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Short communication

Levodopa-induced morphologic changes of prefrontal pyramidal tract-type neurons in a rat model of Parkinson's disease

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ABSTRACT

Long-term administration of levodopa for Parkinson's disease is associated with various motor and non-motor complications. We examined the dendritic spine morphology of pyramidal tract-type neurons in the prefrontal cortex in a rat model of Parkinson's disease chronically treated with levodopa. Dendritic spines showed decreased density and increased average volume after dopamine denervation and levodopa treatment. These morphologic alterations suggest that the prefrontal neurons may maladaptively respond to excitatory input, which might be one of the mechanisms underlying various levodopa-induced complications in patients with Parkinson's disease.

Parkinson's disease (PD) is a neurodegenerative disease with progressive degeneration of nigrostriatal dopamine neurons. The ensuing depletion of dopamine in the brain causes motor deficits such as akinesia, rigidity, tremor and postural dysfunction (Jankovic, 2008). The dopamine precursor levodopa remains the most effective drug for alleviating motor symptoms in PD (Mercuri and Bernardi, 2005). However, chronic levodopa treatment may lead to the development of various motor and non-motor complications, such as wearing-off, levodopa-induced dyskinesia (LID) and dopamine dysregulation syndrome (DDS), which limits the usefulness of levodopa (Aquino and Fox, 2015; Beaulieu-Boire and Lang, 2015). DDS is one of the most severe non-motor fluctuations induced by levodopa (Beaulieu-Boire and Lang, 2015). Patients with DDS increase their levodopa doses beyond those required for motor control, resulting in a pattern of compulsive drug taking. Evans et al. (2006) showed altered levodopa-induced dopamine neurotransmission in the ventral striatum of patients with DDS compared with control PD patients using ^{11}C-raclopride scans, indicating that chronic use of the drug modifies the reward system. The prefrontal cortex forms a part of the reward system (Sesack and Grace, 2010). Most of the neurons in prefrontal cortex are excitatory pyramidal cells (Riga et al., 2014). It has been shown in rats that there are two types of pyramidal cells in the prefrontal cortex. One type, the intratelencephalic (IT-type) neurons, expresses D1 dopamine receptors and projects to contralateral cortex, ipsi/contralateral striatum and ipsilateral amygdala. The other, pyramidal tract (PT-type) neurons, expresses D2 dopamine receptors and projects to the pons, brainstem, ipsilateral striatum and thalamus (Reiner et al., 2010, 2003; Riga et al., 2014). The D2 dopamine receptor family has a significant role in levodopa-associated neuropsychiatric dysfunction in PD patients (Sierra et al., 2015). Dysfunctions of neurons in the prefrontal cortex are related to various neuropsychiatric disorders such as autism, attention-deficit hyperactivity disorder, post-traumatic stress disorder and impulse control disorder (Courtin et al., 2013; Ridderinkhof et al., 2004). Recently, structural and functional abnormalities of the prefrontal cortex in PD patients with LID, which frequently co-exists with DDS, were shown using

Abbreviations: 6-OHDA, 6-hydroxydopamine; ABC, avidin-biotin-peroxidase complex; AIM, abnormal involuntary movement; DDS, dopamine dysregulation syndrome; IT, intratelencephalic; LID, levodopa-induced dyskinesia; PD, Parkinson's disease; PT, pyramidal tract.

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quantitative magnetic resonance imaging (Cerasa et al., 2011, 2012, 2013). Animal models of drug addiction, which is considered to have similar underlying mechanisms to DDS (Linazasoro, 2009), showed altered dendritic spine density in the prefrontal cortex (Kolb and Gibb, 2015). Thus PT-type neurons in prefrontal cortex might have a role in pathogenesis of levodopa-induced non-motor complications such as DDS in PD. Dendritic spines form the post-synaptic compartment of excitatory glutamatergic synapses in the brain. Morphologic changes of dendritic spines are linked with changes in synaptic function (Holtmaat and Svoboda, 2009). We hypothesized that the dendritic spines of PT-type pyramidal neurons in the prefrontal cortex change their morphology in a rat model of PD chronically treated with levodopa. To verify the hypothesis, we conducted the present study to examine the spine pathology of PT-type neurons in the prefrontal cortex of 6-hydroxydopamine (6-OHDA) lesioned hemiparkinsonian rats.

In total, 32 male Wistar rats (Japan Clea Co., Ltd., Tokyo, Japan) were used in this study. All experiments were carried out in accordance with the guidelines of Hirosaki University School of Medicine and regulations for the care and use of animals set out by the National Institutes of Health (publication No. 80-23, revised 1996). We prepared eight 6-OHDA lesioned hemiparkinsonian rats (PD model group), eight 6-OHDA lesioned chronically levodopa treated rats (PD-levodopa group), eight normal rats with chronic levodopa treatment (normal-levodopa group), and eight control rats (control group) according to the methods previously described (Ueno et al., 2014). In brief, each rat was pretreated with desipramine to prevent denervation of noradrenergic neurons, anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg body weight, intraperitoneally; Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan), then 6-OHDA was injected into the right medial forebrain bundle in 16 rats at 10 weeks of age. To evaluate the extent of dopaminergic denervation the rats underwent behavioral testing using apomorphine, indicating a lack of dopaminergic function in the striatum. To confirm the extent of dopaminergic denervation in 6-OHDA lesioned rats, at the conclusion of the experiments brain sections that included the medial prefrontal cortex were immunostained with monoclonal antibodies against tyrosine hydroxylase (TH16; Sigma, St. Louis, MO; 1:3000), using the avidin-biotin-peroxidase complex (ABC) method with a Vectastain ABC kit (Vector, Burlingame, CA) (Fig. 1). Dopaminergic denervation was found not only in the striatum but also in the medial prefrontal cortex (Fig. 1). Four weeks after the apomorphine test, the 16 6-OHDA lesioned rats were randomly allocated to one of two groups. One group received intraperitoneal injections of levodopa methyl ester hydrochloride (Sigma), 50 mg/kg in combination with benserazide hydrochloride (Sigma), 12.5 mg/kg, twice daily for 2 weeks (PD-levodopa group, n = 8). The other group received the same dose of saline (PD model group, n = 8). Eight rats without 6-OHDA lesions received the same levodopa treatment (normal-levodopa group, n = 8). To confirm behavioral sensitization after levodopa treatment, we measured abnormal involuntary movement (AIM) scores on days 1, 4, and 11 of levodopa treatment according to the methods described previously (Ueno et al., 2014). AIMs were classified into three subtypes: axial dystonia, orolingual dyskinesia and forelimb dyskinesia, and the total scores of AIMs exhibited by each animal at each time point were submitted to analyses.

To label cell bodies of PT-type neurons in the prefrontal cortex, on day 11 of the drug treatment we stereotactically injected the retrograde tracer Fast Blue (Polysciences, Warrington, PA) into the right ventral pons where a pyramidal tract runs (Reiner et al., 2003; Riga et al., 2014). All rats were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg body weight, intraperitoneally; Dainippon Sumitomo Pharma Co., Ltd.) and the head was fixed in a stereotactic apparatus (David Kopf Instruments, Tujunga, CA) with the incisor bar set 3.3 mm below the horizontal. A stainless steel needle was inserted through a small burr hole on the right side of the skull, and the needle tip was placed in the right ventral pons (9.6 mm posterior to bregma, 0.5 mm lateral to the sagittal suture, and 10.7 mm ventral to the periosteum surface) according to the atlas of Paxinos and Watson (Paxinos and Watson, 2007). We injected Fast Blue (15 μg/1 μl in saline) over a 1-min period. After injection, the needle was left in place for 2 min to prevent backflow. Four days after the Fast Blue injection (12 h after the last levodopa treatment), all rats were deeply anesthetized with sodium pentobarbital (Nembutal, >75 mg/kg body weight, intraperitoneally; Dainippon Sumitomo Pharma Co., Ltd.), and they were perfused transcardially with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (1200 ml/kg). After perfusion, the brains were removed and cut into serial sections (250 μm thick) through the prefrontal cortex using a Microslicer (DSK DTK-1000; Dosaka EM, Kyoto, Japan). Individual sections were mounted between Millipore filters (Millipore Corporation, Billerica, MA). The slices were then mounted in a Perspex dish on a fixed-stage microscope (Optiphot2-UD microscope; Nikon, Tokyo, Japan) and the labeled neurons in cortical layer 5 of the right prefrontal cortex (prelimbic cortex, according to the atlas of Paxinos and Watson (Paxinos and Watson, 2007)) were observed under ultraviolet excitation (380–420 nm). Lucifer Yellow CH dilithium salt (Sigma) was injected into the cell bodies of the Fast Blue-labeled neurons through a patch pipette. Neurons were filled with Lucifer Yellow until their dendritic spines were sufficiently visible. Sections were mounted onto slides and then coverslipped with SlowFade

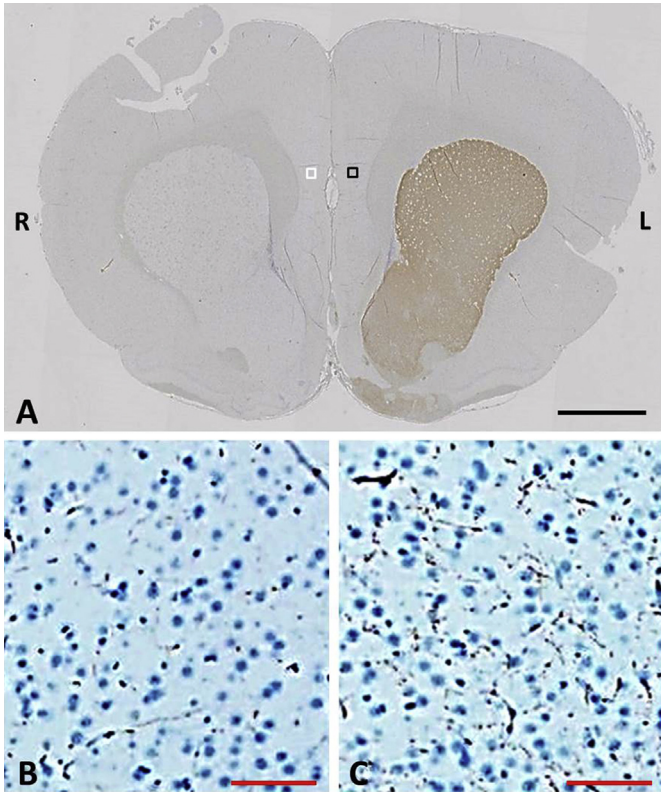

Fig. 1. Tyrosine hydroxylase immunohistochemistry in a brain section including the medial prefrontal cortex of a 6-hydroxydopamine lesioned rat.
(A) Dopamine denervation was found in the right hemisphere, most markedly in the striatum. L, left; R, right; scale bar = 2.5 mm.
(B) Higher magnification view of the area indicated by the white box in (A) showing dopaminergic denervation in the right medial prefrontal cortex (prelimbic cortex). Scale bar = 100 μm.
(C) Higher magnification view of the area indicated by the black box in (A) showing intact dopaminergic innervation in the left medial prefrontal cortex. Scale bar = 100 μm.