Serotonin 5-HT\textsubscript{2A} but not 5-HT\textsubscript{2C} receptor antagonism reduces hyperlocomotor activity induced in dopamine-depleted rats by striatal administration of the D\textsubscript{1} agonist SKF 82958

Christopher Bishop, Gregory S. Daut, Paul D. Walker *

Department of Anatomy & Cell Biology, Wayne State University School of Medicine, 540 East Canfield, Detroit, MI 48201, USA

Received 5 August 2004; received in revised form 7 March 2005; accepted 18 March 2005

Abstract

While recent work has indicated that D\textsubscript{1} receptor agonist-induced hyperlocomotion in DA-depleted rats is reduced by striatal 5-HT\textsubscript{2} receptor antagonism, the 5-HT receptor(s) subtype mediating these effects are not yet known. In the present study, we examined the influence(s) of striatal 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors on locomotor behavior induced by D\textsubscript{1} agonism in neonatal DA-depleted rats. On postnatal day 3, male Sprague–Dawley rats (n = 68) were treated with either vehicle or 6-hydroxydopamine (6-OHDA; 60 \( \mu \)g) which produced >98\% DA depletion. Sixty days later, all rats were fitted with bilateral striatal cannulae. A subset of control and 6-OHDA-lesioned rats (n = 20) was tested for locomotor responses to striatal infusion of the D\textsubscript{1} agonist SKF 82958 (0, 0.1, 1.0, 10 \( \mu \)g/side). The remaining rats (n = 48) were tested for locomotor responses to intrastriatal SKF 82958 (2.0 \( \mu \)g/side) alone or in combination with the 5-HT\textsubscript{2A}- or 5-HT\textsubscript{2C}-preferring antagonists M100907 or RS102221 (0.1 or 1.0 \( \mu \)g/side), respectively. Intrastriatal SKF 82958 dose-dependently increased measures of motor activity within DA-depleted rats. This hyperlocomotor activity was suppressed by co-infusion of M100907, but not RS102221. These results indicate that DA depletion strengthens striatal 5-HT\textsubscript{2A}/D\textsubscript{1} receptor interactions and suggest that 5-HT\textsubscript{2A} receptor antagonists may prove useful in reducing D\textsubscript{1}-related movements.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: 6-Hydroxydopamine; Basal ganglia; Dopamine; Locomotion; Parkinson’s disease; Striatum

1. Introduction

Selective destruction of central dopamine (DA) neurons with the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) produces neuroplasticity within the striatum, altering the regulation and function of DA and serotonin (5-HT) systems (Kostrzewa et al., 1998; Reader and Dewar, 1999). While these compensatory mechanisms may initially mask underlying motor deficiencies that arise as a result of altered striatal output, challenge with DA and 5-HT receptor agonists can produce exaggerated motor responses. For instance, D\textsubscript{1} receptor stimulation within the DA-depleted striatum of rats significantly enhances locomotor activity (Breece et al., 1987; Bishop and Walker, 2003) thought to arise from strengthened cellular transduction mechanisms within the direct striatal pathway (Cai et al., 2002; Gerfen et al., 2002; Papadeas et al., 2004). 5-HT\textsubscript{2} receptor stimulation of
the DA-depleted striatum with the 5-HT2 receptor agonist [(±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-amino-propanol] (DOI) also induces motor behaviors (Bishop and Walker, 2003; Bishop et al., 2004), likely as a consequence of increased 5-HT2A receptor expression in the dorsal striatum (Radja et al., 1993; Laprade et al., 1996; Basura and Walker, 1999) and enhanced 5-HT2 receptor-mediated activation of the direct striatal pathway (Gresh and Walker, 1999a; Basura and Walker, 2000, 2001).

Given that motor behavior likely recruits multiple striatal neurotransmitter systems, recent attention has focused on the interaction between DA and 5-HT receptors. In the intact striatum, studies consistently demonstrate that intrinsic 5-HT2 receptors can modify DA function and have postulated divergent roles for 5-HT2A and 5-HT2C receptor subtypes (Lucas and Spampinato, 2000; Porras et al., 2002). 5-HT2A antagonists reduce hyperlocomotion induced by cocaine, amphetamine and 3,4-methylenedioxymethamphetamine (MDMA) (Kehne et al., 1996; O’Neill et al., 1999), conversely, 5-HT2C receptor antagonists have been shown to enhance or reduce these effects depending upon which compounds and neural sites are studied (Filip and Cunningham, 2002; Fletcher et al., 2002; Filip et al., 2004). While less is known about the interaction of these receptors following DA depletion, DA-related behaviors are also altered by 5-HT2 receptor antagonists. For instance, elevated oral dyskinesia induced by ventral striatal infusion of the partial D1 agonist SKF 38393 is lessened by 5-HT2A/2C receptor antagonism (Gong et al., 1992; Plech et al., 1995). Recent work from our laboratory has also demonstrated that hyperlocomotor activity induced by systemic or intrastriatal administration of the full D1 agonist SKF 82958 to adult DA-depleted rats (O’Boyle et al., 1989) can be reduced by pretreatment with the 5-HT2A/2C receptor antagonist ritanserin (Bishop et al., 2003). However, to this point it is not known which receptor subtype(s), 5-HT2A vs. 5-HT2C, contribute to this effect.

To determine this, the present study examined the effects of 5-HT2A and 5-HT2C receptor antagonism on hyperlocomotor activity elicited by striatal D1 receptor stimulation in neonatal DA-depleted rats. Because 5-HT2A receptor signaling is enhanced under conditions of DA depletion and thus are in a strengthened position to mediate motor activity, we predicted that antagonism with the putative 5-HT2A receptor antagonist M100907 (Kehne et al., 1996), but not the purported 5-HT2C antagonist RS102221 (Bonhaus et al., 1997) would decrease D1-induced hyperlocomotion. The results of this study suggest that 5-HT2A but not 5-HT2C receptors within the DA-depleted striatum significantly reduce the supersensitive locomotor responses to striatal D1 agonism.

2. Methods

2.1. Animals

Male Sprague–Dawley rat pups (Charles River Laboratories, Wilmington, MA) were used and maintained in strict accordance with the guidelines of the Institutional Animal Care and Use Committee of Wayne State University and the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH publication number 85–23, revised 1996). Rat pups arrived on postnatal day 2 and were initially group-housed (n = 12/dam) in plastic cages (22 cm high, 45 cm deep and 23 cm wide) within a temperature controlled colony room (22–23 °C) illuminated on a 12 h light/dark cycle (lights on 0700 hours). Once rats were 3 weeks old, they were weaned from the dams, group-housed (4–5/cage) and fed standard lab chow (Rodent Diet 5001; LabDiet, Brentwood, MO) and water which were available throughout the remainder of the experiment.

2.2. 6-Hydroxydopamine (6-OHDA) lesions and cannulae implantation surgeries

Bilateral intracerebroventricular (icv) 6-OHDA lesions were performed as reported previously (Basura and Walker, 1999). On postnatal day 3, rat pups were anesthetized to effect by hypothermia (body immersion in a foil chamber cooled in wet ice) and a midsagittal incision was made on the dorsal scalp to reveal the skull sutures and obtain reference coordinates from bregma (Paxinos and Watson, 1998). Each rat received a bilateral infusion of 6-OHDA (60 μg; Sigma, St. Louis, MO) or its vehicle solution of saline (0.9% NaCl) containing 0.1% ascorbic acid (Sigma) at a rate of 5 μl over 30 s into each ventricle (AP 0.0 mm, ML ±1.0 mm, DV –2.5 mm relative to bregma) using a 10 μl syringe equipped with a 30 gauge needle. Pups were placed under a heat lamp for recovery prior to being returned to the mother rat. All rats received subcutaneous injections of desipramine (25 mg/kg; Sigma) 30 min prior to surgery to protect norepinephrine (NE) neurons. Sixty days following icv lesions, rats were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg; Lloyd Laboratories, Shenandoah, IO) and xylazine (15 mg/kg; Lloyd Laboratories) mixture and implanted bilaterally with chronic 22 gauge intracranial guide cannulae (C313G/SPC; Plastics One Inc., Roanoke, VA). With the incisor bar positioned 3.3 mm below the interaural line, cannulae were positioned above the dorsal striatum bilaterally using the coordinates AP +0.4 mm, ML ±2.9 mm and DV –3.6 mm relative to bregma (Paxinos and Watson, 1998). Cannulae were fixed in place using liquid and
powder dental acrylic (Lang Dental, Wheeling, IL). At the completion of surgery, guide cannulae were fitted with 28 gauge inner stylets (Plastics One) to maintain patency.

### 2.3. Pharmacological treatments

The following D₁ and 5-HT₂ receptor agents were used in this study: SKF 82958 ((±)-6-chloro-7, 8-dihydroxy-3-allyl-1-phenyl 2,3,4,5-tetra-hydro-(1 H)-3-benzazepine hydrobromide; Sigma); M100907 ((R)-(+)α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl]-4-piperidinemethanol; synthesized as described in Ullrich and Rice, 2000); RS102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylsulpho-amido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione; Tocris, Ellisville, MO). At least 1 week after cannulation, rats were randomly divided into treatment groups and assigned to receive a regimen of bilateral intrastriatal drug infusions on separate testing occasions. One group of intact control (n = 10) and DA-lesioned rats (n = 10) was tested for locomotor effects elicited by intrastriatal drug infusions of vehicle [50% dimethyl sulfoxide (DMSO) in 0.9% NaCl] or SKF 82958 (0.1, 1.0 or 10 μg). To determine whether these effects were more likely to be mediated by 5-HT₂A or 5-HT₂C receptors, a larger group of control (n = 24) and 6-OHDA-lesioned (n = 24) rats was then tested for responses to vehicle or SKF 82958 (2.0 μg) alone or in combination with the 5-HT₂C-preferring antagonist RS102221 (0.1 or 1.0 μg) or the 5-HT₂A-preferring antagonist M100907 (0.1 or 1.0 μg) in a counter-balanced design. Doses of antagonists were based on previous work and they showed pharmacological blockade of behaviors and no intrinsic effects on basal locomotor activity when administered alone (Filip and Cunningham, 2002; Bishop et al., 2004). All compounds were found to have a pH in the range of 6–8 and were administered in a volume of 0.8 μl that preliminary dye tests demonstrated was sufficient to limit the infusion to the striatal region of interest. The number of animals per group ranged from 6 to 8 rats. No rat was tested more than 3 times, and at least 3 days separated each testing session.

### 2.4. Behavioral procedure

Locomotor activity monitoring was conducted in six identical acrylic chambers measuring 40 cm long, 40 cm wide and 30 cm high (Accuscan Instruments, Columbus, OH). Each chamber was surrounded by a 15 × 15 infrared photocell array interfaced with a computer that ran the Versamax and Versadat programs (Accuscan Instruments). These programs tabulated and processed a number of variables related to locomotor behavior. For the present experiment the variables collected over twenty 3 min periods were total distance traveled (in centimeters) and stereotypy activity (beam breaks counted at the same photocell array). Prior to experimentation, rats were acclimated to the behavioral procedure at least 3 times. All testing occurred between 0830 and 1230 hours. On testing days, rats were placed in the activity chambers for 30 min before injections commenced. During injections, rats were lightly restrained with a towel and 16 mm 30 gauge injectors (Plastics One) were slowly lowered to extend 2 mm past the end of the guide cannulae. Once injectors were in place, drug was infused at a rate of 0.63 μl/min by a microinfusion pump (Harvard Apparatus, Boston, MA) that held two-50 μl Hamilton syringes attached to plastic tubing (PE20 Tygon tubing; Plastics One) and the injectors. Following infusions, injectors remained in place for 30 s. Immediately after the injectors were removed, rats were returned to the testing chambers for behavioral recording, the pump was re-started and injectors were inspected for fluid expulsion.

### 2.5. Tissue dissection and cryostat sectioning

When experiments were completed, rats were sacrificed by decapitation and brains were immediately removed and dissected. Tissue from all rats included in the study was examined for verification of striatal placements. To accomplish this, the region surrounding the injection sites was blocked and rapidly frozen in isopentane (−30 °C) and stored at −80 °C. Cresyl violet (FD Neurotechnologies, Baltimore, MD) staining was used to determine injection sites and neuronal viability from cryostat-generated 12 μm coronal sections surrounding the cannulae placements that were post-fixed with 4% paraformaldehyde (Fisher Scientific, Hanover Park, IL). To determine the level of DA depletion, a randomly selected set of control (n = 10) and 6-OHDA-lesioned rat (n = 10) striata were freshly dissected +0.5 mm to −0.5 mm around the injection site, frozen at −80 °C and later subjected to monoamine analysis using high performance liquid chromatography with electrochemical detection (HPLC-ED).

### 2.6. HPLC analysis

HPLC-ED was performed on selected striatal tissue. To do so, samples were homogenized in a cold solution containing 0.1 M perchloric acid, 1.0% ethanol, and 0.02% EDTA and centrifuged for 30 min at 4 °C. Supernatants from individual samples were analyzed with reverse-phase HPLC-ED. The HPLC system consisted of a Waters WISP autoinjector, a Waters 510 dual piston pump, an external pulse dampener (Rainin), a Waters guard pak column, and a C-18 (100 × 3.2 mm, 5 μm packing, Applied Biosystems) column. A mobile phase consisting of 0.75 mM sodium phosphate, 0.5 mM EDTA, 1.4 mM octane sulfonic acid, and 7%
acetonitrile, pH 3.0 was used to separate out NE, DA, 5-HT and their metabolites. A coulometric detector (Model 5011, ESA) configured with three electrodes was used to measure the monoamines and their metabolites. An ESA model 5020 guard cell (+400 mV) was placed before the WISP injector and an ESA model 5011 analytical cell (first electrode at −40 mV; second electrode at +500 mV) was placed immediately after the column. The signal from the second analytical electrode was recorded and analyzed by a Waters Baseline 810 Chromatography Workstation via a Waters Interface Module. The signals produced by the oxidation of monoamines and metabolites were measured and compared with those of known concentrations of high quality standards. The final oxidation current values were adjusted to milligram protein amounts determined by Lowry protein assay and expressed as nanogram monoamine per milligram protein.

2.7. Data analysis

All data collected were expressed as mean ± S.E. Data collected from the SKF 82958 dose-response experiment were analyzed by 2-way analyses of variance (ANOVAs) with lesion and treatment as between subjects factors. Data collected from 5-HT antagonist experiments were analyzed by 1-way ANOVAs with treatment as a between subjects factor. When appropriate, post hoc comparisons were carried out with Fisher’s post hoc tests. For monoamine analysis, single group (lesioned vs. intact) differences were determined by independent 2-tailed t-tests. All analyses were carried out using Statistica software '98 (Statsoft Inc., Tulsa, OK) with alpha values set to p < 0.05.

3. Results

3.1. Representative histology

Fig. 1 shows a schematic representation of a coronal brain section (Paxinos and Watson, 1998) located 0.48 mm anterior to bregma illustrating placements for 20 randomly selected 6-OHDA-lesioned (shaded ovals) and control (clear ovals) rats included in the study. All rats analyzed were found to have injector placements within the dorsal aspects of the striatum. Upon histological examination, data from two rats (1 control; 1 6-OHDA lesion) were removed due to exceptional gliosis.

3.2. Monoamine and metabolite levels

As shown in Table 1, HPLC analyses revealed a significant decline in striatal DA (−99%) and 3,4-dihydroxyphenylacetic acid (DOPAC) (−97%) levels in the 6-OHDA-injected group as compared to intact control rats while DOPAC/DA ratio increased (421%) indicating heightened DA turnover in a small percentage of surviving DA terminals. Increased 5-HT (+74%) and 5-hydroxyindole-3-acetic acid (5-HIAA) (+63%) levels were indicative of the 5-HT hyperinnervation, but 5-HIAA/5-HT ratios remained at control levels. NE amounts in lesioned striata were not significantly different from controls. Such results are consistent with our previous studies (Basura and Walker, 2001; Campbell et al., 2001; Bishop et al., 2004).

3.3. Locomotor effects of intrastriatal SKF 82958

Main effects of lesion ($F_{(1,31)} = 9.88; p < 0.05$) and treatment ($F_{(3,31)} = 5.24; p < 0.05$) on total distance demonstrated that 6-OHDA lesions increased ambulation and that intrastriatal administration of the D$_1$ agonist SKF 82958 produced significant effects on locomotor behavior (Fig. 2A). More importantly, post hoc tests on a significant lesion × treatment interaction ($F_{(3,31)} = 3.19; p < 0.05$) indicated that both doses of SKF 82958 significantly enhanced ambulation in 6-OHDA-lesioned rats compared to all other conditions (all $p < 0.05$). On measures of stereotypy activity (Fig. 2B) similar lesion ($F_{(1,31)} = 13.22; p < 0.05$) and treatment main effects were found ($F_{(3,31)} = 6.56; p < 0.05$). Post hoc analyses of a significant lesion × treatment interaction ($F_{(3,31)} = 3.08; p < 0.05$) demonstrated that the 10 µg dose of SKF 82958 increased stereotypy compared to all other groups (all $p < 0.05$). 6-OHDA-lesioned rats also displayed more stereotypy.
following the 1.0 µg dose of SKF 82958 than either vehicle condition (both \( p < 0.05 \)).

### 3.4. Effects of intrastriatal 5-HT\(_{2A}\) antagonism on \(D_1\)-agonist induced locomotor behavior

As shown in Fig. 3A, a significant main effect of treatment (\( F(7,53) = 7.48; p < 0.001 \)) was found on total distance. Post hoc testing demonstrated that 2.0 µg of SKF 82958 induced locomotion in 6-OHDA-lesioned rats compared to all other conditions (all \( p < 0.05 \)). More importantly, both doses of M100907, when co-infused with SKF 82958 in 6-OHDA-lesioned rats, reduced this effect (both \( p < 0.05 \)). As shown in Fig. 3B, similar treatment effects were found on measures of stereotypy activity (\( F(7,53) = 6.99; p < 0.001 \)). As was observed with total distance, SKF 82958-induced stereotypy in 6-OHDA rats (\( p < 0.05 \) vs. all other groups) was reduced by both doses of co-infused M100907 (both \( p < 0.05 \)).

### Table 1

<table>
<thead>
<tr>
<th>Group</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>Vehicle ((n = 10))</td>
<td>0.03 ± 0.01</td>
<td>3.02 ± 0.29</td>
<td>15.5 ± 0.98</td>
<td>0.19 ± 0.01</td>
<td>1.26 ± 0.08</td>
<td>0.53 ± 0.04</td>
<td>2.71 ± 0.31</td>
</tr>
<tr>
<td>6-OHDA ((n = 10))</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>0.99 ± 0.16</td>
<td>2.05 ± 0.14</td>
<td>0.92 ± 0.08</td>
<td>2.38 ± 0.16</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 independent 2-tailed \( t \)-tests (* \( p < 0.05 \) compared to vehicle group).

Fig. 2. Locomotor effects of local infusion of the \(D_1\) agonist SKF 82958 (0, 0.1, 1.0 and 10 µg/side) into the striatum of intact control and 6-OHDA-lesioned rats. Graphs denote averages of twenty 3 min measurement periods for total distance \( (S.E.) \) (A) and stereotypy activity \( (S.E.) \) (B). Statistical differences were determined by 2-way ANOVAs analyzing lesion and treatment effects. Significant post hoc testing differences are denoted by the following symbols: * \( p < 0.05 \) vs. all but Lesion-1.0, * \( p < 0.05 \) vs. all but Lesion-10.0, * \( p < 0.05 \) vs. all, \# \( p < 0.05 \) vs. Control-0 and Lesion-0.

Fig. 3. Locomotor effects of local co-infusion of the \(D_1\) agonist SKF 82958 (SKF; 2.0 µg/side) and the 5-HT\(_{2A}\) antagonist M100907 (M; 0, 0.1 and 1.0 µg/side) into the striatum of intact control and 6-OHDA-lesioned rats. Graphs denote averages of twenty 3 min measurement periods of testing for total distance \( (S.E.) \) (A) and stereotypy activity \( (S.E.) \) (B). Statistical differences were determined by 1-way ANOVAs analyzing treatment effects. Significant post hoc differences are denoted by the following symbols: * \( p < 0.05 \) vs. Lesion-Veh and Control-SKF, * \( p < 0.05 \) vs. Lesion-SKF.


**Fig. 4.** Locomotor effects of local co-infusion of the D1 agonist SKF 82958 (SKF; 2.0 μg/side) and the 5-HT2C antagonist RS102221 (RS; 0, 0.1 and 1.0 μg/side) into the striatum of intact control and 6-OHDA-lesioned rats. Graphs denote averages of twenty 3 min measurement periods of testing for total distance ± S.E. (A) and stereotypy activity ± S.E. (B). Statistical differences were determined by 1-way ANOVAs analyzing treatment effects. Significant post hoc differences are denoted by the following symbol: *p < 0.05 vs. Lesion-Veh and all control treatments.

### 3.5. Effects of intrastriatal 5-HT2C antagonism on D1-agonist induced locomotor behavior

Analysis of total distance (Fig. 4A) revealed a significant main effect of treatment ($F(7,52) = 7.48; p < 0.001$). As previously shown, post hocs showed that SKF 82958 significantly enhanced locomotion in the 6-OHDA-lesioned rats ($p < 0.05$). However, in contrast to M100907, neither dose of RS102221, when co-infused with SKF 82958 in 6-OHDA-lesioned rats, altered this effect. As shown in Fig. 4B, post hoc testing of a significant main effect of treatment for stereotypy activity ($F(7,52) = 8.80; p < 0.001$) revealed that SKF 82958 increased stereotypy in 6-OHDA rats ($p < 0.05$) that was unaffected by co-infused RS102221.

### 4. Discussion

In concert with previous studies, the results of the present investigation demonstrated that striatal D1 agonist administration to 6-OHDA-lesioned rats induces hyperlocomotor activity. More importantly, we extend what is currently known about D1 and 5-HT2 receptor interactions within the DA-depleted striatum by showing that D1-induced locomotor activity can be reduced by antagonism of striatal 5-HT2A, but not 5-HT2C receptors.

The striatum is a major component of the basal ganglia and plays an integral role in movement initiation and execution. Under normal conditions, DA and 5-HT innervation from nigrostriatal and raphe-striatal afferents, respectively (Graybiel, 1990; Lavoie and Parent, 1990) regulate its function. As such it has been postulated that striatal output is likely mediated by interactions between intrinsic DA and 5-HT receptors. For example, microdialysis studies have demonstrated that 5-HT2 receptor antagonists alter striatal extracellular DA levels (Lucas and Spampinato, 2000; Porras et al., 2002), and locomotion induced by psychomotor stimulants (O’Neill et al., 1999; Fletcher et al., 2002; Filip et al., 2004).

Selective destruction of central DA through the administration of the catecholamine neurotoxin 6-OHDA significantly modifies striatal DA and 5-HT neurotransmission (Kostrzewa et al., 1998; Reader and Dewar, 1999). For example, D1 receptor agonist treatment more potently stimulates ambulation and stereotypy (Breese et al., 1985, 1987; Bishop and Walker, 2003), postulated to occur as a result of strengthened D1-related cellular signal transduction (Cai et al., 2002; Gerfen et al., 2002; Papadeas et al., 2004). 5-HT function is also altered when brain DA levels are significantly reduced. For example, neonatal DA depletion induces 5-HT hyperinnervation (Breese et al., 1984; Stachowiak et al., 1984) and, as shown by the present study and others, increased 5-HT levels in striatum (Basura and Walker, 1999; Bishop et al., 2004). This enhanced 5-HT signaling, thought to contribute to hyperactivity in neonatal DA-depleted rats, can be reduced by 5-HT2 receptor antagonism (Luthman et al., 1991). Interestingly, neither 5-HT2A nor 5-HT2C receptor plasticity is dependent upon 5-HT hyperinnervation (Laprade et al., 1996; Basura and Walker, 1999; Fox and Brotchie, 2000a), suggesting that enhanced 5-HT levels are not required to elicit 5-HT2-specific effects. This is supported by studies showing that striatal infusion of the 5-HT2 receptor agonist DOI increases locomotor behaviors in DA-depleted rats (Bishop and Walker, 2003; Bishop et al., 2004). These effects are thought to arise as a result of upregulated striatal 5-HT2A receptors (Radja et al., 1993; Laprade et al., 1996; Basura and Walker, 1999) and strengthened activation of the direct striatal output pathway (Gresch and Walker, 1999a; Basura and Walker, 2000, 2001).

While it is clear that DA and 5-HT functions are altered after DA depletion, less is known about how these receptors interact under these conditions to
influence motoric behavior. In a series of papers, we have demonstrated that striatal 5-HT₂ receptors are in a strengthened position to influence supersensitive behavioral and cellular responses to D₁ agonist administration. For example, gene expression studies have indicated that combined administration of the D₁ agonist SKF 38393 and 5-HT₂ receptor agonist DOI increases activation of the direct striatongiral output pathway (Gresch and Walker, 1999b; Campbell et al., 2001). These effects were recently corroborated with behavioral studies showing that intrastriatal co-administration of the full D₁ agonist SKF 82958 and DOI, synergistically increased locomotion (Bishop and Walker, 2003). Further studies have also revealed that D₁ agonist supersensitivity following 6-OHDA lesions in adult rats can be reduced by prior administration of the 5-HT₂A/₂C antagonist ritanserin (Bishop et al., 2003). While these studies suggest that 5-HT₂ receptors can influence striatal D₁ receptors, it was not known which receptor subtype(s) mediated these effects.

A number of clues led us to hypothesize that striatal 5-HT₂A receptors were more likely to influence D₁-related hyperlocomotor activity. First, in the intact brain, 5-HT₂A and 5-HT₂C receptors have divergent roles in modulating DA function. For example, 5-HT₂A receptor antagonism decreases striatal DA release (Lucas and Spampinato, 2000) and behavioral activity accompanying psychomotor stimulants (O’Neill et al., 1999; Fletcher et al., 2002). 5-HT₂C receptor antagonism enhances striatal DA levels (Lucas and Spampinato, 2000), but can increase or decrease psychomotor stimulant-induced behavioral activity based on the site (accumbens vs. medial prefrontal cortex) and compound administered (RS102221 vs. SB242084) (Filip and Cunningham, 2002; Fletcher et al., 2002; Filip et al., 2004). Second, DA depletion has been shown to increase levels of striatal 5-HT₂A mRNA without altering 5-HT₂C receptor mRNA (Laprade et al., 1996; Basura and Walker, 1999). Third, gene activation within the direct pathway of the DA-depleted rat stimulated by 5-HT release agents or 5-HT agonism is blocked by 5-HT₂A-preferring antagonists (Basura and Walker, 2000, 2001). Finally, DOI-induced locomotor activity in neonate 6-OHDA-lesioned rats was attenuated by the 5-HT₂A and not 5-HT₂C receptor antagonism (Bishop et al., 2004). Thus, the relationship between 5HT₂A and D₁ receptors in the DA-depleted striatum appears strengthened.

While we did not find that striatal 5-HT₂C receptor antagonism altered D₁-induced locomotor activity, there is evidence that following DA depletion, enhanced oral activity induced by the D₁ agonist SKF 38393 and the 5-HT₂A/₂C agonist m-chlorophenylpiperazine (m-CPP) is mediated through striatal 5-HT₂C receptors (Gong and Kostrzewa, 1992; Plech et al., 1995). Extrastriatal 5-HT₂C receptor effects on movement have also been reported, including increased oral dyskinesia after intra-subthalamic administration of m-CPP in the intact rat (Eberle-Wang et al., 1996). In the DA-depleted brain, the substantia nigra pars reticulata appears to be a particularly important site mediating 5-HT’s effects. For example, nigral 5-HT₂C receptor binding is elevated in the post-mortem tissue of Parkinson’s disease patients (Fox et al., 2000a). Functionally, rodent studies have shown that intranigral administration of the 5-HT₂C antagonist SB206553 induces contralateral rotations in the unilateral DA-depleted rat (Fox et al., 1998). Moreover, systemic administration of SB206553 enhances contralateral rotations elicited by D₁ or D₂ agonists (Fox et al., 1998; Fox and Brodutch, 2000b). Thus, it appears that extrastriatal 5-HT₂C receptors may more readily impact D₁ agonist-induced movement under conditions of DA depletion.

In the present study we employed the putative 5-HT₂A and 5-HT₂C receptor antagonists M100907 and RS102221 to discern the potential contributions of these receptors to D₁ agonist-induced locomotor activity. These compounds have nanomolar binding affinities for 5-HT₂A vs. 5-HT₂C receptors with 100-fold selectivity in discriminating each receptor subtype (Kehne et al., 1996; Bonhaus et al., 1997). As demonstrated by the results of the present study, only M100907 was able to effectively reduce the D₁-induced locomotor activity, indicating that within the DA-depleted striatum, 5-HT₂A receptors more likely influence the function of D₁ receptors. While it is not clear how these receptors interact, it is known that DA depletion increases the coupling of D₁ receptors to Gq/11 proteins which are functionally linked to phosphoinositol hydrolysis and protein lipase C second messenger cascades (Cai et al., 2002). Because 5-HT₂A receptors utilize phosphoinositol hydrolysis as a second messenger (Berg et al., 1994), DA depletion-induced alterations in D₁ signal transduction mechanisms may strengthen 5-HT₂A/D₁ cooperation.

While the present study demonstrated that striatal 5-HT₂A receptors functionally influence supersensitive D₁ receptors in the DA-depleted rodent, these findings may have relevance to excessive movement in humans. For example, it has been demonstrated that 5-HT₂A receptors are highly expressed in the striosomes of the human caudate nucleus and putamen (Waebner and Palacios, 1994; Lopez-Gimenez et al., 1999) and are postulated to play a significant role in the DA-mediated induction of stereotypy and unwanted movements (Graybiel et al., 2000; Henry et al., 2003). Because overactivity of D₁ receptors in the direct striatal output pathway plays a central role in the expression of stereotypy (Starr and Starr, 1986; Chartoff et al., 2001) and the development of levodopa-induced dyskinesia (Andringa et al., 1999; Rascol et al., 2001), reducing supersensitive D₁-mediated signaling through a non-dopaminergic mechanism like 5-HT₂A receptor antagonism may allow for treatment of excessive move-
ments without loss of therapeutic efficacy. For example, the atypical antipsychotic clozapine, which shows, among other receptor families, affinity as an antagonist at 5-HT2 receptor sites (Meltzer, 1999), and the 5-HT2A/2C receptor antagonist ritanserin, are reported to reduce levodopa-induced dyskinesia in Parkinson’s disease patients (Maertens de Noordhout and Delwaide, 1986; Pierelli et al., 1998; Durif et al., 2004).

In summary, this study demonstrated that increased locomotor behaviors induced by striatal administration of the D1 agonist SKF 82958 in rodents with near total DA depletion can be reduced by co-infusion of the selective 5-HT2A antagonist M100907, but not the 5-HT2C selective antagonist RS102221. Coupled with gene expression studies, the results of this study suggest that striatal D1 and 5-HT2A receptors interact to alter direct striatal pathway output. This strengthened relationship, brought about by DA depletion may represent a novel mechanism by which pathological D1-related signaling may be reduced and suggests an alternative pharmacological target for the lessening of excessive movements that can occur with DA replacement therapy.

Acknowledgements

Special thanks to Drs. K.C. Rice and T. Ullrich for the generous donation of the 5-HT2A antagonist M100907. This work was supported by the National Institute of Neurological Disorders & Stroke Grant NS39013 to P.D.W.

References


Campbell, B.M., Gresh, P.J., Walker, P.D., 2001. Neonatal dopamine depletion reveals a synergistic mechanism of mRNA regulation that is mediated by dopamine(D1) and serotonin(2) receptors and is targeted to tachykinin neurons of the dorsomedial striatum. Neuroscience 105, 671–680.


Fox, S.H., Brotchie, J.M., 2000a. 5-HT2C receptor binding is increased in the substantia nigra pars reticulata in Parkinson’s disease. Movement Disorders 15, 1064–1069.


