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Research report

Effects of noradrenergic denervation by anti-DBH-saporin on behavioral responsivity to L-DOPA in the hemi-parkinsonian rat

Corinne Y. Ostock, David Lindenbach, Adam A. Goldenberg, Elias Kampton, Christopher Bishop*

Behavioral Neuroscience Program, Department of Psychology, Binghamton University, Binghamton, NY, USA

HIGHLIGHTS

- αDBH treatment produced severe loss of noradrenergic neurons in the locus coeruleus.
- Norepinephrine loss did not significantly influence dyskinesia in parkinsonian rats.
- Norepinephrine lesions reduced therapeutic effects of L-DOPA in parkinsonian rats.

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ABSTRACT

Dopamine (DA) replacement with L-DOPA remains the most effective pharmacotherapy for motor symptoms of Parkinson's disease (PD) including tremor, postural instability, akinesia, and bradykinesia. Prolonged L-DOPA use frequently leads to deleterious side effects including involuntary choreic and dystonic movements known as L-DOPA induced dyskinesias (LID). DA loss in PD is frequently accompanied by concomitant noradrenergic (NE) denervation of the locus coeruleus (LC); however, the effects of NE loss on L-DOPA efficacy and LID remain controversial and are often overlooked in traditional animal models of PD. The current investigation examined the role of NE loss in L-DOPA therapy by employing the NE specific neurotoxin anti-DA-beta hydroxylase saporin (αDBH) in a rat model of PD. Rats received unilateral 6-hydroxydopamine lesions of the medial forebrain bundle to deplete nigral DA and intraventricular injection of vehicle (DA lesioned rats) or αDBH (DANE lesioned rats) to destroy NE neurons bilaterally. Results indicated that αDBH infusion drastically reduced NE neuron markers within the LC compared to rats that received vehicle treatment. Behaviorally, this loss did not alter the development or expression of L-DOPA- or DA agonist-induced dyskinesia. However, rats with additional NE lesions were less responsive to L-DOPA's pro-motor effects. Indeed, DANE lesioned animals rotated less and showed less attenuation of parkinsonian stepping deficits following high doses of L-DOPA than DA lesioned animals. These findings suggest that severe NE loss may reduce L-DOPA treatment efficacy and demonstrate that degradation of the NE system is an important consideration when evaluating L-DOPA effects in later stage PD.

Abbreviations: 6-OHDA, 6-hydroxydopamine; α2R, α2-noradrenergic receptor; αDBH, anti-dopamine-beta-hydroxylase saporin; ABC, avidin–biotin–peroxidase complex; AIMs, abnormal involuntary movements; ALO, axial, forelimb, orolingual; DA, dopamine; FAS, forepaw adjusting steps; ICV, intraventricular; LC, locus coeruleus; LIDL-, DOPA-induced dyskinesia; MAD, median absolute deviation; MFB, medial forebrain bundle; NE, norepinephrine; NET, norepinephrine transporter; PBS, phosphate buffered saline; PD, Parkinson's disease; SEM, standard error of the mean; SNsubst, antia nigra; TH, tyrosine hydroxylase.

* Corresponding author at: Department of Psychology, Binghamton University, PO Box 6000, Binghamton, NY 13902-6000, USA. Tel.: +1 607 777 3410; fax: +1 607 777 4890.

E-mail addresses: costock1@binghamton.edu (C.Y. Ostock), dlinden1@binghamton.edu (D. Lindenbach), agolden1@binghamton.edu (A.A. Goldenberg), ekampton@gmail.com (E. Kampton), cbishop@binghamton.edu (C. Bishop).

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1. Introduction

Parkinson's disease (PD) is progressive neurodegenerative movement disorder traditionally characterized by the loss of dopamine (DA) neurons in the nigrostriatal pathway. However, over the last century it is has become increasingly apparent that PD pathology extends far beyond the DA system. Indeed, noradrenergic cell loss in the locus coeruleus (LC) precedes and is equal to, if not greater than, DA loss within the substantia nigra pars compacta (SN) in PD [1]. Noradrenergic loss has been linked to a number of cardinal motor symptoms of PD and purportedly exacerbates parkinsonian motor deficits [2–6].

Despite these findings, most pharmacological treatments for PD are aimed solely at restoring central DA function. Long-term DA replacement, especially with the DA precursor L-DOPA, is often complicated by the emergence of debilitating motor side effects, notably L-DOPA-induced dyskinesia (LID), typified by hyperkinetic involuntary movements [7]. LID has largely been attributed to pre- and post-synaptic changes in striatal DA neurotransmission leading to aberrant basal ganglia signaling [8–10]. The noradrenergic system innervates nearly all nuclei of the basal ganglia and has recently been implicated in LID by several lines of research. Recent work indicates that LID severity is positively correlated with basal firing parameters of LC neurons [11] and direct infusion of exogenous norepinephrine (NE) into the striatum induces dyskinesia in L-DOPA-primed hemiparkinsonian rats [12]. There is also evidence that the NE transporter (NET) can take up DA and may play an integral role in clearing L-DOPA-derived DA following the loss of striatal DA-transporters in PD [13]. Finally, several compounds that target the NE system have been shown to reduce LID in experimental and clinical populations [14–16]. Unfortunately, a paucity of systematic basic research exists regarding the direct impact of NE cell loss within the LC on the development and expression of LID.

Animal models of LID have focused on creating severe lesions that are specific to the nigrostriatal DA pathway. However, most do not display or account for NE cell loss typically observed in the human condition. In fact, the NET blocker desipramine is frequently given prior to 6-OHDA infusion to prevent noradrenergic cell loss in rodent models of PD. To date, only a few studies have directly examined whether the state of the noradrenergic system influences LID symptoms. These studies have thus far produced contradictory behavioral effects with some reporting that additional NE loss increased [11,17], decreased [15], or did not change [11,18] the severity or duration of LID expression in experimental PD models.

The selective NE neurotoxin anti-DA beta-hydroxylase saporin (αDBH) effectively destroys NE neurons [19] but has not yet been investigated in a Parkinsonian model. αDBH consists of a monoclonal antibody for DA beta-hydroxylase (DBH) conjugated to the ribosomal-inactivating protein saporin [19,20]. During neurotransmitter release, αDBH molecules bind to vesicular DBH enzymes and following endocytosis undergo retrograde transport to the cell body where saporin inactivates the ribosomes preventing protein synthesis ultimately resulting in NE cell death [21]. Therefore, the goal of the current study was to systematically address the role of NE loss on L-DOPA-mediated behaviors by characterizing differences in the development and expression of LID and motor performance in hemiparkinsonian rats using well established behavioral techniques.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats were used ($N = 30$; 225–250 g upon arrival; Harlan, USA). Animals were pair-housed in plastic cages (22 cm high, 45 cm deep, and 23 cm wide) and had free access to water and standard lab chow (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA). The colony room was maintained on a 12/12 h light/dark cycle (lights on at 0700 h) at a temperature of 22–23 °C. Throughout the study, animals were cared for in full accordance with the guidelines of the Institutional Animal Care and Use Committee of Binghamton University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Systemically administered drugs were given at a volume of 1 ml/kg and were injected subcutaneously unless otherwise noted. All drugs were acquired from Sigma (St. Louis, MO, USA) unless specified differently. 6-Hydroxydopamine (6-OHDA) and l-3,4-dihydroxyphenylalanine methyl-ester + dl-serine 2-(2,3,4-trihydroxybenzyl) hydrazide HCL (benserazide) (L-DOPA) were dissolved in 0.9% NaCl + 0.1% ascorbic acid. Benserazide was always administered at a concentration of 15 mg/kg, regardless of L-DOPA concentration. Buprenorphine HCL (Rechitt Benckiser Pharmaceuticals Inc., Richmond, VA) was suspended in saline (0.9% NaCl). Desipramine HCl, d-amphetamine, and quinpirole were dissolved in dH_2O. SKF81297 was dissolved in 20% DMSO. Anti-DBH saporin (αDBH) was purchased from Millipore (Billerica, MA) and came pre-suspended in phosphate-buffered saline (PBS).

2.3. Stereotaxic surgery

One week after arrival, (day −21) rats were randomly assigned to one of two lesion groups as follows: (1) αDBH-saporin NE lesion + 6-OHDA DA lesion (DANE-lesion; $n = 15$); or (2) sham NE lesion + 6-OHDA DA lesion (DA-lesion; $n = 15$). Briefly, rats were anesthetized with inhalant isoflurane (2–3%) in oxygen (1000 cc/min) and placed in a stereotaxic apparatus. All rats received the analgesic buprenorphine HCl (0.03 mg/kg, ip) prior to surgery and the day after surgery. NE- or sham-lesions were produced by infusing the NE selective neurotoxin αDBH (10 μg/10.2 μl; based on [19]) or its vehicle (10.2 μl) into the left lateral ventricle using the following coordinates relative to bregma: AP, −0.8 mm; ML 1.4 mm; DV, −3.9 mm with the interaural line at 0 [28]. Immediately after, unilateral DA lesions were produced in all rats by infusing the neurotoxin 6-OHDA (12 μg/4 μl) directly into the left medial forebrain bundle (MFB) using the following coordinates relative to bregma: AP, −1.8 mm; ML, 2.0 mm; DV, −8.6 mm. Drugs were infused at a rate of 2 μl/min and the needle was left in place for 5 min after infusion to allow for drug dispersal. All rats received Desipramine HCl (25 mg/kg, ip) 30 min prior to 6-OHDA injection to ensure that destruction of NE neurons was induced by αDBH-saporin-, not 6-OHDA. Stainless steel wound clips were used to close the surgical site and animals were returned to group housing. Animals were allowed to recover with ad lib. food and water and soft chow was provided as needed to facilitate recovery during the first week after surgery.

2.4. Experimental procedure and design

As shown in Fig. 1, following recovery from surgery, rats underwent a battery of behavioral tests in order to characterize the behavioral outcome of NE lesions on L-DOPA-, and DA agonist-induced motor behaviors.

Two weeks after surgery (day −7), all rats were tested using amphetamine-induced rotations in order to assess DA lesion severity and to determine whether additional noradrenergic loss altered responsiveness to acute d-amphetamine treatment (see Section 2.5.1). During the next 5 d (day −6 to −1), rats were acclimated to the forepaw adjusting steps (FAS) testing procedure (see Section