Mécanismes de neuroprotection de stéroïdes neuroactifs de la toxicité du MPTP

Thèse

Mélanie Bourque

Doctorat en pharmacie
Philosophiæ doctor (Ph.D.)

Québec, Canada

Résumé

Les études chez l'humain et les modèles animaux ont montré que les œstrogènes exercent des effets bénéfiques importants sur le risque de développer la maladie de Parkinson. Bien que les œstrogènes soient neuroprotecteurs, leurs actions périphériques limitent actuellement leur utilisation pour le traitement ou la prévention de maladies neurodégénératives, d'où l'importance de trouver des stratégies alternatives qui reproduisent les effets favorables des œstrogènes mais minimisent les effets indésirables. Lors de cette thèse, nous avons montré que l'activation du récepteur membranaire des œstrogènes couplé aux protéines G (GPER1), un récepteur non-féminisant, est aussi puissante que le 17β-estradiol à protéger les neurones dopaminergiques de la toxicité du MPTP chez des souris mâles, un modèle de la maladie de Parkinson. La neuroprotection par le 17β-estradiol est perdue lorsque le GPER1 ou les récepteurs des œstrogènes (ER) α/β sont bloqués, montrant que les ERα/β et le GPER1 sont requis dans la protection des neurones dopaminergiques par le 17β-estradiol. Ces résultats suggèrent une potentielle interaction entre les ERα/β et le GPER1. Utilisant une approche pharmacologique, nos résultats montrent que le ERα interagit avec le GPER1 pour augmenter la signalisation d'Akt ainsi que les niveaux de Bcl-2 et du BDNF, et protéger les neurones dopaminergiques de l'effet toxique du MPTP. L'effet neuroprotecteur du GPER1 se fait indépendamment des ERα/β, quoique le GPER1 requière la collaboration des ERα/β pour augmenter les niveaux du BDNF. L'investigation du mécanisme d'action du raloxifène, un modulateur sélectif des récepteurs des œstrogènes utilisé en clinique, a révélé que le raloxifène agit par le GPER1 pour activer Akt, augmenter les niveaux de Bcl-2 et du BDNF, et protéger les neurones dopaminergiques et les niveaux plasmatiques d'androgènes. La progestérone, un stéroïde non-féminisant, possède également des propriétés neuroprotectrices chez les souris MPTP. De plus, nos résultats montrent que la progestérone, lorsqu'administrée après le MPTP, permet la récupération partielle des neurones dopaminergiques. Lors de cette thèse, nous avons montré l'effet neuroprotecteur de plusieurs composés qui pourraient servir de stratégies alternatives à l'utilisation des œstrogènes. L'investigation des récepteurs oestrogéniques impliqués dans la neuroprotection fournissent d'importantes informations pour le développement de nouvelles thérapies.

Abstract

Studies in humans and animal models have shown that estrogens exert significant beneficial effects on the risk to develop Parkinson's disease. Although estrogens are neuroprotective, their peripheral actions currently limit their use for the treatment or prevention of neurodegenerative diseases, hence the importance of finding alternative strategies that mimic the beneficial effects of estrogen but minimize adverse effects. In this thesis, we have shown that activation of the membrane G protein-coupled estrogen receptor (GPER1), a non-feminizing receptor, is as potent as 17β-estradiol to protect dopaminergic neurons against MPTP toxicity in male mice, a model of Parkinson's disease. Neuroprotection by 17β-estradiol is lost when the GPER1 or estrogen receptors (ER) α/β are blocked, indicating that both ERα/β and GPER1 are required for the protection of dopaminergic neurons by 17β-estradiol. These results suggest a potential interaction between ERα/β and GPER1. Using a pharmacological approach, our results show that ERα interacts with GPER1 to increase Akt signaling, as well as the levels of Bcl-2 and BDNF, and protect dopaminergic neurons from the toxic effect of MPTP. However, the neuroprotective effect of GPER1 is independent of ERα/β, while GPER1 requires collaboration with ERα/β to increase BDNF levels. Investigation of the mechanism of action of raloxifene, a selective estrogen receptor modulator used in the clinic, has revealed that raloxifene acts through GPER1 to activate Akt, increasing the levels of Bcl-2 and BDNF, and protects dopaminergic neurons and plasma androgen levels. Progesterone, a non-feminizing steroid, has neuroprotective properties in MPTP mice. In addition, our results show that progesterone, when administered after MPTP, has rescued effects on dopaminergic neurons. In this thesis, we have shown the neuroprotective capacity of several compounds that could serve as alternative strategies to the use of estrogen. Investigation of estrogen receptors involved in neuroprotection provides important information for the development of new therapies.

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Liste des abréviations

AF : Fonction d'activation
AP-1 : Protéine activatrice-1
AMPA : Acide α-amino-3-hydroxy-5-méthyl-4-isoxazolepropionique
AMPH : Amphétamine
AROM : aromatase
BDNF : facteur neurotrophique dérivé du cerveau
CB : cathepsine B
CD : cathepsine D
COMT : Catéchol-O-méthyltransférase
CREB : Protéine de liaison à l'élément de réponse de l'AMP cyclique
CS : Colliculus supérieur
Cyt c : cytochrome c
DA : Dopamine
DARPP-32 : Phosphoprotéine de 32 kDa régulée par la DA
DAT : Transporteur de la dopamine
DOPAC : Acide 3,4-dihydroxyphénylacétique
DPN : 2,3-bis(4-hydroxyphényl)propionitrile
ER : Récepteur des œstrogènes
ERE : Élément de réponse à l'œstrogène
ERK : Kinases régulées par un signal extracellulaire
GABA : Acide γ-aminobutyrique
GDNF : facteur neurotrophique dérivé de la glie
GFAP : Protéine gliale fibrillaire acide
GPe : Globus pallidus externe
GPi : Globus pallidus interne
GPER1 : Récepteur des œstrogènes couplés aux protéines G
GSK3β : Kinase synthase du glycogène 3β
HVA : Acide homovanillique
HST : sulfotransférase
IGF-1 : Facteur de croissance de l'insuline 1
IMS : espace intermembranaire de la mitochondrie
JNK : kinase c-Jun N-terminal
L-DOPA : L-3,4-dihydroxyphénylalanine
LMP : perméabilisation de la membrane lysosomale
LRRK2 : protéine kinase 2 avec des répétitions riches en leucine
MAO : Monoamine oxydase
MAPK : Protéines kinases activées par les mitogènes
MPDP+ : 1-méthyl-4-phényl-2,3-dihydropyridium
MPP+ : 1-méthyl-4-phénylpyridium
mPR : récepteurs de la progestérone membranaire
MPTP : 1-méthyl-4-phényl-1,2,3,6-tétrahydropyridine
NFκB : Facteur nucléaire κB

NMDA : N-méthyl-D-aspartate
NST : Noyau sous-thalamique
$P450_{7\alpha}$: cytochrome 7α-hydroxylase;
$P450_{C11\beta}$: 11β-hydroxylase;
$P450_{C17}$: cytochrome P450 17α-hydroxylase/C17,20-lyase;
$P450_{C21}$: 21-hydroxylase
P450scc : cytochrome P450 responsable de la coupure de la chaîne latérale du cholestérol;
PI3K : Kinase phosphatidylinositol-3
PKA : Protéine dépendante de l'AMP cyclique
PPN : Noyau pédonculopontine
PPT : 4,4′,4″-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)tris-phénol
PR : récepteur de la progestérone
ROS : dérivés réactifs de l'oxygène
SERM : Modulateur sélectif des récepteurs des œstrogènes
SNc : Substance noire *pars compacta*
SNr : Substance noire *pars reticulata*
STS : sulfatase
TH : Tyrosine hydroxylase
UPS : système ubiquitine-protéasome
VMAT2 : Transporteur vésiculaire des monoamines 2
3-MT : 3-méthoxytyramine
6-OHDA : 6-hydroxydopamine
3α-HSD : 3α-hydroxystéroïde déhydrogénase;
3β-HSD : 3β-hydroxystéroïde déhydrogénase;
5α-R : 5α-réductase;
17β-HSD : 17β-hydroxystéroïde déhydrogénase.

Avant-Propos

Contributions

Durant mon doctorat, j'ai participé à de nombreux projets qui ont porté sur la différence entre les sexes dans l'effet toxique de la methamphétamine, la neuroprotection par les composés oestrogéniques et la progestérone, la neuroprotection avec le dutastéride (un inhibiteur de la 5α-réductase), ainsi qu'un projet sur l'effet neuroprotecteurs des plasmalogènes.

Mon projet principal au doctorat a conduit à la publication de 3 articles, dont je suis première auteur. Le premier article de mon doctorat intitulé «Sex and temporally-dependent effects of methamphetamine toxicity on dopamine markers and signaling pathways» publié dans la revue Neuropharmacology en 2012 n'est pas présenté dans cette thèse. Les deux autres articles publiés sont présenté aux chapitres 4 et 5, et les travaux présentés au chapitre 6 ont été soumis au journal Neurobiology of Aging. Au chapitre 7, nous présentons les premiers résultats de nos expériences avec la progestérone, dont le projet est toujours en cours.

J'ai participé à plusieurs revues de la littérature, soit comme première auteure, ou comme co-auteur :

***Bourque M**, Dluzen DE, Di Paolo T (2012). Signaling pathways mediating the neuroprotective effects of sex steroids and SERMs in Parkinson's disease. Front Neuroendocrinol. 33:169-178.*

*Al Sweidi S, Sánchez MG, **Bourque M**, Morissette M, Dluzen D, Di Paolo T. (2012) Oestrogen receptors and signalling pathways: implications for neuroprotective effects of sex steroids in Parkinson's disease. J Neuroendocrinol. 24:48-61.*

***Bourque M**, Dluzen DE, Di Paolo T (2011). Male/Female differences in neuroprotection and neuromodulation of brain dopamine. Front Endocrinol (Lausanne) 2:35.*

*Dluzen DE, McDermott JL, **Bourque M**, Di Paolo T, Darvesh AS, Buletko AB, Laping NJ (2011). Markers Associated with Sex Differences in Methamphetamine-Induced Striatal Dopamine Neurotoxicity. Current Neuropharmacology 9 :40-44.*

*Sánchez MG, **Bourque M**, Morissette M, Di Paolo T (2010). Steroids-dopamine interactions in the pathophysiology and treatment of CNS disorders. CNS Neurosci Ther 16:e43-71.*

***Bourque M**, Dluzen DE, Di Paolo T (2009). Neuroprotective actions of sex steroids in Parkinson's disease. Front Neuroendocrinol 30:142-57.*

En marge de mon projet principal, j'ai collaboré sur plusieurs projets avec mes collègues Sara (récepteurs des œstrogènes et neuroprotection), Nadhir (neuroprotection avec le dutastéride, manuscrit en préparation) et Édith (neuroprotection avec les plasmalogènes, manuscrit en préparation), ou avec l'équipe du Dr Denis Soulet (effet des composés oestrogéniques contre la toxicité du MPTP en périphérie), qui ont conduit ou qui conduiront à la publication d'articles.

*Al Sweidi S, Morissette M, **Bourque M**, Di Paolo T (2011). Estrogen receptors and gonadal steroids in vulnerability and protection of dopamine neurons in a mouse model of Parkinson's disease. Neuropharmacology. 61:583-91.*

*Côté M, **Bourque M**, Poirier AA, Aubé B, Morissette M, Di Paolo T, Soulet D (2014). GPER1-mediated immunomodulation and neuroprotection in the myenteric plexus of a mouse model of Parkinson's disease. Manuscrit soumis: Brain, Behavior, and Immunity.*

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Je tiens à remercier les Instituts de Recherche en Santé du Canada pour le financement accordé au Dre Di Paolo. Je remercie le Fonds d'enseignement et de recherche (FER) de la Faculté de pharmacie de l'université Laval, les Fonds de recherche du Québec-Santé (FRQS), et le Centre de recherche en endocrinologie moléculaire et oncologique et génomique humaine (CREMOGH) pour leur support financier tout au long de mes études de doctorat.

J'aimerais remercier Laurent Grégoire, qui pose toujours la bonne question. Pour son intérêt dans mes projets, ses conseils, sa présence et ses encouragements. Je tiens à remercier Marc Morissette, pour son implication dans les projets, pour son aide lors de mise au point et pour ses conseils.

Merci à tous mes collègues, Bazoumana, Sara, Maria, Nicolas, Vincent, Nadhir et Édith, qui ont su créer une ambiance de travail exceptionnelle. Merci aussi au Dr. Denis Soulet, pour ses encouragements et son support, et à Mélissa Côté, avec qui nous avons collaboré. Je tiens aussi à remercier le Dr. Dean Dluzen, avec qui nous avons collaboré sur certains projets, pour ses conseils et son support.

Je remercie ma famille et Jean-Samuel, pour leur inconditionnel support et leurs encouragements. À mon père : C'est maintenant terminé!

Introduction

Chapitre 1 : La dopamine

1.1. Synthèse

La dopamine (DA) est un neurotransmetteur de la famille des catécholamines, qui regroupe des neurotransmetteurs ayant un noyau catéchol et une amine. La DA est synthétisée dans le système nerveux central et dans les glandes surrénales à partir de la tyrosine (Figure 1.1). La tyrosine hydroxylase (TH), à partir d'une molécule d'oxygène comme co-substrat et de la tétrahydrobioptérine comme cofacteur, catalyse l'hydroxylation en position 3 de la tyrosine, formant la L-3,4-dihydroxyphénylalanine (L-DOPA) (Elsworth & Roth 1997). Par la suite, la L-DOPA est convertie en DA par la décarboxylase des acides aminés aromatiques (ou DOPA décarboxylase), une enzyme qui requiert le phosphate de pyridoxal (la forme active de la vitamine B6) comme cofacteur (Elsworth & Roth 1997). Sous des conditions normales, la quantité de L-DOPA retrouvée dans une cellule est minime, en raison de la haute activité enzymatique de la DOPA décarboxylase. L'étape limitante de la synthèse de la DA est la conversion de la tyrosine en L-DOPA et la synthèse de DA peut être régulée de plusieurs manières. La DA peut inhiber la TH en compétitionnant avec le site de liaison du cofacteur (Foley 2009). De plus, l'activation des autorécepteurs de la DA amène un changement dans l'état de phosphorylation de la TH, inhibant ainsi son activité (Foley 2009).

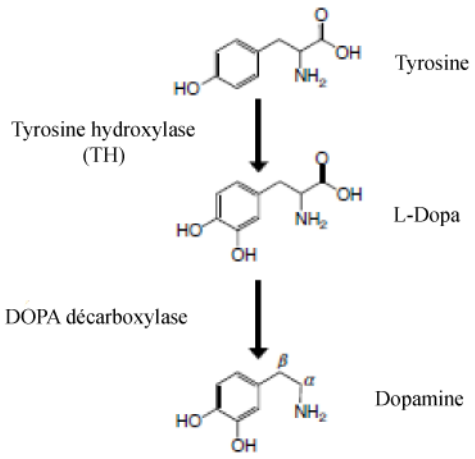

Figure 1.1. Voie de biosynthèse de la dopamine. Schéma tiré de (Foley 2009).

1.2. Stockage et libération

À l'intérieur du neurone, la DA est emmagasinée dans des vésicules, par le transporteur vésiculaire des monoamines 2 (VMAT2), pour une éventuelle libération dans la fente synaptique par exocytose (figure 1.2). Le VMAT2 emmagasine également dans des vésicules synaptiques la sérotonine, la noradrénaline, l'adrénaline et l'histamine dans leur neurone respectif (Hoffman *et coll.* 1998). Le VMAT2 est un membre de la famille des antiporteurs extrudant les toxines et est constitué de douze domaines transmembranaires (Eiden *et coll.* 2004). Deux processus sont impliqués dans l'emmagasinage de la DA à l'intérieur des vésicules (Parsons 2000). La lumière des vésicules est acidifiée et chargée positivement par une ATPase vacuolaire de type H^+ qui permet l'entrée de protons H^+. L'emmagasinage de la DA dans les vésicules se fait par le VMAT2, en échange de deux protons, ce qui permet l'emmagasinage de la DA contre son gradient de concentration. Les concentrations de DA à l'intérieur de la vésicule peuvent atteindre 500 mM, soit environ 10 000 fois la concentration de DA cytoplasmique (Eiden *et coll.* 2004). Le maintien de faible niveau de DA cytoplasmique par son emmagasinage dans des vésicules synaptiques contribue à diminuer le métabolisme de la DA et à réduire son auto-oxydation, deux processus formant des produits toxiques (Guillot & Miller 2009).

Suite à un potentiel d'action, la terminaison nerveuse est dépolarisée, ce qui provoque l'entrée de calcium à l'intérieur de la terminaison par des canaux voltage-dépendants (Foley 2009). L'augmentation des concentrations de calcium provoque la fusion des vésicules avec la membrane cellulaire et, subséquemment, la libération de la DA dans la fente synaptique (Elsworth & Roth 1997). La DA présente dans l'espace extracellulaire peut se lier avec les récepteurs dopaminergiques présents sur les neurones pré- ou post-synaptiques.

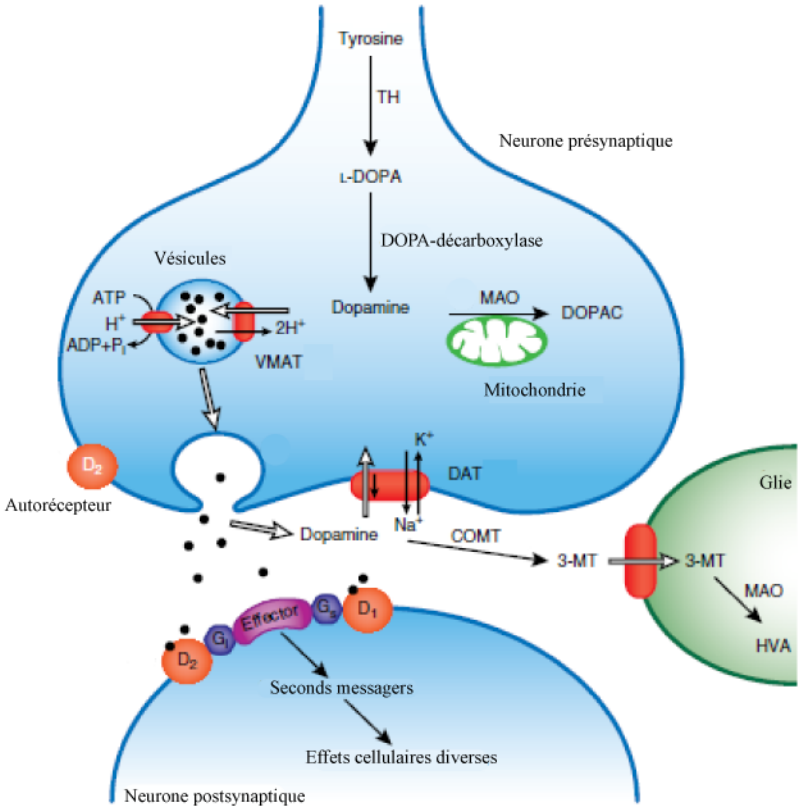

Figure 1.2. Représentation de la synthèse, de l'emmagasinage, de la libération et du métabolisme de la DA. Schéma tiré de (Foley 2009).

1.3. Régulation de la transmission dopaminergique

Le transporteur membranaire de la DA (DAT) régule les concentrations extracellulaires de DA en ré-emmagasinant dans la terminaison dopaminergique la DA présente dans l'espace extracellulaire suite à sa libération (Sotnikova *et coll.* 2006). Le

mécanisme par lequel se fait la recapture de la DA par le DAT implique la liaison séquentielle du substrat et la présence d'ions Na^+ et Cl^-. Le gradient électrochimique est généré par une ATPase Na^+/K^+. Le DAT est un symporteur, c'est-à-dire que la recapture de la DA se fait en co-transport avec deux ions Na^+ et un ion Cl^-, contre le sens du gradient de concentration du substrat et dans le sens du gradient de concentration des ions (Torres *et coll.* 2003).

Le DAT est un membre de la famille des transporteurs des monoamines, dont font aussi parties les transporteurs de la noradrénaline et de la sérotonine (Sotnikova *et coll.* 2006). La structure du DAT contient douze domaines transmembranaires, des domaines N- et C-terminal intracellulaires, trois sites de glycosylation retrouvés sur la deuxième boucle extracellulaire (entre les domaines transmembranaires 3 et 4) et plusieurs sites de phosphorylation (Sotnikova *et coll.* 2006). Ce transporteur fonctionnerait comme une simple sous-unité, quoique l'oligomérisation du DAT soit possible et celle-ci interviendrait dans le transport du DAT à la surface cellulaire (Torres *et coll.* 2003).

L'utilisation d'alpha-méthyl-para-tyrosine, un inhibiteur de la TH, ainsi que les études chez les souris invalidées en DAT, ont montré qu'une grande quantité de la DA emmagasinée dans les vésicules synaptiques à l'intérieur de la terminaison provient de la recapture de la DA extracellulaire (Jones *et coll.* 1998, Benoit-Marand *et coll.* 2000). La recapture de DA joue un rôle important sur la reconstitution du réservoir de DA emmagasinée dans les vésicules synaptiques dans les régions fortement innervées par les terminaisons dopaminergiques, comme le striatum (Sulzer *et coll.* 2010). Par contre, dans la substance noire *pars compacta* (SNc), l'activité du DAT est moindre que dans le striatum, avec un taux de recapture de la DA deux cent fois plus faible que dans le striatum (Rice & Cragg 2008).

Le principal mécanisme de la régulation de la transmission dopaminergique se fait par le DAT. Le DAT est localisé dans des zones extra-synaptiques sur les neurones dopaminergiques (Sotnikova *et coll.* 2006). De plus, les récepteurs dopaminergiques dans le striatum sont majoritairement localisés dans des zones extra-synaptiques et rarement à la synapse (Sulzer *et coll.* 2010). Ceci implique que la DA, une fois libérée dans la fente synaptique, doit diffuser dans des zones extra-synaptiques pour activer ses récepteurs. La transmission dopaminergique se fait donc par une transmission de volume basée sur la

diffusion (figure 1.3) (Rice & Cragg 2008). Dans le striatum, la majorité des récepteurs dopaminergiques D1 seraient dans un état de faible affinité (1 µM) tandis que la plupart des récepteurs dopaminergiques D2 seraient dans un état de haute affinité (10 nM) (Richfield *et coll.* 1989). Dans leur modèle, Rice et Cragg (Rice & Cragg 2008) proposent que la transmission dopaminergique par les récepteurs D1 survient à une distance maximale de 2 µm du site de libération de la DA, et que la recapture de la DA n'a pas d'effet sur l'activation de ce type de récepteur. Les concentrations de DA ne sont pas modulées par sa recapture jusqu'à 1 µm du site de libération. Ceci s'explique par le fait que la diffusion sur une courte distance est plus rapide que la recapture de la DA (Rice & Cragg 2008). Par contre, l'activation des récepteurs D2 peut se faire jusqu'à une distance de 7 µm du site de libération de la DA et est influencée par la recapture de la DA (Rice & Cragg 2008). En effet, le temps durant lequel les concentrations extracellulaires de DA excèdent 10 nM (soit une concentration suffisante pour activer les récepteurs de haute affinité) et la distance de diffusion de la DA sont largement influencés par la recapture de la DA dans le striatum, mais non dans la SNc (Rice & Cragg 2008). Le DAT régule donc la transmission dopaminergique en contrôlant la durée et l'intensité de l'action de la DA sur ses récepteurs.

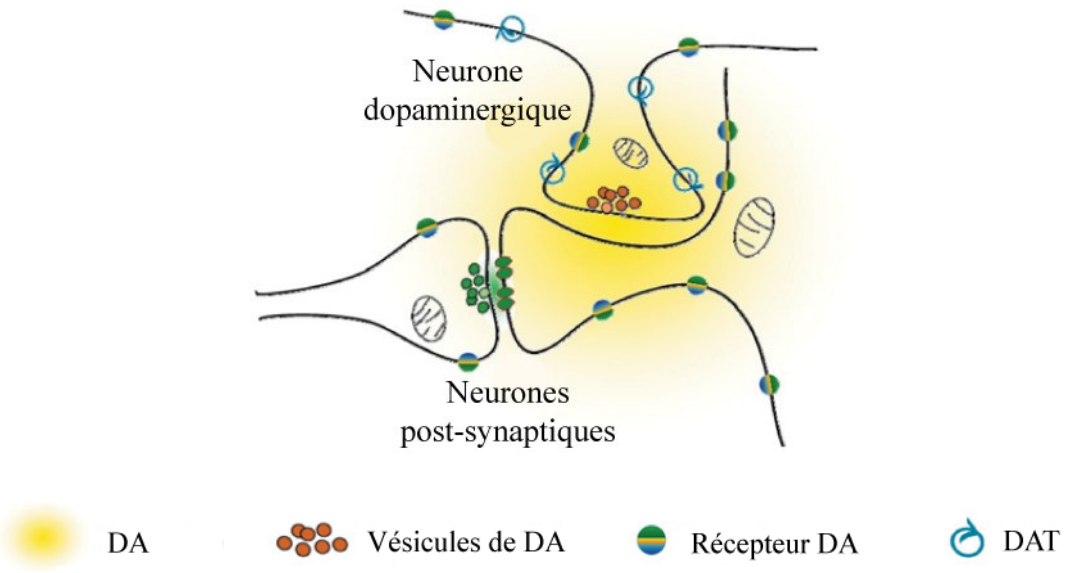

Figure 1.3. Représentation d'une synapse dopaminergique. Schéma tiré de (Rice & Cragg 2008).

Le renouvellement du DAT est un processus lent. Le temps de demi-vie du DAT dans le striatum a été estimé, *in vivo* chez le rat, d'une durée de 2 à 6 jours (Kimmel *et coll.* 2000, Fleckenstein *et coll.* 1996). Par contre, l'activité du DAT est fortement régulée par plusieurs molécules, que ce soit au niveau de son activité, par la régulation de son déplacement entre la membrane plasmatique et les endosomes (« trafficking »), ou par sa dégradation (Eriksen *et coll.* 2010). La régulation dynamique du DAT à la membrane est un processus servant à contrôler l'intensité et la durée de la signalisation dopaminergique, car le temps de demi-vie de la DA dans l'espace extracellulaire est principalement contrôlé par le DAT (Eriksen *et coll.* 2010). L'activation de la protéine kinase C (Vaughan *et coll.* 1997), ainsi que l'inhibition de la kinase phosphatidylinositol-3 (PI3K) (Carvelli *et coll.* 2002) et des protéines kinases activées par les mitogènes (MAPK) (Moron *et coll.* 2003) diminuent la capacité de transport du DAT (V_{max}) sans en affecter l'affinité (K_m), et réduisent l'expression du DAT à la surface cellulaire. La phosphorylation du DAT par la protéine kinase Ca^{2+}/calmoduline-dépendante de type II conduit au transport inverse de la DA, donc à une sortie extracellulaire de la DA (Fog *et coll.* 2006). Une interaction directe du DAT et de la forme courte du récepteur D2 dans le striatum a été rapportée (Lee *et coll.* 2007). Cette interaction faciliterait le recrutement du DAT à la surface cellulaire et la recapture de la DA. En plus de l'interaction directe, la stimulation de la forme courte du récepteur D2 augmente l'activité et l'expression du DAT à la surface membranaire par un mécanisme dépendant des kinases régulées par un signal extracellulaire (ERK1/2) (Bolan *et coll.* 2007). De forte concentration de DA (> 10 µM) diminue la recapture de la DA, un effet associé avec une diminution du DAT à la surface cellulaire (Chi & Reith 2003).

1.4. Métabolisme de la DA

La monoamine oxydase (MAO) et la catéchol-O-méthyltransférase (COMT), retrouvées dans les neurones et les cellules gliales, sont les enzymes responsables du métabolisme de la DA (figure 1.4) (Elsworth & Roth 1997). La MAO est localisée sur la membrane mitochondriale externe et cette enzyme catalyse la désamination oxydative de la DA en acide 3,4-dihydroxyphénylacétique (DOPAC) (Elsworth & Roth 1997). La 3-méthoxytyramine (3-MT) est formée par l'ajout d'un groupe méthyle sur la molécule de DA par la COMT dans la fente synaptique (Elsworth & Roth 1997). Le DOPAC et la 3-MT sont

par la suite métabolisés en acide homovanillique (HVA) par la COMT et la MAO, respectivement (Elsworth & Roth 1997).

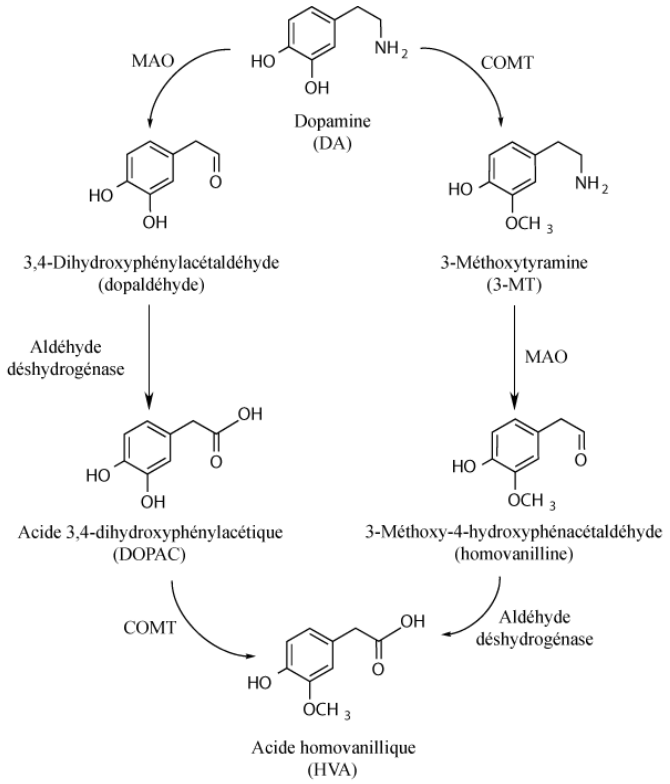

Figure 1.4. Métabolisme de la DA. Schéma tiré de (Foley 2009).

L'inhibition pharmacologique des enzymes de dégradation de la DA a montré que la MAO et la COMT ont peu d'effet sur la clairance de la DA extracellulaire dans le striatum (Jones *et coll.* 1998). Dans les régions fortement innervées en terminaisons dopaminergiques, comme dans le striatum, la clairance de la DA se fait principalement par le DAT (Jones *et coll.* 1998, Benoit-Marand *et coll.* 2000). Par contre, dans d'autres régions cérébrales, moins fortement innervées en terminaisons dopaminergiques, telles que le cortex préfrontal, la métabolisation de la DA est un processus plus actif dans la clairance de la DA (Yavich *et coll.* 2007).

1.5. Voies dopaminergiques

Quatre voies majeures dopaminergiques ont été identifiées au cerveau (figure 1.5). La principalement voie dopaminergique est la voie nigrostriée, dont les neurones dopaminergiques en provenance de la substance noire (A9) projettent vers le striatum (noyau caudé et putamen) (Prakash & Wurst 2006). Cette voie est impliquée dans le contrôle des mouvements. L'organisation topographique des projections dopaminergiques permet de subdiviser cette voie en deux groupes de neurones innervant des régions distinctes du striatum. Chez le rat, les neurones dopaminergiques situés dans la partie latérale de la SNc projettent essentiellement au striatum moteur (partie latérale), tandis que ceux de la partie médiale de la SNc innervent principalement le striatum associatif (partie médiale) (Joel & Weiner 2000, Prensa *et coll.* 2009). Chez les primates, les projections dopaminergiques en provenance de la partie médiale de la SNc innervent les régions associatives et motrices du striatum, alors que les neurones dopaminergiques de la partie latérale et du un-tiers caudal de la SNc projettent principalement vers le striatum moteur (Joel & Weiner 2000, Prensa *et coll.* 2009).

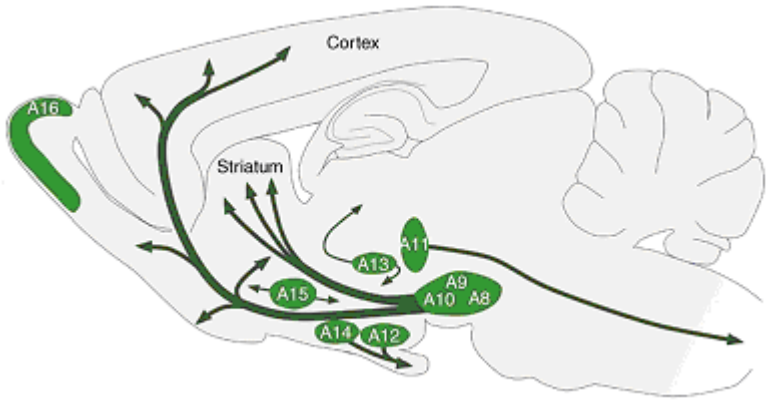

Figure 1.5. Distribution des neuf groupes de cellules dopaminergiques au cerveau chez le rongeur. Les principales projections de ces groupes de cellules dopaminergiques sont illustrées par des flèches. Schéma tiré de (Bjorklund & Dunnett 2007).

Les projections dopaminergiques en partance de l'aire tegmentale ventrale (A10) forment deux voies : la voie méso-limbique et la voie méso-corticale. Les terminaisons

dopaminergiques de la voie méso-limbique innervent plusieurs structures limbiques (principalement le noyau accumbens mais aussi l'amygdale, le septum latéral, la stria terminalis et l'hypothalamus latéral) (Prakash & Wurst 2006). Cette voie est impliquée dans la motivation, les émotions et dans la régulation des comportements de renforcement positif. La voie méso-corticale contient des neurones dopaminergiques qui projettent au niveau du cortex frontal et du cortex cingulaire (Prakash & Wurst 2006). Cette voie est impliquée dans les processus cognitifs. Les neurones dopaminergiques du noyau arqué (A12) et de la zone péri-ventriculaire (A14) projettent vers l'infundibulum et l'hypophyse antérieure, formant la voie tubéro-infundibulaire (Prakash & Wurst 2006). Cette voie régule la sécrétion de prolactine.

1.6. Les récepteurs dopaminergiques

La DA exerce ses effets par l'intermédiaire de cinq types de récepteurs couplés aux protéines G, subdivisés en deux familles. Basés sur leurs propriétés structurales, biochimiques et pharmacologiques, les récepteurs dopaminergiques ont été classifiés comme appartenant à la famille des récepteurs de type D1 (les récepteurs D1 et D5) ou à la famille des récepteurs de type D2 (les récepteurs D2, D3 et D4) (Beaulieu & Gainetdinov 2011).

1.6.1. Les récepteurs de type D1

Les récepteurs D1 et D5 partagent 80% d'homologie au niveau de leurs domaines transmembranaires (Beaulieu & Gainetdinov 2011). Les récepteurs D1 et D5 sont difficilement différentiables sur le plan pharmacologique; les divers agonistes et antagonistes ont une affinité similaire pour les deux sous-types de récepteur (Pivonello *et coll.* 2007). Par contre, la DA a environ dix fois plus d'affinité pour le récepteur D5 que pour le récepteur D1 (Missale *et coll.* 1998).

La stimulation des récepteurs de type D1 induit l'activation de l'adénylate cyclase par l'intermédiaire de la sous-unité $G\alpha_s/\alpha_{olf}$ des protéines G (Fisone 2009). La sous-unité $G\alpha_{olf}$ est fortement exprimée dans le striatum par contre, l'expression de la sous-unité $G\alpha_s$ est, quant à elle, minimale (Fisone 2009). L'adénylate cyclase catalyse la conversion de l'ATP en AMP cyclique, laquelle active la protéine dépendante de l'AMP cyclique (PKA). Parmi

les substrats de PKA, la phosphoprotéine de 32 kDa régulée par la DA (DARPP-32) joue un rôle d'amplification du signal de PKA (Beaulieu & Gainetdinov 2011). L'activation de PKA régule plusieurs protéines impliquées dans la transduction du signal et dans la régulation de l'expression de gènes tels que la protéine de liaison à l'élément de réponse de l'AMP cyclique (CREB), la signalisation des ERK et des canaux ioniques (les récepteurs de l'acide γ-aminobutyrique (GABA), N-méthyl-D-aspartate (NMDA) et de l'acide α-amino-3-hydroxy-5-méthyl-4-isoxazolepropionique (AMPA)) (Fisone 2009). Les récepteurs de type D1 peuvent stimuler la phospholipase C par l'intermédiaire de la sous-unité $G\alpha_q$ et moduler les canaux Na^+, K^+ et Ca^{2+} (Beaulieu & Gainetdinov 2011). De plus, le récepteur D5, mais non le récepteur D1, peut se coupler à l'adénylate cyclase d'une manière indépendante du ligand, suggérant que ce récepteur est constitutivement actif (Rankin *et coll.* 2009).

Le récepteur D1 est le récepteur dopaminergique le plus abondamment exprimé. Le récepteur D1 est fortement retrouvé, entre autres, dans le striatum, la SNc, le noyau accumbens et le cortex frontal, et de manière plus réduite dans le thalamus (Beaulieu & Gainetdinov 2011). Le récepteur D5 est exprimé à de faible niveau et est retrouvé, notamment, dans le striatum, le thalamus, le cortex cérébral et la substance noire (Beaulieu & Gainetdinov 2011). Dans le striatum, les récepteurs D1 et D5 sont principalement localisés sur les neurones épineux de taille moyenne (Missale *et coll.* 1998). Le récepteur D5 est aussi présent sur les interneurones cholinergiques (Missale *et coll.* 1998).

1.6.2. Les récepteurs de type D2

Les récepteurs D2 et D3 partagent 75% d'homologie au niveau de leurs domaines transmembranaires tandis que les récepteurs D2 et D4 ont 53% d'homologie (Pivonello *et coll.* 2007). Le gène codant le récepteur D2 est interrompu par des introns, donnant ainsi lieu à deux variants d'épissage du récepteur D2 : la forme courte (D2S) et la forme longue (D2L) (Beaulieu & Gainetdinov 2011). Pharmacologiquement, il est possible de différentier les sous-types des récepteurs de type D2 à l'aide de ligands ayant une affinité beaucoup plus grande pour un sous-type de récepteur (Pivonello *et coll.* 2007). La DA a une plus grande affinité (environ vingt fois supérieure) pour le récepteur D3 que pour le récepteur D2 (Missale *et coll.* 1998).

La stimulation des récepteurs de type D2 conduit à l'inhibition de l'adénylate cyclase par l'intermédiaire des sous-unités $G\alpha_{i/o}$ des protéines G, inhibant ainsi l'accumulation d'AMP cyclique et empêchant l'activation de PKA (Fisone 2009). Les récepteurs D2 peuvent moduler les récepteurs NMDA et inhiber la voie de signalisation d'Akt (Beaulieu & Gainetdinov 2011). Certains des effets des récepteurs de type D2 se font par l'intermédiaire de la sous-unité $G_{\beta\gamma}$, qui est séparée de la sous-unité G_{α} suite à l'activation du récepteur. Les sous-unités $G_{\beta\gamma}$ activées par les récepteurs D2 peuvent stimuler la phospholipase C et réguler les canaux Ca^{2+} et K^{+} (Beaulieu & Gainetdinov 2011).

Contrairement aux récepteurs de type D1, les récepteurs D2 et D3 sont exprimés sur les neurones pré- et post-synaptiques (Beaulieu & Gainetdinov 2011). L'isoforme D2L est davantage abondant que le récepteur D2S et est exprimé de manière prédominante sur les neurones post-synaptiques (Rankin *et coll.* 2009). À l'opposé, les récepteurs D2S sont retrouvés sur les neurones pré-synaptiques et ont une fonction d'autorécepteur, en exerçant un mécanisme de rétroaction en réponse au changement de concentration extracellulaire de DA. Ainsi, l'activation du récepteur D2 pré-synaptique diminue la libération de DA, inhibe la TH, et augmente la recapture de la DA par le DAT et le VMAT2 en augmentant l'expression de ces transporteurs à la membrane (Schmitz *et coll.* 2003). Les récepteurs D2 sont fortement exprimés sur les neurones épineux de taille moyenne du striatum et à des niveaux plus réduits dans la SNc (Rankin *et coll.* 2009). Le récepteur D3 est environ dix fois moins abondant que le récepteur D2, avec une distribution plus limitée (Rankin *et coll.* 2009). La plus forte expression du récepteur D3 est observée dans les régions limbiques, et une expression modérée de ce récepteur est trouvée dans la SNc (Beaulieu & Gainetdinov 2011). Par contre, il y a peu de récepteur D3 exprimé dans le striatum. Le récepteur D4 a le plus faible niveau d'expression au cerveau et est peu exprimé dans le thalamus, les globus pallidus et la substance noire *pars reticulata* (SNr), où ce récepteur est retrouvé sur les neurones GABA (Rankin *et coll.* 2009).

Les principaux récepteurs dopaminergiques impliqués dans l'activité locomotrice sont les récepteurs D1, D2 et D3 (Beaulieu & Gainetdinov 2011). Quoique le récepteur D1 a un effet stimulateur modéré sur l'activité locomotrice (Missale *et coll.* 1998), la fonction du

récepteur D2 est plus complexe étant donné sa localisation pré- et post-synaptique. L'activation du récepteur D2 pré-synaptique diminue la libération de DA, ce qui conduit à une réduction de l'activité locomotrice (Beaulieu & Gainetdinov 2011). Par contre, l'activation du récepteur D2 en post-synaptique augmente l'activité motrice. Le récepteur D3 post-synaptique, qui est fortement exprimé dans le noyau accumbens, semble exercer un effet inhibiteur sur les mouvements (Missale *et coll.* 1998). Quant aux récepteurs D4 et D5, qui sont faiblement exprimés dans les régions motrices, ils ont un rôle limité dans le contrôle des mouvements (Beaulieu & Gainetdinov 2011). Les études ont montré que l'activation simultanée et l'interaction synergique des récepteurs D1 et D2 sont nécessaire pour la pleine manifestation de l'activité motrice (Missale *et coll.* 1998).

1.7. Régulation des mouvements par les ganglions de la base

Les ganglions de la base sont formés de structures interconnectées impliquées dans la régulation de la motricité. Les noyaux des ganglions de la base sont le striatum, les globus pallidus externe (GPe) et interne (GPi), le noyau sous-thalamique (NST), la SNc et la SNr (Samadi *et coll.* 2007). Ces noyaux forment un réseau de connexions intégrant plusieurs régions du cortex cérébral (moteur, associatif et limbique) et le thalamus (figure 1.6). L'équilibre entre deux voies de transmission, la voie directe et la voie indirecte, régule le bon fonctionnement des ganglions de la base et est essentielle pour le contrôle des mouvements.

Le striatum est le principal noyau d'entrée (« input ») des ganglions de la base et reçoit des projections excitatrices glutamatergiques en provenance du cortex et du thalamus, et des projections dopaminergiques en provenance de la SNc (Gerfen & Bolam 2010). Les cibles principales de ces projections afférentes glutamatergiques sont les neurones épineux de taille moyenne GABAergiques, qui représentent 95 % des neurones du striatum (Gerfen & Bolam 2010). Ces neurones GABAergiques inhibiteurs transmettent l'information aux deux noyaux de sortie des ganglions de la base, soient le GPi et la SNr, par une voie directe ou indirecte (Gerfen & Bolam 2010, Obeso *et coll.* 2008). Les noyaux de sortie agissent sur la voie thalamo-corticale, qui est glutamatergique donc excitatrice.

Les neurones GABAergiques de la voie directe expriment le récepteur D1 ainsi que les neuropeptides substance P et dynorphine (Gerfen & Bolam 2010). Ces neurones projettent

directement sur les neurones GABAergiques du GPi et de la SNr. L'activité résultante de la voie directe est donc une inhibition des noyaux de sortie, une désinhibition du thalamus et un transfert de l'information jusqu'au cortex. La voie directe facilite donc le mouvement.

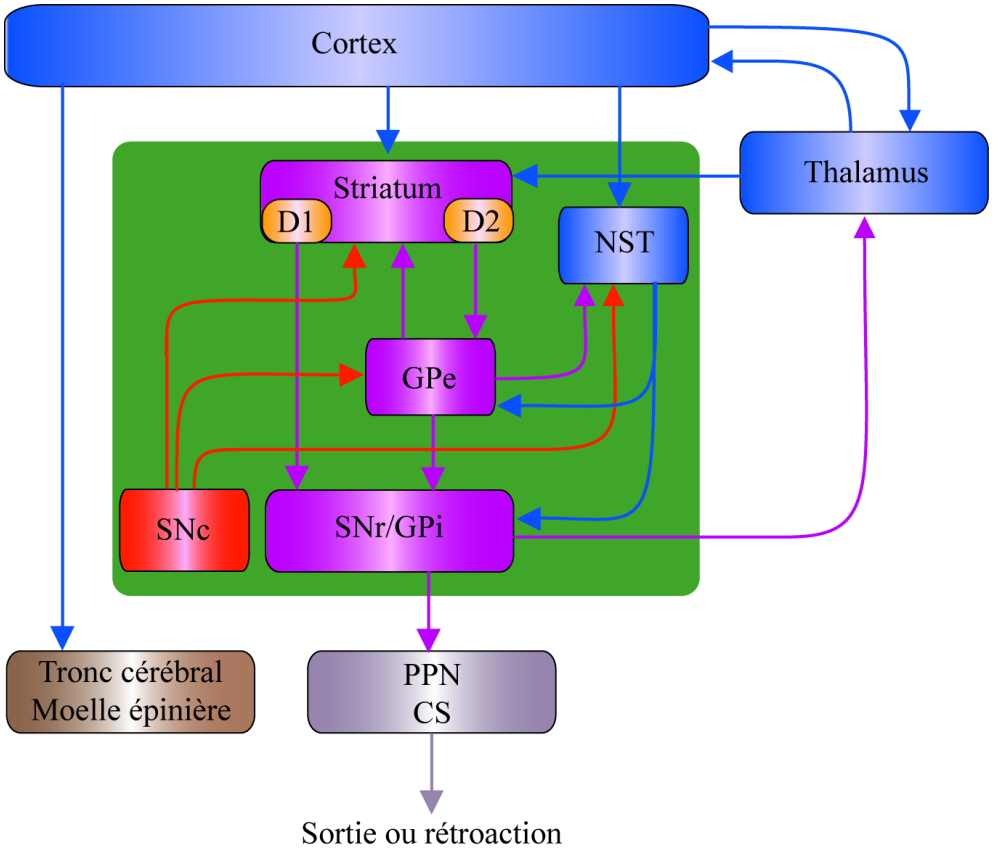

Figure 1.6. Schéma simplifié des principales connexions des ganglions de la base. Les noyaux des ganglions de la base sont représentés dans le carré vert. Les structures glutamatergiques sont indiquées en bleu, les structures GABAergiques sont symbolisées en mauve et les structures dopaminergiques sont représentées en rouge. PPN, noyau pédonculopontine; CS, colliculus supérieur. D'après (Bolam *et coll.* 2009) et (Samadi *et coll.* 2007).

Les neurones GABAergiques de la voie indirecte expriment le récepteur D2 ainsi que le neuropeptide enképhaline (Gerfen & Bolam 2010). Ces neurones projettent directement sur le GPe, dont les projections GABAergiques vont vers le NST ou vers le GPi et la SNr. Le NST envoie des projections glutamatergiques vers le GPi et la SNr mais aussi vers le GPe. L'activité résultante de la voie indirecte est une stimulation des noyaux de sortie (soit par une

désinhibition du NST ou par l'inhibition du GPe), une inhibition du thalamus, et donc une inhibition du transfert de l'information jusqu'au cortex. La voie indirecte inhibe donc les mouvements.

Quoique la DA ait un effet stimulateur par l'activation des récepteurs D1 et un effet inhibiteur par l'activation des récepteurs D2, l'effet de la DA sur la voie directe et indirecte favorise le mouvement. En effet, l'activation de la voie directe (inhibition des noyaux de sortie et désinhibition de la voie thalamo-corticale) et la désinhibition de la voie indirecte (désinhibition du GPe et inhibition du NST et des noyaux de sortie, qui conduit à la désinhibition de la voie thalamo-corticale) par la DA favorise le transfert de l'information du thalamus vers le cortex moteur, et ainsi vers le tronc cérébral et la moelle épinière, ce qui permet au mouvement de se produire (Gerfen & Bolam 2010, Obeso *et coll.* 2008).

La régulation des mouvements par les ganglions de la base est beaucoup plus complexe que ce que décrit dans ce chapitre, qui fait la description d'un schéma simplifié (Gerfen & Bolam 2010, Obeso *et coll.* 2008). L'organisation neuroanatomique et neurophysiologique des ganglions de la base a été en premier lieu définie chez les rongeurs et confirmée par la suite chez les primates. Les principales différences entre les rongeurs et les primates dans l'organisation des ganglions de la base concernent l'anatomie des noyaux. En effet, chez les primates, le striatum est divisé en deux noyaux, le putamen et le noyau caudé, tandis que le rongeur n'a pas cette division anatomique (on parle seulement du striatum) (Gerfen & Bolam 2010). Quoique la division structurelle du striatum chez les rongeurs ne soit pas présente, le striatum montre des différences régionales qui sont fonctionnellement comparable au putamen et au noyau caudé. Une seconde distinction anatomique concerne le GPi. Chez les primates, ce noyau est adjacent au GPe, tandis que chez les rongeurs, le noyau homologue est séparé du GPe et est intégré dans le faisceau de fibres de la capsule interne (Gerfen & Bolam 2010). Ce structure se nomme noyau entopédonculaire et est fonctionnellement comparable au GPi chez les primates. La ségrégation des voies directes et indirectes n'est pas aussi prononcée chez le primate que celle décrite dans ce chapitre. En effet, les études chez le primate rapportent qu'environ 90% des neurones du striatum envoient des projections à la fois dans les trois ciblent principales

du striatum (GPe, GPi, SNr) (Levesque & Parent 2005, Nadjar *et coll.* 2006), ce pourcentage étant d'environ 63% chez le rongeur (Kawaguchi *et coll.* 1990, Wu *et coll.* 2000). Plusieurs voies de rétroaction existent (SNc vers le GPe et le NST; le noyau raphé vers le striatum et la SNc; le GPe vers le striatum; le CS vers le thalamus; et autres) et des innervations dopaminergiques provenant de régions extrastriatales touchent aussi certains noyaux des ganglions de la base (Gerfen & Bolam 2010, Bolam *et coll.* 2009). Environ 5 % des neurones du striatum sont des interneurones (cholinergiques et GABAergiques, ces derniers existant sous plusieurs types) qui reçoivent des informations de plusieurs noyaux et qui influencent les neurones épineux de taille moyenne du striatum (Gerfen & Bolam 2010). De plus, une petite quantité des neurones du striatum co-expriment les récepteurs D1 et D2 (Beaulieu & Gainetdinov 2011). Le striatum contient deux compartiments, les matrices et les striosomes (ou patch) qui sont distinctement ciblés par les neurones dopaminergiques de la substance noire et de l'aire tegmentale ventrale, et qui reçoivent des afférences différentes en provenance du cortex et du thalamus (Gerfen & Bolam 2010). Ainsi, quoique non complet, ce modèle simplifié sert d'assise à la compréhension de la régulation du mouvement par les ganglions de la base.

Chapitre 2 : La maladie de Parkinson

La maladie de Parkinson est la deuxième maladie neurodégénérative en importance, affectant 0,3 % de la population (de Lau & Breteler 2006). La prévalence de cette maladie dans les pays industrialisés est de 1 % chez les personnes de soixante ans et plus, et passe à 4 % chez les personnes de quatre-vingts ans et plus (de Lau & Breteler 2006). Les symptômes moteurs de cette maladie apparaissent vers l'âge de soixante ans par contre, environ 10% des patients ont un début précoce de la maladie, où les symptômes moteurs vont apparaître entre l'âge de 20 et 50 ans (Olanow *et coll.* 2009).

2.1. Différences entre les hommes et les femmes dans la maladie de Parkinson

Plusieurs aspects, incluant le risque de développer la maladie, l'âge de début de la maladie et les symptômes supportent une différence sexuelle dans la maladie de Parkinson. La plupart des études rapportent une plus grande prédisposition de la maladie chez les hommes (Van Den Eeden *et coll.* 2003, Wooten *et coll.* 2004, Shulman 2007), tandis que ceci n'est pas observée dans certaines études (Granieri *et coll.* 1991, de Rijk *et coll.* 1995). Les hommes ont au moins une fois et demie plus de risque de développer la maladie de Parkinson que les femmes (Van Den Eeden *et coll.* 2003, Wooten *et coll.* 2004). L'âge de début de la maladie se produit environ deux ans plus tard chez les femmes comparativement aux hommes (Twelves *et coll.* 2003, Haaxma *et coll.* 2007), quoiqu'une étude ne rapporte pas cette différence (Baba *et coll.* 2005). Le score moteur ne montre pas de différence entre les hommes et les femmes en début de maladie (Baba *et coll.* 2005). Par contre, les femmes ont un meilleur score à un stade plus avancé (Lyons *et coll.* 1998). De plus, au début des symptômes, des niveaux plus élevés de la liaison du [^{123}I]FR-CIT au DAT dans le striatum sont observés chez les femmes, suggérant que le développement des symptômes peut être retardé par de plus haut niveau de DA et que le phénotype de la maladie de Parkinson est plus bénin chez les femmes (Haaxma *et coll.* 2007). Ainsi, des différences sexuelles sont présentes dans la maladie de Parkinson, ce qui suggère que les œstrogènes exercent un effet bénéfique sur le développement et la progression de la maladie.

2.2. Pathophysiologie et symptômes

La maladie de Parkinson est caractérisée par une perte progressive des corps cellulaires dopaminergiques de la substance noire et par la présence d'inclusions protéiniques cytoplasmiques appelées corps de Lewy (qui contiennent la protéine α-synucléine) (Olanow *et coll.* 2009). La destruction des neurones dopaminergiques de la substance noire qui projettent vers le striatum produit une réduction considérable des concentrations striatales de DA, de ses métabolites DOPAC et HVA, du DAT et du VMAT2, ainsi qu'une diminution de l'activité et des niveaux des protéines TH et dopa-décarboxylase (Hornykiewicz 2001). La perte des neurones du tier ventral de la substance noire (projection vers le putamen, perte de 70 à 90 %) est plus importante que dans le tier dorsal (projection vers le noyau caudé et le putamen, perte de 20 à 30 %) (Double *et coll.* 2010). La perte de DA est plus prononcée dans le putamen, une région qui reçoit des afférences principalement des aires motrices et sensorielles du cortex, que dans le noyau caudé, une région recevant particulièrement, mais non exclusivement, des projections des aires associatives du cortex (Parent & Hazrati 1995, Hornykiewicz 2001). La mort des neurones dopaminergiques de la voie nigrostriée se ferait par une dégénérescence rétrograde des neurones, soit des terminaisons vers le corps cellulaire (Cheng *et coll.* 2010, Burke & O'Malley 2012).

La dégénérescence neuronale dans la maladie de Parkinson n'est pas restreinte aux neurones dopaminergiques de la voie nigrostriée et peut toucher d'autres types de neurones. En effet, une dégénérescence et la formation des corps de Lewy ont été observées dans les neurones cholinergiques du noyau basal de Meynert, les neurones sérotoninergiques du raphé, les neurones noradrénergiques du locus coeruleus ainsi que dans les neurones du cortex cérébral, du bulbe olfactif, du tronc cérébral et du système nerveux autonome (Olanow *et coll.* 2009, Ferrer *et coll.* 2012). Basé sur des études pathologiques, Braak et collaborateurs ont suggéré que les inclusions d'α-synucléine présentes dans la maladie de Parkinson évolueraient selon un ordre établi, donnant lieu à l'apparition de six stades (figure 2.1) (Hawkes *et coll.* 2010, Goedert *et coll.* 2013). Selon ces auteurs, l'apparition des inclusions d'α-synucléine se produirait en premier lieu dans le bulbe olfactif et/ou le noyau moteur dorsal des nerfs vague et glossopharyngien du tronc cérébral, ainsi que dans le système nerveux entérique. Dans le stade 2, la pathologie de Lewy se propage au bulbe rachidien et

au tegmentum pontique (locus coeruleus, le noyau magnocellulaire de la formation réticulée et le noyau raphé caudal). Ces deux premiers stades sont asymptomatiques au niveau moteur et pourraient être responsable des troubles olfactif, de constipation et des mouvements oculaires rapides qui sont rapportés en début de maladie de Parkinson, avant l'apparition des symptômes moteurs. Dans le stade 3, la substance noire et l'amygdale sont touchés. Durant le stade 4, les corps de Lewy atteignent le cortex temporal. Le stade 3 ou 4 correspond à l'apparition des symptômes moteurs. Les stades 5 et 6 sont caractérisés par l'apparition des corps de Lewy dans le cortex et pourraient avoir une influence sur les troubles cognitifs présents dans la maladie de Parkinson à un stade avancé (Goedert *et coll.* 2013). Le mécanisme par lequel la progression de la pathologie de Lewy des stades 1 à 6 se produit et atteint le cerveau demeure à élucider.

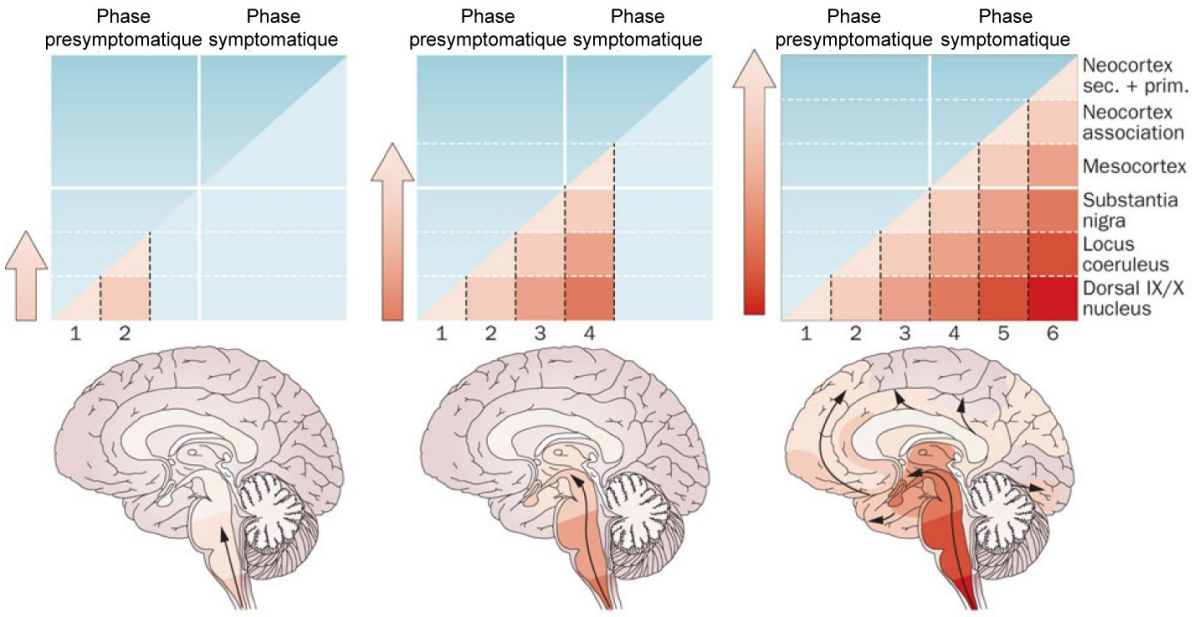

Figure 2.1. Les 6 stades de Braak. Schéma tiré de (Goedert *et coll.* 2013).

Dans la majorité des cas, les stades décrits par Braak et collaborateurs montrent une corrélation adéquate entre la pathologie et l'état clinique (Jellinger 2012, Goedert *et coll.* 2013). Néanmoins, environ 15% des patients ont une progression qui diverge des stades proposés par ces auteurs (Jellinger 2012, Goedert *et coll.* 2013). En effet, certains patients ne

présentent pas d'inclusions dans le noyau des nerfs vague du tronc cérébral, malgré la présence d'inclusions dans la substance noire et le cortex (Jellinger 2012, Goedert *et coll.* 2013).

La perte de DA dans le striatum conduit à un déséquilibre dans la transmission de l'information au niveau des ganglions de la base (figure 2.2). La diminution de la DA dans le striatum cause une augmentation de l'activité de la voie indirecte et une réduction de l'activité de la voie directe (Obeso *et coll.* 2008). Le déséquilibre de ces deux voies résulte en une augmentation de l'activité inhibitrice du GPi et de la SNr vers le thalamus, et une diminution de l'activation des aires corticales motrices (Obeso *et coll.* 2008). L'information se rend donc difficilement au cortex et par le fait même les voies descendantes motrices sont perturbées. Les principaux symptômes de la maladie de Parkinson sont une rigidité, de l'instabilité posturale, une lenteur à initier le mouvement (bradykinésie) et des tremblements au repos (Olanow *et coll.* 2009).

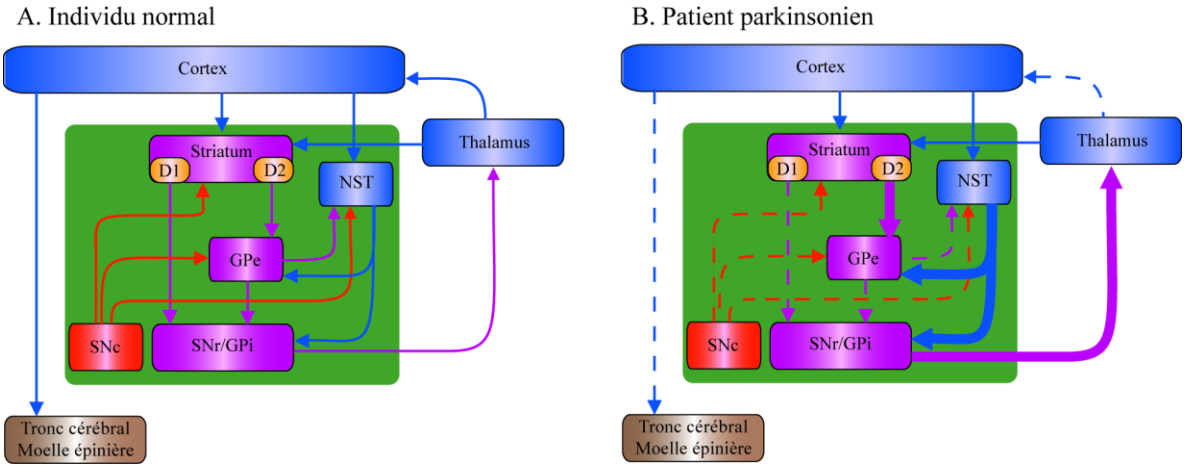

Figure 2.2. Schéma simplifié des principales connexions des ganglions de la base chez un individu normal (A) et chez un patient parkinsonien (B). Les structures glutamatergiques sont indiquées en bleu, les structures GABAergiques sont symbolisées en mauve et les structures dopaminergiques sont représentées en rouge. Les flèches pointillées représentent une diminution de l'activité et les flèches en gras indiquent une augmentation de l'activité. D'après (Bolam *et coll.* 2009) et (Rodriguez-Oroz *et coll.* 2009).

Les symptômes moteurs de la maladie de Parkinson vont se manifester lors d'une réduction de 80% des concentrations de DA dans le putamen et lorsque 50-60% des corps

cellulaires dopaminergiques de la SNc sont perdues (Dauer & Przedborski 2003). Avant ce seuil, une dégénérescence progressive des neurones dopaminergiques se produit, mais les fonctions motrices sont préservées par plusieurs mécanismes de compensation (Bezard *et coll.* 2010). Une augmentation du renouvellement de la DA, des récepteurs D2, de la pré-proenképhaline et des changements thalamo-cortical ont été suggérés comme mécanisme de compensation (Bezard *et coll.* 2010). La phase pré-symptomatique de cette maladie a été initialement estimée d'une durée de trois à six ans, extrapolée à partir de la perte de neurones pigmentés de la substance noire ou à partir d'études mesurant la DA striatale par tomographie à émission de positrons lors d'un vieillissement normal et chez des patients atteints de la maladie de Parkinson (Savica *et coll.* 2010, Gaig & Tolosa 2009). Par contre, l'observation de corps de Lewy extra-nigral (incluant le tronc cérébral, le bulbe olfactif et le système nerveux entérique) précédant l'apparition des corps de Lewy dans la substance noire ainsi que les études épidémiologiques portant sur les symptômes non-moteurs de la maladie de Parkinson suggèrent que la phase pré-symptomatique peut s'étendre jusqu'à vingt ans avant l'apparition des symptômes moteurs (Savica *et coll.* 2010, Gaig & Tolosa 2009, Hawkes *et coll.* 2010).

Plusieurs symptômes non-moteurs sont également observés dans la maladie de Parkinson. Environ 30 % des patients parkinsoniens ont une démence et 20-25 % des patients présentent un déclin cognitif (Svenningsson *et coll.* 2012). L'altération de l'innervation cholinergique au cortex a été associée avec le déclin cognitif et la démence chez les patients parkinsoniens (Svenningsson *et coll.* 2012, Ferrer *et coll.* 2012). La dépression et l'anxiété sont présentes chez environ 40 % des patients (Olanow *et coll.* 2009). Quoique ces symptômes puissent résulter d'une cause exogène (en réaction à la maladie), la dépression et l'anxiété pourraient être causées par la réduction de l'innervation dopaminergique, cholinergique et sérotoninergique (Olanow *et coll.* 2009). Parmi les autres symptômes non-moteurs, une dysfonction olfactive, une dysautonomie, des troubles du sommeil et de l'apathie sont aussi observés (Olanow *et coll.* 2009, Ferrer *et coll.* 2012). Les symptômes non-moteurs peuvent précédés les symptômes moteurs et peuvent être présents à tous les stades de la maladie (Olanow *et coll.* 2009, Ferrer *et coll.* 2012).

2.3. Étiologie de la maladie de Parkinson

Les causes de la maladie de Parkinson idiopathique sont majoritairement inconnues. Des formes familiales de la maladie, causées par des mutations génétiques, sont rapportées dans 5 à 10% des cas (Le *et coll.* 2014). Plusieurs facteurs environnementaux et génétiques ont été associés avec le développement de la maladie de Parkinson.

2.3.1. Le vieillissement

L'âge est le principal facteur de risque de la maladie de Parkinson (Obeso *et coll.* 2010, Schapira & Jenner 2011). Durant le vieillissement, une augmentation des protéines ayant subi des dommages par oxydation se produit, ainsi qu'une diminution de l'activité protéasomale (Floor & Wetzel 1998, Keller *et coll.* 2004, Zeng *et coll.* 2005). En conséquent, une réduction de la dégradation des protéines anormales a lieu, ce qui peut favoriser l'accumulation de protéines et leur agrégation (Olanow & McNaught 2006). Une augmentation de l'activation des microglies est observée dans les neurones du tier ventral de la substance noire lors du vieillissement, suggérant que ces neurones sont soumis à une inflammation modérée chronique qui peut rendre les neurones plus vulnérables (Kanaan *et coll.* 2010).

Les neurones de la substance noire ont une activité autonome qui, avec le vieillissement, utilise davantage les canaux Ca^{2+}, exposant les neurones à de fortes concentrations intracytoplasmiques de Ca^{2+} (Surmeier *et coll.* 2011). Cette activité autonome augmente la production des dérivés réactifs de l'oxygène dans les mitochondries des neurones dopaminergiques de la SNc (Guzman *et coll.* 2010). L'expression de la calbindine, une protéine tamponnant le Ca^{2+}, est élevée dans les neurones du tier dorsal de la substance noire, qui sont les moins affectés par la dégénérescence dans la maladie de Parkinson, comparativement à l'absence de la protéine dans les neurones du tier ventral, qui sont les plus affectés (Damier *et coll.* 1999). Ainsi, les neurones du tier ventral pourraient être exposés à des niveaux de stress oxydatif plus élevés que le tier dorsal et l'aire tegmentale ventrale. Un niveau élevé de stress oxydatif peut causer des mutations dans l'ADN mitochondriale ce qui, par la suite, peut conduire à une déficience dans la chaîne de respiration (Bender *et coll.* 2006, Kraytsberg *et coll.* 2006, Schon & Przedborski 2011). La substance noire accumule des taux

élevés de mutation dans l'ADN mitochondriale durant le vieillissement (Bender *et coll.* 2006, Kraytsberg *et coll.* 2006). De plus, une diminution de l'activité du complexe I de la chaîne de transport d'électron, une augmentation des dommages oxydatifs et une diminution des capacités anti-oxydantes sont aussi observées lors du vieillissement, ce qui contribue à rendre les neurones plus susceptibles aux dommages lors d'une insulte (Venkateshappa *et coll.* 2012).

2.3.2. Facteurs environnementaux

Les études épidémiologiques ont suggéré que plusieurs facteurs environnementaux peuvent augmenter le risque de développer la maladie de Parkinson. L'exposition à des pesticides et à certaines variables reliées (la vie en milieu rural et l'utilisation d'eau de puits), ainsi que l'exposition à certains métaux, ont été associés à une augmentation du risque de la maladie de Parkinson dans certaines études, quoique d'autres ne rapportent pas d'association (Wirdefeldt *et coll.* 2011). Parmi les métaux, une forte exposition au manganèse produit un syndrome parkinsonien (Jankovic 2005). Aucun pesticide spécifique n'a été associé avec le développement de la maladie de Parkinson. De plus, quoiqu'une association entre les pesticides et le développement de la maladie de Parkinson est décrite, aucun lien de cause à effet n'a été établi (Wirdefeldt *et coll.* 2011).

2.3.3. Facteurs génétiques

Dans 5 à 10% des cas, des mutations génétiques ont été associées avec les formes familiales de la maladie de Parkinson. En tout, 18 gènes ont été impliqués avec la forme familiale de cette maladie (Le *et coll.* 2014). Ces gènes codent pour plusieurs catégories de protéines dont des protéines affectant les mitochondries, des protéines qui modifient le stress oxydatif ou les fonctions anti-oxydantes et des protéines impliquées dans le système ubiquitine-protéasome. Ces fonctions sont affectées dans les formes sporadiques de la maladie de Parkinson (Hirsch *et coll.* 2013). Tous les patients atteints d'une des formes génétiques répondent à la L-DOPA et certaines de ces formes ont des caractéristiques cliniques atypiques, en particulier un début de maladie précoce, mais aussi un début de maladie avec des dystonies et l'apparition prématurée de démence et de dysautonomie (Corti *et coll.* 2011).

Les mutations de Parkin, PINK1 et DJ-1 sont responsables des formes autosomiques récessives de la maladie de Parkinson, et sont caractérisées par un début précoce de la maladie (Corti *et coll.* 2011). La mutation de Parkin est la cause la plus fréquente d'un début prématuré de cette maladie (Corti *et coll.* 2011). Parkin est une ligase ubiquitine E3 impliquée dans le système ubiquitine-protéasome (Corti *et coll.* 2011). Parkin est impliqué dans la maintenance mitochondriale et collabore avec PINK1 dans la dégradation macro-autophagique des mitochondries dysfonctionnelles (Gilkerson *et coll.* 2012). PINK1 est impliqué dans la régulation de la dynamique mitochondriale et les fonctions de respiration (Saiki *et coll.* 2012). Plusieurs fonctions sont attribuées à DJ-1, dont le contrôle du stress oxydatif mitochondrial, la régulation de la morphologie mitochondriale et la dégradation des protéines mal repliées (Saiki *et coll.* 2012).

Les formes autosomiques dominantes de la maladie de Parkinson sont causées par des mutations de l'α-synucléine et de la protéine kinase 2 avec des répétitions riches en leucine (LRRK2). La mutation de LRRK2 est la forme génétique de la maladie la plus commune, est caractérisée par un début tardif et reproduit les caractéristiques cliniques et pathologies de la maladie idiopathique (Di Napoli *et coll.* 2007). La surexpression d'une forme mutante de LRRK2 cause une réduction de la libération et de la recapture de la DA, suggérant un rôle de LRRK2 dans la transmission dopaminergique (Le *et coll.* 2014). Des mutations, des duplications et des triplications de l'α-synucléine ont été rapportés (Saiki *et coll.* 2012). La triplication du gène de l'α-synucléine est associée avec une progression rapide de la maladie et une dégénérescence plus importante.

2.4. Pathogénèse

Le processus de dégénérescence dans la maladie de Parkinson serait causé par une multitude de mécanismes. Chacun de ces mécanismes n'agit pas de manière indépendante, mais peut influencer le fonctionnement d'un autre, parfois d'une manière bidirectionnelle, créant ainsi un cercle vicieux (figure 2.3). Les formes génétiques de cette maladie peuvent être causées par la mutation de plusieurs gènes, impliqués dans diverses fonctions, dont le phénotype clinique est similaire, montrant que les mécanismes pathologiques convergents en une voie commune. De plus, les protéines impliquées dans les formes génétiques touchent

des fonctions qui sont affectées dans la forme sporadique de la maladie. Ces observations supportent que les mécanismes de mort neuronale dans la maladie de Parkinson impliquent une combinaison de plusieurs évènements pathologiques multifactoriels (Hirsch *et coll.* 2013).

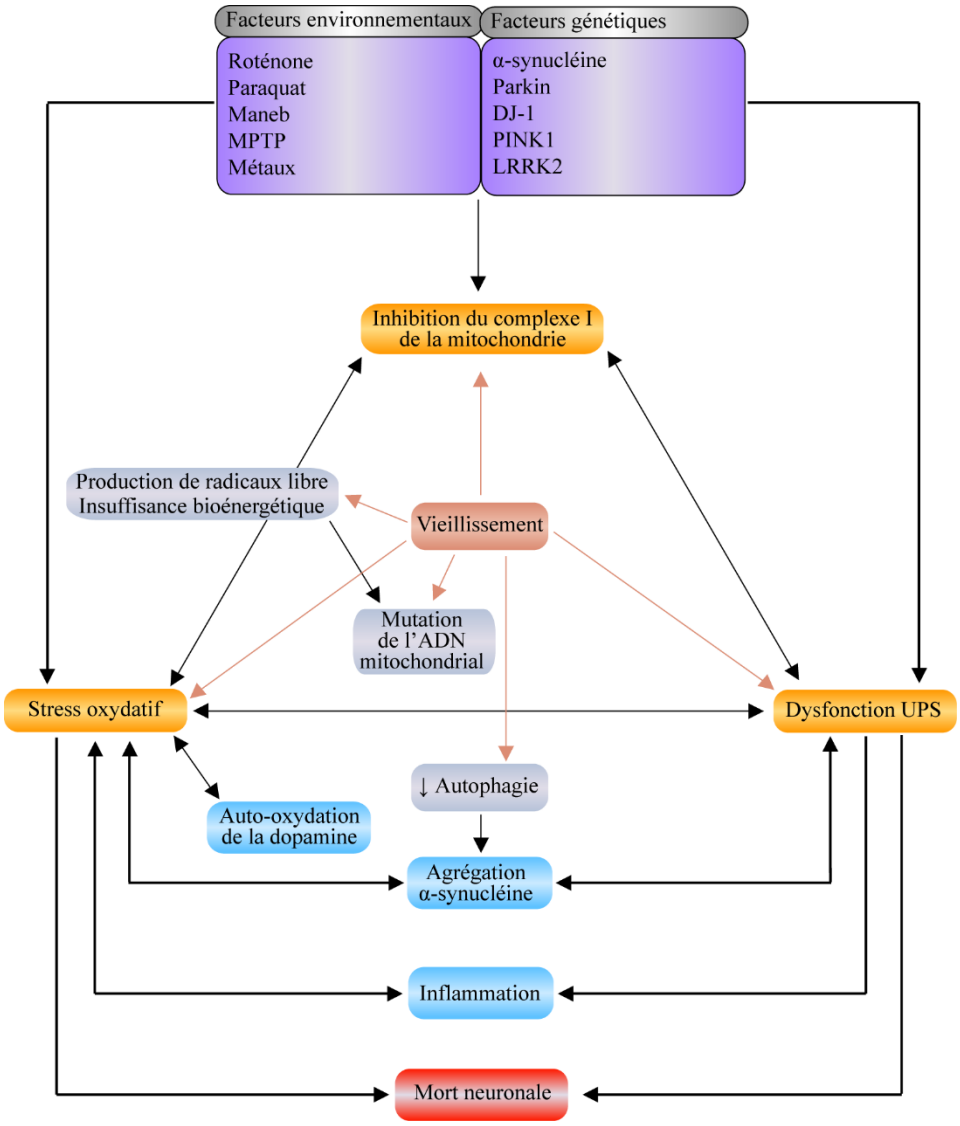

Figure 2.3. Changements survenant durant le vieillissement et les mécanismes impliqués dans la pathogénèse de la maladie de Parkinson. UPS, système ubiquitine-protéasome. D'après (Di Napoli *et coll.* 2007) et (Schapira & Schrag 2011).

2.4.1. Agrégation de protéines

Dans la maladie de Parkinson, les corps de Lewy sont principalement composés de protéines agrégées, dont la principale protéine est l'α-synucléine (Jellinger 2012). Des modifications post-traductionnelles ou des niveaux élevés de l'α-synucléine favorisent un mauvais pliage de cette protéine, ce qui conduit à son agrégation (Rochet *et coll.* 2012). Les dérivés réactifs de l'oxygène ainsi que les dérivés oxydés de la DA (les quinones et le DOPAC) favorisent un mauvais pliage et l'agrégation de l'α-synucléine (Di Napoli *et coll.* 2007). Quoique les fonctions de l'α-synucléine ne soient pas totalement élucidées, un rôle dans la fonction des vésicules synaptiques et dans la régulation du métabolisme de la DA a été rapporté (Venda *et coll.* 2010).

2.4.2. Le système ubiquitine-protéasome

Dans la SNc de patients parkinsonien, une diminution de la fonction des protéasomes est observée (McNaught *et coll.* 2003). Le système ubiquitine-protéasome est la principale voie de dégradation des protéines anormales (Olanow & McNaught 2006). Les protéines agrégées ne sont pas dégradées par ce système et peuvent inhiber la fonction protéosomale et inactiver les protéines de chocs thermiques (dont le rôle est de redonner aux protéines leur conformation native) (Olanow & McNaught 2006). Ainsi, la perturbation du système ubiquitine-protéasome conduit à une réduction de la dégradation des protéines et en une augmentation de leur agrégation. L'inhibition du système ubiquitine-protéasome cause du stress oxydatif, une dysfonction mitochondriale, de l'inflammation et de l'apoptose (Di Napoli *et coll.* 2007). De plus, le système ubiquitine-protéasome, qui fonctionne avec de l'ATP, peut être perturbé par une dysfonction mitochondriale ainsi que par le stress oxydatif (Olanow & McNaught 2006).

2.4.3. Le stress oxydatif et la dysfonction mitochondriale

Une altération des niveaux de plusieurs enzymes anti-oxydantes (catalase, superoxyde dismutase et glutathion peroxydase), ainsi qu'une diminution du glutathion réduit, sont observées dans la substance noire de patients parkinsoniens (Saggu *et coll.* 1989, Sian *et coll.* 1994, Schapira & Jenner 2011). De plus, une augmentation des dommages oxydatifs aux lipides, aux protéines et à l'ADN est présente dans la substance noire de ces

patients (Dexter *et coll.* 1994, Alam *et coll.* 1997a, Alam *et coll.* 1997b). Ces observations montrent qu'un stress oxydatif se produit dans la maladie de Parkinson. Quoique le stress oxydatif est considéré comme contribuant à la mort neuronale dans la maladie de Parkinson, la source et la nature des dérivés réactifs de l'oxygène restent à déterminer (Schapira & Jenner 2011).

Chez les patients parkinsoniens, une diminution de l'activité du complexe I de la chaîne de transport d'électron de la mitochondrie est observée (Keeney *et coll.* 2006). Cette réduction de l'activité du complexe I serait causée par l'auto-oxydation du complexe I, probablement par un mécanisme impliquant une mutation dans l'ADN mitochondrial de ce complexe (Keeney *et coll.* 2006). L'inhibition du complexe I conduit à une diminution de la production d'ATP (perturbant ainsi tous les processus cellulaires dépendants de l'ATP dont le système ubiquitine-protéasome et l'emmagasinage de la DA) et à une augmentation de la production de dérivés réactifs de l'oxygène.

La réduction de l'emmagasinage vésiculaire de la DA produit une augmentation des concentrations cytoplasmiques de DA, laquelle peut être métabolisée et produire du peroxyde d'hydrogène, du radical superoxyde ou s'auto-oxyder en quinone (Napolitano *et coll.* 2011). Les quinones altèrent ou modifient la fonction des protéines en se liant à leur résidu cystéine, créant ainsi de la cytotoxicité, telles qu'une dysfonction de la respiration mitochondriale, l'ouverture du pore de transition de perméabilité mitochondriale et une inhibition des protéasomes (Berman & Hastings 1999, Zhou & Lim 2009, Miyazaki & Asanuma 2009). Le peroxyde d'hydrogène, en présence de fer, peut être converti en radical hydroxyle, un composé plus réactif, et des niveaux élevés de fer sont présents dans la substance noire de patients parkinsoniens, ce qui pourrait contribuer au stress oxydatif (Dexter *et coll.* 1989, Zecca *et coll.* 2004).

2.4.5. Inflammation

La dégénérescence des neurones dopaminergiques dans la maladie de Parkinson est associée avec une activation importante des microglies dans la substance noire (McGeer *et coll.* 1988). Les microglies activées ont une fonction ambivalente; elles ont un rôle

neuroprotecteur mais peuvent aussi induire des dommages neuronaux en relâchant des facteurs pro-inflammatoires.

Une augmentation des niveaux du facteur de nécrose tumorale α, des interleukines 1β, 2 et 6, et de l'interféron γ est rapportée dans le striatum de patients parkinsoniens (Mogi *et coll.* 1994b, Mogi *et coll.* 1994a, Mogi *et coll.* 1996, Mogi *et coll.* 2007). De plus, des niveaux élevés du facteur de nécrose tumorale α, de l'interleukine 1β et de l'interféron γ ont aussi été détectés dans la substance noire (Boka *et coll.* 1994, Hunot *et coll.* 1999, Mogi *et coll.* 2007). Plusieurs enzymes impliquées dans les processus inflammatoires, telles la forme inductible de l'oxyde nitrique synthase, la cyclo-oxygénase 2 et la NAPDH oxydase, sont augmentées dans la maladie de Parkinson (Knott *et coll.* 2000, Wu *et coll.* 2003).

Quoiqu'un processus d'inflammation soit présent dans la maladie de Parkinson, il demeure à déterminer si l'activation des cellules gliales est la cause principale de la mort neuronale ou la conséquence de celle-ci. Néanmoins, l'activation des cellules gliales peut avoir un rôle dans le processus de dégénérescence des neurones. La NAPDH oxydase et la forme inductible de l'oxyde nitrique synthase produisent de l'oxyde nitrique et du superoxyde (Hirsch & Hunot 2009). Ces deux radicaux peuvent se combiner pour former le peroxynitrite, hautement réactif, lequel peut induire des dommages oxydatifs à plusieurs protéines, dont la TH (diminution de la fonction) et l'α-synucléine (favorise l'agrégation) (Ara *et coll.* 1998, Przedborski *et coll.* 2001). L'α-synucléine agrégée est phagocytée par les microglies, conduisant en l'activation de la NAPDH et en la production de dérivés réactifs de l'oxygène (Zhang *et coll.* 2005). De plus, les cytokines pro-inflammatoires peuvent se lier à des récepteurs qui activent des voies de mort neuronale, telles que la signalisation de la kinase c-Jun N-terminal (JNK), du facteur nucléaire κB (NFκB) et p38 MAPK (Hirsch & Hunot 2009).

2.5. Traitements de la maladie de Parkinson

Il n'existe à ce jour aucun traitement curatif ou pouvant ralentir la progression de la maladie de Parkinson. Les traitements ont pour but de diminuer les symptômes moteurs et visent à compenser pour la perte des concentrations de DA. La L-DOPA, le précurseur de la DA, est le médicament le plus utilisé et est administrée conjointement avec un inhibiteur de

la DOPA décarboxylase pour diminuer sa conversion en périphérie (Olanow *et coll.* 2009, Connolly & Lang 2014). L'utilisation d'un inhibiteur de la COMT permet également d'empêcher le métabolisme de la L-DOPA en périphérie (Schapira & Schrag 2011, Connolly & Lang 2014). Les inhibiteurs de la MAO sont utilisés pour empêcher le métabolisme de la DA et ainsi augmenter l'activité de la DA dans la fente synaptique (Schapira & Schrag 2011, Connolly & Lang 2014). Une autre stratégie visant à compenser pour la perte de DA est l'utilisation des agonistes dopaminergiques, qui induisent moins de complications motrices que la L-DOPA, probablement en raison de leur plus longue demi-vie (Olanow *et coll.* 2009). Les anticholinergiques visent à diminuer l'hyperactivité cholinergique dans le striatum, qui est normalement balancée par la transmission dopaminergique (Olanow *et coll.* 2009). Un agent anti-glutamatergique, l'amantadine, a une activité antagoniste sur le récepteur NMDA, ce qui permet de diminuer la transmission glutamatergique excessive (Olanow *et coll.* 2009). De plus, l'amantadine peut augmenter la libération de DA, diminuer sa recapture, stimuler les récepteurs dopaminergiques et pourrait avoir des effets anticholinergiques (Olanow *et coll.* 2009). Bien que la L-DOPA soit le traitement le plus efficace pour les symptômes moteurs, son utilisation peut conduire à l'apparition de fluctuations motrices et de mouvements involontaires, appelés dyskinésies (Olanow *et coll.* 2009).

2.6. Les modèles animaux de la maladie de Parkinson

2.6.1. La réserpine

Dans les années 1950, Carlsson et collaborateurs ont utilisé la réserpine chez le lapin pour diminuer les niveaux des catécholamines au cerveau (Carlsson et coll. 1957a). Un comportement d'akinésie a été observé suite à l'administration de la réserpine, et la diminution des concentrations DA fut identifiée comme étant responsable de ce comportement. L'utilisation de la L-DOPA a permis de renverser le phénotype observé (Carlsson et coll. 1957b). Ces travaux ont permis de montrer le rôle central de la diminution des concentrations de DA dans les ganglions de la base dans la maladie de Parkinson, et que l'utilisation de la L-DOPA, qui demeure le médicament le plus utilisé à ce jour, permet de diminuer les symptômes moteurs. L'utilisation de la réserpine comme modèle de la maladie de Parkinson comporte des limites, puisque plusieurs neurotransmetteurs sont affectés, que

la diminution des catécholamines est temporaire, et qu'aucune perte des neurones dopaminergiques de la voie nigrostrié ne se produit (Cannon & Greenamyre 2010).

2.6.2. Le 1-méthyl-4-phényl-1,2,3,6-tétrahydropyridine (MPTP)

En 1979 et 1982, des cas de syndrome parkinsonien secondaires à l'injection d'un analogue de mépéridine ont été rapportés chez l'humain (Davis *et coll.* 1979, Langston *et coll.* 1983). Il a été découvert que l'analogue de mépéridine administré contenait un contaminant, le MPTP, responsable du syndrome parkinsonien. Ainsi, le modèle MPTP est un modèle expérimental de la maladie de Parkinson découvert chez l'humain. Le MPTP reproduit les symptômes moteurs de la maladie de Parkinson chez l'humain et le singe, incluant une rigidité, une bradykinésie, une instabilité posturale et des tremblements. Cette neurotoxine cause des dommages sélectifs aux neurones dopaminergiques de la voie nigrostriée d'une manière similaire à ce qui est observés dans la maladie de Parkinson, à l'exception de la formation des corps de Lewy (Blandini & Armentero 2012). Le modèle MPTP est le meilleur modèle animal de la maladie de Parkinson (Blandini & Armentero 2012, Jackson-Lewis *et coll.* 2012). Un désavantage de ce modèle est que la dégénérescence des neurones dopaminergiques induite par le MPTP n'est pas progressive, comme rapportée dans la maladie de Parkinson (Blesa *et coll.* 2012).

Le MPTP est une molécule fortement lipophile qui traverse la barrière hémato-encéphalique (Przedborski & Ischiropoulos 2005). Le MPTP est converti en ion 1-méthyl-4-phényl-2,3-dihydropyridium (MPDP+) par la MAO-B localisée dans les cellules gliales et les neurones sérotoninergiques (figure 2.4) (Przedborski & Ischiropoulos 2005). Le MPDP+ est par la suite oxydé en 1-méthyl-4-phénylpyridium (MPP+), le métabolite actif et toxique (Przedborski & Ischiropoulos 2005).

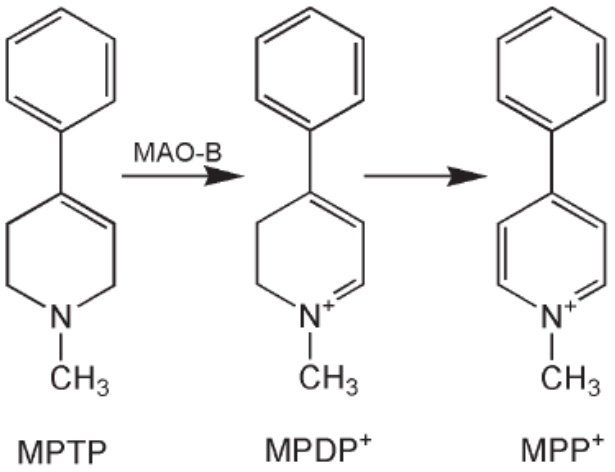

Figure 2.4. Métabolisme du MPTP. Schéma tiré de (Przedborski & Ischiropoulos 2005).

Le MPP+ est un substrat ayant une forte affinité pour le DAT, par lequel il entre dans le neurone dopaminergique (Javitch *et coll.* 1985). L'accumulation de MPP+ à l'intérieur du neurone provoque l'inhibition du complexe I de la chaîne de transport d'électron de la mitochondrie, résultant en une diminution de la production d'ATP et en une augmentation de la production de dérivés réactifs de l'oxygène (figure 2.5) (Przedborski & Ischiropoulos 2005). La réduction de l'ATP peut causer une dysfonction de l'emmagasinage de la DA à l'intérieur des vésicules, provoquant l'augmentation des concentrations cytosoliques de DA et favorisant son oxydation (Dauer & Przedborski 2003). Les dérivés réactifs de l'oxygène présents à l'intérieur de la mitochondrie favorisent l'accumulation de cytochrome c dans l'espace intermembranaire de la mitochondrie (Perier *et coll.* 2012). Les dérivés réactifs de l'oxygène à l'extérieur de la mitochondrie produisent des dommages qui favorisent l'augmentation de la transcription de la protéine Bax, une molécule pro-apoptotique, et sa translocation à la membrane mitochondriale (Perier *et coll.* 2012). Lorsque localisé à la membrane mitochondriale, Bax induit la libération de cytochrome c dans le cytosol, ce qui active les caspases et induit la mort neuronale (Perier *et coll.* 2012).

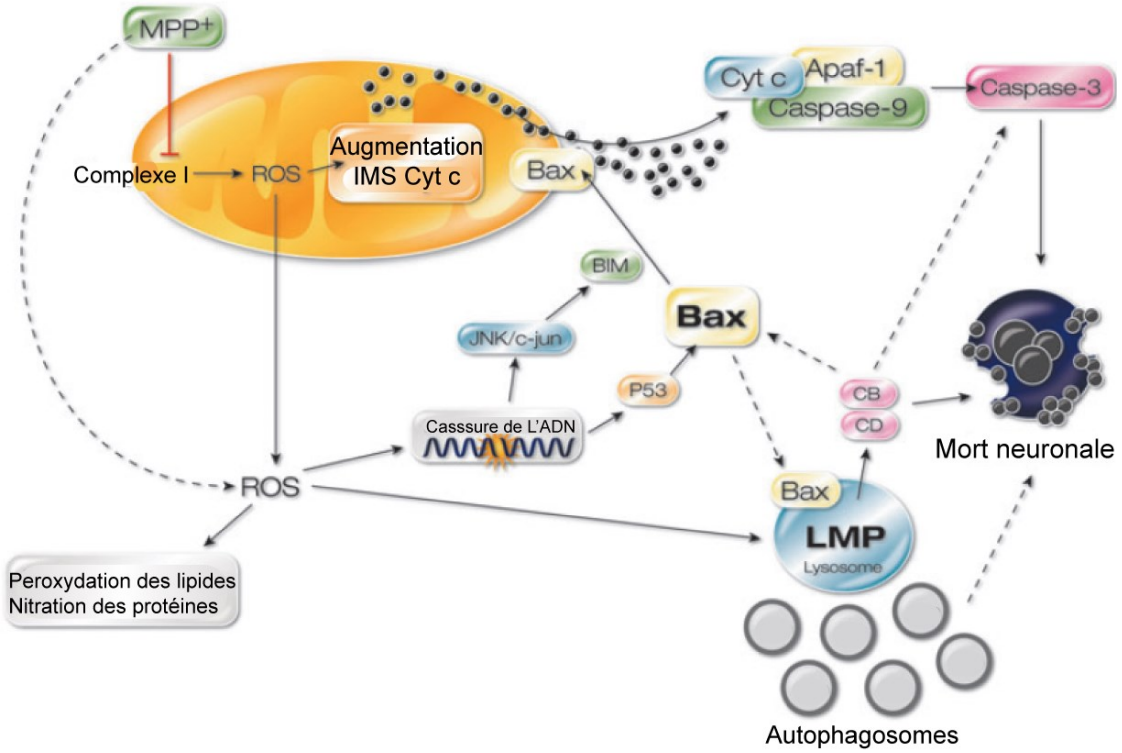

Figure 2.5. Mécanisme d'action du MPP+. CB, cathepsine B; CD, cathepsine D; Cyt c, cytochrome c; IMS, espace intermembranaire de la mitochondrie; LMP, perméabilisation de la membrane lysosomale; ROS, dérivés réactifs de l'oxygène. Schéma tiré de (Perier *et coll.* 2012).

À l'intérieur du neurone, le MPP+ peut être séquestré à l'intérieur des vésicules synaptiques par le VMAT2, prévenant ainsi son accumulation à l'intérieur de la mitochondrie (Guillot & Miller 2009). Cette accumulation vésiculaire de MPP+ serait un mécanisme pour réduire l'effet toxique. En support, les cellules transfectées pour exprimer une plus grande densité de VMAT2 sont résistantes à l'effet toxique du MPP+ (Liu *et coll.* 1992). De plus, les souris hétérozygotes invalidées en VMAT2 sont plus susceptibles à l'effet toxique du MPTP (Gainetdinov *et coll.* 1998). Au contraire, l'invalidation du DAT chez les souris ou l'inhibition pharmacologique du DAT confère une résistance à la toxicité du MPTP (Gainetdinov *et coll.* 1997, Pifl *et coll.* 1993). Ainsi, le DAT et le VMAT2 sont deux composantes importantes de l'effet toxique du MPTP.

2.6.3. La 6-hydroxydopamine (6-OHDA)

Le modèle 6-OHDA est le premier modèle animal de la maladie de Parkinson (Ungerstedt 1968). La pertinence de ce modèle vient du fait que cette neurotoxine est sélective pour les neurones catécholaminergiques et qu'elle induit un stress oxydatif qui cause la dégénérescence de ces neurones (Schober 2004). La structure de la 6-OHDA est similaire à celle de la DA (figure 2.6). Par contre, la présence d'un groupe hydroxyle additionnel rend la molécule de 6-OHDA toxique, en raison de la formation de dérivés réactifs de l'oxygène. La 6-OHDA ne passe pas la barrière hémato-encéphalique et est directement injectée dans la SNc, le striatum ou le faisceau médian du télencéphale (Blesa *et coll.* 2012). L'injection de la 6-OHDA dans la SNc ou le faisceau médian du télencéphale cause une destruction antérograde des neurones dopaminergiques, avec une perte des corps cellulaires dopaminergiques survenant dans les douze heures post-lésion tandis que les dommages aux terminaisons apparaissent dans les deux à trois jours (Blandini & Armentero 2012). L'injection de la toxine dans le striatum produit une dégénérescence rétrograde progressive, qui s'étend de quatre à six semaines suivant l'injection de 6-OHDA (Blandini & Armentero 2012). Ainsi, l'injection de 6-OHDA dans la SNc ou le faisceau médian du télencéphale modèle la maladie de Parkinson à un stade avancé tandis que l'injection de la toxine dans le striatum produit une dégénérescence moins sévère.

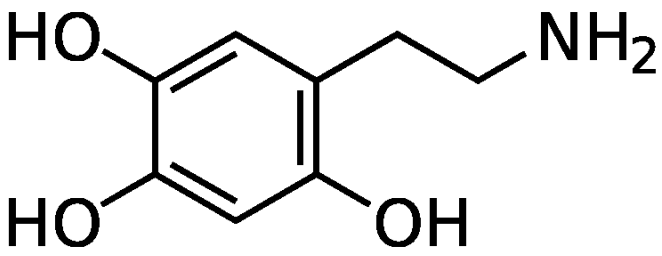

Figure 2.6. Représentation de la molécule de la 6-OHDA.

La 6-OHDA est sélective pour les neurones catécholaminergiques, et est préférentiellement recapturée par le DAT et le transporteur de la noradrénaline (Schober 2004). L'utilisation d'un inhibiteur de la recapture de la noradrénaline ainsi que la spécificité

du site d'injection de la toxine permettent d'atteindre une sélectivité pour les neurones dopaminergiques de la voie nigrostriée (Deumens *et coll.* 2002). La 6-OHDA a une grande affinité pour le DAT, par lequel cette toxine entre dans le neurone dopaminergique (Schober 2004). À l'intérieur du neurone, la 6-OHDA s'accumule dans le cytoplasme et s'auto-oxyde, ce qui induit la formation de radicaux libres (Przedborski & Ischiropoulos 2005). La 6-OHDA peut aussi s'accumuler dans les mitochondries, où cette toxine inhibe le complexe I de la chaîne de respiration mitochondriale (Deumens *et coll.* 2002). Le modèle de lésion unilatérale chez le rat ou la souris est le plus utilisé et produit un comportement moteur asymétrique (rotation) après administration d'amphétamine (AMPH) ou d'apomorphine, causé par le déséquilibre de l'activité dopaminergique entre les deux hémisphères (Deumens *et coll.* 2002). L'avantage du modèle unilatéral est qu'il induit un déficit moteur quantifiable (rotation), qui est corrélé avec le degré des dommages induits à la substance noire (Deumens *et coll.* 2002). Dans le modèle de lésion unilatérale, l'hémisphère intacte de l'animal sert de contrôle à l'hémisphère lésé, quoique certaines compensations puissent survenir dans l'hémisphère non-lésé (Deumens *et coll.* 2002). Les lésions bilatérales produisent des déficits moteurs et offrent un avantage par rapport à une lésion unilatérale à savoir qu'il n'y a pas de mécanisme de compensation d'un hémisphère (Deumens *et coll.* 2002).

2.6.4. La roténone et le paraquat

La roténone, un inhibiteur du complexe I de la chaîne de transport d'électron de la mitochondrie, et le paraquat, donc la structure est similaire au MPP+ et qui génère des dérivés réactifs de l'oxygène, sont deux pesticides utilisés comme modèle animal de la maladie de Parkinson (Jackson-Lewis *et coll.* 2012). L'effet du paraquat sur la dégénérescence du système dopaminergique nigrostrié n'est pas toujours consistant (Blesa *et coll.* 2012, Jackson-Lewis *et coll.* 2012). Par contre, le paraquat augmente l'expression de l'α-synucléine et induit la formation de structures similaires au corps de Lewy dans la SNc, ce qui permet d'étudier le rôle de l'α-synucléine et le processus de développement des corps de Lewy (Blesa *et coll.* 2012). La roténone cause des dommages au système dopaminergique nigrostrié accompagnés de comportements moteurs (bradykinésie, instabilité posturale, démarche instable et tremblement), d'agrégat d'α-synucléine et de la formation de structures similaires au corps de Lewy dans la SNc (Blesa *et coll.* 2012). Par contre, une grande variation dans la

réponse à ce pesticide est observée chez les animaux, ce qui fait que le modèle est difficile à reproduire (Blandini & Armentero 2012, Le *et coll.* 2014).

2.6.5. Les modèles génétiques

La découverte de gènes impliqués dans la maladie de Parkinson a conduit au développement de modèles animaux qui ont les mutations impliquées dans les formes familiales de cette maladie.

Les souris invalidées en Parkin ne présentent pas de dégénérescence de la voie nigrostriée ou ne montrent pas de déficits moteurs (Harvey *et coll.* 2008). Par contre, la surexpression de la mutation de la protéine Parkin dans les neurones dopaminergiques provoque une dégénérescence progressive de la voie nigrostriée, des déficits moteurs ainsi qu'une pathologie α-synucléine (Blandini & Armentero 2012). Les souris déficientes en PINK1 ou en DJ-1 ne présentent pas de dégénérescence de la voie nigrostriée mais sont par contre plus sensibles au stress oxydatif (Le *et coll.* 2014). Une mutation de LRRK2 conduit à un déficit progressif de l'activité motrice qui est corrigé par l'administration d'agents dopaminergiques (Le *et coll.* 2014). Quoique la surexpression de la protéine α-synucléine chez la souris n'induit pas toujours une dégénérescence dopaminergique (dépendamment du promoteur utilisé pour exprimer l'α-synucléine), une altération de la voie nigrostriée, ainsi que des déficits moteurs ont aussi été rapportés (Dauer & Przedborski 2003, Blandini & Armentero 2012). Néanmoins, des dysfonctions non-motrices telles qu'observées en début de maladie de Parkinson (troubles gastro-intestinal et olfactif) sont observées chez certains modèles surexprimant l'α-synucléine (Blandini & Armentero 2012).

Deux groupes ont généré un modèle de souris afin d'investiguer le rôle de la dysfonction mitochondriale dans la dégénérescence dopaminergique. La souris MitoPark est une souris conditionnellement invalidée en facteur de transcription A mitochondrial (Ekstrand *et coll.* 2007, Ekstrand & Galter 2009). L'expression de mito-Pst1 dans les neurones dopaminergique chez la souris (souris PD-mito-Pst1) provoque des dommages à l'ADN mitochondrial, ce qui conduit en une déficience de la phosphorylation oxydative (Pickrell *et coll.* 2011). Ces deux modèles de souris montrent une dégénérescence progressive

de la voie nigrostriée à l'âge adulte, un déficit moteur progressif ainsi qu'une réponse favorable à la L-DOPA. De plus, la présence d'inclusion intraneuronale est observée chez la souris MitoPark. La dégénérescence dopaminergique est plus rapide chez la souris MitoPark que chez la souris PD-mito-Pst1.

Quoique les modèles génétiques basés sur les mutations chez l'humain reproduisent difficilement la dégénérescence dopaminergique, ces modèles sont utiles pour étudier le rôle et les mécanismes moléculaires impliqués dans la dysfonction des gènes dans la maladie de Parkinson. Il a été suggéré que l'absence de mort neuronale dans ces modèles pourrait être causée par un mécanisme de compensation qui pourrait être évité par la délétion conditionnelle des gènes à l'âge adulte (Meissner *et coll.* 2011). De plus, la plupart des modèles génétiques montrent une déficience dans la transmission dopaminergique et peuvent ainsi modeler les phases précoces de la maladie, soit avant l'apparition des symptômes moteurs (Meissner *et coll.* 2011).

Chapitre 3 : Les hormones ovariennes

3.1. Les œstrogènes

3.1.1. Structure et synthèse

Les trois principaux œstrogènes endogènes sont le 17β-estradiol, l'estrone et l'estriol. Le 17β-estradiol est le plus abondant et le plus puissant des œstrogènes. L'estrone est le principal œstrogène retrouvé chez la femme ménopausée (Rannevik *et coll.* 2008) tandis que l'estriol constitue de 60-70% des œstrogènes présents lors de la grossesse (Pasqualini 2005). Les œstrogènes sont des hormones stéroïdiennes composées de 18 carbones, un anneau phénol A et des groupements hydroxyles ou cétones.

Le précurseur de la synthèse des stéroïdes est le cholestérol (figure 3.1). La biosynthèse des œstrogènes est catalysée par le cytochrome P450 aromatase, qui catalyse la conversion de l'androstenedione en estrone et de la testostérone en estradiol (Do Rego *et coll.* 2009). Les ovaires constituent la source la plus importante de production du 17β-estradiol chez la femme non-ménopausée (Simpson 2003). Chez la femme ménopausée et chez l'homme, le 17β-estradiol provient principalement des tissus extra-gonadiques, tels que le cerveau, les muscles, les tissus adipeux, les cellules endothéliales, le foie et les os (Simpson 2003). Dans les gonades, la synthèse de 17β-estradiol provient principalement de l'aromatisation de la testostérone tandis que dans les tissus extra-gonadiques, le 17β-estradiol est formé préférentiellement par l'hydroxylation de l'estrone (Luu-The & Labrie 2010).

3.1.2. Fonctions des œstrogènes

Les œstrogènes ont un rôle majeur dans le développement des organes reproducteurs et dans l'apparition des caractéristiques sexuelles secondaires chez la femme. En plus de leur effet sur le système reproducteur, les œstrogènes exercent une multitude d'actions dans plusieurs tissus. Les œstrogènes ont des effets bénéfiques, entre autres, sur le système cardiovasculaire, maintiennent la densité osseuse, diminuent les lipoprotéines de basse densité et régulent la réponse inflammatoire (Nilsson & Gustafsson 2010). Dans le cerveau, les œstrogènes augmentent la densité des épines dendritiques, régulent la plasticité synaptique, modulent la cognition et accroissent le métabolisme du glucose (Cooke &

Woolley 2005, Sherwin & Henry 2008, Boon *et coll.* 2010, Srivastava *et coll.* 2011). Les œstrogènes ont longtemps été considérés comme des hormones féminines. Par contre, les effets des œstrogènes n'ont pas lieu exclusivement chez la femme et ces hormones exercent aussi un rôle important dans plusieurs tissus chez l'homme. La déficience en aromatase chez les hommes ainsi que le cas d'un homme ayant un récepteur des œstrogènes (ER) α non-fonctionnel supportent que les œstrogènes exercent des effets importants chez les hommes dans divers systèmes (Smith *et coll.* 1994, Rochira & Carani 2009).

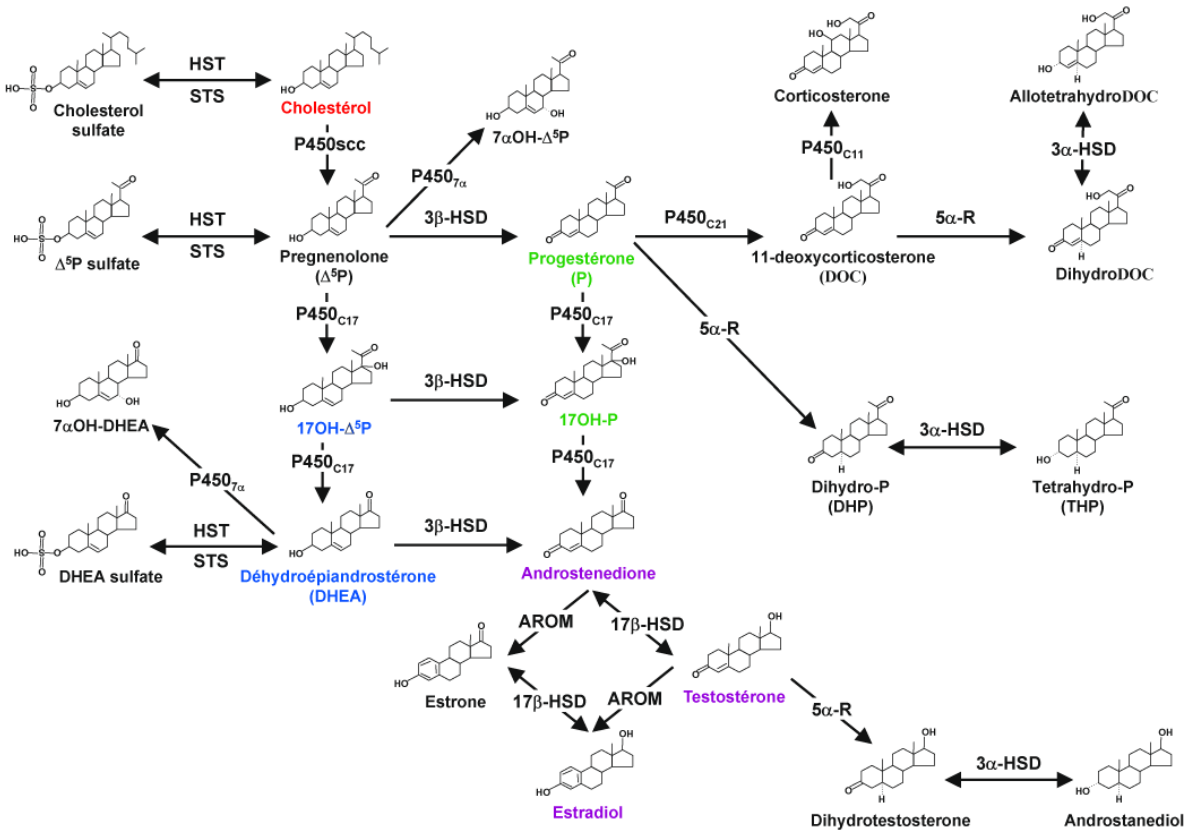

Figure 3.1. Voie de biosynthèse des stéroïdes. La voie Δ5 est représentée en bleue et la voie Δ4 est montrée en verte. AROM, aromatase; HST, sulfotransférase; STS, sulfatase; P450scc, cytochrome P450 responsable de la coupure de la chaîne latérale du cholestérol; $P450_{7\alpha}$, cytochrome 7α-hydroxylase; $P450_{C11\beta}$, 11β-hydroxylase; $P450_{C17}$, cytochrome P450 17α-hydroxylase/C17,20-lyase; $P450_{C21}$, 21-hydroxylase; 3α-HSD, 3α-hydroxystéroïde déhydrogénase; 3β-HSD, 3β-hydroxystéroïde déhydrogénase; 5α-R, 5α-réductase; 17β-HSD, 17β-hydroxystéroïde déhydrogénase. Schéma tiré de (Do Rego *et coll.* 2009).

3.1.3. Les récepteurs des œstrogènes

Les œstrogènes produisent leurs effets en se liant au ERα et au ERβ, tous deux appartenant à la famille des récepteurs nucléaires. Le ERα est encodé part le gène ESR1 du chromosome 6 et le ERβ est encodé par le gène ESR2 du chromosome 14 (Nilsson *et coll.* 2011). Trois isoformes du ERα et cinq isoformes du ERβ ont été identifiés (Heldring *et coll.* 2007). Les isoformes du ERβ sont formés par un épissage alternatif du dernier exon codant (Nilsson & Gustafsson 2011). La plupart des isoformes du ERα diffèrent dans leur région 5' non traduite. Un isoforme du ERα (ERα-36) ne contient ni l'AF-1, ni l'AF-2, tandis que pour le ERα-46, seulement l'AF-1 est manquant (Nilsson & Gustafsson 2011). Quoique les ERs partagent un degré élevé d'homologie de séquence dans leur domaine de liaison à l'ADN, le domaine N-terminal, où est localisé la fonction d'activation (AF)-1, n'est pas préservé entre le ERα et le ERβ (figure 3.2) (Marino *et coll.* 2006). Les AF-1 et AF-2 facilitent la transcription en fournissant des surfaces pour les interactions protéines-protéines avec les co-régulateurs (Nilsson & Gustafsson 2011). L'AF-1 est associée à une activité de transcription qui est indépendante du ligand. En effet, l'AF-1 contient des sites de phosphorylation pour la transcription d'une manière indépendante du ligand par la voie des MAPK (Nilsson & Gustafsson 2011). L'AF-2 est associée à une activité de transcription qui est dépendante du ligand (Nilsson & Gustafsson 2011). Des données *in vitro* ont montré que le ERα est un plus puissant activateur de la transcription que le ERβ étant donné la différence dans leur région où est située l'AF-1 (Delaunay *et coll.* 2000).

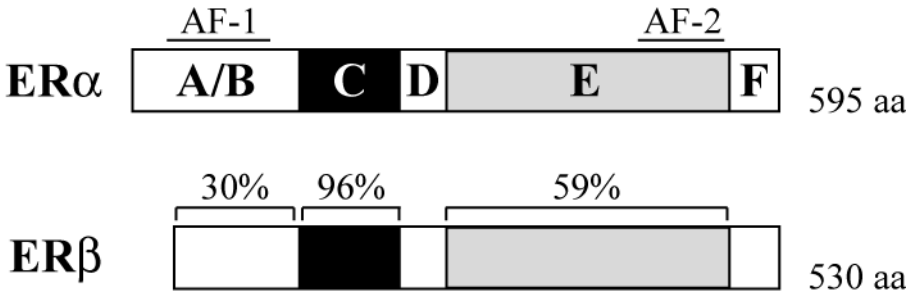

Figure 3.2. Domaines structuraux des récepteurs des œstrogènes. Le ER est composé d'une région N-terminal (les domaines A/B) qui contient l'AF-1, d'un domaine de liaison à l'ADN (le domaine C), d'une région charnière contenant des séquences qui favorisent la localisation nucléaire et les modifications post-traductionnelles (le domaine D), et d'une région C-

terminale, contenant le domaine de liaison du ligand et l'AF-2, qui est impliquée dans le processus de dimérisation (les domaine E/F). Les pourcentages indiquent l'homologie des différents domaines entre le ERα et le ERβ. aa, acides aminés. D'après (Nilsson & Gustafsson 2011).

La différence entre l'homologie du domaine de liaison entre le ERα et le ERβ confère à ces deux sous-types de récepteurs une différence dans leur habileté à lier leur ligand (Kuiper *et coll.* 1997). Le 17β-estradiol se lie aux deux sous-types de récepteurs avec la même affinité cependant le stéréoisomère 17α-estradiol a peu d'affinité pour les ERs et est généralement considéré comme inactif à des concentrations physiologiques (Kuiper *et coll.* 1998). L'estrone et le modulateur sélectif des récepteurs des œstrogènes (SERM), le raloxifène, ont une plus grande affinité pour le ERα que le ERβ (Kuiper *et coll.* 1998). L'estriol lie préférentiellement le ERβ (Kuiper *et coll.* 1997). Des ligands sélectifs pour chaque sous-type de ER ont été développés. Le 2,3-bis(4-hydroxyphényl)propionitrile (DPN) montre une affinité de liaison relative de 70 fois plus importante pour le ERβ et une puissante relative de 170 fois plus grande dans les essais de transcription avec le ERβ comparativement au ERα (Meyers *et coll.* 2001). L'affinité de liaison du 4,4′,4″-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)tris-phénol (PPT) est 410 fois plus grande pour le ERα que pour le ERβ et cet agoniste active la transcription des gènes seulement par le ERα (Stauffer *et coll.* 2000). Quoique le PPT induise préférentiellement la formation d'homodimères ERα, la formation d'hétérodimères ERα-ERβ peut aussi se produire (Powell & Xu 2008). Par contre, le DPN produit seulement la formation d'homodimères ERβ (Powell & Xu 2008).

Une forte affinité du 17β-estradiol pour un récepteur orphelin de la famille des récepteurs à 7 domaines transmembranaires a été décrite en 2005 (Revankar *et coll.* 2005, Thomas *et coll.* 2005). Au départ connu sous le nom de GPR30, le récepteur des œstrogènes couplés aux protéines G (GPER1) est exprimé dans le striatum et dans la substance noire (Brailoiu *et coll.* 2007, Hazell *et coll.* 2009). Le GPER1 est présent au niveau de la membrane plasmatique et une localisation de ce récepteur au niveau de l'appareil de Golgi ainsi que du réticulum endoplasmique a aussi été observée (Funakoshi *et coll.* 2006, Matsuda *et coll.* 2008). Le GPER1 lie le 17β-estradiol avec une forte affinité quoique le 17α-estradiol ne déplace pas la liaison du [^{3}H]estradiol au GPER1 (Thomas *et coll.* 2005). L'estrone et

l'estriol montre très peu d'affinité pour le GPER1 et d'autres stéroïdes tels que le cortisol, la progestérone et la testostérone ne lient pas le GPER1 (Thomas *et coll.* 2005). De plus, l'antagoniste des ERs, soit le ICI 182,780, certain SERM tel le tamoxifène, ainsi qu'un phytoestrogène, la génistéine, sont des agonistes du GPER1 (Thomas *et coll.* 2005, Thomas & Dong 2006). Un agoniste spécifique, le G1 (Bologa *et coll.* 2006), ainsi que des antagonistes spécifiques, le G15 (Dennis *et coll.* 2009) et le G36 (Dennis *et coll.* 2011), du GPER1 ont été développés, sans activité sur le ERα ou le ERβ.

Quoique le ERα et le ERβ soient présents dans le striatum et dans la substance noire, leur distribution est différence entre ces deux régions. L'immunoréactivité du ERα au niveau du striatum est supérieure à celle du ERβ (Mitra *et coll.* 2003, Almey *et coll.* 2012). Inversement, une plus grande immunoréactivité pour le ERβ comparativement au ERα a été rapportée pour la substance noire (Mitra *et coll.* 2003). Les ERα exprimés au niveau du striatum sont majoritairement associés à la membrane plasmatique plutôt que dans la fraction nucléaire (Schultz *et coll.* 2009). Une étude combinant un marquage rétrograde et un double marquage par immunocytochimie pour les neurones de la SNc positifs pour le ERβ et la TH a été menée afin d'investiguer les projections de ces neurones (Creutz & Kritzer 2004). Cette étude a révélé que les projections des neurones de la SNc positifs pour le ERβ et la TH projettent principalement vers le striatum ventral et peu vers le striatum dorsal (Creutz & Kritzer 2004). Une étude récente utilisant la microscopie électronique a montré que le ERα, le ERβ et le GPER1 sont présents dans le striatum et ont une localisation exclusivement extranucléaire (Almey *et coll.* 2012). Un double marquage révèle une absence de localisation du ERα et du GPER1 sur les neurones dopaminergiques, mais une colocalisation est observée sur d'autres types de neurones (cholinergiques et probablement GABAergiques) (Almey *et coll.* 2012). De plus, le ERα, le ERβ et le GPER1 sont présents sur les cellules gliales et la membrane mitochondriale (Almey *et coll.* 2012).

Un récepteur des œstrogènes, désigné ER-X, associé à la membrane et localisé dans les calvéoles, est exprimé dans le néocortex lors du développement post-natal mais non chez l'adulte (Toran-Allerand *et coll.* 2002). Le ER-X serait ré-exprimé suite à un dommage cérébral dans le cerveau adulte, tel que lors d'une ischémie cérébrale (Toran-Allerand *et coll.*

2002). Puisque de plus faible concentration de 17α-estradiol comparativement au 17β-estradiol produise une plus forte activation des ERK, les auteurs concluent que le 17α-estradiol est le ligand préféré du ER-X, quoique ce récepteur lie le 17β-estradiol et le 17α-estradiol avec la même affinité (Toran-Allerand 2004).

3.1.4. Mécanisme d'action génomique

Le mécanisme d'action génomique des ERs implique la transcription des gènes se produisant suite à l'activation des ERs (Marino *et coll.* 2006). Ce mécanisme nécessite des heures et jusqu'à des jours pour exercer son effet. En absence d'un ligand, les ERs sont des monomères associés avec des protéines de choc thermique (Marino *et coll.* 2006). La liaison des œstrogènes à leurs récepteurs induit un changement de conformation du récepteur qui induit la dissociation des protéines de choc thermique et permet l'homo- ou l'hétéro-dimérisation du récepteur et sa translocation au noyau. Lorsque les deux sous-types de ER sont exprimés dans une cellule, la formation d'hétérodimère prédomine (Pettersson *et coll.* 1997, Matthews & Gustafsson 2003) et seulement le ERα serait capable d'induire la formation d'hétérodimère (Powell & Xu 2008). Le dimère ER interagit avec une séquence spécifique de l'ADN (élément de réponse à l'œstrogène (ERE)) dans la région du promoteur du gène cible. Ce complexe interagit avec des co-régulateurs, résultant en l'altération de la transcription. Les gènes de transcription manquant un ERE fonctionnel nécessitent un second facteur de transcription liant l'ADN servant d'intermédiaire à l'association du ER avec l'ADN. L'association indirecte du ER avec l'ADN peut se faire par l'interaction avec le NFκB, la protéine spécificité 1, CREB ou par l'interaction avec les facteurs de transcription fos/jun régulant ainsi la transcription de gènes par la protéine activatrice-1 (AP-1) (Marino *et coll.* 2006).

L'action spécifique des œstrogènes au noyau dépend de plusieurs facteurs. La structure de l'hormone, le sous-type de récepteur impliqué, les particularités des promoteurs des gènes cibles et l'équilibre entre les coactivateurs et les corépresseurs ont une influence sur la spécificité de l'action du complexe œstrogène-ER (Ascenzi *et coll.* 2006). Les gènes régulés par les hétérodimères ERα-ERβ sont différents des gènes régulés par les homodimères (Monroe *et coll.* 2005). De plus, les niveaux de ERα et de ERβ ne sont pas

exprimés de la même manière dans tous les tissus. Quoique la distribution du ERα et du ERβ soit similaire pour plusieurs régions du cerveau, une plus forte proportion de ERα est observée à l'hippocampe, à l'aire préoptique, au striatum et à l'hypothalamus (Mitra *et coll.* 2003). Le ERβ est le sous-type de récepteur prédominant retrouvé au cortex, à la SNc et au cervelet (Mitra *et coll.* 2003). Dans les cellules où les niveaux de ERβ sont supérieurs à ceux du ERα, le ERβ peut inhiber l'activité transcriptionnelle du ERα (Nilsson & Gustafsson 2010).

3.1.5. Mécanisme d'action non-génomique

Le mécanisme d'action non-génomique des œstrogènes se définit par un effet rapide (dans les secondes et les minutes) initié par l'interaction avec des ERs présents dans la membrane plasmatique et/ou le GPER1. Les ERs membranaires et les ERs nucléaires proviennent des mêmes gènes et les ERs membranaires fonctionnent aussi comme des dimères (Razandi *et coll.* 2004). Étant des récepteurs nucléaires, la structure des ERs ne leur permet pas d'être intégrés à la membrane. Le mécanisme par lequel les ERs sont incorporés à la membrane plasmatique n'est pas complètement élucidé, mais la palmitoylation des ERs et l'interaction avec des protéines calvéoline sont des étapes essentielles pour la localisation de ces récepteurs à la membrane et une signalisation efficace (Acconcia *et coll.* 2005, Pedram *et coll.* 2007).

La localisation plasmatique des ERs dans les calvéoles, invagination de la membrane qui forme des microdomaines regroupant des molécules de signalisation, permet leurs interactions avec les protéines de signalisation intracellulaire (Marin *et coll.* 2012). Plusieurs protéines d'échafaudage des ERs et des molécules de signalisation qui sont associées avec les ERs servent à faciliter l'activation des kinases par les œstrogènes. Plusieurs protéines dont les protéines calvéoline, les protéines G, Src, la sous-unité régulatrice p85α de PI3K, Shc et les récepteurs tyrosine kinase (tel que le récepteur du facteur de croissance de l'insuline 1 (IGF-1)) ont tous été identifiés comme servant de composantes à des complexes de protéines interagissant avec les ERs (Marino *et coll.* 2006, Marin *et coll.* 2012). L'activation de la signalisation par le 17β-estradiol peut se faire par une interaction entre les

ERs et le récepteur de l'IGF-1 ou les récepteurs métabotropique du glutamate (Mendez *et coll.* 2003, Quesada & Micevych 2004, Grove-Strawser *et coll.* 2010).

3.1.6. Œstrogènes et neuroprotection

Plusieurs études ont rapporté un effet bénéfique du 17β-estradiol contre les dommages au cerveau. Les études *in vitro* ont démontré le rôle neuroprotecteur du 17β-estradiol contre l'excitotoxicité du glutamate, la toxicité du peptide β-amyloïde, l'oxydation par le peroxyde d'hydrogène, l'apoptose causée par la privation en sérum et la toxicité du MPP+ et de la 6-OHDA (Singer *et coll.* 1996, Green *et coll.* 1997, Sawada *et coll.* 1998, Callier *et coll.* 2002, Fitzpatrick *et coll.* 2002). *In vivo*, le 17β-estradiol protège contre la mort neuronale induite par l'ischémie cérébrale et contre la toxicité du 6-OHDA et de la methamphétamine (Murray *et coll.* 2003, D'Astous *et coll.* 2004a, Lebesgue *et coll.* 2010).

Plusieurs études ont montré que le 17β-estradiol induit un effet neuroprotecteur contre la toxicité du MPTP chez les souris femelles et mâles (Dluzen *et coll.* 1996). L'effet bénéfique du 17β-estradiol chez la souris MPTP est observé sur les concentrations striatales de DA et ses métabolites, sur la liaison spécifique au DAT et au VMAT2 dans le striatum et dans la substance noire, sur les cellules immunoréactives positives pour la TH, ainsi que sur l'activation des astrocytes, observée par une diminution des niveaux de la protéine gliale fibrillaire acide (GFAP) (Miller *et coll.* 1998, Ekue *et coll.* 2002, D'Astous *et coll.* 2004b, Jourdain *et coll.* 2005, Tripanichkul *et coll.* 2006).

Une activation des astrocytes dans le striatum et dans la substance noire a été rapportée chez la souris MPTP, observée par une augmentation de l'immunoréactivité de la GFAP (Morale *et coll.* 2006). Les microglies et les cellules gliales activées expriment des niveaux élevés de la forme inductible de l'oxyde nitrique synthase, responsable de la génération d'oxyde nitrique et du superoxyde. Lorsque ces deux radicaux se combinent, ils forment le radical hautement réactif, le peroxynitrite, responsable des dommages oxydatifs (Morale *et coll.* 2006, L'Episcopo *et coll.* 2010). Le 17β-estradiol diminue l'activation des astrocytes chez les souris MPTP et inhibe l'augmentation de la forme inductible de l'oxyde nitrique synthase induite par le MPTP (Morale *et coll.* 2006). Ainsi, le 17β-estradiol inhibe la réponse anti-inflammatoire néfaste chez la souris MPTP.

Les études ont démontré que l'effet neuroprotecteur du 17β-estradiol chez la souris MPTP se fait probablement par un mécanisme impliquant les ERs. En effet, les composés ayant une faible affinité pour les ERs (Kuiper *et coll.* 1997), soit le 17α-estradiol, l'estrone et l'estriol, montrent peu ou pas d'effet bénéfique (Grandbois *et coll.* 2000, Jourdain *et coll.* 2005). L'activation du ERα avec l'agoniste PPT reproduit l'effet neuroprotecteur du 17β-estradiol quoique l'agoniste du ERβ, le DPN, n'est pas aussi puissant (D'Astous *et coll.* 2004b). Ainsi, le mécanisme par lequel le 17β-estradiol exerce son effet neuroprotecteur semble impliquer de manière prédominante le ERα. En support de cette conclusion, les souris invalidées en ERα sont plus susceptibles à la toxicité du MPTP (Morissette *et coll.* 2007, Al-Sweidi *et coll.* 2011). Par contre, un rôle pour le ERβ dans la neuroprotection par le 17β-estradiol ne peut être exclue, puisque le 17β-estradiol n'induit pas d'effet protecteur chez les souris invalidées en ERα et chez les souris invalidées en ERβ (Morissette *et coll.* 2007, Al-Sweidi *et coll.* 2011).

3.1.7. Signalisation intracellulaire et neuroprotection

Les effets du 17β-estradiol au cerveau ont été associé avec l'activation de deux importants régulateurs de la fonction et de la survie cellulaire, la voie des MAPK/ERK et la voie PI3K/Akt (Raz *et coll.* 2008) (figure 3.3). Ces deux voies ont pour cible des protéines communes, telles que la kinase synthase du glycogène 3β (GSK3β) et la protéine proapoptotique BAD, qui sont phosphorylées, et ainsi inhibées, par Akt et les ERK1/2 (Hetman & Gozdz 2004, Parcellier *et coll.* 2008). Akt contrôle aussi plusieurs membres de la famille Bcl-2 qui est composée de molécules pro- (Bad, Bax) et anti-apoptotique (Bcl-2, Bcl-x) et qui jouent un rôle crucial dans la régulation de l'apoptose mitochondriale (Parcellier *et coll.* 2008). Akt et les ERK1/2 peuvent transloquer au noyau et activer des facteurs de transcription tel que CREB, qui module la transcription de Bcl-2 (Hetman & Gozdz 2004, Parcellier *et coll.* 2008). Le facteur de transcription NF-κB, dont les gènes ciblent sont, entre autres, Bcl-2 et Bcl-x, est aussi activé par Akt (Parcellier *et coll.* 2008).

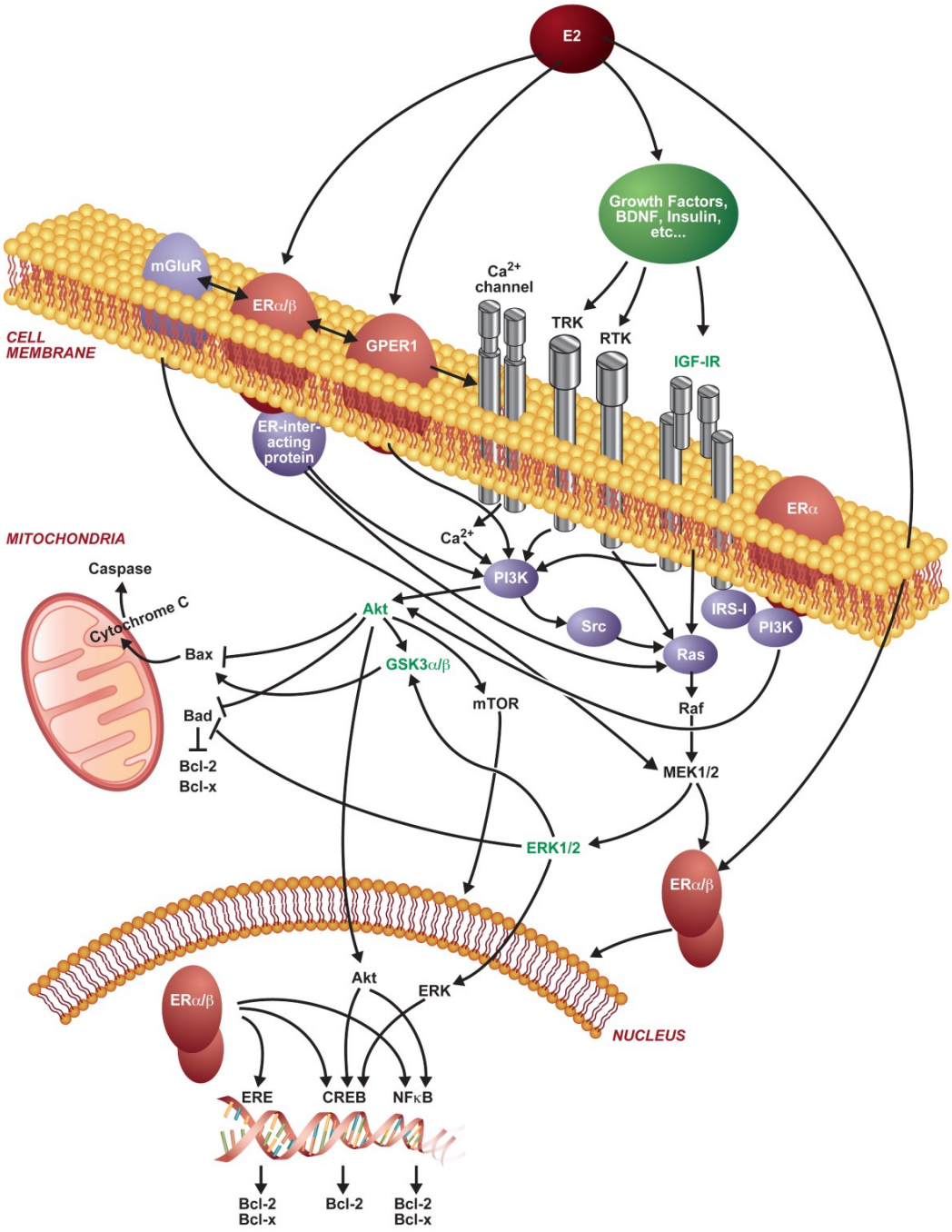

Figure 3.3. Représentation des voies de signalisation activées en réponse au 17β-estradiol et impliquées dans son effet neuroprotecteur. Schéma tiré de (Bourque *et coll.* 2012).

Les mécanismes d'action génomique et non-génomique du 17β-estradiol convergent pour potentialiser la transcription des gènes cibles impliqués dans la survie cellulaire. Ainsi, le 17β-estradiol induit l'expression de Bcl-2 par un mécanisme génomique impliquant la liaison des ERs au site ERE du gène Bcl-2 (Perillo *et coll.* 2000). Le 17β-estradiol peut aussi réguler la transcription de Bcl-2 par l'activation de la signalisation des MAPK (Wang *et coll.* 2011).

Chez la souris MPTP, l'effet neuroprotecteur du 17β-estradiol implique l'activation de la voie PI3K/Akt et un effet anti-apoptotique, mesuré par le rapport de Bcl-2/Bad, par le ERα (D'Astous *et coll.* 2006). En support d'un rôle neuroprotecteur du ERα, une étude chez le rat lésé avec la 6-OHDA a montré que le 17β-estradiol et le PPT, mais non le DPN, diminuent le stress oxydatif dans le striatum, mesuré par l'activité de la peroxydase glutathion, ainsi que l'apoptose, évalué par l'activité de la caspase 3 dans la substance noire (Baraka *et coll.* 2011). La protection des corps cellulaires et des terminaisons des neurones dopaminergiques par le 17β-estradiol chez le rat lésé avec la 6-OHDA est atténuée par l'antagoniste du récepteur de l'IGF-1, suggérant que l'effet du 17β-estradiol dépend de la co-activation des ERs et du récepteur de l'IGF-1 (Quesada & Micevych 2004). De plus, l'effet protecteur du 17β-estradiol contre la toxicité du 6-OHDA dans la substance noire est dépendant de la voie PI3K/Akt, mais non de la signalisation des ERK (Quesada *et coll.* 2008).

Une étude *in vitro* sur des cultures mésencéphaliques a montré que l'effet neuroprotecteur du 17β-estradiol contre la toxicité de la 6-OHDA se fait par un mécanisme impliquant l'activation des ERs et l'augmentation de l'expression de Bcl-2 par la signalisation des MAPK (Wang *et coll.* 2011). Dans des cellules PC12, le 17β-estradiol réduit l'apoptose causé par le MPP+ en augmentant l'expression de Bcl-x et en réduisant l'expression de l'enzyme convertissant l'interleukine 1β (la caspase 1) (Li *et coll.* 2008).

La signalisation par JNK a été impliqué dans l'apoptose (Davis 2000). JNK phosphoryle le facteur de transcription c-Jun, régulant ainsi l'activité de transcription par l'AP-1 (Davis 2000). *In vitro*, le MPP+ cause la perte des neurones dopaminergiques par l'activation de la signalisation JNK/c-Jun/AP-1 (Sawada *et coll.* 2002). L'effet

neuroprotecteur du 17β-estradiol dans ce modèle n'est pas causé par le blocage de JNK mais par l'inhibition de l'activité de transcription de l'AP-1 par le ERβ, suggérant que le 17β-estradiol peut supprimer la régulation de la transcription des gènes impliqués dans la mort neuronale induite par c-Jun/AP-1 (Sawada *et coll.* 2002).

L'effet neuroprotecteur du 17β-estradiol implique une augmentation des niveaux du facteur neurotrophique dérivé du cerveau (BDNF) et du facteur trophique dérivé de la glie (GDNF). Ces facteurs trophiques sont impliqués dans la survie, la fonction et la maintenance des neurones dopaminergiques (Aron & Klein 2011, Fumagalli *et coll.* 2006). Les études ont montré que l'inhibition de la signalisation du BDNF et du GDNF conduit à une dégénérescence dopaminergique nigrostriatale (Baydyuk *et coll.* 2011, Kramer *et coll.* 2007). Chez les rats lésés avec la 6-OHDA, le 17β-estradiol augmente les niveaux du GDNF dans le striatum et dans la substance noire (Campos *et coll.* 2012). De plus, la diminution des niveaux du GDNF abolie l'effet protecteur du 17β-estradiol, montrant l'importance de facteur trophique dans la neuroprotection (Campos *et coll.* 2012). L'augmentation des niveaux du BDNF a aussi été impliquée dans l'effet neuroprotecteur du 17β-estradiol lors d'une ischémie (Yang *et coll.* 2010).

3.1.8. Œstrogènes et neuromodulation du système dopaminergique

Le 17β-estradiol module le système dopaminergique à plusieurs niveaux, dont la synthèse et le métabolisme de la DA, ainsi qu'une modulation des récepteurs et des transporteurs dopaminergiques (Di Paolo 1994, Becker 1999, Sanchez *et coll.* 2010).

3.1.8.1. Le cycle œstral

De nombreuses études effectuées chez les rongeurs ont montré que les différentes étapes de la neurotransmission dopaminergique varient au cours du cycle œstral (Di Paolo 1994). Le striatum est sujet à des fluctuations dans les niveaux de 17β-estradiol et de progestérone au cours du cycle œstral, d'une manière similaire à celle observée dans le plasma (Morissette *et coll.* 1992). Dans le cycle œstral chez la ratte, les niveaux de 17β-estradiol sont élevés dans la matinée et dans l'après-midi du proestrus, et les niveaux de progestérone sont élevés en diestrus I et dans l'après-midi du proestrus (Morissette & Di Paolo 1993b).

Les concentrations de DA, la densité du DAT et du récepteur D1, ainsi que la proportion des sites de haute et de basse affinité du récepteur D2, varient selon les concentrations endogènes de 17β-estradiol dans le striatum durant le cycle œstral (Di Paolo 1994). En effet, les concentrations de DA et de DOPAC, ainsi que la densité du DAT, sont plus élevées en proestrus (lors de l'augmentation des niveaux endogènes de 17β-estradiol), tandis que le rapport des sites de haute et de basse affinité du récepteur D2 et la densité des récepteurs D1 sont augmentés en diestrus (lorsque les niveaux de 17β-estradiol sont bas et que les niveaux de progestérone sont élevés) (Di Paolo *et coll.* 1988, Levesque *et coll.* 1989, Morissette & Di Paolo 1993b). Les concentrations striatales de DA extracellulaire sont plus élevées en proestrus et en œstrus comparativement à la période diestrus (Xiao & Becker 1994). La comparaison directe entre les mesures biochimiques et comportementales n'est pas aussi simple car les tests comportementaux sont effectués sur un cycle lumière-obscurité inversé, contrairement aux mesures biochimiques. Cette différence affecte les niveaux de stéroïdes entre les différentes phases du cycle œstral. Par conséquent, lors d'un cycle lumière-obscurité inversé, les rats en œstrus ont des niveaux plus élevés de17β-estradiol que lors de la diestrus, et les niveaux de progestérone sont à leurs plus hauts (Becker & Cha 1989). Les tests comportementaux montrent que lorsque l'AMPH est administrée pour stimuler le système dopaminergique striatal, la libération de DA et les comportements moteurs induits par l'AMPH sont à leurs plus hauts niveaux chez les rats en œstrus et sont à leurs plus bas niveaux lors de la période diestrus (Becker & Cha 1989). Ainsi, les études biochimiques et comportementales montrent que les variations endogènes des niveaux de 17β-estradiol et de progestérone induisent des changements dans la transmission dopaminergique.

3.1.8.2. L'ovariectomie

Dans des conditions où la source principale de 17β-estradiol est enlevée, soit lors de l'ovariectomie, une diminution de la densité du DAT dans le striatum, sans modification de l'affinité de liaison, est observée à court et à long terme suivant l'ovariectomie (Bosse *et coll.* 1997). Par contre, l'ovariectomie laisse inchangée la densité du VMAT2 dans le striatum (Rehavi *et coll.* 1998, Le Saux & Di Paolo 2006). Une diminution de la densité des récepteurs D1 et D2, sans changement de l'affinité de liaison, est observée (Di Paolo *et coll.* 1988, Landry *et coll.* 2002, Levesque *et coll.* 1989, Le Saux *et coll.* 2006). De plus, la proportion

des sites agonistes de haute affinité des récepteurs D2 augmente, les niveaux extracellulaires de DA sont bas, et la libération striatale de DA ainsi que les comportements moteurs induits par l'AMPH et par la cocaïne sont atténués chez les rattes ovariectomisées (Becker & Ramirez 1981, Camp *et coll.* 1986, Di Paolo *et coll.* 1988, Xiao & Becker 1994). Ainsi, dans une condition où les niveaux de 17β-estradiol et de progestérone endogènes sont bas, telle que se produisant à la ménopause, une diminution de l'activité dopaminergique se produit, observée avec une diminution des mesures comportementales et biochimiques.

3.1.8.3. Traitement avec le 17β-estradiol

Lorsque les niveaux de 17β-estradiol sont rétablis par un traitement chronique avec des concentrations physiologiques de cette hormone chez les rattes ovariectomisées, une augmentation de l'activité locomotrice en réponse à la cocaïne et à l'APMH est observée (Becker 1990, Sell *et coll.* 2000). Une augmentation de la densité du DAT dans le striatum est rapportée lorsque le traitement avec le 17β-estradiol est initié peu de temps après l'ovariectomie (Le Saux & Di Paolo 2006). De plus, la restauration de la densité du DAT est également observée lorsque le 17β-estradiol est administré après une longue période de retrait hormonal, montrant que ce transporteur répond toujours au 17β-estradiol, même après une période prolongée (Le Saux & Di Paolo 2006). La réponse au changement hormonal (effet de l'ovariectomie et du remplacement avec le 17β-estradiol) sur la liaison spécifique au DAT est région spécifique, avec un changement du DAT observé seulement dans le striatum médian alors que le striatum antérieur et postérieur ne sont pas affectés (Le Saux & Di Paolo 2006). Dans la SNc de rattes ovariectomisées, l'ovariectomie et le traitement avec le 17β-estradiol n'ont aucun effet sur les niveaux d'ARNm du DAT (Bosse *et coll.* 1997), ce qui suggère que la modulation du DAT par le 17β-estradiol se fait par un mécanisme non-génomique. Des résultats contradictoires sont rapportés concernant l'effet du 17β-estradiol sur la liaison spécifique au VMAT2. Une étude rapporte une diminution de la densité du VMAT2 dans le striatum avec un traitement chronique de 17β-estradiol (Rehavi *et coll.* 1998) alors qu'une autre étude a observé aucune différence (Le Saux & Di Paolo 2006).

La densité des récepteurs D1 est également corrigée par un traitement chronique avec le 17β-estradiol. Néanmoins, le traitement avec le 17β-estradiol doit être initié peu de temps après l'ovariectomie car ce récepteur n'est plus sensible au 17β-estradiol lorsque cette

hormone est administrée après une longue période d'ovariectomie (Bosse & DiPaolo 1996, Levesque & Di Paolo 1989). Les rattes ovariectomisées traitées avec le 17β-estradiol ont une densité restaurée des récepteurs D2 dans le striatum, mais aucune élévation de l'ARNm de ce récepteur n'est observée, ce qui suggère que l'augmentation de la densité des récepteurs D2 induite par le 17β-estradiol se produit par un mécanisme indépendant de la transcription des gènes (Le Saux *et coll.* 2006). Lorsque les terminaisons dopaminergiques sont endommagées avec la 6-OHDA chez le rat, l'augmentation de la densité des récepteurs D2 par le 17β-estradiol est toujours observée, indiquant que le 17β-estradiol module les récepteurs D2 situés sur les neurones post-synaptiques, et que l'effet observé ne résulte pas de changements dans la transmission dopaminergique pré-synaptique (Di Paolo *et coll.* 1982).

Alors que la majorité des études ont investigué l'effet du 17β-estradiol sur l'activité de neurotransmission dopaminergique chez les rongeurs femelles, il est intéressant d'explorer si le 17β-estradiol pourrait également influencer la transmission dopaminergique dans le cerveau masculin. Il existe une différence entre les sexes dans le comportement moteur induit par l'AMPH, les rongeurs mâles répondent moins que les rongeurs femelles (Robinson *et coll.* 1980). Après l'administration aiguë de 17β-estradiol, aucune augmentation de la libération de DA ou de comportement moteur induit par l'AMPH chez les rongeurs mâles n'est observée (Becker 1990, Castner *et coll.* 1993). Le traitement chronique avec le 17β-estradiol chez les souris mâles laisse inchangé la liaison spécifique au DAT et au VMAT2, à la fois dans le striatum et dans la substance noire (Jourdain *et coll.* 2005). De plus, l'administration chronique de 17β-estradiol ne modifie pas la densité des récepteurs D1 et D2 dans le striatum (Lammers *et coll.* 1999).

3.1.9. Œstrogènes et maladie de Parkinson

3.1.9.1. Effets des œstrogènes sur le risque de la maladie de Parkinson

Plusieurs études ont investigué l'influence des œstrogènes endogènes et exogènes sur le développement de la maladie de Parkinson. Une plus grande période de fertilité (écart entre l'âge à la ménarche et l'âge à la ménopause; plus de 39 ans d'écart) est associée avec une diminution du risque de la maladie de Parkinson (Saunders-Pullman *et coll.* 2009). Une

période de fertilité plus courte que 36 ans est associée avec une augmentation du risque de cette maladie et une ménopause précoce est plus fréquente chez les femmes parkinsoniennes (Ragonese *et coll.* 2004). Les femmes ayant la maladie de Parkinson ont subi une ménopause chirurgicale plus fréquemment que les sujets contrôles, quoiqu'une autre étude rapporte une diminution du risque de cette maladie chez les femmes ayant subi une ménopause chirurgicale (Benedetti *et coll.* 2001, Ragonese *et coll.* 2004).

L'utilisation des thérapies hormonales à la ménopause est associée avec une diminution du risque de la maladie de Parkinson (Benedetti *et coll.* 2001, Currie *et coll.* 2004, Popat *et coll.* 2005). Par contre, cette association n'est pas toujours observée et une étude a rapporté un risque similaire entre les femmes qui ont, ou qui n'ont pas, utilisé des thérapies hormonales à la ménopause (Ascherio *et coll.* 2003). De plus, une augmentation du risque de la maladie de Parkinson a aussi été rapporté chez les femmes ayant subi une hystérectomie et utilisant des thérapies hormonales (Popat *et coll.* 2005, Saunders-Pullman *et coll.* 2009).

3.1.9.2. Effets des œstrogènes sur les symptômes de la maladie de Parkinson

Une détérioration des symptômes parkinsoniens est rapportée chez les femmes durant les périodes prémenstruelles et menstruelles, lorsque les niveaux des œstrogènes et de la progestérone sont bas (Quinn & Marsden 1986, Kompoliti *et coll.* 2000, Tolson *et coll.* 2002). La majorité des femmes rapportent une détérioration des symptômes parkinsoniens durant la grossesse et la période post-partum, et les variations considérables des niveaux d'œstrogènes durant ces périodes ont été suggérées d'être impliquées dans la détérioration des symptômes moteurs (Golbe 1987, Hagell *et coll.* 1998, Shulman *et coll.* 2000, Robottom *et coll.* 2008). Les femmes post-ménopausées en début de maladie de Parkinson, avant l'initiation de la L-DOPA, utilisant des thérapies hormonales ont des symptômes moteurs moins sévères, quoique les œstrogènes n'aient pas d'effet à un stade plus avancé de la maladie (Saunders-Pullman *et coll.* 1999, Strijks *et coll.* 1999). Une réduction de la dose de L-DOPA nécessaire pour améliorer la fonction motrice est rapportée chez les femmes recevant du 17β-estradiol (Blanchet *et coll.* 1999). De plus, une amélioration des symptômes moteurs et des fluctuations motrices est rapportée chez les femmes parkinsoniennes utilisant des thérapies hormonales (Tsang *et coll.* 2000).

Ainsi, ces études cliniques et épidémiologiques supportent que les œstrogènes exogènes et endogènes exercent un effet bénéfique sur le risque de développer la maladie de Parkinson. De plus, ces études suggèrent que les œstrogènes agissent comme un neuromodulateur du système dopaminergique, en diminuant les symptômes parkinsoniens.

3.2. Le raloxifène

Le raloxifène (Evista®) est un SERM de deuxième génération, utilisé en clinique pour le traitement et la prévention de l'ostéoporose chez la femme, ainsi que pour réduire les risques de cancer du sein invasif chez les femmes post-ménopausées (Lee *et coll.* 2008). Le raloxifène est un SERM non-stéroïdien faisant partie de la famille des benzothiophènes (figure 3.4).

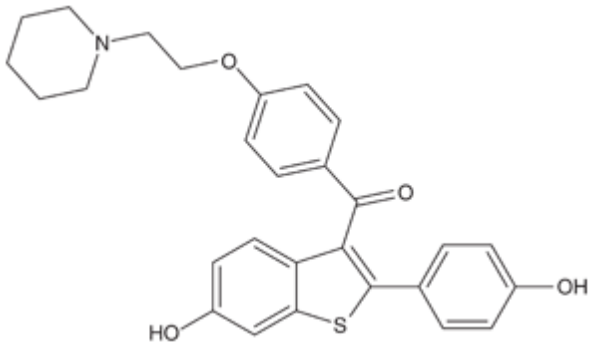

Figure 3.4. Structure du raloxifène.

3.2.1. Mécanismes d'action

Les SERMs possèdent des activités œstrogéniques agonistes ou antagonistes qui sont tissus spécifiques. Le raloxifène possède une activité œstrogénique agoniste sur le métabolisme du cholestérol et sur les os mais a une activité œstrogénique antagoniste sur les glandes mammaires et l'utérus (Dutertre & Smith 2000). L'habileté des SERMs à inhiber ou activer la transcription des gènes dépend de plusieurs facteurs dont le changement de conformation du ER, la sélectivité du ligand pour le sous-type de ER, l'interaction avec les co-régulateurs et l'expression des sous-types de ER selon le tissu (Dutertre & Smith 2000).

Lors de la formation du complexe ERα-raloxifène et ERβ-raloxifène, l'hélice 12 du ER s'oriente dans une position antagoniste, soit d'une manière à bloquer la liaison du co-activateur sur le récepteur (Johnson & O'Malley 2012). Le bon positionnement de l'hélice 12 est crucial pour la liaison avec les co-activateurs et pour l'activation de la transcription par l'AF-2 (Nilsson & Koehler 2005). Ainsi, le raloxifène inactive l'AF-2 du ERα et du ERβ. Le raloxifène a une activité antagoniste sur le ERβ et agoniste partiel sur le ERα lorsque les gènes contiennent un ERE (Lewis & Jordan 2005). Cette différence d'activité du raloxifène entre les sous-types de ER pourrait s'expliquer par la faible activité de l'AF-1 du ERβ, comparativement à l'AF-1 du ERα qui est très actif à stimuler la transcription des gènes par le ERE (Lewis & Jordan 2005).

3.2.2. Neuroprotection par le raloxifène

Les études de neuroprotection ont montré le potentiel protecteur du raloxifène. Le raloxifène protège contre la perte de DA et de la liaison spécifique au DAT induite par le MPTP chez la souris (Grandbois *et coll.* 2000, Callier *et coll.* 2001). Chez le rat lésé avec la 6-OHDA, le raloxifène augmente l'activité striatale de la peroxydase glutathion, diminue l'activité nigrale de la caspase 3, prévient la perte de DA et améliore le comportement moteur (Baraka *et coll.* 2011). Le raloxifène protège contre le stress oxydatif induit par l'acide kaïnique et diminue les déficits moteurs et cognitifs suivant un traumatisme cérébral chez le rat (Kokiko *et coll.* 2006, Armagan *et coll.* 2009). Ces effets sont associés avec l'augmentation de l'expression de Bcl-2 et du glutathion (Armagan *et coll.* 2009). *In vitro*, le raloxifène protège contre l'effet toxique de l'acide kaïnique par l'activation d'Akt et montre un effet bénéfique contre la toxicité du peptide β-amyloïde (Du *et coll.* 2004, Ciriza *et coll.* 2004). De plus, le raloxifène réduit la réponse inflammatoire causée par la lipopolysaccharide (Cerciat *et coll.* 2010).

3.3. La progestérone

La progestérone est l'autre stéroïde ovarien important avec l'estradiol (figure 3.1). La progestérone exerce une multitude d'effets, impliqués dans des mécanismes anti-apoptotiques, anti-inflammatoires, dans la régulation des fonctions des cellules gliales et des

mitochondries, dans la mémoire, dans la neurogénèse et dans la prolifération neuronale (Irwin *et coll.* 2008, Chen *et coll.* 2008, Brinton *et coll.* 2008).

3.3.1. Mécanismes d'action

Les mécanismes d'action génomique de la progestérone se font par l'intermédiaire de deux récepteurs nucléaires, le récepteur de la progestérone (PR)-A et le PR-B, qui sont codés par le même gène (Brinton *et coll.* 2008). La liaison de la progestérone au PR induit un changement de conformation du récepteur, la dissociation des protéines chaperonnes, la dimérisation des récepteurs, et l'interaction avec l'élément de réponse dans la région promotrice du gène cible (Brinton *et coll.* 2008). Le PR-A et le PR-B régulent de manière distincte la transcription des gènes (Brinton *et coll.* 2008). De plus, le PR-B est un plus puissant activateur que le PR-A, et le PR-A peut réprimer l'activité de transcription de PR-B d'une manière spécifique au promoteur et au type de cellule (Brinton *et coll.* 2008).

Les mécanismes d'action non-génomique de la progestérone sont exercés par sa liaison à la composante 1 du récepteur membranaire de la progestérone, et par sa liaison à des récepteurs membranaires couplés à une protéine G, soient les récepteurs de la progestérone membranaire (mPR-α et-β) (Thomas & Pang 2012). De plus, les PR localisés dans le cytoplasme peuvent avoir des effets indépendants de la transcription. En effet, les PR contiennent un motif polyproline dans leur domaine amino-terminal qui interagit avec le domaine SH3 de Src, ce qui conduit à l'activation de la signalisation Src/MAPK (Boonyaratanakornkit *et coll.* 2008). La progestérone peut activer les voies de signalisation des ERK et Akt, diminuer les niveaux des molécules pro-apoptotiques Bad et Bax, et de la caspase 3, et augmenter l'expression de Bcl-2 et du BDNF (Djebaili *et coll.* 2005, O'Connor *et coll.* 2007, Kaur *et coll.* 2007, Guerra-Araiza *et coll.* 2009, Ishrat *et coll.* 2012).

Plusieurs des effets de la progestérone se font par l'action d'un de ses métabolites, soit l'alloprégnanolone (tétrahydroprogestérone), qui ne lie pas les récepteurs nucléaires mais pourrait exercer ses effets par les mPR (Thomas & Pang 2012). De plus, l'alloprégnanolone est un modulateur allostérique du récepteur $GABA_A$ et NMDA, causant ainsi une augmentation de l'activité inhibitrice du récepteur $GABA_A$ et une réduction de

l'excitotoxicité associée au récepteur NMDA (Akk *et coll.* 2007, Charalampopoulos *et coll.* 2008).

3.3.2. Neuromodulation et neuroprotection

La progestérone peut moduler le système dopaminergique nigrostrié. Chez les rats mâles et femelles, un traitement aigu avec la progestérone à des concentrations physiologiques augmente les concentrations striatales de DA, DOPAC et HVA (Di Paolo *et coll.* 1986, Petitclerc *et coll.* 1995). Ces changements sont associés avec une déviation posturale de la tête chez les rats ayant une lésion unilatérale du noyau entopédonculaire, montrant que la progestérone augmente l'activité dopaminergique (Petitclerc *et coll.* 1995). L'effet rapide de la progestérone sur la libération de DA se ferait par la liaison de la progestérone à un site membranaire (Dluzen & Ramirez 1989b), serait dépendant d'un potentiel d'action (Dluzen & Ramirez 1989a) et impliquerait une action par les récepteurs NMDA (Cabrera & Navarro 1996). Un traitement chronique avec la progestérone chez les rattes ovariectomisées augmente la densité du DAT dans le striatum et dans la substance noire (Morissette & Di Paolo 1993a), quoique cet effet ne soit pas observé lors d'un traitement aigu (Morissette *et coll.* 1990). De plus, la densité et l'affinité des récepteurs D2 ne sont pas modifiées lors d'un traitement chronique avec la progestérone chez les rattes ovariectomisées (Levesque & Di Paolo 1993), quoiqu'une diminution de la densité de ce récepteur soit observée après un traitement aigu avec une forte dose de progestérone (Fernandez-Ruiz *et coll.* 1989).

Le potentiel protecteur de la progestérone a été investigué quoique les données disponibles soient plus limitées que celles sur l'effet du 17β-estradiol. *In vitro*, la progestérone protège contre l'effet toxique du glutamate et du peptide β-amyloïde (Goodman *et coll.* 1996, Kaur *et coll.* 2007). Des études *in vivo* ont montré que la progestérone montre des effets bénéfiques lors de dommages ischémiques et contre l'effet toxique de la methamphétamine (Yu & Liao 2000, Ishrat *et coll.* 2012). Chez la souris reproductrice retraitée, la progestérone prévient la perte de DA et de la liaison spécifique au DAT induite par le MPTP (Grandbois *et coll.* 2000, Callier *et coll.* 2001).

Problématique, hypothèses et objectifs

Problématique

Aucun traitement curatif de la maladie de Parkinson n'est disponible à ce jour (Olanow & Schapira 2013, AlDakheel *et coll.* 2014). De plus, les causes de cette maladie sont pour la plupart inconnues et auraient une origine multifactorielle. Ainsi, une intervention basée sur la protection des cellules neuronales est de grand intérêt et les œstrogènes sont des composés intéressants. En effet, les études chez l'humain et les modèles animaux ont montré que les œstrogènes exercent des effets bénéfiques importants sur le risque de développer la maladie de Parkinson (Liu & Dluzen 2007, Bourque *et coll.* 2009).

Quoique les rapports initiaux de la Women's Health Initiative suggèrent que les thérapies hormonales augmentent les risques d'accident vasculaire cérébral et le déclin cognitif (Rossouw *et coll.* 2002, Shumaker *et coll.* 2003), une ré-analyse subséquente suggère que l'initiation d'un traitement hormonal tôt lors de la post-ménopause est en fait bénéfique pour le cerveau (Greendale *et coll.* 2009). En effet, comme présenté à la section 3.1.9.1. du chapitre 3, l'exposition aux œstrogènes endogènes et exogènes est associée avec une diminution du risque de développer la maladie de Parkinson (Liu & Dluzen 2007). Néanmoins, plusieurs effets indésirables subsistent lors de l'utilisation à long terme des thérapies hormonales, incluant une augmentation des risques de cancer, de thrombose et d'accident vasculaire cérébrale (Taylor & Manson 2011). Ainsi, bien que les œstrogènes soient neuroprotecteurs, leurs actions périphériques limitent actuellement leur utilisation pour le traitement ou la prévention de maladies neurodégénératives, d'où l'importance de trouver des stratégies alternatives qui reproduisent les effets favorables des œstrogènes mais minimisent les effets indésirables.

Les agonistes spécifiques des récepteurs des œstrogènes fournissent une approche pharmacologique pour étudier l'implication de chacun de ces récepteurs et leurs utilisations ont fait progresser notre compréhension du rôle des sous-types des récepteurs dans les effets des œstrogènes. Quoique la stimulation du ERα induise un effet neuroprotecteur (D'Astous *et coll.* 2004b), son activation stimule la prolifération cellulaire des glandes mammaires et de

l'utérus (Nilsson & Gustafsson 2010), ce qui augmente les risques de cancer et ce qui rend la conception et le développement des agonistes sélectifs aux ERα une approche moins attrayante et sécuritaire pour le développement de nouvelles thérapies. Au contraire, l'activation du ERβ n'induit pas la prolifération cellulaire des glandes mammaires et de l'utérus (Nilsson & Gustafsson 2010), mais son activation n'est pas impliquée dans la neuroprotection des neurones dopaminergiques (D'Astous *et coll.* 2004b), ce qui en fait aussi une cible thérapeutique moins intéressante. Ainsi, nous devons explorer et comprendre les mécanismes d'action des hormones ovariennes afin de trouver de nouvelles stratégies capables de reproduire les effets bénéfiques des œstrogènes et ce, sans les effets néfastes associés. Afin de répondre à cet objectif, les études de neuroprotection réalisées lors de cette thèse ont mis l'emphase sur le rôle protecteur du GPER1, l'exploration du mécanisme d'action impliqué dans l'effet neuroprotecteur du raloxifène, et l'investigation de l'effet neuroprotecteur et neurorécupérateur de la progestérone.

Le GPER1 est un nouveau récepteur des œstrogènes qui n'est pas impliqué dans la prolifération des cellules épithéliales des glandes mammaires et de l'utérus (Otto *et coll.* 2009, Otto *et coll.* 2008). Ainsi, les agonistes spécifiques qui ciblent le GPER1 sont des composés intéressants car ils sont non-féminisants, pourraient empêcher les effets indésirables des œstrogènes et peuvent être utilisés à la fois chez l'homme et chez la femme. Au moment de commencer cette thèse, le rôle neuroprotecteur du GPER1 était très peu connu. Aucune étude n'avait investigué son effet protecteur sur le système dopaminergique et aucune étude n'avait démontré son implication dans l'effet protecteur du 17β-estradiol.

Une autre alternative à l'utilisation des œstrogènes est le SERM raloxifène. Le raloxifène est utilisé chez les femmes pour le traitement et la prévention de l'ostéoporose ainsi que pour réduire les risques de cancer du sein (Lee *et coll.* 2008). Les études ont rapporté un effet bénéfique de l'utilisation du raloxifène sur la cognition autant chez les femmes que chez les hommes (Goekoop *et coll.* 2006, Goekoop *et coll.* 2005, Jacobsen *et coll.* 2010). L'avantage du raloxifène est que ce composé possède des propriétés agonistes et antagonistes œstrogéniques selon les tissus, permettant ainsi de reproduire les effets bénéfiques des œstrogènes mais de bloquer leurs effets prolifératifs au niveau des organes reproducteurs,

diminuant ainsi les risques de cancer (Dutertre & Smith 2000, Yaffe *et coll.* 2005). Puisque le raloxifène est déjà utilisé en clinique, son application pour le cerveau peut être facilitée. Notre laboratoire a déjà démontré que le raloxifène protège le système dopaminergique nigrostrié chez la souris MPTP (Grandbois *et coll.* 2000, Callier *et coll.* 2001), mais le mécanisme d'action du raloxifène au cerveau est peu connu et pourrait impliquer un effet par le GPER1. En effet, une étude *in vitro* a montré que l'effet protecteur du raloxifène contre la privation en oxygène-glucose se fait par un mécanisme dépendant du GPER1 (Abdelhamid *et coll.* 2011).

La progestérone est parfois utilisée en monothérapie chez les femmes post-ménopausées pour diminuer les symptômes de la ménopause, et ce stéroïde n'augmente pas les risques de cancer (Spark & Willis 2012). La progestérone, ou plus souvent un progestatif synthétique, est aussi utilisée conjointement avec les œstrogènes dans les thérapies hormonales pour diminuer les effets des œstrogènes au niveau de l'utérus (Al-Safi & Santoro 2014). La progestérone est non-féminisante et peut donc être utilisée à la fois chez l'homme et chez la femme. Les études chez les animaux supportent que la progestérone possède des propriétés neuroprotectrices face à différents modèles de dommage au cerveau (Singh & Su 2013b). Une propriété intéressante de la progestérone est sa capacité d'induire un effet bénéfique lorsque des dommages sont présents au cerveau. En effet, deux études clinique de phase III évaluent présentement l'efficacité de la progestérone chez des patients ayant subi un traumatisme cérébrale (Wei & Xiao 2013). Les résultats des études clinique précédentes de phase II ont révélé que l'administration de fortes doses de progestérone peu de temps suivant un traumatisme cérébral chez les hommes et les femmes améliore les résultats neurologiques, et ce, sans effet secondaire (Wright *et coll.* 2007, Xiao *et coll.* 2008). Nous avons choisi la progestérone dans notre étude de neurorécupération puisque plusieurs données de la littérature, tant chez les modèles animaux que chez l'humain, rapportent un effet neurorécupérateur de ce stéroïde. Ainsi des doses, des modes d'administration et des durées de traitements ont été mis au point avec la progestérone pour récupérer après un traumatisme cérébral.

Hypothèses

Une première hypothèse est qu'une activité œstrogénique bénéfique et neuroprotectrice peut être obtenue au cerveau par l'utilisation de différents composés oestrogéniques et ce, par un mécanisme impliquant le GPER1. Une seconde hypothèse est que les neurones dopaminergique peuvent être protégés, mais peuvent aussi être récupérés, par un traitement avec la progestérone.

Objectifs spécifiques

1-Déterminer l'effet neuroprotecteur de l'agoniste du GPER1, soit le G1, et comparer son effet à celui du 17β-estradiol chez des souris MPTP. Déterminer l'implication du GPER1 dans l'effet du 17β-estradiol en utilisant l'antagoniste spécifique, soit le G15. Les résultats sont présentés au chapitre 4.

2-Déterminer le rôle des différents récepteurs des œstrogènes (ERα, ERβ, GPER1) dans l'effet protecteur du 17β-estradiol. Investiguer si une collaboration potentielle entre les ERα/β et le GPER1 est présente dans la protection des neurones dopaminergiques chez la souris MPTP. Déterminer les voies de signalisation activées en réponse à la stimulation de chaque type de récepteur des œstrogènes, plus spécialement la signalisation d'Akt et les facteurs trophiques. Ces objectifs seront investigués par une approche pharmacologique utilisant les divers agonistes et antagonistes des trois récepteurs des œstrogènes. Les résultats sont présentés au chapitre 5.

3-Investiguer le rôle du GPER1 dans l'effet du raloxifène chez les souris MPTP en utilisant l'antagoniste spécifique du GPER1, le G15. Étudier les voies de signalisation cellulaire impliquées dans l'effet neuroprotecteur du raloxifène, plus particulièrement la signalisation d'Akt et l'induction de facteurs trophiques. Les résultats sont présentés au chapitre 6.

4-Investiguer l'effet neuroprotecteur et neurorécupérateur de la progestérone sur les neurones dopaminergiques chez la souris MPTP. Les résultats sont présentés au chapitre 7.

Résultats

Chapitre 4: Implication of GPER1 in neuroprotection in a mouse model of Parkinson's disease

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Mélanie Bourque[a,b], Marc Morissette[a], Mélissa Côté[c], Denis Soulet[c], Thérèse Di Paolo[a,b]

[a]Molecular Endocrinology and Genomic Research Center, Centre de recherche du CHUQ (CHUL), Quebec City, Quebec, G1V 4G2

[b]Faculty of Pharmacy, Laval University, Quebec City, Quebec, G1K 7P4, Canada

[c]Axe Neurosciences, Centre de Recherche du CHUQ (CHUL) and Department of Psychiatry and Neuroscience, Faculty of Medicine, Laval University, Quebec City, Quebec, G1K 7P4, Canada

Contribution des auteurs: La conception et l'organisation du projet fut réalisée par la Dr T. Di Paolo et M. Bourque. M. Bourque a fait les traitements des animaux, les autoradiographies, l'immunohistochimie, l'analyse des résultats et la rédaction du manuscrit. M. Morissette a fait le dosage de la dopamine et de la sérotonine, ainsi que leurs métabolites par HPLC. M. Côté a fait le compte de cellules TH positives immunoréactives. Le manuscrit a été corrigé par les Dr T. Di Paolo, D. Soulet et M. Morissette.

Résumé

Cette étude a investigué la contribution du nouveau récepteur des œstrogènes, le GPER1, dans l'effet neuroprotecteur du 17β-estradiol chez la souris traitée au 1-méthyl-4-phényl-1,2,3,6-tétrahydropyridine (MPTP), un modèle de la maladie de Parkinson. Chez les souris non-lésées, l'administration de l'agoniste du GPER1, le G1, reproduit les effets du 17β-estradiol en augmentant les concentrations striatales des métabolites de la dopamine ainsi que le renouvellement de la dopamine. L'antagoniste du GPER1, le G15, bloque l'effet du G1 sur le rapport de HVA/dopamine et bloque partiellement l'effet du 17β-estradiol. Les souris MPTP traitées avec le G15 sont plus susceptibles à l'effet toxique du MPTP, démontrées par une plus grande diminution des concentrations striatales de dopamine et de la liaison spécifique au DAT. Chez les souris MPTP, l'analyse des concentrations de dopamine ainsi que de la liaison spécifique au transporteur de la dopamine et au transporteur vésiculaire des monoamines 2 montrent que le traitement avec le G1 est aussi puissant que celui avec le 17β-estradiol en protégeant le striatum et la substance noire. Le G15 antagonise complètement l'effet du G1 dans le striatum et la substance noire, ainsi que la protection du 17β-estradiol dans le striatum mais partiellement dans la substance noire. Cette étude montre un rôle important du GPER1 dans la neuroprotection et que le G1 est aussi puissant que le 17β-estradiol pour produire un effet bénéfique.

Abstract

This study investigated the contribution of the new GPER1 in neuroprotection by 17β-estradiol in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice model of Parkinson's disease. In intact mice, administration of GPER1 agonist G1 reproduced the effect of 17β-estradiol in increasing striatal dopamine metabolites concentrations as well as the turnover of dopamine. GPER1 antagonist G15 blocked the effect of G1 on HVA/dopamine ratio and partially for 17β-estradiol. MPTP mice treated with G15 were more susceptible to MPTP toxicity with a greater decrease in striatal dopamine concentration and dopamine transporter specific binding. In MPTP mice, dopamine concentrations as well as dopamine and vesicular monoamine transporter specific binding showed that G1 treatment was as potent as 17β-estradiol in protecting striatum and substantia nigra. G15 antagonized completely the neuroprotective effects of G1 in the striatum and substantia nigra as well as protection by 17β-estradiol in the striatum but partially in the substantia nigra. This study showed an important role of GPER1 in neuroprotection and that G1 is as potent as 17β-estradiol in mediating beneficial effects.

Keywords: GPER1, 17β-estradiol, MPTP, neuroprotection, dopamine, striatum, substantia nigra

4.1. Introduction

Human studies have shown that longer exposure to endogenous ovarian steroids and the use of estrogen therapy were associated with a decrease risk of Parkinson's disease (PD) (Liu and Dluzen, 2007). Those studies support a beneficial role of estrogen exposure on the development of PD and that estrogen could be used as a neuroprotective agent. Nevertheless, there are several limitations to the use of estrogen in humans as unwanted side effects of long term use of hormone therapy have been reported and are associated with activation of estrogen receptors (ERs) (Taylor and Manson, 2011). Indeed, the search for a neuroprotective compound as potent as 17β-estradiol against brain injury and without peripheral adverse effect is of great interest.

Studies performed with the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice model of PD support the neuroprotective capacity of estrogen against brain damage (Bourque et al., 2009). The mechanism of 17β-estradiol neuroprotection against MPTP implicates activation of ERα but the discovery of a new receptor responding to 17β-estradiol, the G protein-coupled estrogen receptor 1 (GPER1), offers the possibility of an additional mechanism of 17β-estradiol action. The contribution of GPER1 in the neuroprotective action of 17β-estradiol has received little attention, but *in vivo* (Lebesgue et al., 2010) and *in vitro* (Gingerich et al., 2010) studies reported a neuroprotective action of GPER1 activation on hippocampal neurons. Interestingly, studies using GPER1 knockout mice and GPER1 agonist G1 have shown that this receptor does not mediate an estrogenic response in reproductive organs (Otto et al., 2009, Otto et al., 2008). As a non-feminizing ER with neuroprotective capacity, the potential contribution of GPER1 in mediating protective effects of 17β-estradiol against MPTP toxicity could be a promising alternative to 17β-estradiol and warrants investigation.

In the present study, the specific GPER1 agonist G1 and antagonist G15, which have no affinity for either ERα or ERβ (Bologa et al., 2006, Dennis et al., 2009), were used to evaluate the implication of GPER1 in neuroprotection by 17β-estradiol against MPTP. We performed a moderate lesion with MPTP in intact male mice to model an early stage of PD. Integrity of DA cell bodies and terminals was evaluated with measures of dopamine (DA),

DA transporter (DAT), vesicular monoamine transporter 2 (VMAT2) and tyrosine hydroxylase (TH) positive cells. These DA markers are decreased in PD (Hornykiewicz, 2001) and were used to evaluate damage and protection of DA neurons.

4.2. Materials and methods

4.2.1. Animals and treatments

C57BL/6 male mice (10 weeks) were purchased from Charles River Canada (Montreal, Qc, Canada). MPTP and 17β-estradiol were purchased from Sigma Chemical (St Louis, MO), G1 and G15 were from Tocris (Ellisville, MO). 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.

4.2.1.1. G1 dose-response experiment

Each group received treatment with G1 (1 or 5 µg, B.I.D., sc), 17β-estradiol (1 µg, B.I.D., sc) or vehicle (0.9% saline with 0.3% gelatin, B.I.D., sc) for 10 days. On day 5, mice received four injections of MPTP (4.75 mg/kg, i.p.) at 2-h intervals. This dose regimen of MPTP was chosen to achieve approximately a decrease of 50% in striatal DA concentration to model the early stage of PD. The early stage of neurodegeneration, where substantia nigra DA neurons are injured but not yet dead, represent an appropriate time to evaluate this capacity for neuroprotection by estradiol given the clinical evidence supporting a beneficial effect of estrogen therapy, particularly in the early stages of PD (Saunders-Pullman et al., 1999). The dose of 17β-estradiol used was previously reported to induce a neuroprotective effect in MPTP mice (Callier et al., 2001, Morissette et al., 2007). On day 11, mice were decapitated, and brains were quickly removed and frozen in isopentane (-40°C). Control groups for the estrogenic treatments were also performed (nonlesioned mice) and mice received estrogenic drug treatments or the vehicle for 10 days at the same dose as described above.

4.2.1.2. Inhibition of GPER1 experiment

According to the results of the G1 dose-response experiment, 5 µg of G1 was used in this experiment. 10 µg of G15 (B.I.D., sc) was used with 17β-estradiol (1 µg, B.I.D., sc) and 50 µg (B.I.D., sc) of G15 was given with G1 (Dennis et al., 2009). The treatment schedule and the dose of MPTP were the same as in the G1 dose-response experiment. Control groups for the estrogenic treatments were also performed (nonlesioned mice) and mice received estrogenic drug treatments or the vehicle for 10 days at the same dose as described above. The effect of G15 alone in MPTP mice was investigated in a separate experiment. Mice were

treated with G15 (10 or 50 µg, B.I.D., sc.) for 10 days and received four injections of MPTP (5.5 mg/kg, i.p.) at 2-h intervals on day 5.

4.2.2. Striatal dopamine assay

The left anterior striata were dissected and the concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as well as serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were assayed by high performance liquid chromatography (HPLC) with electrochemical detection as previously described (D'Astous et al., 2004).

4.2.3. DAT and VMAT2 autoradiography

The striatum (bregma 0.74 at 0.26 mm) (Franklin and Paxinos, 1997) and the substantia nigra (bregma -2.92 at -3.64 mm) of the right hemisphere were cut on a cryostat in 12 µm slices and 6 consecutives brain sections were placed on one glass slide. Slices were kept at – 80 ^{o}C until assayed.

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) and 100 nM of Mazindol (Sandoz Pharmaceuticals, Dorval, Quebec) for non-specific binding, as previously described (Callier et al., 2001). Brain slices were apposed to Kodak films (Biomax), 31 hours for the striatum and 72 hours for the substantia nigra. VMAT2 autoradiography (Kilbourn and Frey, 1996) was performed using the specific ligand [^{3}H]dihydrotetrabenazine ([^{3}H]-TBZ-OH) (20 Ci/mmol and 1 µM of cold TBZ-OH for the non-specific binding, American Radiolabeled Chemicals, St. Louis, MO, USA). 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. One glass slide per animal (containing 6 consecutive brain sections) was used for each binding thus the data presented for each animal was from 6 brain sections. To avoid variability of binding within the same brain structure, the brain slices for each animal were chosen to be at the same bregma. The data presented represent the specific binding of the ligand that was calculated by substracting the value for non-specific binding from the total binding. The data obtained for the striatum

was from measures of the total striatum (lateral and medial part). For the substantia nigra, the data obtained represent also the value of the total substantia nigra pars compacta.

4.2.4. Tyrosine hydroxylase immunohistochemistry

Immunohistochemistry was performed on frozen slices coming from the inhibition of GPER1 experiment. The slices were rinsed two times in a phosphate-buffered saline (PBS, pH 7.4) solution, fixed 20 minutes in paraformaldehyde (4%) at 4ºC and rinsed three times in PBS. Slices were incubated 30 minutes at room temperature with a solution containing 45% ethanol and 0.3% H_2O_2 to block peroxydase activity and rinsed three times in PBS. Slices were preincubated for 30 minutes, at room temperature, in a PBS solution containing 0.05% normal donkey serum and 0.1% Triton X-100. They were then incubated overnight at 4°C in a PBS solution containing 0.05% normal donkey serum, 0.1% Triton X-100, and the primary antibody directed against tyrosine hydroxylase (1:500, rabbit monoclonal antibody; Abcam, Cambridge, MA). After three rinses in PBS, slices were incubated at room temperature during 1 h in a PBS solution containing 0.05% normal donkey serum, 0.1% Triton X-100, and the biotinylated anti-rabbit donkey-made IgG as the secondary antibody (Jackson ImmunoResearch Labs, West Grove, PA). After three additional rinses in PBS, slices were incubated for 1 h at room temperature with avidin–biotin complex (Vectastain ABC kit, Vector Labs, Burlingame, CA). Slices were rinsed 2 times in phosphate-buffered (0.1 M, pH 7.4) solution followed by two rinsed in Tris (50 mM, pH 7.6) solution. The bound peroxidase was revealed by incubating the slices for 3 min, at room temperature, in a 0.05% solution of 3,3′diaminobenzidine (DAB; Sigma, St. Louis, MO) to which 0.005% H_2O_2 was added. Slices were rinsed 2 times in phosphate-buffered solution followed by two rinses in Tris solution. Slices were incubated 3 minutes with hematoxyline solution (Sigma-Aldrich, St. Louis, MO) for nuclei staining, dehydrated and coverslipped with DPX (Sigma-Aldrich, St. Louis, MO).

Numbers of tyrosine hydroxylase positive neurons were assessed in brain cryosections with typical morphology of the substantia nigra pars compacta, as described previously (Li et al., 2008). Four series of 12 µm thick cryosections were collected and used for systematic quantification of tyrosine hydroxylase positive neurons with stereoinvestigator

(Microbrightfield, Colchester, VT) on a Nikon C80i microscope. Briefly, the contours of the substantia nigra were traced as a virtual overlay with a 4× Plan Apochromat objective (NA 0.2) in brightfield mode. Then, the microscope was set with a 40× Plan Apochromat objective (NA 0.95). The average cell soma diameter (the mean length of the long- and short-axis) of the dopaminergic neurons was estimated from 20 randomly selected neurons on each section. All tyrosine hydroxylase positive neurons observed within the selected substantia nigra contour were counted following a systematic meander scan. The formula of Abercrombie (Abercrombie, 1946) was then used to estimate the total number of tyrosine hydroxylase positive neurons per section, and tyrosine hydroxylase positive neuron density was thereafter calculated. An analysis of the ventral and dorsal tier of the substantia nigra pars compacta was performed (Gerfen et al., 1987), since these subdivisions are differently sensitive to MPTP (Liang et al., 1996).

4.2.5. Statistical analysis

Statistical comparisons of the data were performed with analyses of variance (ANOVA) using Stat View 4.51 for Macintosh Computer software, followed by a post-hoc analysis with a Fisher protected least significant difference test. A simple regression model was used to determine the coefficient of correlation. A $p \leq 0.05$ was required for the results to be considered statistically significant.

4.3. Results

4.3.1. G1 mimics most of 17β-estradiol effect on striatal DA metabolism in intact mice and G15 partially antagonized the effect of 17β-estradiol

G1 (1 µg) treatment increased striatal DOPAC and HVA concentrations to a similar extent as 17β-estradiol in intact, nonlesioned male mice (Figure 4.1). Only mice treated with 17β-estradiol had a higher striatal DOPAC/DA ratio but G1 (1 µg) treatment increased HVA/DA ratio as 17β-estradiol-treated mice whereas a trend was observed with 5 µg of G1 ($p = 0.07$). 17β-estradiol and G1 treatment had no effect on striatal 5-HT and 5-HIAA concentrations (Figure 4.1) as well as DAT and VMAT2 specific binding in the striatum and substantia nigra (Table 4.1).

G15 treatment alone had no effect on striatal DA and DA metabolites concentrations and their ratios (Figure 4.2 and data not shown). 17β-estradiol increased striatal DOPAC/DA and HVA/DA ratios while mice treated with the combination of 17β-estradiol and G15 did not differ from both 17β-estradiol treated mice and control mice. G15 treatment antagonized the increase of striatal HVA/DA ratio induced by G1. Striatal 5-HT and 5-HIAA concentrations were unchanged by treatments with 17β-estradiol, G1, G15 or their combination (data not shown). No significant difference was observed for DAT and VMAT2 specific binding in the striatum and the substantia nigra in mice treated with 17β-estradiol, G1 and G15 (Table 4.2).

4.3.2. Dose-response to G1 experiment: G1 is as potent as 17β-estradiol in mediating DA neuroprotective effects

17β-estradiol and both doses of G1 similarly reduced MPTP toxicity as measured with striatal DA concentration and prevented the MPTP-induced increase of HVA/DA ratio (Figure 4.3). G1 at 5µg, but not at 1 µg, as 17β-estradiol diminished the MPTP effect on striatal DOPAC concentrations. MPTP at the dose used showed specificity to the DA system, striatal 5-HT and 5-HIAA concentrations showing no significant difference between groups.

Measures of DAT and VMAT2 specific binding in striatum showed that G1 at 5 µg, but not 1µg, was as effective as 17β-estradiol in reducing MPTP toxicity (Figure 4.4A). DAT specific binding in substantia nigra was slightly reduced in MPTP and MPTP + G1 (1 µg)

treated mice whereas 17β-estradiol and G1 (5 μg) MPTP treated mice were at control levels. Changes observed for striatal DA concentration parallel those found for DAT and VMAT2 specific binding in striatum, as demonstrated by significant positive correlations (Figure 4.4B).

4.3.3. Inhibition of GPER1 prevented completely the 17β-estradiol effect against MPTP toxicity in the striatum and partially in the substantia nigra

The dose of 50 μg of G15 increased MPTP toxicity as observed by a more extensive lost of striatal DA concentration, DAT and VMAT2 specific binding in striatum and a higher HVA/DA ratio in these mice as compared to the MPTP group (Figures 4.5 and 4.6A). Significant positive correlations were found between striatal DAT specific binding and DA concentrations as well as VMAT2 specific binding and DA concentrations (Figure 4.6B).

Administration of G15 in mice completely abolishes the effect of 17β-estradiol and G1 on MPTP toxicity for striatal DA, DOPAC and HVA concentrations as well as HVA/DA ratio (Figure 4.7). DOPAC/DA ratio in 17β-estradiol MPTP treated mice was at control values and this effect of 17β-estradiol was blocked by G15.

G15 treatment completely opposed the effect of 17β-estradiol and G1 on MPTP induced decrease of striatal DAT and VMAT2 specific binding (Figure 4.8A). In the substantia nigra, G15 opposed the effect of G1 on MPTP-induced decrease of DAT specific binding whereas a partial opposition was observed for 17β-estradiol with values for 17β-estradiol + G15 treated mice not significantly different from MPTP ($p = 0.067$) and 17β-estradiol-treated MPTP mice ($p = 0.817$). MPTP mice treated with the combination of G1 and G15 had VMAT2 specific binding significantly lower than mice treated with G1 alone. Significant positive correlations were measured between striatal DAT specific binding and DA concentrations as well as VMAT2 specific binding and DA concentrations (Figures 4.8B).

The moderate conditions of lesion chosen for our paradigm of early Parkinson's disease uses a regimen of MPTP induced damage to striatal DA terminals with limited or no loss of DA cells in substantia nigra. To confirm the sparing of DA cell bodies, assessment of

tyrosine hydroxylase positive cells in substantia nigra was performed. No significant difference between groups for density of total neurons, density of tyrosine hydroxylase positive neurons, the ratio of tyrosine hydroxylase positive neurons to total number of neurons was observed (Figures 4.8C). Analyses performed separately of the ventral and dorsal tier of the substantia nigra pars compacta of Figure 4.8C show no effect of MPTP on tyrosine hydroxylase positive cells in either sub-region (data not shown).

4.4. Discussion

Our results show, for the first time, that GPER1 is implicated in the neuroprotective effect of 17β-estradiol against MPTP toxicity in male mice (summarized in Figure 4.9). We demonstrated that inhibition of GPER1 with G15 completely opposed the effect of 17β-estradiol in striatum. In the substantia nigra, G15 antagonized partially the 17β-estradiol action, suggesting a contribution of both ERs and GPER1. Furthermore, we showed that stimulation of GPER1 with the selective agonist G1 is as potent as 17β-estradiol in inducing neuroprotection against MPTP toxicity in both the striatum and substantia nigra. Moreover, we showed that in intact mice G1, as previously observed with 17β-estradiol (Di Paolo et al., 1985), has a neuromodulator stimulatory role on striatal DA that is antagonized by G15.

The present results demonstrate the contribution of GPER1 in protection by 17β-estradiol against MPTP (Figure 4.9). Indeed, the results presented here and an other study using ERα and ERβ agonists (D'Astous et al., 2004) show that both ERα and GPER1 have a major role in mediating neuroprotection against MPTP toxicity and that stimulation of ERβ with specific agonist fails to induce a neuroprotective effect. ERα and GPER1 activation were also shown to mediate neuroprotection against hippocampal ischemic neuronal damage (Gingerich et al., 2010). Moreover, G15 treated mice and ERα knockout, but not ERβ knockout, mice were more susceptible to MPTP toxicity (Al-Sweidi et al., 2011, Morissette et al., 2007) (Figure 4.9F), suggesting that activity of GPER1 and ERα is significant for neuroprotection. The possibility that GPER1 and ERα could interact to produce 17β-estradiol neuroprotection should be taken into consideration. It was reported that GPER1 could modify ERα phosphorylation signal in the mouse uterus (Gao et al., 2011). ERα can directly interact and activate G proteins to mediate estradiol signaling (Kumar et al., 2007). An in vitro study performed on endothelial cells isolated from the brain capillaries from double knockouts ERα and ERβ mouse, but expressing GPER1, shows that 17β-estradiol fails to activate signaling pathways in these cells (Pedram et al., 2006). Since agonists of ERα, ERβ and GPER1 induce activation of signaling pathways, as 17β-estradiol, in conditional immortalized mouse brain capillary endothelial cell line (Kitamura et al., 2009), results from these two studies suggest that the presence of ERs is necessary for GPER1 activation of 17β-estradiol signaling

pathways. Results from ERα and ERβ knockout mice also point in this direction, as the presence of ERs seems necessary for 17β-estradiol neuroprotective action in MPTP mice (Al-Sweidi et al., 2011, Morissette et al., 2007). Alternatively, others studies show that the effect of 17β-estradiol is observed even in the absence or blockage of both ERα and ERβ (Singh et al., 1999, Ullrich et al., 2008). Indeed, redundant, independent or co-dependant action of 17β-estradiol could be mediated through ERα, ERβ and GPER1 (Prossnitz and Barton, 2011).

GPER1 is required to mediate 17β-estradiol action, even in the presence of ERα and ERβ, since blockage of GPER1 completely antagonizes the neuroprotective effect of 17β-estradiol observed in the striatum (Figure 4.9C and 4.9G). The mechanism of 17β-estradiol action in substantia nigra could implicate an additional mechanism as the one observed for the striatum given that G15 partially opposed the 17β-estradiol effect, suggesting that ERs and GPER1 can act independently in the substantia nigra to activate neuroprotective mechanisms. The different distribution in the striatum and the substantia nigra of both types of classical ER (Mitra et al., 2003) could lead to different mechanisms of neuroprotection of DA terminals and cell bodies and also the possibility that the beneficial effect of 17β-estradiol requires or not the interaction of ERs and GPER1 depending of the brain region.

The DA metabolites concentration ratios over DA show overall a dynamic picture of the DA response with an increase by the MPTP lesion to maintain neuronal activity homeostasis while the estrogenic treatments resulted in maintaining higher levels of striatal DA and metabolites concentrations. DAT and VMAT2 are transporters located on presynaptic DA terminals in the striatum and are used as markers of DA terminal loss and/or protection in the present study. The highly significant correlations between striatal DAT or VMAT2 specific binding and striatal DA concentrations of all the MPTP lesion and estrogen treatment experiments presented here support that the DA changes observed involve estrogenic neuroprotection rather than only activation of synthesis of DA.

Results obtained in intact mice showed that the 17β-estradiol effect on striatal DA metabolism was partially blocked by G15, while G1 (1 µg) mimics most of 17β-estradiol action, suggesting also a redundant neuromodulation mechanism mediated by ERs and GPER1. 17β-estradiol is well documented to modulate nigrostriatal DA activity. 17β-

estradiol has a stimulatory action on DA synthesis mediated by the activation of tyrosine hydroxylase (Pasqualini et al., 1995). Furthermore, 17β-estradiol increases DA turnover (Di Paolo et al., 1985), but has no effect, in the male striatum, on monoamine oxidase and catechol-o-methyltransferase activity (Meyers et al., 2010), two enzymes implicated in DA metabolism. Indeed, we showed that G1 reproduced some effects of 17β-estradiol on DA metabolism and that the role of both ERs and GPER1 seems to be crucial since the effect of 17β-estradiol was partially antagonized by G15.

Most findings in human studies suggest a beneficial effect of estrogen with regard to PD risk (review (Liu and Dluzen, 2007)). Furthermore, estrogen can also act as a neuromodulator of PD symptoms. The use of estrogen therapy is reported to be associated with lower symptom severity scores in postmenopausal women with early stage of PD not yet taking levodopa (Saunders-Pullman et al., 1999). Moreover, a reduction in the dose of levodopa needed to improve motor function was reported in PD women receiving 17β-estradiol (Blanchet et al., 1999). Despite the beneficial effect of 17β-estradiol against brain damage, there are several limitations associated to the use of estrogen therapy (Taylor and Manson, 2011). Indeed, the search for an estrogen analog that mimics the beneficial effect of 17β-estradiol, without its associated adverse effects, may have a significant impact on therapeutic approaches and a non-feminizing compound allows the application in men. A critical outcome of this study is the finding that the GPER1 agonist G1 is as potent as 17β-estradiol in inducing neuroprotection of DA terminals and cell bodies in MPTP mice (Figure 4.9C and 4.9D), as reported in an ischemia model of rats (Lebesgue et al., 2010). G1 has no affinity for ERs (Bologa et al., 2006) and has no effect on reproductive organs (Otto et al., 2008). Selective estrogen receptor modulators (SERM), such as tamoxifen and raloxifene, are also an alternative to estrogen and are commonly used clinically. Some drugs used in humans as “anti-estrogen” such as certain SERMs or used as estrogen replacements, such as phytoestrogens, are GPER1 agonists (Prossnitz and Barton, 2009). Thus, it is likely that some of the clinical effects observed with these compounds implicate, at least in part, an action mediated by GPER1 (Prossnitz and Barton, 2009, Prossnitz and Barton, 2011). Recently, a structure-activity study of raloxifene analogs found a biphenolic pharmacophore for

neuroprotection of cortical neurons against oxygen-glucose deprivation signaling via GPER1 (Abdelhamid et al., 2011). In the past years, many potential role of GPER1 have been described in several systems including nervous, endocrine, immune and cardiovascular systems (review (Prossnitz and Barton, 2011)). Indeed, the therapeutic potential of GPER1 agonist as a neuroprotective agent merits future research to elucidate its role and mechanism in the maintenance and neuroprotection of neurologic functions.

While 17β-estradiol shows neuroprotective effect against MPTP toxicity in both female and male mice (Dluzen et al., 1996), male mice were used in this study to reproduce the epidemiological observations that more men than women develop PD (Shulman, 2007). The androgens testosterone and dihydrotestosterone fail to induce a neuroprotective effect against MPTP in male mice (Ekue et al., 2002) whereas estrogenic compounds (17β-estradiol and raloxifene) show beneficial effects (Callier et al., 2001). While estrogen therapy in men is not appropriate, raloxifene has been used clinically in elderly men, without side effect (Goekoop et al., 2005). Alteration of the nigrostriatal DA system of nonlesioned male mice is observed in ER knockout mice as well as increase susceptibility to the toxins (Al-Sweidi et al., 2011, Morissette et al., 2007), showing the role of ER in DA system and neuroprotection in male mice. Thus, the importance of ER in the nigrostriatal system in male mice offers a neuroprotective target. Estrogen neuroprotection represent an interesting opportunity in both females and males and a better understanding of the role of the estrogen receptors in mediating the neuroprotective effects of 17β-estradiol is crucial for the development of selective agents that are neuroprotective but that lack feminizing effects. Targeting the GPER1 estrogen receptor is especially relevant for male since its stimulation is non-feminizing.

In conclusion, this study reported, for the first time, that the membrane estrogen receptor, GPER1, is implicated in the neuroprotective effect of 17β-estradiol in MPTP mice. Importantly, we demonstrated that the GPER1 specific agonist G1 is as potent as 17β-estradiol in mediating a protecting effect in both the striatum and substantia nigra. The current study provided evidence supporting the consideration of GPER1 agonist as an alternative

therapeutic approach to estrogens to reproduce beneficial effects and to minimize adverse outcomes.

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Table 4.1. Effects of a chronic treatment of male mice with 17β-estradiol and G1 on [^{125}I]-RTI-121 (DAT) and [^{3}H]-TBZ-OH (VMAT2) specific binding in striatum and substantia nigra. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 7-8 mice per group.

Treatment	DAT		VMAT2	
Groups	[^{125}I]-RTI-121 specific binding (fmol/mg of tissue)		[^{3}H]-TBZ-OH specific binding (fmol/mg of tissue)	
	Striatum	Substantia nigra	Striatum	Substantia nigra
Control	0.288 ± 0.025	0.245 ± 0.010	1800.2 ± 35.4	662.2 ± 20.1
17β-estradiol	0.263 ± 0.010	0.204 ± 0.013	1716.5 ± 46.8	561.6 ± 48.3
G1 (1 µg)	0.280 ± 0.013	0.220 ± 0.011	1720.2 ± 40.0	573.2 ± 44.7
G1 (5 µg)	0.294 ± 0.007	0.227 ± 0.005	1646.4 ± 25.4	591.4 ± 43.3

Table 4.2. Effects of a chronic treatment of male mice with 17β-estradiol, G1 and G15 on [^{125}I]-RTI-121 (DAT) and [^{3}H]-TBZ-OH (VMAT2) specific binding in striatum and substantia nigra. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 8-9 mice per group.

Treatment Groups	DAT [^{125}I]-RTI-121 specific binding (fmol/mg of tissue)		VMAT2 [^{3}H]-TBZ-OH specific binding (fmol/mg of tissue)	
	Striatum	Substantia nigra	Striatum	Substantia nigra
Control	1.861 ± 0.073	1.482 ± 0.149	2999.4 ± 148.4	1212.2 ± 42.7
17β-estradiol	1.810 ± 0.072	1.712 ± 0.091	3027.9 ± 124.1	1189.1 ± 21.1
G1 (5 µg)	1.803 ± 0.043	1.631 ± 0.079	2935.8 ± 124.2	1186.7 ± 28.6
G15 (10 µg)	1.946 ± 0.057	1.691 ± 0.120	2928.5 ± 132.9	1249.6 ± 33.8
G15 (50µg)	1.808 ± 0.043	1.577 ± 0.089	2847.6 ± 95.8	1240.6 ± 28.8
17β-estradiol + G15	1.744 ± 0.062	1.537 ± 0.136	3051.9 ± 115.3	1230.2 ± 30.1
G1 + G15	1.668 ± 0.081	1.507 ± 0.051	2920.5 ± 101.0	1192.9 ± 29.9

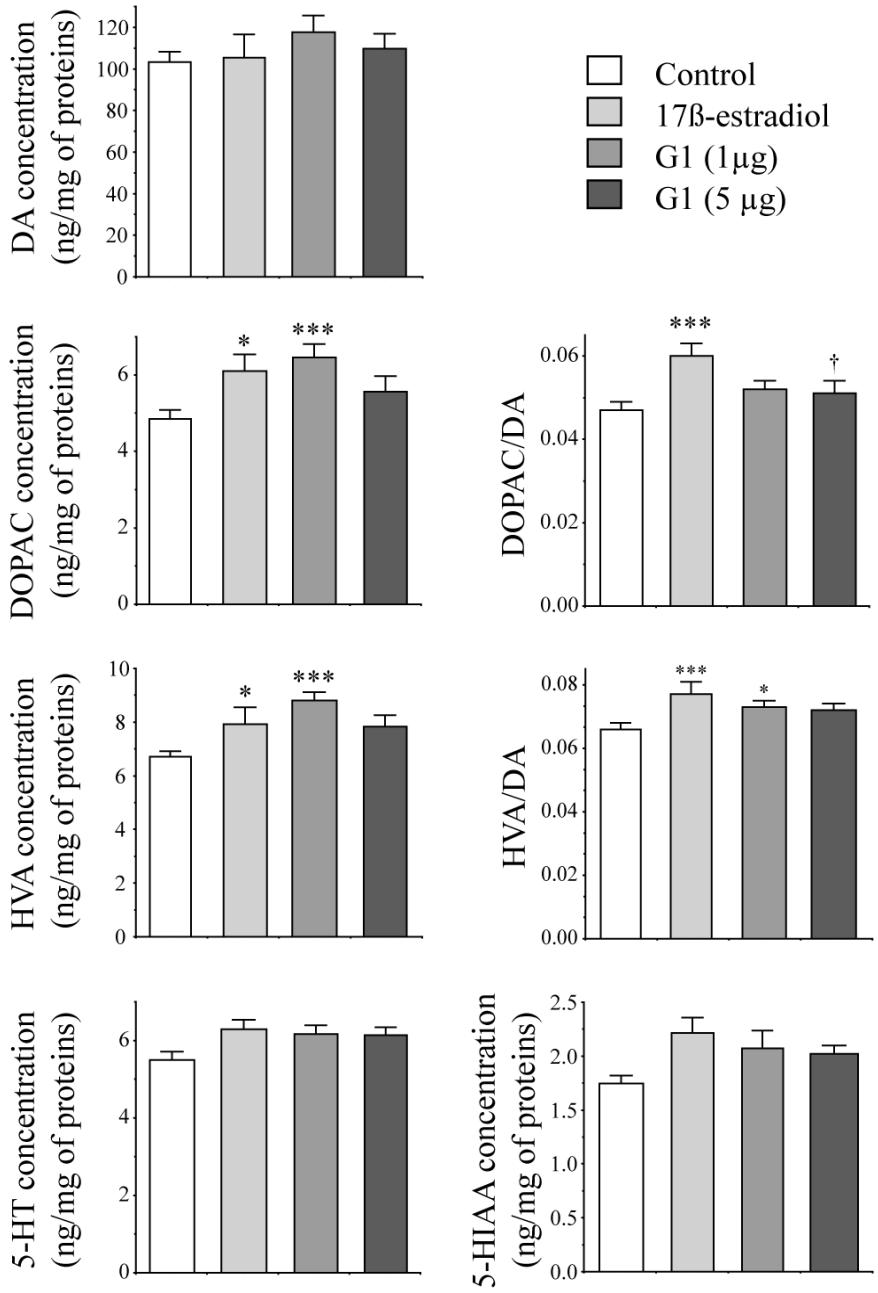

Figure 4.1. Effects of chronic treatment of male mice with 17β-estradiol and G1 on striatal dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), DOPAC/DA and HVA/DA ratios, as well as serotonin (5-HT) concentrations and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Values shown are the means (ng/mg of proteins) ± S.E.M. of 7-8 mice per group. There was a statistically significant effect of treatments for DOPAC ($F_{3,26} = 3.80$, $p = 0.02$), HVA ($F_{3,26} = 4.05$, $p = 0.01$), DOPAC/DA ($F_{3,27} = 3.89$, $p = 0.01$) and HVA/DA ($F_{3,28} = 3.35$, $p = 0.03$) ratios. * $p < 0.05$ and *** $p < 0.005$ vs control; † $p < 0.05$ vs 17β-estradiol.

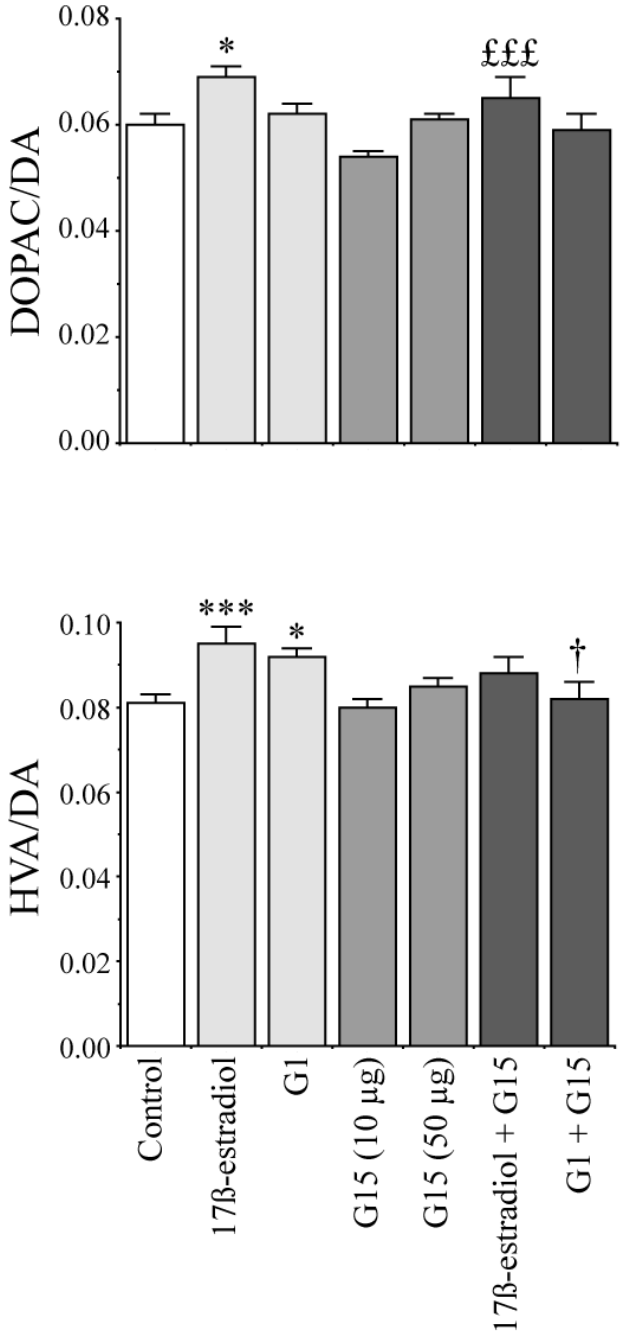

Figure 4.2. Effects of chronic treatment of male mice with 17β-estradiol, G1 and G15 on striatal DOPAC/DA and HVA/DA ratios. Values shown are the means ± S.E.M. of 8-9 mice per group. There was a statistically significant effect of treatments for DOPAC/DA ($F_{6,48}$ = 3.388, p = 0.007) and HVA/DA ($F_{6,51}$ = 3.04, p = 0.012) ratios. * $p < 0.05$ and *** $p < 0.005$ vs control; † $p < 0.05$ vs G1; £££ $p < 0.005$ vs G15 (10 µg). DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA homovanillic acid. As results in experiment shown in figure 4.1, DA concentrations of this experiment remained unchanged (Data not shown).

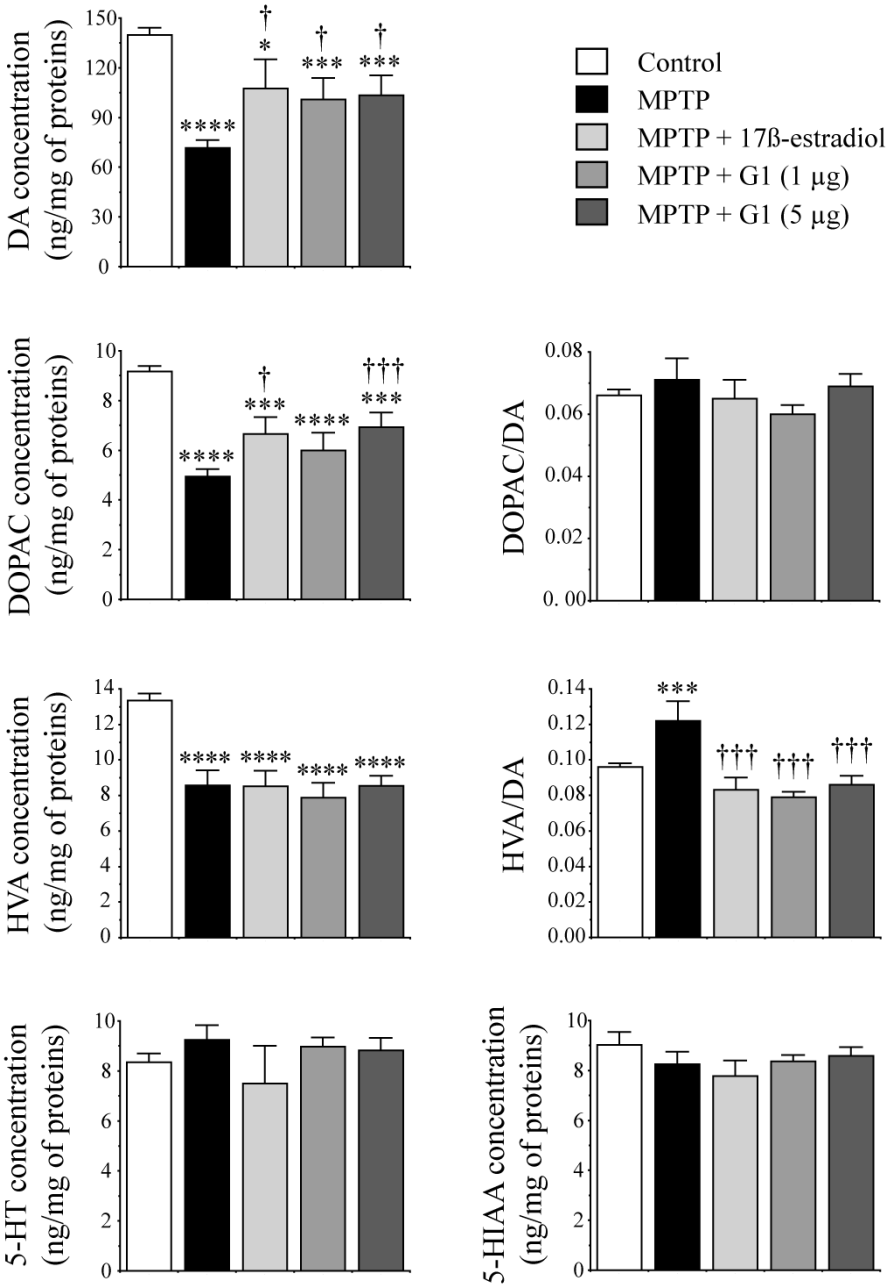

Figure 4.3. Effects of MPTP, 17β-estradiol and G1 chronic treatment of male mice on striatal dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), DOPAC/DA and HVA/DA ratios as well as serotonin (5-HT) concentrations and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Values shown are the means (ng/mg of proteins) ± S.E.M. of 5-13 mice per group. There was a statistically significant effect of treatments for DA ($F_{3,34} = 10.56$, $p < 0.0001$), DOPAC ($F_{3,34} = 18.78$, $p < 0.0001$) and HVA ($F_{3,34} = 15.43$, $p < 0.0001$) as well as HVA/DA ($F_{3,34} = 6.65$, $p = 0.0005$). * $p < 0.05$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$ and ††† $p < 0.005$ vs MPTP.

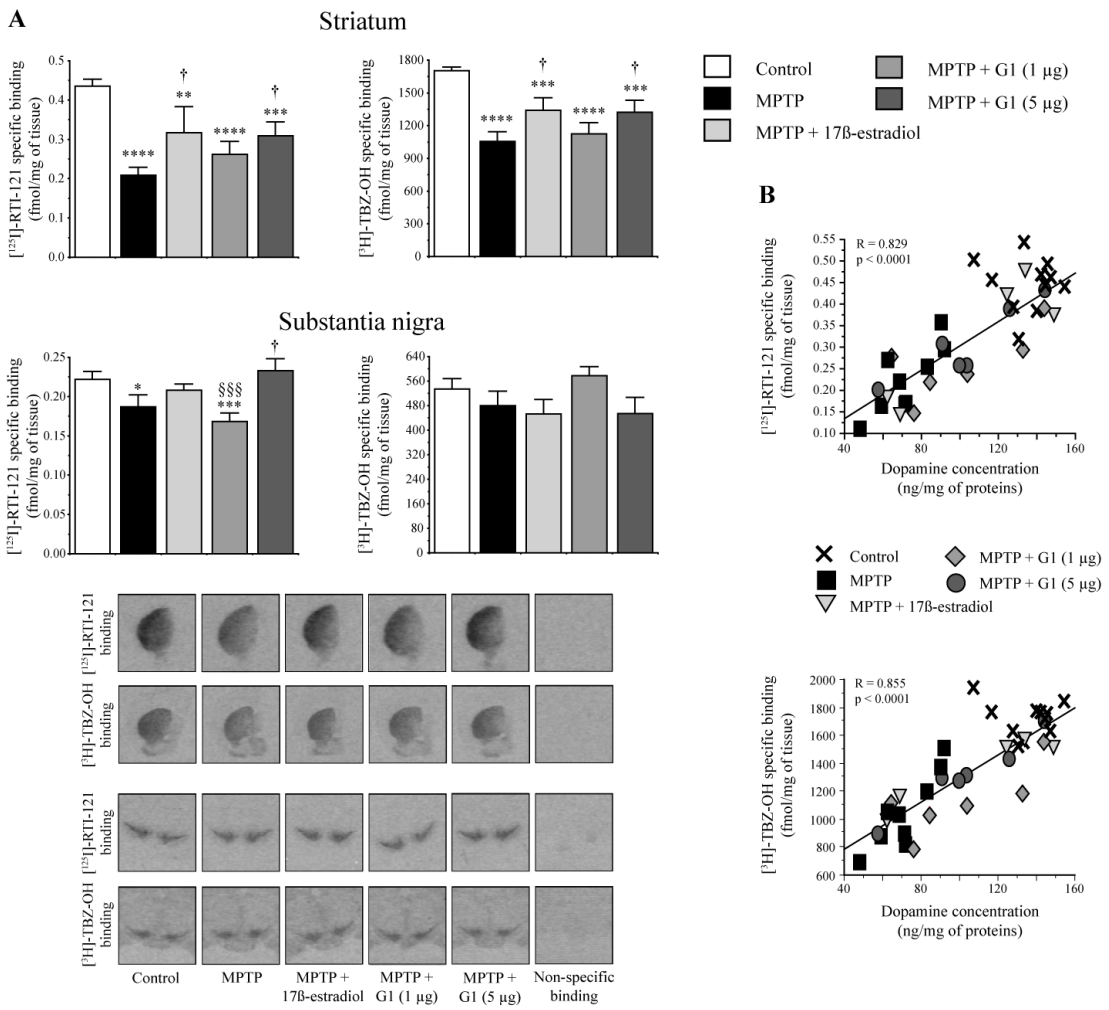

Figure 4.4. A. Effects of MPTP, 17β-estradiol and G1 chronic treatment of male mice on [^{125}I]-RTI-121 DAT and [^{3}H]-TBZ-OH VMAT2 specific binding in striatum and substantia nigra and examples of these binding autoradiography. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 5-13 mice per group. There was a statistically significant effect of treatments for striatal DAT ($F_{3,34}$ = 11.21, $p < 0.0001$) and VMAT2 ($F_{3,34}$ = 13.69, $p < 0.0001$) as well as DAT in substantia nigra ($F_{3,33}$ = 3.92, $p = 0.010$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$ vs MPTP; §§§ $p < 0.005$ vs MPTP + G1 (5µg). B. Correlations between striatal DA concentration and DAT specific binding in striatum, as well as between DA concentration and VMAT2 specific binding in striatum. Each symbol represents an individual mouse.

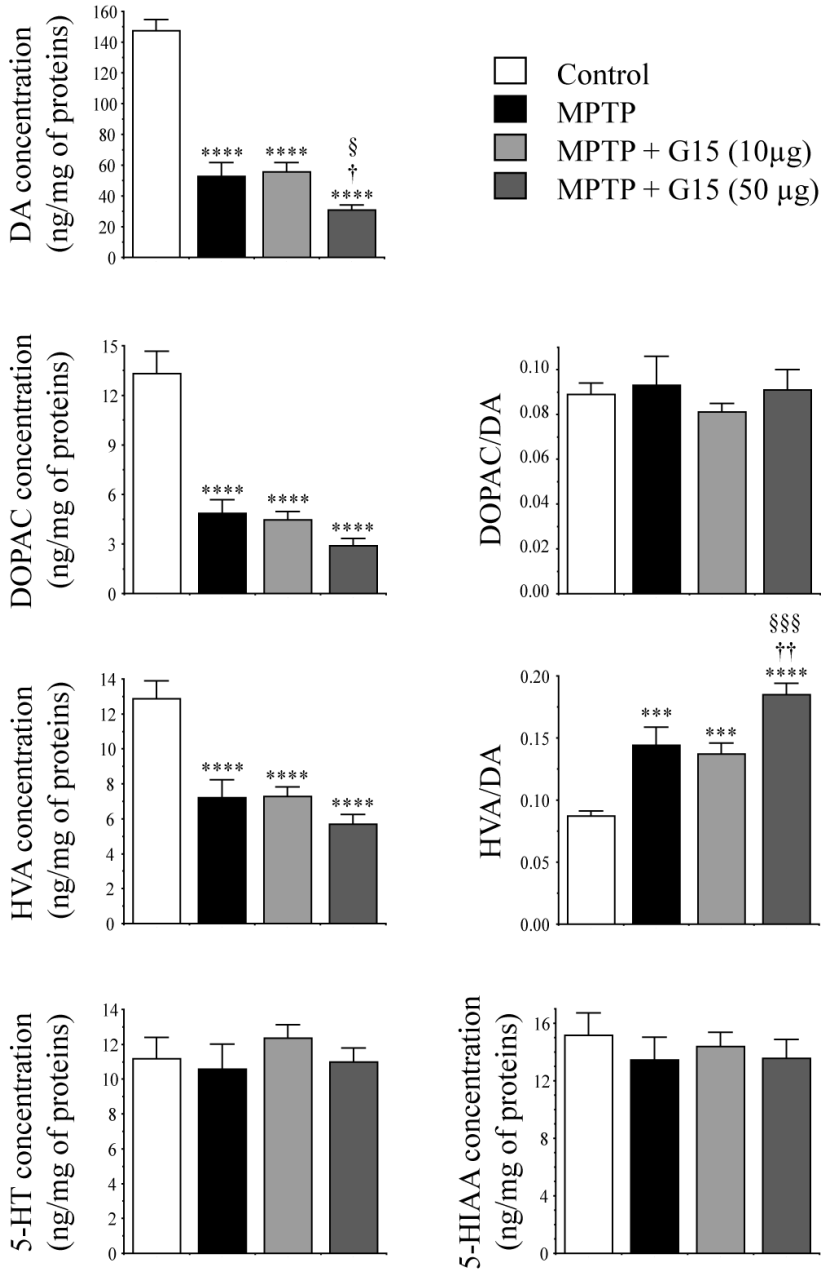

Figure 4.5. Effects of MPTP and G15 chronic treatment of male mice on striatal dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), DOPAC/DA and HVA/DA ratios as well as serotonin (5-HT) concentrations and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Values shown are the means (ng/mg of proteins) ± S.E.M. of 8-9 mice per group. There was a statistically significant effect of treatments for DA ($F_{3,32} = 61.38$, $p < 0.0001$), DOPAC ($F_{3,32} = 32.27$, $p < 0.0001$), HVA ($F_{3,32} = 15.80$, $p < 0.0001$) and HVA/DA ratio ($F_{3,32} = 16.144$, $p < 0.0001$). *** $p < 0.005$ and **** $p < 0.0001$ vs control; † < 0.05 and †† < 0.01 vs MPTP; § < 0.05 and §§§ < 0.005 vs MPTP + G15 (10µg).

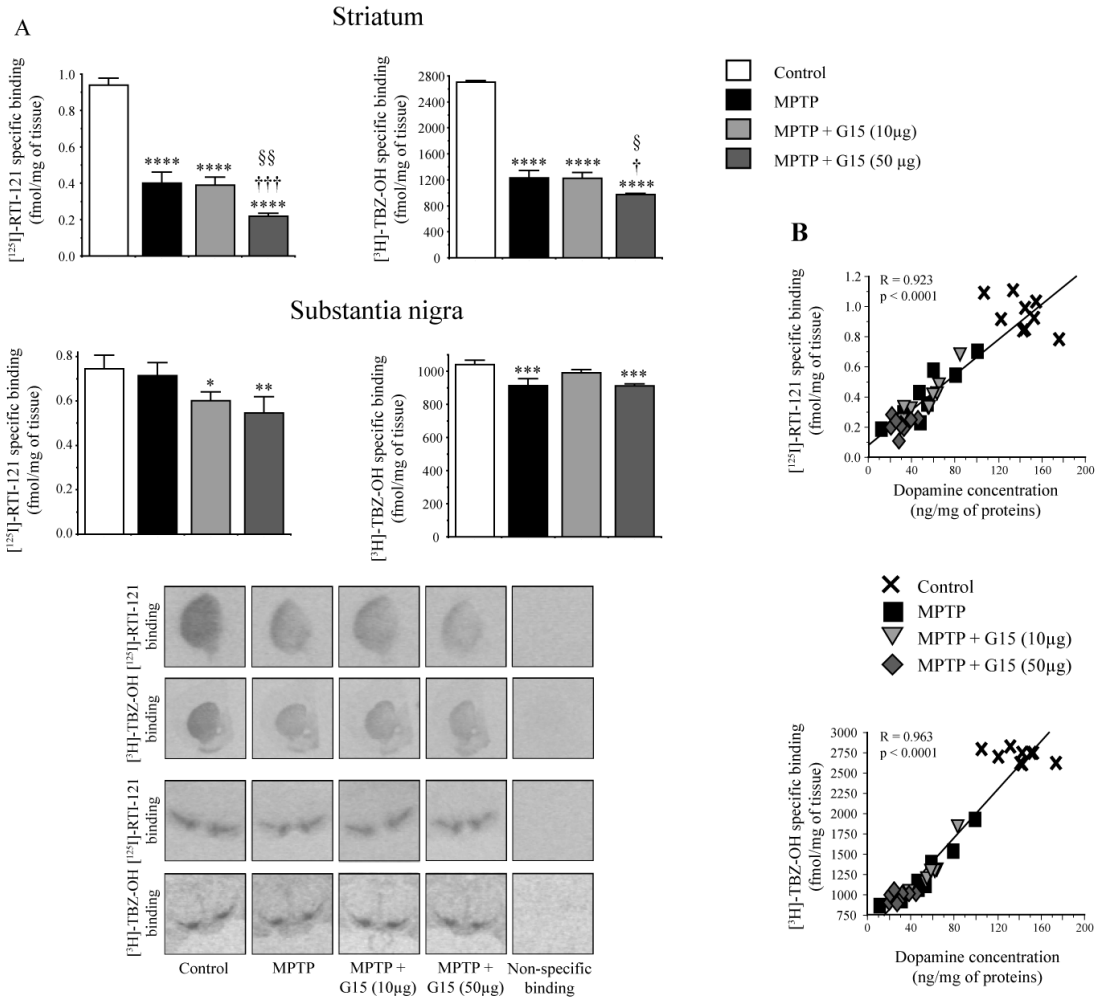

Figure 4.6. A. Effects of MPTP and G15 chronic treatment of male mice on [^{125}I]-RTI-121 DAT and [^{3}H]-TBZ-OH VMAT2 specific binding in striatum and substantia nigra and examples of these binding autoradiography. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 8-9 mice per group. There was a statistically significant effect of treatments for striatal DAT ($F_{3,32} = 54.88$, $p < 0.0001$) and VMAT2 ($F_{3,32} = 115.24$, $p < 0.0001$) as well as DAT ($F_{3,31} = 3.25$, $p = 0.03$) and VMAT2 ($F_{3,31} = 5.10$, $p = 0.005$) in substantia nigra. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$ and ††† $p < 0.005$ vs MPTP; § $p < 0.05$ and §§ $p < 0.01$ vs MPTP + G15 (10µg). B. Correlations between striatal DA concentration and DAT specific binding in striatum, as well as between DA concentration and VMAT2 specific binding in striatum. Each symbol represents an individual mouse.

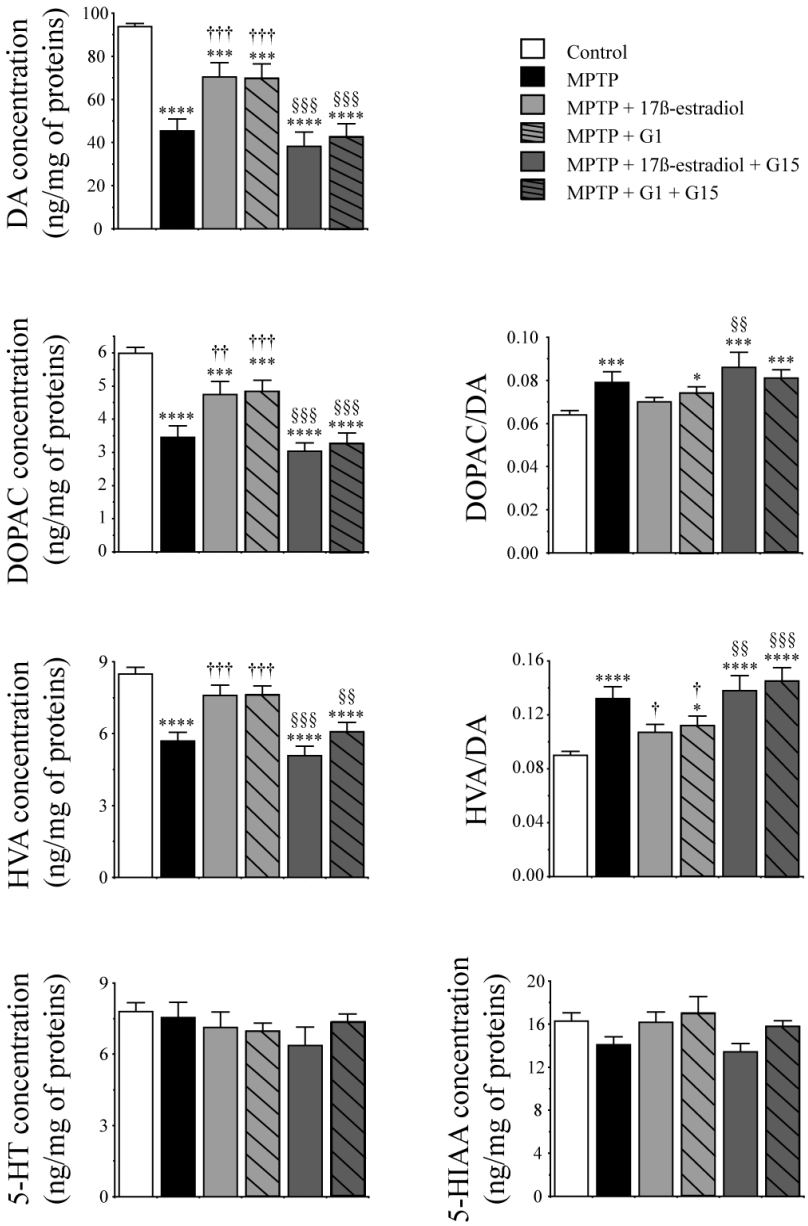

Figure 4.7. Effects of MPTP, 17β-estradiol, G1 and G15 chronic treatment of male mice on striatal dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), DOPAC/DA and HVA/DA ratios as well as serotonin (5-HT) concentrations and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Values shown are the means (ng/mg of proteins) ± S.E.M. of 6-14 mice per group. There was a statistically significant effect of treatments for DA ($F_{5,45}$ = 18.78, $p < 0.0001$), DOPAC ($F_{5,45}$ = 16.64, $p < 0.0001$), HVA ($F_{5,45}$ = 12.99, $p < 0.0001$) as well as DOPAC/DA ($F_{5,45}$ = 4.99, $p = 0.001$) and HVA/DA ($F_{5,45}$ = 9.84, $p < 0.0001$) ratios. * $p < 0.05$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.005$ vs MPTP; §§ $p < 0.01$ and §§§ $p < 0.005$ vs corresponding group without G15.

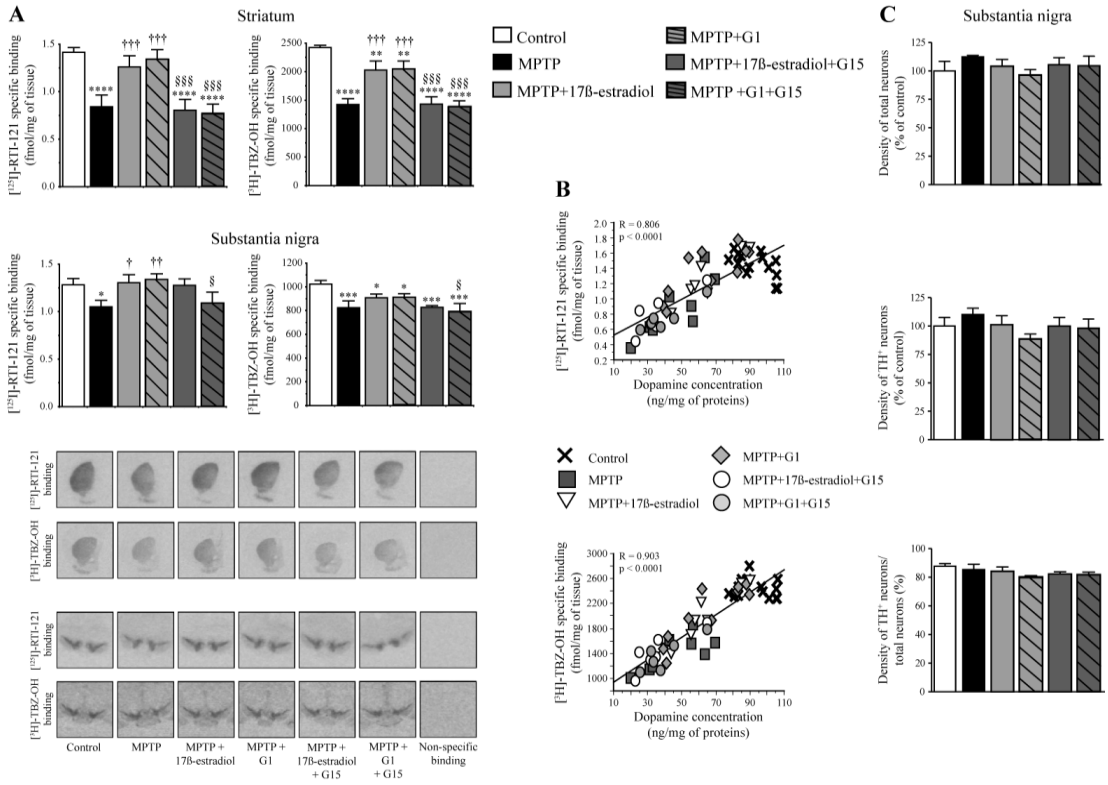

Figure 4.8. A. Effects of MPTP, 17β-estradiol, G1 and G15 chronic treatment of male mice on [^{125}I]-RTI-121 DAT and [^{3}H]-TBZ-OH VMAT2 specific binding in striatum and substantia nigra and examples of these binding autoradiography. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 6-14 mice per group. There was a statistically significant effect of treatments for striatal DAT ($F_{5,45} = 9.86$, $p < 0.0001$) and VMAT2 ($F_{5,45} = 17.09$, $p < 0.0001$) as well as DAT ($F_{5,45} = 2.35$, $p = 0.05$) and VMAT2 ($F_{5,44} = 5.08$, $p = 0.0009$) in substantia nigra. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.005$ vs MPTP; § $p < 0.05$ and §§§ $p < 0.005$ vs corresponding group without G15. B. Correlations between striatal DA concentration and DAT specific binding in striatum, as well as between DA concentration and VMAT2 specific binding in striatum. Each symbol represents an individual mouse. C. Effects of MPTP, 17β-estradiol, G1 and G15 chronic treatment of male mice on tyrosine hydroxylase (TH) positive density in substantia nigra pars compacta.

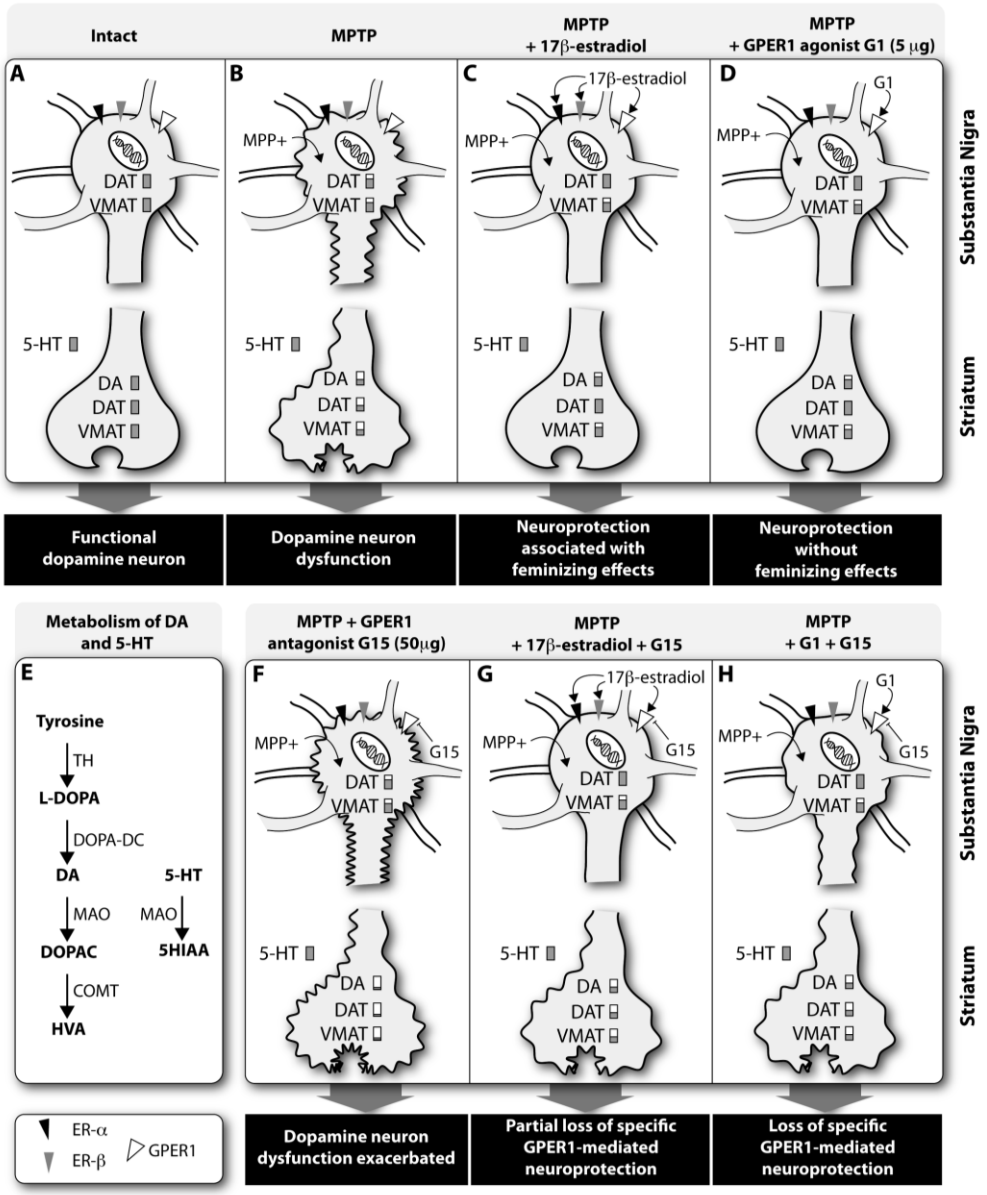

Figure 4.9. A simplified scheme illustrating the effect of an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion on nigrostriatal dopamine (DA) neurons and neuroprotection by estrogens implicating G protein-coupled estrogen receptor 1 (GPER1). Concentrations of DA and serotonin (5-HT) as well as specific binding to the DA transporter (DAT) and the vesicular monoamine transporter 2 (VMAT2) are shown with a gauge representing their relative levels compared with intact mice values. Striatal 5-HT concentrations remained unchanged showing specificity of the lesion and treatments for DA systems. (E) Metabolism of DA and 5-HT. Enzymes involved in synthesis (tyrosine hydroxylase [TH]) and metabolism (3,4-dihydroxyphenylalanine decarboxylase [DOPAC-DC], monoamine oxidase [MAO] and catechol-O-methyltransferase [COMT]) of DA and 5-HT. DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; L-DOPA, levodopa; MPP+, 1-methyl-4-phenylpyridinium.

Chapitre 5: The neuroprotective effect of estrogen receptor α activation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice involves interaction with G protein-coupled estrogen receptor 1

Mélanie Bourque[a,b], Marc Morissette[a], Thérèse Di Paolo[a,b]*

[a]Neuroscience Research Unit, Centre Hospitalier Universitaire de Québec, CHUL, Quebec City, Quebec, G1V 4G2

[b]Faculty of Pharmacy, Laval University, Quebec City, Quebec, G1K 7P4, Canada

Manuscrit soumis « Neurobiology of Aging »

Contribution des auteurs: La conception et l'organisation du projet fut réalisée par la Dr T. Di Paolo et M. Bourque. M. Bourque a fait les traitements des animaux, les autoradiographies, les immunobuvardages, l'analyse des résultats et la rédaction du manuscrit. M. Morissette a fait le dosage de la dopamine et de ses métabolites par HPLC. Le manuscrit a été corrigé par la Dre T. Di Paolo et M. Morissette.

Résumé

Cette étude investigue l'interaction entre les récepteurs des œstrogènes (ER) α/β et le récepteur membranaire des œstrogènes couplé aux protéines G (GPER1), dans le neuroprotection des neurones dopaminergiques chez les souris mâles traitées avec le 1-méthyl-4-phényl-1,2,3,6-tétrahydropyridine, en utilisant une approche pharmacologique. Le ICI 182,780 (un antagoniste des ERα/β) bloque l'effet protecteur du 17β-estradiol, mais non celui du G1 (un agoniste du GPER1) sur les concentrations de dopamine, ainsi que sur la liaison spécifique au DAT et au VMAT2 dans le striatum et la substance noire. La protection par le G1 est accompagnée par une augmentation des niveaux de Bcl-2 et du BDNF au striatum; la co-administration de l'ICI 182,780 bloque l'effet du G1 seulement sur les niveaux du BDNF. L'activation du ERα par le PPT protège les neurones dopaminergiques, un effet associé avec l'activation de la signalisation d'Akt dans striatum et une augmentation des niveaux de Bcl-2 et du BDNF. Ces effets sont inhibés par le G15, un antagoniste du GPER1. Nos résultats suggèrent une collaboration entre le ERα et le GPER1 dans la protection des neurones dopaminergiques et la modulation des voies de signalisation, et que l'effet du GPER1 se fait indépendamment des ERα/β, quoique le GPER1 requière les ERα/β pour augmenter les niveaux du BDNF.

Abstract

This study investigated the interaction between estrogen receptors (ER) a/β and the G protein-coupled estrogen receptor 1 (GPER1) in neuroprotection of dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated male mice, using a pharmacologic approach. The ERα/β antagonist ICI 182,780 blocked the protective effects of 17β-estradiol, but not those of GPER1 agonist G1, on dopamine concentration as well as dopamine transporter and vesicular monoamine transporter 2 specific binding in both the striatum and the substantia nigra. G1 protection was accompanied by an increase in Blc-2 and BDNF levels in the striatum; coadministration of ICI 182,780 blocked the effect of G1 only on BDNF levels. ERα activation by PPT protected dopamine neurons, an effect associated with activation of striatal Akt signaling and an increase in Bcl-2 and BDNF levels; those effects were inhibited by the GPER1 antagonist G15. Our results suggest collaboration between ERα and GPER1 in protection of dopamine neurons and modulation of signaling pathways, and that the effect of GPER1 occurs independently of ERα/β, whereas GPER1 require ERα/β to increase BDNF levels.

Keywords: 17β-estradiol, estrogen receptor, GPER1, MPTP, neuroprotection, Akt

5.1. Introduction

The neuroprotective effect of 17β-estradiol against toxins damaging dopaminergic neurons, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine, is well documented (Bourque et al., 2009; Gillies et al., 2014). Studies in the past years have highlighted some of the receptor mechanisms by which 17β-estradiol mediated neuroprotection of dopamine (DA) neurons. 17β-estradiol exerts its action by binding to estrogens receptors (ER) α and β, both belonging to the family of nuclear receptors, and also binds to the G protein-coupled estrogen receptor 1 (GPER1) (Marino et al., 2006; Revankar et al., 2005; Thomas et al., 2005). The use of specific ER α and β agonists, 4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl)*tris*phenol (PPT) and 2,3-*bis*(4-Hydroxyphenyl)-propionitrile (DPN) respectively, have shown that PPT, but not DPN, reproduces the 17β-estradiol beneficial effect on DA neurons, suggesting that protection is mediated through ERα (D'Astous et al., 2004). Recently, our group has shown that the GPER1 agonist G1 is as potent as 17β-estradiol to induce protection against MPTP toxicity in mice (Bourque et al., 2013). Thus, both ERα activation with its agonist PPT and GPER1 stimulation with G1 reproduce the 17β-estradiol protection observed in MPTP mice. This suggested that both ERα and GPER1 have a major role in mediating protection of DA neurons.

In support of these findings are studies showing that both ERα and GPER1 were required for 17β-estradiol neuroprotective effect as observed by the lack of protection in ER knock-out mice and in mice treated with G15, a GPER1 antagonist (Al-Sweidi et al., 2011; Bourque et al., 2013; Morissette et al., 2007). Further, ERα knock-out mice and mice treated with G15 were more susceptible to MPTP toxicity (Al-Sweidi et al., 2011; Bourque et al., 2013; Morissette et al., 2007). Taken together, these results suggest interplay between ER and GPER1 in the action of 17β-estradiol in protection of dopaminergic neurons.

Several studies reported a similar effect when ERα is activated or after stimulation of GPER1 (Gingerich et al., 2010; Kuo et al., 2010; Liu et al., 2011). Furthermore, 17β-estradiol actions could be inhibited by both ICI 182,780 and G15 (Gingerich et al., 2010; Notas et al., 2012). This suggests an interaction between both receptors in the effect of 17β-estradiol, or a common related mechanism. This is supported by findings showing that GPER1 can modify

ERα phosphorylation signal in the mouse uterus (Gao et al., 2011) and that ERα can directly interact and activate G proteins to mediate 17β-estradiol signaling (Kumar et al., 2007). An in vitro study in endometrial cancer cells reported a physical interaction between GPER1 and ERα that was increased by 17β-estradiol, and prevented by ICI 182,780, suggesting that the interaction between both receptors is ligand dependent (Vivacqua et al., 2009). Nevertheless, the interplay of ERs and GPER1 in neuroprotection of dopaminergic neurons is not known.

With a pharmacological approach, we studied a potential collaboration between ERα/β and GPER1 in the neuroprotection of dopaminergic neurons in MPTP treated male mice. The aim of our study was to investigate the three following questions (1) is GPER1 required for an estrogenic effect through ERα or ERβ, (2) are ERα and ERβ required for the 17β-estradiol effect via GPER1, and (3) are ERα and ERβ required for specific GPER1 neuroprotection (Figure 1). Measures of DA concentration, dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) were performed to assess protection and damage of DA terminals in striatum and cell bodies in substantia nigra. We also investigated major targets involved in 17β-estradiol, Akt signaling as well as trophic factor brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), to verify common or independent signaling between the three estrogen receptors.

5.2. Materials and Methods

5.2.1. Animals and Treatment

C57Bl/6 male mice (10 weeks) were purchased from Charles River Canada (Montreal, Qc, Canada). MPTP and 17β-estradiol were purchased from Sigma Chemicals (St. Louis, MO), and PPT, DPN, G1, G15 and ICI 182,780 were from Tocris (Ellisville, MO, USA). The Laval University Animal Care Committee approved all of the animal studies. All efforts were made to minimize animal suffering and to reduce the number of mice used.

This study used a pharmacological approach to investigate the potential interaction between ERα/β and GPER1 with specific agonists of ERα (PPT), ERβ (DPN) and GPER1 (G1), and antagonists of ERα/β (ICI 182,780) and GPER1 (G15) (Figure 5.1). PPT displays 410-fold selectivity for ERα over ERβ (Stauffer et al., 2000), and activity on GPER1 has been reported in vitro at high concentration (100 nM) (Petrie et al., 2013). DPN is exhibits 70-fold selectivity for ERβ over ERα (Meyers et al., 2001) and does not have activity on GPER1 (Petrie et al., 2013). G1 is a specific agonist to GPER1 and does not bind to ERα/β at concentration up to 10μM (Bologa et al., 2006; Dennis et al., 2011). G15 is a specific antagonist to GPER1 and displays binding to ERα and ERβ only at high concentration (10 μM) (Dennis et al., 2011). ICI 182,780 is a high affinity ER antagonist (Wakeling et al., 1991) but has also an agonist activity on GPER1, with a relative binding affinities approximately 10% of the activity of 17β-estradiol for GPER1 (Thomas et al., 2005).

Each experimental group of mice received twice daily subcutaneous injection of vehicle (0.9% saline with 0.3% gelatin), 17β-estradiol (1 μg), G1 (5 μg), PPT (1 μg), DPN (3 μg) or ICI 182,780 (25 μg) for 10 days. To study the interaction between the different subtypes of estrogen receptors, groups of mice received the following agonist-antagonist combinations: 17β-estradiol and ICI 182,780, G1 and ICI 182,780, PPT and G15 (10 μg), or DPN and G15 for 10 days. On day 5, mice received four injections of MPTP (4.75 mg/kg i.p., Sigma Chemical (St. Louis, MO)) at 2-h intervals, whereas the control groups received saline solution. On day 11, mice were decapitated, and brains were quickly removed and frozen in isopentane (-40°C).

5.2.2. Quantification of steroids in mouse plasma

The plasma levels of testosterone, dihydrotestosterone, 3β-diol, progesterone and 17β-estradiol were measured with gas chromatography mass spectrometry as described (Audet-Walsh et al., 2011). Plasma of 2 or 3 mice from the same group were pooled in order to have enough plasma to quantify the above steroids.

5.2.3. Brain preparation

A unilateral striatum was used to assay DA and its metabolites concentrations. The striatum of the contralateral hemisphere (bregma 1.18 at 0.14 mm) and the substantia nigra (bregma -2.92 at -3.64 mm) (Franklin and Paxinos, 1997) were cut on a cryostat in 12 µm slices for autoradiography and striatal dissection was performed with 50 µm slices for Western blots. Slices and dissections were kept at – 80 ºC until assayed.

5.2.4. Striatal biogenic amines determination

The left anterior striata were dissected, homogenized in 250 µl of 0.1 N $HClO_4$ at 4 °C and then centrifuged at 10 000 × g for 10 min (4 °C) to precipitate proteins. The concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by high performance liquid chromatography (HPLC) with electrochemical detection (D'Astous et al., 2004). Supernatants of striatal tissue were directly injected into the chromatograph consisting of a Waters 717 plus autosampler automatic injector, a Waters 515 pump equipped with a C-18 column (Waters Nova-Pak C18, 3 µm, 3.9 mm × 150 cm), a BAS LC-4C electrochemical detector and a glassy carbon electrode. The mobile phase consisted of 0.025 M citric acid, 1.7 mM 1-heptane-sulfonic acid, and 10% methanol, in filtered distilled water, delivered at a flow rate of 0.8 ml/min. The final pH of 4.1 was obtained by addition of NaOH. The electrochemical potential was set at 0.8 V with respect to an Ag/AgCl reference electrode. Results were expressed in nanograms of amine per milligram of protein. Proteins were assayed with a Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL).

5.2.5. Dopamine transporter autoradiography

DAT autoradiography in the striatum and the 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 exposed to Kodak films (Biomax) 18 hours for the striatum and 30 hours for substantia nigra. Films were analyzed using the software NIH Image 1.63.

5.2.6. Vesicular monoamine transporter 2

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.

5.2.7. Western Blots

Striata were homogenized in RIPA lysis buffer (50 mM TRIS-HCl, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.5% SDS, 2 mM EDTA, 1% phenylmethylsulfonyl fluoride 100 mM) supplemented with protease and phosphatase inhibitors (inhibitor cocktails from Sigma, Oakville, On). Homogenates were kept on ice for 30 minutes to allow solubilization and then centrifuged at 16,000 × g for 15 min. Proteins content of supernatants was measured with a Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL). Proteins were resolved using 12 % SDS-polyacrylamide gel electrophoresis with a triple wide mini-vertical gel system (C.B.S. Scientific Compagny, Del Mar, CA) and electrophoretically transferred to polyvinylidine difluoride (PVDF) membrane. The membranes were blocked with 5 % bovine albumin serum or 5% nonfat dry milk diluted in 0.1 % Tween 20/phosphate-buffered saline and incubated overnight with the primary antibodies. The antibodies (diluted 1:1000) against phosphorylated Akt at serine 473 (pAkt), phosphorylated GSK3β at serine 9 (pGSK3β), Akt and GSK3β were obtained from

Cell Signaling Technology (Pickering, On). Bcl-2, BDNF and GDNF antibodies (diluted 1:1000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). βIII-tubulin antibody (diluted 1:10000) was from Chemicon International (Hornby, On). 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 (KPL LumiGLO Reserve, Gaithersburg, MD, USA). When needed, membranes were stripped using 62.5 mM Tris-HCl, 10 % SDS, 0.5 % β-mercaptoethanol. Densitometric analysis was performed using AlphaView Image Analysis Systems (FluorChem Q Alpha Innotech). The density of each band was normalized to its respective loading control (total proteins or βIII-tubulin). Experiments were repeated two to three times.

5.2.8. 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's protected least significant difference test. A simple regression model was used to determine the coefficient of correlation. A $p \leq 0.05$ was required for the results to be considered statistically significant.

5.3. Results

5.3.1. Dopaminergic markers

MPTP administration decreased striatal concentrations of DA, DOPAC and HVA, and increased DOPAC/DA and HVA/DA ratios (Figure 5.2). Treatment with 17β-estradiol, G1 and PPT decreased MPTP toxicity, an effect that was not observed with the administration of DPN. The use of ICI 182,780 blocked the effect of 17β-estradiol on DA concentrations. Mice treated with the combination of 17β-estradiol and ICI 182,780 had DOPAC and HVA concentrations lower than mice that received 17β-estradiol only. Mice administered PPT or DPN, combined with G15, had concentrations of DA, DOPAC and HVA lower than when only the agonist was used. An increase in the effect of MPTP on HVA concentrations was observed in mice treated with PPT-G15 and DPN-G15 combinations. No significant difference was observed for striatal DA, DOPAC and HVA concentrations as well as metabolites/DA ratios between mice treated with G1 and those treated with the combination of G1 and ICI 182,780. The ratio of HVA/DA was not increased in mice treated with 17β-estradiol, PPT, DPN, G1 or the combination G1 and ICI 182, 780, although the other experimental groups had a ratio of HVA/DA higher than for control mice.

MPTP administration decreased DAT and VMAT2 specific binding in the striatum (Figure 5.3A). Treatment with 17β-estradiol and PPT decreased the effect of MPTP on these markers. This effect was blocked by the administration of ICI 182,780 in mice treated with 17β-estradiol and by G15 in mice that received PPT. Administration of G1 reduced the effect of MPTP on DAT and VMAT2 specific binding in the striatum and this effect was not abolished in the presence of the ERα/β antagonist ICI 182, 780. Changes observed for striatal DA concentration paralleled those found for DAT and VMAT2 specific binding in striatum, as demonstrated by significant positive correlations between these markers (Figure 5.3B).

In the substantia nigra, treatment with 17β-estradiol, PPT and G1 completely prevented the decrease of DAT specific binding induced by MPTP (Figure 3A). This effect was also observed in mice treated only with ICI 182, 780. The effect of 17β-estradiol and PPT was blocked by the use of an antagonist (ICI 182,780 for 17β-estradiol and G15 pout PPT). The use of ICI 182,780 did not antagonize the effect of G1 on DAT specific binding.

DPN did not prevent damage resulting from the administration of MPTP. No significant differences between groups were observed for VMAT2 specific binding in the substantia nigra.

5.3.2. Signaling pathways

We next examined signaling pathways implicated in 17β-estradiol neuroprotection, namely the Akt pathway as well as of trophic factors induction, and also investigated which estrogen receptors was responsible to modulate those molecules (Figure 5.4). We found that only ERα activation with PPT significantly increased Akt activation and a trend was observed for G15 blocking the PPT effect ($p=0.063$). 17β-estradiol, PPT and DPN significantly increased GSK3β phosphorylation at serine 9. The effect of 17β-estradiol was lost when combined with ICI 182,780. Inhibition of GPER1 with G15 blocked the effect of PPT, but not DPN. Only MPTP lesioned mice receiving vehicle showed a decrease in striatal Bcl-2 levels, whereas MPTP mice treated with estrogenic compounds did not show this reduction in Bcl-2 levels. Treatment with G1 or PPT increased the levels of BCl-2 and those effects were not blocked when agonist was combined with an antagonist. MPTP mice treated with 17β-estradiol, G1, PPT and DPN had higher BDNF levels than control mice. The effect of 17β-estradiol and DPN was not antagonized by ICI 182,780 and G15, respectively. When combined with ICI 182,780, the increase in BDNF levels observed with G1 was lost. G15 antagonized the increase in BDNF levels induced by PPT. No significant change in GDNF levels was observed.

5.3.3. Steroid plasma levels

We next measured how steroid plasma levels were changed by MPTP lesion and by treatment with estrogenic compounds (Figure 5.5). The steroid plasma assay showed that MPTP-treated mice had testosterone, dihydrotestosterone and 3β-diol levels lower than control mice. Only DPN treatment reversed the effect of MPTP on androgen levels; mice treated with DPN showed normal plasma androgens levels and G15 antagonized the effect of DPN. Mice treated with the combination of 17β-estradiol and ICI 182,780 had higher levels of testosterone than mice treated only with 17β-estradiol, and a trend was also observed for dihydrotestosterone ($p=0.09$) and 3β-diol ($p=0.1$). Mice treated with PPT had

dihydrotestosterone and 3β-diol levels not significantly different than control and MPTP mice, and G15 treatment did not antagonize the PPT effect. MPTP treatment did not change plasma progesterone levels. MPTP mice treated with the estrogenic agonists 17β-estradiol, G1 and PPT showed normal levels of progesterone, whereas a decrease was observed with DPN. Mice treated with the combination of PPT and G15 had lower levels of progesterone than PPT treated mice. The use of an estrogenic antagonist (ICI 182,780 or G15, alone or combined with agonist) decreased progesterone levels, except for the group where G15 was combined with DPN, where normal progesterone levels was observed. Only mice receiving 17β-estradiol had higher plasma levels of this steroid than control mice.

5.4. Discussion

Our results show that inhibition of GPER1 with G15 completely opposed the beneficial effect of the ERα agonist PPT on dopaminergic neurons, suggesting collaboration between ERα and GPER1. Protection of dopaminergic neurons observed with the GPER1 agonist G1 was not lost when combined with the ERs antagonist ICI 182,780, suggesting that the neuroprotective effect mediated by GPER1 occurs independently of ERα/β. Investigation of signaling pathways revealed some common pathways between the three estrogen receptors but also highlighted some independent mechanism.

ERα, ERβ and GPER1 have been detected in both the striatum and the substantia nigra. In the striatum, immunoreactivity for ERα, ERβ, and GPER1 are most frequently observed in axons and glial cells, and labeling was found exclusively at extranuclear sites (Almey et al., 2012). Whereas investigation of a colocalization between the three estrogen receptors in the striatum has not been done, the immunoreactive profile for ERα and GPER1 were very similar (Almey et al., 2012). The presence of ERα and GPER1 is 5-fold more abundant than ERβ, and none of the three estrogen receptors has been found on dopaminergic neurons (Almey et al., 2012), suggesting that estrogen neuroprotection occurs by a mechanism involving cross-talk signaling between others neurons or glial cells expressing estrogen receptors, and the dopaminergic neurons.

Our results show that both ERα and GPER1 activation confers protection of DA neurons as potent as 17β-estradiol, suggesting a major role for both receptors in neuroprotection. This is supported by studies reporting that ERα knock-out and G15 treated mice are more susceptible to MPTP toxicity (Bourque et al., 2013; Morissette et al., 2007). Whereas activation of GPER1 with G1 and stimulation of ERα with PPT reproduce 17β-estradiol neuroprotective effects, they do not demonstrate the implication of GPER1 or ERα in mediating 17β-estradiol effect. Thus, we used ICI 182,780 to block binding of 17β-estradiol to ERα/β, and investigated if activation of GPER1 by 17β-estradiol is sufficient to induce neuroprotection of dopaminergic neurons.

The present results showed that the 17β-estradiol effect was blocked with the ERα/β antagonist ICI 182,780. This is consistent with previous results obtained from both ERα and ERβ knock-out mice showing an absence of protection of DA neurons by 17β-estradiol, supporting a role of ERs in neuroprotection (Al-Sweidi et al., 2011; Morissette et al., 2007). Previously, we reported that inhibition of GPER1 with G15 opposed the effect of 17β-estradiol (Bourque et al., 2013). Thus, 17β-estradiol binding at either ERα/β or GPER1 is not sufficient to mediate protection, and both ERs and GPER1 are required for 17β-estradiol protection of dopaminergic neurons in MPTP-treated mice.

The interplay of ER and GPER1 is also reported to occur in a rat model of global cerebral ischemia, where antisense knockdown of either ERα or GPER1, but not ERβ, attenuate 17β-estradiol rapid signaling and neuroprotection (Tang et al., 2014; Zhang et al., 2009). An in vitro study in breast cancer cells investigating the ER and the GPER1 related transcription has shown that the majority of transcripts modified by 17β-estradiol were inhibited by both G15 and ICI 182,780, suggesting a collaboration between ERs and GPER1 (Notas et al., 2012). This study also reports that some transcripts were only inhibited by G15, indicating a specific GPER1 effect (Notas et al., 2012). In support, 17β-estradiol effects on neuritogenesis in hippocampal neurons were not mimics by ERα or ERβ agonists, and not blocked by ICI 182,780, but were rather reproduced by G1 and antagonized by G15 or by a small interfering RNA for GPER1 (Ruiz-Palmero et al., 2013; Ruiz-Palmero et al., 2011). On the other hand, specific action of 17β-estradiol through ERα/β, but not via GPER1, has been reported. In hippocampal slice culture, G1 was reported not to reproduce the protective effect of 17β-estradiol against oxygen–glucose deprivation (Lamprecht and Morrison, 2014). Further, 17β-estradiol action is shown to be blocked by ICI 182,780, but not by G15, supporting that 17β-estradiol acts through ERα/β and that GPER1 activation is neither necessary nor sufficient for 17β-estradiol-mediated neuroprotection (Lamprecht and Morrison, 2014). Thus, 17β-estradiol effect could be produced by a specific GPER1 or ERs action, or by collaboration between these receptors, depending on tissues.

We used estrogenic agonists and antagonists in order to investigate a potential interaction between ERs and GPER1. We found that inhibition of GPER1 with G15

completely opposed the beneficial effect of the ERα agonist PPT, suggesting collaboration between ERα and GPER1. Protection of dopaminergic neurons observed with the GPER1 agonist G1 was not lost when combined with the ERs antagonist ICI 182,780, suggesting that the neuroprotective effect mediated by GPER1 occured independently of ERα/β. To our knowledge, this is the first report showing an interaction between ERα and GPER1 in the brain. An in vitro study in cortical neurons reported that protection by G1 against oxidative insult was not blocked by ICI 182,780, supporting a specific GPER1 effect independent of ERα/β (Liu et al., 2011). The interaction between ERα and GPER1 could be by a physical interaction or by a common related mechanism. Whereas these possibilities have not been investigated in the present study, a physical interaction between ERα and GPER1 has been reported to occur in cancer cells (Vivacqua et al., 2009). 17β-estradiol increases GPER1 co-immunoprecipitation with ERα, an effect prevent by ICI 182,780, suggesting that the interaction between both receptors is ligand dependent (Vivacqua et al., 2009). ERs are known to interact with several signaling molecules, including p85α regulatory subunit of PI3K, receptor tyrosine kinase and G proteins (Marino et al., 2006). In striatal neurons, some of the rapid effects of 17β-estradiol are mediated through the interaction of ERs and metabotropic glutamate receptors (Grove-Strawser et al., 2010). The neuroprotective effects of 17β-estradiol is attenuated by blockage of the insulin-like growth factor-1 receptor in substantia nigra, suggesting that the neuroprotective actions of 17β-estradiol depend on the co-activation of both ERs and insulin-like growth factor-1 receptors (Quesada and Micevych, 2004). A functional and physical interaction between ERα, GPER1 and epidermal growth factor receptor has been described in cancer cells (Vivacqua et al., 2009). Thus, estrogen actions are likely to be complex and involve multiple protein interaction. Whereas we reported an interaction between ERα and GPER1 in protection of dopaminergic neurons, we do not exclude the formation of a large complex of protein including ERα, GPER1 and others proteins, since ERs and insulin-like growth factor-1 receptors, as well as GPER1 and insulin-like growth factor-1 receptors, are reported to cross-talk (Lappano et al., 2013; Quesada and Micevych, 2004).

ERβ activation with DPN failed to protect dopaminergic neurons against MPTP toxicity. This is consistent with previous finding in MPTP mice and in 6-hydroxydopamine lesioned rats, reporting a lack of protection of DA neurons with ERβ agonists (Baraka et al., 2011; D'Astous et al., 2004). Whereas ERβ do not show a protective role on dopaminergic neurons, this receptor mediates many of the neuromodulatory effect of 17β-estradiol on dopaminergic neurons, including modulation of dopaminergic activity and preservation of the phenotype of DA neurons (Sanchez et al., 2010). Thus, it is possible that some effects of ERβ activation resulted from an interplay between ERβ and GPER1. A previous study from our group showed that G1 mimics most of the effect of 17β-estradiol on DA metabolism and that G15 antagonized partially the action of 17β-estradiol (Bourque et al., 2013). We found that the increase in GSK3β phosphorylation and in the levels of BDNF induced by DPN was not blocked by G15, suggesting that these effects were mediated only by ERβ. On the other hand, the action of DPN on androgen synthesis seemed to occur in collaboration with GPER1, since G15 decreased the DPN effect. Further, mice treated with the combination of DPN and G15 had lower levels of DA, DOPAC and HVA than mice receiving only DPN, suggesting an effect through GPER1.

Whereas ICI 182,780 is an antagonist of ERα/β, this compound has also an agonist activity on GPER1 (Thomas et al., 2005). This could explain that in the substantia nigra, under conditions of a small lesion, MPTP mice treated with ICI 182,780 had DAT specific binding at control values. Nevertheless, with moderate loss of DA markers in the striatum, the agonist effect of ICI 182,780 on GPER1 did not induce a protective effect. Moreover, the effects of 17β-estradiol on dopaminergic markers were antagonized by ICI 182,780 and those of G1 were not potentiated by this compound.

Whereas 17β-estradiol, G1 and PPT induced a protective effect on DA neurons, investigation of the signaling pathways underlying this effect revealed common mechanisms but also some independent actions. Akt activation has been implicated in the neuroprotective effect of 17β-estradiol in MPTP mice and 6-hydroxydopamine lesioned rats (D'Astous et al., 2006; Quesada et al., 2008) and was also shown to be activated by G1 in neurons (Tang et al., 2014). The 17β-estradiol effect on Akt phosphorylation was reported to be blocked by

G15 in hippocampal neurons (Tang et al., 2014) and a previous study by our group showed that Akt activation induced by raloxifene in the striatum occured by a mechanism dependent of GPER1 (Bourque et al., 2014). In the present study, we have shown that PPT significantly increased Akt phosphorylation and Bcl-2 levels, an effect partially blocked by G15, whereas the inhibition of GKS3β and the increase in BDNF levels induced by PPT was completely inhibited by G15. Akt could regulate transcriptional activity by activating the cAMP response element binding protein, which regulates the expression of Bcl-2 and BDNF (Parcellier et al., 2008). This suggests that PPT increases Akt phosphorylation, GSK3β inhibition and enhance Bcl-2 and BDNF levels by a mechanism implicating both ERα and GPER1 to protect dopaminergic neurons.

Investigation of the signaling pathways implicated in the G1 effect suggests that the mechanism of neuroprotection seems to occur independently of Akt/GSK3β signaling, in contrast to that observed for PPT. G protein coupled receptors are able to show specific signaling in response to a particular agonist and G protein coupled receptor interaction with other receptors also affect the cellular signaling response (Gonzalez-Maeso, 2011; Kenakin and Christopoulos, 2013). This could explain the absence of a significant increase of GSK3β phosphorylation with G1, and that the effect of PPT on this protein is blocked by G15. The increase in Bcl-2 levels induced by G1 likely does not occur by ER binding to estrogen response element in the coding region of Bcl-2 gene, since inhibition of ERs did not affect the effect of G1, but perhaps by a mechanism implicating mitogen-activated protein kinase signaling (Wang et al., 2011).

On all the markers that we have investigated, the G1 effects were not blocked by ICI 182,780, except for BDNF levels. Whereas protection of dopaminergic neurons by GPER1 stimulation occured independently of ERα/β, some interplay seemed to occur between these receptors. An estrogen response element has been found on BDNF gene (Sohrabji et al., 1995), whereas regulation of BDNF expression has also been described through CREB activation (Yang et al., 2010). Our results suggest that GPER1 requires ERs to mediate transcriptional activity, either by an estrogen response element or by CREB activation, and increase BDNF levels in the striatum. Nevertheless, inhibition of GPER1 blocked the

increase in the levels of BDNF induce by PPT. Furthermore, the 17β-estradiol induced increase in BDNF levels was partially antagonized by ICI 182,780. Taken together, these results suggest that both ERα and GPER1 are required to induce an increase in the levels of BDNF. Thus, transcriptional activity of the BDNF gene seems to depend of the co-activation of both ERα and GPER1.

MPTP has been reported to decrease plasma levels of androgen (Bourque et al., 2014; Ruffoli et al., 2008), as shown in the present study, in consequence of damage to Leydig cells (Ruffoli et al., 2008). We also found that 17β-estradiol, G1 and PPT, but not DPN, treatment reduced plasma androgen levels. Estradiol could modulate testosterone levels by two different mechanisms. First, estradiol could suppress luteinizing hormone secretion, which is responsible for the production of testosterone by Leydig cells (Maeda et al., 2010). Second, estradiol could reduce testosterone production by inhibiting the enzyme 17α-hydroxy/C17,20-lyase (responsible for the conversion of pregnenolone to dehydroepiandrosterone, and progesterone to androstenedione, both are precursors of testosterone) (Nozu et al., 1981). Thus, estradiol exerts an inhibitory action on testosterone biosynthesis that seems to be mediated by ERα and GPER1, whereas ERβ is reported to stimulate testosterone production (Gould et al., 2007; Vaucher et al., 2014). The G1 effect on androgen levels is not the result of detrimental effect on Leydig cells (Vaucher et al., 2014), supporting the contribution of GPER1 in 17β-estradiol effect. This is supported by a study showing that GPER1 activation lowered testosterone levels in rat Leydig cells (Vaucher et al., 2014). Our results show that the levels of testosterone in MPTP mice treated with DPN was reduced when G15 was co-administered, suggesting cross-talk between ERβ and GPER1 in testosterone synthesis. This result is surprising considering the inhibitory role of GPER1 activation on androgen levels. Nevertheless, as the inhibitory role of GPER1 activation on testosterone biosynthesis has been observed in vitro in Leydig cells, this could suggest another way of regulation of androgen production mediated by ERβ in association with GPER1, such as modulation of luteinizing hormone secretion. Our results showed that only mice treated with 17β-estradiol had plasma levels of 17β-estradiol higher than control values, supporting that estrogenic agonists effects are mediate through binding to the corresponding receptor rather than by an increase in 17β-estradiol levels. Brain levels of

progesterone have been shown to be modulated by 17β-estradiol and a role of ERα and GPER1, but not ERβ, was reported in this effect (Kuo et al., 2010). We found a decrease in plasma levels of progesterone as compared to control values when estrogenic antagonists G15 or ICI 182,780 were used, suggesting that inhibition of ERs or GPER1 modulated progesterone synthesis.

In conclusion, our results suggest that ERα interacts with GPER1 to induce a nigrostriatal DA protective effect, whereas the beneficial effect of GPER1 is mediated independently of ERα/β. Moreover, our results suggest that ERα interact with GPER1 to activate striatal Akt signaling and increase Bcl-2 and BDNF levels to protect DA neurons. We showed that the beneficial effect of GPER1 was mediated independently of ERα/β, whereas GPER1 required ERα/β to modulate striatal BDNF levels. A better understanding of the interplay between ERs and GPER1, but also how GPER1 signal occurs with or without an interaction with ERs could permit the development of better therapies for brain diseases.

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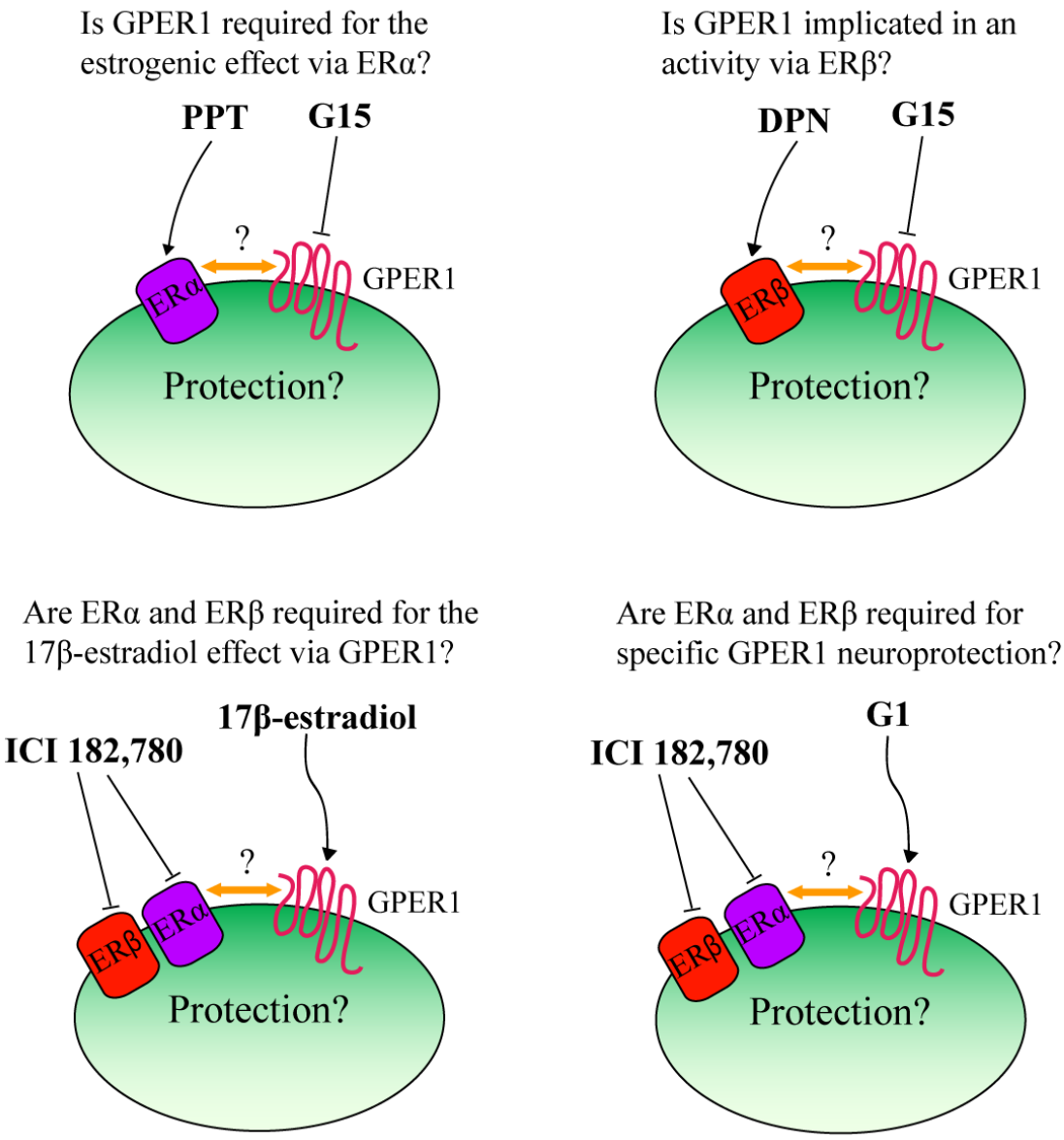

Figure 5.1. Schematic representation of the questions addressed in the present study and the pharmacological approach used to answer them. The objective of this research was to investigate a potential interaction between ERs and GPER1 in neuroprotection of dopaminergic neurons against MPTP toxicity in mice.

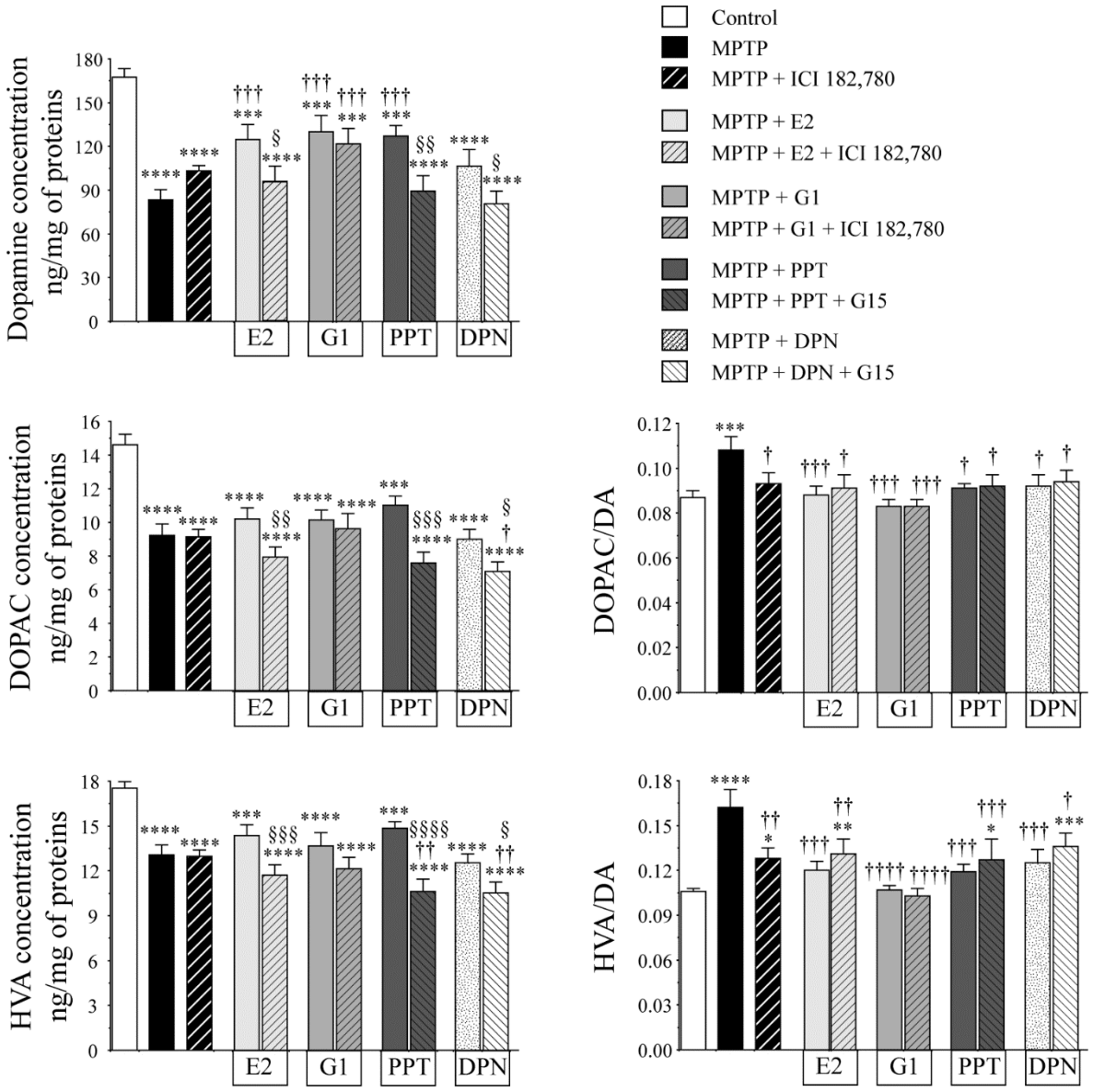

Figure 5.2. Effects of MPTP and estrogenic compounds treatment on striatal dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as DOPAC/DA and HVA/DA ratios. Values shown are the means (ng/mg of proteins) ± S.E.M. of 7-14 mice per group. There was a statistically significant effect of treatment for DA (F[10,84] = 9.61; $p < 0.0001$), DOPAC (F[10,84] = 12.85; $p < 0.0001$), HVA (F[10,84] = 11.63; $p < 0.0001$), DOPAC/DA (F[10,84] = 3.65; $p = 0.0004$) and HVA/DA (F[10,84] = 4.78; $p < 0.0001$). * p < 0.05, ** p < 0.01, *** p < 0.005 and **** p < 0.0001 vs control; † p < 0.05, †† p < 0.01, ††† p < 0.005 and †††† p < 0.0001 vs MPTP; § p < 0.05, §§ p < 0.01, §§§ p < 0.005 and §§§§ p < 0.001 vs corresponding group without G15 or ICI 182,780. E2, 17β-estradiol. The hatched columns of the graphs indicate the presence of an antagonist.

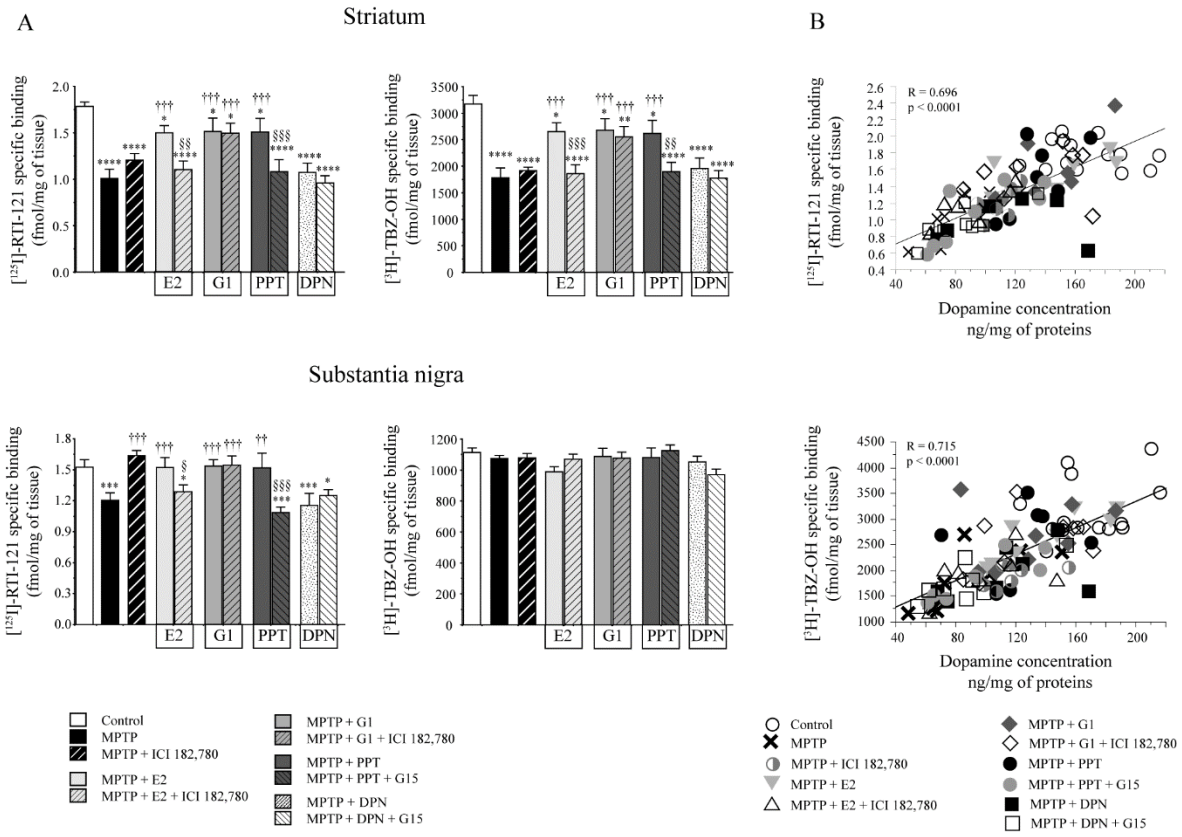

Figure 5.3. Effects of MPTP and estrogenic compounds treatment on [^{125}I]-RTI-121 DAT and [^{3}H]-TBZ-OH VMAT2 specific binding in striatum and substantia nigra. A.Values shown are the means (fmol/mg of tissue) ± S.E.M. of 7-14 mice per group. There was a statistically significant effect of treatment for striatal DAT (F[10,84] = 8.59; $p < 0.0001$), striatal VMAT2 (F[10,83] = 8.46; $p < 0.0001$) and nigral DAT (F[10,89] = 5.38; $p < 0.0001$). * p < 0.05, ** p < 0.01, *** p < 0.005 and **** p < 0.0001 vs control; †† p < 0.01 and ††† p < 0.005 vs MPTP; § p < 0.05, §§ p < 0.01 and §§§ p < 0.005 vs corresponding group without G15 or ICI 182,780. E2, 17β-estradiol. The hatched columns of the graphs indicate the presence of an antagonist. **B.** Correlations between striatal DA concentration and DAT specific binding in striatum, as well as between DA concentration and VMAT2 specific binding in striatum. Each symbol represents an individual mouse.

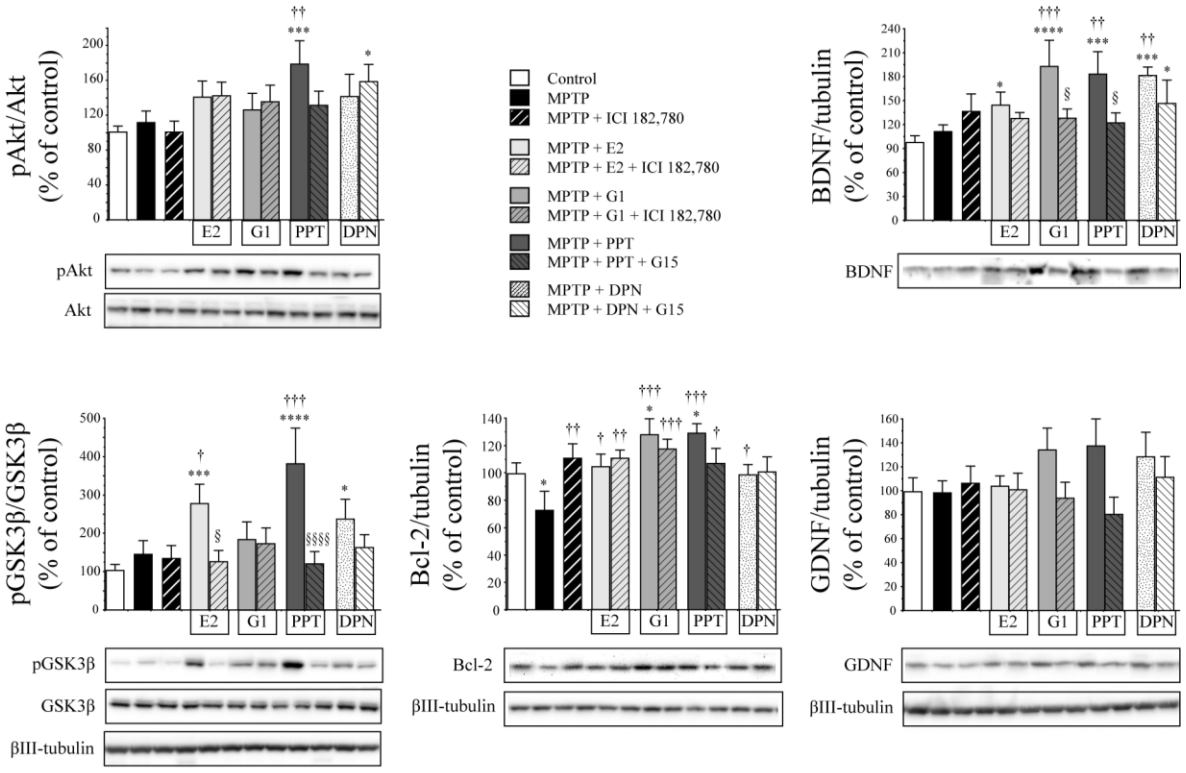

Figure 5.4. Effects of MPTP and estrogenic compounds treatment on striatal pAkt/Akt, pGSK3β/GSK3β, Bcl-2, BDNF and GDNF levels. Values are the means of arbitrary units (expressed as percentage of control) of three independent experiments ± S.E.M. There was a statistically significant effect of treatment for striatal pAkt/Akt (F[10,94] = 1.93; p = 0.04), pGSK3β/GSK3β (F[10,90] = 3.59; p = 0.0005), Bcl-2/tubulin (F[10,77] = 2.38; p = 0.01) and BDNF/tubulin (F[10,83] = 3.25; p = 0.001). * $p < 0.05$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.005$ vs MPTP; § $p < 0.05$ and §§§§ $p < 0.0001$ vs corresponding group without G15 or ICI 182,780. Representative examples of Western blots are shown. E2, 17β-estradiol. The hatched columns of the graphs indicate the presence of an antagonist.

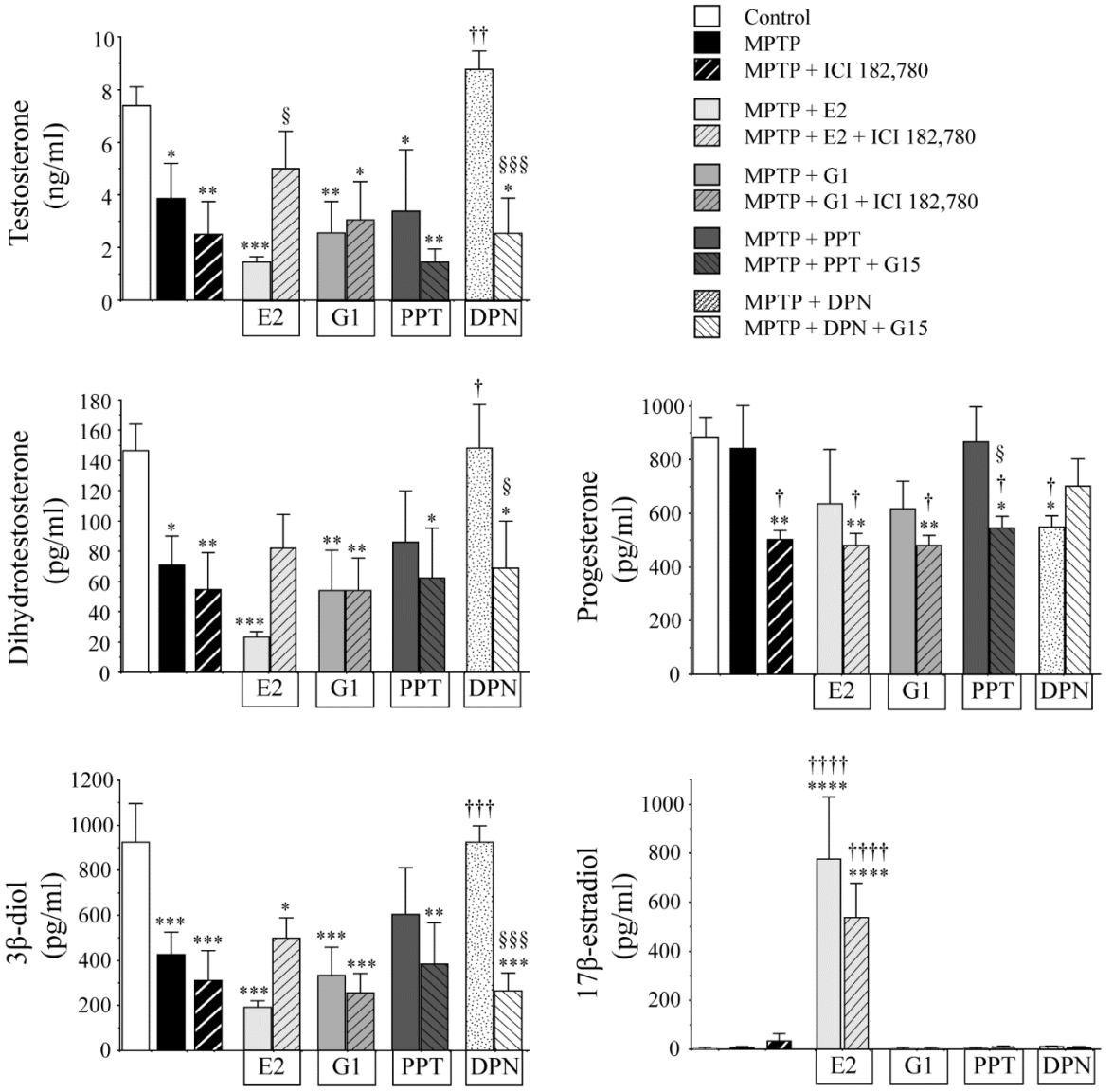

Figure 5.5. Effects of MPTP and estrogenic compounds treatment on plasma levels of testosterone, dihydrotestosterone, 3β-diol, progesterone and 17β-estradiol. Values shown are the means ± S.E.M. There was a statistically significant effect of treatment for plasma levels of testosterone (F[10,31] = 3.12; p = 0.007), dihydrotestosterone (F[10,34] = 2.69; p = 0.01), 3β-diol (F[10,34] = 4.05; p = 0.001), progesterone (F[10,35] = 2.25; p = 0.03) and 17β-estradiol (F[10,34] = 9.50; p < 0.0001). * p < 0.05, ** p < 0.01, *** p < 0.005 and **** p < 0.0001 vs control; † p < 0.05 , †† p < 0.01, ††† p < 0.005 and †††† p < 0.0001 vs MPTP; § p < 0.05 and §§§ p < 0.005 vs corresponding group without G15 or ICI 182,780. E2, 17β-estradiol. The hatched columns of the graphs indicate the presence of an antagonist.

Chapitre 6 : Raloxifene activates G protein-coupled estrogen receptor 1/Akt signaling to protect dopamine neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice

Mélanie Bourque[a,b], Marc Morissette[a], Thérèse Di Paolo[a,b]*

[a]Neuroscience Research Unit, Centre Hospitalier Universitaire de Québec, CHUL, Quebec City, Quebec, G1V 4G2

[b]Faculty of Pharmacy, Laval University, Quebec City, Quebec, G1K 7P4, Canada

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Contribution des auteurs: La conception du projet fut réalisée par la Dr T. Di Paolo. L'organisation du projet a été faite par la Dre T. Di Paolo et M. Bourque. M. Bourque a fait les traitements des animaux, les autoradiographies, les immunobuvardages, l'analyse des résultats et la rédaction du manuscrit. Les analyses statistiques des résultats ont été faites par la Dre T. Di Paolo et M. Bourque. M. Morissette a fait le dosage de la dopamine et de ses métabolites par HPLC. Le manuscrit a été corrigé par la Dr T. Di Paolo et M. Morissette.

Résumé

Le raloxifène, utilisé en clinique, protège les neurones dopaminergiques chez la souris. Une activité du raloxifène par le récepteur des œstrogènes couplé aux protéines G (GPER1) a été rapportée. Nous avons investigué si l'effet neuroprotecteur du raloxifène chez la souris MPTP se fait par le GPER1 en utilisant son antagoniste, soit le G15. Les concentrations striatales de dopamine, de l'acide 3,4-dihydroxyphénylacétique, du rapport de dopamine/acide homovanillique, ainsi que la liaison spécifique au transporteur de la dopamine et au transporteur vésiculaire des monoamines 2 montrent que la neuroprotection des neurones dopaminergiques par le raloxifène est abolie par le G15. La protection par le raloxifène est accompagnée par une activation de la signalisation d'Akt dans le striatum (mais non de la signalisation de ERK1/2) et d'une augmentation des niveaux de Bcl-2 et du BDNF; ces effets sont abolis par le G15. L'effet du raloxifène ne se fait pas par une augmentation des niveaux de 17β-estradiol. Les souris MPTP ont des niveaux plasmatiques réduits de testostérone, de dihydrotestostérone et de 3β-diol; ces effets sont prévenus chez les souris MPTP traitées avec le raloxifène. Nos résultats suggèrent que le raloxifène agit par le GPER1 pour activer Akt, augmenter les niveaux de Bcl-2 et du BDNF, et protéger les neurones dopaminergiques et les niveaux plasmatiques d'androgènes.

Abstract

Raloxifene, used in the clinic, is reported to protect brain dopaminergic neurons in mice. Raloxifene was shown to mediate an effect through the G protein-coupled estrogen receptor 1 (GPER1). We investigated if raloxifene neuroprotective effect in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated male mice is mediated through GPER1 by using its antagonist G15. Striatal concentrations of dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid/dopamine ratio as well as dopamine transporter and vesicular monoamine transporter 2 showed that raloxifene neuroprotection of dopaminergic neurons was blocked by G15. Protection by raloxifene was accompanied by activation of striatal Akt signaling (but not ERK1/2 signaling) and increased Bcl-2 and brain-derived neurotrophic factor levels; these effects were abolished by co-administration with G15. The effect of raloxifene was not mediated through increased levels of 17β-estradiol. MPTP mice had decreased plasma testosterone, dihydrotestosterone and 3β-diol levels; this was prevented in raloxifene–treated MPTP mice. Our results suggest that raloxifene acted through GPER1 to mediate Akt activation, increase Bcl-2 and brain-derived neurotrophic factor levels and protection of dopaminergic neurons and plasma androgens.

Keywords: raloxifene, GPER, MPTP, neuroprotection, Akt, dopamine, striatum

6.1. Introduction

Human studies have shown the beneficial influence of endogenous estrogen exposure and estrogen therapy at menopause on the risk of neurodegenerative disorder such as Parkinson's disease (Liu and Dluzen, 2007). While the use of hormonal therapy shortly after menopause has beneficial effect on brain function (Greendale et al., 2009), there are several limitations associated with the use of estrogen therapy because reproductive organs are targeted, which can promote increase risk of cancer (Taylor and Manson, 2011). Thus, although estrogens are neuroprotective, their peripheral actions currently limit their use for the treatment or prevention of neurodegenerative diseases, hence the importance of finding alternative strategies that mimic the beneficial effects of estrogen but minimize adverse outcomes.

Among alternatives to the use of estrogen are the selective estrogen receptor modulators (SERM), such as raloxifene that is used clinically. Raloxifene displays agonist and antagonist estrogenic response depending of tissues. Thus, raloxifene act as an antagonist on estrogen receptors (ER) in breast and uterus, and shows estrogenic effects on bone, cholesterol metabolism and brain (Dutertre and Smith, 2000; Yaffe et al., 2005). Because raloxifene does not increase risk of cancer in reproductive organs, this SERM could be an interesting alternative option to 17β-estradiol in neuroprotection. The capacity of raloxifene to induce a neuroprotective effect has been show in animal models of Parkinson's disease, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice and 6-hydroxydopamine (6-OHDA) lesioned rats (Baraka et al., 2011; Callier et al., 2001; Grandbois et al., 2000). While raloxifene has a neuroprotective effect, its mechanism of action in neuroprotection of dopamine (DA) neurons has received little attention.

Raloxifene acts by binding the classical ERα and ERβ but has also been reported to mediate an effect through the G protein-coupled estrogen receptor 1 (GPER1) (Abdelhamid et al., 2011). GPER1 is implicated in many estrogen effects in various tissues (Prossnitz and Barton, 2011) but this receptor does not mediate an estrogenic response in reproductive organs (Otto et al., 2009; Otto et al., 2008). GPER1 is expressed in the striatum and the substantia nigra (Almey et al., 2012; Brailoiu et al., 2007) and thus could be involved in the

effect of raloxifene on nigrostriatal dopaminergic neurons. Recently, we reported that GPER1 activation confers protection of dopaminergic neurons, showing the involvement of this receptor in neuroprotection (Bourque et al., 2013). Evidence for a role of GPER1 in raloxifene neuroprotective effect comes from a structure-activity study of raloxifene analogs that found a biphenolic pharmacophore for neuroprotection of cortical neurons against oxygen-glucose deprivation via a GPER1-dependent mechanism (Abdelhamid et al., 2011). Nevertheless, the contribution of GPER1 in the neuroprotective effect of raloxifene on dopaminergic neurons is not known.

In the present study, we evaluated if raloxifene neuroprotection of dopaminergic neurons in MPTP mice is mediated through GPER1 by using the specific antagonist G15. To verify whether the effect of raloxifene is through the molecule per se or by an increase in endogenous steroids, we measured blood plasma levels of testosterone, dihydrotestosterone, 3β-diol, progesterone and 17β-estradiol. To evaluate the damage and protection of dopaminergic neurons, the integrity of DA cell bodies in substantia nigra and terminals in striatum were measured by analysis of DA concentration, dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2). Akt and extracellular signal-regulated kinases (ERK)1/2 signaling, as well as brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), are major targets in cell survival that are implicated in 17β-estradiol neuroprotection (Bourque et al., 2012; Campos et al., 2012; Yang et al., 2010), and were studied in the present experiment to elucidate the mechanism underlying raloxifene protection of dopaminergic neurons.

6.2. Materials and Methods

6.2.1. Animals and Treatment

C57Bl/6 male mice (10 weeks) were purchased from Charles River Canada (Montreal, Qc, Canada). Each group was initially comprised of 8 mice and received treatment with vehicle (0.9% saline with 0.3% gelatin, twice daily, subcutaneous), raloxifene (2.5 mg/kg, twice daily, subcutaneous, Tocris, (Ellisville, MO, USA)) or the combination raloxifene and G15 (10 or 50 µg, twice daily, subcutaneous, Tocris, (Ellisville, MO, USA)) for 10 days. On day 5, mice received four injections of MPTP (4.75 mg/kg i.p., Sigma Chemical (St. Louis, MO)) at 2-h intervals, whereas the control groups received saline solution. On day 11, mice were decapitated, trunk blood was collected, and brains were quickly removed and frozen in isopentane (-40°C). The Laval University Animal Care Committee approved all of the animal studies. All efforts were made to minimize animal suffering and to reduce the number of mice used.

6.2.2. Quantification of steroids in mouse plasma

The plasma levels of testosterone, dihydrotestosterone, 3β-diol, progesterone and 17β-estradiol were measured with gas chromatography mass spectrometry as described (Audet-Walsh et al., 2011).

6.2.3. Brain preparation

A unilateral striatum was used to assay DA and its metabolites concentrations. The striatum of the contralateral hemisphere (bregma 1.18 at 0.14 mm) and the substantia nigra (bregma -2.92 at -3.64 mm) (Franklin and Paxinos, 1997) were cut on a cryostat in 12 µm slices for autoradiography and striatal dissection was performed with 50 µm slices for Western blots. Slices and dissections were kept at – 80 ^{o}C until assayed.

6.2.4. Striatal biogenic amines determination

The left anterior striata were dissected, homogenized in 250 µl of 0.1 N $HClO_4$ at 4 °C and then centrifuged at 10 000 × g for 10 min (4 °C) to precipitate proteins. The concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by high performance liquid chromatography

(HPLC) with electrochemical detection (D'Astous et al., 2004). Supernatants of striatal tissue were directly injected into the chromatograph consisting of a Waters 717 plus autosampler automatic injector, a Waters 515 pump equipped with a C-18 column (Waters Nova-Pak C18, 3 μm, 3.9 mm × 150 cm), a BAS LC-4C electrochemical detector and a glassy carbon electrode. The mobile phase consisted of 0.025 M citric acid, 1.7 mM 1-heptane-sulfonic acid, and 10% methanol, in filtered distilled water, delivered at a flow rate of 0.8 ml/min. The final pH of 4.1 was obtained by addition of NaOH. The electrochemical potential was set at 0.8 V with respect to an Ag/AgCl reference electrode. Results were expressed in nanograms of amine per milligram of protein. Proteins were assayed with a Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL).

6.2.5. Dopamine transporter autoradiography

DAT autoradiography in the striatum and the 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 exposed to Kodak films (Biomax) 18 hours for the striatum and 30 hours for substantia nigra. Films were analyzed using the software NIH Image 1.63.

6.2.6. Vesicular monoamine transporter 2

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.

6.2.7. Western Blots

Striata were homogenized in RIPA lysis buffer (50 mM TRIS-HCl, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.5% SDS, 2 mM EDTA, 1%

phenylmethylsulfonyl fluoride 100 mM) supplemented with protease and phosphatase inhibitors (inhibitor cocktails from Sigma, Oakville, On). Homogenates were kept on ice for 30 minutes to allow solubilization and then centrifuged at 16,000 × g for 15 min. Proteins content of supernatants was measured with a Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL). Proteins were resolved using 12 % SDS-polyacrylamide gel electrophoresis with a triple wide mini-vertical gel system (C.B.S. Scientific Compagny, Del Mar, CA) and electrophoretically transferred to polyvinylidine difluoride (PVDF) membrane. The membranes were blocked with 5 % bovine albumin serum or 5% nonfat dry milk diluted in 0.1 % Tween 20/phosphate-buffered saline and incubated overnight with the primary antibodies. The antibodies (diluted 1:1000) against phosphorylated Akt at serine 473 (pAkt), phosphorylated GSK3β at serine 9 (pGSK3β), Akt, GSK3β, phosphorylated ERK1/2 (pERK1/2), ERK1 and ERK2 were obtained from Cell Signaling Technology (Pickering, On). Bax, Bcl-2, BDNF and GDNF antibodies (diluted 1:1000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). βIII-tubulin antibody (diluted 1:10000) was from Chemicon International (Hornby, On). 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 (KPL LumiGLO Reserve, Gaithersburg, MD, USA). When needed, membranes were stripped using 62.5 mM Tris-HCl, 10 % SDS, 0.5 % β-mercaptoethanol. Densitometric analysis was performed using AlphaView Image Analysis Systems (FluorChem Q Alpha Innotech). The density of each band was normalized to its respective loading control (total proteins or βIII-tubulin). Experiments were repeated three times.

6.2.8. 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's protected least significant difference test. A simple regression model was used to determine the coefficient of correlation. A $p \leq 0.05$ was required for the results to be considered statistically significant.

6.3. Results

6.3.1. G15 opposed raloxifene protective effect against MPTP toxicity on dopaminergic markers

We first evaluated the integrity of dopaminergic neurons following MPTP lesion by measuring DA and its transporters DAT and VMAT2. To investigate whether raloxifene neuroprotection of dopaminergic neurons was mediated through GPER1, we used G15 to antagonize GPER1 and to prevent raloxifene binding to this receptor. Results show that in intact nonlesioned mice, raloxifene treatment had no effect on striatal DA and its metabolites DOPAC and HVA concentrations as well as DOPAC/DA and HVA/DA ratios (Figure 6.1). Administration of MPTP decreased striatal DA concentrations as well as DOPAC and HVA and increased HVA/DA ratio. Treatment with raloxifene decreased MPTP toxicity on DA and DOPAC concentrations and prevented the increase of HVA/DA ratio. Administration of G15 (50 µg) in mice completely abolished the effect of raloxifene on MPTP toxicity for DA and DOPAC concentrations, whereas G15 used at 10 µg did not oppose the raloxifene effect. MPTP mice treated with raloxifene and G15 (50 µg) had a more extensive decrease in HVA concentration than MPTP mice.

Raloxifene treatment had no effect on DAT and VMAT2 specific binding in the striatum of nonlesioned mice (Figure 6.2A). Measures of DAT and VMAT2 specific binding in striatum showed a decrease in MPTP-treated mice as compared to control values. Raloxifene completely prevented this decrease induced by MPTP. G15 (50 µg) treatment in mice totally opposed the effect of raloxifene on MPTP toxicity, whereas this was not observed when 10 µg of G15 was combined with raloxifene. In substantia nigra, no significant difference between groups was observed for measures of DAT and VMAT2 specific binding (Figure 6.2A). Changes observed for striatal DA concentration parallel those found for DAT and VMAT2 specific binding in striatum, as demonstrated by significant positive correlations between these markers (Figure 6.2B).

6.3.2. Raloxifene increased Akt activation and the levels of BDNF and Bcl-2 through GPER1

To explore the mechanism of raloxifene neuroprotection, we examined some of the signaling pathways implicated in 17β-estradiol neuroprotection, including the Akt and ERK1/2 pathways as well as induction of neurotrophic factors. We also investigated how activation of these pathways and modulation of neurotrophic factors by raloxifene depended on GPER1 activation by using G15. Results obtained showed that raloxifene treatment increased phosphorylated Akt levels in the striatum of intact and MPTP-treated male mice (Figure 6.3). The effect of raloxifene in MPTP mice was antagonized with 50 µg of G15 and not at the lower dose of 10 µg. MPTP mice had phosphorylated Akt levels similar to those observed for control mice. Measures of phosphorylation of GSK3β at serine 9 showed no statistically significant changes between groups (Figure 6.3). Striatal Akt and GSK3β levels (non-phosphorylated form) remained unchanged after MPTP lesion or raloxifene treatments. Striatal Bax levels were not changed following MPTP lesion or raloxifene treatment. Intact, nonlesioned, and MPTP mice receiving raloxifene for 10 days presented a significant increase in striatal Bcl-2 levels as compared to control and MPTP mice treated with vehicle. When combined with G15 (50 µg), the augmentation of Bcl-2 levels induced by raloxifene was lost. Raloxifene treatment in intact, nonlesioned mice induced a decrease in the phosphorylated forms of ERK1 and ERK2 as compared to control mice (Figure 6.3). No difference between groups was observed in the levels of non-phosphorylated ERK1/2. Raloxifene increased the levels of striatal BDNF, but not GDNF, in intact and MPTP male mice, an effect blocked by G15 (50 µg) (Figure 6.4). Levels of BDNF and GDNF were not changed following the MPTP lesion.

6.3.3. Raloxifene prevented MPTP induced changes in steroid plasma levels

We next addressed how steroid plasma levels were modulated by MPTP lesion and raloxifene treatment. Measures of steroid plasma levels showed that raloxifene treatment in intact mice induced a decrease in the androgens levels testosterone, dihydrotestosterone and 3β-diol (Figure 6.5). MPTP-treated mice had plasma testosterone, dihydrotestosterone and 3β-diol levels lower than control mice. This decrease in androgens plasma levels induced by MPTP was completely prevented by raloxifene or the combination of raloxifene and G15 (10

μg) treatment. Administration of G15 (50 μg) antagonized the raloxifene effect on plasma testosterone levels, and this group of mice also exhibited a decrease in dihydrotestosterone and 3β-diol levels. No significantly difference between groups was found for plasma 17β-estradiol and progesterone levels.

6.4. Discussion

Our results showed that the neuroprotective effect of raloxifene on dopaminergic neurons was abolished when GPER1 was antagonized with G15 (50 μg), supporting that GPER1 is involved in the neuroprotective actions of raloxifene. Further, we have showed that inhibition of GPER1 blocked Akt activation and the increase of Bcl-2 and BDNF levels induced by raloxifene, suggesting that raloxifene induced activation of signaling pathways and up-regulated neurotrophic factor levels through GPER1 to protect dopaminergic neurons against MPTP toxicity. Thus, our results showed an important role of GPER1 in mediating neuroprotection by raloxifene against MPTP toxicity in mice.

The present results demonstrate the contribution of GPER1 in raloxifene neuroprotective effect against MPTP. G15 has no affinity for either ERα or ERβ (Dennis et al., 2009). Indeed, we used this antagonist to block GPER1, but binding of raloxifene to ERα and ERβ is not compromised. Nevertheless, the neuroprotective effect of raloxifene through ERα and ERβ should not be excluded, as collaboration between GPER1 and ERα/β has been suggested to occur in some tissues (Levin, 2009). In the present study, GPER1 seemed to have a dominant role in mediating raloxifene action because inhibition of this receptor completely abolished raloxifene neuroprotection of dopaminergic neurons and activation of downstream signaling, even in the presence of ERα and ERβ. This is supported by an in vitro study in cortical neurons showing that the raloxifene neuroprotection mechanism is GPER1 dependent and ERα/β independent (Abdelhamid et al., 2011).

We have investigated crucial mediators of 17β-estradiol neuroprotective effect, the Akt and the ERK1/2 signaling pathways, as well as neurotrophic factors, to elucidate the mechanism of action of raloxifene (Bourque et al., 2012; Campos et al., 2012; Yang et al., 2010). Akt is a major contributor of cell survival and targets several proteins including GSK3β and the Bcl-2 family members (Parcellier et al., 2008). Activation of Akt could also regulate transcriptional activity, since this kinase could translocate to the nucleus and activate transcription factors such as cAMP response element-binding protein (CREB), which induces the expression of the anti-apoptotic protein Bcl-2 and the trophic factor BDNF (Parcellier et al., 2008). Previously, our group has shown that activation of the Akt/GSK3β

signaling pathway is implicated in the neuroprotective effect of 17β-estradiol in MPTP mice (D'Astous et al., 2006). In 6-OHDA lesioned rats, 17β-estradiol benzoate neuroprotection of dopaminergic neurons was shown to be dependant of the PI3K/Akt pathway (Quesada et al., 2008). A study using the 6-OHDA mice model of Parkinson's disease revealed that adenovirus associated transduction for the constitutive activated form of Akt confers protection of dopaminergic neurons and that this protection is due to the preservation of the structural integrity of neurons following injury (Cheng et al., 2011; Ries et al., 2006). Akt activation induces pronounced trophic effects on dopaminergic neurons and also produces nigrostriatal axon regeneration (Kim et al., 2011; Ries et al., 2006). Thus, Akt is a crucial mediator of cell survival of dopaminergic neurons and compounds that target this kinase offer a therapeutic opportunity to promote cells survival and to prevent the neurodegenerative process. In our present experiment, we found that the neuroprotective effect of raloxifene implicates activation of Akt as well as up-regulation of Bcl-2 and BDNF proteins. These effects were not observed when raloxifene was administered with G15, suggesting that raloxifene induces Akt phosphorylation and modulation of Bcl-2 and BDNF levels through GPER1. In contrast to 17β-estradiol, raloxifene does not change striatal GSK3β phosphorylation levels (D'Astous et al., 2006). In support of the role of Akt in raloxifene neuroprotection, an in vitro study using cortical neurons demonstrated that the protection induced by raloxifene is abolished in the presence of a PI3K inhibitor (Abdelhamid et al., 2011), showing that Akt is an important mediator of raloxifene beneficial effect.

The function of BDNF and GDNF in the survival, function and maintenance of dopaminergic neurons is well established (Aron and Klein, 2011; Fumagalli et al., 2006). In support, deprivation of BDNF-TrkB or GDNF-Ret signaling provokes nigrostriatal dopaminergic degeneration (Baydyuk et al., 2011; Kramer et al., 2007). Moreover, dopaminergic neurons of the substantia nigra that express TrkB are less sensitive to MPTP toxicity (Ding et al., 2011). BDNF and GDNF have been found to prevent dopaminergic neurodegeneration following 1-methyl-4-phenylpyridinium (MPP+) and MPTP toxicity (Biju et al., 2010; Frim et al., 1994). Neurotrophic factors have received consideration as potential therapeutic agent for Parkinson's disease (Aron and Klein, 2011). Because of the

high challenge of BDNF or GDNF delivery to the brain, one approach to increase BDNF and GDNF levels is through neurotrophic factors-inducing drugs. The regulation of neurotophic factors by 17β-estradiol is well documented in several brain regions. 17β-estradiol increases GDNF levels in both the striatum and substantia nigra and this GDNF up-regulation seems to be essential to the 17β-estradiol neuroprotective effects (Campos et al., 2012). Further, neuroprotection by estrogen against ischemic neuronal injury is reported to be mediated through CREB-BDNF signaling (Yang et al., 2010). To our knowledge, no study has investigated the regulation of BDNF and GDNF by raloxifene. Here, we report that raloxifene increase BDNF, but not GDNF, levels in the striatum of intact and MPTP mice, and that this effect is dependent of GPER1.

ERK1/2 are reported to be rapidly modulated and highly variable temporally (Hetman and Gozdz, 2004; Subramaniam and Unsicker, 2010). The present results did not bring a clear conclusion about the role of ERK1/2 in the neuroprotective effect of raloxifene. We report a decrease activation of ERK1 and ERK2 after 10 days of raloxifene treatment in intact mice and a non-significant reduction is also observed in MPTP mice receiving raloxifene. Similarly, a diminution in ERK phosphorylation occurs in the substantia nigra of rats that received 17β-estradiol for 7 days, but the authors reported that inhibition of ERK activity did not block the survival effects of 17β-estradiol on dopaminergic neurons following 6-OHDA lesion, suggesting that protection by 17β-estradiol is independent of ERK pathway (Quesada et al., 2008).

The site of action of raloxifene or 17β-estradiol in the nigrostriatal system is not completely understood. The localisation of estrogen receptors in the striatum and the substantia nigra pars compacta provides information on the site of action of raloxifene or 17β-estradiol. A recent study showed by electron microscopy that, in the striatum, ERα, ERβ and GPER1 are not expressed in dopaminergic neurons (Almey et al., 2012), suggesting that estrogenic compounds act on estrogen receptors on other neurons or glial cells in this brain region. These authors found that between 22 and 27% of ERα, ERβ and GPER1 immunoreactivity was observed in glia cells in the striatum. The importance of glia cells in neuroprotection against MPP+ comes from an in vitro study showing that removal of glia

from the cultures abolished 17β-estradiol neuroprotection of dopaminergic neurons (Bains et al., 2007). These results suggest that the neuroprotective mechanism of 17β-estradiol requires signal cross-talk between neurons and glial cells. Furthermore, ERα, ERβ and GPER1 have been detected in the substantia nigra (Brailoiu et al., 2007; Mitra et al., 2003). ERβ and tyrosine hydroxylase positive substantia nigra *pars compacta* neurons were found to project to the striatum (Creutz and Kritzer, 2004). Thus, it is also possible that the protection could occur by a direct mechanism of estrogenic compounds on dopaminergic neurons.

The neuromodulatory effect of 17β-estradiol on nigrostriatal DA activity is well documented (Sanchez et al., 2010). Our present results obtained with intact mice showed that raloxifene does not reproduce 17β-estradiol effect on DA metabolism (Bourque et al., 2013). Further, the absence on any change on DA and its metabolites concentrations, as well as DAT and VMAT2 specific binding with raloxifene treatment in intact mice support the protection of dopaminergic neurons observed in MPTP mice receiving raloxifene. Thus, the dopaminergic changes observed in raloxifene-treated MPTP mice involve neuroprotection rather than only activation of DA synthesis. This is also supported by the positive correlations obtained between striatal DAT or VMAT2 specific binding and striatal DA concentrations of all ours experimental groups.

Our results show a decrease in plasma testosterone levels in intact male mice treated with raloxifene as previously reported (Neubauer et al., 1993). Raloxifene is reported to have no effect on 5α-reductase or 17α-hydroxylase activities, does not bind to the androgen receptor as well as does not change luteinizing hormone levels in male rodents (Neubauer et al., 1993), suggesting that raloxifene does not inhibit testicular androgen biosynthesis in rodents. Nevertheless, the mechanism by which raloxifene decreases the levels of androgen in rodents is not well understood, and could perhaps be an agonist estrogenic effect on androgen metabolic clearance (Lee et al., 1975). In healthy elderly men, raloxifene is reported to increase luteinizing hormone, testosterone and 17β-estradiol levels (Birzniece et al., 2010; Birzniece et al., 2012; Duschek et al., 2004), suggesting that raloxifene has an antagonist estrogenic effect on steroid biosynthesis. Thus, raloxifene seems to have a different effect on steroids biosynthesis depending of species. Our results also show that raloxifene

administration in male mice does not increase plasma levels of 17β-estradiol, or modify progesterone levels, which is a major precursor of steroids and neuroactive steroids in rodents, showing that raloxifene action is not mediated through an increase levels of 17β-estradiol but rather by binding to its receptors.

A higher prevalence of low plasma testosterone levels is reported in Parkinson's disease patients as compared with age-matched control subjects (Okun et al., 2002). Similarly, we found decreased plasma testosterone levels as well as reduced levels of its metabolites dihydrotestosterone and 3β-diol. This decrease in testosterone levels induced by MPTP likely occurs in response to damage to Leydig cells (Ruffoli et al., 2008). Raloxifene treatment in MPTP mice completely prevented the decrease in testosterone, dihydrotestosterone and 3β-diol, suggesting that raloxifene has not only a beneficial effect on dopaminergic neurons, but also acts in the periphery to protect against damage induced by MPTP. The effect of raloxifene on testosterone levels was opposed by G15 (50 µg) and GPER1 immunoreactivity has been detected in Leydig cells (Rago et al., 2011) suggesting a mechanism of raloxifene action to prevent gonadal steroid biosynthesis modulation induced by a lesion. We observed a different effect of raloxifene in intact compared to MPTP mice. Hence, the intact and the MPTP mice seem to differ in their capacity to produce steroids, as reported in the present results. Damage to Leydig cells occurs following MPTP (Ruffoli et al., 2008) and the activity of the steroidogenesis enzymes could also be modified by exposure to reactive oxygen species (Allen et al., 2004; Lee et al., 2009), which are induced following MPTP lesion. Thus, the effect observed with raloxifene in MPTP mice could reflect the preservation of steroid biosynthesis. Neuroactive steroids are important modulators of brain function and are involved in different roles such as neuromodulation, in the maintenance and neuroprotection of dopaminergic systems (Sanchez et al., 2010; Zheng, 2009). Thus, in MPTP mice, raloxifene preserves normal levels of neuroactive steroids, which could contribute to maintain the integrity of the dopaminergic system.

Raloxifene and 17β-estradiol show a neuroprotective effect in both male and female rodents against toxins damaging the nigrostriatal dopaminergic system (Baraka et al., 2011; Callier et al., 2001; Dluzen et al., 1996). Whereas ERα, ERβ and GPER1 have been detected

in the striatum, the presence of ERα and GPER1 is predominant as compared to ERβ (Almey et al., 2012). Female mice were reported to have higher levels of striatal ERα than male mice (Rodriguez-Navarro et al., 2008), but no sex difference was observed for GPER1 (Bourque et al., 2011). Alteration of the nigrostriatal dopaminergic system of non lesioned males and females is observed in ERα and ERβ knockout mice (Al-Sweidi et al., 2011; Kuppers et al., 2008; Morissette et al., 2007), showing the role of ER in the function of the dopaminergic system in both male and female mice. Thus, the importance of ER in the nigrostriatal system in both female and male mice offers a neuroprotective target. Neuroprotection with estrogenic compounds represent an interesting opportunity that could be used in both females and males and a better understanding of the role of the estrogen receptors in mediating the neuroprotective effects is crucial to provide selective agents that are neuroprotective but that lack feminizing effects.

The search for alternative compounds as potent as 17β-estradiol against brain injury that could be used in both men and women, but without adverse effect, is of great interest. Raloxifene has been reported to display beneficial effects on the brain of both men and women. Postmenopausal women were shown to be responsive to raloxifene displaying a lower risk of cognitive impairment (Yaffe et al., 2005). Improvement of verbal memory was reported in postmenopausal women receiving raloxifene (Jacobsen et al., 2012; Jacobsen et al., 2010). Furthermore, raloxifene treatment in elderly men was reported to enhance brain activation during encoding of new information and during recognition of familiar items, without side effect, suggesting a beneficial effect on attention, memory and executive function (Goekoop et al., 2006; Goekoop et al., 2005). Raloxifene is an interesting compound because of its antagonist estrogenic effect on reproductive organs, with the result that this SERM does not increase the risk of cancer (Dutertre and Smith, 2000). While estrogen therapy in men is not appropriate, raloxifene represents an interesting option because of its non-feminizing effect. A critical outcome of the present study is the finding that raloxifene neuroprotection is mediated through GPER1, a non-feminizing receptor. We have recently shown that targeting the GPER1 with its specific agonist G1 induced a neuroprotective effect as potent as 17β-estradiol against MPTP, and that 17β-estradiol protection of DA neurons

requires the GPER1 (Bourque et al., 2013). The neuroprotective effect of GPER1 activation has also been reported in an ischemia model of rats and in an in vitro study (Gingerich et al., 2010; Lebesgue et al., 2010). Our present results support the consideration of targeting the GPER1 to prevent neurodegenerative diseases and maintain cognitive function. Furthermore, considering the significant emerging role of GPER1 in neuromodulation and protection against brain damage, neuroprotective effects that are mediated through the GPER1 should be taken into consideration for the development of new SERMs.

In conclusion, this study reported that GPER1 is implicated in the neuroprotective effect of raloxifene on dopaminergic neurons in MPTP mice. Our results show that raloxifene acted through GPER1 to mediate Akt activation, increased the levels of Bcl-2 and BDNF, and protected dopaminergic neurons. This study highlights a new mechanism of raloxifene neuroprotection in MPTP mice and provides support for the consideration of GPER1 in the development of therapeutic neuroprotective agents.

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6.6. References

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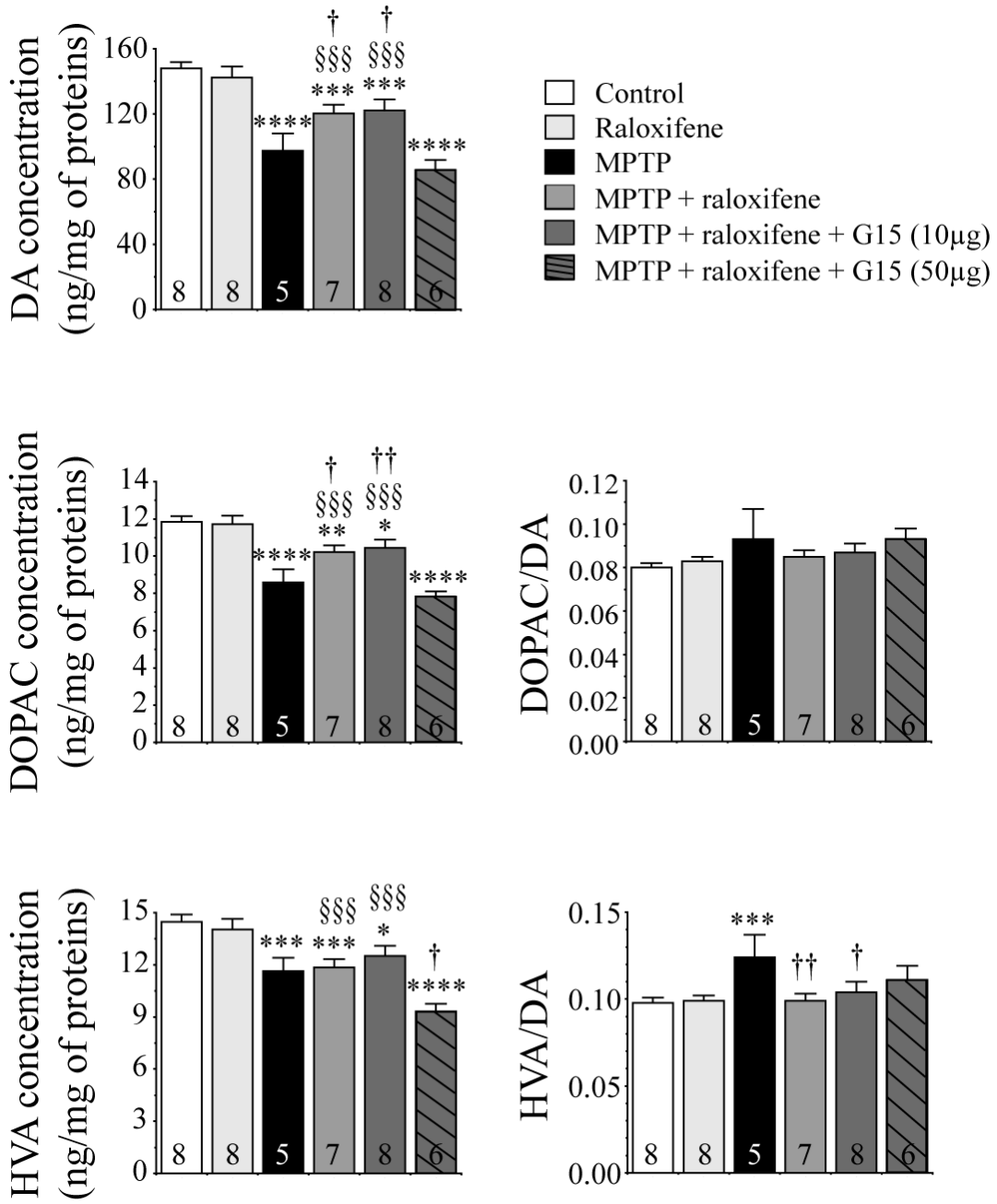

Figure 6.1. Effects of MPTP, raloxifene and G15 treatment on striatal dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as DOPAC/DA and HVA/DA ratios. Values shown are the means (ng/mg of proteins) ± S.E.M. of 5-8 mice per group (number of mice in each group is included in each column of the graphs). There was a statistically significant effect of treatments for DA ($F(5,36) = 13.25$; $p < 0.0001$), DOPAC ($F(5,36) = 13.79$; $p < 0.0001$), HVA ($F(5,36) = 10.89$; $p < 0.0001$), and HVA/DA ratio ($F(5,36) = 2.62$; $p < 0.0001$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$ and †† $p < 0.01$ vs MPTP; §§§ $p < 0.005$ vs MPTP + raloxifene + G15 (50 µg).

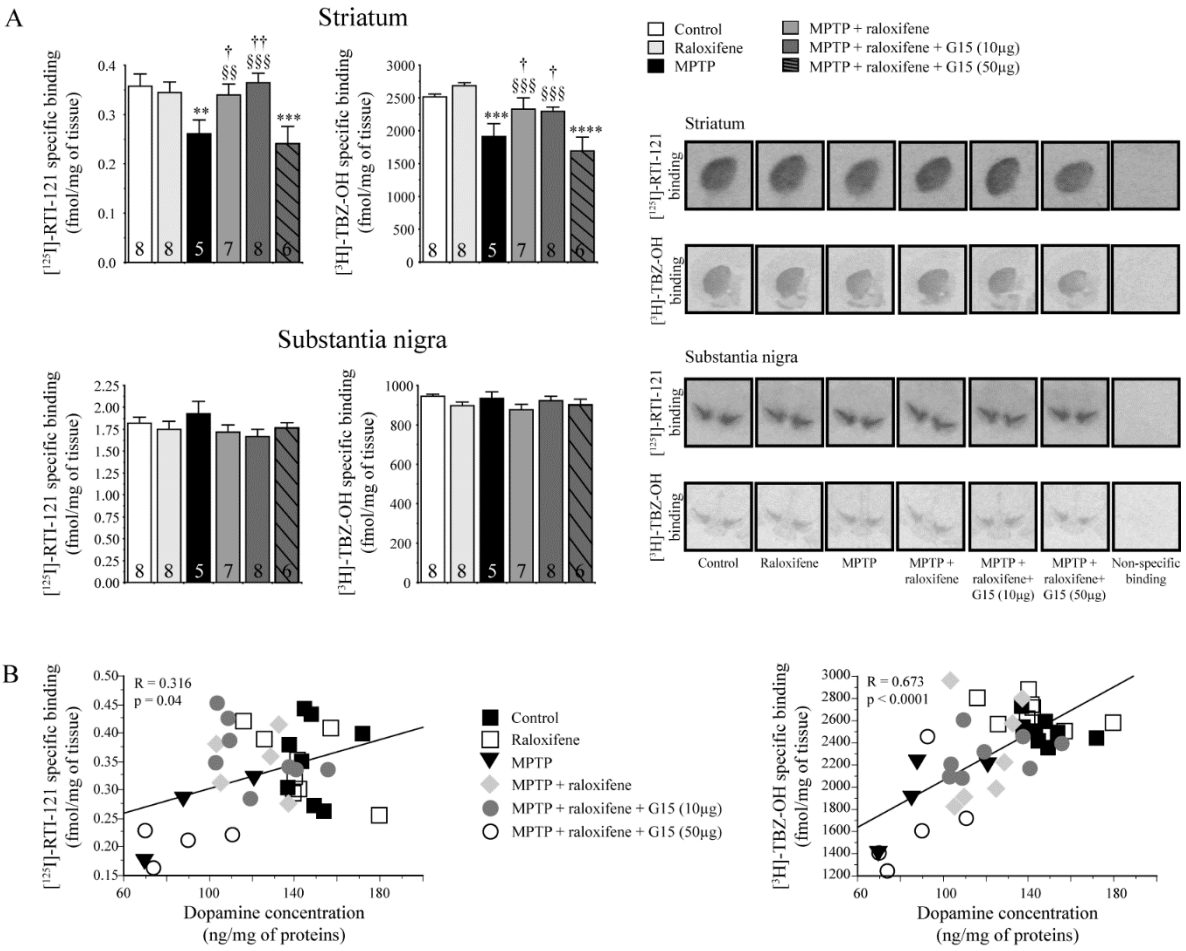

Figure 6.2. A. Effects of MPTP, raloxifene and G15 treatment on [^{125}I]-RTI-121 DAT and [^{3}H]-TBZ-OH VMAT2 specific binding in striatum and substantia nigra and examples of these binding autoradiography. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 5-8 mice per group (number of mice in each group is included in each column of the graphs). There was a statistically significant effect of treatments for striatal DAT ($F(5,34) = 4.13$; $p = 0.004$) and VMAT2 ($F(5,34) = 8.99$; $p < 0.0001$). ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$ and †† $p < 0.01$ vs MPTP; §§ $p < 0.01$ and §§§ $p < 0.005$ vs MPTP + raloxifene + G15 (50 µg).B. Correlations between striatal DA concentration and DAT specific binding in striatum, as well as between DA concentration and VMAT2 specific binding in striatum. Each symbol represents an individual mouse.

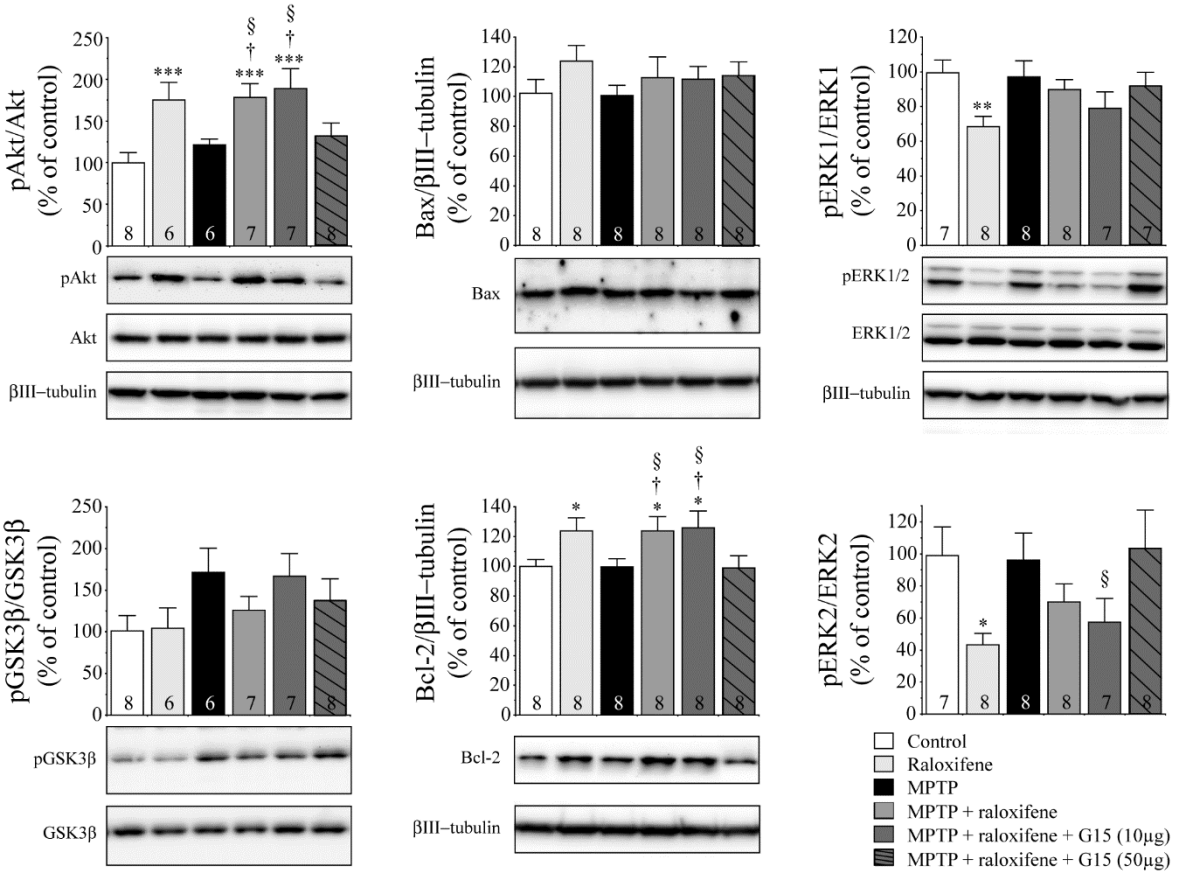

Figure 6.3. Effects of MPTP, raloxifene and G15 treatment on striatal pAkt/Akt, pGSK3β/GSK3β, pERK1/ERK1, pERK2/ERK2, Bax and Bcl-2 levels. Values are the means of arbitrary units (expressed as percentage of control) of three independent experiments ± S.E.M. of 6-8 mice per group (number of mice in each group is included in each column of the graphs). There was a statistically significant effect of treatments for striatal pAkt/Akt ($F(5,36) = 4.73$; $p = 0.002$), pERK1/ERK1 ($F(5,39) = 2.36$; $p = 0.05$), pERK2/ERK2 ($F(5,40) = 2.38$; $p = 0.05$) and Bcl-2 levels ($F(5,42) = 2.7$; $p = 0.03$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.005$ vs control; † $p < 0.05$ vs MPTP; § $p < 0.05$ vs MPTP + raloxifene + G15 (50 μg). Representative examples of Western blots are shown.

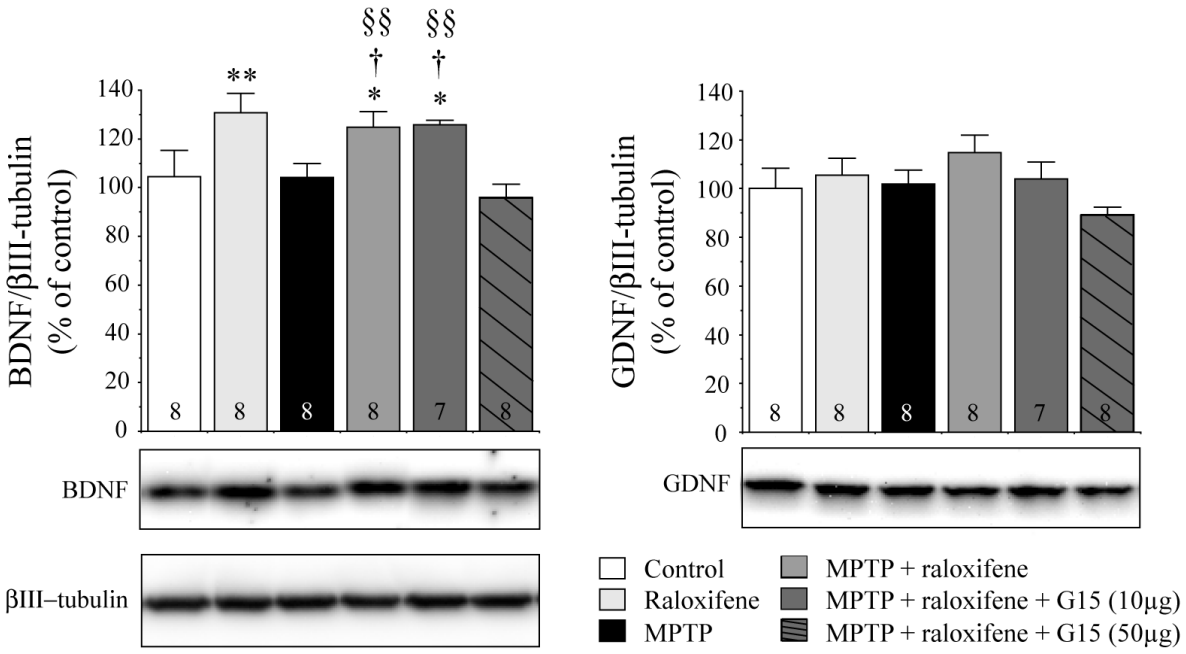

Figure 6.4. Effects of MPTP, raloxifene and G15 treatment on striatal BDNF and GDNF levels. Values are the means of arbitrary units (expressed as percentage of control) of three independent experiments ± S.E.M. of 7-8 mice per group (number of mice in each group is included in each column of the graphs). There was a statistically significant effect of treatments for striatal BDNF levels ($F(5,41) = 4.15$; $p = 0.003$). * $p < 0.05$ and ** $p < 0.01$ vs control; † $p < 0.05$ vs MPTP; §§ $p < 0.01$ vs MPTP + raloxifene + G15 (50 µg). Representative examples of Western blots are shown.

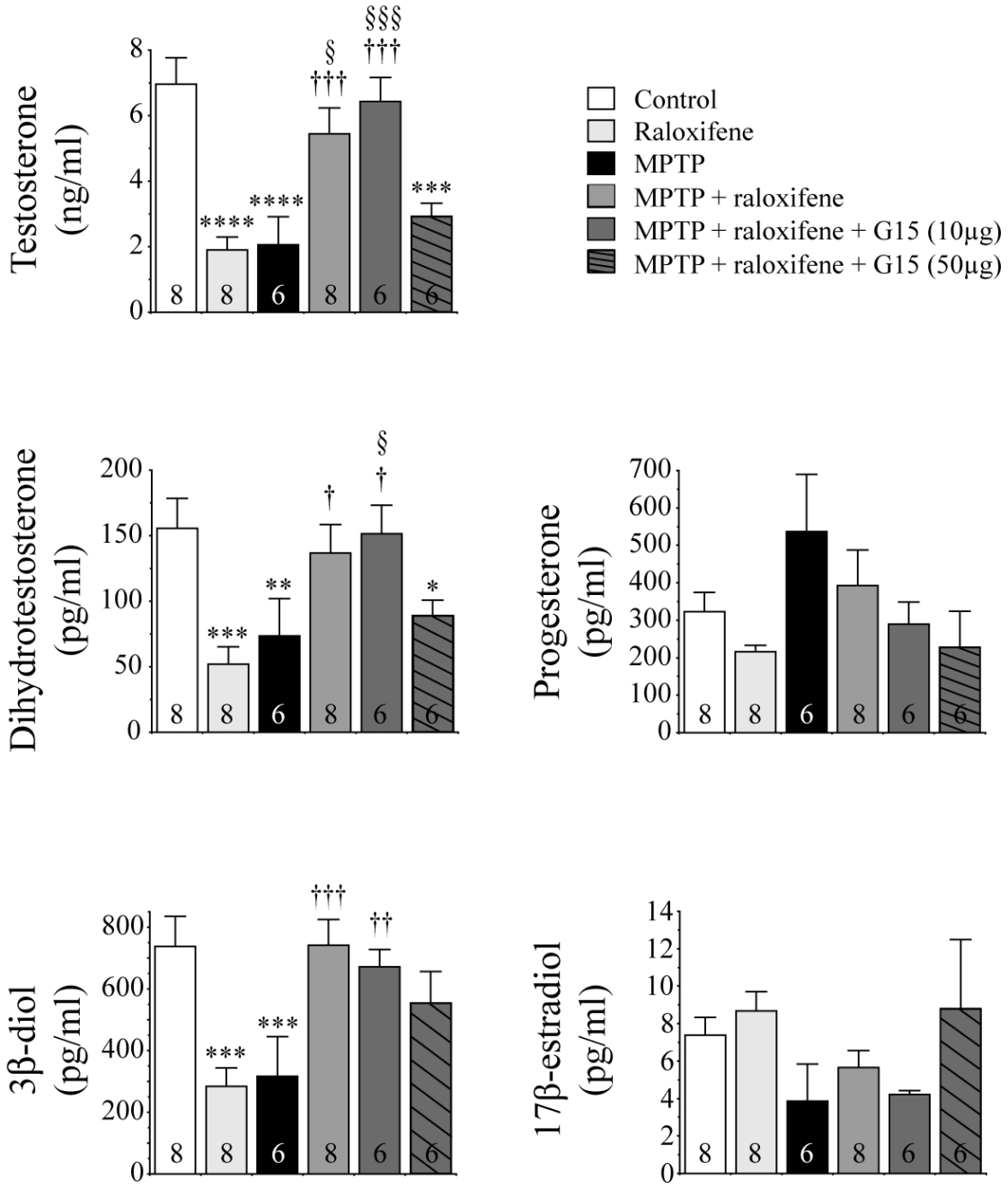

Figure 6.5. Effects of MPTP, raloxifene and G15 treatment on plasma levels of testosterone, dihydrotestosterone, 3β-diol, progesterone and 17β-estradiol. Values shown are the means ± S.E.M (number of mice in each group is included in each column of the graphs). There was a statistically significant effect of treatments for plasma levels of testosterone ($F(5,36) = 10.58$; $p < 0.0001$), dihydrotestosterone ($F(5,36) = 4.7$; $p = 0.002$) and 3β-diol ($F(5,36) = 5.51$; $p = 0.007$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.005$ vs MPTP; § $p < 0.05$ and §§§ $p < 0.005$ vs MPTP + raloxifene + G15 (50 µg).

Chapitre 7: Neuroprotective and rescue effect of progesterone in MPTP-treated male mice

Mélanie Bourque[a,b], Marc Morissette[a], Sara Al Sweidi[a,b], Donatella Caruso[c], Roberto C. Melcangi[c], Thérèse Di Paolo[a,b,*]

[a]Neuroscience Research Unit, Centre Hospitalier Universitaire de Québec, CHUL, Quebec City, Quebec, G1V 4G2

[b]Faculty of Pharmacy, Laval University, Quebec City, Quebec, G1K 7P4, Canada

[c]Department of Pharmacological and Biomolecular Sciences, Center of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano, 20133 Milano, Italy

Projet en cours

Contribution des auteurs : La conception du projet fut réalisée par la Dre T. Di Paolo. L'organisation du projet a été faite par le Dre Di Paolo et M. Bourque. Les traitements des animaux ont été faits par M. Bourque et S. Al-Sweidi. M. Bourque a fait les autoradiographies, l'analyse des résultats et la rédaction du manuscrit. Les analyses statistiques des résultats ont été faites par la Dre T. Di Paolo et M. Bourque. M. Morissette a fait le dosage de la dopamine et de la sérotonine, ainsi que leurs métabolites, par HPLC. Le manuscrit a été corrigé par la Dre T. Di Paolo et M. Morissette.

Résumé

Cette présente étude investigue l'effet neuroprotection d'une faible dose de progestérone chez de jeunes souris mâle C57Bl/6 traitées avec le MPTP, et de le comparer à l'effet de la progestérone chez des souris intactes, non-lésées. Nous avons aussi investigué si l'administration de progestérone à de faible (1 µg) et fortes doses (8 et 16 mg/kg) peut récupérer les neurones dopaminergiques ayant déjà été exposé au MPTP. Pour l'expérience de neuroprotection, nos résultats montrent que la progestérone prévient complètement la toxicité du MPTP sur les concentrations de dopamine, sur l'augmentation du rapport de 3-méthoxytyramine/dopamine, ainsi que sur la liaison spécifique au VMAT2 dans le striatum et la substance noire. La progestérone diminue l'effet du MPTP sur les concentrations de DOPAC et sur la liaison spécifique au DAT dans la partie latérale du striatum antérieur, et dans le striatum médian (les parties médiale et latérale). Le traitement avec la progestérone chez les souris intactes n'a pas d'effet sur les marqueurs investigués. Pour l'expérience de neurorécupération, les mesures des concentrations de dopamine et de ses métabolites, ainsi que de la liaison spécifique au DAT et au VMAT2 dans le striatum, montre que la dose de 8 mg/kg de progestérone est la plus efficace à réduire l'effet du MPTP. Des effets plus limités sont observés avec les doses de 1 µg et 16 mg/kg de progestérone. Dans la substance noire, un effet protecteur est observé chez les souris traitées avec 1 µg de progestérone. L'analyse de plusieurs stéroïdes révèle que les changements dans les concentrations plasmatiques ne reflètent pas toujours les changements dans les niveaux cérébraux. Nos résultats montrent que la progestérone possède des propriétés neuroprotectrice et de neurorécupération des neurones dopaminergiques chez les souris mâles MPTP.

Abstract

The present study sought to investigate neuroprotection with a low dose of progesterone (1 µg) in young C57Bl/6 MPTP male mice and compare them to the effect of this steroid in intact mice. We also investigated if progesterone administered at low (1µg) and high (8 and 16 mg/kg) doses could rescue dopaminergic neurons already exposed to MPTP. For neuroprotection, our results showed that progesterone prevented completely MPTP toxicity on dopamine concentrations, on the increase of 3-methoxytyramine/dopamine ratio as well as VMAT2 specific binding in the striatum and the substantia nigra. Progesterone decreased MPTP effects on DOPAC concentration and DAT specific binding in the lateral part of the anterior striatum and in the middle striatum (medial and lateral parts). Progesterone treatment in intact mice had no effect on the markers investigated. For the rescue experiment, measures of dopamine and its metabolites concentrations, as well as DAT and VMAT2 specific binding in striatum showed that 8 mg/kg of progesterone was the most effective dose to reduce MPTP effects, and more limited effects were observed with 1 µg and 16 mg/kg. In the substantia nigra, a protective effect was observed in mice treated with 1µg of progesterone. Measures of several steroids in both plasma and brain reveal that change in plasma concentration do not always reflect change in brain steroid levels. We found that progesterone treatment at all doses increase levels of brain progesterone and tetrahydroprogesterone. Our result showed that progesterone has neuroprotective and neurorescue effects on dopaminergic neurons in MPTP male mice.

Keywords: neuroprotection, neurorescue, progesterone, MPTP, dopamine

7.1. Introduction

Parkinson's disease is the second most common neurodegenerative disorder and is likely to increase due to the aging population. The major pathology in Parkinson's disease is the selective and progressive death of dopamine (DA) cell bodies in substantia nigra. There is presently no cure for Parkinson's disease and the most common treatments (levodopa and DA agonists) improve motor symptoms without preventing or delaying the degenerative process (Olanow et al., 2009). Nevertheless, as the disease progresses, these therapies eventually lose effectiveness for many patients and major motor complications such as dyskinesia often appear (Olanow et al., 2009). Therefore, neuroprotective compounds that could prevent the loss of DA neurons or reverse the progression of Parkinson's disease are critically needed.

Several studies have reported that men are at greater risk of developing PD than women (Van Den Eeden et al., 2003; Wooten et al., 2004; Shulman, 2007), suggesting that ovarian hormone have a beneficial influence on the risk to develop Parkinson's disease. Animal models supported the neuroprotective effect of 17β-estradiol against the damage to the nigrostriatal neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice and by lesion with 6-hydroxydopamine (6-OHDA) in rats (Bourque et al., 2009; Baraka et al., 2011). The beneficial effect of progesterone has been less studied than that of 17β-estradiol, but several animals and clinical studies have shown that progesterone shows beneficial effect against brain damage. Progesterone has been reported to have protective effects on the dopaminergic terminals in mice expose to MPTP or methamphetamine (Grandbois et al., 2000; Yu and Liao, 2000; Callier et al., 2001). Moreover, studies have highlighted that progesterone has a beneficial effect when administered following traumatic brain injury, both in animal models and in human (Wright et al., 2007; Xiao et al., 2008; Ishrat et al., 2012), showing that progesterone has rescue capacity. There is presently no therapy (drug that slows, stops, or reverses disease progression) recognized to have a neuroprotective effect in Parkinson's disease patients (Olanow and Schapira, 2013; AlDakheel et al., 2014), and base on those human studies (Wright et al., 2007; Xiao et al., 2008), progesterone could be a good candidate to delay the progression or rescue neurons in Parkinson's disease patients.

The present study sought to replicate and extend our previous findings concerning the neuroprotective effect of a low dose of progesterone (1 µg) (Grandbois et al., 2000; Callier et al., 2001). First, given that progesterone is a neuromodulator (Sanchez et al., 2010), we have differentiated the neuroprotective versus neuromodulatory effect of progesterone on the DA system, by investigating and comparing the effect of progesterone in intact, non-lesioned, and in MPTP-treated mice with measures of DA, DA transporter (DAT) and vesicular monoamine transporter 2 (VMAT2). In a second experiment, we investigated if progesterone administered at low (1µg) and higher (8 and 16 mg/kg) doses has the capacity to rescue dopaminergic neurons that have been exposed to MPTP in male mice. Furthermore, we have measured how plasma and brain levels of several neurosteroids are modulated following MPTP lesion and treatment with progesterone.

7.2. Materials and methods

7.2.1. Animals and Treatment

C57Bl/6 male mice (10 weeks) were purchased from Charles River Canada (Montreal, Qc, Canada). MPTP and progesterone were purchased from Sigma Chemical (St. Louis, MO, USA). The Laval University Animal Care Committee approved all of the animal studies. All efforts were made to minimize animal suffering and to reduce the number of mice used.

7.2.2. Neuroprotection experiment

Each group of mice received treatment with progesterone (1 µg, B.I.D., sc) or vehicle (0.9% saline with 0.3% gelatin, B.I.D., sc) for 10 days. On day 5, mice received four intraperitoneal injections of MPTP (11 mg/kg) at 2-h intervals, whereas the control groups received saline solution. On day 11, mice were decapitated, and brains were quickly removed and frozen in isopentane (-40°C).

7.2.3. Neurorescue experiment

Mice received four intraperitoneal injections of MPTP (6.5 mg/kg) at 2-h intervals, whereas the control groups received saline solution. One hour after the first MPTP injection, mice received one intraperitoneal injection of progesterone (1µg, 8 mg/kg or 16 mg/kg). One hour after the last injection of MPTP, mice received one subcutaneous injection with progesterone (1µg, 8 mg/kg or 16 mg/kg). During the next five days, mice received one (8 mg/kg or 16 mg/kg) or twice (1µg) daily subcutaneous injection with progesterone. On day 6, mice were decapitated, and brains were quickly removed and frozen in isopentane (-40°C).

7.2.4. Brain preparation

A unilateral striatum was used to assay DA and its metabolites concentrations. The striatum anterior (bregma 1.54 to 1.18), middle (bregma 0.50 to 0.14) and posterior (bregma –0.34 to -0.70 mm) and the substantia nigra (bregma -2.80 to -3.88 mm) (Franklin and Paxinos, 1997) of the contralateral hemisphere were cut on a cryostat in 12 µm slices. Slices were maintained at -80 °C until assay.

7.2.5. Striatal biogenic amines determination

The left anterior striata were dissected, homogenized in 250 µl of 0.1 N $HClO_4$ at 4 °C and then centrifuged at 10 000 × g for 10 min (4 °C) to precipitate proteins. The concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA), as well as serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), were measured by high performance liquid chromatography (HPLC) with electrochemical detection. Supernatants of striatal tissue were directly injected into the chromatograph consisting of a Waters 717 plus autosampler automatic injector, a Waters 515 pump equipped with a C-18 column (Waters Nova-Pak C18, 3 µm, 3.9 mm × 150 cm), a BAS LC-4C electrochemical detector and a glassy carbon electrode. The mobile phase consisted of 0.025 M citric acid, 1.7 mM 1-heptane-sulfonic acid, and 10% methanol, in filtered distilled water, delivered at a flow rate of 0.8 ml/min. The final pH of 4.1 was obtained by addition of NaOH. The electrochemical potential was set at 0.8 V with respect to an Ag/AgCl reference electrode. Results were expressed in nanograms of amine per milligram of protein. Proteins were assayed with a Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL).

7.2.6. Dopamine transporter autoradiography

DAT autoradiography in the striatum and the 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) 18 hours for the striatum and 30 hours for substantia nigra. Films were analyzed using the software NIH Image 1.63.

7.2.7. Vesicular monoamine transporter 2

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.

7.2.8. Quantification of steroids

7.2.8.1. Quantification of steroids in mouse plasma of neuroprotection experiment

The plasma levels of testosterone, dihydrotestosterone, 3β-diol and progesterone were measured with gas chromatography mass spectrometry as described (Audet-Walsh et al., 2011). Plasma of 2 or 3 mice from the same group were pooled in order to have enough plasma to quantify the above steroids.

7.2.8.2. Quantification of steroids in mouse plasma and brain of neurorescue experiment

Quantitative analysis of steroids (pregnenolone, progesterone, dihydroprogesterone, isopregnanolone, tetrahydroprogesterone, dehydroepiandrosterone (DHEA), testosterone, dihydrotestosterone, 3α-diol and 3β-diol) by liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed as reported (Caruso et al., 2010).

7.2.9. 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's protected least significant difference test. A $p < 0.05$ was required for the results to be considered statistically significant.

7.3. Results

7.3.1. Neuroprotection experiment

7.3.1.1. Dopaminergic markers

In intact nonlesioned mice, progesterone treatment had no effect on striatal DA and its metabolites DOPAC, HVA and 3-MT concentrations as well as on striatal serotonin and its metabolite 5-HIAA concentrations (Figure 7.1). Administration of MPTP decreased striatal DA concentrations (decrease of 49 %) as well as DOPAC (decrease of 48 %), HVA (decrease of 45 %) and 3-MT (decrease of 35 %) concentrations and increased 3-MT/DA ratio. MPTP at the dose used showed specificity to the DA system, striatal serotonin and 5-HIAA concentrations showing no significant difference between groups. Treatment with progesterone prevented completely the MPTP toxicity on DA concentrations as well as the increase of 3-MT/DA ratio and partially the decrease on DOPAC content.

Progesterone treatment had no effect on DAT specific binding in the striatum of nonlesioned mice (Figure 7.2). Measures of DAT specific binding in all region of the striatum showed a decrease in MPTP-treated mice as compared to control values (Figure 7.2). Progesterone was effective in reducing MPTP toxicity on DAT specific binding in the lateral part of the anterior striatum as well as in the medial and lateral parts of the middle striatum. No significant difference between groups was observed for DAT specific binding in substantia nigra (Figure 7.2).

No statistically significant differences in VMAT2 specific binding in the striatum were observed between control mice and progesterone treated, nonlesioned, mice (Figure 7.3). MPTP mice showed a decrease in VMAT2 specific binding in all the areas of the striatum measured (Figure 7.3). Progesterone treatment reduced MPTP toxicity as measured with VMAT2 specific binding in the medial and lateral parts of the anterior, middle and posterior striatum. In substantia nigra, progesterone treatment did not change VMAT2 specific binding in nonlesioned mice (Figure 7.3). Nigral VMAT2 specific binding was slightly reduced in MPTP treated mice whereas progesterone MPTP treated mice were at control levels.

7.3.1.2. Plasma concentration of steroids

Measures of steroid plasma levels showed that progesterone treatment in intact mice induces a decrease in the levels of testosterone, dihydrotestosterone and 3β-diol (Figure 7.4). Lower levels of the androgens testosterone, dihydrotestosterone and 3β-diol were observed in MPTP mice as compared to control mice. MPTP mice receiving progesterone treatment had significantly higher levels of testosterone and dihydrotestosterone than MPTP mice receiving vehicle. An increase in plasma levels of progesterone was observed in MPTP mice treated with progesterone.

7.3.2. Rescue experiment

Administration of MPTP induced a significant decrease in concentrations of DA (65% decrease compared to the control group) (Figure 7.5). A dose-response effect of progesterone was observed when the steroid was administered after MPTP. The dose of 8 mg/kg of progesterone, but not those of 1 µg or 16 mg/kg, significantly reduced the effect of MPTP, with a 53% increase in DA concentrations as compared to MPTP mice. Administration of progesterone (8 mg/kg) also reduced the effect of MPTP on concentrations of DOPAC and on the ratio of 3-MT/DA. Progesterone at all doses tested reduced the increase in the ratio of HVA/DA induced by MPTP. No significantly change in striatal serotonin concentrations was observed (Figure 7.5). MPTP mice and MPTP mice treated with progesterone (all doses) had higher 5-HIAA concentrations and superior 5-HIAA/serotonin ratio than control mice. Mice treated with 16 mg/kg of progesterone had a decrease of striatal 5-HIAA concentrations as compared to MPTP mice. Only the dose of 8 mg/kg of progesterone significantly reduced the effect of MPTP on the 5-HIAA/serotonin ratio.

All doses of progesterone significantly decreased the effect of MPTP on DAT specific binding in the medial part of the anterior striatum, whereas only mice receiving progesterone at doses of 8 and 16 mg/kg had DAT specific binding in the lateral part of the anterior striatum higher than MPTP mice (Figure 7.6). In the medial part of the middle striatum, increased DAT specific binding as compared to MPTP was observed with doses of 1µg and 8 mg/kg of progesterone, whereas a reduction in MPTP effect was observed only with 8 mg/kg of progesterone in the lateral part of the middle striatum. In both the medial and lateral parts of

the posterior striatum, progesterone (8 mg/kg) treatment was effective in reducing the MPTP effect. In the substantia nigra, MPTP mice treated with progesterone (1 µg and 8 mg/kg) had DAT specific binding at control values (Figure 7.6). A decrease in DAT specific binding was observed in MPTP mice and in mice receiving 16 mg/kg of progesterone. Only MPTP mice receiving progesterone at the dose of 1 µg had higher levels of DAT specific binding than MPTP mice.

For VMAT2 specific binding, only progesterone administered at the dose of 8 mg/kg reduced MPTP toxicity in the medial and lateral parts of the anterior striatum, and also in the medial part of the middle and posterior striatum (Figure 7.7). When mice were treated with 16 mg/kg of progesterone, a decrease in MPTP effect was observed in the lateral part of the posterior striatum. Reduction in nigral VMAT2 specific binding was observed in MPTP mice and in mice treated with doses of 8 and 16 mg/kg of progesterone (Figure 7.7). Normal levels of VMAT2 specific binding in substantia nigra were observed in mice receiving 1 µg of progesterone.

7.3.2.2. Plasma and brain concentration of steroids

We next addressd how steroids plasma and brain levels were changed by MPTP lesion and treatment with progesterone. A schematic representation of biosynthesis pathways of steroids assayed is presented in Figure 7.8. In plasma, our results show a dose-dependent increase in progesterone levels with progesterone treatments (Figure 7.9A). Measure of progesterone metabolites revealed that all doses of progesterone increased dihydroprogesterone levels. This was also observed in MPTP mice, where dihydroprogesterone levels were higher than in the control group and with all group of progesterone treated-mice. Only progesterone administered at 1 µg and 8 mg/kg increased isopregnanolone levels, whereas a decrease was observed when mice received 16 mg/kg of progesterone. Tetrahydroprogesterone plasma levels were only increased in mice treated with 1 µg of progesterone. All mice treated with progesterone had lower levels of testosterone than control mice.

In brain, our results showed an increase in pregnenolone levels with 16 mg/kg of progesterone as compared to control and 8 mg/kg of progesterone treated groups (Figure

7.9A). All dose of progesterone significantly increased the levels of progesterone in the brain, with a higher increase observed when progesterone was administered at 16 mg/kg. Significant positive correlations were observed between plasma and brain levels of progesterone, as well as between plasma and brain levels of tetrahydroprogesterone (Figure 7.9B). A significant negative correlation was found between plasma and brain levels of isopregnanolone.

MPTP mice had decreased plasma levels of dihydrotestosterone, 3α-diol and 3β-diol as compared to the control group (Figure 7.10A). Administration of 1 µg and 16 mg/kg of progesterone lowered dihydrotestosterone levels. All doses of progesterone reduced 3β-diol levels whereas only the doses of 8 and 16 mg/kg of progesterone decreased 3α-diol levels.

In brain, dihydroprogesterone levels were only increased with the 1 µg and 16 mg/kg dose of progesterone (Figure 7.10A). Higher levels of isopregnanolone was observed in mice receiving 16 mg/kg of progesterone. All doses of progesterone increased brain levels of tetrahydroprogesterone. Lower levels of DHEA was measured in brain of mice treated with progesterone (all doses). Mice receiving 1 µg of progesterone had lower brain testosterone levels. Dihydrotestosterone levels were increased in MPTP mice and with 16 mg/kg progesterone treatment. Progesterone administered at 1 µg and 16 mg/kg decreased the levels of 3β-diol. Significant positive correlations were observed between plasma and brain levels of DHEA, as well as between plasma and brain levels of testosterone (Figure 7.10B). A significant negative correlation was found between plasma and brain levels of dehydrotestosterone.

7.4. Discussion

The present results showed that a low dose of progesterone has a neuroprotective effect in MPTP-treated mice. In addition, we have shown that progesterone has the ability to rescue dopaminergic markers when administered after MPTP. The present results support our previous publications on the neuroprotective effect of progesterone in MPTP retired breeder mice (Grandbois et al., 2000; Callier et al., 2001). At a dose of 1µg, progesterone reduced the effect of MPTP when a moderate lesion of the nigrostriatal dopaminergic system is present, but had more limited effect in rescue experiment. A higher dose of progesterone was required to be administered after the injury to observe an effect on dopaminergic markers; a dose of 8 mg/kg was neuroprotective while treatment with 1µg and 16 mg/kg progesterone had more limited effects. The beneficial effect of progesterone is also known to be dose-dependent, to have a U-inverted shape, at promoting the recovery from traumatic brain injury in rodents (Chen et al., 1999; Cutler et al., 2007).

Although data on the beneficial effect of progesterone on brain trauma is abundant (Sayeed and Stein, 2009), few studies have investigated the potential rescue role of progesterone on dopaminergic neurons. A study in rats lesioned with 6-OHDA showed that administration of chronic doses of progesterone (8 mg/kg) 24 hours post-injury increases the metabolism of DA in the striatum, as well as motor deficits induced by the lesion (Chao et al., 2011). Moreover, others reported that the administration of high doses of progesterone (4 mg/kg) seven days after the injection of 6-OHDA in the striatum improved motor and cognitive deficits (Casas et al., 2011). Although in the present study the therapeutic window of opportunity of progesterone was not investigated, and that the administration of progesterone was done in a relatively short time after the lesion, the study with rats injured with 6-OHDA (Casas et al., 2011) suggests that the therapeutic window of opportunity of progesterone is relatively long, and that benefit could be seen after a long period following the lesion. This data suggests that progesterone may have a beneficial effect in patients when administered following the diagnosis of Parkinson's disease.

Accumulation of progesterone after its intraperitoneal administration is reported to be much higher in the brain than in plasma (Wong et al., 2012). Thus, high levels of

progesterone is quickly found in the brain after injection, allowing a rapid action of this steroid in the brain. The mechanisms of action of progesterone are multiple. Progesterone increases brain mitochondrial efficiency by reducing leakage and reducing lipid peroxidation, supporting the ability of this steroid to increase the oxidative capacity of brain mitochondria and thus to reduce oxidative stress (Irwin et al., 2008). Tetrahydroprogesterone (allopregnanolone), an active metabolite of progesterone, but not progesterone, has anti-apoptotic effects by inhibiting the opening of the permeability transition pore and inhibiting the mitochondrial release of cytochrome c (Sayeed et al., 2009). In cultured neurons of the striatum, progesterone, but not its active metabolite tetrahydroprogesterone, blocks neuronal death occurring by depolarization induced by opening voltage-gated calcium channels by blocking the entry of calcium in these channels (Luoma et al., 2011). This effect of progesterone requires high concentrations and cannot be blocked by the use of a nuclear receptor antagonist, suggesting a mechanism independent of the activation of these receptors (Luoma et al., 2011). Progesterone increases the activity of Akt and ERK1/2, both major kinases involved in cell survival (Guerra-Araiza et al., 2009; Ishrat et al., 2012). Progesterone also has anti-inflammatory properties and its action reduces the inflammatory response following brain damage (Gibson et al., 2005; Stein, 2005). By activation of nuclear receptors, progesterone increases levels of brain-derived neurotrophic factor (Jodhka et al., 2009), a neurotrophic factor involved in neuroprotection and in maintaining neurons function (Aron and Klein, 2011). Progesterone increases the expression of the mitochondrial anti-apoptotic protein Bcl-2 (Yao et al., 2005), and has also been reported to decrease oxidative stress by increasing the levels of endogenous antioxidants such as glutathione, superoxide dismutase and catalase (Aggarwal et al., 2008).

Progesterone has active metabolites, dihydroprogesterone and tetrahydroprogesterone, which have beneficial effects on brain (Leonelli et al., 2007; Wang et al., 2010). The two enzymes responsible for conversion of progesterone to dihydroprogesterone and, subsequently, to tetrahydroprogesterone, the 5α-reductase and the 3α-hydroxysteroid dehydrogenase, are present in the brain (Do Rego et al., 2009). It is therefore possible that the neuroprotective effect observed in the present study is due to an effect coming from the metabolism of progesterone. Our results shown that an increase in

brain levels of progesterone and tetrahydroprogesterone occurred following progesterone treatment at all dose investigated, while brain levels of dihydroprogesterone were only increased with 1µg and 16 mg/kg of progesterone. A high accumulation of progesterone and its metabolites in the brain was not associated with neuroprotection levels, since more limited effect of progesterone treatment was observed on dopaminergic markers when administered at the dose of 16 mg/kg. In support of the role of progesterone metabolites in neuroprotection, several in vitro and in vivo studies have shown that blockage of the 5α-reductase, the first enzyme involved in the conversion of progesterone into dihydroprogesterone, abolishes the neuroprotective effect of progesterone in various models (Singh et al., 2010; Ishihara et al., 2012; Radley et al., 2012). Furthermore, administration of tetrahydroprogesterone once weekly for two weeks in MPTP mice is reported to restore positive cells for tyrosine hydroxylase in the substantia nigra as well as motor behavior, and induce the generation of new positive neurons for tyrosine hydroxylase in substantia nigra (Adeosun et al., 2012). Tetrahydroprogesterone in this study was administered at a time when the damage induced by MPTP are stable, that is after seven days (L'Episcopo et al., 2011). Thus, tetrahydroprogesterone has potent capacity of recovery and neurogenesis of tyrosine hydroxylase neurons.

High levels of progesterone and its metabolites dihydroprogesterone and tetrahydroprogesterone were found in the human caudate nucleus and substantia nigra (Bixo et al., 1997). A decrease of 5α-reductase protein and mRNA, the enzyme responsible of the conversion of progesterone to its metabolites, were observed in the substantia nigra of Parkinson's disease patients (Luchetti et al., 2010). This reduction could account for the decreased levels of tetrahydroprogesterone and dihydroprogesterone found in the cerebrospinal fluid of Parkinson's disease patients (di Michele et al., 2003). Further work is needed to investigate how the decrease in progesterone metabolites contributes to the degenerative process but current data suggests that progesterone could be an interesting therapeutic compound because tetrahydroprogesterone is known to modulate dopaminergic systems, to act as a positive neuromodulator of the GABA-A receptor and to have neuroprotective effects (Zheng, 2009; Adeosun et al., 2012).

MPTP is reported to induce damage to Leydig cells (Ruffoli et al., 2008) causing a decrease in plasma androgens levels (Ruffoli et al., 2008; Bourque et al., 2014), as shown in the present study. We also observed a reduction in plasma androgens levels in intact and MPTP mice treated with progesterone. The effect of progesterone on androgen biosynthesis is reported to be mediated by an inhibitory effect of progesterone on luteinizing hormone, which is responsible of the production of testosterone by Leydig cells (Jeyaraj et al., 2001). In the brain, an increase of dihydrotestosterone was observed in MPTP mice and with progesterone treatment at 16 mg/kg. A previous study from our group reported a lack of protective effect on dopaminergic markers in MPTP mice receiving androgens treatment (Ekue et al., 2002), suggesting that an increase in the levels of brain androgens is not implicated in the limited protective effect of progesterone observed at the dose of 16 mg/kg.

Progesterone is reported to modulate DA activity as shown by a rapid increase in striatal DA, DOPAC and HVA concentrations in rodents (Di Paolo et al., 1986; Petitclerc et al., 1995). Thus, our results obtained in MPTP mice treated with progesterone could be caused by a hormonal effect on DA synthesis or metabolism. Nevertheless, we reported that chronic treatment with progesterone in intact male mice did not modulated DA and turnover, DAT and VMAT2 specific binding, whereas progesterone treated MPTP mice have higher DA, DAT and VMAT2 than vehicle treated MPTP mice. Thus, the results presented here support that the DA changes observed involve neuroprotection rather than only modulation of DA activity.

Studies in both men and women have shown the beneficial effect of progesterone administration to reduce damage following traumatic brain injury (Wright et al., 2007); (Xiao et al., 2008). No side effects from the use of high dose of progesterone in the short term have been reported in patients with a traumatic brain injury (Wright et al., 2007; Xiao et al., 2008). Progesterone is sometimes used in monotherapy to treat symptoms of menopause in women and few side effects were observed during long-term use with low, medium or high doses (Spark and Willis, 2012). Progesterone represent an interesting option for therapeutic for brain disease that could be used in both men and women.

CHAPITRE VII : Neuroprotective and rescue effect of progesterone

In this study, we have shown that the use of low dose of progesterone had neuroprotective capacities by decreasing the toxic effects of MPTP in dopaminergic neurons. Furthermore, administration of progesterone following the MPTP lesion induced rescue effects on dopaminergic markers. Our results show that progesterone has neuroprotective and rescue properties on dopaminergic neurons.

7.5. Acknowledgments

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7.6. References

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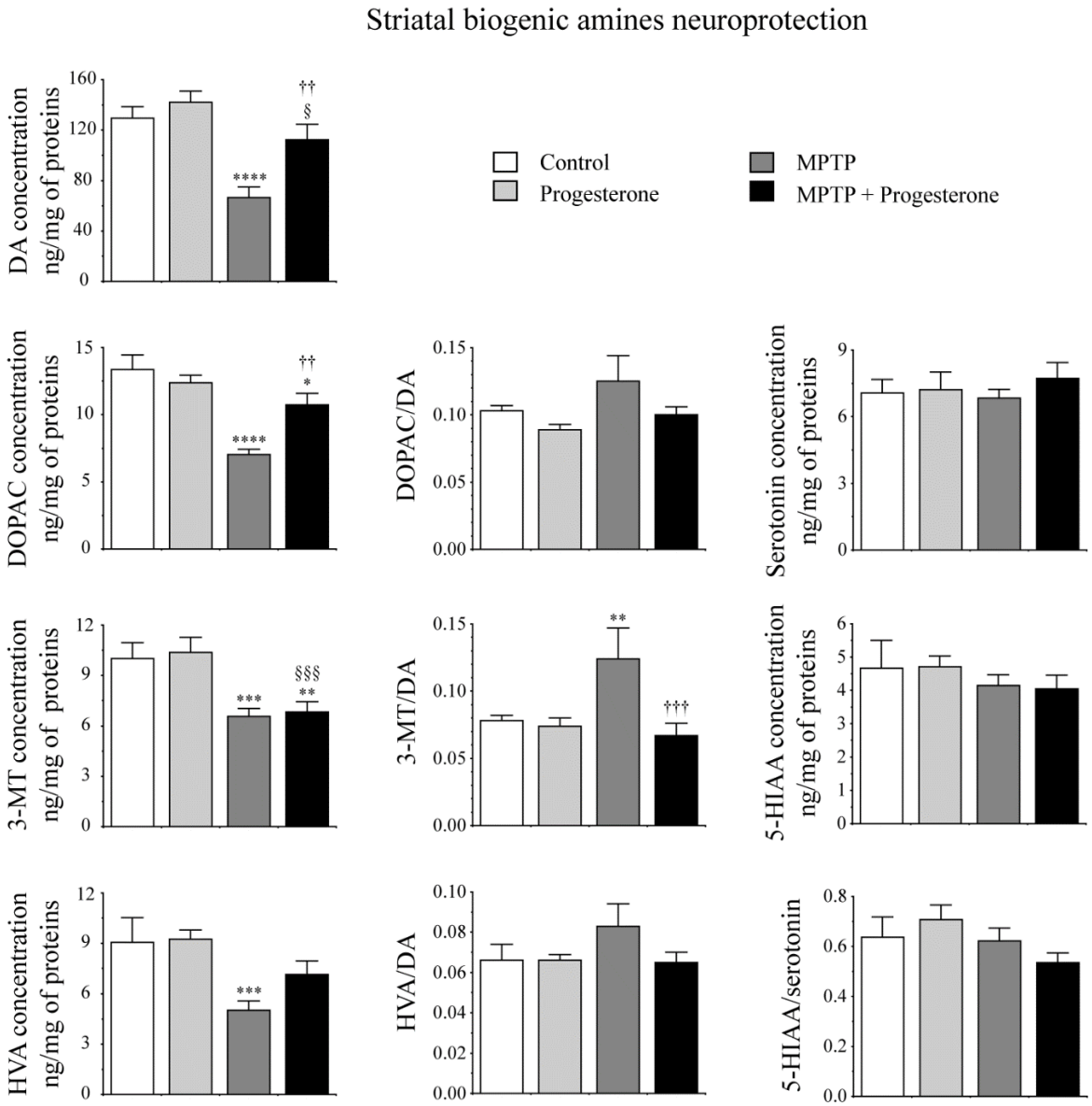

Figure 7.1. Effects of MPTP and neuroprotection with progesterone treatment on dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT), DOPAC/DA, HVA/DA and 3-MT/DA ratios, as well as serotonin concentrations and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Mice were treated with progesterone twice daily (1 µg) for ten days.Values shown are the means (ng/mg of proteins) ± S.E.M. of 10-12 mice per group. There was a statistically significant effect of treatment for DA (F[3,41] = 11.82; $p < 0.0001$), DOPAC (F[3,41] = 13.14; $p < 0.0001$), 3-MT (F[3,41] = 6.78; $p = 0.0008$), HVA (F[3,41] = 4.29; $p = 0.01$) and 3-MT/DA (F[3,41] = 4.13; $p = 0.012$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ and **** $p < 0.0001$ vs control; § $p < 0.05$ and §§§ $p < 0.005$ vs progesterone; †† $p < 0.01$ and ††† < 0.005 vs MPTP.

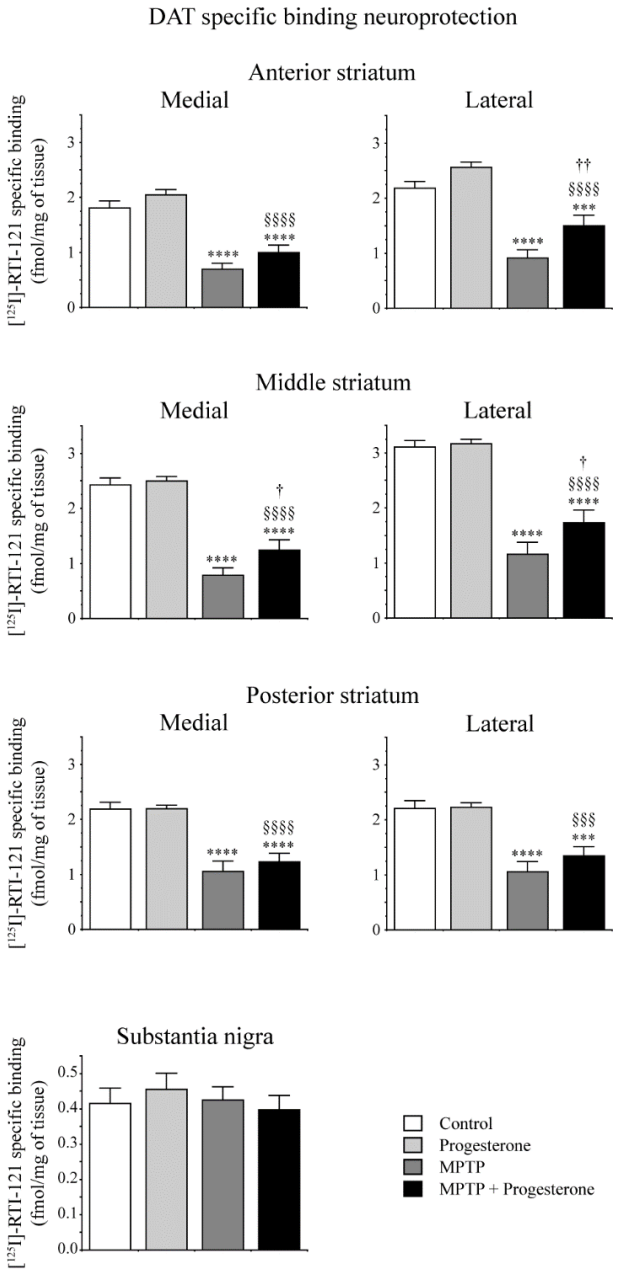

Figure 7.2. Effects of MPTP and neuroprotection with progesterone treatment on [^{125}I]-RTI-121 DAT specific binding in striatum and substantia nigra. Mice were treated with progesterone twice daily (1 µg) for ten days. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 10-12 mice per group. There was a statistically significant effect of treatment for medial part of the anterior striatum (F[3,41] = 30.62; $p < 0.0001$), lateral part of the anterior striatum (F[3,41] = 28.15; $p < 0.0001$), medial part of the middle striatum (F[3,40] = 41.08; $p < 0.0001$), lateral part of the middle striatum (F[3,40] = 36.21; $p < 0.0004$), medial part of the posterior striatum (F[3,41] = 20.04; $p < 0.0001$), and lateral part of the posterior striatum (F[3,41] = 16.56; $p < 0.0001$). *** p < 0.005 and **** p < 0.0001 vs control; §§§ p < 0.005 and §§§§ p < 0.0001 vs progesterone; † p < 0.05 and †† < 0.01 vs MPTP.

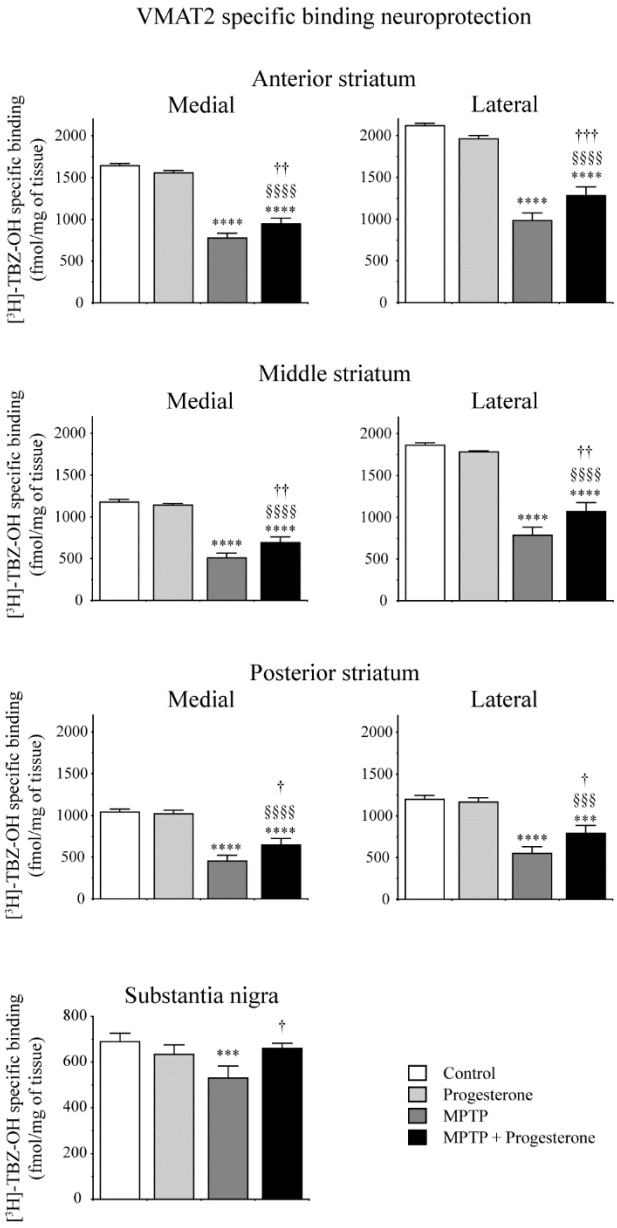

Figure 7.3. Effects of MPTP and neuroprotection with progesterone treatment on [^{3}H]-TBZ-OH VMAT2 specific binding in striatum and substantia nigra. Mice were treated with progesterone twice daily (1 µg) for ten days. Values shown are the means (fmol/mg of tissue) ± S.E.M. of 10-12 mice per group. There was a statistically significant effect of treatment for medial part of the anterior striatum (F[3,41] = 89.74; $p < 0.0001$), lateral part of the anterior striatum (F[3,41] = 62.01; $p < 0.0001$), medial part of the middle striatum (F[3,40] = 50.70; $p < 0.0001$), lateral part of the middle striatum (F[3,40] = 54.29; $p < 0.0004$), medial part of the posterior striatum (F[3,41] = 26.65; $p < 0.0001$), lateral part of the posterior striatum (F[3,41] = 20.03; $p < 0.0001$) and for substantia nigra (F[3,41] = 3.15; $p = 0.035$). *** p < 0.005 and **** p < 0.0001 vs control; §§§ p < 0.005 and §§§§ p < 0.0001 vs progesterone; † p < 0.05, †† p < 0.01 and ††† p < 0.005 vs MPTP.

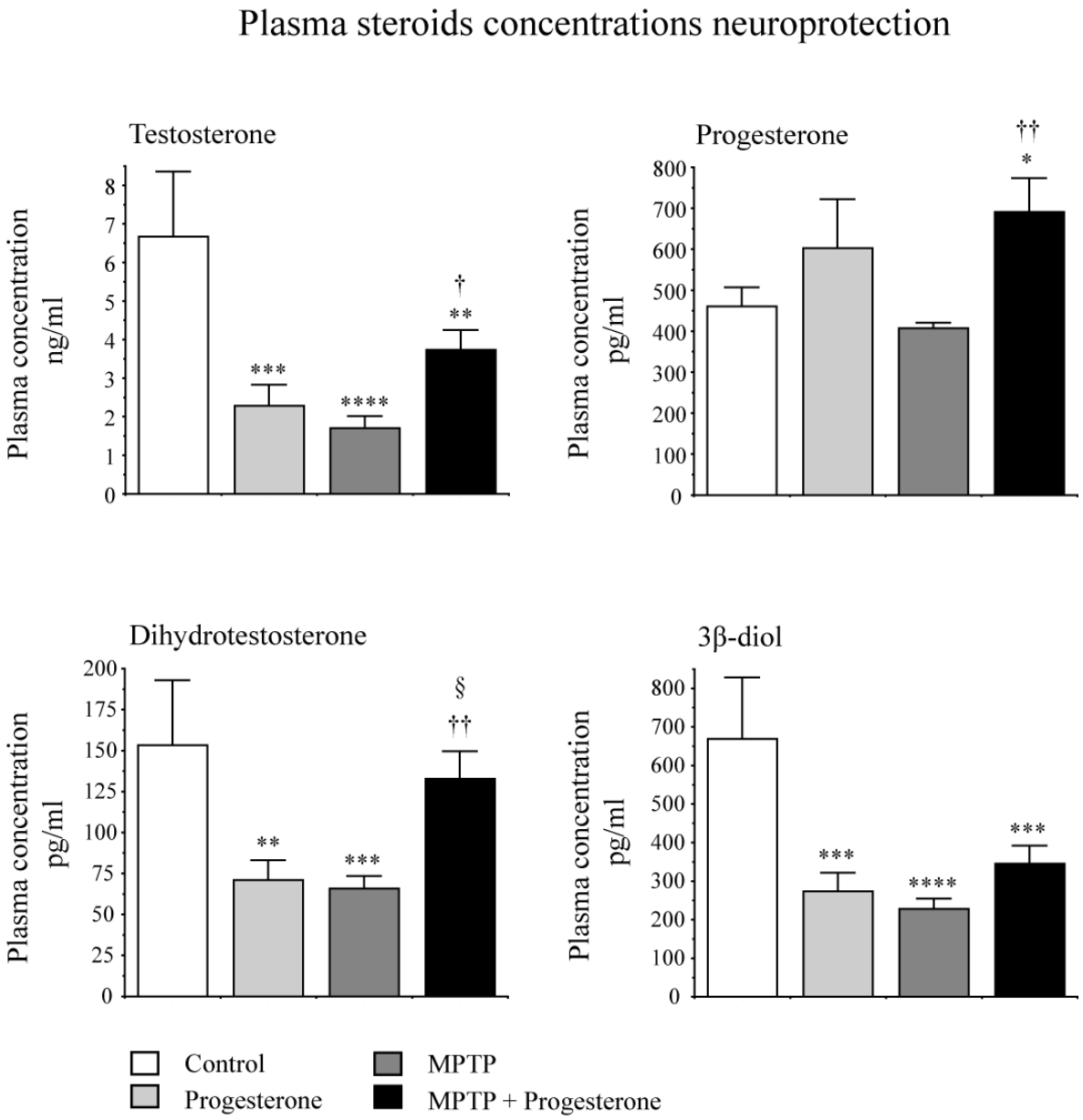

Figure 7.4. Effects of MPTP and neuroprotection with progesterone treatment on plasma concentration of testosterone, dihydrotestosterone, 3β-diol and progesterone. Mice were treated with progesterone twice daily (1 µg) for ten days. Values shown are the means (pg/ml or ng/ml) ± S.E.M. of 8-14 mice per group. There was a statistically significant effect of treatment for testosterone (F[3,44] = 7.76; p = 0.0003), dihydrotestosterone (F[3,44] = 5.46; p = 0.0028), 3β-diol (F[3,44] = 7.17; p = 0.0005) and progesterone (F[3,46] = 3.05; p = 0.0379). * p < 0.05, ** p < 0.01, *** p < 0.005 and **** p < 0.0001 vs control; † p < 0.05 and †† < 0.01 vs MPTP; § p < 0.05 vs progesterone.

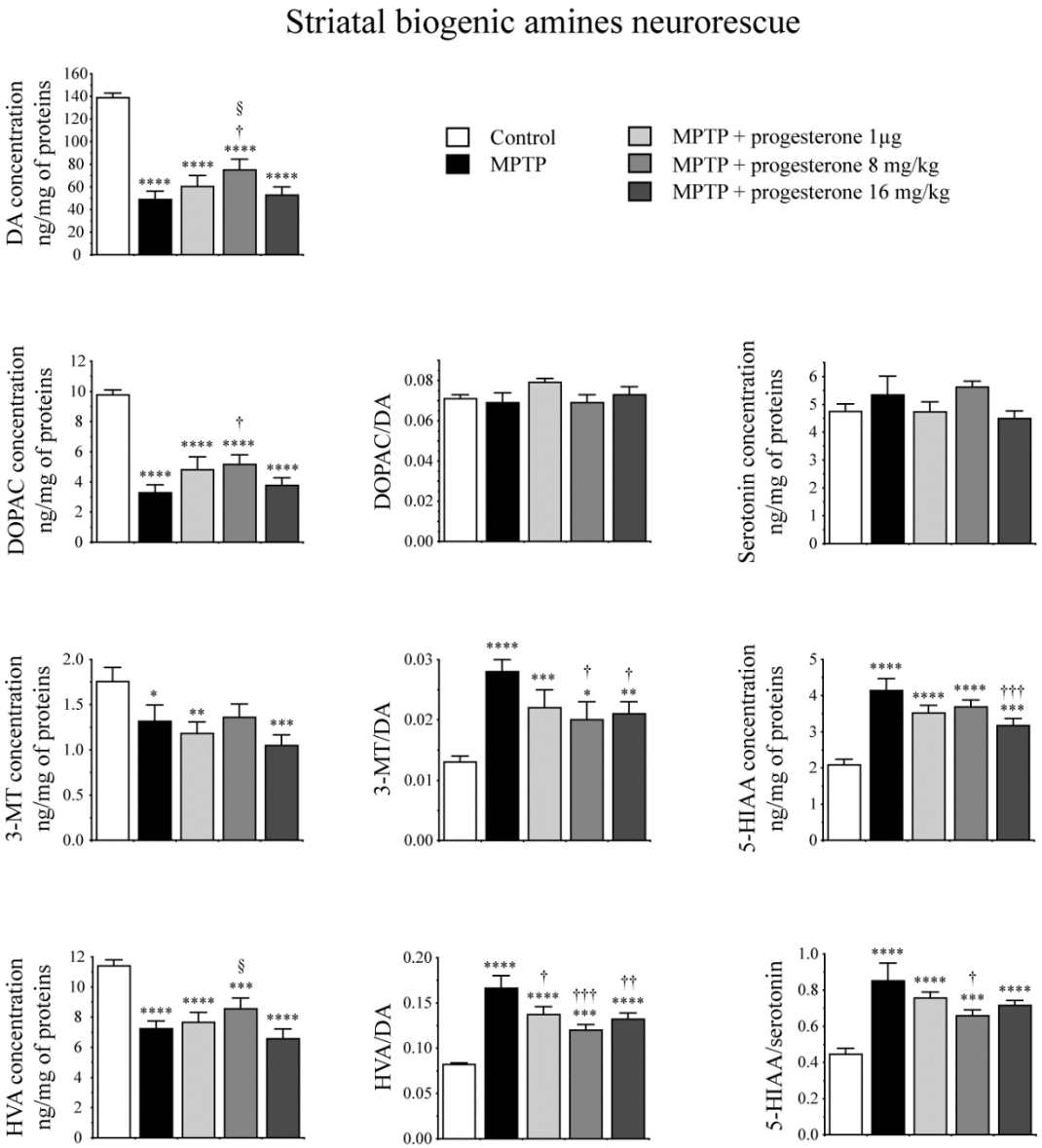

Figure 7.5. Effects of MPTP and neurorescue with progesterone treatment on dopamine (DA) concentrations and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT), DOPAC/DA, HVA/DA and 3-MT/DA ratios, as well as serotonin concentrations and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Mice were treated with progesterone once daily (8 or 16 mg/kg) or twice daily (1 µg). Values shown are the means (ng/mg of proteins) ± S.E.M. of 10-15 mice per group. There was a statistically significant effect of treatment for DA (F[4,51] = 30.93; $p < 0.0001$), DOPAC (F[4,51] = 25.14; $p < 0.0001$), 3-MT (F[4,51] = 3.57; $p = 0.012$), HVA (F[4,51] = 12.63; $p < 0.0001$), 3-MT/DA (F[4,51] = 6.94; $p = 0.0002$), HVA/DA (F[4,51] = 16.53; $p < 0.0001$), 5-HIAA (F[4,51] = 14.73; $p < 0.0001$) and 5-HIAA/serotonin (F[4,51] = 10.99; $p < 0.0001$). * p < 0.05, ** p < 0.01, *** p < 0.005 and **** p < 0.0001 vs control; † p < 0.05, †† p < 0.01 and ††† < 0.005 vs MPTP; § p < 0.05 vs MPTP + progesterone (8 mg/kg).

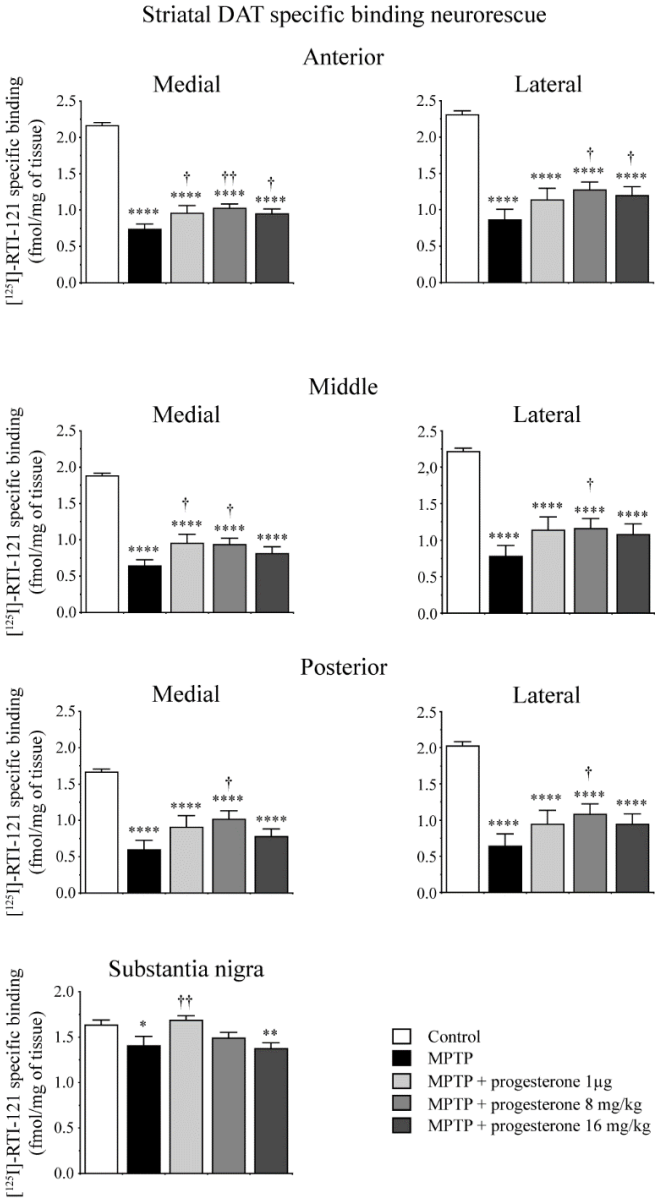

Figure 7.6. Effects of MPTP and neurorescue with progesterone treatment on [^{125}I]-RTI-121 DAT specific binding in striatum and substantia nigra. Mice were treated with progesterone once daily (8 or 16 mg/kg) or twice daily (1 µg). Values shown are the means (fmol/mg of tissue) ± S.E.M. of 10-15 mice per group. There was a statistically significant effect of treatment for medial part of the anterior striatum (F[4,51] = 81.69; $p < 0.0001$), lateral part of the anterior striatum (F[4,51] = 28.42; $p < 0.0001$), medial part of the middle striatum (F[4,51] = 39.79; $p < 0.0001$), lateral part of the middle striatum (F[4,51] = 21.05; $p < 0.0004$), medial part of the posterior striatum (F[4,51] = 16.46; $p < 0.0001$), lateral part of the posterior striatum (F[4,51] = 17.65; $p < 0.0001$) and for substantia nigra (F[4,50] = 4.19; $p = 0.035$). * p < 0.05, ** p < 0.01 and **** p < 0.0001 vs control; † p < 0.05 and †† p < 0.01 vs MPTP.

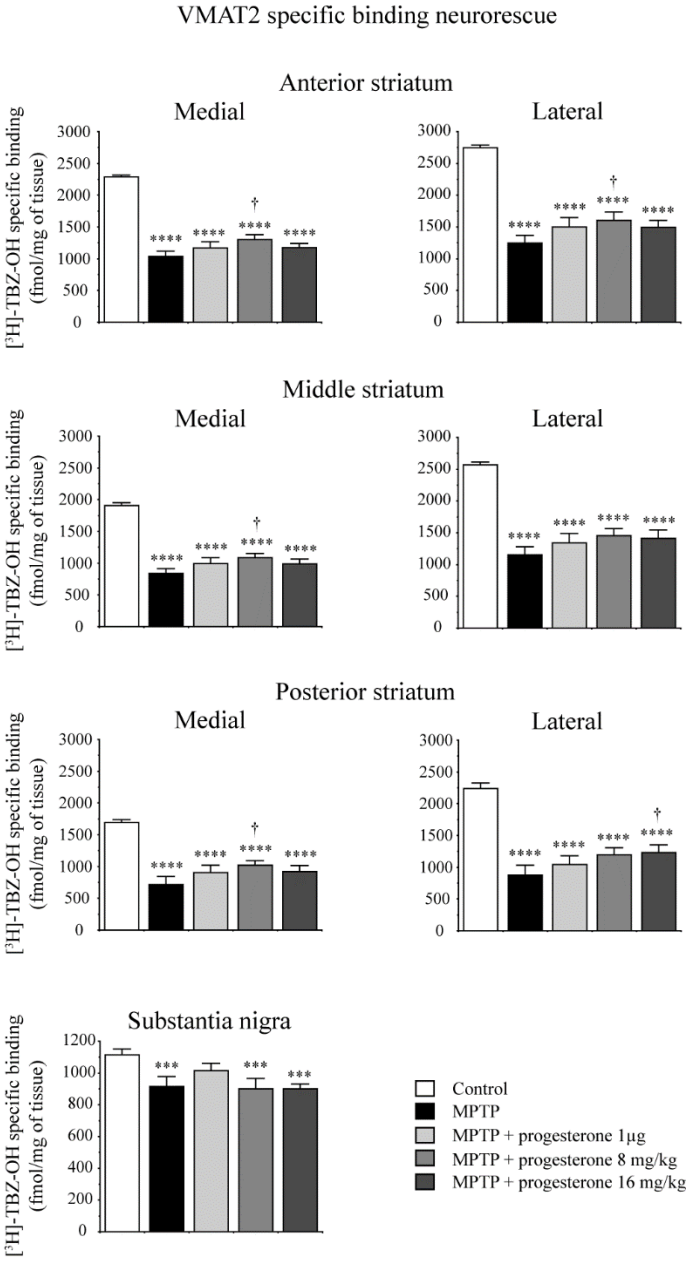

Figure 7.7. Effects of MPTP and neurorescue with progesterone treatment on [^{3}H]-TBZ-OH VMAT2 specific binding in striatum and substantia nigra. Mice were treated with progesterone once daily (8 or 16 mg/kg) or twice daily (1 µg). Values shown are the means (fmol/mg of tissue) ± S.E.M. of 10-15 mice per group. There was a statistically significant effect of treatment for medial part of the anterior striatum (F[4,51] = 69.11; $p < 0.0001$), lateral part of the anterior striatum (F[4,51] = 37.06; $p < 0.0001$), medial part of the middle striatum (F[4,51] = 47.05; $p < 0.0001$), lateral part of the middle striatum (F[4,51] = 32.56; $p < 0.0004$), medial part of the posterior striatum (F[4,50] = 20.77; $p < 0.0001$), lateral part of the posterior striatum (F[4,50] = 23.72; $p < 0.0001$) and for substantia nigra (F[4,48] = 4.41; $p = 0.004$) *** $p < 0.005$ and **** $p < 0.0001$ vs control; † $p < 0.05$ vs MPTP.

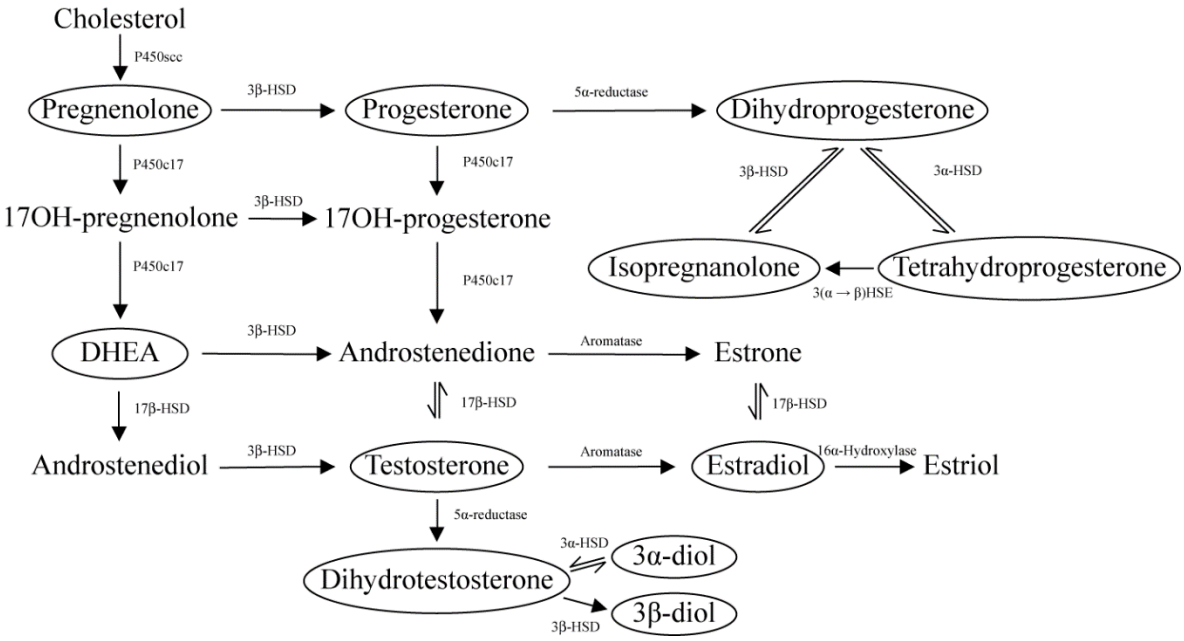

Figure 7.8. Schematic representation of biosynthesis pathways of steroids assayed (encircled steroids were assayed). P450scc, cytochrome P450 side-chain cleavage; P450c17, cytochrome P450 17α-hydroxylase/C17,20-lyase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 3(α → β)HSE, 3(α → β) hydroxysteroid epimerase; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

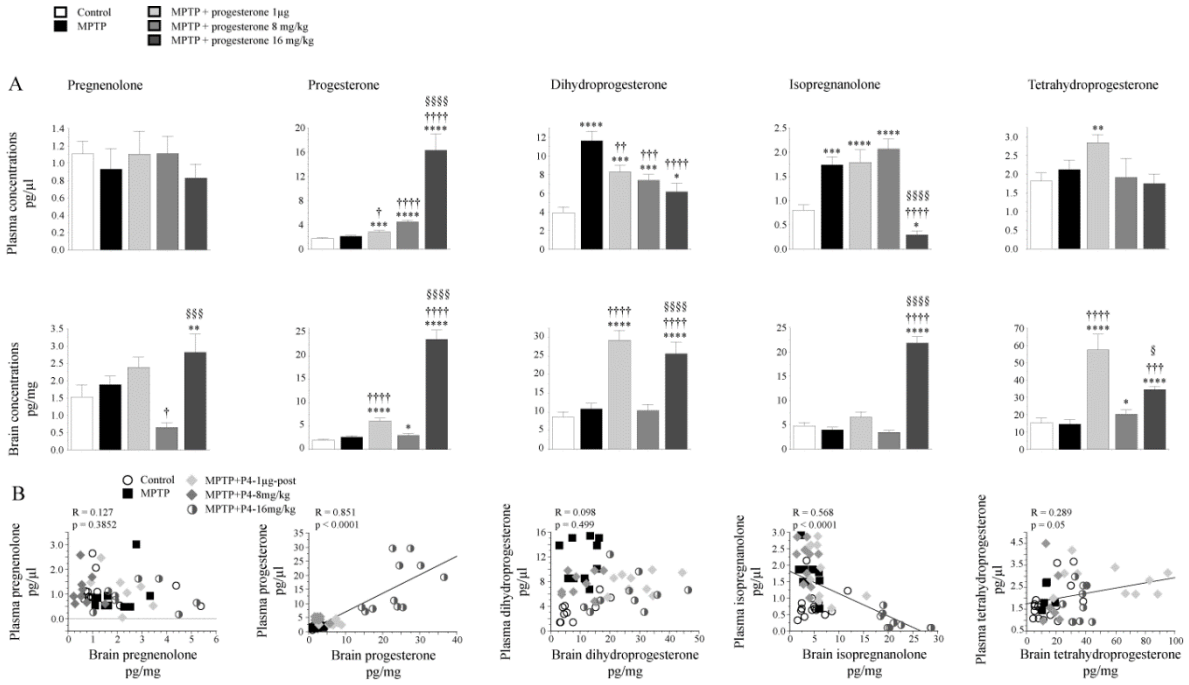

Figure 7.9. A. Effects of MPTP and neurorescue with progesterone treatment on plasma and brain concentration of pregnenolone, progesterone, dihydroprogesterone, isopregnanolone and tetrahydroprogesterone. Mice were treated with progesterone once daily (8 or 16 mg/kg) or twice daily (1 µg). Values shown are the means (pg/µl or pg/mg) ± S.E.M. of 10-15 mice per group. There was a statistically significant effect of treatment for plasma progesterone (F[4,50] = 70.83; $p < 0.0001$), plasma dihydroprogesterone (F[4,47] = 13.08; $p < 0.0001$), plasma isopregnanolone (F[4,49] = 17.76; $p < 0.0001$), plasma tetrahydroprogesterone (F[4,42] = 2.59; $p = 0.05$), brain pregnenolone (F[4,46] = 5.007; $p = 0.002$), brain progesterone (F[4,51] = 65.51; $p < 0.0001$), brain dihydroprogesterone (F[4,49] = 20.05; $p < 0.0001$), brain isopregnanolone (F[4,50] = 74.58; $p < 0.0001$) and brain tetrahydroprogesterone (F[4,49] = 14.11; $p < 0.0001$). * p < 0.05, ** p < 0.01, *** p < 0.005 and **** p < 0.0001 vs control; † p < 0.05, †† p < 0.01, ††† < 0.005 and †††† < 0.0001 vs MPTP; § p < 0.05, §§§ p < 0.005 and §§§§ p < 0.0001 vs MPTP + progesterone (8 mg/kg). B. Correlations between plasma and brain concentration of each steroid. Each symbol represents an individual mouse.

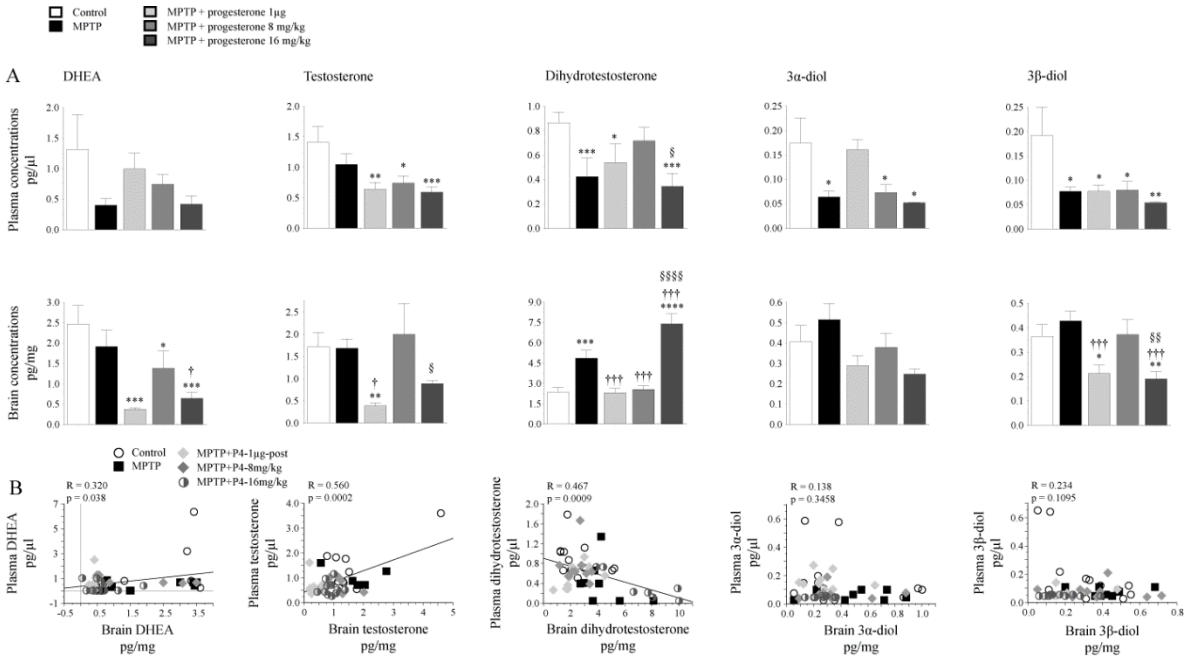

Figure 10. Effects of MPTP and neurorescue with progesterone treatment on plasma and brain concentration of DHEA, testosterone, dihydrotestosterone, 3α-diol and 3β-diol. Mice were treated with progesterone once daily (8 or 16 mg/kg) or twice daily (1 µg). Values shown are the means (pg/µl or pg/mg) ± S.E.M. of 10-15 mice per group. There was a statistically significant effect of treatment for plasma testosterone (F[4,38] = 4.32; p = 0.0056), plasma dihydrotestosterone (F[4,44] = 4.18; p = 0.0059), plasma 3α-diol (F[4,45] = 3.33; p = 0.0179), plasma 3β-diol (F[4,44] = 2.78; p = 0.0379), brain DHEA (F[4,43] = 5.40; p = 0.0013), brain testosterone (F[4,45] = 3.75; p = 0.0103), brain dihydrotestosterone (F[4,49] = 20.39; p < 0.0001) and brain 3β-diol (F[4,50] = 4.93; p = 0.002). * p < 0.05, ** p < 0.01, *** p < 0.005 and **** p < 0.0001 vs control; † p < 0.05 and ††† < 0.005 vs MPTP; § p < 0.05, §§ p < 0.01 and §§§§ p < 0.0001 vs MPTP + progesterone (8 mg/kg). B. Correlations between plasma and brain concentration of each steroid. Each symbol represents an individual mouse.

Chapitre 8 : Discussion et conclusion

Lors du début de mon projet de doctorat en 2009, les connaissances sur les diverses fonctions du GPER1 commençaient à émerger. Ce récepteur, connu au départ sous le nom de GPR30, fut cloné vers la fin des années 1990 par plusieurs groupes, et était un récepteur orphelin, sans ligand connu (Prossnitz & Barton 2011). En 2005, deux groupes indépendants rapportent une liaison du 17β-estradiol au GPR30 (Thomas *et coll.* 2005, Revankar *et coll.* 2005). Le développement d'un agoniste spécifique du GPER1 en 2006, le G1 (Bologa *et coll.* 2006), et d'un antagoniste spécifique en 2009, le G15 (Dennis *et coll.* 2009), a permis d'identifier plusieurs fonctions du GPER1 dans de nombreux systèmes (Prossnitz & Barton 2011).

Notre intérêt pour le GPER1 s'est accru avec la découverte que ce récepteur est présent dans le striatum et la substance noire (Brailoiu *et coll.* 2007), et que le GPER1 n'est pas impliqué dans la prolifération des cellules épithéliales dans les organes reproducteurs (Otto *et coll.* 2009, Otto *et coll.* 2008), faisant de ce récepteur une stratégie alternative à l'utilisation du 17β-estradiol pour diminuer les effets périphériques associés à son utilisation. De plus, étant un récepteur ayant des effets non-féminisant, son application thérapeutique non seulement chez la femme mais aussi chez l'homme devient possible. Au moment où nous concevions notre projet avec le GPER1, seulement deux études avaient investigué le rôle protecteur du GPER1 dans un modèle *in vivo* d'ischémie (Lebesgue *et coll.* 2010) et dans un modèle *in vitro* de toxicité du glutamate (Gingerich *et coll.* 2010), et aucune étude n'avait montré que l'effet protecteur du 17β-estradiol implique le GPER1. Lors de cette thèse, nous avons investigué l'implication du GPER1 dans la neuroprotection, dans le mécanisme d'action du 17β-estradiol et du raloxifène, ainsi que son interaction avec les ERα/β. Une autre stratégie alternative à l'utilisation des œstrogènes que nous avons investiguée est la progestérone. Peu d'études ont investigué son effet neuroprotecteur et neurorécupérateur sur les neurones dopaminergiques. Les effets de la progestérone chez l'humain pour contrer les dommages au cerveau sont bénéfiques et suggèrent une application clinique possible de l'utilisation de la progestérone. En effet, deux études cliniques de phase III sont présentement en cours pour évaluer l'efficacité de la progestérone lors d'un traumatisme cérébral chez

l'homme et la femme (Wei & Xiao 2013), et les études précédentes de phase II ont révélé des effets positifs (Wright *et coll.* 2007, Xiao *et coll.* 2008).

8.1. Des composés neuroprotecteurs ayant plusieurs cibles pour contrer le développement d'une maladie neurodégénérative possédant une pathogénèse multiple

L'étiologie de la maladie de Parkinson idiopathique est inconnue et la pathogénèse de cette maladie est complexe. Comme présenté au chapitre 2, la maladie de Parkinson implique une combinaison de plusieurs évènements pathologiques multifactoriels. Les causes de la maladie de Parkinson idiopathique demeurent à ce jour inconnu et pourraient varier selon les patients, comme observées dans les formes génétiques de la maladie, où les mutations de diverses protéines régulant différentes fonctions sont impliquées dans cette maladie. Quoique le développement de thérapie est souvent basé sur la spécificité du composé (un composé-une cible), ceci n'est pas toujours applicable, spécialement lorsque les cibles sont multiples et les causes d'origine inconnue et probablement différentes d'un individu à l'autre (Geldenhuys *et coll.* 2011). Un des obstacles au développement d'agents neuroprotecteurs pour la maladie de Parkinson est l'incertitude concernant les causes de cette maladie et ce qui doit être ciblé par les composés (Olanow & Schapira 2013, AlDakheel *et coll.* 2014). Les études cliniques n'ont pas réussi a montré l'efficacité d'un agent neuroprotecteur lorsque celui-ci ciblait un seul mécanisme de la pathogénèse de la maladie de Parkinson. C'est le cas des thérapies anti-inflammatoires, anti-apoptotiques, des composés qui augmentent la fonction mitochondriale et des antioxydants (ParkinsonStudyGroup 2006, Investigators 2007, AlDakheel *et coll.* 2014). Ainsi, les stratégies thérapeutiques ayant plusieurs mécanismes d'action, tels les composés oestrogéniques et la progestérone, sont des options intéressantes pour prévenir le développement de cette maladie neurodégénérative (Geldenhuys *et coll.* 2011, Olanow & Schapira 2013, AlDakheel *et coll.* 2014).

8.1.1. Les composés oestrogéniques

Le 17β-estradiol est un stéroïde ayant plusieurs mécanismes d'action et cible de nombreux évènements qui sont dysfonctionnels dans la maladie de Parkinson. Le 17β-estradiol maintien l'intégrité des neurones dopaminergiques, augmente la fonction

mitochondriale, diminue le stress oxydatif, inhibe l'apoptose, augmente l'activation des voies de signalisation impliquées dans la survie cellulaire, supprime les agrégats de protéines, module la réponse inflammatoire et diminue l'activité des canaux calciques (Vegeto *et coll.* 2008, Morissette *et coll.* 2008, Brewer *et coll.* 2009, Simpkins *et coll.* 2010, Sanchez *et coll.* 2010, Arnold *et coll.* 2012, D'Alessandro *et coll.* 2012). La variété des cibles ainsi que la potentialisation des mécanismes non-génomiques et génomiques (Bjornstrom & Sjoberg 2005) font des œstrogènes une stratégie thérapeutique intéressante pour prévenir le développement de la maladie de Parkinson.

Plusieurs évidences supportent que l'effet protecteur du 17β-estradiol ne résulte pas en un effet sur le métabolisme ou la recapture du MPTP. En effet, une étude a montré que le taux striatal de MPP+ chez les souris femelles intactes et chez les souris mâles est similaire, suggérant que le 17β-estradiol ne module pas la conversion du MPTP en MPP+ (Miller *et coll.* 1998). De plus, le 17β-estradiol ne module pas la MAO (Meyers *et coll.* 2010), ni l'affinité de DAT chez les rongeurs mâles (Meyers & Kritzer 2009). Ainsi, ces données suggèrent que le mécanisme d'action du 17β-estradiol est indépendant de la conversion du MPTP en MPP+, et en l'entrée du MPP+ dans les neurones.

Quoique les données de la littérature ont révélé la présence des ERs à la membrane dans le striatum, et que notre figure 5.1. les présente comme tel, nous ne pouvons exclure la présence des ERs au noyau. La faible localisation des ERs dans le striatum peut les rendre difficile à détecter au noyau et ainsi donner l'impression que les ERs soient exclusivement extranucléaires.

Quoique les effets du GPER1 et du raloxifène aient été moins étudiés que ceux du 17β-estradiol, les études suggèrent que ce récepteur et ce SERM reproduisent plusieurs des effets bénéfiques du 17β-estradiol qui permettraient de contrer les processus pathologiques lors d'une dégénérescence. Dans un modèle d'ischémie, l'effet neuroprotecteur du G1 est associé avec l'activation de la signalisation d'Akt et des ERK, et avec la diminution de l'activation de la protéine pro-apoptotique JNK (Tang *et coll.* 2014). Dans un modèle de traumatisme cérébrale, le G1 diminue l'apoptose ainsi que l'activation des astrocytes, et

augmente la survie cellulaire (Day *et coll.* 2013). Le raloxifène reproduit plusieurs des mécanismes d'action du 17β-estradiol incluant la diminution du stress oxydatif, l'inhibition de l'apoptose, l'augmentation de l'activation des voies de signalisation impliquées dans la survie cellulaire, ainsi que la modulation de la réponse inflammatoire (Du *et coll.* 2004, Razmara *et coll.* 2008, Armagan *et coll.* 2009, Baraka *et coll.* 2011, Arevalo *et coll.* 2012).

Lors de cette thèse, nous avons exploré certains mécanismes d'action du 17β-estradiol, du raloxifène et du G1 en investiguant la signalisation d'Akt, un important médiateur de la survie cellulaire (Raz *et coll.* 2008). Nous avons montré que l'effet neuroprotecteur du raloxifène et de la stimulation du ERα implique une augmentation des niveaux d'Akt, de Blc-2 et du BDNF, par un mécanisme dépendant du GPER1. Témoignant de l'importance d'Akt dans la protection des neurones dopaminergiques, les études ont montré que l'activation de cette kinase est suffisante pour induire un effet neuroprotecteur. En effet, l'activation constitutive d'Akt protège les corps cellulaires dopaminergiques de la substance noire et les terminaisons dans le striatum chez les souris lésées avec la 6-OHDA (Ries *et coll.* 2006, Cheng *et coll.* 2011), montrant que Akt est une cible majeure dans la neuroprotection.

Les composés qui ciblent Akt offre une opportunité thérapeutique pour promouvoir la survie cellulaire et prévenir les processus de dégénérescence (Greene *et coll.* 2011, Burke & O'Malley 2012). L'analyse post-mortem de cerveau de patients parkinsoniens et les études chez les animaux avec les modèles MPTP et 6-OHDA ont montré qu'une dysfonction des protéines kinase se produit. Certains haplotypes d'Akt1 ont été associé avec une diminution du risque de maladie de Parkinson (Xiromerisiou *et coll.* 2008). Deux polymorphismes nucléotidiques fonctionnels de GSK3β ont été rapportés dans le cerveau de patients parkinsoniens, associés avec une augmentation des transcrits de GSK3β, et avec une augmentation de la phosphorylation de la protéine Tau (Kwok *et coll.* 2005). Une analyse post-mortem chez des patients parkinsoniens a révélé une augmentation de l'activation de GSK3β dans le striatum (Duka *et coll.* 2009). Dans les neurones dopaminergiques de la substance noire, une diminution de l'activité d'Akt ainsi qu'une augmentation de RTP801,

une protéine impliquée dans la mort neuronale, ont été observées (Malagelada *et coll.* 2008, Malagelada *et coll.* 2006).

Dans le modèle MPTP, les études ont montré que l'inhibition de l'activité de GSK3β bloque l'induction de la mort neuronale induite par le MPTP, montrant que GSK3β est un médiateur important de la neurotoxicité du MPTP (Wang *et coll.* 2007, Petit-Paitel *et coll.* 2009). Les études utilisant la transduction virus adéno-associée, avec un gène codant pour la forme constitutivement active d'Akt (Myr-Akt) injecté dans la substance noire, ont montré à la fois une protection des corps cellulaires et des terminaisons dopaminergiques chez les souris lésées avec la 6-OHDA (Ries *et coll.* 2006, Cheng *et coll.* 2011). De plus, ces études ont montré que Myr-Akt prévient la fragmentation et la formation sphéroïde (gonflement associé avec des dommages) des axones, conservant ainsi leur intégrité, et que l'effet de Myr-Akt sur la protection des axones contre la dégénérescence rétrograde pourrait être causé par la suppression de l'autophagie (Cheng *et coll.* 2011).

Les facteurs trophiques ont des fonctions importantes dans la survie, le maintien et la fonction des neurones dopaminergiques (Fumagalli *et coll.* 2006, Aron & Klein 2011), et leur utilisation comme agent thérapeutique a été considérée dans les maladies neurodégénératives. Les études chez les modèles animaux de la maladie de Parkinson ont montré l'effet protecteur des facteurs trophiques sur les neurones dopaminergiques, et leur potentiel à promouvoir la récupération du système dopaminergique nigrostrié (Rangasamy *et coll.* 2010, Aron & Klein 2011, Allen *et coll.* 2013). Basées sur les résultats des études précliniques, plusieurs études cliniques ont été mené afin d'investiguer l'effet thérapeutique du GDNF chez des patients parkinsoniens, mais ces études n'ont pas donné les résultats escomptés (Rangasamy *et coll.* 2010, Aron & Klein 2011, Allen *et coll.* 2013). L'utilisation des facteurs trophiques comme thérapies impliquent de nombreux défis. En effet, les facteurs trophiques ne passent pas la barrière hémato-encéphalique, et doivent donc être injectés localement (Aron & Klein 2011). Leur faible taux de diffusion suite à l'injection ainsi que leur rapide dégradation limite aussi leur application thérapeutique (Aron & Klein 2011). Une solution pour contourner ces limites consiste en l'utilisation de composés qui induisent l'expression du BDNF et du GDNF, et le 17β-estradiol et la progestérone sont connus pour

augmenter l'expression des facteurs trophiques (Yang *et coll.* 2010, Campos *et coll.* 2012, Singh & Su 2013c). Les résultats obtenus au cours de cette thèse ont montré que les composés œstrogéniques augmentent les niveaux du BDNF. L'effet du raloxifène implique le GPER1, et une interaction bidirectionnelle entre le ERα et le GPER1 est requise dans la modulation des niveaux du BDNF. Nous avons montré un mécanisme par lequel les composés oestrogéniques protègent les neurones dopaminergiques et contribuent à préserver leurs fonctions.

8.1.2. La progestérone

La progestérone module aussi plusieurs fonctions qui sont affectées dans la maladie de Parkinson. La progestérone possède des propriétés anti-apoptotiques, anti-inflammatoires, augmente la survie cellulaire et module les canaux calciques (Djebaili *et coll.* 2005, O'Connor *et coll.* 2007, Kaur *et coll.* 2007, Guerra-Araiza *et coll.* 2009, Luoma *et coll.* 2011, Ishrat *et coll.* 2012, Arevalo *et coll.* 2013). La progestérone peut aussi avoir des effets bénéfiques dans des conditions où des accumulations d'agrégats de protéines sont présentes, tel qu'observé dans les maladies neurodégénératives, puisque cet hormone augmente l'autophagie (Kim *et coll.* 2012). L'administration de progestérone accroit la capacité oxydative des mitochondries, diminue le stress oxydatif (Irwin *et coll.* 2008) et augmente les niveaux d'enzyme anti-oxydante (Aggarwal *et coll.* 2008). Très peu d'études ont investigué l'effet de la progestérone sur le risque de développer la maladie de Parkinson. Une étude a observé une augmentation du risque de maladie de Parkinson chez les femmes utilisant des progestatifs en monothérapie (Simon *et coll.* 2009). Néanmoins, ces résultats doivent être interprétés avec caution, puisque ces observations sont basées sur quatre cas, et que le progestatif utilisé n'est pas spécifié (utilisation de la progestérone ou d'un composé synthétique tel que l'acétate de médroxyprogestérone). Considérant la différence des effets au cerveau entre la progestérone et l'acétate de médroxyprogestérone, ce dernier n'ayant aucun effet protecteur (Singh & Su 2013a), le progestatif utilisé pourrait influencer les résultats de cette étude. Notre étude de neuroprotection avec une faible dose de progestérone supporte l'effet bénéfique de ce stéroïde dans la protection des neurones dopaminergiques. L'effet neuroprotecteur de la progestérone est aussi rapporté dans plusieurs modèles dont la toxicité de la

methamphétamine, du glutamate, dans un modèle d'ischémie, de traumatisme cérébral et de contusion de la moelle épinière (Yu *et coll.* 2002, De Nicola *et coll.* 2009, Singh & Su 2013b). Notre étude de neurorécupération est basée sur les données préclinique et clinique de l'effet de la progestérone lors d'un traumatisme cérébrale, utilisant de fortes doses de progestérone, sur une courte période de temps (Wright *et coll.* 2007, Xiao *et coll.* 2008, Stein 2013). Les études de phase II investiguant l'effet de la progestérone lors d'un traumatisme cérébral ont révélé que l'utilisation de la progestérone réduit les dommages au cerveau chez les hommes et les femmes, et ce, sans effet secondaire (Wright *et coll.* 2007, Xiao *et coll.* 2008). Ces études, ainsi que la capacité de neurogénèse de la progestérone (Wang *et coll.* 2005) et son effet bénéfique dans notre modèle MPTP et chez le rat lésé avec la 6-OHDA (Casas *et coll.* 2011), suggèrent que la progestérone pourrait être un bon candidat pour retarder la progression de la maladie de Parkinson ou récupérer les neurones dysfonctionnels chez les patients parkinsonien.

Une étude récente réalisée sur des cerveaux de patients parkinsoniens suggère que les neurones dopaminergiques en début de maladie de Parkinson (trois ans et moins post-diagnostic), sont dysfonctionnels et non complètement dégénérés, ce qui offre une opportunité pour récupérer ces neurones tôt dans le début de la maladie (Kordower *et coll.* 2013). En effet, la diminution des neurones positifs pour la mélanine est moindre que la réduction des neurones positifs pour la TH, suggérant qu'une importante perte de fonction des neurones dopaminergiques se produit en début de maladie (Kordower *et coll.* 2013). Une étude réalisée chez la souris 6-OHDA montre qu'il est possible de récupérer les neurones dopaminergiques dysfonctionnels (Ries *et coll.* 2006). Cette étude a montré que, quoiqu'une perte de neurones dopaminergiques se produise suite à l'injection de 6-OHDA, la perte du phénotype TH des neurones n'est pas toujours associée à la perte du neurone dopaminergique. Cette étude a montré que l'injection de la forme constitutivement active d'Akt dans la substance noire trois semaines suivant la lésion avec la 6-OHDA augmente les neurones positifs pour la TH dans le striatum et dans la substance noire (Ries *et coll.* 2006). L'étude réalisée par Kordower et coll. (2013) sur des cerveaux de patients parkinsoniens, ainsi que les études chez les animaux sur la récupération des neurones (Ries *et coll.* 2006,

Casas *et coll.* 2011), suggèrent que des composés thérapeutiques peuvent avoir un effet bénéfique lorsqu'administrés suivant le diagnostic de la maladie de Parkinson. Néanmoins, la fenêtre d'opportunité thérapeutique de la progestérone demeure à investiguer, étant donné que dans notre étude, l'administration de la progestérone s'est fait dans un temps relativement court suivant la lésion avec le MPTP.

8.2. L'effet du vieillissement et de la ménopause sur les marqueurs dopaminergique et les fonctions cérébrales

Les données de la littérature clinique et les études chez les animaux ont montré qu'un déclin de l'activité dopaminergique se produit avec l'âge, incluant une diminution de la synthèse de la dopamine, du DAT, des récepteurs D1 et D2, ainsi que des neurones positifs pour la TH (Rollo 2009, Darbin 2012). Chez l'homme et la femme, la diminution reliée à l'âge dans l'activité dopaminergique au cerveau est associée avec un déclin des fonctions cognitives et motrices (Volkow *et coll.* 1998, Volkow *et coll.* 2000, Erixon-Lindroth *et coll.* 2005).

L'âge est le principal facteur de risque de la maladie de Parkinson et un déclin de l'activité dopaminergique en fonction de l'âge se produit. Ainsi, est-ce que ces changements dans l'activité dopaminergique lors du vieillissement rendent les neurones plus vulnérables pour le développement de la pathologie de la maladie de Parkinson? Des auteurs suggèrent que le vieillissement du système dopaminergique induit un état pré-parkinsonien, et que les mécanismes responsables du déclin de l'activité dopaminergique durant le vieillissement sont accélérés durant la maladie de Parkinson, par une combinaison de facteurs génétiques et environnementaux (Collier *et coll.* 2011). Cette hypothèse est basée sur les niveaux de certains marqueurs (l'α-synucléine, les systèmes lysosome et protéasome, le stress oxydatif, les transporteurs de la DA et les cellules gliales) affectés durant le vieillissement et la maladie de Parkinson selon un patron de région spécifique similaire. Au contraire, d'autres auteurs soutiennent qu'aucune évidence ne supporte que la cause principale de la maladie de Parkinson soit le résultat d'un processus dégénératif relié à l'âge, ou que les changements fonctionnels du déclin de l'activité dopaminergique soient associés à un état pré-parkinsonien (Darbin 2012). Ces auteurs soutiennent que la pathologie des corps de Lewy et la perte des

neurones dopaminergiques nigrostriés sont des caractéristiques de la maladie de Parkinson, et ne sont pas des marqueurs du vieillissement. Quoi qu'il en soit, les changements se produisant lors du vieillissement (présentés à la section 2.3.1. du chapitre 2, telles une diminution de la fonction mitochondriale, une augmentation du stress oxydatif et de l'inflammation, ainsi qu'une diminution de l'activité dopaminergique (Collier *et coll.* 2011)) semblent rendre les neurones dopaminergiques plus vulnérables aux injures. Ceci est supporté par les modèles animaux montrant que l'administration de MPTP chez des singes et des souris âgés produit une dégénérescence plus importante des neurones dopaminergiques que lorsque la toxine est administrée chez des animaux plus jeunes (McCormack *et coll.* 2004, Jiang *et coll.* 2014).

Les effets du 17β-estradiol sur la fonction des neurones dopaminergiques, la fonction mitochondriale, le stress oxydatif, la survie cellulaire et l'inflammation (Vegeto *et coll.* 2008, Morissette *et coll.* 2008, Brewer *et coll.* 2009, Simpkins *et coll.* 2010, Sanchez *et coll.* 2010, Arnold *et coll.* 2012, D'Alessandro *et coll.* 2012), suggèrent que les actions de ce stéroïde permettent au système d'être mieux outillé pour répondre à une insulte. Fonctionnellement, l'utilisation de thérapies hormonales à la ménopause a des effets bénéfiques sur la performance cognitive et les habiletés motrices fines (Greendale *et coll.* 2009, Bayer & Hausmann 2010). Les études chez les femmes post-ménopausées utilisant une thérapie hormonale rapportent une augmentation de la densité du DAT dans le putamen antérieur (Gardiner *et coll.* 2004), et que l'utilisation à long terme des thérapies hormonales augmente l'activité dopaminergique (Craig *et coll.* 2004). De plus, chez les femmes post-ménopausées utilisant une thérapie hormonale, une connectivité thalamo-corticale a été observée, suggérant que les œstrogènes peuvent préserver l'intégrité des systèmes dopaminergiques et cholinergique, et que les œstrogènes peuvent influencer les fonctions sensorimotrices (Kenna *et coll.* 2009). De plus, comme présenté à la section 3.1.9.1. du chapitre 3, l'utilisation de thérapies hormonales est associée à une diminution du risque de développer la maladie de Parkinson (Liu & Dluzen 2007).

Un effet bénéfique du G1 sur la cognition est aussi rapporté (Hawley *et coll.* 2014), suggérant que la stimulation du GPER1 peut être bénéfique sur le déclin cognitif qui peut

survenir durant le vieillissement. De plus, des fonctions sérotoninergiques sont régulées par le GPER1, suggérant un effet de ce récepteur dans l'action antidépressive du 17β-estradiol (Xu *et coll.* 2009, McAllister *et coll.* 2012). Les études chez les rattes ovariectomisées ont montré que le raloxifène renverse les effets de l'ovariectomie sur la liaison spécifique au DAT et aux récepteurs D2 dans le striatum (Landry *et coll.* 2002, Le Saux & Di Paolo 2006), suggérant que le raloxifène a un effet bénéfique sur le maintien des fonctions dopaminergiques.

8.3. L'utilisation de composés oestrogéniques et de la progestérone chez l'homme

Lors de cette thèse, nous avons étudié les mécanismes d'actions de composés oestrogéniques et l'effet bénéfique de la progestérone chez des souris mâles. Des études précédentes ont montré que l'utilisation de la testostérone et de la dihydrotestostérone n'induit pas d'effet neuroprotecteur contre la toxicité du MPTP et de la 6-OHDA (Dluzen 1996, Murray *et coll.* 2003, Gillies *et coll.* 2004, Ekue *et coll.* 2002). La testostérone peut être métabolisée en 17β-estradiol et en dihydrotestostérone, ce dernier ne pouvant être converti en 17β-estradiol. L'absence de protection de la testostérone chez les souris MPTP suggère que ce stéroïde n'est pas converti en des concentrations suffisantes de 17β-estradiol pour induire un effet protecteur (Ekue *et coll.* 2002). L'investigation d'un effet androgénique spécifique avec la dihydrotestostérone chez la souris MPTP a montré que la stimulation des récepteurs des androgènes n'induit pas d'effet neuroprotecteur (Ekue *et coll.* 2002). Les études investiguant l'effet d'une thérapie de remplacement avec la testostérone sur les symptômes moteurs de la maladie de Parkinson chez des patients ayant une déficience en testostérone sont peu nombreuses, le nombre de patients inclus dans l'étude est petit et les résultats ne sont pas consistants. Quoique certaines études rapportent une certaine amélioration des symptômes moteurs avec l'utilisation de la testostérone (Okun *et coll.* 2002a, Mitchell *et coll.* 2006), d'autres études n'observent aucune amélioration (Okun *et coll.* 2002b, Okun *et coll.* 2002a, Okun *et coll.* 2006). Ainsi, il y a peu d'évidence suggérant un effet bénéfique de l'augmentation des niveaux de testostérone sur la protection des neurones dopaminergiques et sur les symptômes moteurs de la maladie de Parkinson.

Quoique l'importance des œstrogènes dans le maintien du système dopaminergique ait été davantage étudiée chez les souris femelles, la déficience en ERs a mis en évidence le rôle des œstrogènes sur l'intégrité et la fonction du système dopaminergique chez les souris mâles. La déficience en ERα chez les souris mâles induit une augmentation de la liaison spécifique au DAT dans le striatum (Al-Sweidi *et coll.* 2011). Chez les souris mâles déficientes en ERβ, une réduction de la liaison spécifique au DAT et au VMAT2 dans le striatum, ainsi qu'une diminution dans le renouvellement de la DA, est observée (Morissette *et coll.* 2007, Al-Sweidi *et coll.* 2011), suggérant un rôle dominant du ERβ dans le contrôle de l'activité dopaminergique. Les souris mâles déficientes en ERα ont une réduction dans leurs niveaux d'ARNm et de protéines de la TH à la substance noire (Kuppers *et coll.* 2008). En support du rôle du ERβ dans la survie neuronale et dans le maintien des neurones nigraux, les souris mâles âgées déficientes en ERβ présentent une dégénérescence des corps cellulaires neuronaux, principalement dans la substance noire (Wang *et coll.* 2001). Chaque sous-type de ER a un rôle dans la modulation et la préservation du système dopaminergique, mais chaque ER semble être impliqué dans la régulation de fonctions différentes. Ainsi, étant donné le rôle des ERs dans la fonction du système dopaminergique, ces données suggèrent que l'utilisation de composés œstrogènes pourrait être bénéfique pour le maintien du système dopaminergique lors du vieillissement chez les hommes.

Les études réalisées chez l'humain ont montré que les composés oestrogéniques ont un effet sur les fonctions cérébrales chez les hommes. En effet, un traitement avec le raloxifène chez des hommes âgés en santé augmente l'activation du cerveau lors de l'encodage de nouvelles informations et durant la recognition d'item familier, et ce, sans effet secondaire, suggérant un effet bénéfique sur l'attention, la mémoire et les fonctions exécutives (Goekoop *et coll.* 2006, Goekoop *et coll.* 2005). En plus de moduler les symptômes parkinsoniens chez les femmes (Sanchez *et coll.* 2010), comme présenté à la section 3.1.9.2. du chapitre 3, une étude rapporte un effet bénéfique de l'utilisation du 17β-estradiol chez un homme ayant un diagnostic de la maladie de Parkinson depuis 17 ans, et présentant de sévères fluctuations motrices et des dyskinésies (Adams & Kumar 2013). Dans cette étude, l'administration transdermale de 17β-estradiol a permis de diminuer les

fluctuations motrices et les dyskinésies, et de réduire de 35% les doses de L-DOPA utilisées (Adams & Kumar 2013). L'effet bénéfique du 17β-estradiol s'est maintenu pendant trois ans.

8.4. Effets neuroprotecteurs chez les deux sexes

Les expériences réalisées lors de ce projet de thèse ont été investiguées chez des souris mâles afin de reproduire la plus forte incidence et prévalence de la maladie de Parkinson chez les hommes. Néanmoins, l'effet neuroprotecteur du 17β-estradiol et de la progestérone est rapporté autant chez les souris mâles que chez les souris femelles (Bourque *et coll.* 2009). De plus, les études cliniques ont démontré l'efficacité de la progestérone lors d'un traumatisme cérébrale autant chez l'homme que chez la femme (Wright *et coll.* 2007, Xiao *et coll.* 2008). L'action bénéfique du raloxifène est aussi observé à la fois chez des rongeurs mâles et femelles (Grandbois *et coll.* 2000, Callier *et coll.* 2001, Baraka *et coll.* 2011), et les études chez l'humain rapportent un effet bénéfique du raloxifène sur la cognition chez les hommes et les femmes (Yaffe *et coll.* 2005, Goekoop *et coll.* 2006, Goekoop *et coll.* 2005, Jacobsen *et coll.* 2012, Jacobsen *et coll.* 2010). Les études réalisées dans le cadre de cette thèse et les études précédentes ont davantage investigué les sous-types de récepteurs des œstrogènes impliqués dans la neuroprotection du système dopaminergique chez des souris mâles (D'Astous *et coll.* 2004b, Morissette *et coll.* 2007, Al-Sweidi *et coll.* 2011). Il est possible que les récepteurs œstrogéniques impliqués dans la neuroprotection des neurones dopaminergiques soient différents chez les souris femelles. En effet, des niveaux plus élevés de ERα dans le striatum ont été observés chez les souris femelles, comparativement aux souris mâles (Rodriguez-Navarro *et coll.* 2008). Par contre, aucune différence entre les sexes n'est observée dans les niveaux striatales de GPER1 (Bourque *et coll.* 2011). Quoique le rôle neuroprotecteur du GPER1 chez des souris femelles traitées au MPTP n'ait pas été investigué dans cette thèse, une autre étude rapporte un effet bénéfique de la stimulation du GPER1 chez des rongeurs femelles lors d'une ischémie (Lebesgue *et coll.* 2010). De plus, une localisation différente des sous-types de récepteurs des œstrogènes peut être présente entre les sexes, ce qui peut conduire à un mécanisme d'action différent. Quoique nos résultats montrent une interaction entre le ERα et le GPER1 dans la neuroprotection des neurones dopaminergiques chez les souris mâles, nous ne pouvons confirmer que celle-ci se produit chez les souris

femelles, malgré la présence d'un patron de distribution similaire entre le ERα et le GPER1 dans le striatum (Almey *et coll.* 2012). Néanmoins, cette interaction pourrait aussi se produire chez les rongeurs femelles, puisque la signalisation et la neuroprotection par le 17β-estradiol dans un modèle d'ischémie cérébrale impliquent à la fois le ERα et le GPER1 (Tang *et coll.* 2014).

8.5. Le modèle

Lors des études réalisées au cours de cette thèse, nous avons utilisé le modèle de la souris mâle C57Bl/6 traitée avec le MPTP, pour induire une lésion modérée du système dopaminergique nigrostrié. Les doses de MPTP utilisées produisent une diminution d'environ 50% des marqueurs dopaminergiques au niveau des terminaisons dans le striatum, et peu d'effet sur les corps cellulaires de la substance noire. Ainsi, nous utilisons ce modèle de dégénérescence modérée pour reproduire le stade précoce de la maladie de Parkinson. Ce stade précoce représente un temps approprié pour évaluer la capacité de neuroprotection d'un composé, puisque les neurones dopaminergiques sont endommagés, mais non mort. Les composés oestrogéniques sont actifs durant cette période, puisqu'un effet bénéfique des œstrogènes sur les symptômes parkinsoniens est rapporté dans le stade précoce de la maladie de Parkinson (Saunders-Pullman *et coll.* 1999, Blanchet *et coll.* 1999). Les modèles de toxines (MPTP et 6-OHDA) sont largement utilisés et se sont révélés valide à reproduire la dégénérescence dopaminergique observée dans la maladie de Parkinson. Quoique le modèle MPTP soit largement utilisé pour les études de neuroprotection, ce modèle est critiqué en raison de son faible taux de succès lorsque l'agent protecteur fait l'objet d'études chez l'humain (Olanow & Schapira 2013, Stocchi & Olanow 2013). En effet, la dégénérescence dopaminergique chez ces modèles animaux (MPTP et 6-OHDA) est causée par l'administration aigue de la toxine et ne reflète pas la progression de la maladie, ni l'étiopathogenèse de celle-ci. L'étiologie inconnue de la maladie de Parkinson rend difficile la conception du modèle «parfait», et beaucoup de composés testés jusqu'à maintenant ne ciblaient pas l'ensemble des mécanismes de pathogénèse de cette maladie. Parmi les autres obstacles au développement d'agent neuroprotecteur dans la maladie de Parkinson, on

retrouve une étiologie inconnue (ce qui rend difficile de déterminer quelles sont les cibles thérapeutiques), quelles doses doivent être utilisées dans les études cliniques, la complexité de différentier un effet neuroprotecteur d'un effet symptomatique, et quel groupe de patients étudié (Olanow & Schapira 2013, Stocchi & Olanow 2013). Une étude clinique réalisée chez un groupe de patients à un stade précoce de la maladie est intéressante car la dégénérescence est moins prononcée qu'à un stade plus avancé et la progression de la maladie est plus lente. Par contre, il peut être plus difficile de différentier un effet entre le groupe traité et le groupe placebo. Lorsqu'une étude clinique est réalisée chez un groupe de patient à un stade plus avancé de la maladie, ceci peut faire en sorte que l'effet protecteur d'un composé n'est pas perçu, en raison d'une dégénérescence trop avancée. Malgré que le modèle utilisé lors de cette thèse présente certaines lacunes, les données précliniques et cliniques supportent l'utilisation des œstrogènes comme agent protecteur pour la maladie de Parkinson. En effet, une bonne concordance entre les études chez les modèles animaux et chez l'humain est observée avec les œstrogènes, puisque le 17β-estradiol protège contre l'effet toxique du MPTP et de la 6-OHDA, et que l'exposition aux œstrogènes diminue le risque de maladie de Parkinson chez l'humain (Liu & Dluzen 2007, Bourque *et coll.* 2009).

Les souris que nous avons utilisées étaient de jeunes adultes. Quoique l'apparition des symptômes de la maladie de Parkinson se produise vers l'âge de 60 ans, le processus de dégénérescence commence avant l'apparition des symptômes. Ainsi, l'utilisation d'un modèle de souris âgée ne reflète pas l'âge de début de la maladie. Une autre variable à considérer dans le choix de l'âge des souris est la perte de sensibilité du cerveau aux hormones ovariennes lors du vieillissement ou de la ménopause. Les études chez des rattes ovariectomisées ont montré que pour obtenir un maintien optimal de la fonction dopaminergique, le traitement par le 17β-estradiol doit être initié peu de temps suivant l'ovariectomie (Sanchez *et coll.* 2010). De plus, les études chez les rongeurs âgés supportent qu'une longue période de privation hormonale à un impact négatif sur la capacité d'un traitement oestrogénique, ou avec la progestérone, à exercer un effet bénéfique sur la cognition et la neuroprotection (Singh & Su 2013a, Schreihofer & Ma 2013). Ces conclusions sont aussi supportées par les études cliniques, qui rapportent un effet bénéfique de

l'utilisation des œstrogènes sur les fonctions cognitives et le risque de démence lorsque la thérapie hormonale est initiée tôt lors de la ménopause (Greendale *et coll.* 2009). Au contraire, les études cliniques ont montré une augmentation des risques de démence et de déclin cognitif lorsque les thérapies hormonales sont débutées plusieurs années après la ménopause (Rossouw *et coll.* 2002, Shumaker *et coll.* 2003). Ainsi, avec le vieillissement et la diminution des hormones ovariennes durant la ménopause, le cerveau perd sa sensibilité aux hormones et à leurs effets bénéfiques. Ceci pourrait s'expliquer par une diminution des récepteurs des œstrogènes et de la progestérone dans certaines régions du cerveau lors du vieillissement (Singh & Su 2013a, Schreihofer & Ma 2013). Les études chez des rongeurs jeunes adultes et d'âge moyen ont révélé que le cerveau répond aux hormones et que le 17β-estradiol ou la progestérone est capable d'induire un effet neuroprotecteur (Singh & Su 2013a, Schreihofer & Ma 2013), suggérant un temps propice pour l'initiation des thérapies hormonales.

Les comparaisons des mécanismes d'action du 17β-estradiol avec d'autres modèles de dommages au cerveau (ischémie, traumatisme cérébral) est utile pour l'avancement de notre compréhension des mécanismes de neuroprotection. L'ischémie et le traumatisme cérébral sont des pathologies impliquant plusieurs processus tels que l'inflammation, l'apoptose, le stress oxydatif et la perturbation de l'homéostasie ionique (Kumar & Loane 2012, Kaur *et coll.* 2013). Ainsi, comme rapporté dans la maladie de Parkinson, plusieurs mécanismes de pathogénèse sont observés lors d'une ischémie et d'un traumatisme cérébral. Ceci supporte que le 17β-estradiol et la progestérone soient des composés ayant plusieurs cibles et suggère que plusieurs mécanismes de neuroprotection sont semblables lors de divers dommages au cerveau.

8.6. Conclusion

8.6.1. Contribution

Les risques périphériques associés à l'utilisation des thérapies hormonales limitent actuellement leur utilisation pour la prévention des maladies neurodégénératives. L'investigation des mécanismes impliqués dans la neuroprotection et le rôle des différents

types de récepteurs des œstrogènes fournissent des informations cruciales afin de trouver de nouvelles stratégies pour conserver les effets bénéfiques des œstrogènes, tout en minimisant les risques associés à leur utilisation. En effet, les travaux de recherche effectués dans le cadre de cette thèse ont permis de mettre en évidence le rôle neuroprotecteur du GPER1, son implication dans l'effet protecteur du 17β-estradiol et du raloxifène, ainsi que la collaboration du ERα avec le GPER1. Ainsi, nous avons montré un rôle central du GPER1, un récepteur non-féminisant, dans la neuroprotection, et nos résultats supportent la considération de cibler le GPER1 pour prévenir les maladies neurodégénératives et dans le développement de nouvelle thérapie pour le maintien et la neuroprotection des fonctions neurologiques. De plus, nous avons montré que la progestérone possède des capacités de neuroprotection et de neurorécupération du phénotype des neurones dopaminergiques. Les effets de récupération suggèrent que la progestérone pourrait avoir un effet bénéfique chez les patients lorsqu'administrée suivant le diagnostic de la maladie de Parkinson.

8.6.2. Perspectives

Les travaux réalisés lors de cette thèse ont montré l'effet neuroprotecteur de l'agoniste du GPER1, ainsi que le rôle de ce récepteur dans l'effet du 17β-estradiol et du raloxifène, pour contrer la dégénérescence des neurones dopaminergiques induite par le MPTP. Néanmoins, beaucoup reste à investiguer sur les mécanismes de neuroprotection et de neuromodulation du GPER1. Son rôle sur la fonction mitochondriale, le stress oxydatif et l'inflammation demeure à investiguer. Nous avons montré un rôle du GPER1 dans la neuromodulation du système dopaminergique. L'implication du GPER1 dans le maintien du système dopaminergique et des fonctions cérébrales lors du vieillissement et de la ménopause reste à explorer.

Nous avons montré que l'administration du G1 et du raloxifène a un effet neuroprotecteur lorsque ces composés sont utilisés avant la lésion avec le MPTP. Néanmoins, le diagnostic de la maladie de Parkinson se fait lorsque les symptômes moteurs apparaissent, soit lorsque environ 50 à 60% des projections dopaminergiques sont perdues. Le potentiel de ces composés pour retarder, arrêter ou renverser la progression de la dégénérescence reste à étudier.

Les résultats de cette thèse ont présenté le rôle récupérateur de la progestérone sur les neurones dopaminergiques. Dans une perspective clinique, la fenêtre d'opportunité thérapeutique de la progestérone pour renverser les dommages aux neurones demeure à définir. Le rôle du GPER1 dans l'effet neuroprotecteur de la progestérone requière davantage d'investigation. En effet, quoique la progestérone n'ait pas d'affinité pour le GPER1 (Thomas *et coll.* 2005), une étude a révélé que le GPER1 co-immunoprécipite avec le mPR, suggérant que le GPER1 pourrait être impliqué dans certains effets bénéfiques de la progestérone (Akama *et coll.* 2013).

Un modèle de dégénérescence progressive représenterait mieux la perte graduelle des neurones dopaminergiques observée chez les patients parkinsoniens. Deux groupes ont développé des modèles de souris génétiques, où des dysfonctions mitochondriales conduisent à une perte progressive des neurones dopaminergiques (Ekstrand *et coll.* 2007, Pickrell *et coll.* 2011). Ces modèles seraient pertinents pour étudier l'effet des agents neuroprotecteurs, tels les composés oestrogéniques et la progestérone, à empêcher ou retarder la dégénérescence du système dopaminergique nigrostrié.

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Neuroprotective actions of sex steroids in Parkinson's disease

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

[a] Molecular Endocrinology and Genomic Research Center, Laval University Medical Center, CHUL, 2705 Laurier Blvd, Quebec City, Quebec, Canada G1V 4G2
[b] Faculty of Pharmacy, Laval University, Quebec City, Quebec, Canada G1K 7P4
[c] Department of Anatomy, Northeastern Ohio Universities College of Medicine (NEOUCOM), Rootstown, OH 44272, USA

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ABSTRACT

The sex difference in Parkinson's disease, with a higher susceptibility in men, suggests a modulatory effect of sex steroids in the brain. Numerous studies highlight that sex steroids have neuroprotective properties against various brain injuries. This paper reviews the protective effects of sex hormones, particularly estradiol, progesterone and androgens, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of Parkinson's disease as compared to methamphetamine toxicity. The molecular mechanisms underlying beneficial actions of sex steroids on the brain have been investigated showing steroid, dose, timing and duration specificities and presently focus is on the dopamine signaling pathways, the next frontier. Both genomic and non-genomic actions of estrogen converge to promote survival factors and show sex differences. Neuroprotection by estrogen involves activation of signaling molecules such as the phosphatidylinositol-3 kinase/Akt and the mitogen-activated protein kinase pathways. Interaction with growth factors, such as insulin-like growth factor 1, also contributes to protective actions of estrogen.

1. Introduction

Sex steroids are involved in functions that extend beyond reproduction. Estrogen actions are implicated in cognition, synaptic plasticity, memory, neurogenesis and neuroprotection [22]. Hormone therapy is commonly used to compensate for ovarian hormone deficits at menopause and has been associated with a lower risk of Parkinson's disease (PD) [34] and Alzheimer's disease [80]. Several variables can influence the beneficial effects of hormone therapy [105,162]. Here, we review the effect of estrogen, progesterone and androgens in the brain with an emphasis on animal models of PD as well as the molecular mechanisms implicated in estrogen neuroprotection.

2. Parkinson's disease, estrogens and sex differences

PD is the second most common neurodegenerative disorder mainly characterized by a progressive and selective depletion of dopamine (DA) neurons in the substantia nigra [83]. Typically, a greater incidence and prevalence of PD is described in men [5,20,99,103,112,114,129,177], (meta-analysis: [184] reviews: [35,162]) whereas no sex difference was also reported [43]. This sex difference suggests a beneficial influence of estrogens against the development and progression of PD. Conditions causing an early reduction in endogenous estrogen seem to increase the risk to develop PD. Women with PD tend to have an earlier menopause, are more likely to have undergone hysterectomy and used estrogen therapy less frequently than control subjects [11]. Ragonese et al. [143] found an association between factors reducing estrogen stimulation during life (short length of fertility, a cumulative duration of pregnancies longer than 30 months and an earlier time of menopause) and the development of PD. This was recently confirmed in a large group of 83,482 women participating in the Observational Study of the Women's Health Initiative (WHI-OS) showing that longer fertile lifespan among women with natural menopause (age at menopause – age at menarche) was associated with reduced risk of PD [154]. A case-control study reports that women with PD were less likely to have used postmenopausal estrogen and the use of postmenopausal estrogen was a significant factor in reducing the risk of PD [34]. A worsening of parkinsonian symptoms was associated with menstruation [150]. A more rapid progression of parkinsonian symptoms associated with pregnancy and also worsening of symptoms during or shortly after delivery have been described in young PD women, supporting a complex role of ovarian hormones in the modulation of PD symptoms [150]. Sex differences on the evolution of symptoms and responses to levodopa treatment are also reported [75,107,162,191], with men exhibiting more severe parkinsonian motor features than women and improvement of motor function is greater in women following levodopa administration. Symptoms of PD and levodopa-induced dyskinesias are shown to be modulated by estrogens [7,45,68,148,157], and improvement of dyskinesias

* Corresponding author. Address: Molecular Endocrinology and Genomic Research Center, Laval University Medical Center, CHUL, 2705 Laurier Blvd, Quebec City, Quebec, Canada G1V 4G2. Fax: +1 418 654 2761.
E-mail address: therese.dipaolo@crchul.ulaval.ca (T. Di Paolo).

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and parkinsonian symptoms are observed with estrogen therapy or high levels of endogenous estrogen. Lower severity of PD symptoms are reported in women with early PD undergoing estrogen therapy prior to initiation of levodopa [155] but estrogen therapy had no effect at later stages of the disease [168]. Estrogen was reported to improve motor disability in parkinsonian postmenopausal women with motor fluctuations [175]; a slight antiparkinsonian effect [16] or no effect [168] was also observed.

3. Models of Parkinson's disease

The sex differences and effects of estrogens in PD reviewed in the previous section have fueled research in animal models to decipher which steroid is active and under what conditions. Moreover, hormonal modulation and protection of brain neurotransmission has received much attention in the last two decades [45,48,105,126,127]. The neuroprotective effects of estrogen have been studied in various *in vitro* and *in vivo* experimental models. Beneficial effects of estradiol are observed in several types of neurons. Estradiol protects against neuronal damage induced by glutamate excitotoxicity, β-amyloid peptide and toxicity induced by 6-hydoxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium ion (MPP+) [28,58,164]. Estradiol treatment also decreases apoptosis in response to serum deprivation and oxidation by H_2O_2 [72,156].

3.1. MPTP

The neuroprotective properties of estrogen have been demonstrated in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal models of idiopathic PD. MPTP is a by-product of the chemical synthesis of a meperidine analog that causes parkinsonism in humans [100]. This toxin is highly lipophilic and exerts relatively selective damages upon substantia nigra DA neurons in humans and animals [77,101,138]. In support of the usefulness of the MPTP model, altered gene expression profiles reveal similarities in the substantia nigra of MPTP treated mice, α-synuclein mutant mice and human PD for genes related to DA phenotype, synaptic function and mitochondrial metabolism [121]. Sex differences in sensitivity to MPTP have been reported, with a greater neurotoxic effect in male than in female mice [51,119].

3.2. Methamphetamine

Methamphetamine (MA) is a potent and addictive drug of abuse. This psychostimulant causes degeneration of striatal DA nerve terminals in humans and animals as shown by long-lasting depletion in DA concentration and DA transporter (DAT) [115,128,180,182]. Studies in MA treated mice have shown that male mice are more sensitive to MA-induced toxicity showing greater reductions in striatal DA content than females [119,181,190] as well as 3,4-dihydroxyphenylacetic acid (DOPAC) concentration (Fig. 1). Sex differences to MA toxicity are also observed on [^{125}I]-RTI-121 DAT specific binding in striatum, with a more substantial reduction observed in male mice (Fig. 2). The decrease of DAT specific binding seen in female mice was only present in the lateral part of the striatum and this region is the most affected by MA treatment [19,38,198].

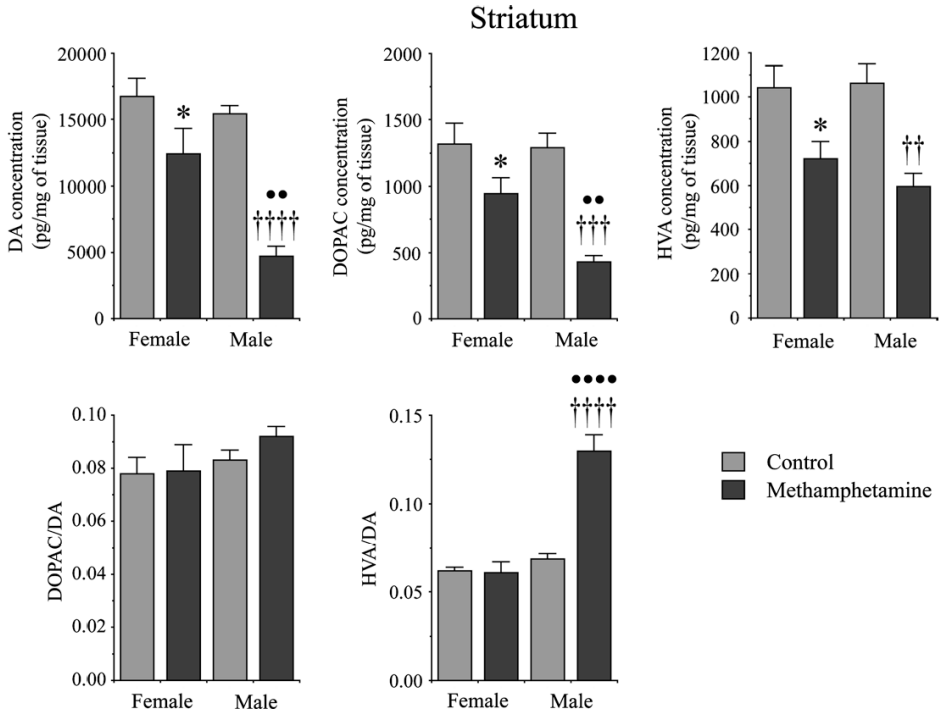

Fig. 1. Effect of methamphetamine (MA) on striatal DA, DOPAC and HVA concentrations measured by HPLC with electrochemical detection under conditions previously described [64] as well as DOPAC/DA and HVA/DA ratios in female and male mice as compared to saline-treated mice (control). Intact mice were treated with 2 intraperitoneal injections of MA (20 mg/kg) at a 2-h interval and euthanasia was a week post-MA. *$p < 0.05$ vs. female control; $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.005$ and $^{\dagger\dagger\dagger\dagger}p < 0.0001$ vs. male control; **$p < 0.01$ and ***$p < 0.005$ vs. female MA. Values are the means (pg/mg of tissue) ± SEM of 4–6 mice per group. 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.

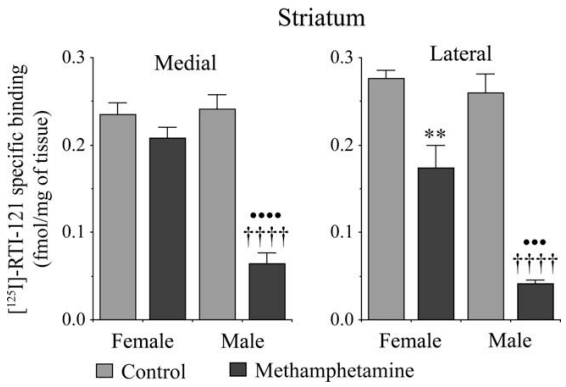

Fig. 2. Effect of methamphetamine (MA) on DAT specific binding measured with 3β-(4-[^{125}I]iodophenyl)tropane-2β-carboxylic acid isopropyl ester [^{125}I]-RTI-121 under conditions previously described [30] in medial and lateral striatum in female and male mice as compared to saline-treated mice (control). Intact mice were treated with two intraperitoneal injections of MA (20 mg/kg) at a 2-h interval and euthanasia was a week post-MA. $^{**}p < 0.01$ vs. female control; $^{††††}p < 0.0001$ vs. male control; $^{•••}p < 0.005$ and $^{••••}p < 0.0001$ vs. female MA. Values are the means (fmol/mg of tissue) ± SEM of 4–6 mice per group. Statistical comparisons of the data were as described in legend to Fig. 1.

4. Neuroprotection by sex steroids

4.1. Estrogens and neuroprotection

Estrogens are steroidal compounds; the main physiological molecules are estradiol, estrone and estriol. Estrone and estriol as well as the stereoisomer 17α-estradiol have less affinity on estrogen receptors (ERs) than 17β-estradiol [95]. The protective properties of these estrogens have been investigated in various experimental models of brain injury. Studies report that estrone has beneficial effects against kainate, $FeCl_2$, NMDA, serum deprivation, glutamate, β-amyloid peptide, glucose deprivation, H_2O_2 toxicities and ischemic injury [4,70,88,89,161,179]. Estriol has been shown to protect against glucose deprivation, $FeSO_4$, β-amyloid peptide, NMDA and glutamate toxicities [70,87]. Our laboratory investigated whether estrone, estriol and 17β-estradiol have neuroprotective effects in MPTP-treated mice [86]. The measure of various DA markers has shown that estriol lacks neuroprotective activity whereas estrone shows some, albeit weak activity compared to 17β-estradiol, supporting the close link between affinity for ER binding and neuroprotective effects against MPTP toxicity within the nigro-striatal DA system. The stereoisomer 17α-estradiol was also assessed in neuroprotection studies. *In vitro* studies report that 17α-estradiol protects neurons against brain injury induced by 6-OHDA, MPP+, glutamate and serum deprivation [28,72,156]. The effect of 17α-estradiol and 17β-estradiol against injury can be because of their antioxidant activity that is observed at higher than physiological levels (micromolar concentrations) [9]. Furthermore, antioxidant properties of estradiol are not stereo-specific, are independent of an activation of ER and are associated with the presence of an hydroxyl group in the C3 position on the A ring of the steroid molecule [9]. In MPTP-treated mice, 17α-estradiol shows no protective activity against the toxin, suggesting that neuroprotective activities are mediated by an ER [29,50,144].

4.1.1. Dose and treatment duration

17β-estradiol pre-treatment prevents MPTP-induced striatal DA depletion in male and female mice [29,30,39,41,51,53,86,119,127,144] (Table 1). Beneficial effects of 17β-estradiol against MPTP DA toxicity are not observed when high doses of the steroid are administered repeatedly or acutely before and after toxin injection [134,144]. Treatment with a high dose of 17β-estradiol (12 μg, 1 injection) 0.75, 2 or 24 h before MPTP administration failed to show any protection [144]. Nor did a high dose of 17β-estradiol (0.5 or 2 mg/kg) in male and female mice shortly prior (30 min) and after (90 min) MPTP insult provide any protection on striatal DA depletion [134]. A dosing regimen that mimics physiological levels of the steroid was found effective to prevent toxicity. Administration of 1 μg of 17β-estradiol, repeated twice a day, or treatment with a 17β-estradiol pellet (0.1 mg, 60 day release) were shown to induce DA neuroprotection [29,30,39,41,51,144]. With 1–10 day durations of pre-treatment and 2–6 days post-treatment, low doses of 17β-estradiol were effective in inducing DA neuroprotection [29,30,39,41,51,127,144], while one study reported no protection [134].

Estrogen was also effective in preventing DA toxicity induced by MA in intact or ovariectomized female mice (Table 2). The protective effect of 17β-estradiol or estradiol benzoate was obtained when the steroid was administered prior to the toxin while a post-treatment administration was not effective [47,63,65,118,187,188]. Low doses of estradiol benzoate administered acutely (only one injection of 1 μg of estradiol benzoate, 24 h prior to MA) or repeatedly (48 pg or 0.47 μg of estradiol benzoate for 3 consecutive days) were effective for a neuroprotective effect [38,118,187,188]. Not only are physiological dosing regimens of estradiol effective as neuroprotectants, but also endogenous estrous cycle modulation can alter MA-induced neurotoxicity with a greater MA-induced DA depletion in diestrus in BALB/c, but not in the C57BL/6J strain of mice [189]. Different time intervals for estradiol benzoate administration prior to MA were also investigated. While protection on several DA markers was observed with a pre-treatment 24 h before MA, a high dose of 10 μg of estradiol benzoate as early as 0.5 and 12 h preceding MA was effective for a limited number of specific DA parameters [36,63]. Studies have shown that treatment with 10 μg of estradiol benzoate ranging from 15 min [63] to 7-days [65] post-MA failed to prevent striatal DA depletion in ovariectomized female mice. Thus, estradiol cannot reverse striatal neurotoxicity induced by MA. Moreover, when estradiol benzoate is administered a week post-MA, that is, when some initial damage is present, estradiol benzoate treatment not only does not protect against a second administration of MA, but increases the amount of striatal DA depletion [104]. This result is in part consistent with the healthy cell bias of estrogen action, which proposes that if neurons are healthy at the time of estrogen treatment, their response to estrogen is beneficial for both neurological function and survival whereas in the case of impaired function, estrogen exposure over time exacerbates neurological injury [23,33]. Prepubertal (25 days of age) ovariectomy in female mice abolishes the capacity of estradiol to produce neuroprotection against MA toxicity when tested in adulthood (55 days of age) [2]. Furthermore, treatment with estradiol benzoate in female mice ovariectomized at 4 weeks of age has no effect on MA-induced DA toxicity as compared to females ovariectomized at 6 weeks of age where a beneficial effect was reported [187]. However, masculinized female mice, as achieved by neonatal ovariectomy (post-natal days 3–5) and testosterone propionate injection (1.25 mg), retained the capacity to show estrogen neuroprotection against MA when tested as adults (55 days of age) [2]. In male mice, gonadectomy either at the neonatal (post-natal days 3–5) or prepubertal (25 days of age) period failed to result in any neuroprotective effects of estrogen against MA when tested as adults (55 days of age) [2], whereas estradiol treatment in intact male mice produces a severe acute toxicity to MA, with a high incidence of mortality [47].

4.1.2. Dopaminergic markers as a measure of toxicity and neuroprotection

Striatal DA and metabolites contents are commonly used as a measure of MPTP toxicity and to assess protection

Table 1
Effects of sex steroids against MPTP toxicity in C57Bl/6 mice.

Dose and treatment duration		Mice sex and age	Assay	Effect	Refs.
Steroid	MPTP				
17β-estradiol					
• 1 μg B.I.D. for 10 days	4 injections of 15 mg/kg on day 6	Retired breeder male	DA, DOPAC and HVA concentrations; DAT specific binding and mRNA levels	Protection of DA, DOPAC and HVA concentrations Protection of DAT specific binding (moderate lesion) No protection of DAT specific binding (extensive lesion)	[29,30,71]
• 1 μg B.I.D. for 10 days	4 injections of 8, 9 or 10 mg/kg on day 5	Male (10–12 weeks)	DA, DOPAC and HVA concentrations; DAT specific binding and TH mRNA levels	Protection of all markers assayed	[37,39–41]
• 1 μg B.I.D. for 10 days	4 injections of 10 mg/kg on day 6	Adult male	DA, DOPAC and HVA concentrations; DAT specific binding and mRNA levels VMAT2 specific binding	Protection of all markers assayed	[53]
• 2 μg B.I.D. for 10 days	4 injections of 7.5 mg/kg on day 5	Male (10–12 weeks)	DA, DOPAC and HVA concentrations; DAT and VMAT2 specific binding; DAT, VMAT2 and TH mRNA levels	Protection of all markers assayed	[86]
• 1 μg B.I.D. 1, 3 or 5 days before MPTP and 5 days after	4 injections of 15 mg/kg on day 6	Male (age NA)	DA, DOPAC and HVA concentrations	Protection of all markers assayed	[127]
• *12 μg 0.75, 2 or 24 h before MPTP	1 injection of 15 mg/kg	Male (age NA)	DA concentrations	No protection	[144]
• 0.1, 0.32, 1 or 3.2 μg B.I.D. for 11 days	1 injection of 15 mg/kg on day 6	Male (age NA)	DA concentrations	Protection only with 1 μg of 17β-estradiol	[144]
• 1 μg B.I.D. for 5 days and until 2, 4 or 6 days after MPTP	1 injection of 15 mg/kg on day 6	Male (age NA)	DA concentrations	Protection of DA concentrations	[144]
• 0.05 or 0.2 mg/kg B.I.D. for 10 days	4 injections of 10 mg/kg on day 6	Male and female (7–12 weeks)	DA, DOPAC and HVA concentrations	No protection	[134]
• † 0.5 or 2 mg/kg 30 min before and 90 min after MPTP	4 injections of 10 mg/kg	Male and female (7–12 weeks)	DA, DOPAC and HVA concentrations; DAT and TH protein levels	No protection of DA concentrations; protection of TH protein levels in male and female mice with 0.5 and 2 mg/kg; Protection of DAT protein levels in male and female mice with 2 mg/kg.	[134]
• § 17β-estradiol pellet (0.1 mg, 60-day release) for 15 days	4 injections of 10 mg/kg on day 10	Retired breeder GDX male and female	DA concentrations	Protection of DA concentrations	[51]
• 100 μg daily for 9 or 16 days	4 injections of 20 mg/kg on day 6	C57/blk6 GDX male (age NA)	TH immunoreactivity	Protection of TH immunoreactivity	[160]
• 2 μg B.I.D. for 11 days	4 injections of 15 mg/kg on day 6	Male (14 weeks)	TH immunoreactivity	Protection of TH immunoreactivity	[174]
• § 17β-estradiol pellet (0.1 mg, 21-day release) for 14 days	4 injections of 10 mg/kg on day 7	Retired breeder OVX female	DA concentrations	Protection of DA concentrations	[50]
• § 17β-estradiol pellet (235 μg/ml) before (time not specified) MPTP and 2 days after	1 injection of 12.5 mg/kg	Retired breeder OVX female	DA, DOPAC and HVA concentrations; Glial fibrillary acidic protein levels (GFAP)	Protection of DA, DOPAC and HVA concentrations Decrease GFAP elevation induced by MPTP	[119]
Estradiol benzoate					
• 50 μg/kg for 8 days	1 injection of 30 mg/kg	OVX female (10–12 weeks)	DA, DOPAC and HVA concentrations; TH immunoreactivity DAT and TH mRNA levels	Protection of all markers assayed	[106]

17α-estradiol					
• 1 μg B.I.D. for 10 days	4 injections of 15 mg/kg on day 6	Retired breeder male	DA, DOPAC and HVA concentrations; DAT specific binding and mRNA levels	No protection	[29,30,71]
• 1 μg B.I.D. for 10 days	4 injections of 10 mg/kg on day 5	Male (10–12 weeks)	DA, DOPAC and HVA concentrations	No protection	[39]
• 0.1, 0.3, 1 or 3.2 μg B.I.D. for 11 days	1 injection of 15 mg/kg on day 6	Male (age NA)	DA concentrations	No protection	[144]
• *12 μg 0.75, 2 or 24 h before MPTP	1 injection of 15 mg/kg	Male (age NA)	DA concentrations	No protection	[144]
• § 17α-estradiol pellet (0.1 mg, 21-day release) for 14 days	4 injections of 10 mg/kg on day 7	Retired breeder OVX female	DA concentrations	No protection	[50]
Estrone					
• 2 μg B.I.D. for 10 days	4 injections of 7.5 mg/kg on day 5	Male (10–12 weeks)	DA, DOPAC and HVA concentrations; DAT and VMAT2 specific binding; DAT, VMAT2 and TH mRNA levels	Protection of DAT specific binding in striatum and VMAT2 mRNA levels in substantia nigra	[86]
Estriol					
• 2 μg B.I.D. for 10 days	4 injections of 7.5 mg/kg on day 5	Male (10–12 weeks)	DA, DOPAC and HVA concentrations; DAT and VMAT2 specific binding; DAT, VMAT2 and TH mRNA levels	No protection	[86]
Progesterone					
• 1 μg B.I.D. for 10 days	4 injections of 15 mg/kg on day 6	Retired breeder male	DA, DOPAC and HVA concentrations; DAT and VMAT2 specific binding and mRNA levels	Protection of DA and HVA concentrations as well as DAT specific binding	[30,71]
• 1 μg B.I.D. for 10 days	4 injections of 15 mg/kg on day 6	Male (12 weeks)	DA concentrations	Protection of DA concentrations	[127]
17β-estradiol + Progesterone					
• 1 μg B.I.D. of 17β-estradiol and 1 μg B.I.D. of progesterone for 10 days	4 injections of 15 mg/kg on day 6	Male (12 weeks)	DA concentrations	Protection of DA concentrations	[127]
Testosterone					
• 50 μg testosterone B.I.D. for 10 days	4 injections of 10 mg/kg on day 6	Adult male	DA, DOPAC and HVA concentrations; DAT specific binding and mRNA levels; VMAT2 specific binding	No protection	[53]
• § Testosterone pellet (0.1 mg, 21-day release) for 17days	4 injections of 10 mg/kg on day 12	Retired breeder GDX male	DA and DOPAC concentrations	No protection	[46]
Dihydrotestosterone					
• 1 or 50 μg B.I.D. for 10 days	4 injections of 10 mg/kg on day 6	Adult male	DA, DOPAC and HVA concentrations; DAT specific binding and mRNA levels; VMAT2 specific binding	No protection	[53]

Steroid treatments were administered s.c. except for Ref. [106,134] (estradiol was injected i.p.). MPTP treatment was administered i.p. except for Ref. [119,160] (MPTP was injected s.c.). Mice were sacrificed 24 h after their last steroid injection except for * (sacrificed 3 days after MPTP treatment, Ref. [144]), for † (sacrificed 5 days after MPTP treatment, Ref. [134]) and § (sacrificed the last day of steroid treatment, Ref. [46,50,51,119]). B.I.D. = twice a day; GDX = gonadectomized; i.p. = intraperitoneal; NA = not available; OVX = ovariectomized; s.c. = subcutaneous.

Effects of sex steroids against methamphetamine toxicity in mice.

Dose and treatment duration		Mice sex and age	Assay	Effect	Refs.
Steroid	Methamphetamine				
17β-estradiol					
• 17β-estradiol pellet (0.1 mg, 21-day release) for 14 days	4 injections of 20 mg/kg on day 7	Retired breeder CD-1 OVX female; Retired breeder CD-1 GDX male	DA concentrations	Protection of DA concentrations in female but not in male mice	[64,65]
• 17β-estradiol pellet (0.1 mg, 21-day release) for 14 days	7 days post-MA (1 × 40 mg/kg) treatment with 17β-estradiol pellet (0.1 mg, 21-day release) for 7 days	Retired breeder CD-1 OVX female	DA concentrations	No protection	[65]
• 17β-estradiol pellet (0.1 mg, 21-day release) for 14 days	4 injections of 20 mg/kg on day 7	Intact and GDX CD-1 female and male (60 days old)	DA concentrations	Protection in female but not in male mice	[47,49]
• 17β-estradiol pellet (0.1 mg, 21-day release) for 14 days. 17β-estradiol treatment at 55 days of age	4 injections of 20 mg/kg on day 7	CD-1 female and male GDX at post-natal days 3–5 or at 25 days of age	DA concentrations	No protection	[2]
Estradiol benzoate					
• 10 μg, 1 injection 24, 12 or 0.5 h before MA	1 injection of 40 mg/kg	Retired breeder OVX CD-1 female	DA concentrations DOPAC/DA ratio; DAT specific binding and mRNA levels	Protection of DA concentrations with estradiol treatment (24 or 12 h); DAT specific binding with estradiol treatment (24 h); DAT mRNA levels (24, 12 or 0.5 h)	[36,63]
• 10 μg, 1 injection 0.25, 0.5, 1 or 2 h after MA	1 injection of 40 mg/kg	Retired breeder CD-1 OVX female	DA concentrations DOPAC/DA ratio	No protection	[63]
• 10 μg, 1 injection at one week post-MA	2 or 4 injections of 20 mg/kg (1 week before estradiol) followed by a second regimen of MA (2 × 20 mg/kg) 24 h after estradiol	GDX CD-1 female and male (2-3 months)	DA and DOPAC concentrations	No protection	[104]
• 1, 10 or 40 μg 24 h before MA injections	1 injection of 40 mg/kg	OVX CD-1 female (2–3 months)	DA concentrations; DAT and VMAT2 specific binding	Protection of all markers assayed	[38,118]
• 48 pg or 0.47 μg for 3 days before MA injections	4 injections of 10 mg/kg	GDX C57Bl/6 male and female (4 or 6 weeks)	DA concentrations	Protection in mice gonadectomized at 6 weeks of age but not 4 weeks	[187,188]
Progesterone					
• 0.47 μg or 0.467 mg for 3 days before MA treatment	4 injections of 10 mg/kg	GDX C57Bl/6 male and female (4 or 6 weeks)	DA and DOPAC concentrations	No protection in mice GDX at 4 weeks of age and in female at 6 weeks with the 0.47 μg dose; protection in OVX female at 6 weeks of age with doses of 0.47 μg and 0.467 mg	[187,188]
Estradiol benzoate + progesterone					
• 2 days of estradiol benzoate treatment (0.467 μg) followed by 1 day progesterone treatment (0.467 mg) before MA	4 injections of 10 mg/kg	OVX C57Bl/6 female (6 weeks)	DA concentrations	Protection of DA concentrations	[188]
Testosterone					
• Testosterone pellet (5 mg, 21-day release) for 14 days	4 injections of 10 mg/kg on day 7	Retired breeder GDX CD-1 female and male; Intact and GDX CD-1 female and male (60 days old)	DA concentrations	No protection	[47,64]
• Concentration ranging from 0.005 to 50 μg (1 injection), 24 h before MA	1 injection of 40 mg/kg	Intact and GDX CD-1 male and female (2–3 months)	DA concentrations	No protection, increase MA toxicity in male mice	[102]

Mice were sacrificed one week post-MA except for Refs. [187,188] (mice were sacrificed two weeks after MA). Steroid treatments were administered s.c. MA treatment was administered i.p. except for Refs. [187,188] (MA was injected s.c.). GDX = gonadectomized; i.p. = intraperitoneal; OVX = ovariectomized; s.c. = subcutaneous.

[29,50,119,186]. Increased DA levels could be caused by increased synthesis or decreased metabolism by a hormonal effect on enzymes in the metabolic pathway of DA. However, such effects do not provide an explanation for all the DA marker changes observed. Hence, DA concentrations were found to correlate significantly with striatal DAT specific binding in intact, MPTP or MA lesioned and MPTP or MA lesioned mice protected by various estrogenic and steroid drugs [19,40,41]. The DAT protein is found on DA nerve terminals in the striatum (Fig. 3) and thus support a role of hormones in neuroprotection of DA terminals. Moreover, estradiol neuroprotection in MPTP mice was associated with reduced striatal glial fibrillary acidic protein (GFAP) levels as an independent biochemical marker of neuronal injury [119].

The vesicular monoamine transporter 2 (VMAT2) is present within striatal DA terminals and its role is to sequester cytoplasmic DA, thus preventing DA oxidation (Fig. 3). VMAT2 specific binding was assessed as related to the neuroprotective effect of 17β-estradiol in MPTP mice. DAT and VMAT2 are both implicated in MPTP-induced toxicity [120,176]. The DAT is involved in MPTP/MPP+ entry and accumulation in DA neurons [13,62] and MPTP/MPP+ can be sequestered by VMAT2 [167]. Hence, the ratio between DAT and VMAT2 provides an index of susceptibility to this neurotoxin, with a high DAT to VMAT2 ratio associated with a greater susceptibility to neurotoxins [120]. Evaluation of VMAT2 and DAT specific binding in mice shows a significant decrease with the MPTP lesion and an increase in 17β-estradiol treated mice as compared to non-17β-estradiol treated MPTP mice [30,92]. This result may be considered somewhat contradictory since a high level of VMAT2 is associated with less susceptibility to damage induced by toxins while a high level of DAT is correlated with increased susceptibility [120]. In tottering mutant mice, the increase expression of VMAT2 binding sites was shown to diminish MPP+ toxicity [93]. However, VMAT2 is only one factor implicated in MPTP toxicity; indeed, strain differences in resistance to MPTP toxicity do not appear to be a result of higher levels of vesicular storage [91]. The lack of change of the DAT/VMAT2 specific binding ratio in MPTP mice with or without hormonal treatment suggests that the lower toxin susceptibility of 17β-estradiol treated MPTP mice was not caused by an increase in VMAT2 levels. This lack of change of the DAT/VMAT2 binding ratio and the positive correlation between DA levels and DAT and VMAT2 levels suggest that binding to the transporters reflects the degree of degeneration. This is consistent with the observation that DAT and VMAT2 specific binding in DA neurons correlate with the extent of DA loss in PD brains [183]. Furthermore, another possibility for the contradictory changes observed for DAT and VMAT2 might be the possibility that these two transporters may be interacting with one another [120]. In this way, considering changes in these two

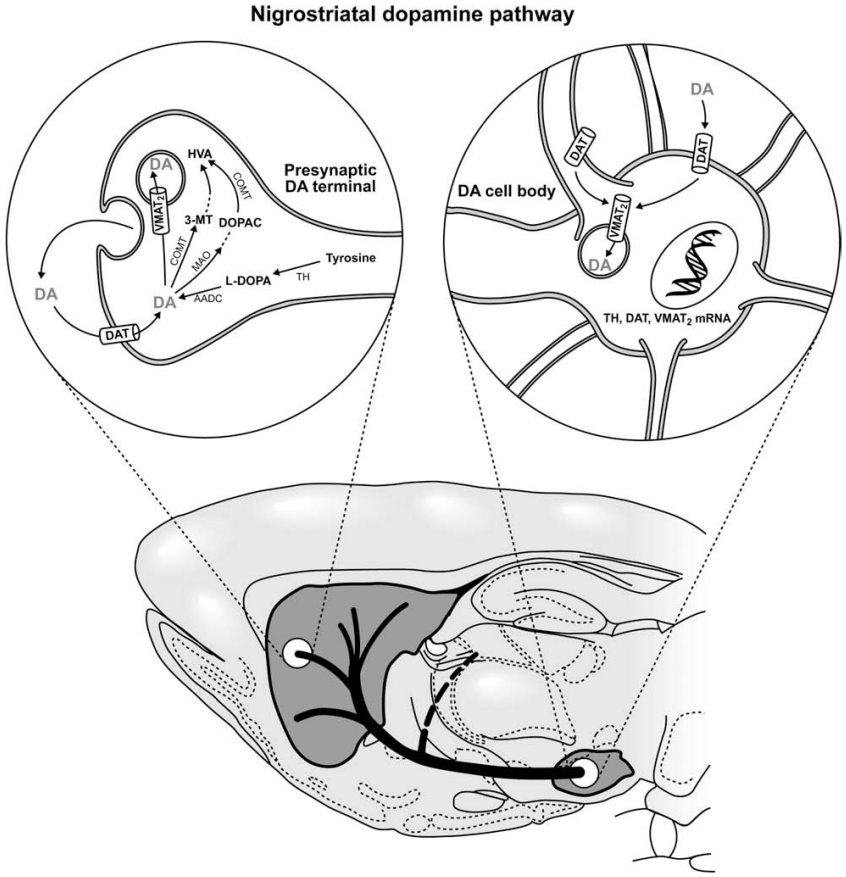

Fig. 3. Schematic illustration of the nigrostriatal dopamine (DA) pathway of the rodent brain and sub-cellular localization of DA, its precursor (L-DOPA) and metabolites (3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA)) as well as the DA membrane transporter (DAT) and vesicular monoamine transporter 2 (VMAT2). Enzymes involved in synthesis (tyrosine hydroxylase (TH)) and metabolism (amino acid decarboxylase (AADC), monoamine oxidase (MAO) and catechol-*O*-mehtyltransferase (COMT)) of DA are shown.

transporters independently may not provide a clear picture of their functions.

DA markers in cell bodies in the substantia nigra were also assessed to probe the extent in MPTP damage. *In situ* hybridization studies show that DAT mRNA levels in the substantia nigra is significantly decreased in MPTP-treated mice [29,30,53] and 17β-estradiol treatment completely prevented the reduction of DAT mRNA levels induced by MPTP [41,53]. The decrease in substantia nigra DAT mRNA levels in MPTP mice is less than that observed in striatal DAT protein binding [53], suggesting that DA neuron cell bodies in the substantia nigra are less affected than their terminals in the striatum [29,30]. The decrease in substantia nigra tyrosine hydroxylase mRNA and DAT mRNA levels [41], as well as VMAT2 mRNA levels [86] induced by MPTP administration is prevented by 17β-estradiol treatment. These observations agree with a report suggesting that with administration of lower concentrations of DA toxins like MPTP, 6-OHDA, ouabain or kainic acid, the loss of dendritic processes in tyrosine hydroxylase positive substantia nigra cells is smaller [27]. At high concentrations of these toxins, the dendritic loss is more extensive and associated with a disintegration of DA cell bodies [27]. These findings suggest that disruption of the dendritic arbor is an early indicator of degeneration irrespective of how it is initiated. This early stage of degeneration, where substantia nigra DA neurons are injured but not dead, may be an appropriate time for neuroprotection by steroids. Indeed, in humans it is in the early stages of PD, before initiating levodopa therapy, that estrogen therapy is reported to be beneficial [155].

Estrogen protection upon measures of DAT and VMAT2 was also evaluated in the MA model of neuronal injury. The DAT is an important target for MA; it is the site by which MA enters the cell terminal and induces release of DA into extracellular space by promoting reverse transport through the DAT [60]. Extra- and intraneuronal accumulation of DA induced by MA leads to formation of reactive oxygen species, which can decrease DAT function [12,59]. DAT and VMAT2 specific binding are reduced in MA treated mice [19,180,198] and estradiol benzoate treatment prevents this decrease in ovariectomized female mice [36,38]. In substantia nigra, DAT specific binding was not significantly reduced following 40 mg/kg MA in female mice whereas a small decrease was observed in male mice [19,38], showing a more extensive MA-induced loss of striatal DAT than in the substantia nigra. These results are consistent with other data showing that damage induced by MA are more effective in altering presynaptic terminals of DA neurons of the striatum than in the cell bodies in the substantia nigra [147]. In agreement, *in situ* hybridization of DAT mRNA in substantia nigra shows a small decrease of DAT mRNA levels in MA lesioned female mice [36]. Estradiol benzoate administration in MA treated female mice completely prevents this decrease [36].

Since estradiol does not induce beneficial effects in MA treated male mice, our research groups have investigated the protective properties of the selective estrogen receptor modulator (SERM) tamoxifen on DA markers [19]. We have shown that tamoxifen pretreatment (1 injection of 50 μg, 24 h before MA insult) diminishes MA induced striatal DA depletion and DAT specific binding and completely prevented the decrease of DAT mRNA levels in male mice.

4.2. Androgens and neuroprotection

In the light of the sex difference in PD and the toxicity of MPTP and MA, the androgens testosterone and dihydrotestosterone were investigated for their neuroprotective role (Tables 1 and 2). Testosterone can be biotransformed in the brain into 17β-estradiol by an aromatization process whereas dihydrotestosterone cannot be converted into 17β-estradiol [117]. In MPTP treated mice, a pre- and post-treatment schedule with testosterone (100 μg/day for 10 days [53] or a 21 day release via a 0.1 mg testosterone pellet for 17days) [46] or dihydrotestosterone (2 or 100 μg/day for 10 days) [53] did not protect against MPTP induced DA depletion in intact or gonadectomized male mice. More specifically, testosterone and dihydrotestosterone do not prevent the decrease induced by MPTP in DA and its metabolites concentrations, DAT and VMAT2 specific binding in striatum and DAT mRNA levels in substantia nigra, whereas testosterone treatment increased the effect of MPTP on DAT mRNA levels [53]. These results suggest that at the doses used, testosterone is not transformed into 17β-estradiol in the brain in sufficient concentrations to reach protective levels. Moreover, androgenic activity, evaluated with dihydrotestosterone, is also not able to protect striatal DA against MPTP toxicity [53]. The potential for a neuroprotective effect of testosterone was also assessed with the MA toxin. Administration of testosterone, acutely (concentrations ranging from 0.005 to 50 μg, 24 h before MA) [102] or chronically (21 day release with a 5 mg testosterone pellet for 14 days) [64] did not protect against MA-induced striatal DA depletion in intact or gonadectomized male and female mice. Moreover, acute treatment with testosterone in male mice enhances MA toxicity as shown by an increased degree of striatal DA depletion [102].

4.3. Progesterone and neuroprotection

While the neuroprotective role of estrogens has been studied in many models, the modulatory activity in the brain of the other main ovarian steroid, progesterone has received less attention. Progesterone effects extend to extrahypothalamic sites and this steroid exerts functions in the brain other than reproduction such as neuroprotection, cognitive function and neurogenesis [23]. Limited data are available on the neuroprotective activity of progesterone in animal models of PD (Tables 1 and 2). A low dose of progesterone (1 μg, twice a day for 10 days) prevents MPTP-induced striatal DA depletion in intact male mice [71,127]. Moreover, when progesterone is co-administered with 17β-estradiol, progesterone did not block the beneficial effect of 17β-estradiol on DA content in MPTP mice [127]. More research is required to investigate possible synergistic neuroprotective effects of combined lower doses of both of these steroids on MPTP toxicity. In MA treated ovariectomized female mice, progesterone pre-treatment attenuated striatal DA depletion when this steroid was used at high (0.467 mg for 3 days) [188] but not at low doses (0.47 μg for 3 days) [187], whereas the low dose decreased MA toxicity in gonadectomized male mice [187]. Two days of estradiol benzoate pre-treatment (0.467 μg) followed by 1 day with progesterone (0.467 mg) resulted in a beneficial effect on DA content in MA treated ovariectomized female mice [188]. In primary hippocampal neuron cultures, 17β-estradiol, progesterone and 19-norprogesterone alone or in combination protected against glutamate toxicity, an effect not observed with medroxyprogesterone acetate (MPA) [131]. Moreover, MPA attenuated 17β-estradiol-induced neuroprotection when these two steroids were co-administered [131]. Hence, different progestins may have divergent effects and the choice of progestin in hormone therapy may have important implications in regards to the maintenance of cognitive function and for neuroprotection against neurodegenerative disease [130,131,165]. Hence, much is left to be done on the structure–activity relationships of progestative neuroprotection of the DA system.

5. Mechanisms of steroid hormone actions

The mechanisms of steroid hormone actions are complex and multifaceted. These mechanisms can vary with dose, site of action

and sex. Since much of the work on mechanisms has been conducted with estradiol and estradiol plays a key role in neuroprotection of the dopaminergic system as described in this review, we will concentrate on this gonadal steroid.

5.1. Genomic actions mediated by ERα and ERβ

It is now well documented that estrogen produces its effects by genomic and non-genomic actions. The genomic mechanism (review [132]) involves gene transcription mediated by activation of two different nuclear ERs: ERα and ERβ [73,96]. In the absence of a ligand, the ER is in an inactivated state, associated with heat-shock proteins [136]. The binding of estrogens to their receptor induces a conformational change in the receptor, allowing receptor homo- or heterodimerization and translocation to nucleus. The receptor dimer interacts with a specific DNA sequence (estrogen responsive element) within the promoter region of the target gene. This complex interacts with coregulators, resulting in the alteration of transcription. Gene transcription lacking a functional estrogen responsive element can also regulate the interaction of ER with the fos/jun transcription factor via the activators protein-1 or by interaction with nuclear factor κB (NFκB) [132].

5.2. Non-genomic actions of ERs

Non-genomic actions of estrogen are defined by rapid effects (within minutes even seconds), initiated generally by the interaction of 17β-estradiol with a plasma membrane-associated ER and likely lead to activation of signaling pathways. In the brain, ultrastructural studies have demonstrated that ERα and ERβ are also localized in plasma membranes [122,123]. Genomic and non-genomic effects of estrogens are documented to interact to potentiate transcriptional activity [14,178].

In addition to the classic ERα and ERβ, a novel seven transmembrane domain G-protein-coupled receptor has been proposed to function as a membrane ER [146,171]. This receptor, called GPR30, is able to mediate both rapid and transcriptional actions in response to estrogens [137]. The ER antagonist ICI 182,780 has a high binding affinity for GPR30 and shows agonist activity on this receptor [171]. GPR30 is involved in activation of extracellular signal-regulated kinase (ERK1/2) mediated by 17β-estradiol in breast cancer cells [56]. In the brain, immunohistochemical studies show a high expression of GPR30 in the hypothalamic-pituitary axis, hippocampus, striatum and substantia nigra [21]. GPR30 is localized in the plasma membrane, endoplasmic reticulum and Golgi apparatus of neurons [61,113]. At this time, no study has assessed the role of GPR30 in estrogen signaling in the brain. GPR30 knockout mice and the selective agonist G-1 [18], which has no detectable activity on ERα and ERβ, may be useful to clarify the role of GPR30 in estrogen-mediated actions and signaling in the brain.

In the neocortex, a plasma membrane-associated ER, called ER-X has been reported [173]. 17α-estradiol is the preferred endogenous ligand for ER-X [172]. ER-X mediates the activation of mitogen-activated protein kinase (MAPK)/ERK by 17α-estradiol and 17β-estradiol [172]. Expression of ER-X in wild-type and ERα knockout mice is reported and this receptor mediated phosphorylation of ERK1/2 in ERα knockout mice [172].

ERs are expressed differently throughout the brain [95]. The neuroanatomical and spatio-temporal organization of brain ERα and ERβ within diverse neuronal groups influences the action of estrogenic compounds. The rapid effect of estrogens in the brain seems to be mediated through a membrane ER and the specific role of ERα, ERβ, GPR30 and ER-X or yet a novel membrane ER remains to be clarified. ERs can be coupled to downstream molecules that may vary with the type of ER, the brain region and the associated scaffolding proteins, resulting in activation of different signaling pathways. Moreover, differential distributions of ERs are reported between male and female rodents [192]. Sex differences are observed in brain non-genomic actions of 17β-estradiol and in intracellular signaling [1]. It remains to be investigated whether estradiol activates the same signaling molecules in males and females or if there are sex differences in downstream signaling molecules linked with ERs. Moreover, neuroprotective effects of estradiol in brain injury can involve different signaling molecules between males and females.

5.3. Role of ERs in neuroprotection

The role of ERs was investigated in estradiol neuroprotection using specific agonists to each subtype and also with ER knockout mice. ERα and ERβ have both been detected in mice striatum [97] and their sparse localization in striatum and substantia nigra does not change after MPTP treatment [160]. Treatment of male mice with specific ER agonists has shown that the ERα agonist 4,4′,4″-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl)tris-phenol (PPT) protects against MPTP induced decreases in DA and DAT specific binding but that the ERβ agonist 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN) and androstenediol (a steroid with more ERβ than ERα activity) fails to show protection, suggesting the involvement of ERα in neuroprotection [40]. Moreover, when MPTP toxicity was investigated using ER knockout mice, results reveal an increase in DA and DOPAC depletion in MPTP treated ERα knockout mice but no change of toxicity in ERβ knockout mice as compared to MPTP treated wild-type mice [125]. Treatment with 17β-estradiol in MPTP lesioned ERα and ERβ knockout mice fails to show striatal DA neuroprotection, suggesting that both ERs are necessary for protection [125]. Other membrane estrogen receptors such as GPR30 and ER-X may be involved in the neuroprotective effects of estrogen in the brain and should be investigated.

5.4. MAPK and PI3K/Akt signaling pathways

Two important signaling pathways are associated with 17β-estradiol actions in the brain: the MAPK and the phosphatidylinositol-3 kinase (PI3K)/Akt pathways [26,44] (Fig. 4). ER can activate MAPK/ERK kinase (MEK 1/2), and ERK 1/2 [8,44,145]. Activated ERK 1/2 translocates to the nucleus where it activates transcription factors such as cAMP-response element-binding protein (CREB), resulting in modulation of transcription in target genes [158]. Activation of ERK 1/2 leads also to non-transcriptional activity such as inhibition of the proapoptotic death protein BAD [199] and glycogen synthase kinase 3β (GSK3β) [79].

ER can also interact with the PI3K pathway, resulting in Akt activation. Akt, also know as protein kinase B, promotes cell survival by inhibiting the function of proapoptotic proteins [111] (Fig. 4). GSK3 is a constitutive active kinase which can be inhibited by Akt phosphorylation on its serines 9 (GSK3β) and 21 (GSK3α) [74]. Activation of GSK3β is associated with neuronal apoptosis [55] and is shown to mediate toxin-induced striatal neuronal death [32] whereas its inhibition promotes cell survival [78]. Downstream from Akt are the prosurvival Bcl-2 and proapoptotic BAD proteins. Akt can modulate Bcl-2 family members. Akt can directly phosphorylate the protein BAD on its serine 136, thereby inhibiting its proapoptotic function [42]. Transcription factors such as NFκB and CREB are also regulated by Akt [52,149]; NFκB induces expression of the antiapoptotic protein Bcl-x and Bcl-2 and brain-derived neurotrophic factor (BDNF) expression is up-regulated by CREB [24,57,139].

Stimulation of tyrosine kinase receptors such as the insulin-like growth factor 1 (IGF-1) receptor can also activate the PI3K/Akt pathway. Interdependence is present between 17β-estradiol and IGF-1 actions in the brain. ERα, but not ERβ, immunocoprecipitates

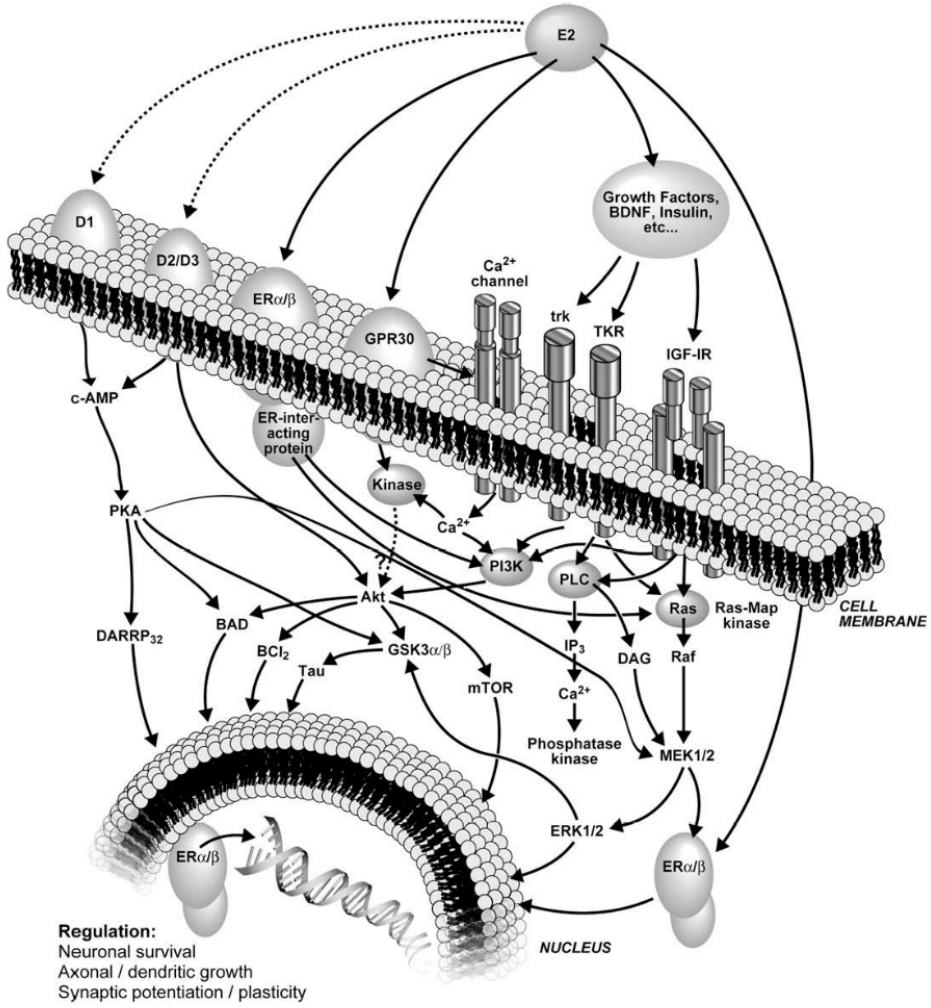

Fig. 4. Schematic presentation of effects of estrogens in neuromodulation and neuroprotection of DA cell survival and plasticity. Dotted lines indicate indirect effects.

with the IGF receptor after 17β-estradiol treatment and ERα can also interact with the p85 subunit of PI3K [116]. A synergistic action of IGF-1 and 17β-estradiol in the brain was reported for activation of Akt, but not on ERK activation [31].

The brain PI3K/Akt and the MAPK/ERK pathways are rapidly activated by 17β-estradiol [25,82,166]. Estrogens can also phosphorylate CREB, inhibit GSK3 activity, up-regulate Bcl-2 expression and attenuate tau hyperphosphorylation in the brain [37,66,69,194,197]. Activation of the PI3K/Akt and the MAPK/ERK pathways is implicated in the neuroprotective effect of 17β-estradiol in various models of brain injury. In cultured neurons, 17β-estradiol protects against glutamate and β-amyloid induced toxicity through a mechanism implicating the PI3K/Akt signaling pathway [81,193]. An *in vitro* study has shown that PPT and DPN increase Bcl-2 levels in hippocampal neurons and promote cell survival against glutamate excitotoxicity [195]. Implication of the MAPK/ERK pathway in the neuroprotective effects of 17β-estradiol against various injuries such as glutamate toxicity, H_2O_2 and quinolinic acid induced cell death is reported [94,98,124,133,163]. In cultured mouse midbrain cells, Akt phosphorylation induced by 17β-estradiol is time and dose-dependent and was inhibited by the PI3K inhibitor LY294002 but not by the ER antagonist ICI 182, 780 [85].

5.5. MAPK and PI3K pathways in neuroprotection against MPTP and MA toxicity

Our laboratory investigated whether neuroprotection by 17β-estradiol against MPTP toxicity is mediated by activation of the PI3K/Akt pathway and implication of ERα and ERβ in the striatum of male mice [37]. We showed that MPTP treatment enhances IGF-1 receptor levels, an effect prevented by the ERα agonist PPT but not by the ERβ agonist androstenediol. Treatment of MPTP mice with 17β-estradiol increased phosphorylation of Akt on serine 473 and a tendency was also observed with PPT. In MPTP mice, levels of phosphorylated GSK3β on serine 9 were decreased and this reduction was completely prevented by PPT treatment and to a lesser extent by 17β-estradiol and androstenediol. The ratio between striatal levels of Bcl-2 and BAD proteins was decreased in MPTP mice and this effect was prevented by PPT treatment. These results suggest that activation of the PI3K/Akt pathway is involved in the neuroprotective effect of 17β-estradiol against MPTP toxicity and

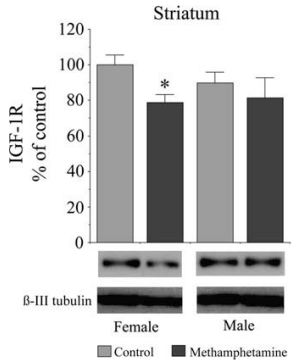

Fig. 5. Effects of methamphetamine (MA) on IGF-1R levels measured by Western blots under conditions previously described [37] in female and male mice as compared with saline-treated mice (control). Intact mice were treated with two intraperitoneal injections of MA (20 mg/kg) at a 2-h interval and euthanasia was a week post-MA. *$p < 0.05$ vs. female control. Values are the means of arbitrary units (normalized to βIII-tubulin) expressed as percentage of female control ± SEM of 4–6 mice per group. Representative examples of Western blots are shown. Statistical comparisons of the data were as described in legend to Fig. 1.

ERα mainly mediates this effect although the ERβ contribution cannot be excluded. In the 6-OHDA lesioned rat, estrogen and IGF-1 are shown to interact with the IGF-1 system to protect nigro-striatal DA neurons and blockage of IGF-1 receptor with JB-1, an IGF-1 receptor antagonist, attenuated the neuroprotective effect of both estradiol benzoate and IGF-1 [141]. The DA protection observed in substantia nigra of the 6-OHDA lesioned rat mediated by IGF-1 and estradiol benzoate is dependent on the PI3K/Akt, but not on the MAPK/ERK pathway, as shown by blockade of survival by both estradiol benzoate and IGF-1 with the PI3K inhibitor LY294002 but not the MEK inhibitor PD98059 [140]. In the substantia nigra *pars compacta*, IGF-1 receptor colocalizes with ERβ, but not ERα, in DA neurons and glial cells, hence, the interaction of signaling pathways between IGF-1 receptor and ER mediating neuroprotection may also involve ERβ [142].

Since MA produces different effects on DA markers in female and male mice, our groups investigated the implication of the PI3K/Akt pathway in this sex-dependent MA toxicity. MA can induce long-term effects on intracellular signaling in male and female mice. IGF-1 receptor levels are decreased in female mice, but not male, with MA treatment (Fig. 5). Phosphorylation of Akt at serine 473 was significantly reduced in male mice treated with MA whereas the total level of GSK3β was diminished in both male and female mice following MA administration (Fig. 6). Interestingly, inhibition of Akt activity by amphetamine has been implicated in the decrease of DAT cell surface expression [67]. Treatment with MA left unchanged Bcl-2 levels in female mice, whereas an increase was shown in MA-treated male mice (Fig. 7). BAD levels remained unchanged by MA in both female and male mice. The phosphorylated forms and basal levels of ERK1/2 were unchanged 7 days post-MA in mice of both sexes (Fig. 6). These results show that MA toxicity induced differences in specific signaling molecules than that of MPTP as well as between female and male mice and that the lesion-induced modulations remain long after MA administration for the PI3K but not ERK1/2 pathway, the latter was reported to change rapidly and transiently after MA or amphetamine [76,159]. Female mice show lower degeneration of DA terminals than male mice and this difference is also noted downstream with differences in signaling molecules.

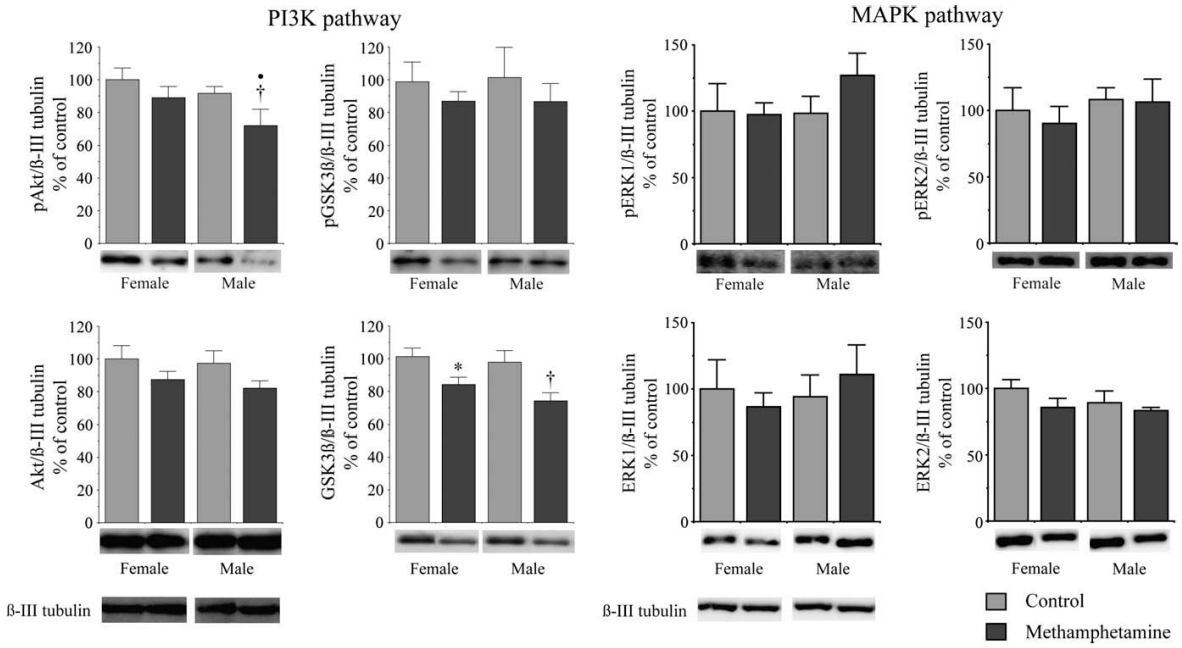

Fig. 6. Effects of methamphetamine (MA) on pAkt (serine 473), Akt, pGSK3ß (serine 9), GSK3ß, pERK1, pERK2, ERK1 and ERK2 levels measured by Western blots under conditions previously described [37] in female and male mice as compared with saline-treated mice (control). Intact mice were treated with two intraperitoneal injections of MA (20 mg/kg) at a 2-h interval and euthanasia was a week post-MA. *$p < 0.05$ vs. female control; †$p < 0.05$ vs. male control; •$p < 0.05$ vs. female MA. Values are the mean of arbitrary units (normalized to βIII-tubulin) expressed as percentage of female control ± SEM of 4–6 mice per group. Representative examples of Western blots are shown. Statistical comparisons of the data were as described in legend to Fig. 1.

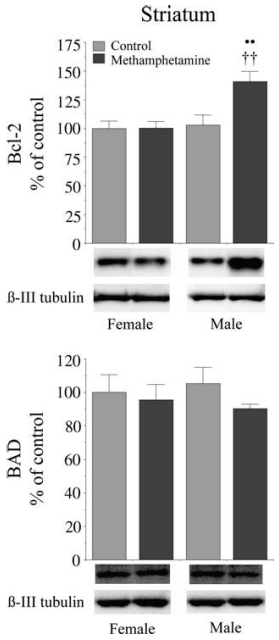

Fig. 7. Effects of methamphetamine (MA) on Bcl-2 and BAD levels measured by Western blots under conditions previously described [37] in female and male mice as compared with saline-treated mice (control). Intact mice were treated with two intraperitoneal injections of MA (20 mg/kg) at a 2-h interval and euthanasia was a week post-MA. $^{\dagger\dagger}p < 0.01$ vs. male control; $^{\bullet\bullet}p < 0.01$ vs. female MA. Values are the mean of arbitrary units (normalized to βIII-tubulin) expressed as percentage of female control ± SEM of 4–6 mice per group. Representative examples of Western blots are shown. Statistical comparisons of the data were as described in legend to Fig. 1.

6. Akt signaling in human diseases

Akt is a major regulator of metabolism, cell growth, cell proliferation, transcription and cell survival [111,152] and was shown to be modulated by estrogens (previous section). Dysfunction of this protein kinase is associated with various diseases in human including cancer [10], diabetes [3] and schizophrenia [90]. A positive association of the Akt1 haplotype to methamphetamine-use disorder has been reported in Japanese [84]. Akt1 variants were associated with risk of schizophrenia [170] and reduction in Akt activity or protein levels was also found in the brain of schizophrenic patients [54,196]. Furthermore, antipsychotic drugs and lithium have been shown to enhance Akt signaling [6]. Certain Akt1 haplotypes have been associated with reduced risk to develop PD [185]. Recently, Malagelada et al. [108] have shown that phosphorylated Akt is decreased in post-mortem substantia nigra DA neurons of PD patients and RTP801, a protein promoting neuronal death, is increased [109]. The upregulation of RTP801 may mediate neuronal degeneration through suppression of the serine–threonine protein kinase mammalian target of rapamycin (mTOR) and Akt activity. Indeed, the mTOR complex 2 phosphorylates Akt on its serine 473 and facilitate phosphorylation of threonine 308 [153]. Moreover, Akt can activate another mTOR complex called mTOR complex 1, also called raptor mTOR, which regulates protein synthesis [169].

It remains of clinical relevance to find active drugs that selectively target the PI3K/Akt pathway for treatment of diseases showing deregulation of Akt signaling. The inhibitor of monoamine oxidase B, rasagiline, was found to slow the functional decline in patients with an early, mild form PD [17,135]. In MPTP-treated mice, rasagiline was shown to protect against the toxin, a neuroprotective effect associated with activation of Akt and inhibition of GSK3β [110,151].

7. Conclusion

Significant sex differences exist in nigro-striatal DA neurodegeneration as observed in animal models as well as clinical and epidemiological reports on PD. Since sex steroid hormones represent the most salient factor related to sex differences, the influences of estrogens, androgens and progesterone were assessed in regards to their contributions to these sex differences in nigro-striatal DA neurodegeneration. The female sex steroids 17β-estradiol and progesterone but not the male steroids testosterone and dihydrotestosterone are neuroprotectants. The effects of estrogens were summarized, as most of the studies on this topic have concentrated on this gonadal steroid. Pretreatment with estradiol doses in a physiological range, but not higher doses, are active against MPTP toxicity in mice of both sexes and MA toxicity against female mice whereas estradiol does not protect male mice against MA toxicity. Alternative estrogenic neuroprotection for both sexes include SERMs [19,71] selective ER agonists [40] and phytoestrogens [15,106]. Evidence from a variety of studies shows that estrogens function as neuroprotectants against nigro-striatal DA neurodegeneration and the various means by which estrogen can exert this effect were presented with the most recent data pertaining to DA signaling pathways.

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Male/female differences in neuroprotection and neuromodulation of brain dopamine

*Mélanie Bourque[1,2], Dean E. Dluzen[3] and Thérèse Di Paolo[1,2]**

[1] Molecular Endocrinology and Genomic Research Center, Centre de recherche du CHUQ (CHUL), Quebec City, QC, Canada
[2] Faculty of Pharmacy, Laval University, Quebec City, QC, Canada
[3] Department of Anatomy and Neurobiology, Northeastern Ohio Universities College of Medicine and Pharmacy, Rootstown, OH, USA

Edited by:
Hubert Vaudry, University of Rouen, France

Reviewed by:
Charlotte A. Cornil, University of Liege, Belgium
Luis Miguel Garcia-Segura, Consejo Superior de Investigaciones Científicas, Spain

***Correspondence:**
Thérèse Di Paolo, Molecular Endocrinology and Genomic Research Center, Centre de recherche du CHUQ (CHUL), 2705 Laurier Boulevard, Quebec City, QC, Canada G1V 4G2.
e-mail: therese.dipaolo@crchul.ulaval.ca

The existence of a sex difference in Parkinson's disease (PD) is observed as related to several variables, including susceptibility of the disease, age at onset, and symptoms. These differences between men and women represent a significant characteristic of PD, which suggest that estrogens may exert beneficial effects against the development and the progression of the disease. This paper reviews the neuroprotective and neuromodulator effects of 17β-estradiol and progesterone as compared to androgens in the nigrostriatal dopaminergic (NSDA) system of both female and male rodents. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice model of PD and methamphetamine toxicity faithfully reproduce the sex differences of PD in that endogenous estrogen levels appear to influence the vulnerability to toxins targeting the NSDA system. Exogenous 17β-estradiol and/or progesterone treatments show neuroprotective properties against NSDA toxins while androgens fail to induce any beneficial effect. Sex steroid treatments show male and female differences in their neuroprotective action against methamphetamine toxicity. NSDA structure and function, as well as the distribution of estrogen receptors, show sex differences and may influence the susceptibility to the toxins and the response to sex steroids. Genomic and non-genomic actions of 17β-estradiol converge to promote survival factors and the presence of both estrogen receptors α and β are critical to 17β-estradiol neuroprotective action against MPTP toxicity.

Keywords: 17β-estradiol, androgens, dopamine, neuroprotection, neuromodulation, sex difference, MPTP, methamphetamine

PARKINSON'S DISEASE

SEX DIFFERENCES

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive and selective loss of dopamine (DA) cell bodies in substantia nigra (Dauer and Przedborski, 2003). The death of nigral DA neurons results in striatal DA decrease, leading to a dysfunction in basal ganglia process, and the appearance of clinical symptoms such as resting tremor, rigidity, and bradykinesia (Obeso et al., 2008).

Several variables (including susceptibility to the disease, age at onset, symptoms) support the existence of a sex difference in PD (Miller and Cronin-Golomb, 2010). While most studies report a higher predisposition of PD in men (Van Den Eeden et al., 2003; Wooten et al., 2004; Shulman, 2007), sex is not considered a risk factor in a few studies (Granieri et al., 1991; de Rijk et al., 1995). Men have at least a 1.5-fold greater risk than women of developing PD (Van Den Eeden et al., 2003; Wooten et al., 2004). The age at onset in women occurs about 2 years later compared to men in the majority of studies (Twelves et al., 2003; Haaxma et al., 2007), while no difference has also been reported in one study (Baba et al., 2005). Moreover, differences in the profile of motor symptoms between men and women have also been reported. Total motor scores on the modified unified Parkinson's disease rating scale (UPDRS) do not differ between men and women however, men have more advanced rigidity and women present with greater instability scores (Baba et al., 2005). When UPDRS motor scores were examined as a function of disease progression, sex differences were not observed in early PD (less than 5 years duration) however, women have better motor scores than men in advanced PD (disease duration more than 5 years; Lyons et al., 1998). Not only do men exhibit more severe parkinsonian motor features than women but a greater improvement of motor function following levodopa therapy is reported in women (Growdon et al., 1998; Lyons et al., 1998; Zappia et al., 2005). Haaxma et al. (2007) found that women have a higher frequency of presentation with the tremor dominant form of PD, which has been associated with a slower disease progression. [^{123}I]FR-CIT single photon emission computed tomography (SPECT) measurements show that, at symptom onset, women had superior levels of striatal DA binding than men, suggesting that the development of symptomatic PD may be delayed by higher physiological striatal DA levels (Haaxma et al., 2007). In light of these findings, the authors suggested that the phenotype of PD in women is more benign (Haaxma et al., 2007). It should be noted however, that differences in disease presentation, for example, the initial symptom of tremor in women, was not consistently reported (Baba et al., 2005). Thus, sex differences represent a notable characteristic of PD, which suggests that estrogens may exert beneficial effects against the development and

progression of the disease in women and/or that androgens may exert destructive effects upon the development and progression of the disease in men.

INFLUENCE OF ENDOGENOUS AND EXOGENOUS ESTROGENS

Several studies have been conducted to investigate how endogenous estrogen status and estrogen therapy influence the risk of PD. A longer fertile lifespan (difference between age at menarche and age at menopause) was associated with a decreased risk of PD in women with natural menopause (>39 years of fertile lifespan) compared to the lowest fertile lifespan (<33 years) as reported among 83,482 women participating in the Observational Study of the Women's Health Initiative (WHI-OS; Saunders-Pullman et al., 2009). A fertile lifespan shorter than 36 years was associated with an increased risk of PD and an earlier menopause was more common among women with PD (Ragonese et al., 2004). A case–control study reported that women with PD had undergone hysterectomy (with or without unilateral oophorectomy) more frequently than control subjects (Benedetti et al., 2001), however data from another report indicated that surgical menopause was associated with a decreased incidence of PD (Ragonese et al., 2004). Most findings suggest a beneficial effect of estrogen with regard to PD risk as the use of either postmenopausal estrogen (Currie et al., 2004; Popat et al., 2005) or oral contraceptives (Ascherio et al., 2003; Simon et al., 2009) were associated with a reduced risk of PD, and women with PD were less likely to use estrogen therapy (Benedetti et al., 2001); though there are also data relating that a similar risk exists between women who have or have not used estrogen therapy (Ascherio et al., 2003). The use of estrogen therapy on the risk of PD varies depending on the type of menopause, with increased risk in women with hysterectomy (Popat et al., 2005; Saunders-Pullman et al., 2009) and decreased risk in women with natural menopause (Popat et al., 2005). No association between exogenous or endogenous estrogens exposure on risk of PD has also been reported (Simon et al., 2009).

In women with PD experiencing regular menstrual cycles a worsening of parkinsonian symptoms was associated with premenstrual and menstrual periods, when estrogens and progesterone are at a low level (Quinn and Marsden, 1986; Kompoliti et al., 2000; Tolson et al., 2002). A reduction in levodopa effectiveness was also observed before and during the menstruation period in young PD women (Quinn and Marsden, 1986; Giladi and Honigman, 1995). Although it was thought that estrogen was the basis for these symptom fluctuations, a study conducted in a 5-week period in 10 PD women with menstrual cycles found that serum estrogens and progesterone levels were not related to PD severity (Kompoliti et al., 2000). Nevertheless, Horstink et al. (2003) report fluctuations of dyskinesias, a motor complication induced by levodopa treatment, as associated with estradiol levels in a young PD woman, with an increase in dyskinesia when estradiol levels were highest, followed by a diminution of dyskinesia and a worsening of parkinsonism in the premenstrual period. The majority of women with PD report a worsening of parkinsonian symptoms during pregnancy and into the postpartum period, while some women do not experience any deterioration of their symptoms (Golbe, 1987; Hagell et al., 1998; Shulman et al., 2000; Robottom et al., 2008). Case reports have documented an increase in total and motor UPDRS scores during and after pregnancy (Shulman et al., 2000), with a faster symptom progression than that observed in the comparison cohort (Robottom et al., 2008). The substantial variations in estrogen levels during and after pregnancy were proposed to be implicated in the worsening of parkinsonian symptoms during these periods (Rubin, 2007; Robottom et al., 2008).

An amelioration of PD symptoms and dyskinesia was reported to be present under conditions of estrogen therapy or high levels of endogenous estrogens (Villeneuve et al., 1978; Session et al., 1994; Giladi and Honigman, 1995). Postmenopausal women with early PD using estrogen therapy prior to initiation of levodopa have lower symptom severity scores (Saunders-Pullman et al., 1999) but estrogen therapy had no effect at later stages of the disease (Strijks et al., 1999). Results from double-blind studies reported a reduction of the dose of levodopa required to improve motor function in women receiving 17β-estradiol (Blanchet et al., 1999) and an improvement of motor disability in PD women with motor fluctuations when treated with estrogens (Tsang et al., 2000) whereas no effect of estrogen on motor function was also observed (Strijks et al., 1999).

While in some cases conflicting information is reported from the above human studies, a longer fertile lifespan seems to be consistently associated with a decreased risk of PD, suggesting that longer exposure to endogenous ovarian steroids exerts a beneficial effect against PD. PD symptoms seem to be modulated by endogenous variation of steroids as reported by the majority of women experiencing menstrual cycles and pregnancy. The most conflicting data comes from the use of estrogen therapy (most of the studies support a beneficial effect of estrogens) but many factors could influence the conclusion of these studies. First, a variety of hormonal therapy compounds are available and the studies do not always state the specific molecule used by women. Further, the dose, timing of initiation of treatment and duration of estrogen therapy can vary substantially. In addition, estrogen therapy could be used alone or in combination with progestin and the type of hormone therapy prescribed could differ between women with a hysterectomy and women with natural menopause. Indeed, women within the same cohort could have used different preparations and doses of estrogen therapy and could have experienced a different duration of treatment. Taken together, the results from these studies suggest that exposure to endogenous and exogenous estrogens seem to influence the risk and symptoms of PD while further research concerning the exact regimen of hormonal therapy will require careful analyses to enable a definitive conclusion.

ESTROGENS RECEPTOR, STEROIDS, AND PARKINSON'S DISEASE

Estrogens receptor (ER) α and ERβ polymorphism were found to be unrelated with an increased risk of PD (Maraganore et al., 2002; Westberg et al., 2004; Li et al., 2009). Alternatively, ERβ polymorphism is reported more frequently in PD patients with an early age at onset (Westberg et al., 2004; Hakansson et al., 2005). A small number of studies have investigated alterations of neurosteroid synthesis in PD. Reduced levels of allopregnanolone and 5α-dehydroprogesterone were found in the cerebrospinal

fluid of PD patients (di Michele et al., 2003). In the substantia nigra, reductions of 5α-reductase protein and mRNA were observed (Luchetti et al., 2010), which could lead to decreased synthesis of 5α-dehydroprogesterone and subsequently allopregnanolone (di Michele et al., 2003). Expression of sulfotransferase 2B1, an enzyme catalyzing the conversion of pregnenolone and dehydroepiandrosterone into their sulfated esters, in the substantia nigra was also downregulated and in the caudate nucleus, expression of 3α-hydroxysteroid dehydrogenase type 3, an enzyme catalyzing the synthesis of allopregnanolone, was upregulated (Luchetti et al., 2010), suggesting a compensatory mechanism in response to the loss of input from the substantia nigra. The authors suggest an involvement of neurosteroids in the neurodegenerative process of PD (Luchetti et al., 2010).

INFLUENCE OF SEX STEROIDS IN EXPERIMENTAL MODELS OF PARKINSON'S DISEASE

SEX DIFFERENCES AND INFLUENCE OF ENDOGENOUS HORMONES

Two animal models of induced degeneration affecting the nigrostriatal dopaminergic (NSDA) system are reviewed here as related to the influence of sex steroids – the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD and methamphetamine (MA)-induced DA toxicity. While both agents primarily target the NSDA system, they differ with regard to their mechanisms of action. For MPTP, conversion to its active metabolite, 1-methyl-4-phenylpyridinium (MPP+), is required followed by uptake into the DA neuron via the DA transporter (DAT). The interaction of MPP+ with mitochondrial complex 1 results in a disruption of the respiratory chain and eventual cell destruction. The exact mechanisms for toxicity resulting from MA remain elusive, but it is believed to produce a reversal in DAT function thus producing excessive amounts of DA which have the potential of generating free radicals and oxidative stress. Studies in these animal models have shown a greater neurotoxic effect in male than female mice, as observed by more extensive striatal DA reduction and a greater decrease in DAT specific binding in striatum (Wagner et al., 1993; Dluzen et al., 1996; Miller et al., 1998; Yu and Liao, 2000b; Bourque et al., 2009, 2011). Moreover, the levels of endogenous sex steroids have been reported to affect the susceptibility of female mice to the toxin. In female BALB/c mice treated with MA, a greater DA depletion was observed when the toxin was administered at diestrus (when estrogens levels are low) whereas lower DA loss occurs at proestrus (when estrogens levels are high; Yu and Liao, 2000b). This effect of estrous cycle variation seems to be strain dependent since this difference in MA sensibility was not observed in the C57BL/6J strain of mice (Yu and Liao, 2000b). These animal models faithfully reproduce the sex differences of PD in that endogenous estrogen levels appear to influence the vulnerability to toxins targeting the NSDA system and support a beneficial role of estrogens against NSDA neurodegeneration.

EFFECTS OF ESTROGENS AND PROGESTERONE IN FEMALE AND MALE RODENTS

Experimental studies have been conducted in order to investigate the potential beneficial effect of exogenous sex steroid treatments against NSDA toxins. A summary of these studies showing steroids, dose, toxin, and DA markers investigated is listed in **Tables 1–4**. These studies are separated by sex to highlight the observed sex differences and we refer the reader to the tables for details and a complete reference to relevant studies. Beneficial effects of 17β-estradiol and estradiol benzoate on DA concentrations against MPTP toxicity in female and male mice (intact or gonadectomized) are shown when physiological concentrations of this gonadal steroid are administered prior to the toxin (**Tables 1** and **3**; Dluzen et al., 2001b; D'Astous et al., 2004b; Liu et al., 2008). A protective effect of 17β-estradiol or estradiol benzoate against MA-induced DA loss was also obtained with a pre-treatment in female mice (Dluzen et al., 2002; D'Astous et al., 2005a). In contrast, 17β-estradiol failed to show any protective effect on DA content in gonadectomized male mice treated with MA (Anderson et al., 2005) and produced a severe acute toxicity to MA in intact male mice (Dluzen et al., 2002). Timing of treatment with 17β-estradiol or estradiol benzoate seems to be critical to reach a maximal beneficial effect, with at least 24 h required for the pre-treatment whereas shorter time intervals fail to achieve optimal protection (Gajjar et al., 2003). In support of this finding are data showing that treatment with a high dose of 17β-estradiol shortly prior to and after MPTP injection failed to protect striatal DA depletion whereas some effects were observed on DAT and tyrosine hydroxylase proteins levels (Ookubo et al., 2008). Moreover, in a study investigating several markers of dopaminergic function, the time required to obtain maximal protection against MA toxicity was observed with a 24-h pre-treatment of estradiol benzoate while pre-treatments of 30 min or 12 h showed incomplete and varying degrees of neuroprotection (Gajjar et al., 2003), suggesting different mechanisms of estradiol action (D'Astous et al., 2004a). Since estradiol is a neuromodulator of DA metabolism (Sanchez et al., 2010), investigations of the integrity of DA neurons using other DA markers to demonstrate the beneficial effect of estradiol are critical. The DAT and the vesicular monoamine transporter 2 (VMAT2) are both important modulators of DA neurotransmission (Sotnikova et al., 2006; Guillot and Miller, 2009) and both play a pivotal role in MPTP and MA toxicities (Gainetdinov et al., 1997, 1998; Fumagalli et al., 1998, 1999). A decrease in DAT and VMAT2 binding in DA neurons of the PD brain is associated with the loss of DA (Wilson et al., 1996). The protective effect of 17β-estradiol on DA content observed in MPTP and MA mice has also been shown on DAT and VMAT2 specific binding (Callier et al., 2001; D'Astous et al., 2004a, 2005a; Jourdain et al., 2005). Moreover, positive correlations exist between DA concentrations and DAT and VMAT2 levels, suggesting that binding to the transporters reflects the degree of degeneration or protection (Jourdain et al., 2005).

While it has received less attention than 17β-estradiol, the neuroprotective effect of the other major ovarian steroid progesterone has also been investigated in animal models of PD. Like 17β-estradiol, progesterone shows beneficial effects against MPTP and MA toxicities (Grandbois et al., 2000; Yu and Liao, 2000a; Callier et al., 2001; Yu et al., 2002; Morissette et al., 2008). In contrast to 17β-estradiol, progesterone is neuroprotective in male mice treated with MA (Yu et al., 2002). Sex differences in the dose of progesterone used to be effective as a neuroprotectant against MA are also reported, with male mice requiring lower doses than females (Yu et al., 2002). Interestingly, co-administration of 17β-estradiol and progesterone (Morissette et al., 2008), or

Table 1 | *In vivo* studies and neuroprotection in female rodents: studies showing protection of DA markers.

Steroid	Toxin	Species	Assay	References
ESTROGENS				
17β-estradiol				
0.5 or 2 mg/kg 30 min before and 90 min after MPTP	MPTP	Intact mice	DOPAC concentrations (with 2 mg/kg) DAT (with 2 mg/kg) and TH protein levels	Ookubo et al. (2008)
Pellet (0.1 mg, 60-day release) for 15 days	MPTP	OVX mice	DA concentrations	Dluzen et al. (1996)
Pellet (0.1 mg, 21-day release) for 14 days	MPTP	OVX mice	DA concentrations	Dluzen et al. (2001b)
Pellet (235 μg/ml) before MPTP and 2 days after	MPTP	OVX mice	DA, DOPAC, and HVA concentrations	Miller et al. (1998)
50 μg/kg for 19 days	MPTP	OVX mice	DAT mRNA levels	Liu et al. (2004)
Single injection of 10 μg, co-administered with MPP+	MPP+	Intact rats	DA concentrations TH immunoreactivity	Tomas-Camardiel et al. (2002)
Pellet (0.1 mg, 21-day release) for 14 days	MA	Intact and OVX mice	DA concentrations	Dluzen et al. (2001a, 2002), Gao and Dluzen (2001b)
Estradiol benzoate				
50 μg/kg for 8 days	MPTP	OVX mice	DA, DOPAC, and HVA concentrations TH immunoreactivity DAT and TH mRNA levels	Liu et al. (2008)
48 pg or 0.47 μg for 3 days	MA	OVX mice (6 weeks)	DA concentrations	Yu and Liao (2000a), Yu et al. (2002)
1, 10, or 40 μg 24 h before MA	MA	OVX mice	DA concentrations DAT and VMAT2 specific binding	Mickley and Dluzen (2004), D'Astous et al. (2005a)
10 μg 0.5, 12, or 24 h before MA	MA	OVX mice	DA concentrations (at 12 and 24 h) DOPAC/DA ratio DAT specific binding (at 24 h) DAT mRNA levels	Gajjar et al. (2003), D'Astous et al. (2004a)
PROGESTERONE				
0.467 mg for 3 days	MA	OVX mice	DA concentrations	Yu and Liao (2000a)
17β-ESTRADIOL BENZOATE + PROGESTERONE				
2 days of estradiol benzoate treatment (0.467 μg) following by 1 day progesterone treatment (0.467 mg)	MA	OVX mice	DA and DOPAC concentrations	Yu and Liao (2000a)

DOPAC, 3,4-dihydrophenylacetic acid; HVA, homovanillic acid; OVX, ovariectomized; TH, tyrosine hydroxylase.

administration of progesterone following 17β-estradiol treatment (Yu and Liao, 2000a) does not oppose the beneficial effect of 17β-estradiol, as has been observed with medroxyprogesterone acetate (Nilsen and Brinton, 2002).

17β-estradiol clearly shows a neuroprotective capacity when administered as a pre-treatment, that is, under conditions of a non-injured brain. Whether 17β-estradiol could retain this capacity within an impaired dopaminergic system has also been investigated. Estradiol treatment after a MA-induced lesion has been introduced lacks a protective capacity against MA toxicity in female mice (Gao and Dluzen, 2001b; Gajjar et al., 2003) and could even worsen the extent of observed damage (Liu and Dluzen, 2006). This is, in part, consistent with the healthy cell bias of estrogen's effect, which proposes that if neurons are healthy at the time of estrogen treatment, their response to estrogen is beneficial for both survival and neurological functions whereas in the presence of impaired function, estrogen exposure over time exacerbates brain injury (Chen et al., 2006; Brinton et al., 2008). Accordingly, 17β-estradiol can prevent but does not seem to have the capacity of regeneration nor is it protective under conditions of an impaired system.

EFFECTS OF ANDROGENS IN FEMALE AND MALE RODENTS

Androgenic compounds have also received attention in order to investigate their effect in neuroprotective studies. Steroids could be synthesized in the brain, as supported by the presence and

Table 2 | *In vivo* studies and neuroprotection in female rodents: studies showing no protection of DA markers.

Steroid	Toxin	Species	Assay	References
ESTROGENS				
17β-estradiol				
0.5 or 2 mg/kg 30 min before and 90 min after MPTP	MPTP	Intact mice	DA, DOPAC (with 0.5 mg/kg), and HVA concentrations DAT protein levels (with 0.5 mg/kg)	Ookubo et al. (2008)
50 μg/kg for 19 days	MPTP	OVX mice	TH mRNA levels	Liu et al. (2004)
Pellet (0.1 mg, 21-day release) for 7 days (7 days after MA)	MA	OVX mice	DA concentrations	Gao and Dluzen (2001b)
Estradiol benzoate				
10 μg, 1 injection 0.25, 0.5, 1, or 2 h after MA	MA	OVX mice	DA concentrations, DOPAC/DA ratio	Gajjar et al. (2003)
10 μg, 1 injection at 1 week post-MA	MA	OVX mice	DA and DOPAC concentrations	Liu and Dluzen (2006)
0.46 μg for 3 days	MA	OVX mice (4 weeks)	DA and DOPAC concentrations	Yu et al. (2002)
17α-estradiol				
Pellet (0.1 mg, 21-day release) for 14 days	MPTP	OVX mice	DA concentrations	Dluzen et al. (2001b)
PROGESTERONE				
0.47 μg for 3 days	MA	OVX mice	DA and DOPAC concentrations	Yu et al. (2002)
ANDROGENS				
Testosterone				
10 μg, 1 injection	MPP+	Intact rats	DA concentrations TH immunoreactivity	Tomas-Camardiel et al. (2002)
Pellet (5 mg, 21-day release) for 14 days	MA	Intact and OVX mice	DA concentrations	Gao and Dluzen (2001a), Dluzen et al. (2002)
0.005–50 μg (1 injection), 24 h before MA	MA	Intact and OVX mice	DA concentrations	Lewis and Dluzen (2008)

DOPAC, 3,4-dihydrophenylacetic acid; HVA, homovanillic acid; OVX, ovariectomized; TH, tyrosine hydroxylase.

distribution of neurosteroidogenic enzymes (Do Rego et al., 2009). Thus, estradiol and testosterone could be formed in the brain. Aromatase, the enzyme converting testosterone to estradiol and androstenedione to estrone, and 5α-reductase, the enzyme converting testosterone into dihydrotestosterone, are present in the brain (Do Rego et al., 2009). While testosterone is considered an important precursor of estradiol biosynthesis and could also be 5α-reduced into dihydrotestosterone (important in testis and ovary), a direct effect of testosterone by itself is also possible as has been described in intracrine tissues (Luu-The and Labrie, 2010). As a result of such testosterone biotransformation it is oftentimes not certain whether any modulatory effects upon toxicity from this steroid result from testosterone, its metabolites and/or its possible conversion into estradiol. To investigate specifically the androgenic potential in neuroprotective activity, dihydrotestosterone, the most potent androgen, is a more appropriate compound since it is not aromatized.

In MPTP-treated male mice, testosterone treatment fails to show any protective effect (Dluzen, 1996; Ekue et al., 2002), suggesting that testosterone is not biotransformed into estradiol in the brain, more specifically in the basal ganglia, in adequate concentrations to reach protective levels. The lack of effect of testosterone is not sex-dependent since this steroid does not protect against MA toxicity in either female or male mice (Gao and Dluzen, 2001a; Lewis and Dluzen, 2008). Not only does testosterone lack a neuroprotective function but further exacerbates DA depletion in male mice receiving a chronic (Dluzen et al., 2002) or acute (Lewis and Dluzen, 2008) administration of testosterone. These findings of an exacerbation of MA-induced DA toxicity with testosterone are supported by data from the 6-hydroxydopamine rat model which shows that greater amounts of striatal DA depletion are obtained in intact versus gonadectomized male rats subjected to 6-hydroxydopamine (Murray et al., 2003; Gillies et al., 2004). Dihydrotestosterone also lacks any beneficial effect against loss of striatal DA concentrations in MPTP-lesioned male mice (Ekue et al., 2002), suggesting that stimulation of androgen receptors was not effective in inducing a protective effect. Indeed, these studies show that androgens do not play a protective role but may actually intensify toxicity in the NSDA pathway. Such effects may be relatively specific to the NSDA system as testosterone is reported

Table 3 | *In vivo* studies and neuroprotection in male rodents: studies showing protection of DA markers.

Steroid	Toxin	Species	Assay	References
ESTROGENS				
17β-estradiol				
1 μg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations DAT and VMAT2 specific binding DAT, VMAT2, and TH mRNA levels	Callier et al. (2000. 2001), Grandbois et al. (2000), Ekue et al. (2002), D'Astous et al. (2003, 2004b, 2005b, 2006), Morissette et al. (2007), Al-Sweidi et al. (2011)
2 μg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations DAT and VMAT2 specific binding DAT, VMAT2, and TH mRNA levels	Jourdain et al. (2005)
1 μg B.I.D. 1, 3, or 5 days before MPTP and 5 days after	MPTP	Intact mice	DA, DOPAC, and HVA concentrations	Morissette et al. (2008)
1 μg B.I.D. for 11 days	MPTP	Intact mice	TH immunoreactivity	Tripanichkul et al. (2006, 2010)
1 μg B.I.D. for 11 days	MPTP	Intact mice	DA concentrations	Ramirez et al. (2003)
1 μg B.I.D. for 5 days and until 2, 4, or 6 days after MPTP	MPTP	Intact mice	DA concentrations	Ramirez et al. (2003)
0.5 or 2 mg/kg 30 min before and 90 min after MPTP	MPTP	Intact mice	DOPAC concentrations DAT (with 2 mg/kg) and TH protein levels	Ookubo et al. (2008)
Pellet (0.1 mg, 60-day release) for 15 days	MPTP	GDX mice	DA concentrations	Dluzen et al. (1996)
100 μg daily for 9 or 16 days	MPTP	GDX mice	TH immunoreactivity	Shughrue (2004)
Estrone				
2 μg B.I.D. for 10 days	MPTP	Intact mice	DAT specific binding VMAT2 mRNA levels	Jourdain et al. (2005)
PROGESTERONE				
1 μg B.I.D. for 10 days	MPTP	Intact mice	DA and HVA concentrations DAT specific binding	Grandbois et al. (2000), Callier et al. (2001), Morissette et al. (2008)
0.47 μg for 3 days	MA	Intact mice	DA and DOPAC concentrations	Yu et al. (2002)
17β-estradiol + progesterone				
1 μg B.I.D. of both 17β-estradiol and progesterone for 10 days	MPTP	Intact mice	DA concentrations	Morissette et al. (2008)

B.I.D., twice a day; DOPAC, 3,4-dihydrophenylacetic acid; HVA, homovanillic acid; GDX, gonadectomized; TH, tyrosine hydroxylase.

to exert neuroprotective effects in others models (Bialek et al., 2004).

COMPARISON BETWEEN CLINICAL AND ANIMAL REPORTS

There is a relatively good consistence between clinical and animal data concerning the neuroprotective activity of ovarian steroids. Longer exposure to ovarian steroids is associated with a reduce risk of PD in women as presented in Section "Influence of Endogenous and Exogenous Estrogens." In animal models, pre-treatment with both 17β-estradiol and progesterone shows neuroprotective activity against MPTP and MA, while 17β-estradiol lacks neuroprotective effect when administered under condition of impaired NSDA system (see Effects of Estrogens and Progesterone in Female and Male Rodents).

While animal studies show that 17β-estradiol does not exert any neuroprotective effect when administered after the lesion, clinical reports suggest that estrogens can act as a neuromodulator of DA system on PD symptoms. Women with PD experience worsening of their symptoms during periods of low endogenous steroids exposure and estrogen therapy has been reported to improve PD symptom (see Influence of Endogenous and Exogenous Estrogens). To our knowledge no

Table 4 | *In vivo* studies and neuroprotection in male rodents: studies showing no protection of DA markers.

Steroid	Toxin	Species	Assay	References
ESTROGENS				
17β-estradiol				
1 μg B.I.D. for 10 days	MPTP	Intact mice	DAT specific binding (extensive lesion)	Callier et al. (2000)
12 μg 0.75, 2, or 24 h before MPTP	MPTP	Intact mice	DA concentrations	Ramirez et al. (2003)
0.1, 0.32, or 3.2 μg B.I.D. for 11 days	MPTP	Intact mice	DA concentrations	Ramirez et al. (2003)
0.05 or 0.2 mg/kg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations	Ookubo et al. (2008)
0.5 or 2 mg/kg 30 min before and 90 min after MPTP	MPTP	Intact mice	DA and HVA concentrations DAT protein levels (with 0.5 mg/kg)	Ookubo et al. (2008)
Pellet (0.1 mg, 21-day release) for 14 days	MA	GDX mice	DA concentrations	Dluzen et al. (2001a, 2002), Gao and Dluzen (2001a,b)
Pellet (0.1 mg, 21-day release) for 7 days (7 days after MA)	MA	GDX mice	DA concentrations	Gao and Dluzen (2001b)
Estradiol benzoate				
0.47 μg for 3 days	MA	GDX mice	DA and DOPAC concentrations	Yu et al. (2002)
10 μg, 1 injection at 1 week post-MA	MA	GDX mice	DA concentrations	Liu and Dluzen (2006)
17α-estradiol				
1 μg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations DAT specific binding and mRNA levels	Callier et al. (2000, 2001), Grandbois et al. (2000), D'Astous et al. (2005b)
0.1, 0.3, 1, or 3.2 μg B.I.D. for 11 days	MPTP	Intact mice	DA concentrations	Ramirez et al. (2003)
12 μg 0.75, 2, or 24 h before MPTP	MPTP	Intact mice	DA concentrations	Ramirez et al. (2003)
Estriol				
2 μg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations DAT and VMAT2 specific binding DAT, VMAT2, and TH mRNA levels	Jourdain et al. (2005)
Estrone				
2 μg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations VMAT2 specific binding DAT and TH mRNA levels	Jourdain et al. (2005)
0.05 or 0.2 mg/kg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations	Ookubo et al. (2008)
0.5 or 2 mg/kg 30 min before and 90 min after MPTP	MPTP	Intact mice	DA, DOPAC, and HVA concentrations DAT and TH protein levels	Ookubo et al. (2008)

(Continued)

Table 4 | Continued

Steroid	Toxin	Species	Assay	References
PROGESTERONE				
1 μg B.I.D. for 10 days	MPTP	Intact mice	DOPAC concentrations DAT mRNA levels	Grandbois et al. (2000), Callier et al. (2001)
0.47 μg for 3 days	MA	GDX mice (4 weeks)	DA concentrations	Yu et al. (2002)
ANDROGENS				
Testosterone				
50 μg testosterone B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations DAT and VMAT2 specific binding DAT mRNA levels	Ekue et al. (2002)
Pellet (0.1 mg, 21-day release) for 17 days	MPTP	GDX mice	DA and DOPAC concentrations	Dluzen (1996)
Pellet (5 mg, 21-day release) for 14 days	MA	Intact and GDX mice	DA concentrations	Gao and Dluzen (2001a), Dluzen et al. (2002)
0.005–50 μg (1 injection), 24 h before MA	MA	Intact and GDX mice	DA concentrations	Lewis and Dluzen (2008)
Dihydrotestosterone				
1 or 50 μg B.I.D. for 10 days	MPTP	Intact mice	DA, DOPAC, and HVA concentrations DAT and VMAT2 specific binding DAT mRNA levels	Ekue et al. (2002)

B.I.D., twice a day; DOPAC, 3,4-dihydrophenylacetic acid; HVA, homovanillic acid; GDX, gonadectomized; TH, tyrosine hydroxylase.

study performed in MPTP-treated rodents has investigated the effect of estrogen treatment on symptoms. In female ovariectomized hemiparkinsonian MPTP monkeys where clear PD symptoms are present we have shown that acutely 17β-estradiol and dehydroepiandrosterone (DHEA, a neurosteroid and precursor of 17β-estradiol) potentiate the motor response obtained with a low dose of levodopa the most common treatment for PD (Belanger et al., 2003, 2006). Moreover, after a long washout of the acute treatments, a chronic 17β-estradiol treatment in these hemiparkinsonian MPTP monkeys increased striatal DA and metabolites concentrations in the intact and lesioned side while the DAT was only increased in the intact side of these very extensively denervated monkeys (Morissette and Di Paolo, 2009). The prodopaminergic effects of estradiol within the NSDA system were observed on DA release and metabolism, DA receptor, DAT, tyrosine hydroxylase, and monoamine oxidase (Sanchez et al., 2010).

MECHANISM OF ACTION OF ESTRADIOL IN FEMALE AND MALE RODENTS

Dopaminergic system

Sex differences in brain structure and function may affect the susceptibility to the toxins. Striatal DA concentration shows no difference between females and males whereas the number of tyrosine hydroxylase immunoreactive cells in the substantia nigra pars compacta were observed to be higher in male rodents, with a sex difference in the topographical distribution (McArthur et al., 2007a; Gillies and McArthur, 2010). In female rats, nigrostriatal DA neurotransmission seems to be more regulated by autoreceptor and transporter mechanisms (Walker et al., 2006). Moreover, although DAT affinity is observed to be the same in males and females, in female rodents superior DA uptake and vesicular storage is present (Morissette and Di Paolo, 1993b; Walker et al., 2000; Bhatt and Dluzen, 2005; Ji et al., 2007; Dluzen et al., 2008), suggesting a greater functional activity of DAT and VMAT2 in females. Further evidence demonstrating a sex difference in DAT function has been indicated from data showing that DA responses to MA infusion in the presence of the DAT inhibitor nomifensine were not abolished in female striatal tissue whereas the response was eliminated in males (Kunnathur et al., 2006). The DAT is considered a critical component of MPTP and MA toxicities since this transporter is the predominant site by which MA and MPP+ enter DA cells (Sotnikova et al., 2006). The importance of the DAT in MA and MPTP toxicities has been revealed in DAT knockout mice that show no striatal DA neurotoxicity when treated with MA or MPTP (Gainetdinov et al., 1997; Fumagalli et al., 1998), thus substantiating that decreased DAT activity is beneficial against toxins. This finding may seem inconsistent with the decreased susceptibility of female mice to the toxins and the greater efficiency of their DAT activity. In contrast to the effects resulting from a decreased number DAT, that is, a reduced potential for uptake of neurotoxins, a decreased number of VMAT2, has the potential for increasing toxicity responses to MA and MPTP (Gainetdinov et al., 1998; Fumagalli et al., 1999; Guillot et al., 2008). With fewer

VMAT2, there would be increased amounts of MPP+ available to interact with mitochondrial complex 1, as less sequestering of MPP+ would occur (Guillot and Miller, 2009). Similarly, the high amount of extra- and intra-cellular DA release by MA favors the production of reactive species (Fleckenstein et al., 2007) which could be sequestered by the VMAT2. Thus, a more efficient DA uptake as well as sequestering of excessive DA and MPP+ into vesicular storage via the VMAT2, as reported in females, could decrease the degree of oxidative stress and favor a more efficient protection of DA terminals when exposed to toxins that utilize these transporters (Guillot and Miller, 2009).

Aromatase activity and mRNA expression show no sex difference in the mouse striatum (Kuppers and Beyer, 1998). However, a sex difference in the expression and activity of aromatase is reported in astrocytes, suggesting that astrocytes of females possess the potential to produce more estradiol than astrocytes of males (Liu et al., 2007). Moreover, aromatase expression in astrocytes is induced following lesion and this aromatase activity has been shown to be neuroprotective (Garcia-Segura, 2008). Male rats treated with an aromatase inhibitor show increased susceptibility to a dopaminergic toxin (McArthur et al., 2007b). In addition, increased vulnerability to MPTP has been reported in aromatase knockout female mice as compared to ovariectomized wild-type females, indicating the contribution of extra-gonadal 17β-estradiol synthesis in the neuroprotection of the NSDA pathway (Morale et al., 2008). The induction of aromatase expression, and its neuroprotective effect, following lesion seem contradictory with the increase toxicity in female mice when 17β-estradiol is given after MA and the lack of protective effect when 17β-estradiol is given shortly after toxins. It should be noted that increased aromatase expression seems to be localized close to the lesion site (Carswell et al., 2005), promoting local synthesis of estradiol, as compared to systemic administration of 17β-estradiol given after toxins. Furthermore, the "amount/degree" of the initial lesion seems to be critical since the capacity for estrogen to enhance the neurotoxicity response in the previously lesioned MA-treated mice was more prominent under conditions where less initial damage was present (Liu and Dluzen, 2006). In addition, the timing/duration of the initial lesion could also influence the effect of estrogen on neurotoxicity.

The finding that estradiol protects DAT specific binding in MPTP- and MA-treated mice could also be considered contradictory since increased susceptibility to toxins is associated with higher DAT levels. Inconsistencies in the literature have been reported concerning the effect of estradiol treatment on DAT. Our group reported that 17β-estradiol treatment in ovariectomized female rats left the affinity of [^{3}H]GBR 12935 binding unchanged whereas DAT density increased (Morissette and Di Paolo, 1993a). A study using [^{3}H]WIN35,428 has also reported unchanged affinity for DAT binding by 17β-estradiol treatment in the striatum of gonadectomized male rats (Meyers and Kritzer, 2009). In contrast, decreased DA uptake in ovariectomized female rats treated with 17β-estradiol has been reported using [^{3}H]DA and a decrease of DAT density measured by [^{3}H]BTCP binding (Attali et al., 1997). [^{3}H]DA uptake from striatal synaptosomes of ovariectomized rats was also shown to be dose-dependently inhibited by 17β-estradiol (Disshon et al., 1998). While the issue regarding these effects of estrogen upon the DAT remains controversial, a more consistent result that emerges from studies of several laboratories is that 17β-estradiol treatment of ovariectomized rats restores DA uptake and DAT density to levels observed in intact female rats. Ovariectomized female rats treated with 17β-estradiol have equivalent DA uptake and DAT density as that of intact females not treated with 17β-estradiol (Attali et al., 1997; Le Saux and Di Paolo, 2006; McArthur et al., 2007b). Considering that females have a superior function of DAT and VMAT2 (Morissette and Di Paolo, 1993b; Walker et al., 2000; Bhatt and Dluzen, 2005; Ji et al., 2007; Dluzen et al., 2008) and are less susceptible to NSDA toxins than males (Miller et al., 1998; Bourque et al., 2011), the preservation of a integral and optimal DA system seems to be important in the neuroprotection process. In addition, DAT affinity and density were not modulated by 17β-estradiol treatment in male rodents (Jourdain et al., 2005; Meyers and Kritzer, 2009) but protection against MPTP is observed (Bourque et al., 2009). While it has been suggested that inhibition of the DAT by estradiol could be an important mechanism for neuroprotection in females (Disshon and Dluzen, 1999), the findings that 17β-estradiol can be effective as a neuroprotectant against MPTP in both males and females, in the apparent absence of any effects upon the DAT in males, suggests alternative or supplementary mechanisms for neuroprotection by this gonadal steroid hormone. As one possibility, there may exist an important interaction among 17β-estradiol, its binding with ERs and the DAT that contributes to this ability to display neuroprotection. Specifically, data resulting from an *in vitro* study have demonstrated that a physiological concentration of 17β-estradiol does not change the membrane and total DAT levels, whereas estrone and estriol cause removal of membrane DAT, with a reduction of total cellular DAT content also being observed with estriol (Alyea and Watson, 2009). Furthermore, 17β-estradiol and estrone, but not estriol, differently changed the subcellular localization of the ERs (Alyea and Watson, 2009). Despite the reduction in membrane DAT caused by estrone and estriol, estrone shows some weak protective activity against MPTP whereas estriol lacks a neuroprotective effect (Jourdain et al., 2005) and these two estrogens are weak agonist of ERs (Kuiper et al., 1997), supporting a close link between affinity for ER binding and a neuroprotective effect. Whereas DAT knockout mice have shown an important role for the DAT in MPTP and MA toxicities (Gainetdinov et al., 1997; Fumagalli et al., 1998), the effect of 17β-estradiol in preserving the affinity and the density of the DAT seems to be an important aspect of the neuroprotective effect. Furthermore, higher DAT density has been reported in females than males (Morissette and Di Paolo, 1993b) and females are less affected by toxins than males (Bourque et al., 2009). Clinical imaging studies in healthy participants have shown that striatal DAT binding is higher in women than men (Lavalaye et al., 2000; Staley et al., 2001) and a lower incidence and prevalence of PD is observed in women. Studies in healthy postmenopausal women reported that a 6-week period of estrogen therapy increases [^{99m}Tc]TRODAT-1 binding to DAT in the anterior putamen (Gardiner et al., 2004), that long-term use of estrogen therapy increases dopaminergic function (Craig et al., 2004), and estrogen therapy has been associated with a decreased risk of PD (Currie et al., 2004; Popat et al., 2005).

An intriguing sex specific effect of estradiol is observed in MA-treated male mice. While male rodents demonstrate a neuroprotective action of 17β-estradiol in response to MPTP (Bourque et al., 2009) a lack of any apparent beneficial effect from estrogen is seen in MA-treated mice (Dluzen et al., 2002). MPTP and MA mechanisms of action differ markedly, with MPTP affecting the mitochondrial complex 1 (Smeyne and Jackson-Lewis, 2005) and MA producing excessive amounts of DA release likely leading to reactive species production (Fleckenstein et al., 2007). The failure of 17β-estradiol to function as a neuroprotectant against MA in the male mouse is not readily obvious. A critical component of MA toxicity is body temperature, which influences the oxidation process (LaVoie and Hastings, 1999). MA itself produces hyperthermia and variations of body temperature have been reported to influence the extent of degeneration (LaVoie and Hastings, 1999). While female mice experience reductions of body temperature in response to estradiol treatment (Gao and Dluzen, 2001b), this effect was not present in male mice (Dluzen et al., 2002), where no change in body temperature was observed. The differential between female and male mice with regard estradiol's effect on body temperature could be involved in the sex difference response to the toxin. Interestingly, MPTP or MPP+ also produce a brief initial period of hyperthermia in mice, but this is followed by a more prolonged period of hypothermia (Satoh et al., 1987). Moreover, mice maintained at 4°C show a greater accumulation of striatal MPP+ along with greater depletions of striatal DA as compared with mice maintained at 22°C (Moy et al., 1998). Essentially opposite results are obtained with MA, where greater striatal DA concentration depletions are obtained in mice maintained at 22°C (Moy et al., 1998) and hypothermia diminished MA-induced striatal DA toxicity in the rat (Bowyer et al., 1992). Accordingly, the inability for estradiol to decrease body temperature may represent a particularly critical variable with regard to moderating MA-, but not MPTP-, induced striatal DA toxicity in the male mouse.

A critical point to consider is whether developmental effects of steroids (organizational effects) influence the neuroprotective response against toxin observed in adults. Anderson et al. (2005) attempted to address this issue of organizational effects in the MA model. Briefly, female mice gonadectomized at 3–5 days of age and immediately treated with testosterone propionate (1.25 mg), that is, masculinized females, continued to show an estrogen neuroprotection response to MA when tested as adults. Male mice gonadectomized at 3–5 days of age and immediately treated with sesame oil, that is, feminized males, failed to show an estrogen neuroprotection response when tested as adults. Therefore, attempts to masculinize female mice or feminize male mice did not alter the sexually dimorphic effect of estrogen (i.e., neuroprotection in females and no effect in males) upon MA-induced neurotoxicity responses. These results suggest that the long-term and even organizational effects of steroids may not necessarily (or adversely) affect the responses obtained in adults.

Estrogens receptors

The presence of 17β-estradiol within the brain at the time of injury is a critical component of the neuroprotective effect of this steroid since post-treatment fails to protect DA neurons (Gajjar et al., 2003; Liu and Dluzen, 2006). The timing of 17β-estradiol treatment also seems to influence the extent of this response since different durations of treatment produce varying degrees of neuroprotection (Gajjar et al., 2003), with increased effectiveness being associated with a longer treatment intervals ($24 > 12 > 0.5$ h), suggesting that different mechanisms of 17β-estradiol are operating. Estradiol produces its actions by genomic and non-genomic effects. Genomic mechanisms involve gene transcription mediated by activation of nuclear receptors, ERα and ERβ, and require periods of hours to days to exert their effects (Vasudevan and Pfaff, 2008). Non-genomic actions are defined by rapid effects (within minutes even seconds) of 17β-estradiol initiated by interaction with membrane ER and/or G protein-coupled estrogen receptor 1 (GPER1), leading to activation of signaling pathways (Vasudevan and Pfaff, 2008). Genomic and non-genomic actions of 17β-estradiol are known to act together to potentiate transcriptional activity (Vasudevan and Pfaff, 2008). 17β-estradiol can activate Akt and extracellular signal-regulated kinase (ERK1/2) signaling; both of which have been implicated in 17β-estradiol neuroprotective effects (Bryant et al., 2006; Raz et al., 2008). 17β-estradiol action can promote the up-regulation of neurotrophic factors such as brain-derived neurotrophic factor, the anti-apoptotic molecule Bcl-2 and/or inhibition of pro-apoptotic proteins such as BAD and Bax (Kipp et al., 2006; Brann et al., 2007). Interactions with growth factors, such as insulin-like growth factor 1, can also contribute to the protective effects of estradiol (Garcia-Segura et al., 2010) since these actions promote survival and have been implicated in the neuroprotective effect of 17β-estradiol.

Non-genomic actions of 17β-estradiol show sex differences in the activation of intracellular mechanisms in the mouse brain (Abraham and Herbison, 2005). Moreover, a sex-related difference in ERK1/2 activation by 17β-estradiol is reported in male and female rat astrocytes (Zhang et al., 2002b). Differential distributions of ERs are reported between female and male rodents. ERα has been detected in the striatum of both female and male rodents (Merchenthaler et al., 2004; Shughrue, 2004; Rodriguez-Navarro et al., 2008; Schultz et al., 2009) whereas the levels of ERα, as quantified by Western blot, are higher in the striatum of female mice (Rodriguez-Navarro et al., 2008). Furthermore, ERα seems to be primarily associated with the membrane fraction rather than the nuclear part when extracted from homogenized striatal tissue of female rats (Schultz et al., 2009). ERβ is not found in the striatum of male mice but both presence and absence have been reported in females (Mitra et al., 2003; Merchenthaler et al., 2004; Shughrue, 2004). Both ERα and ERβ are present in female mice substantia nigra pars compacta and striatum (Mitra et al., 2003) but are absent in male mice (Shughrue, 2004) whereas their presence has been detected in male rats (Zhang et al., 2002a). In female mice, the immunoreactivity of ERβ in substantia nigra pars compacta seems to be more prominent than ERα whereas the opposite seems true of the striatum (Mitra et al., 2003). GPER1 has been detected in the striatum and substantia nigra pars compacta with a similar pattern of distribution between female and male rodents (Brailoiu et al., 2007; Hazell et al., 2009; Bourque et al., 2011). The literature seems to be more consistent concerning ERα showing only species difference between male rats and mice in substantia nigra.

More discrepancies appear for the distribution of ERβ. Differences in species, the antibody used and cellular localization (nuclear; Mitra et al., 2003; Merchenthaler et al., 2004 versus extranuclear; Mitra et al., 2003) could provide some explanation for the divergent distribution of the ERβ described. Thus, when referring only to studies with mice, a different distribution seems to be present between females and males, as ERs are present in female mice substantia nigra pars compacta but absent in males. ERα has been detected in the striatum of both female and male mice, whereas the levels of this receptor seem higher in females. ERβ is not found in the striatum of male mice but both presence and absence have been reported in females. Colocalization of ERβ and tyrosine hydroxylase in substantia nigra pars compacta has been observed in both female and male rats (Creutz and Kritzer, 2004; Quesada et al., 2007). Studies combining double-label immunocytochemistry for ERβ and tyrosine hydroxylase positive substantia nigra pars compacta neurons with retrograde tract tracing revealed a defined topographical organization strongly favoring projection to the ventral striatum, whereas few ERβ and tyrosine hydroxylase positive substantia nigra pars compacta neurons were found to project to the dorsal striatum (Creutz and Kritzer, 2004). No androgen receptor and tyrosine hydroxylase positive substantia nigra pars compacta neurons were found to project to the striatum (Creutz and Kritzer, 2004). It seems reasonable to postulate that the sex differences observed in the distribution and levels of ERs could influence the dopaminergic system and mechanisms of 17β-estradiol action. It remains to be investigated whether estradiol activates the same signaling molecules in females and males and/or if a sexual dimorphism in downstream signaling proteins is linked with the ERs that are present in the dopaminergic system. Neuroprotective effects of 17β-estradiol against toxins can involve different signaling pathways between females and males.

In vitro studies have shown that ERs subtypes (including GPER1) have different effects in 17β-estradiol-mediated DA efflux (Alyea et al., 2008) and that ERα and ERβ, but not GPER1, are associated with the plasma membrane DAT (Alyea and Watson, 2009). Moreover, modulation of tyrosine hydroxylase transcription by 17β-estradiol is regulated in opposite directions depending on the ER subtype (Maharjan et al., 2005). Using ERα and ERβ knockout male mice, our group and others have investigated the role of each ER on NSDA markers in the neuroprotective effect of 17β-estradiol against MPTP toxicity (Morissette et al., 2007; Al-Sweidi et al., 2011). While ERα and ERβ knockout mice show normal striatal DA concentrations, DAT specific binding was increased in ERα knockout mice and normal VMAT2 specific binding was measured (Morissette et al., 2007; Al-Sweidi et al., 2011). By contrast, ERβ knockout mice display lower DA turnover as well as a reduction in striatal DAT and VMAT2 specific binding (Morissette et al., 2007; Al-Sweidi et al., 2011). Female ERα knockout mice exhibit higher D1 DA receptor expression levels and reduced expression of tyrosine hydroxylase and brain-derived neurotrophic factor in the midbrain of both female and male ERα knockout mice (Kuppers et al., 2008). Whereas both ERα and ERβ male knockout mice display normal serum 17β-estradiol levels, higher levels of testosterone, dihydrotestosterone, and 3β-diol were measured in ERα male knockout mice (Al-Sweidi et al., 2011). The highest susceptibility to MPTP was observed in ERα male knockout mice and the levels of testosterone and 3β-diol were inversely correlated with the loss of DA concentration (Al-Sweidi et al., 2011). The significance of these findings remains to be elucidated, however it is interesting to note that not only does testosterone fail to induce a neuroprotective effect in MPTP and MA-treated mice (Dluzen, 1996; Ekue et al., 2002; Lewis and Dluzen, 2008) but may exacerbate NSDA neurotoxicity responses as described below in Section "Mechanism of Action of Androgens in Female and Male Rodents." Exogenous 17β-estradiol does not protect ERα or ERβ male knockout mice from MPTP toxicity, showing that both ERs are necessary for neuroprotection (Morissette et al., 2007; Al-Sweidi et al., 2011). Moreover, the levels of ERs were not modulated by MPTP lesion (Shughrue, 2004). In contrast, we have recently reported increased GPER1 levels in male mice with a moderate MA-induced lesion, while the levels of this receptor remains unchanged in MA-treated female mice (Bourque et al., 2011). Thus, the sex difference in ERs distribution as well as the different roles for each of the ERs in the DA system could be associated with the sex differences present in the susceptibility to toxin and also influence their responses to 17β-estradiol.

MECHANISM OF ACTION OF ANDROGENS IN FEMALE AND MALE RODENTS

In contrast to that of estradiol, there exists relatively little information on the mechanisms of testosterone action within the NSDA system as related to striatal DA toxicity. To a large extent this disinterest stems from the apparent absence of any neuroprotectant effects of this gonadal steroid. However, with the advent of data suggesting that this male gonadal steroid may contribute to an aggravation of toxins that target the NSDA system (Gao and Dluzen, 2001a; Dluzen et al., 2002; Murray et al., 2003; Gillies et al., 2004; Lewis and Dluzen, 2008), it might be worthwhile to re-consider these testosterone effects and mechanisms.

With regard to the NSDA system, treatment of male rats with the anabolic-androgen steroid, nandrolone decanoate, leads to decreases in D1-like receptor labeling and an increase in D2-like binding sites within the caudate putamen (Kindlundh et al., 2001), which creates a type of hypodopaminergic condition. Moreover, a reduction in locomotor behavior and rearing are also observed in these rats treated with nandrolone decanoate (Johansson et al., 2000). Further support for this attenuation in striatal dopaminergic function by testosterone is provided from data showing that spontaneous (Dluzen and Ramirez, 1989) and amphetamine-stimulated locomotor and stereotyped behaviors (Menniti and Baum, 1981; Savageau and Beatty, 1981; Beatty et al., 1982; Dluzen et al., 1986) are decreased in the presence of testosterone. Moreover, basal (Dluzen and Ramirez, 1989) and stimulated (Hernandez et al., 1994; Shemisa et al., 2006) striatal DA release are decreased in testosterone treated rodents. Collating these parameters leads to the conclusion that the presence of testosterone is associated with a generalized reduction in NSDA activity, like that seen in PD.

The exact mechanisms involved in producing this testosterone-dependent reduction in NSDA function are not know, but some tangential data are available that can provide some perspective. Testosterone treatment of neuroblastoma cells induces apoptosis through activation of a Ca^{2+} signaling pathway, an effect that cannot be attributable to conversion into estrogens (Estrada et al., 2006). With the use of N27 cells, which might represent a more relevant model for testing toxicity on dopaminergic neurons, it was also demonstrated that testosterone contributes to an apoptotic cascade, impairing mitochondrial function and increasing oxidative stress to produce a caspase-3-dependent cleavage which would activate protein kinase Cδ (Cunningham et al., 2009). Additional work with this model revealed that this testosterone effect involved an intracellular androgen receptor and was not attributable to conversion into estradiol. The fact that, at least in the rat, androgen receptors are mainly expressed in the substantia nigra pars compacta versus the substantia nigra pars reticulata (Kritzer, 1997), combined with data from the cortex of the mouse that androgen receptors show increased phosphorylation levels to testosterone in aged males (Thakur et al., 2000), provides particularly relevant implications regarding the capacity for testosterone to exert age-related, adverse consequences within the NSDA system as related to PD. This testosterone-dependent increase in oxidative stress and free radical production may, in part, result from modulation of VMAT2 function by this gonadal steroid. Significantly greater amounts of reserpine-evoked DA release are obtained from the striatum of orchidectomized mice treated with testosterone versus those not receiving testosterone (Shemisa et al., 2006). Such results suggest that testosterone may be working like and/or synergistically with reserpine to inhibit VMAT2 function, thereby producing excessive DA levels available for metabolism to free radicals. Testosterone produced a non-significant decrease in vesicular DA uptake in cocaine-treated orchidectomized rats (Chen et al., 2003), and the VMAT2 of males is more sensitive to the toxic effects of MA, as substantia nigra VMAT2 mRNA is significantly decreased in male, but not female, mice receiving either low (20 mg/kg) or high (40 mg/kg) doses of this NSDA toxin (Bourque et al., 2011).

Taken together, the capacity for testosterone to induce a generalized hypodopaminergic state combined with an age-related enhanced potential for oxidative stress in critical NSDA sites, suggests a role and mechanism for this gonadal steroid in PD. In this way, the sex difference in the incidence of PD may involve a combination of neuroprotective effects of estrogens within women and neurodestructive effects of testosterone in men. While much work remains to be performed on this topic, these bidirectional sex and hormonal responses to NSDA toxins may comprise a rewarding direction of investigation to understand the pathology of conditions like PD.

CONCLUSION

Clinical and epidemiological reports on PD as well as animal models show a sex difference in neurodegeneration of the NSDA system. Most of the experimental studies focused on a 17β-estradiol neuroprotective effect, showing that low doses, but not high doses, are effective to protect the NSDA pathway against toxins. Furthermore, increased effectiveness of 17β-estradiol is associated with a longer treatment interval. While having received less attention, progesterone shows interesting neuroprotective actions in both male and female mice and does not oppose the effect of 17β-estradiol on the NSDA system when these two steroids were co-administered. Androgens lack neuroprotective properties in NSDA pathway and may even worsen the extent of the lesion. Genomic and non-genomic actions of 17β-estradiol are implicated in neuroprotection of NSDA system and the presence of both ERα and ERβ are critical for a beneficial effect. The contribution of GPER1 in the neuroprotective effect of 17β-estradiol in the NSDA pathway remains to be investigated but recent *in vitro* (Gingerich et al., 2010) and *in vivo* (Lebesgue et al., 2010) studies have reported a neuroprotective action of GPER1 activation.

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Review

Signaling pathways mediating the neuroprotective effects of sex steroids and SERMs in Parkinson's disease

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, QC, Canada G1V 4G2
[b] Faculty of Pharmacy, Laval University, Quebec City, QC, Canada G1K 7P4
[c] Department of Anatomy and Neurobiology, Northeast Ohio Medical University (NEOMED), Rootstown, OH 44272, USA

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ABSTRACT

Studies with the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of Parkinson's disease have shown the ability of 17β-estradiol to protect the nigrostriatal dopaminergic system. This paper reviews the signaling pathways mediating the neuroprotective effect of 17β-estradiol against MPTP-induced toxicity. The mechanisms of 17β-estradiol action implicate activation of signaling pathways such as the phosphatidylinositol-3 kinase/Akt and the mitogen-activated protein kinase pathways. 17β-estradiol signaling is complex and integrates multiple interactions with signaling molecules that act to potentiate a protective effect. 17β-estradiol signaling is mediated via estrogen receptors, including GPER1, but others receptors, such as the IGF-1 receptor, are implicated in the neuroprotective effect. Glial and neuronal crosstalk is a critical factor in the maintenance of dopamine neuronal survival and in the neuroprotective action of 17β-estradiol. Compounds that stimulate GPER1 such as selective estrogen receptor modulators and phytoestrogens show neuroprotective activity and are alternatives to 17β-estradiol.

1. Parkinson's disease and estrogens

Selective and progressive death of dopamine (DA) neuronal cell bodies in substantia nigra characterizes Parkinson's disease (PD). As a result, an imbalance in the control of movement by the basal ganglia appears, leading to manifestation of clinical symptoms such as resting tremor, rigidity and bradykinesia [88]. Several aspects including susceptibility to develop the disease, age at onset and symptoms support the existence of a sex difference in PD. These differences benefit women, as a higher predisposition of the disease is reported in men (with at least 1.5-fold greater risk) [129,131] and the age at onset occurs about 2 years later in women [47,128]. Different profiles of motor symptoms have also been described. Women present a PD phenotype with greater instability scores while men have worsened rigidity [8]. Although motor scores do not show sex differences in early PD, women have better scores than men in advanced PD (disease duration more than 5 years) [73]. Furthermore, at symptom onset, higher levels of striatal [^{123}I]FP-CIT binding to DA transporter (DAT) are observed in women, suggesting that the development of symptomatic PD may be delayed by higher endogenous striatal DA levels and that the phenotype of PD in women is more benign [47].

Various studies have been conducted to investigate how endogenous estrogen exposure and how the use of estrogens therapy could exert a positive effect on PD risk (reviewed in [71]). A longer fertile lifespan [104,112] and the use of estrogen therapy [24,97] are associated with a reduced risk of PD in most of the studies, suggesting a beneficial effect of estrogen with regard to PD risk. In addition to the beneficial effect of estrogens exposure on PD risk, data from clinical reports show that endogenous and exogenous estrogens can modulate PD symptoms. A worsening of PD symptoms in menstrual women was reported in pre-menstrual and menstrual periods, when estrogen and progesterone levels are low [55,127]. Several women with PD report a deterioration of their symptoms and the extensive variations in estrogen levels during and after pregnancy were suggested to be implicated in worsening of PD symptoms [107,109]. Lower symptom severity scores were reported in women with early PD taking estrogen therapy, but not yet taking levodopa [113]; an effect that was not observed at later stages of the disease [122]. Thus, clinical and epidemiological studies support the conjecture that endogenous and exogenous estrogen exposure exerts a beneficial effect upon the risk of PD. Moreover, these studies also suggest that estrogens can act as a neuromodulator of the DA system with the result being a diminution in PD symptoms. The neuromodulatory effects of estrogens may or may not share the same mechanisms as that of

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

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the neuroprotective effects. Since the focus of this review is on the neuroprotective effect of estrogen, we refer readers to a recent review on the neuromodulatory effect of estrogen on DA neurotransmission for more details on this aspect of estrogen action [110].

2. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of PD

Discovered as by-product of an analog of the narcotic meperidine, MPTP produces an irreversible parkinsonian syndrome in humans that mimics the main characteristics of PD including rigidity, tremor, bradykinesia, postural instability and freezing [29,64]. After crossing the blood brain barrier, MPTP is converted into its active metabolite 1-methyl-4-phenyl pyridinium (MPP^+) in glial cells by monoamine oxidase B. MPP^+ then enters the neuron through the DAT [28]. Inside the DA neurons, MPP^+ concentrates within mitochondria where it impairs mitochondrial respiration by inhibiting complex I of the electron transport chain, leading to an increased production of free radicals, which causes oxidative stress and activation of cell death pathways [28].

In mice, MPTP impairs the nigrostriatal DA system by decreasing DA concentrations as well as DAT and vesicular monoamine transporter 2 (VMAT2) densities [54], effects similar to that observed in PD [50]. The DAT and VMAT2 are both important modulators of DA neurotransmission and localize in striatal DA terminals and DA cell bodies in the substantia nigra. The DAT controls synaptic and extrasynaptic DA levels by re-uptake of released neurotransmitter from the presynaptic neuronal terminals [121], while the role of the cytoplasmic VMAT2 is to sequester DA within vesicles [46]. Thus, DAT and VMAT2 play crucial roles in the maintenance of DA homeostasis and DA, DAT and VMAT2 are valuable markers to evaluate integrity of DA terminals and cell bodies. With a moderate MPTP lesion, DA markers in substantia nigra are less intoxicated than that observed in striatum [54], suggesting that DA neuronal cell bodies in the substantia nigra are less affected than their terminals in striatum. Low doses of neurotoxins cause a loss of tyrosine hydroxylase positive terminals whereas high doses of neurotoxins are required to damage DA cell bodies, suggesting that damage to DA terminals is an early indicator of degeneration [17].

Studies in MPTP-treated mice have shown a greater neurotoxic effect in males as observed by more extensive striatal DA reduction [81]. Thus, the MPTP mouse model faithfully mimics the sex difference of PD. Moreover, this sex difference to MPTP suggests that endogenous estrogen levels appear to influence the vulnerability to MPTP toxicity and support a beneficial role of estrogen against nigrostriatal DA neurodegeneration (as described in Section 4). Given this protective potential of estrogen in the MPTP model and the clinical evidence supporting a beneficial effect of estrogen, particularly in the early stages of PD, these early stages of neurodegeneration, where substantia nigra DA neurons are injured but not yet dead, may represent an appropriate time to evaluate this capacity for neuroprotection by steroids.

3. Estrogen receptors

Estrogens produce their effects by binding to estrogen receptors (ER), ERα and ERβ, both belonging to the nuclear receptor family [76]. 17β-estradiol mediates its effect through two mechanisms. A genomic mechanism of ER action involves gene transcription mediated by activation of ERs, and requires hours to days to exert their effects (reviewed in [76]). ER-direct DNA association is mediated through an estrogen responsive element (ERE) and an ER-indirect DNA association could also result by interaction with the nuclear factor κB (NFκB), specificity protein 1 (SP1), the cAMP-response element binding protein (CREB) and/or by interaction with fos/jun transcription factors thereby regulating gene transcription via the activator protein-1 (AP-1) site. A non-genomic mechanism of estrogen action is also present and is defined as a rapid effect (within minutes even seconds) initiated by interaction with plasma membrane-associated ER and/or G protein-coupled estrogen receptor 1 (GPER1), leading to activation of signaling pathways. The activation of signaling pathways by 17β-estradiol could also result in transcriptional activity. Thus, genomic and non-genomic actions could converge to potentiate transcriptional activity.

Both ERα and ERβ are found in the striatum and substantia nigra while a difference in their distribution is described for these brain regions. The immunoreactivity of ERα in the striatum seems to be higher than ERβ whereas superior immunoreactivity of ERβ relative to ERα is reported in the substantia nigra [82]. Furthermore, ERβ and tyrosine hydroxylase positive substantia nigra pars compacta neurons were found to project to the striatum [23]. Striatal ERα has been shown to be primarily associated with the membrane versus the nuclear fraction of the neuron [115].

In addition to the classic ERα and ERβ, a high binding affinity of 17β-estradiol for an orphan receptor of the 7-transmembrane receptor family was described in 2005 [106,126]. This receptor, first known as GPR30 and now called GPER1, is expressed in the brain including the striatum and the substantia nigra [15,48]. Plasma membrane expression of GPER1 as well as Golgi apparatus and reticulum endoplasmic localization for this receptor have been found [38,77] and GPER1 levels have been detected in astrocytes [59]. GPER1 is able to mediate both rapid and transcriptional effects in response to 17β-estradiol in the brain and peripheral tissues [99].

GPER1 has been the subject of some controversies related to its role as an estrogen receptor due to its inability to mediate estrogenic responses in reproductive organs [89,90]. Nevertheless, it has been shown that the GPER1 agonist, G1, can reproduce many estrogen effects [99]. Additional discussions on the role of GPER1 as an estrogen receptor also come from data showing the lack of estrogenic responses by GPER1 in the absence of both ERα and ERβ as well as the absence of any changes in 17β-estradiol effects when GPER1 was not present [93]. But these data could also be interpreted to suggest that GPER1 acts as a collaborator of ERs and that the presence of ERs in some tissues is required to induce a GPER1 effect [68]. It was reported that GPER1 could change ERα phosphorylation signals in the mouse uterus [39] and that ERα can directly interact and activate G proteins to mediate estradiol signaling [58], supporting the potential collaboration of ER and GPER1. In addition to a potential collaboration of GPER1 and ERs, some 17β-estradiol effects in the brain are observed even when both ERα and ERβ are blocked [92,120], suggesting another mechanism of 17β-estradiol action through an alternative receptor, perhaps via GPER1. Experiments using the GPER1 agonist G1 have shown that GPER1 activation mediates many estrogen effects in various tissues (reviewed in [99]) and that G1 is as potent as 17β-estradiol in mediating neuroprotection following ischemia [65]. The mechanisms of 17β-estradiol signaling are complex, tissue specific and could include independent as well as co-dependent effects through ERα, ERβ and GPER1 [99]. Thus, further research is required to determine the contribution of GPER1, and its potential role as an ERs collaborator, in mediating estradiol effects in the brain.

4. Neuroprotective effects of estradiol against MPTP

Findings from several studies have shown that 17β-estradiol treatment is protective against MPTP toxicity in both female and male mice (reviewed in [12]). Beneficial effects of 17β-estradiol

against MPTP toxicity are observed on DA and its metabolites, DAT and VMAT2 specific binding as well as tyrosine hydroxylase positive immunoreactive cells [12]. In addition to neuroprotection, estrogen can function as a neuromodulator. A critical issue to resolve regarding these estrogen effects within the nigrostriatal dopaminergic system is whether they represent neuroprotection or neuromodulation. 17β-estradiol modulates nigrostriatal DA activity and increases DA turnover in ovariectomized rats [33] whereas nigrostriatal DAT and VMAT2 specific binding were not modulated by 17β-estradiol treatment in intact male mice [54]. The DA turnover provides an overall dynamic representation of the DA response showing an increase by the MPTP lesion in a compensatory mechanism to maintain neuronal activity homeostasis while the estrogenic treatments result in maintaining higher levels of both striatal DA and metabolite concentrations. The highly significant correlations between striatal DA concentrations and striatal DAT or VMAT2 specific binding in 17β-estradiol-treated MPTP mice support the conclusion that the DA modifications observed implicate estrogenic neuroprotection rather than only an activation of DA synthesis [54]. Thus, 17β-estradiol protects DA neuronal integrity and the signaling pathways mediating this effect are presented in Section 6.

The mechanism of 17β-estradiol action, when administered at low concentrations, is likely mediated via ERs. This is supported by findings showing a weak or lack of neuroprotective properties of 17α-estradiol, estrone or estriol *in vivo* [44,54]; all of which have a low affinity for ERs [56]. ERα seems to play the major and critical role in mediating 17β-estradiol's protective effect. In support of this conclusion are data showing that activation of ERα with the selective agonist 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)tris-phenol (PPT) reproduces the neuroprotective effect of 17β-estradiol in MPTP-treated mice whereas the ERβ agonist 2,3-bis(4-hydroxyphenyl)propionitrile (DPN) is not as potent [27]. The use of ERα and ERβ knock-out mice also point in this direction, as ERα knock-out mice are more susceptible to MPTP toxicity and are not protected by 17β-estradiol treatment [2,86]. However, a role for ERβ in neuroprotection cannot be excluded, and the presence of this receptor does seem to play a role since 17β-estradiol fails to protect against MPTP in ERβ knock-out mice [2,86].

Neuronal and glial cells interact in the nervous system. Both nigrostriatal neurons and glial cells express ERs and the response to 17β-estradiol is likely influenced by interaction of these cells. Neuronal signals are reported to modulate estradiol effects on astrocytes and, in turn, glia to neuronal signaling is influenced by estradiol [5]. Moreover, estradiol may also regulate glia-to-glia signaling [5]. Thus, the interaction between neurons and glial cells could also contribute to the neuroprotective process. Indeed, the sensibility of DA neurons to MPP^+ is increased in glia-free mesencephalic tissue cultures [9]. Furthermore, 17β-estradiol neuroprotective effects are not observed in cell cultures lacking glial cells, again suggesting a role for glia in 17β-estradiol-mediating neuroprotection [9].

Glial cells produce a variety of pro- and anti-inflammatory molecules that are implicated in the dualistic role (detrimental versus neuroprotective) of these cells [63]. The local production of 17β-estradiol as well as endogenous 17β-estradiol seems to induce a change in activated glial cells that promotes neuronal survival rather than favor neurodegeneration (reviewed in [6,63]). Overproduction of pro-inflammatory cytokines by glial cells could be responsible for neuronal death or increased vulnerability to cell death [123]. Neuroinflammation processes are observed in MPTP mice [84] and in PD [123]. An activation of astrocytes in the striatum and substantia nigra is reported following MPTP lesion, as shown by an increase in glia fibrillary acidic protein (GFAP) immunoreactivity [84]. Activated microglial cells and astrocytes express high levels of inducible-nitric oxide synthase (iNOS), responsible for generation of NO, and represent a main cytotoxic mediator of DA neuronal injury [84]. MPTP mice that had been treated with 17β-estradiol showed lower levels of astrocyte activation, inhibition of MPTP-induced iNOS-derived nitrites as well as a reduction in cell death and DA toxicity [84]. Thus, 17β-estradiol can modulate an inflammatory response by potentially inducing a change in detrimental inflammatory pathways to exert a beneficial action as mediated by glial cells [84].

Glial cells are a major source of enzymes implicated in the protection of damaged neurons. Aromatase is an enzyme converting testosterone to estradiol and also androstenedione to estrone [34]. Since aromatase expression is increased in reactive astrocytes following brain injury and is localized close to the lesion site, it is possible that a local synthesis of estradiol may result at the lesion site [40]. This aromatase activity has been shown to be neuroprotective and contributes to limiting neural damage after brain injury, as increased susceptibility to a dopaminergic toxin is observed in rats treated with an aromatase inhibitor [78]. Furthermore, increased vulnerability to MPTP has been reported in aromatase knock-out mice, suggesting the contribution of extra-gonadal 17β-estradiol synthesis in the neuroprotection of nigrostriatal DA system [83]. Astrocytes express ER and the local synthesis of 17β-estradiol could act in an autocrine manner by reducing the production of pro-inflammatory molecules and increasing the production of trophic factors that will support neuronal survival [6].

There are limited studies reporting the effects of MPTP lesion on brain ERs levels. Nigrostriatal ERα and ERβ were not increased following lesion with MPTP or treatment with 17β-estradiol [118]. In contrast, our group has recently reported an increase in GPER1 levels in the striatum of MPTP- and methamphetamine-treated male mice as well as in the MPTP-lesioned putamen of hemiparkinsonian monkey [3,14]. Furthermore, 17β-estradiol treatment did not change GPER1 levels in the intact or lesioned striatum of hemi-parkinsonian monkeys [3]. This increase of GPER1 levels following lesion could have significant implications in the vulnerability to toxins targeting the striatum and in the neuroprotective response mediated by 17β-estradiol. ERα levels were reported to increase in mice after stroke injury and ERα is required to mediate 17β-estradiol neuroprotection [36].

5. Estrogen signaling

5.1. PI3K/Akt and ERK1/2 signaling pathways

Dysfunctions of protein kinase have been shown to occur in PD and in MPTP animal models. Certain Akt1 haplotypes have been associated with reduced risk to develop PD [132] whereas two functional single nucleotide polymorphisms of glycogen synthase kinase 3 β (GSK3β), a downstream protein of Akt, are reported in PD brains [60]. Post-mortem substantia nigra DA neurons from PD patients show decreased Akt activity and RTP801, a protein promoting neuronal death, is increased [74,75]. Increased activation of GSK3β in post-mortem striata from PD patients has also been observed [37]. *In vitro* and *in vivo* models of MPTP toxicity have shown increased activity of GSK3β, associated with mitochondrial impairment, caspase activation and cell death [95]. Furthermore, inhibition of GSK3β activity blocks MPTP induced cell death, showing that GSK3β is a significant mediator of MPTP neurotoxicity [95]. Thus, targeting these kinases offers a therapeutic opportunity to promote cell survival and counteract the neurodegenerative process.

17β-estradiol is such a candidate and its effects in the brain have been associated with activation of two central regulators of cell functions and cell survival, the phosphatidylinositol 3 kinase

(PI3K)/Akt and the extracellular signal-regulated kinase (ERK1/2) pathways [105]. These two pathways converge downstream to common targets, such as the constitutive active kinase GSK3β and the proapoptotic protein Bad, which are phosphorylated, and thereby inhibited, by Akt and ERK1/2 [49,91]. Downstream, Akt targets also include several members of the Bcl-2 family, critical checkpoints in the mitochondrial pathway of apoptosis, and are comprised of both pro- (Bad, Bax) and anti-apoptotic (Bcl-2, Bcl-x) molecules (reviewed in [91]). Furthermore, activation of Akt and ERK1/2 could also induce transcriptional activity as these kinases have the possibility to translocate to the nucleus where they can activate transcription factors such as CREB, which induces the expression of Bcl-2 [49,91]. Akt also activates NF-κB, resulting in the modulation of transcription of target genes such as Bcl-2 and Bcl-x [91].

5.2. ER activation of PI3K/Akt and ERK1/2 pathways through multiple interactions with signaling molecules

The mechanism by which ERs activate Akt and ERK1/2 signaling is expected to involve multiple interactions with signaling proteins and is also shown to converge onto common downstream targets (Fig. 1). The plasma membrane localization of ERs in calveolae, microdomains at the plasma membrane that assemble signaling proteins, allows their interaction with PI3K/Akt and ERK1/2 pathways. Many ER-scaffold proteins and signaling molecules that are associated with membrane ERs may serve to facilitate activation of kinases by 17β-estradiol. Calveolin proteins, G proteins, Src, the p85α regulatory subunit of PI3K, Shc and receptor tyrosine kinases (i.e. insulin-like growth factor-1 (IGF-1) receptor) have all been reported to serve as components of complexes of interacting proteins with ERs [76].

17β-estradiol is also reported to interact with growth factor signaling in the brain, including IGF-1 signaling [41]. An immunohistochemistry study has shown that ERs and IGF-1 receptors colocalize in neurons and glial cells [19]. Although both IGF-1 and 17β-estradiol signal through PI3K/Akt and ERK1/2, a synergic activation of Akt by IGF-1 and 17β-estradiol is reported while ERK activation occurs in response to either IGF-1 or 17β-estradiol [20]. 17β-estradiol increases the interaction between the p85 subunit of PI3K and the docking protein insulin receptor substrate-1 (IRS-1), one of the first events in the IGF-I receptor signal transduction, suggesting that the increase in IGF-I receptor phosphorylation induced by 17β-estradiol reflects functional activation of this receptor [79]. Furthermore, administration of either 17β-estradiol or IGF-1 enhances the interaction between ERα, but not ERβ, and the p85 subunit of PI3K [79]. Taken together, these findings demonstrate a clear interaction between ERα and IGF-1 receptors which implicates an additional mechanism mediating brain signaling by 17β-estradiol and IGF-1. This conclusion is supported by the observation that an IGF-1 receptor antagonist blocks 17β-estradiol action in the brain and that the IGF-1 effect is inhibited by an ER antagonist [7].

Some of the rapid effects of 17β-estradiol were suggested to act through G protein activation [80]. Candidates for 17β-estradiol mediating G protein signaling are the metabotropic glutamate receptors (mGluR). Colocalization and co-immunoprecipitation experiments have suggested that ERs can directly interact with mGluR [31]. In striatal neurons, a bidirectional effect of 17β-estradiol on CREB phosphorylation has been described, which appears to be dependent upon the specific ER and mGluR subtype activated, and involves a mechanism independent of glutamate binding to mGluR. Thus, ERα alone activates mGluR5 signaling to mediate mitogen-activated protein kinase (MAPK)-dependent CREB phosphorylation [45]. In contrast, both ERα and ERβ can activate mGluR3 to inhibit L-type calcium channels mediating CREB activation [45]. These mechanisms occur through membrane ERs and require calveolin proteins, which play an important role in the trafficking of ERs to the membrane. The interaction of ERα with mGluR5 requires calveolin-1, and calveolin-3 is essential for coupling of ERα and ERβ with mGluR3 [45].

6. Estradiol signaling, neuroprotection and ERs

As described in the previous sections, 17β-estradiol produces a neuroprotective effect on DA terminals and cell bodies against MPTP toxicity. Molecules implicated in 17β-estradiol signaling and neuroprotection are presented in Tables 1 and 2. We have previously shown that the protection of 17β-estradiol against MPTP implicates activation of the PI3K/Akt pathway [25]. 17β-estradiol treatment increases phosphorylation of Akt in MPTP mice and PPT treatment, but not the ERβ agonist androstenediol, tends to reproduce this effect. Furthermore, only PPT treatment completely prevents the activation of GSK3β induced by MPTP whereas 17β-estradiol and androstenediol were less effective in preventing this response. Investigation of mitochondrial proteins shows that MPTP decreases the ratio of Bcl-2/Bad, suggesting a pro-apoptotic effect. PPT treatment completely abolished while 17β-estradiol and androstenediol to some extent reduced this effect, suggesting a positive regulation of cell survival by modulation of pro- and anti-apoptotic proteins. Taken together, the present results suggest that activation of the PI3K/Akt signaling pathway is involved in the neuroprotective effect of 17β-estradiol. This effect is mainly mediated by ERα, although an ERβ contribution cannot be excluded. Support for a neuroprotective role of ERα comes from a study showing that 17β-estradiol and PPT, but not DPN, decrease oxidative stress, measured by striatal glutathione peroxidase activity, as well as apoptosis, assessed by nigral caspase 3 activity, in 6-hydroxydopamine (6-OHDA) lesioned rats [11].

The interaction of IGF-1 and estradiol signaling in neuroprotection has been assessed in ovariectomized 6-OHDA rats [101]. 17β-estradiol benzoate or IGF-1 treatment prevented 6-OHDA-induced loss of substantia nigra pars compacta neurons and tyrosine hydroxylase immunoreactivity in DA terminals in the striatum [101]. Blockade of IGF-1 receptor with JB-1, an IGF-1 receptor antagonist, attenuated the neuroprotective effects of both 17β-estradiol benzoate and IGF-1, suggesting that the neuroprotective actions of 17β-estradiol depend on the co-activation of both ERs and IGF-I receptors [101]. The DA protection observed in the substantia nigra of 6-OHDA lesioned rats mediated by IGF-1 and 17β-estradiol benzoate is dependent on the PI3K/Akt, but not on the MAPK/ERK, pathway [100]. In the substantia nigra pars compacta, localization of IGF-1 receptor and ERβ, but not ERα, in DA neurons and glial cells has been observed [102]. Hence the interaction of signaling pathways between IGF-1 receptor and ER mediating neuroprotection may also involve ERβ.

Data from studies using mesencephalic cultures provides support for the notion that neuroprotection by estradiol in DA neurons could also be mediated by the MAPK pathway [130]. The ability for 17β-estradiol to protect against the loss of tyrosine hydroxylase immunoreactive cells induced by 6-OHDA is lost in the presence of the ER antagonist ICI 182,780. 17β-estradiol increased Bcl-2 expression and this effect was inhibited by the use of an ER antagonist and a MAKP inhibitor, whereas inhibition of PI3K with wortmannin did not inhibit 17β-estradiol upregulation of Bcl-2. These results suggest that Bcl-2 transcription regulation induced by 17β-estradiol is mediated by MAPK signaling and requires activation of classical ERs. In PC12 cells, 17β-estradiol improves cell viability and survival rate, and reduces apoptosis induced by MPP^+ by increasing the expression of Bcl-x and reducing the expression of IL-1β converting enzyme, a protein mediating apoptosis [69].

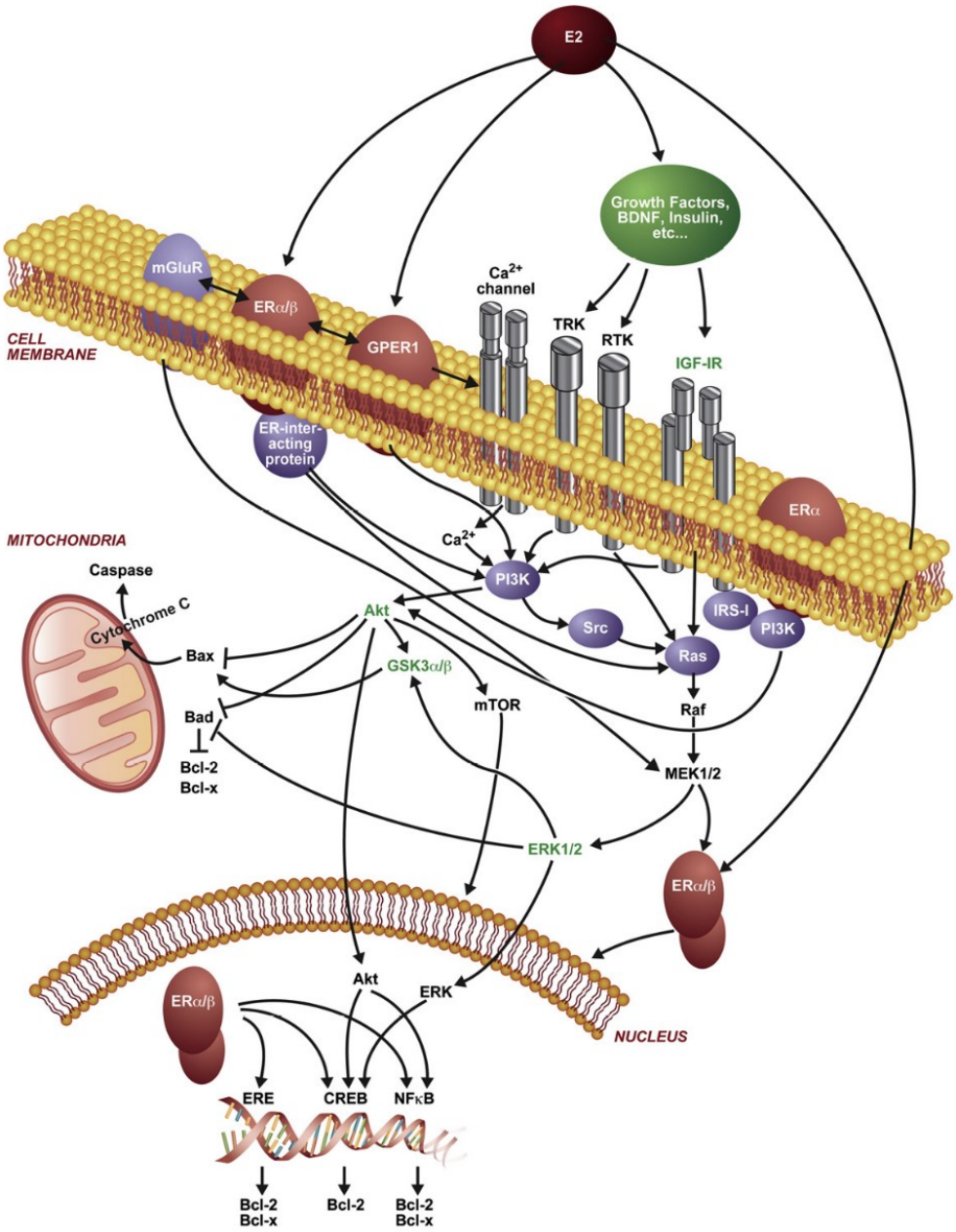

Fig. 1. Schematic presentation of 17β-estradiol signaling in neuroprotection of DA neurons. Arrows indicate stimulatory effects and lines indicate inhibitory effects.

While the results by Wang et al. [130] support the concept that 17β-estradiol regulation of Bcl-2 transcription occurs through a non-genomic mechanism mediated by MAPK signaling, a genomic action mediated by ER binding to ERE sites in the coding region of the Bcl-2 and Bcl-x gene has also been described [94,96]. Thus, 17β-estradiol promotes the expression of Bcl-2 and Bcl-x by different mechanisms and there is convergence of genomic and non-genomic actions of 17β-estradiol to potentiate transcription of target genes implicated in cell survival. An *in silico* analysis in the promoter sequences of Akt, GSK3β, ERK1 and ERK2 for transcription factor binding sites was performed to locate matches in DNA sequences (matrix similarity $\geqslant 0.85$) (MatInspector 8.0.5 software, Genomatix) [21]. Analyses have identified ERE promoter sequences of Akt, GSK3β, ERK1 and ERK2 and several matrix of SP1 ($\geqslant 8$) have been found on the promoter sequence of all genes. While this analysis is a theoretical model, we recently reported an increase in Akt levels following chronic treatment with 17β-estradiol in the putamen of ovariectomized monkeys [111], suggesting that Akt synthesis could be mediated by ERE (0.988 of matrix similarity, MatInspector 8.0.5 software, Genomatix) or by SP1 (28 matrix found, MatInspector 8.0.5 software, Genomatix). Thus, the matrix similarity for ERE and the number of potential response

Table 1
Molecules implicated in estrogen signaling in nigrostriatal system in animal and cellular models.

Assays	Treatment	Effect	Brain region, cell type	References
Akt	17β-estradiol benzoate	Increases activity	Substantia nigra	[100]
	IGF-1	Increases activity		
	LY294002	Inhibits 17β-estradiol and IGF-1 effect		
Akt	17β-estradiol	Increase activity	Striatum	[25]
	PPT	Increase activity		
	Androstenediol	No effect		
Akt	17β-estradiol	Increases activity	Midbrain neurons in culture	[53]
	ICI 182,780	Does not block 17β-estradiol effect		
	LY294002	Inhibits 17β-estradiol effect		
Akt	17β-estradiol	No effect	Midbrain astrocytes	[53]
Akt	17β-estradiol	Increases activity	Midbrain neuronal-enriched cultures	[108]
GSK3β	17β-estradiol	Decreases activity	Striatum	[25]
	PPT	Decreases activity		
	Androstenediol	Decreases activity		
GSK3α/β	17β-estradiol	Decreases activity	Midbrain neuronal-enriched cultures	[108]
Src-ERK	17β-estradiol	Increases activity	Midbrain astrocytes	[92]
	ICI 182,780	Does not block 17β-estradiol effect		
ERK	17β-estradiol	Increases activity	Midbrain neuronal-enriched cultures	[108]
CREB	17β-estradiol	ERα activates mGluR5 to increase CREB activity via MAPK	Striatum	[45]
		ERα and ERβ activate mGluR3 to inhibit CREB activity via L-type calcium channel		

Table 2
Molecules implicated in estrogen signaling in animal and cellular models of Parkinson's disease.

Assays	Treatment	Effects	Brain region, cell type	Toxin	References
Akt	17β-estradiol	Increases activity	Striatum	MPTP	[25]
	PPT	Increases activity			
	Androstenediol	No effect			
PI3K	17β-estradiol benzoate + LY294002 (PI3K inhibitor)	Blocks 17β-estradiol benzoate neuroprotective effect	Striatum and substantia nigra	6-OHDA	[100]
GSK3β	17β-estradiol	Decreases activity	Striatum	MPTP	[25]
	PPT	Decreases activity			
	Androstenediol	Decreases activity			
Bcl-2/Bad	17β-estradiol	Increases ratio	Striatum	MPTP	[25]
	PPT	Increases ratio			
	Androstenediol	Increases ratio			
Bcl-2	17β-estradiol	Increases expression	Mesencephalic cell culture	6-OHDA	[130]
	ICI 182,780	Blocks 17β-estradiol effect			
	PD98059 (MAPK inhibitor)	Blocks 17β-estradiol effect			
	Wortmannin (PI3K inhibitor)	Does not inhibited 17β-estradiol effect			
Bcl-2	17β-estradiol	Increases levels	Mesencephalic cell culture	MPP^+	[114]
IGF-1 receptor	17β-estradiol benzoate + JB-1 (IGF-1 receptor antagonist)	Block 17β-estradiol benzoate neuroprotective effect	Striatum and substantia nigra	6-OHDA	[101]
Bcl-x	17β-estradiol	Increases expression	PC12 cells	MPP^+	[69]
Caspase 3	17β-estradiol	Decreases activity	Substantia nigra	6-OHDA	[11]
	PPT	Decreases activity			
	Raloxifene	Decreases activity			
	DPN	No effect			
	Tamoxifen	No effect			

elements for SP1 strongly support that increased levels of Akt, as reported *in vivo* following 17β-estradiol treatment, could be because of increased synthesis mediated through ERE or SP-1.

The c-Jun N-terminal kinase (JNK) signaling has been implicated in apoptosis signaling [30]. JNK phosphorylates the transcription factor c-Jun, thereby regulating AP-1 complex transcriptional activity [30]. c-Jun is activated in mesencephalic DA neurons in both post-mortem human PD brain and MPTP mice and transcription of JNK-dependent target genes is a required step in DA cell death induced by MPTP [51]. In mesencephalic neurons, MPP^+ caused dopaminergic neuronal death by activation of the JNK/c-Jun/AP-1 cascade [114]. The 17β-estradiol neuroprotective effect is not mediated by inhibition of JNK activation induced by MPP^+ but rather by the inhibitory transcriptional regulation at the AP-1 element through ERβ, suggesting that 17β-estradiol could suppressed the c-Jun/AP-1 regulation of gene transcription implicated in cell death [114].

There is growing evidence that glial cells are targets for 17β-estradiol (Fig. 2). Midbrain astrocytes show nuclear localization of both ERα and ERβ whereas only ERα is detected in the membrane/cytoplasmic fraction [92]. 17β-estradiol treatment of these cells activates a Src-ERK1/2 signaling pathway [92]. While the rapid effect of 17β-estradiol on activation of Src-ERK1/2 pathways suggested a membrane-initiated action likely through ERα, the use of the ER antagonist ICI 182,780 did not inhibit 17β-estradiol phosphorylation of these kinases [92]. Thus, the rapid action of 17β-estradiol appears to be initiated by additional mechanisms differing from classical ERs mediation.

Support for the importance of glia-neuronal interactions in the maintenance of DA neuronal survival comes from recent studies

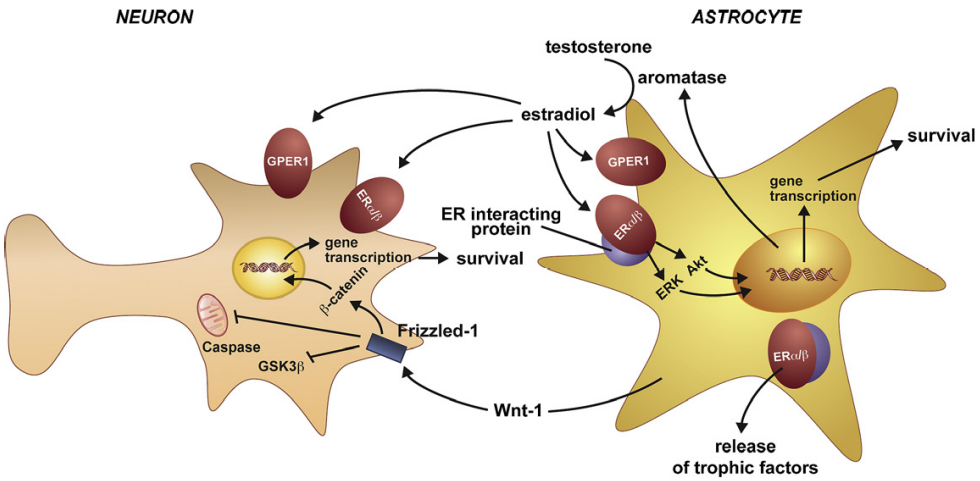

Fig. 2. Schematic presentation of dopaminergic neuron–astrocyte crosstalk in neuroprotection. Arrows indicate stimulatory effects and lines indicate inhibitory effects.

defining a wingless-type MMTV integration site1 (Wnt1)/Frizzled-1/β-catenin pathway as a novel astrocyte–neuron signaling system required for survival and protection of adult substantia nigra DA neurons against MPTP [61,62]. Post-injury activation of Wnt signaling is associated with stabilization of β-catenin, inhibition of GSK3β and caspase 3 activities whereas inhibition of Wnt signaling counteracted DA neuroprotection [61,62]. Dysfunction of Wnt signaling has been implicated in the pathogenesis of several neurodegenerative and neurological diseases, including PD, and potential role for the Wnt pathway as a pro-survival signaling cascade has been described [52]. Interestingly, 17β-estradiol has been shown to regulate Wnt/β-catenin signaling and this effect is implicated in the neuroprotective mechanism of 17β-estradiol against brain injury [103,134].

7. SERMs, phytoestrogen and the GPER1 agonist as interesting alternatives to 17β-estradiol

Whereas 17β-estradiol shows potent neuroprotective effects in various experimental models of brain injury, there are several limitations for its use in the treatment or prevention of neurodegenerative diseases because of its peripheral actions [124]. GPER1 represents an interesting target as no identified role upon reproductive organs has been associated with this receptor and recent studies have reported a neuroprotective effect of GPER1 activation [42,65]. Thus, a specific agonist of GPER1 could represent an interesting alternative to 17β-estradiol and such a non-feminizing estrogenic response could allow for a therapeutic utilization in both women and men.

SERMs, such as tamoxifen and raloxifene, also represent viable alternatives to estrogen and are commonly used clinically. Raloxifene shows estrogenic antagonist activity in mammary and uterine tissues. Raloxifene has been used in healthy elderly men and was reported to enhance brain activation in several cortical areas, without side effects [43]. Tamoxifen and raloxifene, or other drugs used in humans as estrogen replacements, such as phytoestrogens, are all GPER1 agonists [98]. Thus, it is likely that some of the clinical effects observed with these compounds implicate, at least in part, an action mediated by GPER1 [98,99]. Recently, a structure–activity study of raloxifene analogs found a biphenolic pharmacophore for neuroprotection of cortical neurons against oxygen–glucose deprivation via a GPER1-dependent, but ER-independent, mechanism mediated via PI3K, Src, and ERK dependent signaling pathways [1]. Thus, in neurons, GPER1 is able to mediate activation of signaling pathways implicated in the 17β-estradiol neuroprotective effect.

Raloxifene, but not tamoxifen, shows neuroprotective effects against MPTP in mice [18,44,85] and in 6-OHDA lesioned rats [11]. It has been shown that tamoxifen is neuroprotective against methamphetamine-induced toxicity of the nigrostriatal DA system [13,26]. Tamoxifen and raloxifene act on neurons and glial cells to induce neuroprotection by a mechanism implicating activation of a number of signaling pathways, including: MAPK/ERK and PI3K/Akt [35,66,67], the control of pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) expression [116], the regulation of transcription factors, such as CREB [117] and NF-κB [22], the increase of transforming growth factor-β expression and release [32], the reduction of the inflammatory response of glial cells [4] and the decrease of caspase 3 activity [11] (Tables 1 and 2).

Another compound that shows interesting neuroprotective capacity and targets GPER1 is the phenolic isoflavone genistein, a phytoestrogen found in soy products. Genistein displays a higher binding affinity for ERβ than ERα, stimulates the transcriptional activity of both ER subtypes [57] and activates GPER1 [125]. Genistein has neuroprotective effects against MPTP-induced loss of striatal DA concentration, tyrosine hydroxylase positive cells and Bcl-2 expression in the substantia nigra of mice [72]. Furthermore, genistein treatment in rats protects substantia nigra neurons against 6-OHDA lesion [10] and an *in vitro* study reported that genistein suppresses caspase 8 and partially blocks caspase 3 activation induced by 6-OHDA [70].

8. Conclusion

The neuroprotective mechanism of 17β-estradiol is complex and involves not only signaling pathways but integrates multiple protein interactions that act to potentiate a protective action through genomic and non-genomic actions, along with the collaboration of various cell types, such as interactions between neurons and glia (Figs. 1 and 2). The effect of 17β-estradiol in the brain depends on the neuroanatomical organization of the ERα and ERβ, which has been shown to differ between striatum and substantia nigra, and the plasma membrane interaction of ERs with proteins that may serve to facilitate activation of signaling pathways. Whereas ERα seems to have a major role in mediating 17β-estradiol neuroprotective effects against MPTP toxicity, the role of ERβ is less clear but cannot be excluded. There could be synergistic effects of ERα and ERβ activation involving different

signaling pathways which result in protection of the nigrostriatal system against MPTP toxicity. The contribution of GPER1 in 17β-estradiol mediating neuroprotection, as well as its potential interaction with ER, remains to be clarified and represents an interesting alternative approach to 17β-estradiol as GPER1 does not mediate estrogenic responses in reproductive organs. A better understanding of 17β-estradiol signaling, as well as the exact ER subtype (including GPER1) mediating these effects, in neuroprotection against MPTP will allow for the development of novel therapeutic approaches to reproduce 17β-estradiol beneficial effects in the brain while avoiding adverse effects in periphery. Strategy for the development of NeuroSERMs, with neuroprotective properties, that selectively target and activate estradiol mechanisms of action in the brain while avoiding activation of peripheral ER have been suggested [16,136]. A hybrid structure of 17β-estradiol and vitamin E, as well as nonfeminizing estrogenic analogs, have been shown to be neuroprotective [119,133,135]. Development of subtype-selective ER-based therapeutics has also been suggested [87] and show great potential for future therapies.

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