Levodopa-induced dyskinesia and striatal signaling pathways

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Parkinson's disease (PD) is the most common movement disorder, affecting ≈2% of individuals over age 60 (1). The cardinal clinical features of PD are resting tremor, rigidity, bradykinesia, and postural instability, which are often accompanied by autonomic, cognitive, and emotional disturbances. The neuropathological hallmark of PD is the degeneration of dopaminergic neurons in the substantia nigra pars compacta. The loss of dopaminergic input to the striatum results in the depletion of dopamine and ultimately leads to the appearance of the motor deficits. The dopamine precursor, L-DOPA, has been the most effective drug to treat the clinical symptoms of PD. However, most patients develop abnormal involuntary movements, also termed dyskinesia, within a few years of L-DOPA treatment. Dyskinesia is induced by both nigral dopaminergic degeneration, which triggers a complex cascade of changes in the basal ganglia circuitry, and chronic administration of L-DOPA (2, 3). A number of molecular changes that are thought to underlie clinical manifestation of dyskinesia have been reported, including alterations in striatal dopamine receptors and their downstream signaling targets (summarized in Table 1) (3–7). The article in a recent issue of PNAS by Ann Graybiel and colleagues (8) reports that CalDAG-GEFI and CalDAG-GEFII, regulators of the ERK signaling pathway, may be key mediators of dyskinesia expression in the striatum.

L-DOPA Induced Dyskinesia. Dopamine replacement therapy represents the most effective treatment for PD. However, with disease progression the duration of clinical benefits derived from L-DOPA shortens and patients start experiencing motor fluctuations, known as "end of dose deterioration" or the "wearing off" phenomena. The clinical response to L-DOPA becomes unpredictable and the patient may frequently switch from "on" condition to "off" states. The decrease in the clinical response demands a progressive increase in the dosage of L-DOPA, which ultimately fails to provide stable control of motor symptoms, resulting in the appearance of involuntary movements or dyskinesia (9, 10).

Table 1. Dopamine receptor-related changes in L-DOPA-induced dyskinesia

Receptor	Effect	Model
D1	Increased density and sensitization	MPTP primates
	Activation of ERK1/2 and CREB	6-OHDA rats, MPTP primates and mice
	Increased DARPP-32 phosphorylation	
D2	Heterodimerization with A_{2A} adenosine receptors	MPTP primates and 6-OHDA rats
	Reduced RGS9–2 signaling	
D3	Increased expression	MPTP primates

The past decade has witnessed significant advances toward better understanding of the neural mechanisms underlying L-DOPA-induced dyskinesias (2, 3, 7). An essential progress has been the development of animal models. The neurotoxin 6-hydroxydopamine (6-OHDA), a hydroxylated derivative of dopamine, was introduced in the 1960s to produce the first animal model of PD (11). Because 6-OHDA does not cross the rat blood–brain barrier, it is administered by direct injection into either the substantia nigra pars compacta or the medial forebrain bundle, which causes irreversible damages of the ipsilateral nigrostriatal dopaminergic pathway and unilateral hypokinesia (11). The extent of the lesion can be quantified in vivo by measuring the asymmetric turning behavior of the rat after systemic administration of dopaminergic drugs. When rats with unilateral 6-OHDA lesions were treated with therapeutic doses of L-DOPA, they gradually developed abnormal involuntary movements affecting the limbs, the trunk, and the orofacial musculature contralateral to the injected side, resembling the dyskinetic movements observed in PD patients (12). These abnormal involuntary movements could be quantified on the basis of their distribution, duration, and amplitude. Interestingly, the time course of the motor deficits exhibited by 6-OHDA-treated rats is similar to that of dyskinetic PD patients; the deficits are most severe after a drug dose when L-DOPA reaches a peak level in the plasma and the brain, suggesting that 6-OHDA-treated rats represent a valuable experimental tool for investigating the mechanisms of dyskinesia.

Molecular Mechanisms Underlying Dyskinesia. Motor complications are most commonly observed in patients on L-DOPA therapy and are less common in patients taking other antiparkinsonian medications. Hence, current views suggest that aberrant dopamine metabolism has a prominent role in L-DOPA-induced motor fluctuations (13). Indeed, the notion that the capacity of the nigrostriatal terminals to synthesize and store dopamine diminishes with disease progression suggests a presynaptic mechanism underlying the generation of motor fluctuations. However, several reports have indicated that molecular changes occurring at dopamine receptors and their downstream signaling targets during dyskinesia may be responsible for the modifications ultimately leading to the occurrence of motor complications in PD (Table 1). For example, increased dopamine D1 receptor sensitivity and downstream signaling have been reported in primates with L-DOPA-induced dyskinesia (14). Sensitization of D1 receptors results in enhanced activation of cAMP-dependent protein kinase A, which phosphorylates various downstream proteins, including DARPP-32. In 6-OHDA-lesioned rats, DARPP-32 phosphorylation at Thr-34 is dramatically increased, an effect that is reverted by L-DOPA treatment (15). Conversely, such an increase in phosphorylation persists in dyskinetic animals, suggesting that DARPP-32 plays a permissive role in the induction of dys-

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kinesia by sensitizing D1 receptors to L-DOPA. This assumption is further confirmed by the alterations of corticostriatal synaptic plasticity observed in striatal slices from dyskinetic rats, in which loss of synaptic depotentiation was accompanied by abnormally high levels of phospho[Thr-34]-DARPP-32 (16). Accordingly, genetic inactivation of DARPP-32 reduced the occurrence of L-DOPA-induced dyskinesia (15). In addition, DARPP-32, through 2 mitogen-activated protein kinases (MAPKs), ERK1 and ERK2, participates in the regulation of gene expression observed in the striatum of dyskinetic animals. Through different and complex mechanisms, activation of ERK leads to the activation of the transcription factors cAMP-responsive element-binding (CREB) protein (6, 17).

Chronic L-DOPA treatment has been shown to cause profound changes in striatal glutamatergic signaling. NMDA receptor subunits undergo profound adaptive modifications (18), with an abnormal redistribution of the NR2B subunit between synaptic and extrasynaptic membranes (19). A growing body of evidence suggests that dopamine D1 receptors closely interact with glutamate NMDA receptors in striatal neurons. D1 receptor activation has been shown to enhance trafficking of NMDA receptor subunits, promoting clustering of NR1 and NR2 subunits with the postsynaptic density scaffolding protein PSD-95 and enhancing receptor surface expression (20).

Graybiel and coworkers (8) describe a fundamental modification occurring in the striatum of dyskinetic rats induced by unilateral injection of 6-OHDA into the medial forebrain bundle followed by L-DOPA treatment. All of their dopamine-depleted rats treated with L-DOPA developed abnormal motor responses, including repetitive movements and dystonic postures of the limbs contralateral to the 6-OHDA-induced lesion. Interestingly, they found an inverse dysregulation of CalDAG-GEFI and CalDAG-GEFII occurring in 2 distinct regions of the striatum in the dyskinetic rat, such that both CalDAG-GEFI mRNAs and proteins were significantly down-regulated in the matrix compartment, whereas the striosome-enriched CalDAG-GEFII mRNAs and proteins were up-regulated.

CalDAG-GEFI and CalDAG-GEFII were identified as guanine nucleotide exchange factors (GEFs) containing domains that bind calcium and diacylglycerol (DAG) (21, 22). Calcium and DAG can activate Ras and Rap1 through these factors via their GEF domains and further regulate the ERKs. The striosome/matrix organization of the striatum is based on specific differences in the content of neurochemical markers, afferent inputs, and efferent outputs. In general, striatal neurons located within a given compartment would receive synaptic inputs only from those afferents that innervate the compartment within which they are located. This idea implies that such segregation of information flow through the 2 compartments is maintained and transmitted to the output structures of the basal ganglia. Graybiel and coworkers (8) showed that the opposing changes of the 2 CalDAG-GEFs mirror this type of segregation, suggesting that abnormal motor patterns may be functionally segregated. Of note, only the combination of 6-OHDA-induced lesion and the L-DOPA treatment, which produced abnormal movements, were able to cause major modifications in the striatal expression of these 2 CalDAG-GEFs. Conversely, neither the 6-OHDA-induced lesion nor the L-DOPA treatment alone, which did not cause involuntary movements, was able to modify the expression of CalDAG-GEFs. More importantly, Graybiel and coworkers found a strong correlation between the severity of the involuntary movements caused by L-DOPA-induced dyskinesia and the extent of the alteration in the expression of each CalDAG-GEF. These findings raised a tantalizing possibility that dysregulation of CalDAG-GEFs may underlie or contribute to the abnormal involuntary movements caused by L-DOPA-induced dyskinesia.

In summary, the work by Graybiel and coworkers (8) provides an intriguing molecular clue as to how striatal signaling pathways implicated in dyskinesia may be modulated by dopamine depletion and replacement. Future investigation will be needed to determine how L-DOPA treatment elicits opposing changes in the levels of CalDAG-GEFI and CalDAG-GEFII, and whether these opposing changes do indeed lead to enhanced ERK signaling in the dopamine-depleted striatum. Ultimately, it will be important to identify the effector mechanisms operating downstream of the ERK signaling pathway that mediate L-DOPA-induced dyskinesia. Better understanding of the complex molecular cascade underlying abnormal patterns of motor activity may yield novel therapeutic targets for combating motor complications affecting PD patients.

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