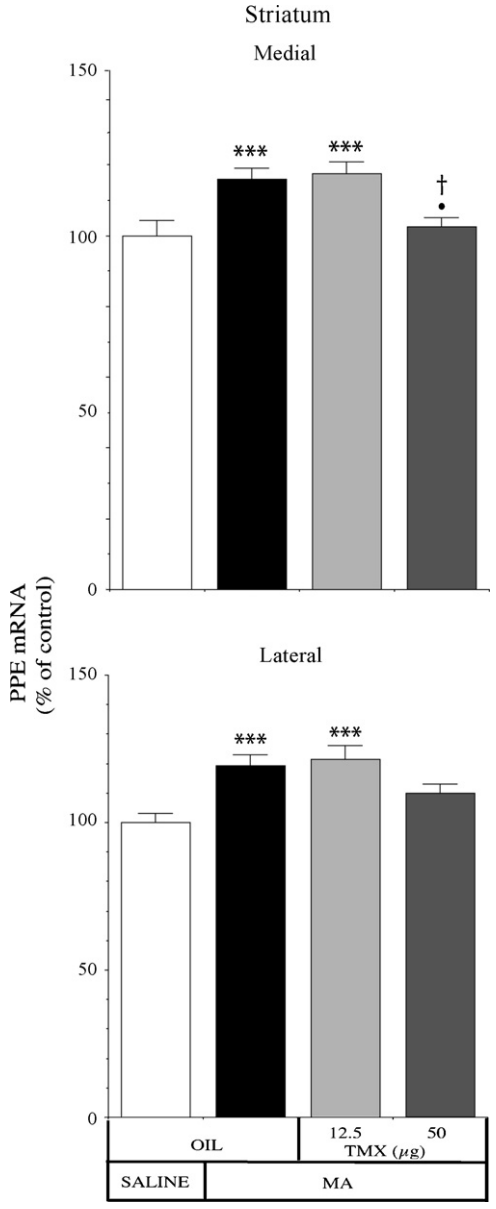

Fig. 7 – Effects of MA and TMX on PPE mRNA levels in the striatum of male mice as compared with saline-treated mice. $^{***}p < 0.005$ vs. saline-treated mice (control); $^{\bullet}p < 0.05$ vs. MA; $^{\dagger}p < 0.05$ vs. TMX (12.5 μg). Values are the means (expressed as percentage of relative optical density) ± S.E.M. of 8–16 mice per group.

terminals of dopaminergic neurons of the striatum than the cell bodies in the SN [10,21]. Accordingly, the present results show a more extensive MA-induced loss of striatal DAT (medial: 60%, lateral: 84%) than the SN (18%). By contrast, the extent of VMAT2 loss in the SN is similar to the loss observed in the striatum, suggesting that this transporter is relatively more sensitive to degeneration in the SN and/or this reflects greater degeneration of these neurons. A partial DAT but no VMAT2 protection was observed. This finding could serve as a specific/unique marker of partial neuroprotection resulting from TMX; indeed this is not seen with estradiol where both markers show protection [11]. Alternately, it could also represent a difference in the time frame of sampling and/or dose—as no significant DAT protection was seen with the lower dose of TMX, but only achieved with the higher. In this regard, perhaps an even higher dose of TMX would be required for a protection upon the VMAT2. To a certain extent this can be seen for VMAT2 in the DM as shown in Fig. 3, where there is a trend ($p = 0.0881$ versus MA) for a dose response protection by TMX 50 μg.

MA administration has been shown to enhance PPE mRNA levels [11,31]. Increases in PPE mRNA levels could represent a compensating mechanism for DA loss and could be considered as a presymptomatic marker of neurodegeneration [24,32]. Moreover, correlations measured in the striatum and SN of DA, DAT, VMAT2 and PPE mRNA show that the modulation of these dopaminergic markers is related to DA depletion, and that overall, these markers reflect degeneration and/or protection of the terminals of the nigrostriatal DA neurons.

MA has been shown to induce hyperthermia and administration of MA at cold temperature prevents DA neurotoxicity [16,33]. Accordingly, there exists an important relationship among MA, body temperature and DA neurotoxicity. This may be particularly relevant to hormonal modulation of MA-induced DA neurotoxicity as treatment with estrogen reduces body temperature and is associated with neuroprotection against MA in female mice while neither body temperature changes nor neuroprotection are observed within estrogen-treated male mice [12,17]. In the present study, TMX did not modulate basal body temperature in male mice and is unlikely implicated in the observed TMX protective effects. Similarly, a neuroprotection study with 4-hydroxytamoxifen reporting a decrease in MA toxicity found no change in body temperature in both female and male mice [34]. These authors found that the metabolite of tamoxifen, 4-hydroxytamoxifen, protected both male and female C57Bl/6 mice striatal DA and DOPAC concentrations against MA toxicity, no other dopaminergic markers were measured. A different time of euthanasia after MA administration, age and strain of the mice as well as in the pharmacokinetic and metabolism of tamoxifen into 4-hydroxytamoxifen could explain the differences with our results showing more protection in female [11] than male (the present data) mice with tamoxifen. Nevertheless, since the drug used in humans is tamoxifen we believe that our results are of clinical relevance.

TMX has estrogenic agonist and antagonist activity depending on the tissue [35]. In the present study, TMX showed differential effects depending on the dose. A differential dose-related agonist/antagonist activity of TMX has previously been proposed with regards to neuroprotection against MA toxicity in female mice [11]. The mechanisms of TMX action in the central nervous system remains to be clarified and may be multiple. Direct and genomic actions of TMX via classical estrogen receptors are likely; both ERα and ERβ have been detected in the mouse striatum and SN [36,37]. TMX exerts estrogenic antagonist or agonist activity depending of the subtypes of the receptor, of the activation domain and of the interaction with corepressors and coactivators (see Refs. [35,38] for review). In addition, various non-estrogenic activities of TMX on the nigrostriatal dopaminergic system have been reported. *In vitro* studies on superfused striatal

Table 1 – Linear correlation between dopaminergic markers in the striatum and the substantia nigra (SN)

Region	Correlated variables	R	p
Striatum	DA concentration vs. medial DAT specific binding	0.837	<0.0001
	DA concentration vs. lateral DAT specific binding	0.944	<0.0001
	DA concentration vs. medial VMAT2 specific binding	0.795	<0.0001
	DA concentration vs. lateral VMAT2 specific binding	0.839	<0.0001
	DA concentration vs. medial PPE mRNA	0.485	0.0013
	DA concentration vs. lateral PPE mRNA	0.605	<0.0001
	Medial VMAT2 specific binding vs. medial DAT specific binding	0.876	<0.0001
	Lateral VMAT2 specific binding vs. lateral DAT specific binding	0.826	<0.0001
	Medial PPE mRNA vs. medial DAT specific binding	0.470	0.0019
	Lateral PPE mRNA vs. lateral DAT specific binding	0.559	0.0001
	Medial PPE mRNA vs. medial VMAT2 specific binding	0.390	0.0118
	Lateral PPE mRNA vs. lateral VMAT2 specific binding	0.438	0.0042
Striatum/SN	DA concentration vs. DAT specific binding	0.450	0.0041
	DA concentration vs. VMAT2 specific binding	0.505	0.0009
	Medial DAT specific binding vs. DAT specific binding	0.438	0.0053
	Lateral DAT specific binding vs. DAT specific binding	0.498	0.0012
	Medial VMAT2 specific binding vs. VMAT2 specific binding	0.358	0.0235
	Lateral VMAT2 specific binding vs. VMAT2 specific binding	0.427	0.0060
	Medial DAT specific binding vs. DAT mRNA	0.580	<0.0001
	Lateral DAT specific binding vs. DAT mRNA	0.511	0.0008
	Medial VMAT2 specific binding vs. VMAT2 mRNA	0.648	<0.0001
	Lateral VMAT2 specific binding vs. VMAT2 mRNA	0.560	0.0002
SN	DAT mRNA vs. VMAT2 mRNA	0.400	0.0117
	DAT specific binding vs. VMAT2 specific binding	0.574	0.0002
	DAT specific binding vs. DAT mRNA	0.355	0.0289
	VMAT2 specific binding vs. VMAT2 mRNA	0.375	0.0188

tissue fragments of mice report that TMX can modulate DA output through a direct, non-genomic effect and this effect does not appear to involve alterations of DAT function [14,39]. Moreover, TMX has been shown to affect membrane fluidity [40], to induce an anti-inflammatory response in mouse microglia cells [41] and to protect glia cells against glutamate toxicity [42]. It has been reported that MA enhances glutamate release [43,44] and that microglia activation is associated with MA neurotoxicity [45,46]. TMX has been shown to inhibit protein kinase C activity [47]. It has been reported that protein kinase C activation, by phosphorylating DAT, reduces DAT activity thus increasing internalization and decreasing delivery to the membrane [48,49]. Hence, TMX could also promote DAT activity by inhibiting DAT phosphorylation through its capacity to inhibit PKC activity.

In conclusion, the present investigation revealed partial protective effects of TMX on MA-induced neurotoxicity of the nigrostriatal dopaminergic pathway in male mice. The effects of TMX are dose dependent and differentially affect various markers of the nigrostriatal dopaminergic system. In this way, the present results reveal the specific components of this brain system modulated by the interaction between TMX and MA-induced neurotoxicity. The identification of specific parameters of nigrostriatal dopaminergic function, which are modulated by MA–TMX interactions, serves as an important foundation for future research directed at understanding the mechanistic basis for these effects. The present findings have two important implications: (1) that TMX can serve as an agent that diminishes DA depletion to a nigrostriatal dopamine neurotoxin and therefore can be related to a neuroprotectant for PD and (2) the fact that this occurs in males and females, whereas estrogen only works in females, suggests that this agent could be beneficial to both sexes.

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