Intrastriatal Serotonin 5-HT2 Receptors Mediate Dopamine D1-Induced Hyperlocomotion in 6-Hydroxydopamine-Lesioned Rats

CHRISTOPHER BISHOP, DEV P. KAMDAR, AND PAUL D. WALKER*

Department of Anatomy and Cell Biology, Wayne State University, School of Medicine, Detroit, Michigan 48201

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ABSTRACT Striatal dopamine (DA) and serotonin (5-HT) functions are altered following DA denervation. Previous research indicates that intrastriatal coadministration of D1 and 5-HT2 receptor agonists synergistically increase locomotor behavior in DA-depleted rats. In the present study, we examined whether striatal 5-HT2 mechanisms also account for supersensitive D1-mediated locomotor behavior following DA denervation. Adult male Sprague-Dawley rats were subjected to bilateral striatal cannulation and then received either intracerebroventricular (i.c.v.) or intrastriatal 6-hydroxydopamine (6-OHDA; 200 µg or 20 µg/side, respectively). After at least 3 weeks, i.c.v.-lesioned rats received intrastriatal infusions of the 5-HT2 receptor antagonist ritanserin (2.0 µg/side) or its vehicle (DMSO) followed by systemic SKF 82958, a D1 agonist (1.0 mg/kg, i.p.) and locomotor activity was monitored. In another experiment, intrastriatal sham and 6-OHDA-lesioned rats received bilateral intrastriatal infusions of ritanserin (2.0 µg/side) or its vehicle (DMSO) followed by intrastriatal infusions of SKF 82958 (5.0 µg/side) or vehicle (0.9% saline). Rats with DA loss demonstrated supersensitive locomotor responses to both systemic and intrastriatal SKF 82958. Ritanserin pretreatment blunted systemic SKF 82958-induced hyperlocomotion and returned intrastriatal D1-mediated hyperactivity to sham lesion levels. The results of this study suggest that striatal 5-HT2 receptors contribute to D1-mediated hyperkinesias resulting from DA loss and suggest a pharmacological target for the alleviation of dyskinesia that can develop with continued DA replacement therapy. Synapse 50:164–170, 2003.

INTRODUCTION

The striatum is one of the major components of the basal ganglia which plays an important role in the initiation and execution of movement (Albin et al., 1989; Alexander et al., 1990; Graybiel et al., 1994). Several intrinsic neurotransmitters interact to regulate its function, including dopamine (DA) from the nigrostriatal pathway and serotonin (5-HT) from afferent raphe projections (Lindefors et al., 1990; Walker et al., 1991). Studies investigating 5-HT/DA receptor interactions within the intact striatum have consistently demonstrated that intrinsic 5-HT2 receptors can modify DA function that may in turn affect behavior (Schmidt et al., 1992; Laprade et al., 1996; Lucas and Spampinato, 2000; Porras et al., 2002). For example, systemically administered 5-HT2 receptor antagonists have been shown to alter hyperlocomotion in rodents induced by cocaine, amphetamine, and 3,4-methylenedioxymethamphetamine (MDMA) (Kehne et al., 1996; O’Neill et al., 1999; Fletcher et al., 2002).

Selective destruction of central DA neurons through the administration of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) can produce striatal neuroplasticity that significantly alters the regulation and function of DA and 5-HT systems (Kostrzewa et al., 1998; Reader and Dewar, 1999). For instance, D1 receptors within the DA-depleted striatum show an en-

*Correspondence to: Paul D. Walker, Department of Anatomy and Cell Biology, Wayne State University, 540 E. Canfield Ave., Detroit, MI 48201.
E-mail: pdwalker@med.wayne.edu

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enhanced response to D1 agonist treatment thought to arise from strengthened cellular signal transduction (Reader and Dewar, 1999; Cai et al., 2002; Gerfen et al., 2002). As a result, D1 receptor agonists produce significant hyperkinesia and stereotypy when administered to neonate and adult DA-depleted rats (Breese et al., 1985; Kostrzewa et al., 1998). Neuroadaptations in response to DA loss also produce changes in striatal 5-HT2 receptor function (el Mansari et al., 1994; Basura and Walker, 1999; Greshc and Walker, 1999) including increased striatal 5-HT2A mRNA levels (Numan et al., 1995; Laprade et al., 1996; Basura and Walker, 1999). These studies suggest that 5-HT2 receptors may play a more prominent role in mediating striatal dopamine signaling following DA denervation. Indeed, recent experiments from our laboratory have demonstrated that intrastratal costimulation of D1 and 5-HT2 receptors synergistically enhances locomotor behavior in 6-OHDA-lesioned rats (Bishop and Walker, in press). Moreover, in 6-OHDA-lesioned rats, behavioral hyperactivity accompanying neonatal DA depletion and elevated oral dyskinesia induced by ventral striatal infusions of the D1 agonist SKF38393 can be blocked by 5-HT2A/2C receptor antagonists (Luthman et al., 1991; Gong et al., 1992; Plech et al., 1995). To this point, however, a number of variables remain unexplained, including whether these effects are similar in adult DA-depleted rats and to what extent these effects also extend to the dorsal striatum. If upregulated dorsal striatal 5-HT2 receptors contribute to D1-receptor sensitization within the DA-depleted striatum, we would expect that intrastratal infusions of the 5-HT2 antagonist ritanserin should blunt the lesion-induced D1 supersensitivity. To test this hypothesis, the present study examined how systemic and intrastratal D1-mediated hyperactivity is altered by 5-HT2 receptor antagonism in the intact and 6-OHDA-lesioned adult rat by using multiple movement parameters.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats were used (225–250 g; Charles River Laboratory, Wilmington, MA). All animals were housed 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 0600 h). Standard lab chow (Rodent Diet 5001; LabDiet, Brentwood, MO) and water were available throughout the experiment. Animals were maintained in strict accordance with the guidelines of the Institutional Animal Care and Use Committee of Wayne State University and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH publication number 85-23, revised 1996).

Cannula implant surgeries and 6-OHDA lesions of DA neurons

One week after arrival in the lab and adaptation to housing conditions, all rats (n = 23) were anesthetized with chloral hydrate (400 mg/kg; i.p.), placed in a stereotaxic apparatus, and implanted bilaterally with chronic 22-gauge intracranial guide cannulae (C313G/SPC, Plastics One, Roanoke, VA). With the incisor bar positioned 3.3 mm below the interaural line, cannulae were positioned above the 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.

Just prior to cannula implantation, one group of rats (n = 6) received 6-OHDA (200 μg/side in 0.9% saline and 0.1% ascorbic acid; Sigma; St. Louis, MO) infused bilaterally into the lateral ventricles at a rate of 4 μl/min through a 50 μl Hamilton syringe to a final volume of 10 μl. The injector needle remained in place for 1 min after the infusions were completed. The stereotaxic coordinates for intracerebroventricular (i.c.v.) infusions were AP –0.8 mm, ML ±1.5 mm and DV –3.9 mm relative to bregma with the incisor bar position 3.3 mm below the interaural line (Paxinos and Watson, 1998). All rats received desipramine (25 mg/kg; i.p.; Sigma) 30 min prior to 6-OHDA to protect brain noradrenaline neurons from its neurotoxic effects. A second group of rats (n = 17), 1 week after recovery from guide cannula implantation, received bilateral infusions of 6-OHDA (20 μg/side; Sigma) or its vehicle (0.9% saline and 0.1% ascorbic acid) into the striatum through 30-gauge microinjectors (Plastics One) attached to Teflon tubing and 50 μl Hamilton syringes placed directly into the implanted guide cannulae at a rate of 1.24 μl/min for 1 min 40 sec to reach a volume of 2 μl. The injector needle remained in place for 2 min after infusions were completed. The stereotaxic coordinates for intrastriatal infusions were AP +0.4 mm, ML ±2.9 mm and DV –5.6 mm relative to bregma with the incisor bar position 3.3 mm below the interaural line (Paxinos and Watson, 1998).

Pharmacological treatments

To allow time for striatal neuroadaptations to occur as a result of 6-OHDA lesions, rats were tested at least 21 days postlesion. I.c.v. 6-OHDA-lesioned rats were randomly assigned to receive a regimen of intrastratal and systemic drug infusions on separate testing occasions. During a session, a rat could receive a pretreatment bilateral intrastratal infusion of the 5HT2 receptor antagonist ritanserin (Baxter et al., 1995) (2.0 μg/side in 0.8 μl/side) or its vehicle (50% DMSO in 0.9% saline) followed by a systemic injection of the D1 ago-
nist SKF 82958 (O’Boyle et al., 1989) (1 mg/kg i.p. in 1.0 ml saline). Intrastriatal 6-OHDA-lesioned rats were assigned to receive a regimen of intrastriatal drug infusions. In each session a rat could receive a pretreatment of ritanserin (2.0 μg) bilaterally in a volume of 0.8 μl/side or its vehicle (50% DMSO in 0.9% saline) followed by a bilateral intrastriatal infusion of either SKF82958 (5.0 μg/side) or its vehicle (0.9% saline) in a volume of 0.8 μl/side. No rat was tested more than three times and at least 3 days separated each testing session.

Behavioral procedure
Prior to testing, rats were acclimated to the testing procedure at least three times. All testing occurred between 0900–1200 h. On testing days, all rats were placed in the activity chambers for 30 min before injections commenced. During pretreatment injections, rats were lightly restrained with a towel and 30-gauge injectors were slowly lowered into the cannulae. Once injectors were in place, ritanserin or vehicle 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 injectors. Following infusions, the injectors remained in place for 30 sec. Immediately after the injectors were removed rats were returned to the activity chambers for 30 min monitoring and the pump was restarted for inspection of fluid displacement from injector tips. After this initial monitoring period, rats with i.c.v. 6-OHDA lesions received an i.p. injection of SKF 82958 or its vehicle and then locomotor activity was measured for another 30-min period. Rats with intrastriatal sham or 6-OHDA lesions were removed from the chambers and were bilaterally infused with either SKF 82958 or its vehicle and returned to the chambers for another 30-min measurement period.

Equipment
Locomotor activity testing 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. The locomotion variables measured and reported here were horizontal activity, distance traveled (in centimeters), movement bouts (times a rat ended and then initiated another movement period), and stereotypy count (number of beam breaks at the same photocell array) over 10 3-min periods.

Tissue dissection and cryostat sectioning
When experiments were completed, rats were sacrificed by decapitation and brains were immediately removed and dissected. For rats subject to i.c.v. lesions, striatum was dissected caudal to the block (0 to −2.0 mm from bregma), frozen at −80°C, and later assayed for DA and DOPAC measurements. hematoxylin and eosin staining was used to determine injection sites and neuronal viability from 12 μm coronal sections surrounding the cannulae placements. For rats with intrastriatal lesions, one hemisphere was removed and the area directly around the lesion site was dissected for high-performance liquid chromatography (HPLC) analysis while the other hemisphere was frozen at −80°C and later stained to determine correct injection site and neuron viability.

High performance liquid chromatography
HPLC coupled to electrochemical detection was performed on striatal tissue as previously described (Gresch and Walker, 1999). 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 mg protein amounts determined by Lowry assay (Lowry et al., 1951) and expressed as ng monoamine per mg protein (mean ± SE).

Data analysis
Data were analyzed by using Statistica software ’98 (Statsoft, Tulsa, OK). Independent, one-tailed t-tests were used to analyze locomotor behavior in i.c.v.-lesioned rats. One-way ANOVAs were used to analyze HPLC data and locomotor behavior following drug treatments in striatal-lesioned rats. All post-hoc comparisons were carried out with Fisher’s least squares design post-hoc tests.

RESULTS
Attrition and histology
All rats were found to have injector placements within the dorsal striatum. Figure 1A shows a representative hemisection of striatum with a dashed oval denoting the target area for drug injection. Schematic representations of a coronal brain hemisection identifying placements for rats processed for histology in the study are shown in Figure 1B.

HPLC results
Analyses of HPLC data revealed significant lesion-specific alterations in both DOPAC and DA levels. I.c.v. and intrastriatal 6-OHDA lesions produced significant decreases (35.8% and 62.1% of sham control, respectively) in DOPAC levels (F(1,12) = 26.76; P < 0.001 and F(1,15) = 13.28; P < 0.005, respectively). Similarly, DA
levels were significantly lowered in the striatum in i.c.v. \( (F(1,12) = 27.88; P < 0.001) \) and intrastriatal \( (F(1,15) = 38.52; P < 0.001) \) 6-OHDA-lesioned rats (23.9% and 46.2% of sham controls, respectively).

Effects of 5-HT2 antagonism on systemic D1 agonist-stimulated behavior

As shown in Figure 2, which demonstrates the 30-min test following SKF 82958 or vehicle, a number of locomotor activities in i.c.v. 6-OHDA-lesioned rats induced by the systemic SKF 82958 were significantly blunted by intrastriatal pretreatment with the 5-HT2 receptor antagonist ritanserin. Analysis of horizontal activity revealed an effect of drug treatment \( (T(10) = 2.34; P < 0.05) \) showing that rats were less active after receiving SKF 82958 following a pretreatment of ritanserin. Ritanserin also decreased SKF 82958-induced total distance traveled \( (T(10) = 2.23; P < 0.03) \), movement bouts \( (T(10) = 2.06; P < 0.03) \), and stereotypy counts \( (T(10) = 2.21; P < 0.04) \) in each case demonstrated an attenuation of D1-induced locomotor activity.

Attenuation of intrastriatal D1 hyperlocomotion by intrastriatal 5-HT2 antagonism

To determine whether the dorsal striatum is an active site for 5-HT2 receptor antagonism of D1-mediated effects on behavior, intrastriatally lesioned rats were tested for locomotor responses to intrastriatal SKF 82958 subsequent to pretreatment with ritanserin (see Fig. 3). Ritanserin itself had no intrinsic effects during the first 30-min measurement period (data not shown). Analysis of horizontal activity found a main effect of treatment \( (F_{(5,31)} = 2.91; P < 0.03) \). Post-hoc testing revealed that rats with 6-OHDA lesions receiving intrastriatal SKF 82958 subsequent to DMSO pretreatment demonstrated more horizontal activity than any other group (all \( P < 0.05 \)), while ritanserin pretreat-
ment blunted these effects. Similar effects were seen upon analysis of movement bouts (F_{5,31} = 4.31; P < 0.005), such that rats with 6-OHDA lesions receiving a pretreatment of DMSO followed by SKF 82958 recorded more movement periods than all other groups (all P < 0.05) and ritanserin pretreatment attenuated the D1-mediated supersensitivity. No significant main effects were seen after analyses of total distance and stereotypy counts.

**DISCUSSION**

The present experiment supports previous work showing that locomotor behavior in 6-OHDA-lesioned rats is enhanced following D1 receptor agonist treatment. More importantly, the novel finding of the present investigation indicates that this supersensitivity is, in part, dependent on 5-HT2 receptor function located within the dorsal striatum such that local antagonism of these receptors can attenuate D1-mediated effects on locomotor behavior.

Previous work has demonstrated that DA loss within the basal ganglia output pathways produces DA receptor agonist-induced behavioral responses in 6-OHDA-lesioned rats that are of greater magnitude than that observed in sham or unlesioned rats. Systemic administration of the D1 agonist SKF38393 increases locomotor activity and stereotypy when administered to neonate and adult DA-depleted rats (Breese et al., 1985; Kostrewa et al., 1998). Similarly, we found that i.c.v. 6-OHDA-lesioned rats appear hyperlocomotive following systemic administration of the full D1 agonist SKF 82958. Others have also shown that direct infusions of D1 agonists into the DA-depleted striatum can induce increases in locomotion, exploratory, and oral behavior (Breese et al., 1987; Gong et al., 1992; Plech et al., 1995; Bishop and Walker, in press). In the present study, administration of SKF 82958 into the dorsal striatum increased behavioral responses on a number of measures, including horizontal activity and movement bouts. These effects were pronounced in rats with 6-OHDA lesions and have been hypothesized to arise as a result of strengthened cellular transduction mechanisms that occur following DA loss (Reader and Dewar, 1999; Cai et al., 2002; Gerfen et al., 2002).

5-HT mechanisms within the striatum are also altered by DA loss within the basal ganglia, including strengthened striatal 5-HT2 receptor function (el Mansari et al., 1994; Basura and Walker, 1999; Gresch and Walker, 1999) and increased striatal 5-HT2A mRNA levels (Numan et al., 1995; Laprade et al., 1996; Basura and Walker, 1999). As a result, we hypothesized that striatal 5-HT2 receptors may play a more important role in DA-induced striatal function following DA denervation. While ritanserin appeared to produce no intrinsic effects on its own, 5-HT2 receptor-mediated effects may depend on activation of DA receptors within the striatum. We have recently shown that DA-lesioned rats are particularly sensitive to D1/5-HT2 receptor costimulation. Intrastriatal coadministration of D1 and 5-HT2 agonists synergistically enhanced locomotion in 6-OHDA-lesioned rats, indicating that up-regulated 5-HT2 receptors may promote D1-mediated supersensitivity (Bishop and Walker, in press). This appears to be particularly true of the 5-HT2A receptor, which has been shown to be significantly increased in the DA-depleted anterior and dorsal striatum (Laprade et al., 1996; Basura and Walker, 1999). Work by a
number of groups has demonstrated that 5-HT2A receptor agonists positively modulate DA release, preferentially under conditions of DA neuron activity (Schmidt et al., 1992; Lucas and Spampinato, 2000). This is in contrast to 5-HT2C receptors, which appear to exert inhibitory control on both basal and stimulated DA release (Di Giovanni et al., 1999; Hutson et al., 2000; Lucas et al., 2000). Further studies with specific 2A vs. 2C antagonists are warranted to discern these differences.

Because DA and 5-HT2 receptors are likely to interact within the striatum (Schmidt et al., 1992; De Deurwaredere et al., 1996; Laprade et al., 1996; Lucas and Spampinato, 2000; Porras et al., 2002), we were particularly interested in determining whether intrastriatal D1 receptor-stimulated behavior is altered by intrastriatal 5-HT2 antagonism in the DA-depleted rat. While systemically administered 5-HT2 antagonists have been shown to attenuate the locomotor effects of cocaine, amphetamine, and MDMA (Kehne et al., 1996; O'Neill et al., 1999; Fletcher et al., 2002) in the intact rat, no studies to date have addressed whether dorsal intrastriatal infusions of 5-HT2 antagonists block DA-mediated locomotor behavior in the adult DA-depleted rat. We show here that intrastriatal 5-HT2 antagonist pretreatment not only blunts systemic D1-mediated hyperactivity, but that this same pretreatment attenuates D1-mediated supersensitivity accompanying 6-OHDA-lesioned rats after intrastriatal D1 agonist infusion.

While the mechanisms of these effects are not fully understood, recent studies have suggested that D1 supersensitivity following DA depletion may involve a shift in signal transduction mechanisms in addition to increased cyclic nucleotide phosphorylation events and enhanced D1 coupling to Gs-proteins (Cole et al., 1994; Cai et al., 2002). For example, Cai et al. (2002) demonstrated that DA depletion increased coupling of D1 receptors to Gs/11 proteins which are linked to PLC and PI hydrolysis. Because 5-HT2A and 5-HT2C receptors are functionally coupled to PI hydrolysis (Conn and Sanders-Bush, 1986; Berg et al., 1994), altered PI hydrolysis coupling may indeed be one mechanism by which D1 and 5-HT2 receptors interact to modify locomotor behavior.

While the results of the present study suggest that 5-HT2 receptor antagonism blunts the D1 receptor-mediated increases in locomotor activity within the DA-depleted rat, clinical work with Parkinson's disease (PD) patients indicates that 5-HT pharmacotherapeutics may be useful for the treatment of L-DOPA-induced dyskinesias. For example, administration of clozapine, an atypical antipsychotic with high affinity as an antagonist at central 5-HT2 receptor sites (Travis et al., 1998; Meltzer, 1999), to PD patients has been reported to dose-dependently decrease L-DOPA-induced dyskinesia without affecting relief from parkinsonism (Bennett et al., 1994; Pierrelli et al., 1998). Ritanserin administration has also been shown to reduce dyskinesia, in part through 5-HT2 receptor antagonism (de Noordhout and Delwaide, 1986). As such, it is possible that upregulated 5-HT2 receptors in concert with supersensitive D1 receptors may be responsible for the development of dyskinesia in L-DOPA-treated patients and a potential target for the attenuation of these effects.

In summary, data presented here demonstrate that antagonism of 5-HT2 receptors within the DA-depleted striatum attenuates D1-stimulated hyperlocomotor behavior. These effects implicate striatal 5-HT2 receptors as potential mediators of D1-related supersensitivity occurring after DA loss. Future studies should focus on the 5-HT subtype specificity of these effects and the signal transduction mechanisms that converge to produce synergy following DA depletion. Such investigations may aid in the development of novel pharmacological agents for the treatment of PD and the alleviation of L-DOPA-induced dyskinesia in PD patients.

REFERENCES


