Behavioral and cellular dopamine D1 and D3 receptor-mediated synergy: Implications for L-DOPA-induced dyskinesia

Kathryn Lanza a, Samantha M. Meadows a, Nicole E. Chambers a, Emily Nuss a, Molly M. Deak a, Sergi Ferré b, Christopher Bishop a, *

a Behavioral Neuroscience Program, Department of Psychology, Binghamton University, 4400 Vestal Parkway East, Binghamton, NY 13902, USA
b National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Department of Health and Human Services, 251 Bayview Blvd #200, Baltimore, MD 21224, USA

Abstract

Individually, D1 and D3 dopamine receptors (D1R and D3R, respectively) have been implicated in L-DOPA-induced dyskinesia (LID). Of late, direct D1R-D3R interactions have been linked to LID yet remain enigmatic. Therefore, the current research sought to characterize consequences of putative D1R-D3R interactions in dyskinesia expression and in LID-associated downstream cellular signaling. To do so, adult male Sprague-Dawley hemi-parkinsonian rats were given daily L-DOPA (6 mg/kg; s.c.) for 2 weeks to establish stable LID, as measured via the abnormal voluntary movements (AIMs) scale. Thereafter, rats underwent dose-response AIMs testing for the D1R agonist SKF38393 (0, 0.3, 1.0, 3.0 mg/kg) and the D3R agonist, PD128907 (0, 0.1, 0.3, 1.0 mg/kg). Each agonist dose-dependently induced dyskinesia, implicating individual receptor involvement. More importantly, when threshold doses were co-administered, rats displayed synergistic exacerbation of dyskinesia. Interestingly, this observation was not mirrored in general locomotor behaviors, highlighting a potentially dyskinesia-specific effect. To illuminate the mechanisms by which D1R-D3R co-stimulation led to in vivo synergy, levels of striatal phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2) were quantified after administration of SKF38393 and/or PD128907. Combined agonist treatment synergistically drove striatal pERK1/2 expression. Together, these results support the presence of a functional, synergistic interaction between D1R and D3R that manifests both behaviorally and biochemically to drive dyskinesia in hemi-parkinsonian rats. © 2018 Elsevier Ltd. All rights reserved.

1. Introduction

For over half a century, dopamine (DA) replacement therapy by means of the DA precursor, L-DOPA, has been the most efficacious and non-invasive method of Parkinson’s Disease (PD) symptom management. However, chronic L-DOPA treatment often leads to the development of a spectrum of side effects including motor fluctuations and L-DOPA induced dyskinesia (LID) characterized by abnormal involuntary movements (AIMs; (Smith et al., 2012). About 90% of PD patients develop LID within their first decade of treatment and by year 15, a staggering 95% of patients display some LID (Ahlskog and Mueenter, 2001; Hely et al., 2005). Understanding the mechanisms that portend LID is crucial in the pursuit of better pharmacotherapy with less aversive side effects.

Abbreviations: DA, Dopamine; DOPAC, Dihydroxyphenylacetic acid; PD, Parkinson’s disease; LID, L-DOPA-Induced Dyskinesia; D1R, Dopamine D1 Receptor; D2R, Dopamine D2 Receptor; D3R, Dopamine D3 Receptor; PLA, Proximity ligation assay; pERK1/2, phosphorylated extracellular signal-regulated kinase; ERK1/2, Extracellular signal-regulated kinase; cAMP, cyclic adenosine monophosphate; 6-OHDA, 6-hydroxydopamine hydrobromide; MFB, Medial forebrain bundle; AIMs, Abnormal involuntary movements; ALD, Axial limb and orolingual; FAS, Forepaw adjusting steps; HPLC, High performance liquid chromatography; M.A.D., Mean absolute deviation; S.E.M., Standard error of the mean.

* Corresponding author.
E-mail addresses: KLanza1@binghamton.edu (K. Lanza), Smeadow3@binghamton.edu (S.M. Meadows), Nchambe4@binghamton.edu (N.E. Chambers), Enuss1@binghamton.edu (E. Nuss), Mdeak@binghamton.edu (M.M. Deak), Sferrere@intra.nida.nih.gov (S. Ferré), Cbishop@binghamton.edu (C. Bishop).
therapeutic and dyskinetic components of L-DOPA therapy (Chiken et al., 2015; Gomez-Mancilla and Bedard, 1991; Grondin et al., 1999).

Until more recently, the role of D2 receptors (D2R) in LID has been understudied. This is, in part, because within the intact dorsal striatum of the rat, D2R is sparsely expressed (Ridray et al., 1998). However, D2R is significantly upregulated within this area following L-DOPA administration where they co-localize with D1R-expressing neurons (Bordet et al., 1997; Farre et al., 2015; Solis et al., 2015). This shift is of particular importance considering D2R displays the highest affinity for DA and consequently, expression changes can significantly influence dopaminergic tone (Sokoloff et al., 1990). In fact, overexpression of D2R in rats lacking DA lesions is sufficient to precipitate L-DOPA-induced dyskinesia (Cote et al., 2014; Cote and Kuzhiikandathil, 2015). Furthermore, mice lacking D2R have tempered LID development (Cote et al., 2014; Solis et al., 2015). Although D2R is clearly involved in dyskinesia manifestation, pharmacological interrogation of its role has been both challenging and inconsistent. Some report that LID is resistant to D2R antagonism or interferes with L-DOPA efficacy (Hsu et al., 2004; Mela et al., 2010; Silverdale et al., 2004), whereas others report that normalizing D2R activity can attenuate LID expression (Bordet et al., 2005; Sebastianutto et al., 2016; Visanji et al., 2009). Fortunately, new DA receptor targets have emerged that have reinvigorated the field (Cortes et al., 2016). More specifically, direct interactions between G-protein-coupled receptors have emerged as promising therapeutic targets (Ferre et al., 2010, 2014). There is strong evidence that D1R and D2R interact as one of these complexes. Upregulation of D2R mRNA is induced by D1R agonist treatments alone and L-DOPA-induced changes in D2R expression are attenuated with the co-administration of a D1R antagonist, providing strong evidence of not only an interaction between these two receptors, but also a possible link between their activation states and cross receptor mediated effects (Bordet et al., 1997). More recently, in situ proximity ligation assay (PLA) showed D1R and D2R co-localization increased within the striatum of dyskinetic rats and monkeys (Farre et al., 2015). That D1R and D2R could form heteromers in the striatum was also suggested by previous co-immunoprecipitation and radioligand binding experiments (Fiorentini et al., 2008; Marcellino et al., 2008). In those studies, D1R-D2R heteromerization was demonstrated in transfected cells with bioluminescence and fluorescence resonance energy transfer techniques (BRET and FRET, respectively). Taken together, there is a substantial amount of evidence supporting the presence of striatal D1R-D2R cooperativity, potentially in the form of heteromers, and their role in LID expression, but precise behavioral consequences have yet to be characterized.

Despite compelling in vitro support for D1R-D2R interactions, in vivo work has been limited. Therefore, the present study utilized a unilateral 6-OHDA-lesioned rat model to investigate the behavioral consequences of D1R-D2R co-activation via receptor specific agonists. We also evaluated signaling changes in phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2), as enhanced pERK1/2 expression has been consistently observed in the dyskinetic striatum across multiple models of LID and PD (Fasano et al., 2010; Lindgren et al., 2009; Pavon et al., 2006; Santini et al., 2007; Westin et al., 2007). We hypothesized that D1R and D2R agonist co-administration would produce synergistic increases in dyskinesia expression. In line with previous in vitro work, we also predicted that animals co-administered both D1R and D2R agonists would display synergistic enhancement of pERK1/2 compared to the additive effect of those treated with D1R or D2R agonist alone. Ultimately, the present research provides in vivo support of D1R-D2R cooperativity and resultant synergistic behavioral and biochemical properties.

2. Methods and materials

2.1. Animals

Adult male Sprague–Dawley rats (300–400 g) were used throughout the experiments (N = 55). Animals were pair housed in plastic cages (22 × 45 × 23 cm) and were allowed free access to both food (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA) and water. Rats were maintained on a 12/12 light/dark cycle beginning at 07:00 h in a temperature-controlled room (22–23 °C). Rats were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Binghamton University and the “Guide for the Care and Use of Laboratory Animals” (Institute for Laboratory Animal Research, National Academies Press, 2011).

2.2. 6-Hydroxydopamine lesion surgeries

All rats underwent a unilateral DA lesion with 6-hydroxydopamine hydrobromide (6-OHDA; Sigma) 6-OHDA; Sigma, St Louis, MO, USA) infused in the left medial forebrain bundle (MFB), a procedure previously described in (Conti et al., 2014; Meadows et al., 2017). In brief, 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) with the incisor bar positioned at 5.0 mm below the interaural line. The targeted site, relative to bregma, was: AP, −1.8 mm; ML, + 2.0 mm; DV, −8.6 mm. A 10 μL Hamilton syringe attached to a 26 gauge needle was lowered into the target through a small hole in the skull and then used to deliver 6-OHDA (3 μg/μL; Sigma) dissolved in 0.9% NaCl + 0.1% ascorbic acid at a rate of 2 μL/min, for a total volume of 4 μL. Five minutes later, the needle was withdrawn. To minimize pain and discomfort buprenex (buprenorphine HCL; 0.03 mg/kg, i.p.; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA) was administered before surgery and the following day. After surgery, animals were pair-housed, placed in warmed, clean cages and monitored for a post-operative period of 10 days in which they received soft food, fruit, and fluid replacement as needed to facilitate recovery. All experiments began 3 weeks post-surgery to allow for sufficient recovery time.

2.3. Experimental designs

2.3.1. Pharmacological treatments

Three weeks after surgery, all lesioned animals received a daily subcutaneous injection of L-DOPA methyl ester (6 mg/kg; Sigma) + DL-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride (benserazide; 15 mg/kg; Sigma), dissolved in vehicle (0.9% NaCl + 0.1% ascorbic acid), for a 2-week priming period in order to establish stable LID expression (Fig. 1; (Lindgren et al., 2007; Putterman et al., 2007). D1R and/or D2R were stimulated individually by agonist administration. The D1R partial agonist, SKF38393 (SKF; Sigma) has been shown to induce dyskinesia individually, with selective D1R antagonists attenuating this effect (Monville et al., 2005). D3R agonist PD128907 (PD; Tocris) has been shown to bind to the D3R receptor preferentially over other DA subtypes. Most importantly, it displays higher D3R affinity compared to the pharmacologically similar DA D2 receptor (D2R; Cote and Kuzhiikandathil, 2014; Pugsley et al., 1995). Both agonists were dissolved in dH2O (vehicle) and administered at an injection volume of 1 ml/kg subcutaneously.

2.3.2. Experiment 1: establishment of a dose response for DA agonist-induced dyskinesia

The timeline of Experiment 1 is described in Fig. 1A. Following surgery, recovery, and priming, rats (n = 9) entered Experiment 1 to...