Effect of tricyclic antidepressants on L-DOPA-induced dyskinesia and motor improvement in hemi-parkinsonian rats

Melissa M. Conti, Adam A.A. Goldenberg, Alexandra Kuberka, Mohamed Mohamed, Satie Eissa, David Lindenbach, Christopher Bishop *

Behavioral Neuroscience Program, Department of Psychology, Binghamton University, 4400 Vestal Parkway East, Binghamton, NY 13902-6000, USA

Summary

Although dopamine replacement therapy with L-DOPA in Parkinson’s disease initially reduces motor symptoms, its chronic use often leads to the development of abnormal involuntary movements known as L-DOPA-induced dyskinesia. Increasingly, research has indicated that non-dopaminergic neurons gain function in the parkinsonian brain, taking up and converting L-DOPA into dopamine and releasing it as a “false neurotransmitter”. Although less explored, promiscuity between monoamine transporters may also modulate these processes. Therefore, in order to examine the differential roles of monoamine transporters in L-DOPA’s behavioral effects, three tricyclic antidepressants (TCA) with graded affinity for serotonin (SERT) vs. norepinephrine (NET) transporters were tested in hemi-parkinsonian rats: clomipramine (SERT > NET), amitriptyline (SERT = NET), and desipramine (SERT < NET). Rats received 6-hydroxydopamine lesions and were primed with L-DOPA (12 mg/kg, s.c.) to develop stable dyskinesia (N = 19 of 26). In a series of studies, rats were administered TCAs (0, 7.5, 15 or 30 mg/kg, i.p.) followed by L-DOPA (6 mg/kg, s.c.) and were measured for dyskinesia using the abnormal involuntary movements scale as well as motor performance and activity using the forepaw adjusting steps test and locomotor chambers, respectively. Clomipramine, the compound with the highest affinity for SERT was most effective in attenuating L-DOPA-induced dyskinesia without altering L-DOPA’s stimulatory effects. In contrast, desipramine, the TCA with the highest NET affinity deferred L-DOPA’s effects to later time points in testing. The current results indicate divergent roles for non-dopaminergic neuronal transporters in L-DOPA’s mechanisms of action and point to novel targets for improving Parkinson’s disease treatment.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Over the past fifty years, dopamine (DA) replacement therapy with L-DOPA has been commonly employed to moderate the akinesia and bradykinesia resulting from Parkinson’s disease (PD) (Smith et al., 2012). However, long-term L-DOPA treatment often leads to uncontrollable abnormal involuntary movements (AIMs) termed L-DOPA-induced dyskinesias (LID) (Jankovic, 2005), which affect approximately 90% of its users after a decade (Ahlskog and Muenter, 2001). As irregular movements resulting from Parkinson’s disease (PD) (Smith et al., 2005), but lack D2 autoreceptors and DA transporters (DAT) which regulate late DA efflux (Carta et al., 2007; Eskow et al., 2009; Navailles et al., 2010). Recent research has suggested that the 5-HT transporter (SERT) may modulate unregulated DA release as it can transport DA (Kannari et al., 2006; Larsen et al., 2011; Yamato et al., 2001), is upregulated in dyskinesic rats, primates, and humans (Rylander et al., 2010), and can reduce LID when blocked, in part via stimulation of 5-HT1A receptors (Bishop et al., 2012; Carta et al., 2007; Conti et al., 2014; Eskow et al., 2009). In addition, evidence has suggested that norepinephrine (NE) transporters (NET) may also play an enhanced role in DA clearance when striatal DAT levels wane in PD (Arri et al., 2008; Chotibut et al., 2012; Chotibut et al., 2014). Therefore, targeting SERT and NET may provide symptomatic relief to PD patients.

Tricyclic antidepressants (TCAs) are popular pharmacotherapy for PD patients suffering from depression (Chung et al., 2010; Menza et al., 2009). According to the meta-analysis by Liu et al. (2013), TCAs...
are better tolerated among patients than selective monoamine uptake inhibitors. TCAs also allow for the investigation of opposing transporter mechanisms given the varying balance of NET:SERT affinity across compounds. Therefore, we compared the effects of clinically relevant TCAs clomipramine (CLOM; SERT > NET), amitriptyline (AMI; SERT = NET), and desipramine (DES; SERT < NET) (Antonini et al., 2006; Devos et al., 2008; Millan et al., 2001a; Owens et al., 1997) on L-DOPA-induced behaviors to uncover the potential of SERT and NET as L-DOPA adjuncts. It was thus hypothesized that greater SERT blockade would provide greater anti-dyskinetic benefit, while greater NET blockade would accentuate L-DOPA’s motor enhancing effects in hemi-parkinsonian rats.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats were used (225–250 g upon arrival; Taconic Farms, NY, USA). Animals were housed in plastic cages (22 × 45 × 23 cm) and had free access to standard laboratory chow (Roden Diet 5001; Lab Diet, Brentwood, MO, USA) and water. The colony was maintained on a 12/12 h light/dark cycle (lights on at 07:00 h) at a temperature of 22–23 °C. Animals were maintained and protocols were approved in accordance with the guidelines of the Institutional Animal Care and Use Committee of Binghamton University and the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academic Press, 2011).

2.2. 6-Hydroxydopamine lesion surgeries

One week after arrival, all rats (N = 26) received unilateral 6-hydroxydopamine HBr (6-OHDA) (Sigma, St Louis, MO, USA) lesions of the left medial forebrain bundle to destroy DA neurons (Bishop et al., 2009). DES (25 mg/kg, i.p.; Sigma) was given 30 min prior to 6-OHDA injection to prevent 6-OHDA-induced loss of NE neurons. Rats were anesthetized with inhalant isoflurane (2–3%; Sigma) in oxygen (2.5 L/min), and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The lesion coordinates were AP: −1.8 mm, ML: +2.0 mm, and DV: −8.6 mm relative to bregma with the incisor bar positioned 5 mm below the interaural line (Paxinos and Watson, 1998). Using a 10 μL Hamilton syringe attached to a 26 gauge needle, 6-OHDA (12 μg) dissolved in 0.5% NaCl + 0.1% ascorbic acid was infused through a small hole in the skull at a rate of 2 μL/min to a volume of 4 μL. The needle was withdrawn 5 min later. Following surgery, all rats were placed in clean cages on a warming pad for recovery, after which they were pair-housed. Soft chow was provided to facilitate recovery during the first week after surgery. Analgesic buprenorphine HCl (0.03 mg/kg, i.p.; Hospira Inc, Lake Forest, IL, USA) was given pre-emptively and the remaining cohort of dyskinetic rats (n = 10) were acclimated to an automated locomotor chambers at least 4 times over 2 weeks for 180 min off treatment. On test days, rats received injections of either CLOM, AMI, or DES (0 or 15 mg/kg, i.p.) 30 min prior to L-DOPA (6 mg/kg, s.c.). Motor performance was examined using the FAS test, as described below, 60 min after L-DOPA, since this time point coincides with peak L-DOPA efficacy and LID (Bishop et al., 2012).

2.4. Experimental design

2.4.1. Effects of TCAs on L-DOPA-induced AIMS and rotations

Using a counterbalanced within-subjects design, the first cohort of rats (n = 9) received clomipramine HCl (CLOM; SERT > NET), amitriptyline HCl (AMI; SERT = NET) or DES (SERT < NET) (0, 7.5, 15 and 30 mg/kg, i.p.; Sigma) to determine the influence of NET and SERT inhibition on L-DOPA-induced ALO AIMS and rotations. Thirty min after TCAs treatment, L-DOPA (6 mg/kg, s.c.) was administered and AIMS and rotations were scored as noted below every 10 min for 3 h. TCAs were tested separately and doses were randomized across rats with water used as vehicle; therefore, all rats received all doses of all compounds by the last test day. There was a 2 day wash out period between each testing session which allowed for full drug and metabolite clearance (Bae et al., 2009; Kozisek et al., 2007; Weigmann et al., 2000). TCA timing and doses were chosen based on previous work demonstrating the pharmacological profiles of each compound (Chotibut et al., 2014; Millan et al., 2001b; Paumier et al., 2015a).

2.4.2. Effects of TCAs on L-DOPA efficacy

To examine whether SERT and NET blockade alter the anti-parkinsonian efficacy of L-DOPA, rats tested on AIMS and rotations were also tested for modifications to motor performance. To do so, rats received counterbalanced doses of CLOM, AMI, or DES (0 or 15 mg/kg, i.p.) 30 min prior to L-DOPA (6 mg/kg, s.c.). Motor performance was examined using the FAS test, as described below, 60 min after L-DOPA, since this time point coincides with peak L-DOPA efficacy and LID (Bishop et al., 2012).

2.4.3. Impact of TCAs on spontaneous motor activity

The remaining cohort of dyskinetic rats (n = 10) were acclimated to an automated locomotor chambers at least 4 times over 2 weeks for 180 min off treatment. On test days, rats received injections of either CLOM, AMI, or DES (0 or 15 mg/kg, i.p.) 30 min prior to vehicle (0.9% NaCl containing 0.1% ascorbic acid) or L-DOPA (6 mg/kg, s.c.). Rats were then immediately placed in locomotor chambers for analysis. The order of treatments was counterbalanced across test days. All testing was performed between 10:00–16:00 h and rats were placed in the same chambers for each session. Locomotor activity was assessed for a period of 180 min with data grouped into 3 blocks of 60 min.

2.5. Behavioral analyses

2.5.1. AIMS and rotations

The AIMS model of dyskinesia utilizes distinct behavioral measures and demonstrates face validity with known anti-dyskinetic compounds (Lundblad et al., 2002; Taylor et al., 2005). After treatment with L-DOPA, rats were placed in plastic cylinders and rated by trained observers blind to the experimental condition for 1 min every 10 min over a 180-min period. Individual dyskinesia severity scores ranging from 0 (not present) to 4 (severe and not interruptible) were recorded for axial, limb, and orolingual (ALO) as detailed in (Eskow et al., 2009; Lindenbach et al., 2011). The 3 AIMS subtypes were combined to create a single ALO AIMS score for data analyses.

2.5.2. Forelimb adjusting steps

The effect of DA lesion and subsequent drug therapy on motor ability was assessed using the FAS test, which assesses akinesia, a cardinal symptom of PD (Chang et al., 1999; Olsson et al., 1995). Rats were held such that they had only 1 free forelimb; for each trial, rats were moved laterally across a table at a steady rate of 90 cm/10 s. Each stepping test consisted of 6 trials for each forepaw, alternating between directions. “Percent intact” stepping was derived by dividing the total steps taken by the lesioned forelimb by the total steps taken by the intact forelimb and multiplying by 100. Overall stepping was determined.
by adding the total steps taken by the lesioned and intact forelimbs. Lower scores indicate greater parkinsonian impairment.

2.5.3. Locomotor activity

Locomotor activity testing was conducted in 6 identical acrylic chambers measuring $40 \times 40 \times 30$ cm (Accuscan Instruments, Columbus, OH, USA) in a dimmed room. Each chamber was surrounded by a $15 \times 15$ infrared photocell array interfaced with a computer that ran Versamax and Versadat programs, which tabulated and processed behaviors in the test field. Chambers were cleaned with 15% ethanol/water and new shavings were applied prior to each use. For the present experiment the variables examined included locomotor measures such as total distance (measured in cm), movement number (number of discrete movements punctuated by $\geq 1$ s of rest), and stereotypy counts. Changes in total distance often reflect parkinsonian status; alterations in movement number indicate initiation of movements while modification of stereotypy activity often corresponds with non-ambulatory behaviors such as dyskinesia (Bishop et al., 2005; Lindenbach et al., 2011).

2.6. High-performance liquid chromatography

Following a 2–3 day washout period following the last day of testing, striatal tissue was obtained from all rats to quantify lesion-induced monoamine and metabolite loss via reverse-phase HPLC-ED analysis (Kilpatrick et al., 1986), a method for semi-automated catecholamine analysis with coulometric detection, as reported previously (Eskow et al., 2009; Eskow Jaunarajs et al., 2010). The limit of detection was $10^{-10}$ M for the monoamines and the metabolites measured which included NE, 3,4-Dihydroxyphenylacetic acid (DOPAC), DA, 5-Hydroxyindoleacetic acid (5-HIAA) and 5-HT. The final oxidation current values were plotted on a standard curve of known concentrations from $10^{-6}$ M to $10^{-8}$ M, adjusted to respective tissue weights and expressed as picogram (pg) of monoamine or metabolite per milligram (mg) tissue.

2.7. Data analyses

Overall treatment effects for summed ALO AIMs and individual ALO AIM time points (expressed as medians ± median absolute deviation [MAD]) were analyzed by employing non-parametric Friedman tests and significant differences between treatments were determined by Wilcoxon signed-rank post hoc comparisons. Parametric data (expressed as means ± standard mean error [SEM]) derived from HPLC were analyzed with independent t-tests (by hemisphere), rotations were analyzed with 2-way (treatment × time) ANOVAs, FAS data was analyzed with a 1-way ANOVA (treatment) while 3-way (L-DOPA × TCA × time) ANOVAs were used for analyses of locomotor data. Mauchley’s test of sphericity was used to assess heteroschedasticity and degrees of freedom were lowered using Huyn–Feldt corrections when appropriate. When appropriate, LSD post hoc tests were employed. Analyses were performed with Statistica software 98 (Statsoft Inc., Tulsa, OK, USA). Alpha was set at 0.05 for main effects and interactions and 0.01 for post hoc comparisons.

3. Results

3.1. 6-OHDA lesions reduce striatal DA and DOPAC levels

HPLC analysis of the intact (right) and lesioned (left) striatal tissue was employed to observe the effects of 6-OHDA lesion on the monoamines NE, DA, 5-HT, and the metabolites DOPAC and 5-HIAA. Unilateral administration of 6-OHDA into the medial forebrain bundle severely depleted DOPAC ($t_{15} = 4.74$, $p < 0.001$) and DA ($t_{15} = 6.04$, $p < 0.001$) 96% and 99%, respectively, in lesioned striata (Table 1). No significant differences were observed for NE, 5-HIAA, or 5-HT.

3.2. TCAs differentially reduce ALO AIMs and rotations

L-DOPA primed rats received 3 doses of each TCA in order to determine their dose-dependent effects on L-DOPA-induced ALO AIMs.
and rotations. Statistical analyses of dose (bar graph insets) and dose at each time point (time courses) depicted in Fig. 2A, C, and E revealed that CLOM, AMI, and DES pretreatments produced unique behavioral profiles against LID. Post hoc analyses demonstrated that CLOM pretreatment attenuated ALO AIMs with increasing doses enhancing and prolonging the anti-dyskinetic effects and pre-treatment with AMI resulted in a non-significant reduction of LID only at the highest dose tested. Interestingly, at moderate and high doses of DES, the onset of LID appeared to be delayed, rather than reduced.

CLOM, AMI, and DES (Fig. 2B, D, and F) also modified L-DOPA-induced rotations with analyses demonstrating dose × time interactions for each (F51,408 = 3.8, p < 0.001; F51,408 = 2.00, p < 0.001; F51,408 = 2.20 p < 0.001, respectively). Post-hoc analyses of each TCA revealed somewhat different behavioral patterns than what was observed with AIMs. CLOM effects on rotations mirrored the time course effects of AIMs, AMI appeared to more robustly suppress rotations than AIMs, and deferral of dyskinesia to later time points in DES-treated subjects was not reflected in the general suppression of rotations brought about by NET blockade.

3.3. TCA administration differentially affects L-DOPA-induced improvements in forepaw stepping

The maintenance L-DOPA's anti-parkinsonian efficacy was assessed by assays of motor performance with the FAS test. Analyses of the percent intact data (Fig. 3A) demonstrated a main effect of treatment (F4,28 = 3.26, p < 0.026). Further post hoc analyses revealed that L-DOPA significantly reversed lesion induced stepping deficits (p < 0.05), and that CLOM and AMI, but not DES, when combined with L-DOPA, maintained this effect (p < 0.05). Planned comparisons of total forepaw steps (Fig. 3B) revealed that DES reduced overall number of steps at the dose and time tested (p < 0.05).

3.4. Effects of L-DOPA and TCAs on locomotor activity

3.4.1. Total distance traveled

Analysis of total distance traveled revealed a main effects of L-DOPA (F1,10 = 10.75, p < 0.01), TCA (F1.4,14.2 = 9.66, p < 0.01), and time

<table>
<thead>
<tr>
<th>Striatum</th>
<th>NE (pg/mg)</th>
<th>DOPAC (pg/mg)</th>
<th>DA (pg/mg)</th>
<th>5-HIAA (pg/mg)</th>
<th>5-HT (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>54.0 ± 21.7</td>
<td>1681.7 ± 357.6</td>
<td>5095.1 ± 870.4</td>
<td>346.1 ± 85.2</td>
<td>272.1 ± 55.6</td>
</tr>
<tr>
<td>Lesion</td>
<td>24.7 ± 12.1</td>
<td>43.6 ± 8.3*</td>
<td>47.3 ± 12.1*</td>
<td>497.9 ± 140.9</td>
<td>207.2 ± 48.1</td>
</tr>
<tr>
<td>Percent intact</td>
<td>69.6 ± 16.4</td>
<td>4.2 ± 0.9</td>
<td>1.0 ± 0.2</td>
<td>160.7 ± 33.4</td>
<td>76.1 ± 8.2</td>
</tr>
</tbody>
</table>

Table 1

Monoamine and metabolite levels in the intact and lesioned striatum for cohorts 1 and 2. After the last test day, rats (n = 19) were sacrificed off treatment and bilateral striatal tissue was dissected for monoamine (norepinephrine [NE], dopamine [DA], serotonin [5-HT]) and metabolite (3,4-Dihydroxyphenylacetic acid [DOPAC] and 5-Hydroxyindoleacetic acid [5-HIAA]) analysis via HPLC. Raw values are presented as mean picogram of monoamine per milligram of tissue (pg/mg) ± standard mean error (S.E.M.). Percent intact values were calculated as monoamine content on lesion side divided by monoamine content on intact side then multiplied by 100 (mean ± S.E.M.). Significant differences between lesion and intact striatal tissue was determined using independent samples t-test. *p < 0.01 vs Intact.
indicating overall stimulatory effects of L-DOPA, reduced activity with TCAs, and an overall reduction in ambulation over time. Importantly a 3-way interaction between L-DOPA, TCA, and time was also demonstrated (F3.9, 39.3 = 5.00, p < 0.01). Post hoc comparisons revealed that during the first hour DES significantly reduced total distance traveled relative to vehicle (p < 0.005), and co-administration of CLOM, AMI, or DES with L-DOPA attenuated L-DOPA's stimulatory effects on ambulation in the first hour of testing (Fig. 4A; all p < 0.01).

3.4.2. Movement number

Analyses of movement number revealed main effects of L-DOPA (F1,10 = 48.57, p < 0.001), TCA (F3,30 = 3.71, p < 0.05), and an overall reduction in ambulation over time (F2,20 = 11.82, p < 0.02) as well as a 3-way interaction (F3,60 = 4.39, p < 0.01; Fig. 4B). Post hoc comparisons of main effects indicated stimulation of movement bouts with L-DOPA and suppressive effects with TCAs (p < 0.01). Post hoc comparisons of the third order effect demonstrated that L-DOPA significantly increased movement number relative to vehicle during the first and second hours of treatment (both p < 0.01). Like total distance, TCA co-administration with L-DOPA significantly attenuated L-DOPA's effect during the first hour of testing (all p < 0.01). Interestingly, the combination of DES and vehicle significantly increased movement number compared to vehicle alone during the second hour of testing (p < 0.01).

3.4.3. Stereotypy

A similar pattern of effects was observed for stereotypy with main effects of L-DOPA (F1,10 = 27.96, p < 0.001), TCA (F3,30 = 12.67 p < 0.001), and an overall reduction in ambulation over time (F2,20 = 6.96, p < 0.01) as well as a 3-way interaction between L-DOPA, TCA and time (F2.0,19.8 = 6.15, p < 0.01). Post hoc comparisons revealed that L-DOPA significantly increased stereotypy relative to vehicle during the first and second hours of treatment (both p < 0.01). During the first hour of treatment, the combination of CLOM, AMI, or DES with L-DOPA significantly decreased stereotypy relative to L-DOPA alone (all p < 0.01). This effect was prolonged through the second hour of testing by the combinations of CLOM or DES with L-DOPA (Fig. 4C; p < 0.01).

4. Discussion and conclusions

Accumulating research over the past several years has suggested that the fate of L-DOPA-derived DA uptake and release is mediated by non-DA neurons when DA cell loss is severe. The present study extends these findings by demonstrating that L-DOPA's behavioral effects are also dramatically and differentially altered by uptake mechanisms of non-DA monoamine transporters. Using hemi-parkinsonian rats, we show for the first time that TCAs with higher affinity for SERT blockade (CLOM > AMI > DES) convey the most pronounced anti-dyskinetic effects, while those with higher affinity for NET blockade (DES > AMI > CLOM) prolong LID expression. As important, the effects of SERT inhibition...
appear specific to LID and L-DOPA-induced stereotypy and do not deleteriously alter L-DOPA-related improvement on motor performance. These findings have immediate implications for patients prescribed medications targeting transporters and broader implications for novel targets that could improve PD treatment.

It has become increasingly clear that both 5-HTergic and noradrenergic neurons modulate L-DOPA's neurobiological effects once central DA loss is severe; however, research is beginning to indicate that their respective roles may differ. For example, it has been suggested that 5-HTergic neurons are integral to L-DOPA uptake, its conversion to DA, and resultant DA release (Arai et al., 1995; Carta et al., 2007; Navailles et al., 2010), whereas NE neurons appear to play a central role in DA clearance (Arai et al., 2008; Chotibut et al., 2014; Moron et al., 2002).

In PD, DA cell loss positively correlates with DAT loss (Kraemmer et al., 2014). Thus, following L-DOPA, increased extracellular DA due to reduced DAT expression likely requires alternative transporters to remove DA from the peri-synaptic milieu. The current study sought to parse the role of SERT and NET in L-DOPA-mediated behaviors using pharmaceutical compounds with graded affinity for each monoamine transporter.

Although not as severe as DAT, SERT loss occurs in later disease stages (Kim et al., 2003; Kish et al., 2008; Strecker et al., 2011) and has been associated with non-motor aspects of PD, including cognition and mood (Fahn et al., 1987; Kerenyi et al., 2003; Mura et al., 2001; Politis et al., 2010). Interestingly, reports of increased SERT concurrent with elevated striatal DA release in dyskinetic PD patients supports L-DOPA-induced 5-HT structural and functional plasticity (Kish et al., 2008; Rylander et al., 2010; Politis et al., 2014). With this knowledge, we set out to determine how CLOM, with the highest affinity for SERT (Millan et al., 2001a), would alter L-DOPA's effects. CLOM dose-dependently reduced LID in a manner consistent with the anti-dyskinetic effects of selective SERT blockade with SSRIs (Conti et al., 2014; Fidalgo et al., 2015; Inden et al., 2012; Kuan et al., 2008). While CLOM did not deleteriously alter L-DOPA's anti-parkinsonian benefit on the FAS test, it appeared to reduce L-DOPA-induced hyperlocomotion comparable to past effects from acute, but not chronic, treatment with high dose SSRIs (Bishop et al., 2012; Fidalgo et al., 2015). The ability of SERT blockade to reduce LID while maintaining L-DOPA efficacy has great potential, although the underlying mechanisms responsible for SERT-L-DOPA interactions remain unclear. Though speculative, SERT inhibition may increase 5-HT1A autoreceptors to normalize DA release (Conti et al., 2014; Eskow et al., 2009; Yamato et al., 2001). Interestingly, SERT blockade may concomitantly reduce DA uptake via SERT and prolong extracellular DA (Kannari et al., 2006; Larsen et al., 2011; Lindenbach et al., 2015). Although the literature is mixed regarding targeting SERT for PD motor symptoms (Menza et al., 2004; Palhagen et al., 2009; Paumier et al., 2012), SSRI exposure has been associated with alleviating dyskinesia and delaying LID onset (Durif et al., 1995; Mazzucchi et al., 2014). Future work to examine these mechanisms should be enlightening.

Melanin-containing noradrenergic neurons within the rostral and dorsal planes of the locus coeruleus innervate the basal ganglia (Ostock et al., 2014). NE loss in PD can be severe as the disease progresses (Marien et al., 2004; McMillan et al., 2011; Smythies, 2005; Soldani and Fornai, 1999). The MFB-6-OHDA model mimics late stage PD, given the severe DA loss induced (Truong et al., 2006), but in the current study prophylactic DES was used to block NET’s uptake of 6-OHDA during the lesion surgery. This allowed us to test the role in L-DOPA-derived DA uptake through NET in DA-depleted brain. To test this, we employed DES in later behavioral tests, to block NET function (Owens et al., 1997). Unlike SERT-acting compounds, DES deferred dyskinesia, interfered with L-DOPA’s anti-parkinsonian efficacy at high doses, and reduced basal locomotor activity, effects consistent with previous work showing that chronic DES with L-DOPA prolonged LID expression (Chotibut et al., 2014). However, DES reduced stereotypic behavior similar to the other TCAs tested. While the NET mechanism(s) remains elusive, NET appears capable of DA uptake (Arai et al., 2008; Takeda et al., 2002). For example, when DAT levels are reduced, blocking NET in hemi-parkinsonian rats prolongs L-DOPA-derived striatal DA (Arai et al., 2008; Moron et al., 2002; Navailles et al., 2014). Under some circumstances increasing extracellular DA via NET blockade may provide some benefit. For example, NET inhibition with the selective norepinephrine reuptake (SNRI) nisoxetine improved motor disability in MPTP-treated primates, but like DES, worsened motor deficit at higher doses (Hansard et al., 2002). Given that NET is a pervasive therapeutic target for non-motor treatment of PD, a better understanding of NET’s contribution to L-DOPA’s motor effects is needed.

The current investigation utilized TCA compounds with varying SERT and NET affinities to determine the relative contribution of SERT and NET in L-DOPA-induced motor behaviors. By utilizing TCAs, we are able to strike a balance between SERT and NET’s purported actions as well as make inferences regarding compounds that are primarily prescribed as treatment for highly prevalent depression in PD patients (Chung et al., 2010; Menza et al., 2009; Ravina et al., 2007). Although their efficacy on motor symptoms remains questionable (Chen et al., 2007; Liu et al., 2013), more recent evidence has suggested that TCA treatment appears to promote neuroprotection for DA neurons and motor behavior (Hwang et al., 2008; Paumier et al., 2015a,b; Valera et al., 2014) and delay increases in anti-parkinsonian medication without interfering with anti-parkinsonian treatment (Devos et al., 2008; Meco and Bernardi, 2007; Paumier et al., 2012; van de Vijver et al., 2002). Such findings have led to a renewed interest in TCAs despite their many adverse side effects, including dry mouth, headache, and constipation (Devos et al., 2008; Paumier et al., 2015a,b). In the current investigation, we also included the mixed SERT = NET TCA AMI. Although we found AMI effects to be limited, there has been a concerted effort to leverage individual transporter effects to optimize therapeutic benefit for PD patients (Huot et al., 2015). For instance, dual DAT and SERT inhibition increases L-DOPA’s anti-parkinsonian efficacy without exacerbating LID or DA-induced psychosis when the compound has greater affinity for DAT (Huot et al., 2012, 2014). Dual DAT and NET inhibition produces mixed effects on motor deficit, but worsens LID (Bedard et al., 1977; Teychenne et al., 1976). Although the optimal balance between transporter blockade requires further investigation, future work in this field is highly promising for these pharmaceutical targets.

Overall, the present results show for the first time that treatment with TCAs of varied monoamine transporter affinity differentially modulate L-DOPA’s motor effects and add to the growing evidence that monoamine transporters play a pivotal role in efficacy and side effects of L-DOPA. Future work understanding long-term effects of TCA administration and the mechanisms by which these transporters convey their effects has the potential to improve treatment for PD and thus patients’ quality of life.

Acknowledgments

Michael J. Fox Foundation as funding source.

References


