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# Local modulation of striatal glutamate efflux by serotonin 1A receptor stimulation in dyskinetic, hemiparkinsonian rats

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ABSTRACT

Serotonin 1A receptor (5-$HT_{1A}$R) agonists reduce both L-DOPA- and D1 receptor (D1R) agonist-mediated dyskinesia, but their anti-dyskinetic mechanism of action is not fully understood. Given that 5-$HT_{1A}$R stimulation reduces glutamatergic neurotransmission in the dopamine-depleted striatum, 5-$HT_{1A}$R agonists may diminish dyskinesia in part through modulation of pro-dyskinetic striatal glutamate levels. To test this, rats with unilateral medial forebrain bundle dopamine or sham lesions were primed with L-DOPA (12 mg/kg + benserazide, 15 mg/kg, sc) or the D1R agonist SKF81297 (0.8 mg/kg, sc) until abnormal involuntary movements (AIMs) stabilized. On subsequent test days, rats were treated with vehicle or the 5-$HT_{1A}$R agonist ±8-OH-DPAT (1.0 mg/kg, sc), followed by L-DOPA or SKF81297, or intrastriatal ±8-OH-DPAT (7.5 or 15 mM), followed by L-DOPA. In some cases, the 5-$HT_{1A}$R antagonist WAY100635 was employed to determine receptor-specific effects. *In vivo* microdialysis was used to collect striatal samples for analysis of extracellular glutamate levels during AIMs assessment. Systemic and striatal ±8-OH-DPAT attenuated L-DOPA-induced dyskinesia and striatal glutamate efflux while WAY100635 reversed ±8-OH-DPAT's effects. Interestingly, systemic ±8-OH-DPAT diminished D1R-mediated AIMs without affecting glutamate. These findings indicate a novel anti-dyskinetic mechanism of action for 5-$HT_{1A}$R agonists with implications for the improved treatment of Parkinson's disease.



## Introduction

Chronic dopamine (DA) replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) for Parkinson's disease (PD) patients often results in abnormal and excessive movements known as L-DOPA-induced dyskinesia (LID; Jankovic, 2005). Although the mechanisms of LID are not fully understood, it is believed that following DA depletion, serotonergic neurons of the raphe nuclei convert exogenously administered L-DOPA to DA and release it into the striatum in a pulsatile, unregulated manner (Carta et al., 2007; Eskow et al., 2009; Lindgren et al., 2010). This aberrant release of DA is believed to stimulate supersensitive DA D1 (D1R) and D2 (D2R) receptors located in the DA-depleted striatum (Pavese et al., 2006; Cenci, 2007). While both receptor subtypes appear to be involved in LID, it is likely that striatal D1R have a more prominent role (Westin et al., 2007). For instance, striatal D1R expression and signaling have been shown to be significantly enhanced in dyskinetic animals and humans (Cenci et al., 1998; Gerfen et al., 2002; Aubert et al., 2005; Guigoni et al., 2007), and D1R agonists induce dyskinesia in both experimental and clinical models of PD (Rascol et al., 2001, 2006; Delfino et al., 2007; Dupre et al., 2007, 2008a).

It is well known that serotonin (5-HT) 1A receptor (5-$HT_{1A}$R) agonists diminish LID (Dekundy et al., 2007; Eskow et al., 2007, 2009) and these results have been mostly attributed to stimulation of raphe 5-$HT_{1A}$R that temper striatal DA release. Interestingly, there is also evidence that stimulation of 5-$HT_{1A}$R located directly within the striatum attenuates both L-DOPA- (Bishop et al., 2009) and D1R-mediated dyskinesia (Dupre et al., 2008a) and improves movement in DA-depleted rats (Mignon & Wolf, 2002; Matsubara et al., 2006; Dupre et al., 2008a). The mechanism(s) surrounding these striatally-

*Abbreviations:* aCSF, Artificial cerebral spinal fluid; AIMs, abnormal involuntary movements; Benserazide, DL-Serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride; Cc, corpus callosum; Cpu, caudate putamen; D1R, D1 receptor; D2R, D2 receptor; DA, Dopamine; DMSO, Dimethyl sulfoxide; DOPAC, 3,4-dihydroxyphenylacetic acid; DPAT, ±8-OH-DPAT; EDTA, ethylenediaminetetraacetic acid; HPLC-ED, high performance liquid chromatography coupled to electrochemical detection; 5-HT, Serotonin; 5-$HT_{1A}$R, Serotonin 1A receptor; L-DOPA, L-3,4-dihydroxyphenylalanine methyl ester; LID, L-DOPA-induced dyskinesia; LV, lateral ventricle; β-ME, β-mercaptoethanol; MFB, medial forebrain bundle; mg, milligram; NaCl, sodium chloride; ng, nanogram; OPA, *o*-phthaldialdehyde; ±8-OH-DPAT, (±)-8-Hydroxy-2-(dipropylamino)tetralin hydrobromide; 6-OHDA, 6-hydroxydopamine hydrobromide; PFA, paraformaldehyde; PD, Parkinson's disease; SKF, SKF81297; SKF81297, R(+)-SKF-81297 hydrobromide; VEH, vehicle; WAY, WAY100635; WAY100635, *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt.

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mediated 5-$HT_{1A}$R effects are not yet known. One possibility is that activation of these receptors, located presynaptically on corticostriatal glutamate neurons, attenuate the release of glutamate into the striatum (Antonelli et al., 2005; Mignon & Wolf, 2005). Indeed, upon DA depletion and subsequent L-DOPA or D1R agonist treatment, augmentation of striatal glutamate levels (Jonkers et al., 2002; Robelet et al., 2004) and increased expression of striatal glutamate receptors have been postulated to result in dyskinetic behaviors (Calon et al., 2002; Ouattara et al., 2010). In support of this, a number of ionotropic and metabotropic glutamate receptor antagonists have been shown to reduce LID and improve D1R-mediated locomotor activity (Goodwin et al., 1992; Ferré et al., 1994; Bibbiani et al., 2005; Rylander et al., 2010; Kobylecki et al., 2010). Thus, whether striatal 5-$HT_{1A}$R stimulation attenuates LID and D1R agonist-induced dyskinesia through modulation of local glutamate release remains an important mechanistic and translational question.

The aim of the current study was to investigate the effects of systemic and local 5-$HT_{1A}$R stimulation on extracellular striatal glutamate levels in hemiparkinsonian rats rendered dyskinetic by either L-DOPA or the D1R agonist SKF81297. Using in vivo microdialysis, the full 5-$HT_{1A}$R agonist (±)-8-Hydroxy-2-(dipropylamino) tetralin hydrobromide (±8-OH-DPAT) was administered systemically or striatally perfused prior to L-DOPA (12 mg/kg, sc + benserazide, 15 mg/kg, sc) or administered systemically prior to R(+)-SKF-81297 hydrobromide (SKF81297; 0.8 mg/kg, sc) in unilaterally DA-depleted and sham-lesioned rats. Striatal sample fractions were collected for analysis of glutamate and dyskinesia was measured using the abnormal involuntary movements (AIMs) scale (Lundblad et al., 2002). The present results indicate that the anti-dyskinetic effect of 5-$HT_{1A}$R stimulation coincides with a reduction in extracellular striatal glutamate levels with L-DOPA, but not D1R agonist, treatment. These findings implicate a novel glutamatergic mechanism by which 5-$HT_{1A}$R agonists work to reduce LID with implications for the treatment of PD.

## Materials and methods

### *Animals*

Adult male Sprague–Dawley rats ($N = 61$) 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.

### *Medial forebrain bundle 6-hydroxydopamine lesion and microdialysis guide cannulae implantation surgeries*

One week after arrival, rats in Experiments 1 and 2 received unilateral DA ($n = 24$) or sham ($n = 18$) lesions of the left medial forebrain bundle (MFB). All rats in Experiment 3 received DA lesions of the left MFB ($n = 20$). Each rat was administered desipramine HCl (25 mg/kg, ip; Sigma, St. Louis, MO, USA) 30 min prior to surgery in order to protect norepinephrine 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 5.0 mm below interaural line (Paxinos & 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 vehicle (0.9% sodium chloride (NaCl) + 0.1% ascorbic acid) or 6-hydroxydopamine hydrobromide (6-OHDA; 3 µg/µl; Sigma) was injected at a rate of 2 µl/min for rats in Experiments 1 and 2, whereas all rats in Experiment 3 received 6-OHDA. The needle was withdrawn 5 min later. During the same surgery, rats were fitted unilaterally with plastic microdialysis guide cannulae (CMA 12 Elite; Stockholm, Sweden) targeting the dorsal striatum ipsilateral to the lesioned side (AP, +1.2 mm; ML, +2.5 mm; DV, −3.7 mm; relative to bregma; Paxinos & Watson, 1998). Cannulae were positioned and affixed to the skull with screws and liquid and powder dental acrylic (Lang Dental, Wheeling, IL). At the completion of surgery, animals were single housed, placed in clean cages and allowed to recover with ad lib food and water. Five minute pre-surgery and 1 h and 1 day post-surgery, rats received an injection of Buprenex (buprenorphine HCl; 0.03 mg/kg, ip; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA) as analgesic treatment. Soft chow was also provided and rats were monitored and handled twice per week for 3 weeks post-surgery in order to ensure full recovery and acclimation to experimenters.

### *Pharmacological treatments and in vivo microdialysis procedure*

#### *Experiment 1: effects of systemic 5-$HT_{1A}$R stimulation on extracellular striatal glutamate levels in L-DOPA-induced dyskinesia*

Three weeks after 6-OHDA ($n = 14$) or sham ($n = 11$) lesions of the MFB and unilateral striatal microdialysis cannulations, rats in the first experiment received injections of L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA; 12 mg/kg, sc; Sigma) + DL-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride (benserazide; 15 mg/kg, sc; Sigma) once daily for 7 days. The dose of L-DOPA and the length of priming have been extensively utilized in our lab to produce prominent and stable AIMs expression (Eskow et al., 2007; Dupre et al., 2008b; Bishop et al., 2009). L-DOPA and benserazide were dissolved in vehicle (0.9% NaCl + 0.1% ascorbic acid). On the final day of priming, AIMs (see description below) were observed every 20 min for 3 h immediately after L-DOPA injections. 6-OHDA-lesioned rats displaying an AIMs score of ≥25 by the 7th day of L-DOPA priming were retained for further study ($n = 12$).

Microdialysis testing commenced 2 days after the last day of L-DOPA priming. On test day, striatal probes (CMA 12 Elite; membrane length = 3 mm; 20 000 Dalton; Stockholm, Sweden) were inserted into rats' guide cannulae and locked into place so that the dialysis membrane extended −3.7 to −6.7 ventral to bregma within the striatum. After 60 min of probe stabilization (2.0 µl/min of artificial cerebral spinal fluid (aCSF) (in mM: 128 NaCl, 2.5 KCl, 1.3 $CaCl_2$, 2.1 $MgCl_2$, 0.9 $NaH_2PO_4$, 2.0 $Na_2HPO_4$, and 1.0 glucose, brought to a pH of 7.4), striatal dialysate samples were collected every 20 min for 40 min to determine baseline levels of glutamate. At this point, rats received a systemic treatment injection of vehicle (0.9% NaCl + 0.1% ascorbic acid, sc) and sample fractions were collected every 20 min for 2 h to determine any changes in extracellular glutamate levels due to systemic injection. Following this, using a counter-balanced design, rats received systemic treatment of: Vehicle (0.9% NaCl), the full 5-$HT_{1A}$R agonist ±8-OH-DPAT (1.0 mg/kg, sc; Sigma), or combined ±8-OH-DPAT (1.0 mg/kg, sc) + the 5-$HT_{1A}$R antagonist *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt (WAY100635; 0.5 mg/kg, sc; Sigma), immediately followed by L-DOPA (12 mg/kg, + benserazide, 15 mg/kg, sc). Sample fractions were collected every 20 min for 3 h and AIMs were concurrently observed during this time. Each rat underwent this microdialysis procedure for 2 consecutive days. No differences in glutamate nor AIMs were found in animals treated with Vehicle + L-DOPA on microdialysis test day 1 versus test day 2 (data not shown).