ERK Phosphorylation and FosB Expression Are Associated with L-DOPA-Induced Dyskinesia in Hemiparkinsonian Mice

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Background: *The dopamine precursor 3,4-dihydroxyphenyl-l-alanine (L-DOPA) is currently the most efficacious noninvasive therapy for Parkinson's disease. A major complication of this therapy, however, is the appearance of the abnormal involuntary movements known as dyskinesias. We have developed a model of L-DOPA-induced dyskinesias in mice that reproduces the main clinical features of dyskinesia in humans.*

Methods: *Dyskinetic symptoms were triggered by repetitive administration of a constant dose of L-DOPA (25 mg/kg, twice a day, for 25 days) in unilaterally 6-hydroxydopamine (6-OHDA) lesioned mice. Mice were examined for behavior, expression of FosB, neuropeptides, and externally regulated kinase (ERK) phosphorylation.*

Results: *Dyskinetic symptoms appear toward the end of the first week of treatment and are associated with L-DOPA-induced changes in ΔFosB and prodynorphin expression. L-DOPA also induces activation of ERK1/2 in the dopamine-depleted striatum. Interestingly, elevated FosB/ΔFosB expression occurs exclusively within completely lesioned regions of the striatum, displaying an inverse correlation with remaining dopaminergic terminals. Following acute L-DOPA treatment, FosB expression occurs in direct striatal output neurons, whereas chronic L-DOPA also induces FosB expression in nitric oxide synthase-positive striatal interneurons.*

Conclusions: *This model provides a system in which genetic manipulation of individual genes can be used to elucidate the molecular mechanisms responsible for the development and expression of dyskinesia.*

Key Words: Dopamine, dynorphin, dyskinesia, ERK1/2 phosphorylation, FosB, Parkinson's disease, striatum

Treatment with the dopamine precursor 3,4-dihydroxyphenyl-l-alanine (L-DOPA) is currently the most effective noninvasive therapy for Parkinson's disease. The response to this treatment changes as the disease progresses, however, and chronic L-DOPA administration causes abnormal involuntary movements known as dyskinesias (Jenner 2004; Marsden 1994). Dyskinesias are manifested in a wide range of motor symptoms ranging from orofacial movements to abnormal movements of the extremities similar to ballism or chorea and dystonic postures (Jenner 2004; Obeso et al 2000, 2004). Generally, dyskinesia occurs when dopamine concentration in the brain is the highest and is therefore called peak-dose dyskinesia (Olanow et al 2004); it can also occur throughout the "on" time period (the period of response of L-DOPA treatment). The appearance of dyskinesia represents a challenge to Parkinsonian therapy because it can be severe enough to warrant reducing the L-DOPA dose below optimal therapeutic levels.

Despite the high incidence and clinical relevance of dyskinesia, little is known about the molecular mechanisms that underlie it. This is in part due to the lack of animal models that accurately reproduce the range of motor symptoms observed in patients. In the last few years, however, it has been shown that dyskinesia-like changes in motor responses consistently occur in parkinsonian rats undergoing chronic administration of L-DOPA or other dopaminomimetic compounds (Canales and Graybiel 2000; Cenci et al 1998; Lundblad et al 2002). These rats exhibit abnormal involuntary movements and motor fluctuations or "wearing off" effects similar to those observed in parkinsonian patients (Cenci et al 1998; Perier et al 2003; Marín et al 2004).

Short- and long-term changes in gene expression following repeated intermittent administration of L-DOPA have been considered to be part of the neural plasticity underlying dyskinesias induced in rats and monkeys (Calon et al 2000; Cenci et al 1998). Repeated exposure to L-DOPA triggers a molecular cascade of signaling molecules including the activation of cyclic adenosine monophosphate (CAMP)-dependent kinase, phosphorylation of N-methyl-D-aspartate (NMDA) receptors and other D1-related proteins (Picconi et al 2003) and induction of immediate-early and late response gene expression (Lee et al 2000). These molecular alterations serve as anatomic and molecular markers of striatal plasticity induced by chronic intermittent dopamine receptor stimulation and appear to be an integral part of the molecular cascade that causes behavioral sensitization and dyskinesias.

In addition, changes in mitogen-activated protein (MAP) kinase signaling pathways have been shown to be associated with the supersensitive response to dopaminergic stimulation in dopamine-depleted striatum (Gerfen et al 2002). For instance, SKF38393, a partial dopamine D1 receptor agonist, dramatically increases externally regulated kinase (ERK)1/2 phosphorylation in the 6-hydroxydopamine (6-OHDA)-lesioned striatum (Gerfen et al 2002). Moreover, ERK1/2 phosphorylation has also been implicated in many forms of synaptic plasticity in the brain, particularly in long-lasting forms of synaptic plasticity such as "late long-term potentiation (LTP)" and memory processes (Kelleher et al 2004; Thomas and Huganir 2004), and because dyskinesia may be an abnormal form of motor learning, it is possible that ERK1/2 phosphorylation is also implicated in dyskinesia.

Despite the well-documented association between these molecular markers and the occurrence of dyskinesia, it is still unclear which molecular changes mediate the development and expression of dyskinesia during chronic L-DOPA treatment. Transgenic and knockout mice already exist for many components of dopaminergic signaling pathways and it is now straight-

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forward to produce new transgenic or null mice. A reliable mouse model of L-DOPA-induced dyskinesia would allow use of transgenic and null mice to investigate specific signaling molecules in the development and production of dyskinesias.

We set out to establish a dyskinesia model and to determine whether molecular markers of dyskinesia seen in other species are evident in mice. During preparation of this article, a mouse model was reported in which dyskinesia was triggered by escalating dosages of L-DOPA (Lundblad et al 2004). Our model uses a paradigm that is more representative of human therapeutic L-DOPA use: chronic intermittent delivery of a constant dose of L-DOPA (25 mg/kg, intraperitoneal (IP), twice a day, for 25 days) to mice unilaterally lesioned with 6-OHDA. We used this novel model to examine the expression and activity of several known components of dopamine signaling in dopamine-depleted striatum after acute and chronic L-DOPA treatment. We report that chronic L-DOPA induces dyskinesia in hemiparkinsonian mice and activates direct striatal output pathway neurons, inducing ΔFosB and prodynorphin expression in the lesioned striatum. We also show activation of the ERK1/2/MAP kinase pathway and the nitric oxide synthase (NOS)-positive interneurons in the lesioned striatum. Finally, we document an inhibition of ΔFosB expression in the remaining dopaminergic terminals after 6-OHDA lesion.

Methods and Materials

Unilateral 6-Hydroxydopamine Lesion of the Striatum

The study was carried out in male C57/BL6/JOlaHsd mice weighing 24–30 g at the start of the experiment. Mice were anesthetized with tribromoethanol, secured in a stereotaxic frame, and injected with 10 μg of 6-OHDA-HBr (Sigma, Madrid, Spain) dissolved in 4 μL of saline containing .02% ascorbic acid. Two 2-μL injections were made at a rate of 1 μL/min, at the following coordinates (mm from bregma and dura: AP +.5, L +2.4, and V −4.0 and 3.0) targeting the dorsolateral striatum (Paxinos and Franklin 1997).

L-DOPA Treatment

At 3 to 4 weeks after 6-OHDA lesions, animals received intraperitoneal injections of L-DOPA methyl ester hydrochloride (25 mg/kg, Sigma-Aldrich, Madrid, Spain) combined with benserazide-HCl (10 mg/kg, Sigma), a DOPA-decarboxilase inhibitor, twice a day (at 9 AM and 6 PM), for 25 days. Control animals received saline instead of L-DOPA.

Behavioral Measurements

Behavioral analysis was conducted at 30 and 60 min following the morning dose of L-DOPA. Rotational behavior and abnormal involuntary movements (AIM) were studied in the same group of animals, on alternate days, twice a week for each behavior. The AIMs were divided into four categories, as in the rat model:

1. Orofacial—discrete vertical (open and close) jaw movements toward the contralateral side; tongue protrusion is also observed.
2. Forelimb—jerky movements of the forelimb contralateral to the lesion with choreic (nonrhytmic, spasmodic) or ballistic (choreic movements with larger amplitude) nature (Fahn 2000).
3. Locomotive—circular locomotion with contralateral side bias. The mice bend laterally over their own dorsoventral axis, making a tight turn.
4. Dystonic—lateral deviation of the trunk, neck, and head toward the contralateral side, leading to a loss of the orthostatic equilibrium.

Because the behavioral manifestation of AIMs in mice were similar to that described for the rat, the frequency and the intensity of each AIM was evaluated using the rat scale (Cenci et al 1998). This scale (0 to 4) scores as follows: 0 = *not present*, 1 = *present during less than half of the observation period,* 2 = *present during more than half of the observation period,* 3 = *present all the time but suspended by a tactile stimuli,* and 4 = *present all the time, regardless of the stimuli*. For the evaluations, each mouse was introduced into a glass cylinder (4 cm diameter) and observed for a 2-min period for orofacial dyskinesias and another 2 min for the rest of the AIMs. Rotational tests were carried out in a rotometer system and only completed turns (360°) were counted. Mice were placed in rotometer bowls and, after 5 min of habituation, rotational data was registered every 10 min for 2 hours after L-DOPA treatment.

Tissue Preparation and In Situ Hybridization

Following behavioral analysis, animals were euthanized 1 hour after the last morning injection of L-DOPA; their brains were removed, frozen in powered dry ice, and cut in coronal sections (12 μm thick) in a cryostat (Leica, Wetzlar, Germany). Sections were subjected to hybridization as described in Fredduzzi et al (2002). The following ^{35}S-labeled rat riboprobes were used: tyrosine hydroxylase (Dr. J. Mallet, Hôpital de la Salpetriere, Paris, France); preproenkephalin (Dr. S.L. Sabol, National Institutes of Health [NIH], Bethesda, Maryland); prodynorphin (Dr. J. Douglass, Vollum Institute, Portland, Oregon); and FosB (Dr. J. Morgan, St Jude Children's Research Hospital Memphis, Tennessee).

Western Blot and Immunocytochemistry

ERK phosphorylation and FosB expression were studied in another group of lesioned mice that received acute or chronic L-DOPA treatment as described earlier. The presence of dyskinesia was evaluated at least once a week. For Western blots, protein extracts from striata of hemiparkinsonian mice were prepared as described (Grande et al 2004) and proteins (20 μg) were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and blotted onto a nitrocellulose membrane. Immunodetection was carried out with rabbit polyclonal primary antisera (1:1000 dilution) against FosB/ΔFosB, CAMP-responsive element binding protein (CREB; both from Santa Cruz Biotechnology, Santa Cruz, California) and p-ERK1/2 (Cell Signaling Technology, Beverly, Massachusetts). Bands were visualized by enhanced chemiluminescence (ECL, Amersham, Buckinghamshire, England) and quantified by densitometric scanning of films (Quantity One, BioRad, Madrid, Spain).

Immunostaining was carried out in free-floating sections with standard avidin-biotin immunocytochemical protocols (Hiroi and Graybiel 1996; Rivera et al 2002a) with specific FosB/ΔFosB or tyrosine hydroxylase (TH) antisera (Chemicon, Temecula, CA). After incubation with the primary (overnight) and secondary (2 hour) antisera, peroxidase reactions were developed in diaminobenzidine. Two-color dual antigen immunostaining was carried out as in Rivera et al (2002a) to visualize FosB/ΔFosB-positive nuclei in parvalbumine-, choline-acetyltransferase-, and calretinin-positive neurons. These proteins correspond to markers characteristic of various types of striatal neurons (Hiroi 1995; Rivera et al 2002b), and the antibodies used to identify these