Dopamine D1 and D2 receptor contributions to L-DOPA-induced dyskinesia in the dopamine-depleted rat

Jennifer L. Taylor a, Christopher Bishop b, Paul D. Walker a,b,*

aCellular and Clinical Neurobiology Program, Department of Psychiatry and Behavioral Neurosciences, USA
bDepartment of Anatomy and Cell Biology, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI, 48201, USA

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Abstract

Using a rat model of L-DOPA-induced dyskinesia (LID), the contributions of dopamine D1 and D2 receptors to axial, limb, and orolingual (ALO) abnormal involuntary movements (AIMs) elicited by L-DOPA were examined. Chronic L-DOPA-treated rats received the D1 receptor antagonist SCH23390 (0.01, 0.1, and 1.0 mg/kg; ip), the D2 receptor antagonist Eticlopride (0.01, 0.1, and 1.0 mg/kg; ip), a mixture of both antagonists (0.01, 0.1, 1.0 mg/kg each; ip), or vehicle 30 min prior to L-DOPA (6 mg/kg; ip)+Benserazide (15 mg/kg; ip). SCH23390 (0.1 and 1.0 mg/kg) significantly reduced axial and limb AIMs, while the same doses of Eticlopride significantly decreased axial, limb, and orolingual AIMs. Co-administration of SCH23390 + Eticlopride significantly reduced axial (0.01, 0.1 and 1.0 mg/kg), limb (0.1 and 1.0 mg/kg ), and orolingual (0.1 and 1.0 mg/kg) AIMs. These results indicate the importance of D1 and D2 receptors to LID and further validate the rat AIMs model.

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1. Introduction

L-DOPA is the standard pharmacological therapy for the treatment of Parkinson’s Disease (PD). While it is efficacious in alleviating the motor impairments associated with the disease, the majority of patients eventually develop debilitating, uncontrollable movements known as dyskinesias (Nutt, 1990; Ahlskog and Mueuter, 2001). The primary cause of L-DOPA-induced dyskinesia (LID) is unknown, but stimulation of sensitized dopamine (DA) D1 and D2 classes of receptors seems to play a key role (Nutt, 1990). For example, D1 and D2 receptor agonists trigger dyskinesia in human PD patients (Klawans and Weiner, 1974; Rinne, 1989; Rascol et al., 2000, 2001) and non-human primates rendered parkinsonian through exposure to the toxin MPTP (Boyce et al., 1990; Gomez-Mancilla and Bedard, 1991; Blanchet et al., 1993 ). Additionally, D1 or D2 receptor antagonism reduces LID, but also lessens the anti-parkinsonian benefit of L-DOPA (Boyce et al., 1990; Elliott et al., 1992; Grondin et al., 1999). As such, D1 or D2 receptor antagonists are not used in the treatment of LID.

Advancements in the understanding of consequences of DA depletion in PD have been aided by studies involving the unilateral 6-hydroxydopamine (6-OHDA) lesioned rat. In recent years the introduction of the abnormal involuntary movements (AIMs) model of LID, in which 6-OHDA-lesioned rats are exposed to chronic L-DOPA, has facilitated investigations into LID (Cenci et al., 1998; Lee et al., 2000; Lundblad et al., 2002; Johansson et al., 2001; Picconi et al., 2004).
2003; Konradi et al., 2004). To this point however, the effects of D$_1$ or D$_2$ receptor antagonism have not been examined in the rat AIMs model of LID. Showing that both D$_1$ and D$_2$ receptors contribute to rodent axial, limb, and orolingual (ALO) AIMs, as they do to LID in PD patients, would enhance the validity of the rat model. Thus, we sought to determine the consequences of administering a D$_1$ receptor antagonist, a D$_2$ receptor antagonist, or a mixture of both antagonists to DA-depleted rats that exhibited AIMs in response to chronic L-DOPA.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats were used (225–250 g upon arrival; Charles River Laboratories; Wilmington, MA). Animals were housed in plastic cages (22 cm high, 45 cm deep and 23 cm wide) and had free access to standard lab chow (Rodent Diet 5001; Lab Diet, Brentwood, MO) and water. The colony room was maintained on a 12 h light/dark cycle (lights on at 0700 h) at a temperature of 22–23 °C. Animals were maintained in strict accordance with the guidelines of the Institutional Animal Care and Use Committee of Wayne State University and the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number 85-23, revised 1996).

2.2. 6-hydroxydopamine lesion surgeries

One week after arrival, 23 rats were subjected to a unilateral 6-OHDA lesion of the right medial forebrain bundle to destroy DA neurons. Desipramine HCl (25 mg/kg; ip; Sigma, St. Louis, MO) was given 30 min prior to the 6-OHDA injection to protect norepinephrine (NE) neurons. Rats were anesthetized with ketamine (5–15 mg/kg; ip; Lloyd Laboratories, Shenendoah, IA) and xylazine (5–15 mg/kg; ip; Lloyd Laboratories), then placed in a stereotoxic apparatus. The coordinates for 6-OHDA injections were AP: −2.5 mm, ML: −2.0 mm, DV: −9.0 mm relative to bregma with the incisor bar positioned 3.3 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; Sigma) dissolved in 0.9% NaCl containing 0.1% ascorbic acid was infused through a small burr hole in the skull at a rate of 2 μl/min for a total volume of 4 μl. The needle was withdrawn one min later. Rats were placed in clean cages on warming pads to recover from the surgery, after which they were returned to group-housing. Fresh fruit and soft chow were provided as needed to facilitate recovery during the first week after surgery.

2.3. Pharmacological treatments

Beginning 3 weeks after the lesion surgery, all rats were primed with L-DOPA methyl ester (6 mg/kg; ip; Sigma)+Benserazide HCl (15 mg/kg; ip; Sigma) by receiving injections once daily for 10 days. L-DOPA was dissolved in 0.9% NaCl containing 0.1% ascorbic acid and administered at a volume of 1 ml/kg. Rats displaying AIMs during the priming period (n = 10) were retained for the remainder of the study. At that point, daily L-DOPA was discontinued and AIMs were maintained by giving rats L-DOPA (6 mg/kg)+Benserazide (15 mg/kg) once per week. Studies examining the effects of D$_1$ and/or D$_2$ antagonist pre-treatment on L-DOPA-induced AIMs began one week after the end of L-DOPA priming. Using a repeated measures design, rats received each antagonist pre-treatment once. The order of treatment was randomized for each rat. Behavioral testing was performed between 0700–1300 h, every 3–4 days over the course of 5 weeks. Rats were given a pre-treatment of the D$_1$ receptor antagonist R-(+)SCH23390 (0.01, 0.1, or 1.0 mg/kg; ip; Sigma), the D2 receptor antagonist S-(−)Eticlopride HCl (0.01, 0.1, or 1.0 mg/kg; ip; Sigma), a mixture of SCH23390+Eticlopride (0.01, 0.1, or 1.0 mg/kg of each; ip) or vehicle (water) 30 min prior to L-DOPA (6 mg/kg)+Benserazide (15 mg/kg) and monitored for AIMs.

2.4. Behavioral procedure

Rats were monitored for AIMs using a procedure slightly modified from that described in Lundblad et al. (2002). On test days, rats were placed individually in plastic cages of the same style as those used to house the animals. Every 20th min for 2 h following the L-DOPA injection, a trained observer blind to treatment condition scored each rat for exhibition of the following 3 categories of AIMs: 1) axial (twisting of the neck and posturing of the torso away from the lesioned side), 2) limb (repetitive motions of the contralateral forelimb), and 3) orolingual (jaw movements and tongue protrusions without the presence of food or other objects). For each 1-min observation period, a score of 0–4 was assigned for each behavioral category based on the following criteria: 0 = not present, 1 = present for 1–29 s, 2 = present for 30–59 s, 3 = present for 60 s and interrupted by a loud stimulus (a tap on the wire cage lid), or 4 = present for 60 s but not interrupted by the stimulus. Scores of the axial, limb, and orolingual (ALO) AIMs were individually summed for each rat per testing period. While the maximum score obtainable for each category using this scoring procedure is 24 (4 × 6 periods), the highest score observed in the current study was 17 for each ALO AIM. Group averages are represented in bar graph form for each individual behavioral category or as a sum of the 3 AIMs categories.
Table 1
Effects of unilateral medial forebrain bundle 6-OHDA lesions on concentrations of norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), and serotonin (5-HT) in the anterior striatum

<table>
<thead>
<tr>
<th>Side</th>
<th>NE</th>
<th>DOPAC</th>
<th>DA</th>
<th>5-HIAA</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (Left)</td>
<td>0.08±0.02</td>
<td>2.28±0.20</td>
<td>19.08±1.26</td>
<td>0.64±0.04</td>
<td>1.28±0.32</td>
</tr>
<tr>
<td>6-OHDA lesioned (Right)</td>
<td>0.06±0.03* (75%)</td>
<td>0.02±0.00* (88%)</td>
<td>0.11±0.09* (58%)</td>
<td>0.72±0.07 (112%)</td>
<td>1.41±0.43 (110%)</td>
</tr>
</tbody>
</table>

Values are nanogram of monoamine or metabolite per milligram protein (mean ± SE) with percent of intact side indicated in parentheses. Differences between lesioned and intact side were determined by independent t-tests (* p < 0.05).

2.5. High performance liquid chromatography

After the completion of experiments, rats were sacrificed by decapitation. The anterior striatum was dissected, immediately frozen on dry ice, and then stored at −80 °C. Reverse-phase high performance liquid chromatography coupled to electrochemical detection (HPLC-EC) was performed on the tissue according to the protocol of Kilpatrick et al. (1986), a method for semi-automated catecholamine and indoleamine analysis with coulometric detection. The system included a Waters WISP autoinjector, an external pulse dampener (Rainin), a Waters Guard PAK column, and a C-18 (100 × 4.6 mm, 5 μm packing) column (Perkin-Elmer). Samples were homogenized in ice-cold perchloric acid (0.1 M), 1% ethanol, and 0.02% EDTA. The homogenates were spun for 30 min at 16,100 g with the temperature maintained at 4 °C. Aliquots of supernatant were then analyzed for abundance of DA, serotonin (5-HT), norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA). Samples were separated using a mobile phase composed of 100 mM sodium phosphate (monobasic, anhydrous), 0.05 mM EDTA, 1.4 mM octane sulfonic acid, and 9% acetonitrile, adjusted to pH 3.0 with orthophosphoric acid. A coulometric detector configured with 3 electrodes (Model 5011, ESA) measured content of monoamines and metabolites. An ESA model 5020 guard cell (+400 mV) was positioned prior to the WISP injector. The analytical cell (Model 5011, ESA) measured content of monoamines and metabolites. An ESA model 5020 guard cell (+400 mV) was positioned prior to the WISP injector. The analytical cell (ESA model 5011; first electrode at +400 mV, second electrode at +500 mV) was located immediately past the column. The second analytical electrode emitted signals that were recorded and analyzed by a Waters Baseline 810 Chromatography Workstation via a Waters Interface Module. The final oxidation current values were adjusted to protein amounts determined by Lowry assay (Lowry et al., 1951) and expressed as nanogram (ng) of monoamine or metabolite per milligram (mg) protein.

2.6. Data analyses

Monoamine and metabolite levels were analyzed by independent t-tests. ALO AIMs data were evaluated by repeated measures 1-way ANOVA. Significant differences between days or treatments were determined by Fisher’s least significance difference (LSD) post hoc tests. Statistica software ’98 (Statsoft Inc., Tulsa, OK, USA) was used. Alpha was set at p < 0.05.

3. Results

3.1. Monoamine and metabolite levels

The effects of the 6-OHDA lesion on concentrations of monoamine and metabolite levels in the ipsilateral (right) versus contralateral (left) striata are shown in Table 1. As anticipated, injection of 6-OHDA into the right MFB produced a significant unilateral reduction in striatal DA (t16 = 15.96; p < 0.001) and DOPAC (t16 = 11.80; p < 0.001) levels. There were no significant differences between the lesioned and intact striata for NE, 5-HIAA, and 5-HT.

3.2. L-DOPA priming and maintenance

ALO AIMs were not present prior to the first day of L-DOPA priming. Fig. 1 shows the average ALO AIMs scores of the 10 dyskinetic rats for Day 1 and Day 10 of L-DOPA (6 mg/kg)+Benserazide (15 mg/kg) priming. The final maintenance administration of L-DOPA (Day 53) is also represented. A repeated measures 1-way ANOVA indicated a main effect of treatment day on AIMs (F(2,18)=10.52; p<0.001). LSD post hoc testing revealed that Day 10 of L-DOPA priming was higher than Day 1 of priming.
Additionally, Day 53 was different from Day 1 of priming \((p<0.01)\), but not Day 10 of priming. These results suggest that L-DOPA priming caused an induction of and initial rise in ALO AIMS that were maintained through the end of the antagonist testing period.

3.3. \(D_1\) receptor antagonism reduces AIMS

A repeated measures 1-way ANOVA revealed a main effect of \(D_1\) antagonist pre-treatment with SCH23390 on L-DOPA (6 mg/kg)-induced axial \((F_{(3,27)}=3.94; p<0.05; \text{Fig. 2A})\) and limb \((F_{(3,27)}=3.52; p<0.05; \text{Fig. 2B})\) AIMS. LSD post hoc comparisons indicated that pre-treatment with the low (0.01 mg/kg) dose of SCH23390 did not have a significant effect in either case. However, axial AIMS were significantly reduced by both the middle \((p<0.05; 70\%\) decline) and high \((p<0.05; 78\%\) decline) doses of SCH23390. Limb AIMS were also reduced by pre-treatment with SCH23390 at the 0.01 mg/kg \((p<0.05; 68\%\) decline) and 1.0 mg/kg \((p<0.05; 76\%\) decline) doses. The effect of SCH23390 on orolingual AIMS did not reach statistical significance \((F_{(3,27)}=2.91; p=0.053; \text{Fig. 2C})\).

3.4. \(D_2\) receptor antagonism reduces AIMS

A repeated measures 1-way ANOVA indicated a main effect of pre-treatment with the \(D_2\) receptor antagonist Eticlopride on L-DOPA (6 mg/kg)-induced axial \((F_{(3,27)}=3.73; p<0.05; \text{Fig. 3A})\), limb \((F_{(3,27)}=5.13; p<0.01; \text{Fig. 3B})\), and orolingual AIMS \((F_{(3,27)}=3.30; p<0.05; \text{Fig. 3C})\). LSD post-hoc comparisons indicated that the low (0.01 mg/kg) dose of Eticlopride did not significantly affect any...
category of AIMs. However, axial scores were significantly reduced by pre-treatment with the middle \((p<0.05; 66\% \text{ decline})\) and high \((p<0.05; 66\% \text{ decline})\) doses of Eticlopride. Limb AIMs were also lowered by the 0.1 mg/kg \((p<0.05; 63\% \text{ decline})\) and 1.0 mg/kg \((p<0.01; 76\% \text{ decline})\) doses. Finally, orolingual scores significantly decreased after pre-treatment with the middle \((p<0.05; 59\% \text{ decline})\) and high \((p<0.05; 52\% \text{ decline})\) doses of Eticlopride.

3.5. Concurrent antagonism of \(D_1\) and \(D_2\) receptors reduces AIMs

A repeated measures 1-way ANOVA revealed a main effect of SCH23390 + Eticlopride co-administration on L-DOPA-induced axial \((F_{(3,27)}=6.74; p<0.01; \text{Fig. 4A})\), limb \((F_{(3,27)}=5.25; p<0.01; \text{Fig. 4B})\), and orolingual \((F_{(3,27)}=5.38; p<0.01; \text{Fig. 4C})\) AIMs. LSD post hoc comparisons indicated that axial AIMs were significantly reduced by co-administration of the low \((p<0.05; 51\% \text{ decline})\), middle \((p<0.001; 91\% \text{ decline})\), and high \((p<0.001; 96\% \text{ decline})\) doses of the antagonists. Limb AIMs were not decreased by pre-treatment with the 0.01 mg/kg doses, but were lowered by the 0.1 mg/kg \((p<0.01; 93\% \text{ decline})\) and 1.0 mg/kg \((p<0.01; 94\% \text{ decline})\) doses of SCH23390 + Eticlopride. Finally, a reduction in orolingual scores resulted from co-administration of the middle \((p<0.01; 89\% \text{ decline})\) and high \((p<0.01; 93\% \text{ decline})\), but not low doses, of SCH23390 + Eticlopride.

4. Discussion

The unilateral 6-OHDA lesioned rat model of PD has been useful for the study of compensatory processes that occur following DA depletion (Ungerstedt, 1971; Miller and Beninger, 1991). The L-DOPA-induced AIMs model developed by Cenci et al. (1998) utilizes the unilateral DA-depleted rat to study dyskinesia associated with chronic L-DOPA treatment. This model has been touted as a clinically relevant measure of LID (Cenci et al., 2002; Lundblad et al., 2002). For an animal model to become widely accepted, it is important to demonstrate similarities to the human condition. In the dyskinetic rat, a number of non-dopaminergic pharmacological agents known to reduce LID in humans also diminish AIMs (Lundblad et al., 2002). However, while it is clear that \(D_1\) and \(D_2\) receptors contribute to LID, their respective roles in the rodent AIMs model had not been thoroughly examined. One group has demonstrated that \(D_2\) receptor agonism induces AIMs in the rat (Delfino et al., 2004), but the effects of DA receptor antagonism have not been explored until now. This study was undertaken in order to confirm that \(D_1\) and \(D_2\) receptor antagonism reduces AIMs, thereby enhancing the validity of the rat model of LID by assuring that similar underlying DA receptor-mediated mechanisms play a role in each phenomenon.

The results of this study verified that \(D_1\) and \(D_2\) receptors contribute to ALO AIMs in the rat model of LID by showing that pre-treatment with either the \(D_1\) antagonist SCH23390 or the \(D_2\) antagonist Eticlopride reduced abnormal movements induced by L-DOPA. These data support the suggestion that the rat ALO AIMs model of LID is similar to the clinical condition (Cenci et al., 2002). The idea that stimulation of \(D_1\) and \(D_2\) receptors is an important contributing factor to LID is substantiated by evidence from pharmacological studies involving PD patients and MPTP-intoxicated non-human primates (Klawans and Weiner, 1974; Rinne, 1989; Boyce et al., 1990; Gomez-Mancilla and Bedard, 1991; Elliott et al., 1992; Blanchet et al., 1993; Grondin et al., 1999; Rascol et al., 2000, 2001; Delfino et al., 2004).
The means by which these antagonists reduce ALOMs is likely to occur by preventing DA stimulation of supersensitive D1 and/or D2 receptors. Supersensitivity refers to the increased responsiveness of DA receptors to agonism following experimental DA depletion (Ungerstedt, 1971; Breese et al., 1987; Miller and Beninger, 1991; Bishop and Walker, 2003). While DA receptor number may or may not change after DA denervation, DA receptor agonists cause increases in signal transduction mechanisms and gene expression in 6-OHDA-lesioned striatum to an extent not observed in the intact striatum (Berke et al., 1998; Cai et al., 2000, 2002; Gerfen et al., 2002). This excessive response is thought to contribute to overstimulation of the “direct” striatal output pathway, which predominantly expresses D1 receptors (Albin et al., 1989; Gerfen et al., 1990; Harrison et al., 1990), but understimulation of the “indirect” pathway, which predominantly expresses D2 receptors (Albin et al., 1989; Gerfen et al., 1990; Harrison et al., 1992). Imbalance of the striatal output pathways has been proposed to result in excessive movement (Albin et al., 1989).

Some controversy remains regarding the relative contribution of each DA receptor class to LID. In this study, there was no clear indication that one DA receptor antagonist was more effective than the other in reducing ALOMs in the rat. Hence, we could not conclude that there was a greater contribution of D1, as opposed to D2, receptors to the production of ALOMs. Individual administration of antagonists would be expected to primarily mediate function of one striatal output pathway while leaving the other pathway dysregulated, thus not completely restoring the imbalance between the direct and indirect pathways. As such, the lack of complete suppression of L-DOPA-induced ALOMs by either antagonist alone may be due to the remaining stimulation of the other class of DA receptors since D1 or D2 receptor stimulation is sufficient to induce dyskinesia (Klawans and Weiner, 1974; Rinne, 1989; Boyce et al., 1990; Gomez-Mancilla and Bedard, 1991; Blanche et al., 1993; Rascol et al., 2000, 2001). Combined administration of the middle and high doses (0.1 and 1.0 mg/kg) of SCH23390 + Eticlopride, were effective at suppressing all 3 categories of ALO ALOMs. The lowest dose (0.01 mg/kg) of SCH23390 and Eticlopride did not have effects when administered individually, but did significantly reduce axial ALOMs when administered together. The combination of the SCH23390 + Eticlopride seemingly did not enhance the ability of the each antagonist to reduce limb or orolingual ALOMs, but it is possible that an enhancement would have been revealed had doses between 0.01 and 0.1 mg/kg been examined.

Interestingly, overall there was not a strong preference for D1, D2, or combined D1 + D2 antagonism to selectively reduce one ALO ALOM over another. At least 2 doses of each antagonist pre-treatment caused a significant decline in both axial and limb ALOMs. Orolingual ALOMs were also reduced by D2 or D1 + D2 antagonism. Although there was a trend for D1 antagonism to reduce orolingual ALOMs, the effect was not powerful enough to reach statistical significance. These results suggest that the suppressive effects of D1 and D2 antagonism are similar across the different ALOMs measurements.

The antagonists used in this study are effective at discriminating between D1 and D2 classes of receptors (Hyttel, 1983; Hall et al., 1985; Sunahara et al., 1991). However, more than two DA receptors exist and these are classified as being D1-like or D2-like (for review, see Missale et al., 1998). It should be noted that SCH23390 does not differentiate between D1 and D2 receptors (Sunahara et al., 1991; Tiberi et al., 1991) and Eticlopride does not differentiate among D2, D3, or D4 receptors (Tang et al., 1994). Thus, the effects of the antagonists used here cannot be attributed to the inhibition only one type of DA receptor. There is some indication that the D3 receptor is involved in dyskinesia. Antagonism or partial agonism of the D3 receptor has been found to reduce dyskinesia in primates (Bezard et al., 2003). As more selective DA receptor compounds become available and information for the roles of each DA receptor is revealed, compounds selective for each DA receptor will be useful to determine which subtypes, alone or combination, underlie mechanisms that are responsible for LID.

In conclusion, antagonism of D1 or D2 receptors reduces L-DOPA-induced ALOMs in a rat model of LID, thus confirming that dopaminergic mechanisms underlie ALOMs. Continued use of this rodent model should be beneficial for determining the effectiveness of compounds which target LID.

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References
