The partial 5-HT$_{1A}$ agonist buspirone reduces the expression and development of l-DOPA-induced dyskinesia in rats and improves l-DOPA efficacy

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Abstract

Dopamine (DA) replacement therapy with l-DOPA remains the standard pharmacotherapy for Parkinson’s disease (PD). Unfortunately, chronic l-DOPA treatment is accompanied by development of motor fluctuations and l-DOPA-induced dyskinesias (LID). While serotonin (5-HT)$_{1A}$ agonists acutely reduce these complications, their prophylactic and long-term effects are not well-delineated. To test this, male Sprague-Dawley rats received unilateral 6-hydroxydopamine (6-OHDA) lesions. In experiment 1, l-DOPA-primed rats were pre-treated with Vehicle (0.9% NaCl), various doses of the partial 5-HT$_{1A}$ agonist, buspirone (0.25, 1.0 or 2.5 mg/kg, ip) or buspirone (2.5 mg/kg, ip)+the 5-HT$_{1A}$ antagonist, WAY100635 (0.5 mg/kg, ip) 5 min prior to l-DOPA (12 mg/kg+15 mg/kg benserazide, ip). Rats were tested for LID using the abnormal involuntary movements (AIMs) scale and motor performance using the forepaw adjusting steps test (FAS). In experiment 2, l-DOPA-naïve rats received co-administration of l-DOPA+buspirone (1.0 or 2.5 mg/kg, ip) for 2 weeks. AIMs and FAS were measured throughout. In l-DOPA-primed rats, buspirone dose-dependently reduced LID and improved l-DOPA-related motor performance due to action at the 5-HT$_{1A}$ receptor. In l-DOPA-naïve rats, buspirone delayed LID development while improving l-DOPA’s anti-parkinsonian efficacy indicating the potential long-term benefits of 5-HT$_{1A}$ agonists for reduction of l-DOPA-related side effects.

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Keywords: Rat; Serotonin; 6-Hydroxydopamine; Buspirone; Dyskinesia; Motor fluctuations; Parkinson’s disease

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1. Introduction

Dopamine (DA) replacement therapy with 1-3,4-dihydroxyphenylalanine (l-DOPA) remains the standard pharmacother-apy for the treatment of movement deficit in patients with Parkinson’s disease (PD; Obeso et al., 2000). Unfortunately, most PD patients eventually experience motor fluctuations, including “wearing off” and l-DOPA-induced dyskinesias (LID; Jankovic, 2005). The current necessity of DA replacement therapy and the debilitating nature of its side effects make non-dopaminergic adjunct treatments for the reduction of l-DOPA-induced motor complications indispensable for the health of PD patients.

One potential non-dopaminergic therapeutic target may prove to be the serotonin (5-HT) system. Following severe DA denervation, neuroadaptive changes in 5-HT raphestriatal projections and upregulated 5-HT receptors allow this system to more readily influence basal ganglia activity (Fox et al., 1998; Maeda et al., 2003; Bishop et al., 2004). Such findings have led to the suggestion that compounds targeting 5-HT neurotransmission may have therapeutic value for the reduction of problems that
accompany chronic 1-DOPA treatment (Nicholson and Broottie, 2002; Scholtissen et al., 2006). In support of this assertion, the 5-HT/DA releaser, 3,4-methylenedioxymethamphetamine and the 5-HT releaser, fenfluramine have been shown to convey both anti-parkinsonian and anti-dyskinetic effects in experimental models of PD (Iravani et al., 2003; Bishop et al., 2006). While multiple 5-HT receptor subtypes may have contributed to these beneficial effects, accumulating evidence suggests an integral role for the 5-HT1A receptor.

In recent years, investigations into the beneficial effects of 5-HT1A receptor stimulation have yielded encouraging, though occasionally conflicting results. In rats with unilateral DA lesions, acute administration of the full 5-HT1A agonists, ±8-OH-DPAT and sarizotan was reported to reduce peak l-DOPA-induced rotations, but prolong response duration (Bibbiani et al., 2001; Ba et al., 2007). In MPTP-lesioned primates, acute administration of sarizotan was also shown to attenuate LID without affecting l-DOPA’s efficacy (Bibbiani et al., 2001). These results were found to be specific to the drug’s action at the 5-HT1A receptor. In contrast, Iravani et al. (2006) reported that the more potent and selective enantiomer for the 5-HT1A receptor, +8-OH-DPAT squelched LID, but worsened movement disability, likely reflecting induction of a 5-HT-like syndrome. Clinically, various 5-HT1A agonists such as tandospirone (Kanari et al., 2002) and sarizotan (Olanow et al., 2004) have been employed with moderate success. For example, sarizotan has been reported to attenuate LID at low doses (Goetz et al., 2007) and prolong l-DOPA’s efficacy in patients with advanced PD (Bara-Jimenez et al., 2005). However, similar to results in preclinical work, higher doses of these compounds can worsen parkinsonian features (Kanari et al., 2002; Goetz et al., 2007) and may have influenced the recent decision to halt the development of sarizotan as an l-DOPA adjunct therapy following Phase III clinical trials.

Given these challenges, essential questions remain concerning the utility of chronic 5-HT1A adjunct therapy for the treatment of l-DOPA-related side effects. One understudied question in this area of research is the differential effects of adjunct treatments on induction (initial development) and subsequent expression (continued behavioral manifestation) of LID. While the effect of adjunct treatments on expression of LID has been extensive, effects on induction of LID have been relatively ignored. For example, 5-HT1A agonists may have prophylactic utility on the development of motor fluctuations and subsequent LID expression (Tomiyama et al., 2005; Hauser et al., 2006). Moreover, partial 5-HT1A agonists may convey greater benefit with less risk by reducing the likelihood of side effects related to potent 5-HT1A receptor stimulation. To answer these questions, we systematically investigated the effects of 5-HT1A receptor stimulation on l-DOPA-related side effects in both l-DOPA-primed and l-DOPA-naïve unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats. We employed the partial 5-HT1A agonist, buspirone, a common anxiolytic that has been used with some success as an experimental adjunct therapy with l-DOPA in humans (Kleedorfer et al., 1991; Bonifati et al., 1994). To measure LID, we utilized the well-validated abnormal involuntary movements procedure (Lundblad et al., 2002) and the forelimb adjusting steps test (Olsson et al., 1995; Chang et al., 1999) to quantify changes in motor performance. The current findings suggest that in this preclinical model, buspirone administration can reduce both the development and expression of l-DOPA-induced motor complications.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats were used (225–250 g upon arrival; Taconic Farms, Hudson, NY, USA). 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, USA) and water. The colony room was maintained on a 12/12 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 Binghamton 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, rats received unilateral 6-hydroxydopamine (6-OHDA) lesions of the left medial forebrain bundle to destroy DA neurons. Desipramine HCl (25 mg/kg, ip; Sigma, St. Louis, MO, USA) was given 30 min prior to the 6-OHDA injection to protect norepinephrine (NE) neurons. Rats were anesthetized with inhalant isoflurane (2–3%; Sigma) in oxygen (2.5 l/min), then placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The coordinates for 6-OHDA injections were AP: –1.8 mm, ML: ±2.0 mm, DV: –8.6 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 + 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 1 min later. Rats were placed in clean cages on warming pads to recover from the surgery, after which they were returned to group-housing (2 rats/cage). Soft chow was provided as needed to facilitate recovery during the first week after surgery.

2.3. Pharmacological treatments

All rats were allowed to recover 3 weeks after lesion surgery and were then assigned a priori to equal treatment groups based on forepaw adjusting steps (FAS) performance (Chang et al., 1999) and amphetamine-induced rotations (2.5 mg/kg, ip; Sigma) before pharmacological treatment commenced.

In order to test the effects of buspirone in 1-DOPA-primed rats, all rats in the first study (n = 15) received 3,4-dihydroxyphenyldiac acid methyl ester (l-DOPA; 12 mg/kg, ip; Sigma) + dl-Serine 2-(2,3,4-trihydroxybenzyl) hydroxide hydrochloride (benserazide; 15 mg/kg, ip; Sigma) once daily for 7 days.
1-DOPA and benzerazide were dissolved in Vehicle (0.9% NaCl containing 0.1% ascorbic acid) and administered at a volume of 1.0 ml/kg. Rats were tested for abnormal involuntary movements (AIMs) on days 1, 5, and 7 of 1-DOPA priming. Only rats displaying total AIMs of greater than 15 on day 5 of priming were included in the study, which corresponded with approximately 95% DA depletion upon HPLC analysis of striatal tissue samples. Thereafter, rats were tested for AIMs every 3–4 days in a within-subjects design, receiving a pre-treatment of busipirone Vehicle (dH2O), various doses of the 5-HT1A partial agonist, busipirone HCl (busipirone; 0.25, 1.0, or 2.5 mg/kg, ip; Sigma), the specific 5-HT1A antagonist, N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl(ethyl)]-N-2-pyrindinylcyclohexanecarboxamide maleate salt (WAY100635; 0.5 mg/kg, ip; Sigma) or busipirone+WAY100635 (2.5+0.5 mg/kg, ip), 5 min prior to injection of 1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip) by random assignment. Immediately following 1-DOPA injections, rats were monitored for AIMs and rotations for 2 h.

The FAS test was also employed throughout the study in order to test the effects of busipirone on the motor performance of 1-DOPA-primed rats. All rats received the following three treatments by random assignment: Vehicle+Vehicle, Vehicle+1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip) or busipirone (2.5 mg/kg, ip)+1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip). To allow for peak 1-DOPA plasma levels (Sato et al., 1994), testing began 1 h after 1-DOPA injection. FAS testing occurred every 3–4 days following termination of AIMs testing.

In order to determine whether busipirone impacts the development of LID, all rats in the second study (n=30) were 1-DOPA naïve. Upon commencement of treatment, rats were assigned one of three daily pre-treatments by random assignment: Vehicle+Vehicle, Vehicle+1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip) or busipirone (1.0 or 2.5 mg/kg, ip)+1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip) in a between-subjects design. AIMs measures were taken on days 1, 5, 8, 11, and 14 of treatment. Treatment was terminated on day 16. On day 19, all rats were injected with 1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip) alone and immediately monitored for AIMs and rotations to investigate post-treatment responsivity.

Rats in the second study were also tested for motor performance to investigate the effects of daily busipirone on 1-DOPA efficacy. Baseline stepping measurements were obtained before initiation of the study (pre-test). On days 3, 9, and 15 following commencement of treatment, rats were treated with the following: Vehicle+1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip) or busipirone (1.0 or 2.5 mg/kg, ip)+1-DOPA (12 mg/kg, ip)+benzerazide (15 mg/kg, ip). Testing began 1 h after 1-DOPA injection.

### Table 1

<table>
<thead>
<tr>
<th>Side</th>
<th>NE</th>
<th>DOPAC</th>
<th>DA</th>
<th>DOPAC/DA</th>
<th>5-HIAA</th>
<th>5-HT</th>
<th>5-HIAA/5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (right)</td>
<td>0.15±0.02</td>
<td>2.58±0.33</td>
<td>11.2±1.06</td>
<td>0.28±0.03</td>
<td>0.54±0.09</td>
<td>0.62±0.09</td>
<td>1.14±0.26</td>
</tr>
<tr>
<td>Lesion (left)</td>
<td>0.14±0.02</td>
<td>1.91±0.03*</td>
<td>5.55±0.10*</td>
<td>0.68±0.05*</td>
<td>0.62±0.08</td>
<td>0.62±0.07</td>
<td>1.30±0.25</td>
</tr>
</tbody>
</table>

Values are nanogram monoamine or metabolite per milligram protein or ratios of metabolite to monoamine (mean ± S.E.) with percent of Vehicle group in parentheses. Differences between group means were determined by paired t-tests (*p<0.05 compared to the intact side).
and drug treatment on motor performance. Rats were moved laterally across a table at a steady rate of 90 cm/10 s. The rear part of the torso and the hindlimbs were lifted from the table and one forepaw was held by the experimenter so as to bear weight on the other forepaw. Each stepping test consisted of six trials for each forepaw, alternating between directions both forehand (defined as compensating movement toward the body) and backhand (defined as compensating movement away from the body) on the table. Data was derived by summing steps (forehand and backhand) of the lesioned forelimb and dividing them by the sum of steps (forehand and backhand) of the intact forelimb and multiplying by 100. This calculation yields a percentage of the intact side indicating the degree of forepaw disability.

2.6. High performance liquid chromatography

One week after the completion of experiments, rats were sacrificed by decapitation. The striatum was dissected, immediately frozen on dry ice, and then stored at −80 °C. Reverse-phase high performance liquid chromatography coupled to electrochemical detection was performed on striatal tissue obtained from 24 randomly selected rats (14 from the first study, 10 from the second study) according to the protocol of Kilpatrick et al. (1986), a method for semi-automated catecholamine and indoleamine analysis with coulometric detection. The system included an ESA autoinjector (Model 542), an ESA solvent delivery system (1582), an external pulse dampener (ESA), an ESA Guard-Pak column, and a C-18 (100 × 4.6 mm, 5 μm packing) column (ESA). 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, 5-HT, NE, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA). Samples were separated using a mobile phase composed of 90 mM sodium dihydrogen phosphate (monobasic, anhydrous), 0.05 mM EDTA, 1.7 mM octane sulfonic acid, and 10% acetoniitriile, adjusted to pH 3.0 with o-phosphoric acid. A coulometric detector configured with 3 electrodes (Coulochem III, ESA) measured content of monoamines and metabolites. An ESA model 5020 guard cell (+350 mV) was positioned prior to the autoinjector. The analytical cell (ESA model 5011A; first electrode at −100 mV, second electrode at +250 mV) was located immediately after the column. The second analytical electrode emitted signals that were recorded and analyzed by EZChrom Elite software via a Scientific Software, Inc. (SS420χ) module. The final oxidation current values were plotted on a standard curve of known concentrations from 10⁻⁵ M to 10⁻⁹ M and adjusted to striatal tissue.
weights and expressed as nanogram (ng) of monoamine or metabolite per milligram (mg) tissue (mean± S.E.).

2.7. Data analyses

Monoamine and metabolite levels in the striatum were analyzed using paired t-tests. Non-parametric Chi-squared median tests determined treatment effects (expressed as means± S.E.) for axial, limb, orolingual, locomotor, and total AIMs (each of the aforementioned subcategories summed). Significant differences between treatments were examined by Mann–Whitney post hoc comparisons. One-way ANOVAs and least significant differences (LSD) post hoc tests were employed for analyses of rotations and FAS results in both acute and chronic treatments. Alpha was set at \( p<0.05 \). Statistical analyses were conducted with Statistica Software '98 (Statsoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Monoamine and metabolite levels

The effects of the 6-OHDA lesion on concentrations of monoamine and metabolite levels and turnover ratios (metabolite/monoamine) in the intact (right) versus lesioned (left) striata are shown in Table 1. As anticipated, unilateral 6-OHDA injection into the medial forebrain bundle produced significant reductions in lesioned striatal DOPAC (\(t_{23}=7.72, p<0.05\)) and DA levels (\(t_{23}=10.32, p<0.05\)), 92.6% and 95.1% respectively, compared to intact striatum. The denervated side also showed an increased DOPAC/DA turnover rate (246%) compared to control (\(t_{23}=7.97, p<0.05\)). There were no significant differences between intact and lesioned striata for any other monoamine measures.

3.2. Buspirone dose-dependently reduced AIMs expression

Various doses of buspirone were tested in l-DOPA-primed rats to determine their effects on AIMs and rotations. As shown in Fig. 1, significant treatment effects were observed on measures of axial (\( \chi^2 = 24.08, p<0.05 \)), forelimb (\( \chi^2 = 31.61, p<0.05 \)), orolingual (\( \chi^2 = 19.93, p<0.05 \)), locomotor (\( \chi^2 = 11.35, p<0.05 \)), and total AIMs (\( \chi^2 = 20.01, p<0.05 \)). Post hoc analyses demonstrated that the 0.25 mg/kg dose of buspirone diminished axial AIMs (\( p<0.05 \)). The 1.0 mg/kg dose of buspirone reduced AIMs on all significant measures (all \( p<0.05 \)) and the 2.5 mg/kg buspirone dose attenuated AIMs in every category (all \( p<0.05 \)) with the exception of locomotor AIMs.

3.3. 5-HT1A receptor antagonism reversed buspirone’s anti-dyskinetic effects

As shown in Fig. 1, rats also received a pre-treatment with either the 5-HT1A antagonist WAY100635 (1.0 mg/kg) or buspirone (2.5 mg/kg)+WAY100635 in order to investigate the contribution of 5-HT1A receptors to the anti-dyskinetic effects of buspirone. On measures of axial, forelimb, orolingual, and total AIMs, post hoc analyses revealed that co-administration of WAY100635 significantly reversed the anti-dyskinetic effects of buspirone (all \( p<0.05 \)). In each case, AIMs rebounded to levels similar to those in the Vehicle pre-treatment group.
3.4. Buspirone treatment improved l-DOPA efficacy on the FAS test

In order to ascertain whether administration of buspirone affects l-DOPA’s efficacy, l-DOPA-primed rats were tested for motor performance using the FAS test. Results are shown in Fig. 2. One-way ANOVA indicated a significant effect of treatment ($F_{2,30}=8.56; p<0.05$). Post hoc analyses revealed that co-administration of buspirone (2.5 mg/kg + l-DOPA, but not Vehicle + l-DOPA improved stepping of the lesioned forepaw of l-DOPA-primed rats ($p<0.05$).

3.5. Co-administration of buspirone with l-DOPA reduced AIMs development

As demonstrated in Fig. 3, various doses of buspirone were co-administered with l-DOPA to l-DOPA-naïve rats to determine their effects on the development of AIMs and rotations. On days 5, 8, and 14, significant treatment effects were observed on measures of axial ($\chi^2=6.81, 7.21,$ and 13.82, respectively; all $p<0.05$), forelimb ($\chi^2=6.81, 7.21,$ and 13.82, respectively; all $p<0.05$), and total AIMs ($\chi^2=6.59, 6.78$ and 8.31, respectively; all $p<0.05$). An effect of treatment was also observed for orolingual AIMs on days 5 and 14 ($\chi^2=8.31$ and 7.21, respectively; both $p<0.05$). Post hoc analyses revealed a reduction of AIMs in every significant category following co-administration of 2.5 mg/kg buspirone + l-DOPA versus l-DOPA alone (all $p<0.05$).

3.6. Buspirone maintained the efficacy of l-DOPA on the FAS test

In order to investigate whether buspirone maintains the efficacy of l-DOPA, motor performance was measured throughout the development study. Results are shown in Fig. 4. Co-administration of Vehicle ($F_{3,17}=6.27, p<0.05$), buspirone (1.0 mg/kg; $F_{3,23}=4.44, p<0.05$), and buspirone (2.5 mg/kg; $F_{3,29}=6.05, p<0.05$) with l-DOPA improved motor performance compared to pre-test levels. Post hoc analyses showed that while Vehicle + l-DOPA proved efficacious on days 3 and 15 (both $p<0.05$), there was a distinct reduction in stepping to pre-test levels on day 9. Co-administration of either dose of buspirone maintained the beneficial effects of l-DOPA on all days tested (all $p<0.05$).

4. Discussion

In the present study, we demonstrate several findings that support exploration of partial 5-HT$_{1A}$ receptor agonists as adjuncts to l-DOPA pharmacotherapy. First, in corroboration with other preclinical models, acute administration of the partial 5-HT$_{1A}$ receptor agonist buspirone conveyed anti-dyskinetic effects in l-DOPA-primed rats that were specific to the 5-HT$_{1A}$ receptor. Second, acute buspirone improved the efficacy of l-DOPA on measures of motor performance. Most importantly, we demonstrated that co-administration of buspirone with l-DOPA to l-DOPA-naïve rats prophylactically suppressed AIMs development and expression while improving l-DOPA efficacy.

The 6-OHDA rat model of PD has proven extremely useful for the study of PD and the side effects of l-DOPA treatment (Ungerstedt, 1971; Miller and Beninger, 1991; Schallert et al., 2000). Traditionally, investigations using this model have measured l-DOPA-induced rotations as an indication of the anti-dyskinetic efficacy of various pharmacological treatments (Carey, 1991). In recent years, the pertinence of rotational behavior has been called into question (Castaneda et al., 2005) and alternative methods that resemble clinical manifestations of LID have been developed (Hagell and Widner, 1999; Lundblad et al., 2002). The AIMs procedure used in the present study employs discrete behavioral measures, displays face validity with known anti-dyskinetic compounds, and shows consistency throughout treatment in l-DOPA-primed rats (Lundblad et al., 2002; Taylor et al., 2005; Bishop et al., 2006).

While examining the anti-dyskinetic properties of potential l-DOPA adjuncts remain the paramount goal of most preclinical studies, the modulation of l-DOPA efficacy by these compounds is often overlooked. In order to fully characterize the effects of buspirone in the l-DOPA-treated hemiparkinsonian rat, we also employed the FAS test (Olsson et al., 1995; Chang et al., 1999; Schallert et al., 2000). The FAS test has been extensively utilized as a measure of forelimb akinesia, demonstrating sensitivity to DA loss and reversal of deficit by DA replacement therapy, stem cell transplantation, and surgical intervention (Chang et al., 1999; Cho et al., 2006). In this study, the FAS test facilitated the investigation of 6-OHDA-induced motor deficit and the recovery of motor function upon treatment with l-DOPA alone or in conjunction with buspirone.

In the first experiment we were interested in determining the acute anti-dyskinetic effects and pharmacological specificity of buspirone on AIMs in the l-DOPA-primed rat. As demonstrated in Fig. 1, buspirone dose-dependently reduced AIMs...
expression, but did not significantly alter l-DOPA-induced rotations. Since rotations were not affected by buspirone administration, the effect of buspirone on locomotor AIMs was also weak. More importantly, co-administration of the 5-HT₁₅A agonist, WAY100635 completely reversed the anti-dyskinetic effects of buspirone treatment. While extant reports indicate that adjunct buspirone therapy lessens LID symptoms in human patients (Kleedorfer et al., 1991; Bonifati et al., 1994), we demonstrate that the anti-dyskinetic effects of buspirone are primarily due to its action on the 5-HT₁₅A receptor. These results corroborate earlier studies in which the effects of direct or indirect 5-HT agonists with anti-dyskinetic properties were blocked by administration of selective 5-HT₁₅A antagonists (Bibbiani et al., 2001; Iravani et al., 2003; Bishop et al., 2006; Ba et al., 2007).

While the acute anti-dyskinetic effects of 5-HT₁₅A receptor stimulation have been shown in both preclinical (Bibbiani et al., 2001; Bishop et al., 2006) and clinical investigations (Olanow et al., 2004; Bara-Jimenez et al., 2005; Goetz et al., 2007), to date the prophylactic anti-dyskinetic efficacy of these compounds remains largely unknown. Preliminary results have suggested that 5-HT₁₅A agonists may have some utility in this role since Tomiyama et al. (2005) observed that chronic pretreatment with the full 5-HT₁₅A agonist, ±8-OH-DPAT reduced the development of l-DOPA-induced rotational behavior. However, rotations are a controversial measure of l-DOPA-related side effects and have limited face validity with the actual human disorder (Cenci et al., 2002; Castaneda et al., 2005). Therefore, in the second experiment we investigated whether buspirone co-administration prophylactically reduces the development of l-DOPA-induced AIMs. As shown in Fig. 3, the high dose of buspirone both reduced the development of AIMs and maintained these effects when chronically co-administered with l-DOPA over a 2 week period. Interestingly, when rats were re-tested for AIMs after a 4 day wash-out period, all groups displayed similar levels of dyskinesia. These results suggest that while buspirone may decrease the expression of LID, the underlying mechanism may be perpetuated. This corroborates an anecdotal report by Bonifati et al. (1994), who reported that one individual who discontinued adjunct buspirone treatment suffered a complete resurfacing of previous LID.

l-DOPA treatment in humans is often beneficial to motor performance for a time until the therapeutic window shrinks resulting in progressive motor fluctuations (Obeso et al., 2000). Optimal adjunct treatment should prolong the beneficial aspects of DA replacement therapy while concurrently reducing deleterious side effects, such as LID and wearing off (Jankovic, 2005). The present study shows that buspirone exerts some promise in this dual role, as demonstrated by the results on the FAS test. In l-DOPA-primed rats, treatment with l-DOPA alone produced a non-significant increase in lesioned forelimb stepping compared to control forelimb (see Fig. 2). Acute co-administration of buspirone with l-DOPA resulted in significantly improved lesion-side stepping. More importantly, we show that chronic adjunct treatment with buspirone may also convey beneficial motor effects (see Fig. 4). While l-DOPA administration alone reversed stepping deficit, this effect was variable which is consistent with motor fluctuations often seen in PD patients receiving chronic l-DOPA therapy (Jankovic, 2005). Buspirone consistently improved motor fluctuations at both doses tested.

This augmentation of l-DOPA efficacy supports previous work in rodents showing an attenuation of shortened rotational motor response duration in chronic l-DOPA-treated rats co-administered the full 5-HT₁₅A receptor agonists, sarizotan (Bibbiani et al., 2001) and ±8-OH-DPAT (Ba et al., 2007). Kannari et al. (2001) observed that 5-HT₁₅A stimulation with ±8-OH-DPAT augmented the half-life of DA concentration in the striatum of 6-OHDA-treated rats. Prolonging optimal DA levels may lead to rehabilitation of motor performance and amelioration of the pulsatile stimulation of DA receptors that leads to motor fluctuations (Jankovic, 2005). Additionally, these results may reflect primary effects of 5-HT₁₅A receptor stimulation since previous studies have reported 5-HT₁₅A receptor stimulation alone can convey anti-parkinsonian effects (Mignoni and Wolf, 2002, 2007; Bezdai et al., 2006).

In the present investigation, we employed the partial 5-HT₁₅A agonist, buspirone (Tunnicliff, 1991). This decision was made based on previous research showing the obvious detriments of engaging full 5-HT₁₅A receptor occupation, including immobility and stereotyped movements (Kannari et al., 2001; Iravani et al., 2006). These drawbacks may have been due to the induction of serotonin syndrome characterized by lower lip retraction, hindlimb abduction and flat body posture caused by an overstimulation of 5-HT₁₅A receptors (Goodwin et al., 1987). However, buspirone displays affinity for additional receptor subtypes in addition to 5-HT₁₅A receptors. For example, buspirone also has moderate affinity for D₂ receptors acting as an antagonist at high doses (McMillan et al., 1983; McCall et al., 1994; Cobert et al., 1999). We do not believe the current results reflect actions at this receptor. First, based on current models of dyskinesia, antagonism of corticostriatal D₂ autoreceptors would most likely promote LID by disinhibiting glutamatergic output to the striatum of these neurons. Second, antagonism of striatal postsynaptic D₂ receptors with eticlopride causes a reduction in AIMs behaviors but most likely at the expense of l-DOPA efficacy (Taylor et al., 2005). Neither of these effects was observed in the current study confirming that buspirone was not likely acting by antagonism of D₂ receptors to affect the development and expression of AIMs. While the 5-HT₁₅A antagonist, WAY100635 also purportedly exhibits antagonistic properties at D₂ receptors at high doses (Ahlenius et al., 1999), the dose employed in the current study was too low to exhibit noticeable effects at the D₂ receptor. There are reports that WAY100635 and its metabolite can act as D₂ agonists (Chemel et al., 2006). In the present study there was no evidence that WAY100635 produced any behavioral effects of its own via action at either of these DA receptors. Collectively, these data suggest that buspirone’s effects on l-DOPA-induced motor complications were unique to its pharmacological profile as a partial agonist at the 5-HT₁₅A receptor.

Despite this knowledge, the mechanisms by which 5-HT₁₅A receptor agonists convey their effects are largely unknown. Buspirone’s anti-dyskinetic action may be the result of 5-HT₁₅A...
receptor stimulation at one or more areas that impact movement. For example, 5-HT1A receptors are found postsynaptically within the motor cortex on corticostriatal glutamate projections (Antonelli et al., 2005; Saigal et al., 2006). There is also evidence of an upregulated 5-HT1A receptor population within striatal striosomes following MPTP lesions in primates (Frechilla et al., 2001; Bezard et al., 2006). Furthermore, 5-HT1A receptors also densely populate the dorsal raphe nucleus, where they act as somatodendritic autoreceptors (Hjorth and Sharp, 1991; Knobman et al., 2000). 5-HT1A receptor populations in these three areas may modulate DA output in several different ways. First, stimulation of postsynaptic 5-HT1A receptors in the glutamatergic corticostriatal pathway may assuage dyskinesia by reducing excessive glutamate release in the striatum (Mignon and Wolf, 2002, 2007). Antonelli et al. (2005) observed that microinfusion of sarizotan into the motor cortex reduced glutamate levels in the striatum via this pathway. Alternatively, presynaptic 5-HT1A receptors within striatal striosomes may squelch glutamate release into the striatum, where they may act to curb pathological striatal output that is responsible for dyskinetic movements. Recent studies further indicate that following extensive DA depletion, serotonergic raphespinal neurons may usurp the role previously held by the nigrostriatal pathway, converting exogenous L-DOPA into DA and releasing it into the striatum (Tanaka et al., 1999; Maeda et al., 2005). 5-HT1A receptor stimulation in the dorsal raphe nucleus may modulate output of L-DOPA-derived DA into the striatum, thereby prolonging DA half-life in the Parkinsonian brain (Tanaka et al., 1999; Olanow and Obeso, 2000; Kannari et al., 2001). Understanding of the mechanism(s) that underlie these effects is urgently needed.

In conclusion, the current preclinical findings further implicate the 5-HT1A receptor as a promising therapeutic target for the reduction of LID and motor fluctuations. As important, these results suggest that partial 5-HT1A receptor agonists may be employed prophylactically to reduce both the induction and expression of motor complications that may arise with continued DA replacement therapy, without a loss in L-DOPA efficacy as seen upon more complete 5-HT1A receptor occupation. Such findings collectively support continued investigations with 5-HT1A agonists that may improve the health of the PD patient. In future studies, we hope to look more closely at the prophylactic effects of 5-HT1A receptor agonists and their benefits in a wider array of behavioral motor performance tests.

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