Priority Communication

Biological Psychiatry

Activation of DREAM (Downstream Regulatory Element Antagonistic Modulator), a Calcium-Binding Protein, Reduces L-DOPA-Induced Dyskinesias in Mice

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ABSTRACT

BACKGROUND: Previous studies have implicated the cyclic adenosine monophosphate/protein kinase A pathway as well as FosB and dynorphin-B expression mediated by dopamine D_1 receptor stimulation in the development of 3,4-dihydroxyphenyl-L-alanine (L-DOPA)–induced dyskinesia. The magnitude of these molecular changes correlates with the intensity of dyskinesias. The calcium-binding protein downstream regulatory element antagonistic modulator (DREAM) binds to regulatory element sites called DRE in the DNA and represses transcription of target genes such as *c-fos*, fos-related antigen-2 (*fra-2*), and prodynorphin. This repression is released by calcium and protein kinase A activation. Dominant-active DREAM transgenic mice (daDREAM) and DREAM knockout mice ($DREAM^{-/-}$) were used to define the involvement of DREAM in dyskinesias.

METHODS: Dyskinesias were evaluated twice a week in mice with 6-hydroxydopamine lesions during long-term L-DOPA (25 mg/kg) treatment. The impact of DREAM on L-DOPA efficacy was evaluated using the rotarod and the cylinder test after the establishment of dyskinesia and the molecular changes by immunohistochemistry and Western blot.

RESULTS: In daDREAM mice, L-DOPA-induced dyskinesia was decreased throughout the entire treatment. In correlation with these behavioral results, daDREAM mice showed a decrease in FosB, phosphoacetylated histone H3, dynorphin-B, and phosphorylated glutamate receptor subunit, type 1 expression. Conversely, genetic inactivation of DREAM potentiated the intensity of dyskinesia, and $DREAM^{-/-}$ mice exhibited an increase in expression of molecular markers associated with dyskinesias. The DREAM modifications did not affect the kinetic profile or antiparkinsonian efficacy of L-DOPA therapy.

CONCLUSIONS: The protein DREAM decreases development of L-DOPA-induced dyskinesia in mice and reduces L-DOPA-induced expression of FosB, phosphoacetylated histone H3, and dynorphin-B in the striatum. These data suggest that therapeutic approaches that activate DREAM may be useful to alleviate L-DOPA-induced dyskinesia without interfering with the therapeutic motor effects of L-DOPA.

Keywords: Abnormal involuntary movements, Dopaminergic denervation, Dynorphin-B, FosB, Parkinson's disease, Phospho-GluR1

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Despite extensive research focused on discovering therapeutic alternatives, the dopamine precursor molecule 3,4-dihydroxyphenyl-L-alanine (L-DOPA), remains the most effective and widely used noninvasive therapy for Parkinson's disease. Chronic administration of L-DOPA and disease progression cause the appearance of abnormal involuntary movements known as dyskinesias in most patients. L-DOPA-induced dyskinesia (LID) is causally linked with hyperstimulation of dopamine D_1 receptors (D1Rs) located on direct pathway medium spiny neurons of the severely denervated striatum (1–3). In the dopamine-depleted striatum, L-DOPA activates a cyclic adenosine monophosphate (cAMP)–dependent signaling cascade via protein kinase A (PKA) activation (4,5), resulting in abnormally increased phosphorylation of cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) and activation of the Ras/extracellular signal-regulated kinase signaling pathway in the same neurons (1,6–9). These events induce transcriptional changes resulting in increased expression of FosB, phospho-(Ser10)-acetyl-(Lys14)-histone-3 (P-AcH3), and dynorphin-B (10,11).

In addition, the increased activation of the cAMP/PKA/DARPP-32 pathway observed in dyskinesia induces persistent phosphorylation of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptor subunit, type 1 (GluR1) at Ser845 (6,7) via protein phosphatase-1 inhibition, enhancing excitatory glutamatergic transmission in D1R-containing neurons and increasing *N*-methyl-D-aspartate receptor (NMDAR) activation (12–15). Enhanced synaptic

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transmission in LID has been associated with altered redistribution and trafficking of D1Rs (16) along with a massive accumulation of postsynaptic density protein 95 (PSD-95) in the synaptic membrane (17). It has been shown that PSD-95 modulates LID in rats and monkeys by its direct interaction with D1Rs (18). In addition, D1R-mediated activation of adenylyl cyclase/PKA triggers calcium entry through voltage-dependent calcium channels (19), and treatment with the L-type calcium channel antagonist isradipine diminishes dyskinesias (20), further supporting the idea that calcium entry into direct pathway striatal neurons after D1R stimulation contributes to LID.

Despite the progress made in recent years, the intracellular signaling mechanism downstream of D1R-PKA–dependent activation is not fully established. The downstream regulatory element antagonist modulator (DREAM), also known as calsenilin and KChIP3, is a calcium-binding protein that mediates calcium-dependent and cAMP-dependent transcriptional responses (21,22). In striatal neurons, moderate levels of DREAM are present in the neuropil and cell soma (23–25). In basal conditions, DREAM binds to a regulatory element called DRE, located downstream from the transcription initiation site, repressing the transcription of target genes, including prodynorphin, *c-fos*, and Fos-related antigen-2 (*fra-2*) (26,27). Repression mediated by DREAM is reversed by calcium and by its PKA-dependent interaction with phospho-CREM, the cAMP-response element modulator (26,28–30). Outside the nucleus, DREAM directly interacts with several proteins (31), including NR1 subunit (32) and PSD-95 (33), inhibiting NMDAR function and its surface expression.

Based on these observations and on our previous data demonstrating a close association between LID and overexpression of FosB and dynorphin-B (1,10), we propose that DREAM, located downstream of D1R-dependent cAMP/PKA activation, plays an important role in the cascade of molecular events leading to LID. If this hypothesis is true, DREAM could provide a site for intervention to improve the efficacy of L-DOPA treatment, while preventing the upregulation of target genes associated with dyskinesia. To investigate this possible role of DREAM in LID, we used DREAM knockout and dominant active DREAM transgenic mice in a mouse model of dyskinesias.

METHODS AND MATERIALS

Animals

This study was carried out in transgenic mice 3–6 months old expressing a dominant active mutant DREAM (daDREAM) (34,35) or in DREAM-deficient mice (22,36). Both genetically modified animals and wild-type (WT) controls were maintained in a C57BL/6 background. In the daDREAM mice, expression of the transgene, a bi-cistronic construct including the daDREAM mutant, an internal ribosome entry site sequence, and the *lacZ* reporter, was driven by the calcium/calmodulin-dependent protein kinase type II alpha promoter (37). The transgenic line (JN26) used in this study has a telencephalic-specific expression with high levels of transgene expression in the striatum (Figure S1 in Supplement 1). Homozygous daDREAM and DREAM knockout ($DREAM^{-/-}$) mice were derived from mating the corresponding heterozygous mice. Genotyping was performed by polymerase chain reaction (PCR) as described previously (35,36). Bacterial artificial chromosome–transgenic D1R-tomato mice (38) were used to study DREAM striatal localization. All animal procedures followed guidelines from the European Union Council Directive (86/609/European Economic Community), and experimental protocols were approved by the Consejo Superior de Investigaciones Científicas Ethics Committee.

6-Hydroxydopamine Lesion and L-DOPA Treatment

Animals received unilateral stereotaxic injections (2 × 2 μL) of 6-hydroxydopamine (6-OHDA) hydrobromide (20 mmol/L, containing .02% ascorbic acid; Sigma-Aldrich, St. Louis, Missouri) as described previously (39). After 3 weeks of recovery, mice received a daily intraperitoneal injection of 25 mg/kg of L-DOPA methyl ester (Sigma-Aldrich) 20 min after benserazide hydrochloride (10 mg/kg; Sigma-Aldrich) for a 3-week period.

Behavioral Analysis

Locomotor Activity and Motor Coordination. Horizontal and vertical activity and total distance traveled were recorded in mice as described previously (40). Motor coordination was measured in the Rota-Rod (Ugo Basile, Varese, Italy) following an accelerating protocol, with increasing speed from 4–40 rpm over a 5-min period as described (41). Mice were tested in six consecutive trials 20 min apart. Measurements were done before injection of 6-OHDA (naïve); 3 weeks after lesion (parkinsonian); and during L-DOPA treatment (dyskinetic) on day 9, 24 hours after the last L-DOPA injection to avoid exhaustion and the peak dyskinesia.

Dyskinetic Score. Dyskinesias induced by L-DOPA were evaluated twice a week for 3 weeks, 40 minutes after L-DOPA injection, using a 0–4 severity scale as described previously (1,38). The time course of L-DOPA response for each genotype was evaluated on day 20, for 1 min every 20 min during 160 min after L-DOPA injection.

Cylinder Test. The cylinder test was performed according to Espadas *et al.* (42) before the 6-OHDA lesion; 3 weeks after lesion; and on day 17 the chronic L-DOPA treatment, 140 min after L-DOPA injection to avoid dyskinetic symptoms. Spontaneous ipsilateral and contralateral forelimb touches to the cylinder were counted for 3 min to assess forelimb asymmetry.

Immunohistochemistry and Image Analysis

Mice were killed 1 hour after the last L-DOPA injection, and immunohistochemistry studies were performed as described previously (43,44) using the following antibodies: tyrosine hydroxylase (TH) (1:1000; Millipore, Temecula, California), FosB (1:7500; Santa Cruz Biotechnology, Santa Cruz, California), dynorphin-B 1-29 (leumorphin) (1:10;000; Serotec, Oxford, United Kingdom), P-AcH3 (1:1500; Upstate Cell Signaling Solutions, Lake Placid, New York), DREAM (FL-214) (1:250; Santa Cruz Biotechnology), and Hoechst (1 μg/mL; Sigma-Aldrich).

The extent of dopaminergic lesions was quantified using Neurolucida software (MBF Bioscience, Williston, Vermont),