Archival Report

Biological Psychiatry

A Role for Mitogen- and Stress-Activated Kinase 1 in L-DOPA–Induced Dyskinesia and ΔFosB Expression

Michael Feyder, Erik Södersten, Emanuela Santini, Vincent Vialou, Quincey LaPlant, Emily L. Watts, Giada Spigolon, Klaus Hansen, Jocelyne Caboche, Eric J. Nestler, and Gilberto Fisone

ABSTRACT

BACKGROUND: Abnormal regulation of extracellular signal-regulated kinases 1 and 2 has been implicated in 3,4-dihydroxy-L-phenylalanine (L-DOPA)-induced dyskinesia (LID), a motor complication affecting Parkinson's disease patients subjected to standard pharmacotherapy. We examined the involvement of mitogen- and stress-activated kinase 1 (MSK1), a downstream target of extracellular signal-regulated kinases 1 and 2, and an important regulator of transcription in LID.

METHODS: 6-Hydroxydopamine was used to produce a model of Parkinson's disease in MSK1 knockout mice and in ΔFosB- or ΔcJun-overexpressing transgenic mice, which were assessed for LID following long-term L-DOPA administration. Biochemical processes were evaluated by Western blotting or immunofluorescence. Histone H3 phosphorylation was analyzed by chromatin immunoprecipitation followed by promotor-specific quantitative polymerase chain reaction.

RESULTS: Genetic inactivation of MSK1 attenuated LID and reduced the phosphorylation of histone H3 at Ser10 in the striatum. Chromatin immunoprecipitation analysis showed that this reduction occurred at the level of the *fosB* gene promoter. In line with this observation, the accumulation of ΔFosB produced by chronic L-DOPA was reduced in MSK1 knockout. Moreover, inducible overexpression of ΔFosB in striatonigral medium spiny neurons exacerbated dyskinetic behavior, whereas overexpression of ΔcJun, which reduces ΔFosB-dependent transcriptional activation, counteracted LID.

CONCLUSIONS: Results indicate that abnormal regulation of MSK1 contributes to the development of LID and to the concomitant increase in striatal ΔFosB, which may occur via increased histone H3 phosphorylation at the *fosB* promoter. Results also show that accumulation of ΔFosB in striatonigral neurons is causally related to the development of dyskinesia.

Keywords: Dopamine D_1 receptor, Histone, Medium spiny neurons, Mouse, Parkinson's disease, Striatum

http://dx.doi.org/10.1016/j.biopsych.2014.07.019

Dyskinesia is a frequent and debilitating motor side effect produced in Parkinson's disease (PD) patients by prolonged administration of 3,4-dihydroxy-L-phenylalanine (L-DOPA) (1). Several lines of evidence indicate that L-DOPA–induced dyskinesia (LID) is caused by long-term modifications of signaling in striatal neurons. These changes occur in concert with the loss of dopamine innervation and include a pronounced sensitization of dopamine D_1 receptors (D_1Rs) (2–4). In rodent and non-human primate models of PD, such sensitization confers to L-DOPA the ability to promote 3′,5′-cyclic adenosine monophosphate (cAMP) signaling and to activate the extracellular signal-regulated protein kinases 1 and 2 (ERK), which have been implicated in the development of dyskinetic behavior (5–11).

L-DOPA–induced activation of ERK is accompanied by increased phosphorylation of mitogen- and stress-activated kinase 1 (MSK1) (8,10). This effect occurs selectively in the gamma-aminobutyric acidergic medium spiny neurons (MSNs) of the striatonigral pathway, which express D_1Rs (8). The activation of MSK1 associated with LID leads to phosphorylation of histone H3 at Ser10 (5,8,10), which has been proposed to play a permissive role in gene expression (12–16). Although it is possible that alterations in gene transcription produced by MSK1-mediated phosphorylation of histone H3 may contribute to the long-term changes involved in dyskinesia, there has to date been no investigation of the involvement of MSK1 in LID.

LID is accompanied by increased expression of several immediate early genes, including *fosB* and its truncated splice product ΔFosB (17–20). The latter product is a highly stable transcription factor, which in combination with JunD, induces long-lasting effects by promoting the expression of several

SEE COMMENTARY ON PAGE 338

 ISSN: 0006-3223

late response genes through binding to their activator protein-1 consensus sites (21). Accumulation of ΔFosB has been linked to the development of dyskinetic behavior in animal models (17,22,23). Interestingly, inhibition of ERK signaling decreases the accumulation of ΔFosB induced by L-DOPA (24). However, the exact mechanism underlying this effect remains to be established.

In this study, we tested the hypothesis that MSK1 was involved in the accumulation of ΔFosB in response to repeated exposure to L-DOPA and in the concomitant development of dyskinesia.

METHODS AND MATERIALS

Animals

Male MSK1 knockout (KO) mice (25), mice overexpressing ΔFosB, mice overexpressing ΔcJun (see below), and wild-type littermates were maintained in a 12-hour light–12-hour dark cycle at a stable temperature of 22°C with food and water ad libitum. Male bitransgenic mice derived from neuron-specific enolase-tetracycline transactivator (NSE-tTA) (line A) × TetOp-ΔFosB (line 11) and NSE-tTA (line A) × TetOp-FLAG-ΔcJun (line E) mice (26–28) were conceived and raised on 100 μg/ml doxycycline to suppress ΔFosB or ΔcJun expression during development. Importantly, in line A, tTA expression is driven by the NSE promoter specifically in striatonigral MSNs, thereby generating mice in which ΔFosB and ΔcJun are selectively overexpressed in these neurons (26,29). Littermates were divided at weaning: half remained on doxycycline and half were switched to water, and the animals were used 8 to 11 weeks later when transcriptional effects of ΔFosB and ΔcJun were maximal (29,30). MSK1 KO mice and line 11A mice were fully backcrossed on C57BL/6N and C57BL/6J backgrounds, respectively. Line EA is a roughly 50:50 mixture of Friend Virus B-Type and 129 backgrounds. These differences in background most likely explain some of the variation in dyskinetic behavior observed between the controls of the three transgenic lines. Therefore, for every experiment, littermate wild-type controls were used to avoid any effects of genetic background. Heterozygous bacterial artificial chromosome transgenic mice expressing enhanced green fluorescent protein (EGFP) under the control of the promoter for the dopamine D_2 receptor (*Drd2*-EGFP) or the dopamine D_1R (*Drd1a*-EGFP) were generated by the Gene Expression Nervous System Atlas Project at Rockefeller University (New York, New York) (31) and were crossed on a C57BL/6 background for more than 10 generations. Experiments were carried out during the light phase, in accordance with the guidelines of Research Ethics Committee of Karolinska Institutet, the Swedish Animal Welfare Agency, the Society for Neuroscience, and the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai.

Drugs

All drugs were purchased from Sigma-Aldrich (St. Louis, Missouri). 6-Hydroxydopamine-HCl (6-OHDA) was dissolved in saline containing .02% ascorbic acid. L-DOPA was dissolved in saline and injected intraperitoneally in combination with the peripheral DOPA decarboxylase inhibitor benserazide hydrochloride in a volume of 10 mL/kg of body weight.

6-OHDA Lesions

Mice were lesioned by using 6-OHDA in a well-established protocol (5,8,10), described in detail in Supplement 1.

Cylinder Test

The cylinder test was conducted as previously described (10,48) at the end of the 3-week recovery period to assess limb akinesia. The effect of L-DOPA was assessed the following day, which corresponded to the first day of treatment. This time point was chosen due to the low levels of observable dyskinesia, which would otherwise interfere with test performance.

Abnormal Involuntary Movements

6-OHDA-lesioned mice were treated for 9 days (MSK1 KO mice and wild-type littermates) or for 14 days (mice overexpressing ΔFosB or ΔcJun and controls) with one injection per day of 10 mg/kg L-DOPA plus 7.5 mg/kg benserazide (MSK1 KO mice and wild-type littermates) or 20 mg/kg L-DOPA plus 12 mg/kg benserazide (mice overexpressing ΔFosB or ΔcJun and respective controls). These doses of L-DOPA and benserazide were selected within a range used in previous studies (10,20,24,32,33). Abnormal involuntary movements (AIMs) were assessed after the last injection of L-DOPA (day 9 or day 14) by an observer blind to mouse genotype or treatment, using a pharmacologically validated mouse model of LID (32). These durations of treatment with L-DOPA were chosen in order to ensure a sufficient expression of ΔFosB, which has been previously shown to occur in response to chronic drug administration (20,34). Briefly, 20 minutes after L-DOPA administration, mice were placed in separate cages, and individual dyskinetic behaviors were assessed for 1 minute (monitoring period) every 20 minutes, over a period of 2 hours. Purposeless movements, clearly distinguishable from natural stereotyped behaviors (such as grooming, sniffing, rearing, and gnawing), were classified into three different subtypes: axial AIMs (contralateral dystonic posture of the neck and upper body toward the side contralateral to the lesion), limb AIMs (jerky and fluttering movements of the limb contralateral to the side of the lesion), and orolingual AIMs (vacuous jaw movements and tongue protrusions). Axial and limb AIMs were scored on a severity scale from 0 to 4: 0, absent; 1, occasional; 2, frequent; 3, continuous; 4, continuous and not interruptible by external stimuli. Orofacial AIMs were assigned a score of 0, absent, or 1, present.

Western Blotting

Mice with a unilateral 6-OHDA lesions were treated with L-DOPA (10 mg/kg) plus benserazide (7.5 mg/kg) and killed 30 minutes postinjection by decapitation. Striatal tissue punches (1-mm thickness, 2-mm diameter; 3 punches per hemisphere) were taken using a stainless steel mouse brain matrix, sonicated in 1% SDS and boiled for 10 minutes. Immunoreactivity corresponding to total or phosphorylated histone H3, dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), ERK, and tyrosine hydroxylase was determined as described in detail in Supplement 1.