Research Report

The differential effects of 5-HT_{1A} receptor stimulation on dopamine receptor-mediated abnormal involuntary movements and rotations in the primed hemiparkinsonian rat

Kristin B. Dupre, Karen L. Eskow, Giselle Negron, Christopher Bishop *

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

**ARTICLE INFO**

Article history:
Accepted 2 May 2007
Available online 8 May 2007

Keywords:
Basal ganglia
Dopamine
Dyskinesia
L-DOPA
Parkinson’s disease
Serotonin

**ABSTRACT**

Serotonin 1A receptor (5-HT_{1A}R) agonists have emerged as valuable supplements to l-DOPA therapy, demonstrating that they can decrease side effects and enhance motor function in animal models of Parkinson’s disease (PD) and human PD patients. The precise mechanism by which these receptors act remains unknown and there is limited information on how 5-HT_{1A}R stimulation impacts striatal dopamine (DA) D1 receptor (D1R) and D2 receptor (D2R) function. The current study examined the effects of 5-HT_{1A}R stimulation on DA receptor-mediated behaviors. Male Sprague–Dawley rats were rendered hemiparkinsonian by unilateral 6-OHDA lesions and primed with the D1R agonist SKF81297 (0.8 mg/kg, i.p.) in order to sensitize DA receptors. Using a randomized within subjects design, rats received a first injection of: Vehicle (dH2O) or the 5-HT_{1A}R agonist ±8-OH-DPAT (0.1 or 1.0 mg/kg, i.p.), followed by a second injection of: Vehicle (dimethyl sulfoxide), the D1R agonist SKF81297 (0.8 mg/kg, i.p.), the D2R agonist quinpirole (0.2 mg/kg, i.p.), or L-DOPA (12 mg/kg + benserazide, 15 mg/kg, i.p.). On test days, rats were monitored over a 2-h period immediately following the second injection for abnormal involuntary movements (AIMs), analogous to dyskinesia observed in PD patients, and contralateral rotations. The present findings indicate that 5-HT_{1A}R stimulation reduces AIMs induced by D1R, D2R and L-DOPA administration while its effects on DA agonist-induced rotations were receptor-dependent, suggesting that direct 5-HT_{1A}R and DA receptor interactions may contribute to the unique profile of 5-HT_{1A}R agonists for the improvement of PD treatment.

© 2007 Elsevier B.V. All rights reserved.
Parkinson’s disease (PD) is a neurodegenerative disease associated with degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (Mink, 1996), leading to loss of DA in the striatum. As a result, PD patients experience motor deficits, such as resting tremor, rigidity and slowness of movement. The standard treatment for PD consists of DA replacement therapy using L-3,4-dihydroxyphenylalanine (L-DOPA). Unfortunately, over time L-DOPA loses its efficacy and deleterious side effects appear. One such side effect is L-DOPA-induced dyskinesia (LID), characterized by abnormal and excessive movements, appearing in 40% of PD patients after 4–5 years of treatment and 90% of patients after 9–15 years (Stocchi et al., 1997; Ahlskog and Muenter, 2001).

Although the precise mechanisms of LID remain unknown, there is evidence to suggest that it is a result of the development of supersensitised striatal D1 (D1R) and D2 (D2R) receptors following DA depletion (Ungerstedt, 1971; Breese et al., 1987; Miller and Beninger, 1991). For example, repeated exposure of DA receptor agonists increase striatal signal transduction, gene expression and DA-mediated behaviors to an extent not portrayed in intact animals (Berke et al., 1998; Cai et al., 2002; Bishop and Walker, 2003). Moreover, chronic treatment with D1R and D2R agonists can induce dyskinesia in both experimental models of PD and human PD patients (Rinne, 1989; Rascol et al., 2000; Taylor et al., 2006). Thus it is not surprising that D1R and D2R antagonists reduce LID; however, the anti-parkinsonian benefit of L-DOPA is also squelched making the identification of nondopaminergic adjuncts to L-DOPA a paramount goal (Boyce et al., 1990; Monville et al., 2005; Taylor et al., 2005).

In recent years, 5-HT1A receptor (5-HT1AR) agonists have emerged as promising supplements to L-DOPA therapy. For example, in animal models of PD, the full 5-HT1AR agonist (+)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide (+8-OH-DPAT) has been shown to decrease rotational behavior and the frequency with which animals fail to respond to L-DOPA administration (Tomiyama et al., 2005; Ba et al., 2006), while the partial 5-HT1AR agonist buspirone and the full 5-HT1AR agonist sarizotan reduce LID while concurrently improving the efficacy of L-DOPA treatment (Bibbiani et al., 2001). Adjunct 5-HT1AR pharmacotherapy also demonstrates beneficial effects in clinical studies. Buspirone, sarizotan and the partial 5-HT1AR agonist tandospirone have been reported to convey anti-dyskinetic effects in PD patients (Bonifati et al., 1994; Kannari et al., 2002; Bara-Jimenez et al., 2005).

Despite the clear potential for more widespread uses of 5-HT1AR agonists, the mechanism by which these compounds produce their anti-parkinsonian and anti-dyskinetic effects remains unknown. It has been postulated that under conditions of severe DA depletion, 5-HT1AR agonists may act at the level of the dorsal raphe, where L-DOPA-derived DA release may be modulated by extant somatodendritic 5-HT1AR (Araki et al., 1996; Tanaka et al., 1999). More recently, evidence has implicated that 5-HT1AR agonists might also directly influence DA receptor-mediated behaviors. For example, Matsubara et al. (2006) reported that the partial 5-HT1AR agonist tandospirone augmented contralateral rotations induced by the D1/D2R agonist apomorphine, while Iravani et al. (2006) demonstrated that the potent 5-HT1AR agonist +8-OH-DPAT reduced D2/D3 receptor-mediated motor behaviors. Accordingly, alternative extraraphe mechanisms may account for these effects at DA receptors.

Therefore, in order to better understand the influence of 5-HT1AR stimulation on DA receptor-mediated behaviors, the current study examined the effects of the 5-HT1AR agonist, +8-OH-DPAT, prior to L-DOPA, the D1 agonist SKF81297 and the D2R agonist quinpirole. Using the abnormal involuntary movements (AIMs) rodent model of LID and drug-induced rotations as measures, we predicted that 5-HT1AR stimulation would differentially modify DA receptor-mediated behaviors, which would signify discrete interactions between 5-HT1AR and supersensitive DA receptors.

### 2. Results

#### 2.1. 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 ($t_8=6.48; p<0.001$) and DA levels ($t_8=4.46; p<0.001$, 92.8% and 97.4% respectively, compared to intact striatum. The denervated side also showed an increased DOPAC/DA turnover rate (+180%) compared to control ($t_8=14.12; p<0.001$). There were no significant differences between intact and lesioned striata for any other monoamine levels.

#### 2.2. Effects of +8-OH-DPAT on ALO AIMs and rotations

In order to discover the effects of +8-OH-DPAT exclusively on AIMs and rotations, animals were administered +8-OH-DPAT (8 mg/kg) 30 min prior to intraperitoneal injections of the ALO AIMs and rotations.

### Table 1 - Effects of unilateral medial forebrain bundle (MFB) 6-OHDA lesions on concentration of norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), serotonin (5-HT), and their metabolites/monoamines ratios in the striatum

<table>
<thead>
<tr>
<th>Side</th>
<th>NE (±SE)</th>
<th>DOPAC (±SE)</th>
<th>DA (±SE)</th>
<th>DOPAC/DA (±SE)</th>
<th>5-HIAA (±SE)</th>
<th>5-HT (±SE)</th>
<th>5-HIAA/5-HT (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (right)</td>
<td>0.23 ±0.04</td>
<td>2.65 ±0.38</td>
<td>13.6 ±1.16</td>
<td>0.20 ±0.02</td>
<td>0.55 ±0.07</td>
<td>0.84 ±0.15</td>
<td>0.70 ±0.05</td>
</tr>
<tr>
<td>Lesion (left)</td>
<td>0.14 ±0.03 (60.9)</td>
<td>0.19 ±0.04* (7.2)</td>
<td>0.35 ±0.09* (2.6)</td>
<td>0.56 ±0.03 (280.0)</td>
<td>0.48 ±0.13 (88.1)</td>
<td>0.55 ±0.12 (65.4)</td>
<td>0.91 ±0.12 (129.0)</td>
</tr>
</tbody>
</table>

Values are nanogram monoamine or metabolite per milligram protein or ratios of metabolite to monoamine (mean ± SE) 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).
AIMs at the 20th, 30th, and 50th min compared to Vehicle (all and from the 50th to 90th min, as well as the 110th and 120th ALO AIMs from the 20th to 120th min compared to Vehicle, the high dose of ±8-OH-DPAT (1.0 mg/kg) significantly reduced contralateral rotations revealed main effects of treatment and time was found (data not shown).

2.4. Effects of ±8-OH-DPAT on D1 agonist-induced ALO AIMs and rotations

Main effects of ±8-OH-DPAT on D1R agonist SKF81297-induced ALO AIMs were found at min 20–70 (all p<0.05). As shown in Fig. 3A, post hoc analyses of these significant effects revealed that the high dose of ±8-OH-DPAT (1.0 mg/kg) significantly reduced ALO AIMs at the 20th, 30th, and 60th min compared to Vehicle, and from the 30th to 70th min compared to the low dose of ±8-OH-DPAT (0.1 mg/kg). The low dose of ±8-OH-DPAT (0.1 mg/kg) significantly reduced ALO AIMs at the 20th, 30th, and 50th min compared to Vehicle (all p<0.05).

The effects of ±8-OH-DPAT on D1R agonist-induced contralateral rotations revealed no main effect of treatment but a main effect of time (F(2,11)=4.91; p<0.001). More importantly, a significant interaction between treatment and time was found (F(2,11)=2.36; p<0.001). Subsequent post hoc analysis of this interaction, demonstrated in Fig. 3B, revealed that the high dose ±8-OH-DPAT (1.0 mg/kg) increased SKF81297-induced rotations from the 20th to 120th min compared to Vehicle, and from the 30th to 100th min compared to the low dose of ±8-OH-DPAT (0.1 mg/kg) while the low dose of ±8-OH-DPAT (0.1 mg/kg) significantly increased SKF81297-induced rotations at the 40th min compared to Vehicle (all p<0.05).

2.5. Effects of ±8-OH-DPAT on D2 agonist-induced ALO AIMs and rotations

Analyses revealed main effects of ±8-OH-DPAT on D2R agonist quinpirole-induced ALO AIMs at min 30 and 60 (both p<0.05). Post hoc analyses of these significant effects (Fig. 4A) revealed that the high dose of ±8-OH-DPAT (1.0 mg/kg) decreased ALO AIMs at the 30th and 60th min compared to Vehicle, and the low dose of ±8-OH-DPAT (0.1 mg/kg) decreased ALO AIMs at the 30th min compared to Vehicle (all p<0.05). As shown in Fig. 4B, analyses of the effects of ±8-OH-DPAT on D2R agonist-induced contralateral rotations revealed no main effects of treatment, time or treatment×time interaction.

3. Discussion

In the present study we demonstrate that 5-HT1AR stimulation influences a diverse number of DA-mediated behaviors within the hemiparkinsonian rat. These effects were directly related to the receptor actions of the compounds tested and the behaviors that were monitored. The 5-HT1A agonist, ±8-OH-DPAT, dose dependently reduced AIMs and altered contralateral rotations induced by l-DOPA treatment. In contrast, 5-HT1A stimulation dose dependently attenuated AIMs induced by D1R stimulation but significantly augmented D1R-induced contralateral rotations. Finally, D2R-induced AIMs but not contralateral rotations were reduced by 5-HT1A R stimulation. The current results implicate an important functional interaction between D1, D2 and 5-HT1A receptors that may have important clinical implications for direct and indirect DA agonist treatment of PD.

The unilateral 6-OHDA rat model of PD has proven extremely useful for the study of PD, including l-DOPA-induced side effects like LID (Ungerstedt, 1971; Miller and Beninger, 1991; Schallert et al., 2000). The AIMs model, which was utilized in the current study, is an alternative method to drug-induced rotations that employs discrete behavioral measures and displays face validity with known antidysskinetic compounds (Lundblad et al., 2002; Taylor et al., 2005). Despite concerns of loss of responsivity, the current study and others have shown that AIMs can be maintained over repeated testing by separating experimental days after initial priming (Bishop et al., 2006; Taylor et al., 2006). In the current study, we corroborated AIMs induction by l-DOPA but also D1R and D2R agonists (Monville et al., 2005; Delfino et al., 2007), providing us with the opportunity to investigate the effects of 5-HT1AR stimulation on receptor-specific behaviors. As portrayed in Fig. 1, the current study used D1R priming instead of l-DOPA to sensitize both D1 and D2 receptors (Pollack and Yates, 1999). This was done in order to address the mechanistic questions that surround the specific effects of the interactions between D1, D2, and 5-HT1A receptors. l-DOPA priming may have disrupted this investigation by stimulating multiple DA receptor classes. As such, l-DOPA treatment was performed during the last 3 test days. A post-test with the D1R agonist confirmed that D1R-induced AIMs remained stable throughout the post-priming testing period (data not shown).
The first goal of the present study was to confirm that 5-HT<sub>1A</sub>R stimulation would decrease L-DOPA-mediated AIMs and rotations. As shown in Fig. 2, ALO AIMs were potently attenuated by the high dose of ±8-OH-DPAT and moderately reduced by the low dose of ±8-OH-DPAT. L-DOPA-induced contralateral rotations were uniformly diminished by the high dose of ±8-OH-DPAT. Interestingly, rotations were initially reduced by the low dose of ±8-OH-DPAT but augmented after approximately 40 min of treatment. The present results support previous work demonstrating that 5-HT<sub>1A</sub>R stimulation can reduce LID in both experimental models of PD (Bibbiani et al., 2001; Tomiyama et al., 2005; Ba et al., 2006), and clinical populations (Kannari et al., 2002; Bara-Jimenez et al., 2005; Goetz et al., 2007). While the mechanism for these actions is unclear, the most prominent theory postulates that in the DA-depleted brain, serotonergic raphe-striatal neurons usurp the role of the nigrostriatal pathway, converting L-DOPA into DA and releasing it into the striatum (Arai et al., 1996; Tanaka et al., 1999). Stimulating 5-HT<sub>1A</sub> somatodendritic autoreceptors on raphe-striatal neurons decreases pulsatile stimulation of DA receptors in the striatum and prolongs DA half-life by regulating raphe-striatal-derived DA (Tanaka et al., 1999; Olanow and Obeso, 2000; Kannari et al., 2001).

While this surrogate serotonergic neuroplasticity is compelling and deserves continued investigation, recent evidence also indicates that 5-HT<sub>1A</sub>R stimulation may also directly influence post-synaptic DA receptor-mediated behaviors that are likely raphe-independent (Iravani et al., 2006; Matsubara et al., 2006). Thus the paramount goal of the present study was to characterize the effects of 5-HT<sub>1A</sub>R stimulation on D1R- and D2R-mediated behaviors. In order to accomplish this goal, we employed the D1R agonist, SKF81297 (Peacock et al., 1990) and the D2R agonist, quinpirole (Tsuruta et al., 1981) at doses that appear to produce equivalent AIMs expression. Numerous studies have implicated D1 receptors in dyskinesia expression and development. For example, studies have shown that D1R antagonists block LID (Boyce et al., 1990; Elliot et al., 1992; Grondin et al., 1999; Monville et al., 2005; Delfino et al., 2007). In the present study, we found that D1R-induced ALO AIMs were robustly attenuated by the high dose of ±8-OH-DPAT and time dependently reduced by the low dose of ±8-OH-DPAT (Fig. 3). Contralateral rotations induced by D1R agonism were potently enhanced by the high dose of ±8-OH-DPAT and moderately enhanced by the low dose of ±8-OH-DPAT. This differential effect of ±8-OH-DPAT on D1R-mediated behavior is clearly a departure from 5-HT<sub>1A</sub>R stimulation on L-DOPA-mediated effects that involve reductions in both ALO AIMs and rotations and may reflect the reported anti-parkinsonian effects of 5-HT<sub>1A</sub>R stimulation (Matsubara et al., 2006; Mignon and Wolf, 2007).

D2 receptors have also been implicated in the expression and development of dyskinesia (Nutt et al., 2000; Lundblad et al., 2002; Delfino et al., 2004). For example, studies have found LID can be blocked by D2R antagonists (Boyce et al., 1990; Grondin et al., 1999; Taylor et al., 2005), and D2R...
agonists have been used to induce dyskinesia in various clinical and experimental investigations (Rinne, 1989; Monville et al., 2005; Delfino et al., 2007). In support of recent work showing 5-HT1AR stimulation reducing D2/D3-induced behavior in MPTP-treated monkeys (Iravani et al., 2006), we found D2R-induced ALO AIMS were mildly attenuated by the high and low dose of ±8-OH-DPAT (Fig. 4). Contralateral rotations induced by D2R stimulation were not affected by either dose of ±8-OH-DPAT, differing from the effects of 5-HT1AR stimulation on t-DOPA and D1R-mediated effects. Taken together, the present findings support a stronger interaction between D1 and 5-HT1A receptors than one between D2 and 5-HT1A receptors.

Although the mechanism of action for the effects of 5-HT1AR stimulation on D1R- and D2R-mediated behaviors remains unknown, there is evidence to suggest that the striatum is involved. Chronic administration of t-DOPA and D1R agonists generates D1R and D2R supersensitivity in the DA-lesioned striatum and site-directed DA agonists can induce dyskinesia like behaviors (Gerfen et al., 2002; Taylor et al., 2005; Carta et al., 2006). In addition, an upregulated population of striatal 5-HT1A R may be acting as intrinsic heteroreceptors on glutamatergic corticostriatal afferents to modify DA receptor function (Frechilla et al., 2001; Bezard et al., 2006). This may be particularly relevant given the suggested role of striatal D1R and NMDAR in the genesis of LID (Chase and Oh, 2000; Kreipke and Walker, 2004; Brodie and Walker, 2005; Fiorentini et al., 2003, 2006) and may account for the more potent effects of 5-HT1AR stimulation on D1R- versus D2R-mediated behaviors. Cortical mechanisms may also account for the influence of 5-HT1AR stimulation on DA-mediated behaviors. For example, the motor cortex is implicated in LID (Antonelli et al., 2005; Mignon and Wolf, 2005; Delfino et al., 2007), and anti-parkinsonian and anti-

Fig. 3 – Effects of ±8-OH-DPAT on D1 receptor agonist SKF81297-induced ALO AIMS and contralateral rotations. Five minutes after pretreatments with ±8-OH-DPAT (0.1 mg/kg, 1.0 mg/kg, or its vehicle), rats (n=9) received treatments of SKF81297 (0.8 mg/kg, i.p.). Symbols denote (A) the average ALO AIMS ± SE and (B) the average contralateral rotations ± SE for unilateral 6-OHDA-lesioned rats every 10 min for 2 h. Main effects were determined by Kruskal–Wallis tests for ALO AIMS and two-way ANOVAs for rotations. Post hoc comparisons denoted significant differences between treatments at the time points indicated. *p<0.05 for 8-OH-DPAT (1.0 mg/kg) versus Vehicle. +p<0.05 for 8-OH-DPAT (1.0 mg/kg) versus 8-OH-DPAT (0.1 mg/kg). #p<0.05 for 8-OH-DPAT (0.1 mg/kg) versus Vehicle.

Fig. 4 – Effects of ±8-OH-DPAT on D2 receptor agonist quinpirole-induced ALO AIMS and contralateral rotations. Five minutes after pretreatments with ±8-OH-DPAT (0.1 mg/kg, 1.0 mg/kg, or its vehicle), rats (n=9) received treatments of the D2 receptor agonist quinpirole (0.2 mg/kg, i.p.). Symbols denote (A) the average ALO AIMS ± SE and (B) the average contralateral rotations ± SE for unilateral 6-OHDA-lesioned rats every 10 min for 2 h. Main effects were determined by Kruskal–Wallis tests for ALO AIMS and two-way ANOVAs for rotations. Post hoc comparisons denoted significant differences between treatments at the time points indicated. *p<0.05 for 8-OH-DPAT (1.0 mg/kg) versus Vehicle. +p<0.05 for 8-OH-DPAT (0.1 mg/kg) versus Vehicle.
dyskinetic effects have been attributed to an extant 5-HT_{1A}R population in the cortex (Mignon and Wolf, 2002, 2007) and its contribution in the reduction of glutamate release (Antonelli et al., 2005; Mignon and Wolf, 2005). These striatal and cortical mechanisms alone or in concert may account for some of the effects of 5-HT_{1A}R stimulation on DA-mediated AIMS and rotations.

The administration of ±8-OH-DPAT alone has been shown to induce several different types of behaviors, including rotational behavior (Mignon and Wolf, 2005) and 5-HT syndrome (Blanchard et al., 1993). Thus, an additional goal of the present study was to determine whether ±8-OH-DPAT administration alone would cause ALO AIMS and/or rotations in the hemiparkinsonian rat. For example, other studies have found that 5-HT_{1A}R stimulation induces either ipsilateral turning (Mignon and Wolf, 2005) or contralateral turning (Matsubara et al., 2006) in 6-OHDA-lesioned animals depending on the dose and compound employed. In the present study, we did not find any significant rotational or dyskinetic behavior with the administration of ±8-OH-DPAT at the doses tested. We did, however, observe flat body posture and lower lip retraction characteristic of 5-HT syndrome (Tricklebank et al., 1984; Berendsen et al., 1989) with the high dose of ±8-OH-DPAT in a minority of the animals in each treatment condition but these responses were transient. Since the effect did not appear detrimental to the animals and remained for only a short period of time (<30 min), 5-HT syndrome did not appear to significantly influence the results of the present study.

Finally, there is some disconnect between the literature of 5-HT_{1A}R effects in rodents versus those seen in humans and primates. Most rodent models describe reduced dyskinesia without additional motor disability (Mignon and Wolf, 2005; Tomiyama et al., 2005; Matsubara et al., 2006); whereas some studies involving humans or primates have indicated that 5-HT_{1A}R stimulation decreased LID but at the expense of increased motor disability (Kannari et al., 2002; Iravani et al., 2006). Therefore, the use of 5-HT_{1A}R agonists as adjuncts to l-DOPA therapy for humans may be limited by the potency of the compound. While reversing LID, full agonists like ±8-OH-DPAT, and the more potent enantiomer +8-OH-DPAT, may reduce l-DOPA efficacy by receptor specific induction of 5-HT syndrome. The use of 5-HT_{1A}R agonists as adjuncts to l-DOPA may also be restricted or in some cases improved by the off-receptor effects of the compound being employed. For example, most partial agonists also act to a lesser extent at DA receptors. Therefore, there are certain limits to the model employed in the present study.

In conclusion, clinical and experimental evidence suggests that 5-HT_{1A}R stimulation may have beneficial effects including anti-parkinsonian, improved l-DOPA efficacy, and anti-dyskinetic effects. The results of the present study suggest another possible use for this class of compounds. 5-HT_{1A}R stimulation may be useful as an adjunct to monotherapy by providing a way to augment DA agonist efficacy and reduce the development of dyskinesia, thereby delaying the need for l-DOPA and prolonging the efficacious treatment of the symptoms of PD. Future research investigating the specific site(s) of action and mechanism(s) may help to further support this potential approach.

4. Experimental procedure

4.1. Animals

Adult male Sprague-Dawley rats were used (225–250 g upon arrival; Taconic Farms, Hudson, NY, USA). Rats were kept in plastic cages (22 cm high, 45 cm deep and 23 cm wide) and given free access to food (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA) and water. The colony room was kept on a 12-h light/dark cycle (light on at 0700 h) and maintained at 22–23 °C. 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) were maintained throughout the study.

4.2. 6-Hydroxydopamine lesion surgeries

One week after arrival, rats received unilateral DA lesions of the left medial forebrain bundle. Each rat was administered desipramine HCl (25 mg/kg, i.p.; Sigma) 30 min prior to surgery in order to protect norepinephrine (NE) neurons. Rats were anesthetized with inhalant isoflurane (2–3%; Sigma) in oxygen (2.5 l/min) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The following coordinates relative to bregma were used for the site of injection: AP, −1.8 mm; ML, +2.0 mm; DV, −8.6 mm, with the incisor bar positioned at 3.3 mm below interaural line (Paxinos and Watson, 1998). After drilling a small hole in the skull above the site of injection, a 10-μl Hamilton syringe attached to a 26-gauge needle was lowered into the target. At that point, 4 μl of 6-hydroxydopamine hydrobromide (6-OHDA; 3 μg/μl; Sigma), dissolved in 0.9% NaCl+0.1% ascorbic acid, was injected at a rate of 2 μl/min. The needle was withdrawn 1 min later. Stainless steel wound clips were used to close the surgical site. Animals were placed in clean cages and allowed to recover with ad lib food and water. Wound clips were removed 3–4 days post-surgery and rats were monitored and handled twice per week for 3 weeks to ensure full recovery and acclimation to experimenters.

4.3. Pharmacological treatments

A time course including surgery, drug administration and behavioral testing is shown in Fig. 1. Three weeks after 6-OHDA lesions, rats received injections of the D1R agonist R(+)SKF-81297 hydrobromide (SKF81297; 0.8 mg/kg, i.p.; Sigma) on 3 separate occasions 2 to 3 days apart in order to sensitize both D1 and D2 receptors (Pollack and Yates, 1999). Contralateral rotations and AIMS (see description below) were observed immediately after injections. Rats displaying a total AIMS score of ≥15 by the 3rd day of D1R priming were retained for further study (n=9 of 12, 75%).

The pharmacological treatment regimen commenced 2 days after the last day of SKF81297 priming. Using a randomized within subjects design, 3 groups of 3 rats/group received different treatment combinations in a given day in order to reduce time effects. Test days were separated by a
period of 2 to 3 days. On the first 8 test days, rats received 1 of 3 pretreatments: Vehicle (dH2O) or the 5-HT₁₄ receptor agonist 8-OH-DPAT (0.1 or 1.0 mg/kg, i.p.; Sigma). These pretreatments were followed 5 min later by injections with: Vehicle (DMSO), the D1R agonist SKF81297 (0.8 mg/kg, i.p.), or the D2R agonist (+)-quinpirole dihydrochloride (quinpirole; 0.2 mg/kg, i.p.; Sigma). One week later, rats were tested with pretreatments of Vehicle (dH2O) or 8-OH-DPAT (0.1 or 1.0 mg/kg, i.p.) 5 min prior to injection with 1-3,4-dihydroxyphenylalanine methyl ester hydrochloride (l-DOPA; 12 mg/kg, i.p.; Sigma) + α-m-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride (benserazide; 15 mg/kg, i.p.; Sigma) in a randomized order. On each of the aforementioned test days, all rats were monitored for AIMS and rotations immediately following the second injection as described below. In total there were 12 combinations tested and all animals received all treatments.

4.4. Behavioral procedures

Rats were monitored for AIMS using a procedure slightly modified from that described in Lundblad et al. (2002) and Bishop et al. (2006). On test days (09.00–14.00 h), rats were individually placed in plastic trays (60×75 cm) 5 min prior to pretreatments. Following SKF81297, quinpirole, or l-DOPA injection, a trained observer blind to treatment condition assessed each rat for exhibition of axial, limb, orolingual and locomotor AIMS. In addition, contralateral rotations, defined as complete 360° turns away from the lesioned side of the brain, were tallied. No ipsilateral rotations, defined as completed 360° turns toward the lesioned side of the brain, were observed during testing at the doses used. Dyssmetric posturing of the neck and torso, involving positioning of the neck and torso in a twisted manner directed toward the side of the body contralateral to the lesion, were referred to as ‘axial’ AIMS. ‘Limb’ AIMS were defined as rapid, purposeless movements of the forelimb located on the side of the body contralateral to the lesion. ‘Orolingual’ AIMS were composed of repetitive openings and closings of the jaw and tongue protrusions. The movements were considered abnormal as they occurred at times when the rats were not chewing or gnawing on food or other objects. Rats occasionally performed ‘locomotor’ AIMS, in which they ambulated in a contralateral circular direction. Every 10th min for 2 h, rats were observed for 2 consecutive min. Rats were rated for AIMS during the 1st min and rotational behavior in the 2nd min. During the AIMS observation periods (beginning 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 min postinjection), a severity score of 0–4 was assigned for each AIMS category: 0, not present; 1, present for <50% of the observation period (i.e., 1–29 s); 2, present for >50% or more of the observation period (i.e., 30–59 s); 3, present for the entire observation period (i.e., 60 s); and interrupted by a loud stimulus (a tap on the wire cage lid), or 4, present for the entire observation period but not interrupted by a loud stimulus. Thus, the theoretical maximum score for each type of AIM was 48 (4×12 periods) although observed scores were never this severe. For three of the AIMS subcategories (axial, forelimb, and orolingual; ALO AIMS), the scores were summed for the entire 2-h period. ALO AIMS (with a maximum potential of 144) and rotations were tallied for the entire 2-h period. Locomotor AIMS were also rated but not included in the analyses given the explicit collection of rotational data.

4.5. High performance liquid chromatography

Rats were sacrificed by decapitation on the final day of the study and the striatum was dissected out, immediately frozen on dry ice and stored at −80 °C. Reverse-phase high performance liquid chromatography coupled to electrochemical detection (HPLC-EC) was performed on striatal tissue, obtained from all rats, according to protocol of Kilpatrick et al. (1986), a method for semiautomated 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 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) with 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 sodium phosphate (monobasic, anhydrous), 100 mM; EDTA, 0.05 mM; octane sulphonic acid, 1.4 mM; and acetonitrile, 9% adjusted to pH 3.0 with o-phosphoric acid. A coulometric detector configured with three electrodes (Gouloumich III; ESA) measured the content of monoamines and metabolites. An ESA model 5020 guard cell (+300 mV) was positioned prior to the autoinjector. The analytical cell (ESA model 501LA; 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 Scientific Software Inc. module (SS420). The final oxidation current values were adjusted to striatal tissue weights and expressed as nanograms (ng) of monoamine or metabolite per milligram (mg) tissue (mean ± SEM).

4.6. Data analyses

Monoamine and metabolite levels in the striatum were analyzed using paired t-tests (comparing intact versus lesioned striata). Treatment effects (expressed as means ± SEM) for ALO AIMS and rotations were analyzed by employing non-parametric Kruskal–Wallis tests and two-way ANOVAs, respectively. Significant differences between treatments were determined by Mann–Whitney post hoc comparisons for ALO AIMS, and Fisher’s LSD post hoc tests for rotations. Analyses were performed with the use of Statistica software (Statsoft Inc., Tulsa, OK, USA). Alpha was set at p < 0.05.

Acknowledgments

This work was supported by funds from the American Parkinson’s Disease Association (C.B.), the Binghamton University Research Foundation (C.B.) and the Center for Developmental Psychobiology at Binghamton University (C.B.).
REFERENCES


Kilpatrick, I.C., Jones, M.W., Phillipson, O.T., 1986. A semiautomated analysis method for catecholamines, indoleamines, and some prominent metabolites in microdissected regions of the


