L-DOPA Treatment Selectively Restores Spine Density in Dopamine Receptor D2–Expressing Projection Neurons in Dyskinetic Mice

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Background: L-3,4-dihydroxyphenylalanine (L-DOPA)–induced dyskinesia is an incapacitating complication of L-DOPA therapy that affects most patients with Parkinson's disease. Previous work indicating that molecular sensitization to dopamine receptor D_1 (D1R) stimulation is involved in dyskinesias prompted us to perform electrophysiological recordings of striatal projection "medium spiny neurons" (MSN). Moreover, because enhanced D1R signaling in drug abuse induces changes in spine density in striatum, we investigated whether the dyskinesia is related to morphological changes in MSNs.

Methods: Wild-type and bacterial artificial chromosome transgenic mice (D1R-tomato and D2R–green fluorescent protein) mice were lesioned with 6-hydroxydopamine and subsequently treated with L-DOPA to induce dyskinesia. Functional, molecular, and structural changes were assessed in corticostriatal slices. Individual MSNs injected with Lucifer-Yellow were detected by immunohistochemistry for three-dimensional reconstructions with Neurolucida software. Intracellular current-clamp recordings with high-resistance micropipettes were used to characterize electrophysiological parameters.

Results: Both D1R-MSNs and D2R-MSNs showed diminished spine density in totally denervated striatal regions in parkinsonian mice. Chronic L-DOPA treatment, which induced dyskinesia and aberrant FosB expression, restored spine density in D2R-MSNs but not in D1R-MSNs. In basal conditions, MSNs are more excitable in parkinsonian than in sham mice, and excitability decreases toward normal values after L-DOPA treatment. Despite this normalization of basal excitability, in dyskinetic mice, the selective D1R agonist SKF38393 increased the number of evoked action potentials in MSNs, compared with sham animals.

Conclusions: Chronic L-DOPA induces abnormal spine re-growth exclusively in D2R-MSNs and robust supersensitization to D1R-activated excitability in denervated striatal MSNs. These changes might constitute the anatomical and electrophysiological substrates of dyskinesia.

Key Words: Behavioral sensitization, dyskinesia, L-DOPA, medium spiny neuron, Parkinson's disease, striatum, three-dimensional neuronal reconstruction

An almost complete loss of dopaminergic fibers in the motor region of the striatum results in the profound akinesia that characterizes advanced Parkinson's disease (PD) (1). At this stage most patients need chronic L-3,4-dihydroxyphenylalanine (L-DOPA) therapy, and many will develop L-DOPA-induced dyskinesia (2). Knowledge gained in recent years about the molecular mechanisms underlying dyskinesias has not yet resulted in improved therapies.

This might be due in part to the occurrence of structural changes in the striatal microcircuit. Postmortem studies have shown decreased total length of medium spiny neuron (MSN) dendrites in patients with advanced PD treated with L-DOPA (3). Subsequent studies revealed a decrease in spine density in MSNs of L-DOPA-treated patients (4,5). In parallel, Ingham *et al.* (6,7) showed a reduction of spine density in MSNs in rats with unilateral nigrostriatal lesion, a finding confirmed in additional animal models of PD (8–10). Although pruning of dendritic spines in striatal neurons at late stages of PD and in animal models of PD has been repeatedly reported, it is not clear whether it is modified by L-DOPA therapy.

Importantly, sensitization of dopamine receptor D_1 (D1R) signaling cascade in MSNs is causally related to the dyskinesias (11–17). A very similar D1R sensitization occurs in drug abuse (18,19), but enhanced D1R signaling in addiction induces an increase in spine density in nucleus accumbens MSNs (20–25). Thus, D1R sensitization might induce similar structural changes in dyskinesias and drug abuse. Here we ask whether the dyskinesias are related to changes in the dendritic arbor of striatal MSNs. Moreover, because molecular sensitization to D1R stimulation is associated with an enhanced metabolic response of striatum to D1 agonists (15), we tested the hypothesis that striatal MSNs are more excitable in dyskinetic mice. We used a mouse model of parkinsonism induced by unilateral injection of 6-hydroxydopamine (6-OHDA) in the adult striatum (16,26). The restricted pattern of nigrostriatal degeneration affecting the motor district of the striatum in this animal model allowed us to correlate morphological and functional changes in MSN to the degree of striatal denervation and to test whether the dyskinesias are associated with any additional alteration of MSN dendrites.

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Methods and Materials

This study was carried out in 3–4-month-old male C57BL6 wild-type (WT) and bacterial artificial chromosome (BAC)-transgenic mice (D1R-tomato or D2R–enhanced green fluorescent protein [eGFP]). Animals were housed and maintained following the guidelines from European Union Council Directive (86/609/European Economic Community). Mice received an intrastriatal 6-OHDA (Sigma-Aldrich, Madrid, Spain) injection (parkinsonian group) or vehicle (sham group), as described previously (26). The mice recovered for 2–3 weeks and then began saline or L-DOPA treatment for 15 days (dyskinetic group). The treatment consisted of a daily intraperitoneal injection of 10 mg/kg benserazide hydrochloride (Sigma-Aldrich) followed 20 min later by an intraperitoneal injection of 25 mg/kg of L-DOPA (Sigma-Aldrich) or two saline injections 20 min apart.

Behavior

Dyskinesias were studied in WT, D1R-tomato, and D2R-eGFP mice three times/week, 30 min after treatment, as described previously (26). We measured orofacial and limb dyskinesias as well as axial dystonia individually with a 0–4 score scale for each animal. After the last L-DOPA injection, total axial, limb, and orofacial dyskinesia were measured every 20 min over a period of 180 min to see the dyskinetic profile over time.

Immunohistochemistry

Anesthetized animals were transcardially perfused with 4% paraformaldehyde (pH 7.4) 1 hour after the last L-DOPA injection. Brains were postfixed for 24 hours, and coronal sections were obtained on a vibratome (Leica, Madrid, Spain). The cutting sequence was 200-, 30-, 30-, and 30-μm-thick sections throughout the entire striatum. The thicker sections were used for Lucifer-Yellow injections, whereas the 30-μm sections were used for tyrosine hydroxylase (TH) and FosB immunostainings. Immunostaining was performed in free-floating sections with standard avidin-biotin protocols (27) with the following rabbit antisera: TH (1:1000; Chemicon, Temecula, California); FosB (1:7,500; Santa Cruz Biotechnology, Santa Cruz, California); and Lucifer-Yellow (1:100,000) (28).

Morphology

We used 200-μm sections from D1R-tomato and D2R-eGFP mice for morphological neuron reconstruction. The D1R- and D2R-positive MSNs from BAC-transgenic mice were visualized with the Cherry (for D1R-positive) or GFP (for D2R-positive neurons) fluorescence filters. To visualize D1R- or D2R-negative MSNs, striatal slices were labeled with 4′,6-diamidino-2-phenylindole (Sigma-Aldrich). The MSNs were injected individually with Lucifer-Yellow (8% in .05% Tris-buffer, pH 7.4; Sigma-Aldrich) by passing a hyperpolarizing current (10–20 nA). Then, sections were processed for immunocytochemistry with the anti-Lucifer-Yellow antibody (see preceding text). Neurolucida (MicroBrightField, Williston, Vermont) was used to trace dendritic arbors of MSNs three-dimensionally and to mark spines. Only neurons that were completely filled were included for analysis. Filled neurons located near the lesion needle track were discarded. Quantifications were done by a researcher blind to the experimental conditions.

Electrophysiology

The WT mice (3–4–month–old) were decapitated, and their brains were removed and dropped into ice-cold Krebs-Ringer-bicarbonate solution containing (in mmol/L): 119 sodium chloride, 26.2 sodium bicarbonate, 2.5 potassium chloride, 1 potassium dihydrogenorthophosphate, 1.3 magnesium sulphate, 2.5 calcium chloride, and 11 glucose, gassed with 95% oxygen and 5% carbon dioxide. Transverse corticostriatal slices (400-μm-thick) were cut and stored for >1 hour at room temperature. Recordings were performed in a submersion-type chamber continuously perfused (1.8–2 ml/min) with standard Krebs-Ringer-bicarbonate solution at 31–32°C. Stock solutions of picrotoxin (5 mmol/L; Sigma-Aldrich), SKF38393 hydrochloride (15 mmol/L; Sigma-Aldrich), and SCH23390 hydrochloride (1 mmol/L; Tocris, London, United Kingdom) were diluted to their final concentrations in the perfusion solution immediately before use. Intracellular recordings of dorsal striatal neurons were obtained with borosilicate glass micropipettes (1.5 mm outer diameter) pulled on a Flaming/Brown puller (P-87; Sutter Instrument, Novato, California) and filled with 2 mol/L potassium chloride (50–80 MΩ), which were connected to an Axoclamp-2B amplifier (Axon Instruments, Foster City, California) used in bridge mode. The MSNs were identified by firing patterns (29). Input resistance (Rin) was calculated from the voltage defection produced by hyperpolarizing current pulses (.1–.2 nA) of 100-msec duration. Action potentials were elicited by 200-msec-long rectangular .3–.8-nA depolarizing pulses.

Statistical Analysis

Data were expressed as mean ± SEM unless stated otherwise. Statistical differences (Systat-Software, San Jose, California; IBM SPSS Statistics version 20, Madrid, Spain) were assessed by one- or two-way analysis of variance followed by Bonferroni *t* test; a value of $p < .05$ was considered statistically significant. Comparisons between cumulative frequency distribution were done with the Friedman test.

Results

Induction of Dyskinesias in 6-OHDA-Lesioned Mice

To quantify L-DOPA-induced dyskinesias in parkinsonian WT, D1R-tomato, or D2R-eGFP mice, we scored orofacial, limb, and locomotive dyskinesia 30 min after L-DOPA or saline injection as previously described (14,16,26). After L-DOPA, parkinsonian BAC-transgenic mice developed marked dyskinetic movements affecting the contralateral forelimb and orofacial structures, together with trunk dystonia, similar to those observed in WT mice (Figure 1A). These symptoms were not observed in the saline group. In all three genotypes, dyskinesias were maximal 30–60 min after L-DOPA, as observed in the time response curve performed on Day 14, and gradually declined to baseline levels over 180 min (Figure 1B), consistent with previous results (16,26,30).

Previous studies have shown that dyskinesias in parkinsonian mice are related to increased phosphorylation of extracellular signal-regulated kinase–1/2 and augmented expression of ΔFosB and prodynorphin in the striatum [(26,31) reviewed in (19)]. We now show that, as shown previously in WT (16,26), in the BAC-transgenic mice, FosB induction only takes place in severely denervated striatal areas where the density of remaining dopaminergic fibers falls to <10% of control levels (referred to here as the "totally denervated area"). Dyskinesias only appear when portions of the dorsolateral striatum are almost completely denervated, inducing substantial changes in FosB expression (Figure 1C) in all types of mice. There were no significant differences in TH or FosB expression between the different genotypes.