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PARALLEL DOPAMINE D1 RECEPTOR ACTIVITY DEPENDENCE OF L-DOPA-INDUCED NORMAL MOVEMENT AND DYSKINESIA IN MICE

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Abstract—L-3,4-Dihydroxyphenylalanine (L-Dopa)-induced dyskinesia (LID) in Parkinson's disease (PD) is a major clinical problem. The prevailing view is that in PD patients and animal PD models dyskinesia develops after repeated L-dopa use or priming, independent of L-dopa's anti-PD therapeutic effect that occurs immediately. Here we show that in mice with severe and consistent dopamine (DA) loss in the dorsal striatum, rendered by transcription factor Pitx3 null mutation, the very first injection of L-dopa or D1-like agonist SKF81297 induced both normal ambulatory and dyskinetic movements. Furthermore, the robust stimulating effects on normal and dyskinetic movements had an identical time course and parallel dose–response curves. In contrast, D2-like agonist ropinirole stimulated normal and dyskinetic movements relatively modestly. These results demonstrate that severe DA loss in the dorsal striatum sets the stage for dyskinesia to occur on the first exposure to L-dopa or a D1 agonist without any priming. These results also indicate that L-dopa stimulated both normal and dyskinetic movements primarily via D1 receptor activation and that proper D1 agonism is potentially an efficacious therapy for PD motor deficits.

Key words: basal ganglia, L-3,4-dihydroxyphenylalanine (L-dopa), dopamine D1 receptor, dyskinesia, medium spiny neuron, Parkinson's disease.

INTRODUCTION

In Parkinson's disease (PD), the degeneration of the nigrostriatal dopamine (DA) system causes motor control dysfunction (Hornykiewicz, 2001). Since its introduction more than 40 years ago, the DA precursor L-3,4-dihydroxyphenylalanine (L-dopa) has been the most effective treatment for the motor symptoms of PD (Cotzias et al., 1969; Standaert and Young, 2006; LeWitt, 2009). L-Dopa's therapeutic anti-PD effect occurs immediately upon the first dose of L-dopa and is mediated by activating DA receptors. However, precisely which DA receptor type is primarily responsible for L-dopa's anti-PD effect is usually not defined in the literature (Fahn and Przedborki, 2005; Standaert and Young, 2006; LeWitt, 2009; Olanow et al., 2009). The use of D2-like agonists such as ropinirole but not D1-like agonists (Jenner, 2003) to treat PD motor symptoms gives an impression that DA D2-type receptors (D2Rs) in the indirect pathway are the primary mediator of the therapeutic effects of L-dopa. Experimental studies, however, have demonstrated an overwhelming importance of the D1R-expressing medium spiny neurons (D1-MSNs) in the direct pathway to promoting motor activity and designated the direct pathway as the Go pathway (Chevalier and Deniau, 1990; DeLong, 1990; Hikosaka et al., 2000; Bateup et al., 2010; Kravitz et al., 2010; Berthet et al., 2012). Therefore, we reason that D1Rs may be primarily responsible for L-dopa's anti-PD effect.

Another important question is about the mechanisms underlying the motor side effects of L-dopa. In advanced stage PD patients, L-dopa often triggers abnormal involuntary movements, known as L-dopa-induced dyskinesias (LID) (Ahlskog and Muenter, 2001; Fahn, 2008), thus limiting the use of an otherwise efficacious drug (Olanow et al., 2009). Although D1Rs are known to be critically involved, the mechanisms underlying LID are not fully understood (Cenci, 2010; LeWitt, 2010; Iravani and Jenner 2011; Smith et al., 2012). Because LID often occurs after long-term, repeated use of L-dopa, it is commonly thought that L-dopa's therapeutic effect and dyskinetic side effect are mediated by separate mechanisms with the therapeutic effect occurring immediately whereas the dyskinetic effect developing only after repeated L-dopa use or priming (Jenner, 2008). This prevailing view advocates delaying L-dopa use, allowing the DA-depleted basal ganglia to undergo compensatory and also detrimental maladaptive changes (Schapira and Obeso, 2006). It also leads to a considerable research effort seeking to either stimulate or suppress one effect without affecting the other (Bezard et al., 2003; Santini et al., 2009; Gottwald and Aminoff, 2011; Huot et al., 2011). However, since L-dopa/DA likely activates the same DA receptors in the basal ganglia, the normal and dyskinetic motor control signals may be generated by at least partially shared mechanisms and thus intrinsically linked and difficult to separate. Indeed, clinical studies indicate that measures that reduce LID often worsen PD symptoms, i.e., dyskinetic and normal movements

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Abbreviations: AMPT, α-methyl-DL-tyrosine methyl ester hydrochloride; DA, dopamine; L-Dopa, L-3,4-dihydroxyphenylalanine; FCV, fast cyclic voltammetry; LID, L-dopa-induced dyskinesias; NAc, nucleus accumbens core; PBS, phosphate-buffered saline; PD, Parkinson's disease; SE, standard error.

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change in the same direction (Katzenschlager et al., 2008; Nutt, 2008). Further, it has been reported that after LID's first appearance, L-dopa's anti-PD effect and dyskinetic effect occurred with identical temporal profile (Nutt et al., 2010). These clinical observations suggest a common pharmacological mechanism that stimulates both normal and dyskinetic movements. However, because pharmacological manipulation is not practical in PD patients, the underlying pharmacological mechanism was not investigated. In this study, we seek to determine this shared pharmacological mechanism. Based on literature data and our reasoning in the preceding section that D1Rs may be primarily responsible for L-dopa's therapeutic effect, we now further hypothesize that D1Rs are the primary mediator of both LID and the therapeutic effect such that these two effects have identical time courses and dose–response curves. We will perform our experiments in transcription factor Pitx3 mutant mice, taking advantage of their consistent DA loss in the dorsal striatum and their robust L-dopa-induced motor effects.

EXPERIMENTAL PROCEDURES

Animals

Pitx3 mutant mice were used for the following reasons. In the brain, the transcription factor Pitx3 gene is expressed only in midbrain DA neurons (Smits et al., 2006). Loss of Pitx3 gene function leads to the death of the vast majority of nigral DA neurons shortly after their birth, whereas many DA neurons in the ventral tegmental area survive (Nunes et al., 2003; van den Munckhof et al., 2003; Smidt et al., 2004; Smits et al., 2006). Consequently, these mice have a severe and consistent DA deficiency in the dorsal striatum (Fig. 1), leading to robust and consistent L-dopa responses including LID (Hwang et al., 2005; Ding et al., 2007, 2011). Thus, these mice are suitable for our questions.

Two breeding pairs of heterozygous Pitx3+/− mice were purchased from the Jackson Laboratory (Bar Harbor, ME), resulting in a small colony of homozygous Pitx3−/− (PitxHomo), heterozygous Pitx3+/−, and wild-type Pitx3+/+ (PitxWT) mice. The genotypes were determined by PCR-based genotyping to identify WT, homozygotes, and heterozygotes. PitxHomo mice are also aphakia and thus clearly identified. PitxHomo mice are highly viable and fertile. Mice had free access to food and water. Male mice of 11–12 weeks old were used for testing. All procedures were approved by The Institutional Animal Care and Use Committee of The University of Tennessee Health Science Center in Memphis, Tennessee.

Treatment regimen

L-3,4-Dihydroxyphenylalanine methyl ester hydrochloride (L-dopa), benserazide hydrochloride, D1-like agonist SKF81297 hydrobromide, tyrosine hydroxylase inhibitor α-methyl-DL-tyrosine methyl ester hydrochloride (AMPT) and ropinirole hydrochloride were either purchased from Sigma–Aldrich (St. Louis, MO) or supplied by the Drug Supply Program of the National Institute of Mental Health. These drugs were dissolved in 0.9% saline and delivered to the mice via intraperitoneal (IP) injection. L-dopa was always injected together with 5 mg/kg

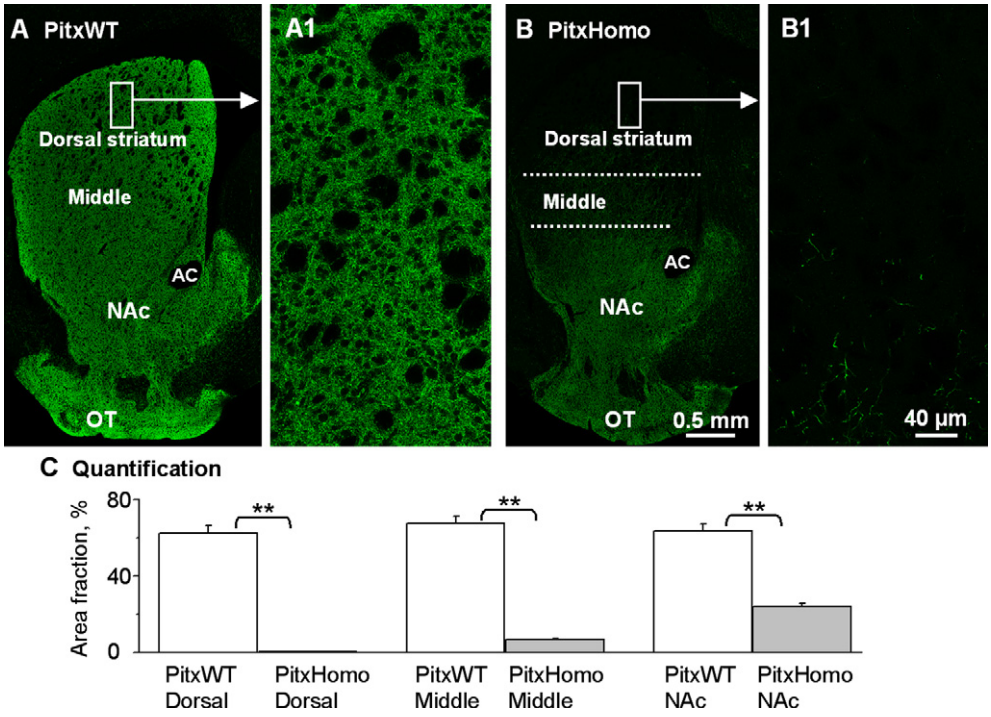

Fig. 1. Severe DA loss in the dorsal striatum in PixtHomo mice. (A) A confocal image of 3-μm optical section showing the typical intense DA axons as labeled by tyrosine hydroxylase (TH) immunostain in the entire striatum in a PitxWT mouse. The box area is expanded and displayed in A1. (B) A confocal image, obtained under identical conditions as in A, evidenced by the identical background signal in the non-striatal areas, showing the typical gradient pattern of DA axon loss in the striatum in PitxHomo mice. The dorsal striatum is largely void of DA axons, the middle striatum retains a significant number of DA axons, whereas the ventral striatum or the nucleus accumbens retains substantial amounts of DA axons. The box area is expanded and displayed in B1. (C) The intensity of DA axons in the striatal subregions was quantified by calculating the fractional area occupied by DA axons in 4 PitxWT mice (16 sections) and 4 PitxHomo mice (16 sections). All sections were from the middle part of the striatum on the anterior-posterior axis. AC, anterior commissure. OT, olfactory tubercle. $^{**}p < 0.01$, *t*-test.