Modulation of L-DOPA’s antiparkinsonian and dyskinetic effects by α2-noradrenergic receptors within the locus coeruleus

Corinne Y. Ostock, Joy Hallmark, Noel Palumbo, Nirmal Bhide, Melissa Conti, Jessica A. George, Christopher Bishop*

Behavioral Neuroscience Program, Department of Psychology, Binghamton University, Binghamton, NY, USA

A R T I C L E   I N F O

Article history:
Received 19 December 2014
Received in revised form 12 February 2015
Accepted 9 March 2015
Available online 25 March 2015

Keywords:
Locus coeruleus
L-DOPA-induced dyskinesia
Norepinephrine
Clonidine
Atipamezole
α2-adrenoceptor

A B S T R A C T

Long-term L-DOPA use for Parkinson’s disease (PD) is frequently complicated by the emergence of a debilitating motor side effect known as L-DOPA-induced dyskinesia (LID). Accumulating evidence has implicated the norepinephrine (NE) system in the pathogenesis of LID. Here we used the unilateral 6-hydroxydopamine rat model of PD to determine the role of the α2-adrenoceptors (α2R) in L-DOPA’s therapeutic and detrimental motor-inducing effects. First, we characterized the effects of systemic α2R stimulation with clonidine, or blockade with atipamezole, on LID using the rodent abnormal involuntary movements scale, and L-DOPA’s therapeutic benefits using the forepaw adjusting steps test and locomotor activity chambers. The anatomical locus of action of α2R in LID was investigated by directly infusing clonidine or atipamezole into the locus coeruleus prior to systemic L-DOPA administration. Results showed systemic clonidine treatment reduced LID and locomotor activity but did not interfere with L-DOPA’s antiparkinsonian benefits. Conversely, systemic atipamezole pretreatment prolonged LID and locomotor activity but did not modulate L-DOPA’s antiparkinsonian benefits. Intra-LC infusions of clonidine and atipamezole mirrored systemic effects where clonidine reduced, and atipamezole increased, LID. Collectively, these results demonstrate that α2R play an important modulatory role in L-DOPA-mediated behaviors and should be further investigated as a potential therapeutic target.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Dopamine (DA) replacement with L-DOPA has remained the most widely used and effective treatment for Parkinson’s disease (PD) since its introduction in the 1960’s (Katzschlager and Lees, 2002; Hauser, 2009). However, long-term L-DOPA use is associated with the emergence of a debilitating, hyperkinetic motor side effect known as L-DOPA-induced dyskinesia (LID) (Jankovic, 2005). While the primary cause of LID is not fully understood, it is generally accepted that LID involves excessive release of L-DOPA-derived DA and stimulation of sensitized DA receptors leading to aberrant striatal signaling pathway activity (Huot et al., 2013). Attempts to reduce LID by pharmacologically manipulating the DA system, either by blocking DA receptors or reducing L-DOPA dosage, are frequently complicated by the return of primary PD symptoms (Grondin et al., 1999; Elliott et al., 1992; Goetz et al., 1982). An alternative to this has been the exploration of non-dopaminergic targets which interact with central motor circuits.

There is a growing body of evidence implicating the norepinephrine (NE) system in the expression of LID. NE levels are frequently reduced in the PD brain (Zarow et al., 2003) and L-DOPA treatment has been shown to enhance central NE concentrations (Chalmers et al., 1971; Bianco et al., 2008). Recent experimental evidence reported LID expression temporally coincides with L-DOPA-derived striatal NE efflux in L-DOPA-primed, hemiparkinsonian rats (Wang et al., 2014). As such, a number of compounds targeting the NE system, and α2-adrenoceptors (α2R) specifically, have shown promise for the treatment of LID. Paradoxically, systemic treatment with either agonists or antagonists for the α2R has been shown to reduce LID symptoms, but demonstrate equivocal effects on L-DOPA’s therapeutic benefits. The classic α2R agonist clonidine relieves LID but blocks L-DOPA’s antiparkinsonian motor effects (Gomez-Mancilla and Bedard, 1993; Dekundy et al., 2007). In contrast, several classic α2R antagonists including idazoxan, yohimbine, and atipamezole reduce the severity...
or duration of LID in experimental and clinical populations (Dekundy et al., 2007; Lundblad et al., 2002; Buck et al., 2010; Barnum et al., 2012; Savola et al., 2003; Grondin et al., 2000; Rascol et al., 2001) without interfering with i-DOPA’s anti-parkinsonian motor benefits (Johnston et al., 2010; Henry et al., 1999). In fact, co-treatment with the z2R-antagonist idoxan actually extends i-DOPA’s antiparkinsonian benefits.

The precise anatomical site of action for these effects has not yet been determined. z2R are abundantly expressed throughout central motor nuclei including the striatum and the rest of the basal ganglia (Rosin et al., 1996; Alachkar et al., 2011); however, it has been suggested that z2R regulate i-DOPA’s effects via a presynaptic mechanism since z2R antagonists influence i-DOPA-, but not DA-agonist-, induced dyskinesias (Fox et al., 2001). The locus coeruleus (LC), the main noradrenergic nucleus in the brain, is a promising candidate since basal firing activity of NE neurons in the locus coeruleus (LC) was positively correlated with dyskinesia severity in a rodent model of LID (Migueléz et al., 2011). NE neurotransmission from the LC is regulated by a class of inhibitory somatodendritic z2- autoreceptors (Norenberg et al., 1997) and stimulation or blockade of these receptors have been shown to influence monoamine efflux and neurotransmitter signaling in motor regions implicated in LID (Yachi et al., 1997, 2003; Nuri et al., 1994; Bucheler et al., 2002).

Progress in understanding the role of z2R in LID has been slowed due to the limited number of receptor-specific ligands. Atipamezole is a highly potent and selective z2R antagonist demonstrating 100 times greater affinity for the z2R than other z2R antagonists commonly investigated in LID like idoxan or yohimbine (Pertovaara et al., 2005). In order to clarify the pharmacological and neuroanatomical underpinnings of z2R action in LID and PD, we first evaluated the consequence of systemic administration of atipamezole, or the classic z2R agonist clonidine, on i-DOPA’s dyskinetic, antiparkinsonian, and general motor-activating properties. The second goal was to determine whether these effects were mediated by z2R within the LC using site-specific microinusions. Collectively, the current work demonstrated that z2R modify i-DOPA’s motor actions in part due to a population of receptors located in the LC.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (N = 43; 225–250 g upon arrival; Harlan, USA) were housed in plastic cages (22 cm high, 45 cm deep, and 23 cm wide) with free access to water and standard lab chow (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA). The colony room was maintained at 22 ± 2 ºC on a 12 h light/dark cycle (lights on at 0700 h). Animals were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee at Binghamton University and the “Guide for the Care and Use of Laboratory Animals” (Institute for Laboratory Animal Research, National Academies Press 2011). Throughout this work, all efforts were made to minimize animal suffering, reduce the number of animals used and utilize alternatives to in vivo techniques when available.

2.2. Drugs

All systemic injections were administered at a volume of 1 ml/kg. All drugs were acquired from Sigma (St. Louis, MO, USA) unless otherwise noted. Buprenorphine (Rechitt Benckiser Pharmaceuticals Inc., Richmond, VA) was suspended in saline (dH2O containing 0.9% NaCl) i-DOPA methyl ester · benzersaide (hereafter denoted as i-DOPA) and 6-hydroxydopamine (6-OHDA) were dissolved in saline with 0.1% ascorbic acid (a-DOPA vehicle). Varying doses of i-DOPA were administered throughout this study; however, it should be noted that i-DOPA was always co-administered with the peripheral decarboxylase inhibitor benzersaide in the same vehicle at a dose of 15 mg/kg. Desipramine and clonidine were dissolved in dH2O for systemic treatments. Clonidine was dissolved in saline when administered intracranially. Atipamezole was dissolved in a 25% dimethyl sulfoxide, 75% dH2O solution.

2.3. Surgery

Prior to surgery, rats were treated with desipramine HCI (25 mg/kg, ip) in an attempt to protect NE neurons and buprenorphine (0.03 mg/kg) ip as pre-emptive analgesia. Under isoflurane anesthesia (2–3% in oxygen, 1000 cc/min), rats were a given unilateral DA lesion by infusing 6-OHDA (12 ug in 4 ul) directly into the medial forebrain bundle (AF, −1.8 mm; ML, 2.0 mm, DV, −8.6 mm from bregma; Paxinos and Watson, 1998) as described in detail previously (Ostock et al., 2011). For rats in experiment 1, stainless steel wound clips were used to close the surgical site (n = 11). During 6-OHDA surgery, an additional two cohorts of rats in experiment 2 (N = 32; clonidine treated: n = 12; atipamezole treated: n = 18) were also implanted with a 30 gauge microinfusion guide cannulae (Plastics One, Roanoke, VA) above the LC, ipsilateral to the 6-OHDA lesion (AF, −9.8 mm; ML, +1.36, DV, −6.42 from bregma). Cannulae were fixed in place using dental acrylic (Lang Dental, Wheeling, IL) and 4 screws. Dummy cannulae (Plastics One) cut 1 mm shorter than the guide cannulae were inserted in the guide cannulae to preserve patience. Rats in experiment 1 were pair-housed while cannulated rats in experiment 2 were single-housed.

2.4. Behavioral tests

2.4.1. Abnormal involuntary movements and rotations

The abnormal involuntary movements (AIMs) test is a well-established metric of rodent dyskinesia (Lundblad et al., 2002; Cenci, 2007). Rats were monitored for AIMs and rotations by trained observers (≥95% agreement between experimenters) using a procedure modified from Cenci and Lundblad (2007) and described in detail in Lindeinbach et al. (2011). During each rating period, individual dyskinesia severity scores ranging from 0 (not present) to 4 (severe and not interruptible) were given for axial, forelimb, and orolingual (ALO) dyskinesia subtypes. The sum of these subtypes was then reported as the ALO AIMs score for each time-point. Higher ALO AIMs scores are indicative of greater LID severity. During AIMS ratings, contralateral rotations, defined as complete 360° turns away from the lesioned side of the brain, were recorded.

2.4.2. Forepaw adjusting steps

Rodent akinesia manifests as deficient stepping ability on the side of the body contralateral to brain lesion in the forepaw adjusting steps (FAS) test (Chang et al., 1998). FAS treatment reverses stepping deficits and can be used to determine whether an adjunctive therapy is interfering with i-DOPA’s anti-parkinsonian effects. Using a procedure similar to that described previously (Dupre et al., 2008), the experimenter held the rat’s rear torso and one forelimb while the free forelimb was forced to bear the rat’s body weight. Rats were moved laterally across a table at a steady rate of 90 cm/10 s and the number of adjusting steps taken by each forelimb to compensate for lateral movement was counted by a trainer observer. Each session consisted of six trials per forelimb alternating between directions where forehand steps were defined as weight-bearing steps made towards the body. Stepping data were expressed as % intact stepping (lesioned steps/intact steps). Lower percent intact scores reflect greater forelimb akinesia.

2.4.3. Locomotor chambers

Drug-induced changes in spontaneous and i-DOPA-induced locomotor behav- iors were assessed using activity chambers (Accuscan Instruments, Columbus, OH) as described previously (Lindenbach et al., 2011). Six Acrylic chambers (41 × 41 × 30.5 cm) surrounded by infrared photocell arrays synched with Versamax and Versadat software were used to measure locomotor activity. Photo beam breaks were recorded to assess horizontal and vertical patterns of movement.

2.5. Tissue collection and processing

One week after behavioral analyses were completed, all rats were killed by rapid decapitation and brains were immediately removed. Left and right striatal tissue was freshly dissected and stored at −80 °C for analysis of monoamine content using high-performance liquid chromatography (HPLC-ED) according to the protocol of Bishop et al. (Bishop et al., 2009) based on Kilpatrick et al. (Kilpatrick et al., 1986). Samples were homogenized in ice cold perchloric acid (0.1 M) with 1% ethanol and 0.02% EDTA and spun for 45 min at 14,000 g at 4 °C. Aliquots of supernatant were then analyzed for abundance of NE, DA, and the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) using HPLC-ED. The final oxidation current values were compared to known standards (1e9 1e9) and adjusted according to striatal tissue weights.

Verification of proper cannulae placement for rats in experiment 2 was accomplished using cresyl violet staining. Posterior portions of the brain were removed and rapidly frozen in methylbutane (−30 °C) then stored at −20 °C for evaluation of LC cannulae placements. Cresyl violet (FD, Neurotechnologies, Balti- more, MD) staining was used to determine injection sites from cryostat-generated, 20-μm coronal sections post-fixed with 4% paraformaldehyde (Fisher Scientific, Hanover Park, IL).

2.6. General experimental procedures

A flowchart of the experimental design is shown in Fig. 1.
2.6.1. Experiment 1: effect of systemic α2R stimulation or blockade on L-DOPA-mediated behaviors

The effects of the α2R agonist clonidine and the α2R antagonist atipamezole on L-DOPA’s dyskinetic and therapeutic effects were evaluated using a counterbalanced, within-subjects design by investigators blind to treatment condition. Three weeks after surgery, rats were treated with L-DOPA (12 mg/kg, sc) for 7 days to rapidly induce stable LID (Ostock et al., 2011). Starting 4 days later and continuing until all rats received all treatment combinations, the effects of clonidine or atipamezole on L-DOPA mediated behaviors was first assessed for LID and then for akinesia and motor activity. On AIMs test days (days 32–65), rats were treated with clonidine (0, 0.01, 0.1 mg/kg, ip) or atipamezole (0, 0.3, 1.0 mg/kg, sc) followed 30 min later by L-DOPA (4, 6 mg/kg, sc) and were rated for ALO AIMs and rotations every 10 min for 3 h post L-DOPA treatment. The effects of clonidine and atipamezole on akinesia and motor performance were assessed using the FAS test and locomotor activity chambers, respectively. Rats were habituated to the FAS testing parameters and the locomotor chambers off treatment during the 2 weeks preceding this testing. On test days, rats were injected with either clonidine (0, 0.1 mg/kg, ip) or atipamezole (0, 1.0 mg/kg, sc) followed by L-DOPA (0, 4, 6 mg/kg, sc) 30 min later. On FAS test days, the effects of clonidine or atipamezole were evaluated 60 min post L-DOPA treatment. For tests of motor activity, rats were placed into the activity chambers immediately following L-DOPA injection. Two animals died prior to completing all FAS and activity chamber test days and their data was excluded from those analyses.

2.6.2. Experiment 2: effect of intra-LC α2R stimulation and blockade on LID

Two separate cohorts of rats were implanted with cannulae over the left LC in addition to the unilateral 6-OHDA MFB lesion. Only rats with correct cannula placement, as determined by cresyl violet histology at the experiment, were retained for final data analyses (clonidine rats: n = 8; atipamezole treated rats, n = 11). Three weeks after surgery all rats were primed with L-DOPA (12 mg/kg) for 1 week to rapidly induce stable ALO AIMs. Rats were tested with the dose of L-DOPA used throughout test days 2 days later (clonidine treated rats: 1 mg/kg; atipamezole treated rats: 4 mg/kg, sc; atipamezole treated rats: 4 mg/kg, sc) and rated for ALO AIMs. L-DOPA doses were chosen for clonidine and atipamezole microinfusion tests by selecting the doses of L-DOPA that were modified by systemic α2R-stimulation and produced a similar magnitude of effect in experiment 1. The effects of direct LC infusions of clonidine or atipamezole on dyskinesia were tested in two separate cohorts of rats. Treatments were counterbalanced across days for each cohort using a Latin-square design for the doses described in Table 1. Intra-LC clonidine microinfusion tests occurred on 4 separate test days where clonidine or its vehicle was administered into the LC prior to systemic L-DOPA (6 mg/kg) treatment (C0-LD6, C2-LD6, C20-LD6) or the high dose of clonidine was administered into the LC prior to systemic L-DOPA vehicle treatment (C20-LD0). Since a floor effect was expected for clonidine treatment based on previous behavioral data, intra-LC clonidine followed by systemic L-DOPA vehicle (C20-LD0) treatment was used as the control group. Intra-LC atipamezole tests occurred on 5 separate test days as follows: atipamezole or its vehicle was injected into the LC 5 min prior to systemic L-DOPA (4 mg/kg) (A0-LD4, A15.1-LD4, A15.2-LD4) or L-DOPA vehicle (A0-LD0) treatment. Given the possibility that atipamezole itself might induce dyskinesia, a comparison between intra-LC atipamezole without L-DOPA (A0-LD0) and vehicle—vehicle (A0-LD0) was also examined. During LC microinjections, rats were restrained with a towel and an injector was lowered 1 mm past the end of the guide cannula. Drugs were infused at a rate of 0.4 μl/min for a total volume of 1.0 μl in 2.5 min using a microinfusion pump (Harvard Apparatus, Boston, MA) holding one 10 μl Hamilton syringe attached to plastic tubing (PE20 Tygon tubing; Plastics One) and the injector. The injector was removed 5 min after the flow was stopped. Rats were then immediately injected with L-DOPA (clonidine treated rats: 0, 6 mg/kg; atipamezole treated rats: 0, 4 mg/kg, sc) and were rated for ALO AIMs and rotations every 10 min for 3 h.

2.7. Statistical analyses

Non-parametric ALO AIMs data were evaluated using within-subjects Friedman tests at each time point, and overall effects for experiments 1 and 2. Wilcoxon sign-rank post hocs were used to distinguish significant differences. Parametric rotation, FAS, and locomotor chamber data were analyzed using repeated-measures, within-subjects ANOVAs. Fisher’s LSD post hoc comparisons were used to evaluate significant differences. Striatal monoamine and metabolite concentrations were analyzed using dependent samples t-tests. Analyses were performed with the use of Statistica software 88 (Statsoft Inc., Tulsa, OK, USA) and alpha was set at 0.05.

3. Results

3.1. Monoamine and metabolite levels

The effects of 6-OHDA lesion on concentrations of NE, DA, and DOPAC levels in the lesioned (left) versus intact (right) striatum are reported in Table 2.

As expected, unilateral 6-OHDA injection into the MFB produced significant (~99%) reductions in DA and the DA metabolite DOPAC in the lesioned striatal hemisphere of all rats compared to the intact striatal hemisphere (t25 = 9.51; p < 0.001). Despite desipramine pretreatment ~80% reductions in striatal NE levels were also observed ipsilateral to MFB lesion (t25 = 4.33, p < 0.001).
Table 1
Drug treatment schedule.

<table>
<thead>
<tr>
<th>Intra-LC treatment (dose)</th>
<th>Systemic treatment (dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine (0.0 µg)</td>
<td>L-DOPA (6 mg/kg)</td>
</tr>
<tr>
<td>Clonidine (0.2 µg)</td>
<td>L-DOPA (6 mg/kg)</td>
</tr>
<tr>
<td>Clonidine (2.0 µg)</td>
<td>L-DOPA (6 mg/kg)</td>
</tr>
<tr>
<td>Clonidine (20.0 µg)</td>
<td>L-DOPA (0 mg/kg)</td>
</tr>
<tr>
<td>Clonidine (20.0 µg)</td>
<td>L-DOPA (0 mg/kg)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intra-LC treatment (dose)</th>
<th>Systemic treatment (dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atipamezole (0.0 µg)</td>
<td>L-DOPA (0 mg/kg)</td>
</tr>
<tr>
<td>Atipamezole (0.1 µg)</td>
<td>L-DOPA (4 mg/kg)</td>
</tr>
<tr>
<td>Atipamezole (1.5 µg)</td>
<td>L-DOPA (4 mg/kg)</td>
</tr>
<tr>
<td>Atipamezole (15.0 µg)</td>
<td>L-DOPA (0 mg/kg)</td>
</tr>
</tbody>
</table>

Table 2
Effects of unilateral MFB lesion on concentrations of striatal NE, DA, and DOPAC.

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>NE</th>
<th>DA</th>
<th>DOPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion (left)</td>
<td>7.83 ± 1.38*</td>
<td>55.37 ± 13.06*</td>
<td>28.94 ± 4.38*</td>
</tr>
<tr>
<td>Intact (Right)</td>
<td>39.32 ± 6.80</td>
<td>4544.93 ± 449.03</td>
<td>1677.81 ± 173.77</td>
</tr>
</tbody>
</table>

Values represent picogram monoamine or metabolite per milligram protein (mean ± standard error of the mean). *p < 0.05 vs. intact hemisphere.

3.2. Experiment 1: effect of systemic α2R stimulation or blockade on L-DOPA-mediated behavior

3.2.1. ALO AIMS and rotations

Upon examination of the effects of the α2R agonist clonidine, time-point analysis revealed significant differences in 4 mg/kg L-DOPA-induced ALO AIMS expression at the 20–100, 120, and 170 min time-points, as well as for the total ALO AIMS sum (χ² > 6.46; p < 0.05 for all). As shown in Fig. 2A, pretreatment with the high dose of clonidine (0.1 mg/kg; C0.1-LD) significantly reduced ALO AIMS expression compared to the low dose (0.01 mg/kg; C0.01-LD) or vehicle (0 mg/kg; C0-LD) throughout the first 100 min of testing. Additionally, the high dose of clonidine also reduced ALO AIMS at 120 and 170 min time-points versus vehicle. The antipsychotic effects of clonidine on the 6 mg/kg dose of L-DOPA were less pronounced. As shown in Fig. 2C, significant differences in ALO AIMS expression were found at the 30 and 40 min time-points as well as the total ALO AIMS sum (χ² = 10.12, 10.32, 10.82, respectively; p < 0.01 for all). Post hoc analyses revealed pretreatment with both doses of clonidine (0.01, 0.1 mg/kg) significantly reduced ALO AIMS expression at both the 30 min time-point, and overall, when compared to vehicle. Additionally, the high dose of clonidine also reduced ALO AIMS expression at the 40 min time-point compared to vehicle pretreatment (p < 0.05 for all).

For rotations, the 3(treatment) x 18(time) within-subjects repeated-measures ANOVAs revealed significant main effects of clonidine treatment (F5, 45 = 4.17, p < 0.01) and time (F17, 153 = 8.32, p < 0.01), but not treatment, and no interaction for L-DOPA 4 mg/kg (Fig. 2F). Post hoc analyses determined co-treatment of 4 mg/kg L-DOPA with the 0.1 mg/kg dose of atipamezole significantly enhanced contralateral rotations, compared to vehicle (p < 0.05; Fig. 2F, insert). A significant effect of time (F17, 153 = 5.96, p < 0.01), but not treatment, and no interaction was observed with 6 mg/kg L-DOPA (Fig. 2H).

3.2.2. FAS

The FAS test was used to determine whether systemic treatment with clonidine or atipamezole altered L-DOPA’s restorative motor benefits. Percent-intact forearm stepping was analyzed using one-factor, repeated-measures ANOVAs and demonstrated main effects of treatment for both clonidine (F5, 45 = 6.79, p < 0.001) and atipamezole (F5, 45 = 9.72, p < 0.001). A limited number of post-hoc comparisons were examined further. As shown in Fig. 3A and B, forearm stepping deficits were dose-dependently reversed by both the low (C0-LD4, A0-LD4) and high (C0-LD6, A0-LD6) doses of L-DOPA compared to vehicle (C0-LD0, A0-LD0).

Treatment with clonidine or atipamezole alone (C0.1-LD0; A1.0-LD0) did not alter forehand stepping compared to L-DOPA vehicle treatment alone (A0-LD0; C0-LD0). Moreover, when combined with either dose of L-DOPA (4, 6 mg/kg) neither clonidine nor atipamezole altered L-DOPA’s motor restorative effects on forehand stepping. For percent backhand stepping, a significant effect of treatment was found for clonidine (F5, 45 = 8.32, p < 0.001), but not atipamezole (Fig. 3D). As shown in Fig. 3C, the high dose of L-DOPA-enhanced backhand stepping compared to vehicle. In addition, co-administration of clonidine with L-DOPA 4 mg/kg (C0.1-LD4) improved backhand stepping beyond the motor benefits achieved with L-DOPA 4 mg/kg treatment alone (C0-LD4).

3.2.3. Locomotor activity

The effects of clonidine and atipamezole pretreatment on motor behaviors were assessed in locomotor activity chambers (Fig. 4).
Data were collapsed into 3 1 h time blocks for each metric and analyzed using 6(treatment) x 3(time) within-subjects repeated-measures ANOVAs. A limited number of post hoc comparisons were conducted to further evaluate significant effects.

For clonidine treatment, analysis of distance traveled demonstrated a significant effect of treatment (F5, 35 = 8.87, p < 0.001) and a significant treatment by time interaction (F10, 70 = 2.83, p < 0.01). As shown in Fig. 4A, regardless of dose, L-DOPA alone (C0-LD4, C0-LD6) enhanced distance traveled compared to vehicle treatment (C0-LD0) in the first 2 h. C0-LD6 also increased distance traveled during the third hour compared to C0-LD0. Administration of clonidine with the low dose of L-DOPA (C0.1-LD4) attenuated movement to control (C0.1-LD0) levels for the entirety of the test. A similar reduction in movement was seen during the first hour when clonidine was administered with the higher dose of L-DOPA (C0.1-LD6; p < 0.05 for all). Close inspection revealed that significantly more vertical activity occurred following the low dose of L-DOPA (C0.1-LD4), than C0-LD0 during the first and second hours. Surprisingly, C0-LD6 treatment did not significantly augment vertical activity compared to C0-LD0 treatment in either the first or second hour. However, clonidine

---

**Fig. 2. Effect of Systemic α2R treatment on ALO AIM and Rotations.** Rats (n = 10) were treated with clonidine (0, 0.01, 0.1 mg/kg, ip; C0-LD, C0.01-LD, C0.1-LD) or atipamezole (0, 0.3, 1.0 mg/kg, sc; A0-LD, A0.3-LD, A1.0-LD) followed 30 min later by L-DOPA 4 mg/kg (top panels) or 6 mg/kg (bottom panels) using a counterbalanced, within-subjects design. Graphs depict the timecourse of ALO AIMs (expressed as medians ± median absolute deviation, MAD; Clonidine: A, C; Atipamezole E, G) and rotations (expressed as means ± standard error of the mean, SEM; Clonidine: B, D; Atipamezole F, H). Insets represent total ALO AIMs or rotations summed over the 3 h testing period. Treatment effects were analyzed with Friedman tests for AIMs and two-way repeated measures ANOVAs for rotations. Post-hoc comparisons denote significant differences between treatments at the time-points. Clonidine: *p < 0.05 C0.1-LD vs. C0-LD; + p < 0.05 C0.01-LD vs. C0-LD; ^ p < 0.05 C0.1-LD vs. C0.01-LD. Atipamezole: *p < 0.05 A1.0-LD vs. A0-LD; + p < 0.05 A0.3-LD vs. A0-LD; p < 0.05 A0.1-LD-A0.3-LD.
pretreatment reduced vertical activity during the first hour when given alone (C0.1-LD0) versus C0-LD0, and in conjunction with either dose of l-DOPA (C0.1-LD4, C0.1-LD6) compared to C0-LD4, and C0-LD6, respectively. In the second hour, the C0.1-LD4-induced suppression of vertical activity was still observed compared to V-LD4 (p < 0.05 for all). Analysis of stereotypy count revealed significant effects of treatment (F5, 35 = 15.63, p < 0.001), time (F2, 14 = 6.24, p < 0.05), and a treatment by time interaction (F10, 70 = 4.47, p < 0.001). As shown in Fig. 4C, both C0-LD4 and C0-LD6 enhanced stereotypic behaviors during the first 2 h, compared to C0-LD0. l-DOPA (4, 6 mg/kg)-induced stereotypy was reduced in the first 2 h when clonidine 0.1 mg/kg (C0.1-LD4, C0.1-LD6) was administered. This effect was most pronounced in the first hour, where C0.1-LD4 and C0.1-LD6 treatment did not differ from C0-LD0. In the third hour, stereotypic behaviors were still elevated in rats that received C0-LD6. As in the first 2 h, C0.1-LD6 treatment significantly reduced stereotypic behaviors compared to C0-LD6 (p < 0.05 for all). For atipamezole, analysis of distance traveled (Fig. 4D) revealed a significant effect of treatment (F5, 35 = 7.73, p < 0.001), time (F2, 14 = 4.45, p < 0.05), and a treatment by time interaction (F10, 70 = 2.08, p < 0.05). Compared to vehicle treatment (A0-LD0), all treatments with l-DOPA (A0-LD4, A0-LD6, A1.0-LD4, A1.0-LD6) enhanced the distance traveled in the first two hours. However, distance traveled in the third hour of testing was no longer elevated in rats that received l-DOPA 4 mg/kg (V-LD4, A1.0-LD4) compared to A0-LD0. Interestingly, during the second hour, A1.0-LD6 treatment further enhanced distance traveled compared to l-DOPA alone (A0-LD6; p < 0.05 for all). Analysis of vertical activity (Fig. 4E) revealed a significant main effect of time (F2, 14 = 6.31, p < 0.05), but no effect of treatment and no interaction. Significant main effects of treatment (F5, 35 = 4.33, p < 0.001), time (F2, 14 = 27.11, p < 0.05), and a treatment by time interaction (F10, 70 = 10.81, p < 0.001) were found for stereotypy count (Fig. 4F). Analysis of the interaction revealed that regardless of pretreatment, l-DOPA induced significantly more stereotypic behaviors in the first 2 h compared to vehicle (A0-LD0). In the 3rd h, stereotypic behaviors remained elevated only in rats that received the high dose of l-DOPA (A0-LD6, A1.0-LD6) or the low dose of l-DOPA with atipamezole (A1.0-LD4) (p < 0.05 for all).

3.3. Experiment 2: effect of intra-LC a2R stimulation or blockade on l-DOPA-mediated behavior

3.3.1. ALO AIMS and rotations

Friedman tests at each individual time-point were used to evaluate the time-course of effects of intra-LC clonidine and atipamezole infusion on l-DOPA-induced ALO AIMS (for representative cannulae placements see Fig. 5A). Direct infusions of clonidine into the LC reduced dyskinesia during the first 50 min and overall (\(\chi^2 > 6.00\), all p < 0.05; Fig. 5B). The effects of intra-LC clonidine were dose-dependent. The high dose of clonidine (20 \(\mu\)g) reduced ALO AIMS at the 10, 20, 40, and 50 min time-points as well as the total ALO AIMS sum (inset) compared to vehicle. The low dose reduced ALO AIMS at the 50th min as well as the total overall ALO AIMS sum. While there was a significant effect of time on rotations (F17, 119 = 3.15; p < 0.001), there was not an effect of treatment, nor a time by treatment interaction (Fig. 5D).

For atipamezole treated rats, significant treatment effects were observed for the first 150 min and the total sum (\(\chi^2 > 11.43\); p < 0.05 for all). A limited number of Wilcoxon sign-rank post-hoc tests were performed including comparisons of: intra-LC atipamezole (1.5,
15 μg) plus systemic α-DOPA (4 mg/kg; A1.5-LD, A15.0-LD) versus intra-LC vehicle plus systemic α-DOPA (4 mg/kg; A0-LD). Since systemic atipamezole alone had activational effects on motor behaviors, the effects of atipamezole by itself (intra-LC atipamezole (15 μg) plus systemic α-DOPA vehicle (A15.0-LD0) treatment) on ALO AIMS were compared to the control, intra-LC vehicle plus systemic vehicle (A0-LD0). As depicted in Fig. 5C, the high dose of atipamezole (15 μg) significantly enhanced α-DOPA-induced ALO AIMS expression at the 30 and 50 min time-points as well as overall compared to A0-LD4 treatment. The low dose of atipamezole (1.5 μg) increased α-DOPA-induced ALO AIMS at the 40 and 120 min, and showed a non-significant trend towards increased total ALO AIMS compared to A0-LD4 (p = 0.055; Fig. 5C, insert). Interestingly, intra-LC atipamezole alone actually induced mild dyskinesia during the first 20 min compared to A0-LD0 treatment. Analysis of contralateral rotations using a 5(treatment) x 18(time) within-subjects repeated measures ANOVA demonstrated no significant main effect of treatment or time, and no interaction (Fig. 5E).

4. Discussion

Although α2R have received attention in recent years as a target for modulating α-DOPA’s effects in the PD brain, their mechanism(s) of action have remained elusive. In the present work, employing the selective α2R antagonist atipamezole allowed us to differentiate the specific contribution of α2R in α-DOPA’s dyskinesia- and motor-inducing effects. Systemic α2R blockade with atipamezole extended the dyskinetic and pro-locomotor actions of a low dose of α-DOPA. Conversely, α2R stimulation with clonidine reduced α-DOPA-mediated locomotor and dyskinetic behaviors. For the first time, we demonstrated these effects are partially mediated by α2R within the LC since direct infusions of clonidine or atipamezole reduced, and enhanced LID expression, respectively.

Several noradrenergic compounds have shown promise for the treatment of LID (Dekundy et al., 2007; Lundblad et al., 2002; Buck et al., 2010; Barnum et al., 2012; Savola et al., 2003; Colosimo and Craus, 2003; Buck and Ferger, 2010; Visanji et al., 2009). Some α2R antagonists have even made it as far as Phase II clinical trials with limited success (Rascol et al., 2001; Manson et al., 2000; Lewitt et al., 2012). Paradoxically, both agonists and antagonists for the α2R display antidyskinetic actions in preclinical settings. Therefore, the first goal of the present work was to characterize the behavioral effects of two compounds with opposing actions on the α2R. The effects of atipamezole on LID had not previously been evaluated, however an extensive body of literature previously demonstrated α2R antagonists reduce LID symptoms (Dekundy et al., 2007; Lundblad et al., 2002; Buck et al., 2010; Buck and Ferger, 2010; Lewitt et al., 2012). In the current investigation, we
Fig. 5. The effect of intra-LC α2R treatment on LID. Schematic representation of coronal rat brain section containing the LC adapted from Paxinos and Watson (Bregma: −10.04 mm) and a representative cresyl violet-stained LC image portraying typical injector placement is displayed (A). Relevant anatomical structures: Cerebelum (Cer), Fourth ventrical (4V), Mesencephalic trigeminal nucleus (Me5). The effects of intra-LC clonidine or atipamezole on ALO AIMs (clonidine: B; atipamezole: C) and rotations (clonidine: D; atipamezole: E) were assessed in 2 separate groups of rats (clonidine treated: n = 8; atipamezole treated (n = 11). Clonidine (0, 2, 20 μg) or atipamezole (0, 1.5, 15.0 μg) was injected into the LC followed by systemic injection of L-DOPA (clonidine treated: 0, 6 mg/kg; atipamezole: 0, 4 mg/kg, sc). Graphs depict the timecourse of ALO AIMs (expressed as medians ± median absolute deviation, MAD; A) and rotations (expressed as means ± standard error of the mean, SEM; B). Insets represent total ALO AIMs and rotations summed over the 3 h testing period. Treatment effects were analyzed with Friedman tests for AIMs and two-way repeated measures ANOVAs for rotations. Post-hoc comparisons denote significant differences between treatments at the time-points indicated. Clonidine: *p < 0.05 C0-LD6 vs. C20-LD6; ^p < 0.05 C0-LD6 vs. C2-LD6; ‘p < 0.05 C0-LD6 vs. C2-LD6. Atipamezole: p < 0.05, A0 -LD4 vs. A15.0 -LD4; + p < 0.05, A0-LD4 vs. A1.5-LD4; ‘p < 0.05, A0-LD0 vs. A15-LD0.

Previous experimental evidence demonstrates α2R stimulation with clonidine suppresses LID symptoms (Gomez-Mancilla and Bedard, 1993; Dekundy et al., 2007). In the current investigation, we confirmed that systemic clonidine reduced L-DOPA-induced ALO AIMs and stereotypic behaviors. This effect was especially pronounced when clonidine was administered with the low dose of L-DOPA. While often reported to be a specific α2R agonist, clonidine has also shown to have actions at imidazoline receptors, α1 noradrenergic receptors, and 5-HT1AR (Newman-Tancredi et al., 1998; Ernsberger et al., 1987; Coupry et al., 1989). In the rat, clonidine is 56 times more selective for α2R than 5-HT1AR, where it displays weak agonist properties (Newman-Tancredi et al., 1998). While not evaluated in the current investigation, high doses of clonidine could be influencing LID through interactions with the 5-HT system, which could be tested in future studies by simultaneously blocking the 5-HT1AR.

Coincident with the dyskinesia investigation, we employed multiple behavioral assays to examine whether α2R-targeting treatments interfered with L-DOPA’s motor-restoring properties, since such actions would limit the usefulness of these compounds as L-DOPA adjuncts. One key feature of α2R antagonists which has led to continued scientific interest is that they generally do not interfere with L-DOPA’s motor benefits. In fact, α2R blockade with idazoxan or fipamezole has been shown to extend the length of L-DOPA’s antiparkinsonian benefits in response to a single dose of L-DOPA (Savola et al., 2003; Johnston et al., 2010; Henry et al., 1999; Fox et al., 2001). In the present work, systemic atipamezole treatment did not change L-DOPA’s antiparkinsonian stepping effects on the FAS test. Atipamezole potentiated L-DOPA’s motor-enhancing effects for distance traveled in the locomotor chambers and for contralateral rotations observed during the AIMS test when given in conjunction with the low dose of L-DOPA. These activating effects support past work showing that α2R blockade augments L-DOPA’s rotational behavior in hemiparkinsonian rats (Haapalina et al., 2001). In contrast, clonidine pretreatment reduced L-DOPA’s motor-stimulating effects on rotations during the AIMS test, and found atipamezole mildly enhanced LID severity and L-DOPA-induced activity. Systemic atipamezole when combined with low dose L-DOPA actually induced an earlier onset and slower decline of LID symptoms compared to L-DOPA alone. In addition, L-DOPA-induced stereotypic behaviors, indicative of LID, were enhanced by atipamezole treatment in the locomotor chambers. This is similar to what was shown in a primate model of LID where the α2R antagonist fipamezole exacerbated non-disabling dyskinesia associated with prolonged L-DOPA “on-time” (Johnston et al., 2010). In rats, others have demonstrated α2R blockade using idazoxan reduced LID severity and L-DOPA-induced rotations during the first hour of testing, but actually extended the duration of LID symptoms during the last hour of testing (Barnum et al., 2012). It should be noted that many of the α2R compounds previously used to investigate LID also display agonist actions at 5-HT 1A receptors (5-HT1A,R) and/or antagonistic properties at DA D2 receptors (DA D2R) (Pertovaara et al., 2005; Convents et al., 1989; Millan et al., 2000; Newman-Tancredi et al., 1998; Scatton et al., 1980), both of which are highly implicated in LID (Dupre et al., 2008; Bishop et al., 2009; Taylor et al., 2005; Carta et al., 2010; Huot and Brotchie, 2011; Koprich et al., 2013; Eskow et al., 2007). In contrast, atipamezole is a highly potent and selective α2R antagonist with virtually no affinity for DA D2R or 5-HT1A,R (Pertovaara et al., 2005; Newman-Tancredi et al., 1998).

Previous experimental evidence has demonstrated α2R stimulation with clonidine or atipamezole augments L-DOPA’s antiparkinsonian effects in the FAS test. Atipamezole potentiated L-DOPA’s motor-enhancing effects for distance traveled in the locomotor chambers and for contralateral rotations observed during the AIMS test when given in conjunction with the low dose of L-DOPA. These activating effects support past work showing that α2R blockade augments L-DOPA’s rotational behavior in hemiparkinsonian rats (Haapalina et al., 2001). In contrast, clonidine pretreatment reduced L-DOPA’s motor-stimulating effects on rotations during the AIMS test, and
distance traveled and vertical activity in the locomotor chambers, two metrics of general motor activity. This is in agreement with previous studies demonstrating the antiparkinsonian and rotational behaviors induced by I-DOPA or other dopaminergic drugs is reduced by concomitant 2R stimulation (Haapalinna et al., 2003; Mavridis et al., 1991; Juhila et al., 2003; Huotari et al., 2000; Chopin et al., 1999). Interestingly, systemic clonidine pretreatment did not hinder the low dose of I-DOPA’s antiparkinsonian effects on the FAS test at a time when dyskinesia symptoms were still suppressed.

Inhibitory 2R are abundantly expressed throughout the brain, especially in regions implicated in PD like the basal ganglia and LC (Rosin et al., 1996; Lee et al., 1998a, 1998b; MacDonald and Scheinin, 1995), however the role of 2R within motor circuits in modulating I-DOPA’s actions are not fully understood and may well be site-specific. Here, we demonstrate a discrete population of 2R within the LC influence LID. 2R are abundantly expressed on NE neurons of the LC where they act as autoreceptors to control the release of NE (Alachkar et al., 2011; Norenberg et al., 1997; Lee et al., 1998a). We administered clonidine and atipamezole directly into the LC prior to systemic I-DOPA treatment. While in the same direction as the systemic effects, the magnitude of the intra-LC clonidine and atipamezole effects on LID were less pronounced. There was significant (~80%) loss of striatal NE levels ipsilateral to 6-OHDA infusion in the current investigation which may have led to the dampened responses. However, intra-LC clonidine did reduce I-DOPA-induced ALO AIMs expression during the first 50 min, with the greatest suppression seen in the first half-hour. In contrast, intra-LC atipamezole infusion induced an earlier onset of LID. Interestingly, intra-LC infusion of atipamezole by itself induced mild, but significant ALO AIMs expression during the first 20 min of AIMs testing compared to rats that received intra-LC vehicle followed by systemic vehicle. This supports a presynaptic mechanism through which 2R can mediate the behavioral effects of I-DOPA. However, different populations of 2R may play opposing roles in I-DOPA’s motor effects. 2R are also found in great number on GABAergic medium spiny projecting neurons in the striatum (Nicholas et al., 1993; Holmberg et al., 1999; Scheinin et al., 1994) and direct infusion of the 2R antagonist idazoxan into the striatum reduced LID in a rodent model of LID (Wang et al., 2014). While not directly tested in the current work, it is important to determine whether the antidyskinetic and antiparkinsonian effects of 2R-targeting compounds can be dissociated to improve patient outcomes.

The mechanisms through which 2R influence I-DOPA’s behavioral effects have not yet been determined; however, NE transmission is thought to be involved. Indeed, LID has been shown to temporarily coincide with I-DOPA-derived striatal NE efflux (Wang et al., 2014) and direct infusion of NE alone into the striatum has been shown to induce dyskinesia in I-DOPA-primed, hemiparkinsonian rats (Wang et al., 2014; Buck and Ferger, 2009). This is further supported by work showing basal firing activity in the LC positively correlates with LID severity in a rodent model of LID (Miguelez et al., 2011). At the level of the LC, 2R function as autoreceptors to control the release of NE. Stimulation of 2R reduces while blockade enhances central NE release (Lapiz et al., 2007; Svensson et al., 1975; Langer, 1974). The current body of work supports a potential NE-dependent mechanism by which 2R modulate I-DOPA-mediated behaviors.

There is evidence 2R in the LC influence striatal DA signaling, however it is not clear how this is occurring. 2R blockade with atipamezole has been shown to potentiate I-DOPA’s effects on evoked DA release in the striatum, and may enhance DA transmission via increasing DA accumulation and the size of the readily releasable DA storage pool (Yavich et al., 2003). Moreover, local and systemically administered 2R antagonists have been shown to enhance striatal DA content and overflow in intact and DA-lesioned rats (Nutt et al., 1994; Hudson et al., 1999; Hertel et al., 1999) while 2R agonism reduced striatal DA release in experimental models (Yavich et al., 1997; Trendelenburg et al., 1994). In contrast to this, a recent experiment using microdialysis has shown striatal infusion of the 2R antagonist idazoxan reduced LID and striatal peak I-DOPA-induced striatal DA efflux (Wang et al., 2014). LC NE neurons terminating in the striatum may influence striatal DA levels by modifying DA uptake. Indeed, evidence shows that striatal NE transporters (NET) can take up I-DOPA-derived DA in the DA-depleted brain (Arai et al., 2008) and subsequent NET blockade exacerbated LID expression in a rodent model of LID (Chotibut et al., 2014). NE from the LC could also modulate striatal DA signaling indirectly by regulating striatal DA release from the SNC (Anden and Grabowska, 1976; Fujimoto et al., 1981). However, in the 6-OHDA rat model used in the current investigation, nigrostriatal DA projections are greatly depleted and I-DOPA-derived DA release is thought to be mediated by non-dopaminergic neurons. In fact, 5-HT neurons of the dorsal raphe nucleus (DRN) take up, convert, and release exogenously administered I-DOPA-derived DA into the striatum (Tanaka et al., 1998; Carta et al., 2007; Navailles et al., 2011). The LC and DRN are linked by reciprocal projections. 1R noradrenergic receptors are abundantly expressed on 5-HT cell bodies while 2R found presynaptically on NE neurons synapsing in the dorsal raphe nucleus (DRN) (Rosin et al., 1996; Talley et al., 1996; Day et al., 1997). As such, the firing activity of 5-HT neurons in the DRN is mediated by tonic activation from noradrenergic neurons that mediated by 1R- and 2R (Baraban and Aghajanian, 1980; Haddjeri et al., 2004). Given the DRN is a major source of I-DOPA-derived DA, it is plausible the LC may modulate LID indirectly by altering 5-HT neurotransmission in the PD brain. Direct confirmation of the impact of 2R stimulation or blockade on striatal DA or NE efflux, and whether these effects occur trans-synaptically through the DRN are clearly warranted.

It has become increasingly apparent that I-DOPA’s effects are conveyed via complex interactions between many neurotransmitter systems. In the present investigation we evaluated the role of 2R in I-DOPA-mediated behaviors, and showed 2R stimulation reduced, while blockade enhanced, LID symptoms and motor activity. These effects were at least partially mediated by a population of 2R located within the LC. How this translates into clinical use remains an open question. To date, two 2R antagonists, pipamazole and idazoxan, have proceeded to clinical trials without complete understanding of the mechanism(s) through which they are acting. These have shown only mild, if any, antidyskinetic efficacy (Rascal et al., 2001; Manson et al., 2000; Lewitt et al., 2012). Thus, more work determining how and where 2R alter motor circuits in the dyskinetic brain should provide valuable information to optimize long-term use of I-DOPA.

Acknowledgments

This work was supported by funds from R01-NS059600 (CB) and the Center for Development and Behavioral Neuroscience at Binghamton University (CB).

References


Cytokine responses in differentially activated human mononuclear cells and their effects on macrophage polarization and cytokine production in human peripheral blood mononuclear cell cultures. Journal of Immunology. 165, 2202–2211.


Millan, M.J., Newman-Tancredi, A., Audinot, V., Cussac, D., Lejeune, F., Nicholas, J.P., et al., 2000. Agonist and antagonist actions of yohimbine as compared to fluparoxan at adrenergic receptors (AR), serotonin (5HT(1A), 5-HT(1B), 5-HT(1D) and dopamine D(2) and D(3) receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states. Synapse 35, 79–95.


