Neurobiology of Disease 64 (2014) 142–149

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Morphological and electrophysiological changes in intratelencephalic-type pyramidal neurons in the motor cortex of a rat model of levodopa-induced dyskinesia

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ARTICLE INFO

Article history:
Received 27 September 2013
Revised 21 December 2013
Accepted 26 December 2013
Available online 4 January 2014

Keywords:
Parkinson's disease
Dyskinesia
Levodopa
Motor cortex
Spine
Plasticity
Animal model
6-Hydroxydopamine
Whole-cell patch clamp
Miniature excitatory postsynaptic currents

ABSTRACT

Levodopa-induced dyskinesia (LID) is a major complication of long-term dopamine replacement therapy for Parkinson's disease, and becomes increasingly problematic in the advanced stage of the disease. Although the cause of LID still remains unclear, there is accumulating evidence from animal experiments that it results from maladaptive plasticity, resulting in supersensitive excitatory transmission at corticostriatal synapses. Recent work using transcranial magnetic stimulation suggests that the motor cortex displays the same supersensitivity in Parkinson's disease patients with LID. To date, the cellular mechanisms underlying the abnormal cortical plasticity have not been examined. The morphology of the dendritic spines has a strong relationship to synaptic plasticity. Therefore, we explored the spine morphology of pyramidal neurons in the motor cortex in a rat model of LID. We used control rats, 6-hydroxydopamine-lesioned rats (a model of Parkinson's disease), 6-hydroxydopamine-lesioned rats chronically treated with levodopa (a model of LID), and control rats chronically treated with levodopa. Because the direct pathway of the basal ganglia plays a central role in the development of LID, we quantified the density and size of dendritic spines in intratelencephalic (IT)-type pyramidal neurons in M1 cortex that project to the striatal medium spiny neurons in the direct pathway. The spine density was not different among the four groups. In contrast, spine size became enlarged in the Parkinson's disease and LID rat models. The enlargement was significantly greater in the LID model than in the Parkinson's disease model. This enlargement of the spines suggests that IT-type pyramidal neurons acquire supersensitivity to excitatory stimuli. To confirm this possibility, we monitored miniature excitatory postsynaptic currents (mEPSCs) in the IT-type pyramidal neurons in M1 cortex using whole-cell patch clamp. The amplitude of the mEPSCs was significantly increased in the LID model compared with the control. This indicates that the IT-type pyramidal neurons become hyperexcited in the LID model, paralleling the enlargement of spines. Thus, spine enlargement and the resultant hyperexcitability of IT-type pyramidal neurons in M1 cortex might contribute to the abnormal cortical neuronal plasticity in LID.

Introduction

Oral L-3,4-dihydroxyphenylalanine (levodopa, L-DOPA) replacement therapy remains the most effective strategy for the symptomatic relief of Parkinson's disease (PD). However, chronic levodopa treatment is often complicated by a variety of involuntary movements, termed levodopa-induced dyskinesia (LID), which represent a major limitation in the treatment of PD (Fabbrini et al., 2007). LID mainly develops in response to activation of sensitized D1 receptors on medium spiny neurons in the direct striatonigral pathway (Feyder et al., 2011). Indeed, corticostriatal synapses in an LID model have been shown to exhibit abnormalities in synaptic plasticity (Belujon et al., 2010; Picconi et al., 2003, 2008, 2011). The corticostriatal synapse in this model lacks depotentiation after induction of long-term potentiation (LTP) (Picconi et al., 2003). Depotentiation leads to a resetting of corticostriatal synapses, to avoid synaptic saturation and is implicated in the mechanisms of physiological *forgetting*. Consequently, the absence of depotentiation might result in the storage of unessential motor information (Picconi et al., 2011). In addition, it has been shown that induction of long-term depression (LTD) is also lost in the dyskinetic model (Picconi et al., 2011). Thus, corticostriatal synapses appear to be electrophysiologically supersensitive in dyskinesia, and dyskinetic movements might be induced by changes in the molecular mechanisms regulating corticostriatal excitatory synaptic transmission (Picconi et al., 2011).

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Available online on ScienceDirect (www.sciencedirect.com).

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http://dx.doi.org/10.1016/j.nbd.2013.12.014

Direct investigation of corticostriatal plasticity in humans is not currently possible. Instead, abnormal neuroplasticity in patients with PD has been identified at the level of the motor cortex using transcranial magnetic stimulation (TMS) (Rothwell, 2007). Using this approach, PD patients with LID exhibit a lack of depotentiation-like cortical plasticity (Huang et al., 2012). Levodopa fails to effectively normalize the excitability of inhibitory systems and does not restore motor cortical plasticity in PD patients with dyskinesia, in contrast to its effects in PD patients without dyskinesia (Morgante et al., 2006). The electrophysiological changes in PD patients with LID are similar to those at corticostriatal synapse of the LID model (Belujon et al., 2010; Picconi et al., 2003, 2008, 2011). It has been shown that abnormal motor cortical plasticity may arise from a loss of dopaminergic terminals in the primary motor cortex (M1) (Luft and Schwarz, 2009). Thus, abnormal synaptic plasticity, resulting in an increase in excitability to glutamate, occurs in the motor cortex of PD patients with LID, a structure that receives indirect inputs from the basal ganglia. However, the cellular mechanisms underlying the hyperexcitability of the motor cortex remain to be determined.

Dendritic spines form the postsynaptic compartment of the majority of excitatory glutamatergic synapses in the brain. The morphological properties of dendritic spines are intimately linked with synaptic functions; i.e., changes in synaptic plasticity are often accompanied by changes in spine size (Kasai et al., 2010a, 2010b). Accordingly, morphological examinations of dendritic spines in pyramidal neurons of the motor cortex in animal models should shed light on the mechanisms underlying the cortical synaptic abnormalities observed in PD patients with LID (Huang et al., 2012; Kishore et al., 2012; Morgante et al., 2006; Suppa et al., 2011).

The striatum of rodents receives excitatory inputs from two types of cortical pyramidal neurons in layer 5 of the cerebral cortex—those with intratelencephalic connections and those sending their axons to the brainstem via the pyramidal tract (Lei et al., 2004; Reiner et al., 2010). It has been demonstrated that the intratelencephalic (IT)-type neuron preferentially innervates contralateral and ipsilateral striatal neurons of the direct pathway, whereas the pyramidal tract (PT)-type neuron preferentially innervates ipsilateral striatal neurons of the indirect pathway (Reiner et al., 2010). The direct pathway plays a central role in the development of LID (Feyder et al., 2011). On the other hand, there is not sufficient evidence to support a role of the indirect pathway for LID (Cenci and Konradi, 2010). Thus, we investigated the morphological and electrophysiological changes in IT-type pyramidal neurons of M1 in rat models of PD and LID in the present study.

Materials and methods

The present study consists of two parts. The first part is the morphological examination of dendritic spine of IT-type neurons. The second part is the electrophysiological examination of IT-type neurons. The study designs are summarized in Fig. 1.

Experimental animals

Male Wistar rats (Japan Clea Co. Ltd, Tokyo, Japan) were housed in a temperature-controlled room (around 25 °C) with a 12-h day/night cycle, with free access to food and water. The experimental procedures employed in this study complied with the guidelines for animal research issued by the Physiological Society of Japan and by Hirosaki University School of Medicine, and all efforts were made to minimize the number of animals used and their suffering.

Creation of rat models

We prepared 14 6-hydroxydopamine (6-OHDA)-lesioned hemiparkinsonian rats (the PD model), 20 6-OHDA-lesioned hemiparkinsonian rats with chronic levodopa treatment (the LID model), 8 control rats with chronic levodopa treatment (levodopa-treated control) and 17 control rats with saline treatment (control) (Fig. 1).

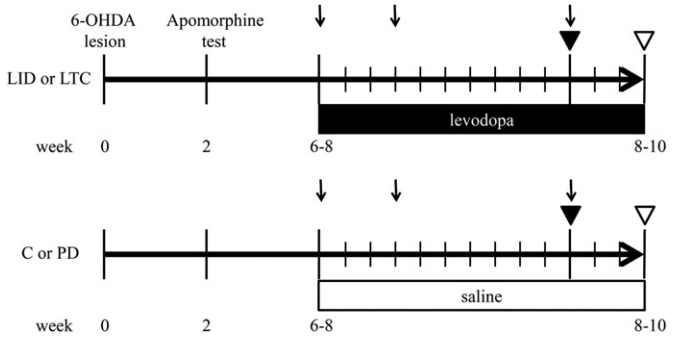

Fig. 1. Time chart and experimental design of the study. We injected 6-hydroxydopamine (6-OHDA) or saline into the medial forebrain bundle to make hemiparkinsonian (LID: levodopa-induced dyskinesia model; PD: Parkinson disease model) or sham-operated rats (Levodopa-treated control: LTC; control: C), respectively. The number indicates number of weeks post-6-OHDA lesion. Dopaminergic denervation in 6-OHDA-injected rats was confirmed by apomorphine test. LID and LTC started to receive daily levodopa treatment and PD and C started to receive daily saline. Arrows indicate abnormal involuntary movement rating sessions. Closed and open triangles indicate the days of the tracer injection and sacrifice, respectively.

At 10 weeks of age, rats underwent stereotactic infusion of 6-OHDA (the PD and LID models) or saline (levodopa-treated control and control) into the medial forebrain bundle (MFB) on the right side as previously described (Tanaka et al., 1999) (Fig. 1). The rats were pretreated with desipramine (25 mg/kg, intraperitoneally) 30 min before the injection into MFB to prevent denervation of noradrenergic neurons. A stainless steel needle (0.4 mm diameter) was inserted through a small burr hole on the right side of the skull and the needle tip was placed in the right MFB (4.5 mm posterior to the bregma, 1.2 mm lateral to the sagittal suture, and 8.5 mm ventral to the dural surface) according to the atlas of Paxinos and Watson (1998). We injected 6-OHDA (8 mg/4 mL in saline with 0.01% ascorbic acid) over 4 min. After injection, the needle was left in place for 2 min to prevent backflow leakage from the site of injection.

To evaluate the extent of dopaminergic denervation, 2 weeks after the 6-OHDA injection, the rats were challenged with apomorphine (in saline with 0.1% ascorbic acid, 0.05 mg/kg, subcutaneously) (Fig. 1). The 6-OHDA-treated animals that made more than 20 contralateral (to the left) turns during a 5-min period between 15 and 20 min after apomorphine injection, indicating lack of dopaminergic function in the striatum (we previously showed that rats meeting this criterion had lost more than 99% of dopamine in the striatum; Maeda et al., 1999), were included in the present study. To confirm the dopaminergic denervation, brain sections of 6-OHDA-lesioned rats were immunostained with monoclonal antibodies against tyrosine hydroxylase (1:3000), using the avidin–biotin–peroxidase complex (ABC) method with a Vectastain ABC kit (Fig. 2). Dopaminergic denervation was mostly complete in the striatum, and was also found in M1 (Fig. 2). Four weeks after the apomorphine test, 6-OHDA-lesioned rats with dopaminergic denervation and sham-operated rats received 50 mg/kg levodopa methyl ester with 12.5 mg/kg benserazide (the LID model and levodopa-treated control) or saline (the PD model and control), twice daily (morning and evening) for 14 consecutive days (Fig. 1). To evaluate the effects of levodopa, we measured the abnormal involuntary movement (AIM) score in the left side of the body on days 1, 4 and 11 (Cenci and Lundblad, 2007; Cenci et al., 1998) (Fig. 1). AIMs are considered to be comparable to LID in patients with PD (Cenci and Lundblad, 2007; Cenci et al., 1998). We observed and scored the rats every 20 min during the 2-h period following the injection of levodopa (Fig. 3). Scores are based on the duration and persistence of the dyskinetic behavior during the 1-min observation period. Movements were recognized as dyskinetic when they fulfilled the following criteria: (i) were induced by L-DOPA; (ii) affected the left side of the body; (iii) were repetitive, purposeless and not ascribable to any