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Research Paper

Characterizing the differential roles of striatal 5-HT$_{1A}$ auto- and hetero-receptors in the reduction of L-DOPA-induced dyskinesia

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ABSTRACT

L-DOPA remains the benchmark treatment for Parkinson's disease (PD) motor symptoms, but chronic use leads to L-DOPA-induced dyskinesia (LID). The serotonin (5-HT) system has been established as a key modulator of LID and 5-HT$_{1A}$ receptors (5-HT$_{1A}$R) stimulation has been shown to convey anti-dyskinetic effects. However, 5-HT$_{1A}$R agonists often compromise clinical efficacy or display intrinsic side effects and their site(s) of actions remain debatable. Recently, highly selective G-protein biased 5-HT$_{1A}$R agonists, F13714 and F15599, were shown to potently target 5-HT$_{1A}$ auto- or hetero-receptors, respectively. The current investigation sought to identify the signaling mechanisms and neuroanatomical substrates by which 5-HT$_{1A}$R produce behavioral effects. In experiment 1, hemi-parkinsonian, L-DOPA-primed rats received systemic injections of vehicle, F13714 (0.01 or 0.02 mg/kg), or F15599 (0.06 or 0.12 mg/kg) 5 min prior to L-DOPA (6 mg/kg), after which LID, motor performance and 5-HT syndrome were rated. Both compounds significantly reduced LID, without affecting motor performance, however, acute administration of F13714 significantly induced 5-HT syndrome at anti-dyskinetic doses. In experiment 2, we elucidated the role of striatal 5-HT$_{1A}$R in the effects of F13714 and F15599. Hemi-parkinsonian, L-DOPA-primed rats received bilateral intra-striatal microinjections of either F13714 (0, 2 or 10 μg/side) or F15599 (0, 10 or 30 μg/side) 5 min prior to systemic L-DOPA (6 mg/kg). Intra-striatal effects mimicked systemic effects, suggesting that striatal 5-HT$_{1A}$R subpopulations play an important role in the anti-LID and pro-5-HT syndrome profiles of F13714 and F15599. Finally, in experiment 3, we examined the effects of F13714 and F15599 on D$_1$ receptor (D$_1$R) agonist-induced dyskinesia by administering either compound 5 min prior to SKF 38393 (2 mg/kg). While F13714 resulted in a mild delay in D$_1$R-mediated dyskinesia, F15599 had no effect. Collectively these data suggest that the F-series compounds articulate their anti-LID effects through activation of a diverse set of striatal 5-HT$_{1A}$ hetero-receptor populations.

1. Introduction

Since the 1960s, dopamine (DA[1]) replacement therapy, with the DA precursor L-3,4-dihydroxyphenylalanine (L-DOPA), has remained the gold standard for alleviating akinetic motor symptoms in Parkinson's disease (PD; Smith et al., 2012). However, in a majority of PD patients, chronic L-DOPA use is complicated by the development of abnormal involuntary movements (AIMs), referred to as L-DOPA-induced dyskinesia (LID; Obeso et al., 2000).

Over the past several years, mounting evidence has implicated raphe-striatal serotonin (5-HT) neurons as surrogates to the compromised DA system, since they possess the necessary machinery to take up exogenous L-DOPA and convert it into DA for synaptic release (Arai et al., 1994; Carta et al., 2007; Kannari et al., 2003; Keber et al., 2015; Nahimi et al., 2012; Nevalainen et al., 2014; Rylander et al., 2010; Tanaka et al., 1999; Yamada et al., 2007). However, 5-HT neuron release of DA becomes unregulated due to a lack of feedback mechanisms, like DA transporters (DAT) and DA D$_2$ auto-receptors (Maeda et al., 1999), prolonging activation of sensitized DA receptors and exacerbating LID expression with chronic L-DOPA treatment (Maeda et al., 2003; Maeda et al., 2005). As such, researchers have sought to temper L-DOPA-

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[1] DA, dopamine; L-DOPA, L-3,4-dihydroxyphenylalanine; PD, Parkinson's disease; AIMs, abnormal involuntary movement; LID, L-DOPA-induced dyskinesia; 5-HT, serotonin; DAT, dopamine transporter; 5-HT$_{1A}$R, serotonin 1A receptor; MSNs, medium spiny neurons; ChI, cholinergic interneuron; 6-OHDA, 6-hydroxydopamine; FAS, forepaw adjusting steps; ALO, axial, limb, and orolingual; HPLC-ED, high performance liquid chromatography with electrochemical detection; DOPAC, 3,4-dihydroxyphenlyacetic acid; M.A.D., median absolute deviation; S.E.M., standard mean error; DRN, dorsal raphe nucleus; PET, positron emission topography; PPD, preprodynorphin; GAD67, glutamic acid decarboxylase 67 kDa isoform.

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derived DA release from striatal 5-HT terminals in order to alleviate LID expression.

The most studied candidate for modulating raphe-striatal function has been the 5-HT_{1A} receptor (5-HT_{1A}R). These inhibitory, G-protein coupled receptors are ubiquitously expressed throughout the brain (Barnes and Sharp, 1999). 5-HT_{1A}R agonists have been shown to substantially reduce LID in parkinsonian rat and primate models (Bibbiani et al., 2001; Carta et al., 2007; Eskow et al., 2007; Lindenbach et al., 2015). However, the clinical use of 5-HT_{1A}R agonists, like sarizotan and tandospirone, has been limited by modest effects and worsening parkinsonism in some patient populations (Goetz et al., 2007; Kannari et al., 2002; Merck KGaA, 2006; Olanow et al., 2004; Svenningsson et al., 2015). These findings are likely attributable to off-target antagonistic effects at DA D_2, D_3, and D_4 receptors and excessive agonist stimulation of 5-HT_{1A} auto-receptors which may suppress L-DOPA-derived 'false neurotransmitter' DA release to an extent that compromises L-DOPA efficacy (Bartoszyk et al., 2004; Eskow et al., 2009; Hamik et al., 1990; Kannari et al., 2001). The pre-clinical effects of more selective 5-HT_{1A}R agonists, like +8-OH-DPAT, are also complicated by increased rigidity and akinesia, though this may, in part, reflect the induction of 5-HT syndrome, a debilitating motor side effect seen in rodents and primates attributed to post-synaptic 5-HT_{1A}R stimulation (Diaz and Maroteaux, 2011; Iravani et al., 2006; Jacobs and Klemfuss, 1975; Lindenbach et al., 2015). Although 5-HT_{1A}R-mediated 5-HT syndrome is rare in humans (Habberzettl et al., 2013), such collective limitations have slowed the translation of 5-HT_{1A}R agonists to clinical employment.

5-HT_{1A}R populations express functional G-protein heterogeneity, likely responsible for regionally-specific 5-HT_{1A}R agonist effects. Agonists could act at auto-receptors on 5-HT neurons, directly blunting 'false neurotransmitter' DA release, or at hetero-receptors residing on non-5-HT neurons, reducing LID by altering other neurotransmitter systems, like glutamate (Dupre et al., 2011; Eskow et al., 2007; Lindgren et al., 2010; Ostock et al., 2011). Relatedly, 5-HT_{1A} auto- and hetero-receptors have differential G-protein-mediated effects on downstream signal transduction, allowing the identification of functionally selective 'biased' 5-HT_{1A}R agonists for more precise pharmacologic and neuroanatomical manipulations (Newman-Tancredi, 2011; Kenakin, 2011). While 5-HT_{1A} auto-receptors reside on raphe-striatal projection neurons, there is little to no evidence that they exist in the striatum (Azmitia et al., 1996). On the other hand, striatal 5-HT_{1A} hetero-receptors are more abundant and seem to be located on a combination of cortico-striatal glutamatergic neurons (Cruz et al., 2004; Huot et al., 2012), medium spiny neurons (MSNs; Ghavami et al., 1999), and cholinergic interneurons (ChIs; Virk et al., 2016). Given the central role of the striatum in LID, precise pharmacologic tools, such as the G-protein biased agonists F13714 and F15599, are invaluable for clarifying the role(s) of these local 5-HT_{1A}R populations. Initial characterizations of F13714 and F15599 revealed regionally specific electrophysiological and neurochemical effects in raphe and cortex, respectively, leading to the conclusion that at low doses these compounds are selective for either 5-HT_{1A} auto- or hetero-receptor (Assié et al., 2006; Buritova et al., 2009; Lladó-Pelfort et al., 2010). Each compound exhibits a unique control of signal transduction responses whereby F13714 shows secondary potency for stimulating h5-HT_{1A} receptor internalization, purportedly inducing rapid onset of 5-HT_{1A} desensitization, while F15599 has secondary potency for G-protein activation in extra-raphe structures (Assié et al., 2006; Newman-Tancredi et al., 2009). Previous studies have assessed the ability of these compounds to modify LID (Iderberg et al., 2015) and elicit rodent 5-HT syndrome (Assié et al., 2010), yet very little work has examined the temporal profile for 5-HT syndrome and its association with LID reductions. Moreover, while some striatal microdialysis data has been collected on these compounds, no previous studies have delivered these drugs directly into the striatum to determine site-specific contributions of 5-HT_{1A}R sub-populations to LID reduction.

The present study sought to determine whether systemic and/or local striatal administration of functionally selective 5-HT_{1A}R biased agonists would provide anti-LID effects, with minimal side-effect profiles. Additionally, the DA D_1R agonist SKF38393 was used as a tool to determine the contribution of 5-HT_{1A} heteroreceptors on D_1R-bearing MSNs to the anti-dyskinetic actions of the F-compounds. The current findings demonstrate that both compounds exert anti-LID effects in the striatum, but with varying 5-HT syndrome profiles and differential effects on D_1R agonist stimulated dyskinesia highlighting the roles of 5-HT_{1A}R populations in the dyskinetic brain.

2. Materials and methods

2.1. Animals

The study utilized adult male Sprague Dawley rats (N = 35; 225–250 g upon arrival, Harlan Farms, NY, USA) housed in plastic cages (22 cm high, 45 cm deep, and 23 cm wide) with free access to standard laboratory chow (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA) and water. The colony room was maintained on a 12 h light/dark cycle (light on at 0700 h) at a temperature of 22–23 °C. Animals 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 of Laboratory Animal Resources, National Academic Press, 2011).

2.2. Medial forebrain bundle 6-hydroxydopamine lesion and bilateral striatal cannulation surgery

All rats underwent surgery to receive unilateral 6-hydroxydopamine (6-OHDA) lesions to the left medial forebrain bundle. Five minutes prior to surgery and the morning after, rats were injected with buprenex (buprenorphine HCl; 0.03 mg/kg, i.p.; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA) to minimize pain. 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 (Paxinos and Watson, 1998). A 10 μL Hamilton syringe attached to a 26 gauge needle was lowered into the target through a small burr 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. The needle was withdrawn 5 min post-injection to allow time for fluid diffusion. During the same surgery, animals in experiment 2 were fitted with bilateral 15 mm guide cannulae (22 gauge, C313/G/SPC; Plastics One Inc., Roanoke, VA), targeting the dorsal striatum (AP, +0.4 mm; ML, ±2.9 mm; DV, −3.6 mm; relative to bregma; Paxinos and Watson, 1998). Cannulae were affixed to the skull with screws and Jet Denture Repair Acrylic (Lang Dental, Wheeling, IL). The guide cannulae were then fitted with 28-gauge inner stylets (Plastics One) to avoid cannulae obstruction and infection. Upon completion, animals in experiment 1 and 3 were dual-housed, while rats in experiment 2 were single-housed, placed in clean cages and given a 21-day recovery period with ad lib food and water. Prior to all testing, animals were handled to minimize stress during the experiment.

2.3. Pharmacological treatments and priming

Three weeks post-lesion surgery, rats were tested on the Forepaw Adjusting Steps test (FAS; see below) on 2 separate occasions to establish baseline motor performance and lesion severity. Lesioned animals in experiments 1 (Fig. 1A), 2 (Fig. 1B), and 3 (Fig. 1C) were administered L-DOPA methyl ester (L-DOPA; 6 mg/kg, s.c.; Sigma) + DL-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride (benserazide;