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Research Paper

The 5-alpha reductase inhibitor finasteride reduces dyskinesia in a rat model of Parkinson's disease

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ABSTRACT

Levodopa-induced dyskinesia (LID) is a disabling motor complication occurring in Parkinson's disease patients (PD) after long-term L-DOPA treatment. Although its etiology remains unclear, there is accumulating evidence that LID relies on an excessive dopamine receptor transmission, particularly at the downstream signaling of D_1 receptors. We previously reported that the pharmacological blockade of 5-alpha reductase (5AR), the rate limiting enzyme in neurosteroids synthesis, rescued a number of behavioral aberrations induced by D_1 receptor-selective and non-selective agonists, without inducing extrapyramidal symptoms. Thus, the present study was designed to verify whether the 5AR inhibitor finasteride (FIN) may counteract the dyskinesias induced by dopaminergic agonists in 6-hydroxydopamine (6-OHDA)-lesioned rats. First, we assessed the acute and chronic effect of different doses of FIN (30–60 mg/kg) on LID, in male 6-OHDA-lesioned dyskinetic rats. Thereafter, to fully characterize the therapeutic potential of FIN on LID and its impact on L-DOPA efficacy, we assessed abnormal involuntary movements and forelimb use in hemiparkinsonian male rats chronically injected with FIN (30–60 mg/kg/24 days) either prior to- or concomitant with L-DOPA administration. In addition, to investigate whether the impact of FIN on LID may be ascribed to a modulation of the D_1- or D_2/D_3-receptor function, dyskinesias were assessed in L-DOPA-primed 6-OHDA-lesioned rats that received FIN in combination with selective direct dopaminergic agonists. Finally, we set to investigate whether FIN may produce similar effect in female hemiparkinsonian rats, as seen in males.

The results indicated that FIN administrations significantly dampened LID in all tested treatment regimens, without interfering with the ability of L-DOPA to ameliorate forelimb use in the stepping test. The antidyskinetic effect appears to be due to modulation of both D_1- and D_2/D_3-receptor function, as FIN also reduced abnormal involuntary movements induced by the selective D_1 receptor agonist SKF-82958 and the D_2/D_3 receptor agonist ropinirole. Significant dampening of LID was also observed in female rats, although only at the higher tested dose. Clinical investigations are warranted to assess whether similar protection from dyskinesia is seen in PD patients.

1. Introduction

L-3,4-Dihydroxyphenylalanine (L-DOPA) is the gold-standard treatment for the motor symptoms of Parkinson's disease (PD); however, its chronic use induces severe motor complications that eventually limit its long-term efficacy (Bastide et al., 2015). The most disabling side effect is represented by the onset of dyskinesias, involuntary choreo-dystonic movements that dramatically affect patients' quality of life. L-DOPA-induced dyskinesias (LIDs) have been shown to reflect abnormal dopaminergic transmission (Berthet and Bezard, 2009; Bastide et al., 2015), and, in particular, dysfunctions in downstream signaling of D_1 receptors (Fiorentini et al., 2016; Aubert et al., 2005; Feyder et al., 2011; Westin et al., 2007).

Converging evidence shows that dopamine signaling is modulated by neuroactive steroids (NASs) (Di Paolo, 1994; Sánchez et al., 2010); in particular, we previously reported that the behavioral effects of dopaminergic agonists are influenced by inhibition of 5-alpha reductase (5AR), a key rate-limiting enzyme in NASs synthesis/metabolism (Paba et al., 2011). Specifically, we showed that, in rodent models, the 5AR inhibitors finasteride (FIN) and dutasteride attenuated the severity

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of a broad set of behavioral alterations induced by D_1 receptor-selective and non-selective agonists, such as hyperactivity, stereotyped behaviors and prepulse inhibition deficits (Frau et al., 2016; Frau et al., 2013). Of note, these antidopaminergic effects were not accompanied by extrapyramidal symptoms, even at the higher tested doses (Bortolato et al., 2008). Furthermore, recent evidence has shown that 5AR inhibition may elicit neuroprotective effects in animal models of PD (Litim et al., 2015, 2016).

Based on this evidence, we postulated that 5AR inhibition may exert therapeutic properties on LID, and tested this hypothesis investigating whether FIN may impact on the development and expression of dyskinesia induced by L-DOPA in male and female 6-hydroxydopamine (6-OHDA)-lesioned rats. In addition, we investigated whether FIN may interfere with the ability of L-DOPA to ameliorate rat forelimb use in the stepping test, and whether reduction of LID takes place via modulation of D_1- or D_2/D_3-receptor function.

2. Material and methods

2.1. Animals

This study was conducted on male (n = 180, 275–300 g) and female (n = 21, 275–300 g) Sprague Dawley rats (Envigo, Italy). Animals were housed 3–4 per cage in standard conditions. All experiments were carried out in accordance with the European Union directive (EEC Council 86/609; D.P.R. 116/92).

2.2. Drugs

Finasteride was purchased from Carbosynth Limited (UK) and suspended in a vehicle (VEH) solution containing 5% Tween80 and 95% sterile saline (SAL; 0.9% NaCl). Apomorphine hydrochloride was purchased from Tocris Bioscience (UK), dissolved in SAL containing 0.1% (v/v) ascorbic acid to prevent oxidation. SKF-82958 hydrochloride was purchased from Carbosynth Limited (UK) and dissolved in distilled water. Ropinirole was purchased from Tocris Bioscience (UK) and diluted in SAL. 6-OHDA was purchased from Sigma-Aldrich (Italy), dissolved in SAL plus 0.02% ascorbic acid, and locally infused into the medial forebrain bundle (MFB). L-DOPA methyl-ester and benserazide were purchased from Research Organics (USA) and Sigma-Aldrich, respectively, and dissolved in SAL. A 20:1 mixture of Fentanest (Pfitzer, Italy) and Domitor® (Orion Pharma, Italy) in a volume range of 1.4–1.6 ml, IP, was used to induce general anaesthesia. Antisedan® (0.37 mg/kg, SC, Orion Pharma, Italy) was injected to reverse the sedative effect of the anesthetics.

2.3. 6-OHDA lesion

Male and female Sprague-Dawley rats were injected with 16 μg of 6-OHDA (4 μg/μl free base in SAL with 0.02% ascorbic acid) into the right MFB (AP: −4.4, ML: −1.2, DV: −7.8), according to Paxinos and Watson (2007), at the rate of 0.38–0.5 μl/min.

2.4. Stepping test

To investigate the severity of the dopaminergic lesion and the possible interference of FIN with the antiparkinsonian effect of L-DOPA, we evaluated the forelimb use in the stepping test. As previously described in Tronci et al. (2013), the rat was held by the experimenter fixing its hindlimbs with one hand and the forelimb not to be monitored with the other, while the unrestrained forepaw was touching the table. The number of adjusting steps was counted while the rat was moved sideways along a table surface (90 cm in 5 s), in the forehand and backhand direction for both forelimbs, and the average of the steps in the two directions was considered.

2.5. Assessment of abnormal involuntary movements (AIMs)

Abnormal involuntary movements (AIMs) were evaluated, as detailed previously (Lundblad et al., 2002; Cenci and Lundblad, 2007; Tronci et al., 2014). Rats were scored for 1 min every 20 min for 120 min after L-DOPA injection, by an experimenter blind to the treatment groups. AIMs were classified into three subtypes: forelimb, orolingual and axial, and the severity assessed using scores from 0 to 4.

2.6. Immunohistochemistry

The animals belonging to the chronic studies were sacrificed 24 h after the last drug injection and the brains were harvested for immunohistochemistry analyses. As described in Tronci et al. (2012), coronal sections from striatal and substantia nigra were processed for tyrosine hydroxylase (TH) staining to verify the level of dopaminergic lesion. Rats with less than 90% of TH depletion in the substantia nigra and striatum were excluded from the study (data not shown).

2.7. Experimental design

The first set of experiments was aimed at investigating the acute effects of FIN on LIDs in 6-OHDA-lesioned L-DOPA primed rats. After 3 weeks from the 6-OHDA injection, rats received daily L-DOPA/benserazide (6/6 mg/kg, SC) treatment for 3 further weeks, so to induce stable dyskinesias. Animals were then assigned to 3 treatment subgroups with equivalent average AIMs scores, and acutely injected with vehicle (VEH, Sal/Tween80) or different doses of FIN (30–60 mg/kg, IP), 40 min prior to L-DOPA treatment (6 mg/kg plus benserazide 6 mg/kg, SC).

Similarly to the acute studies, a separate group of 6-OHDA-lesioned animals were chronically treated with L-DOPA (6 mg/kg plus benserazide 6 mg/kg, SC) for 4 weeks, and AIMs were scored until stable expression of dyskinesia was achieved. Based on AIMs score, rats were allocated into 3 different groups and daily treated with FIN at different doses (30–60 mg/kg, IP), or VEH, followed by L-DOPA (6 mg/kg plus benserazide 6 mg/kg, SC) for further 3 weeks, and then were tested for AIMs.

The following set of experiments was aimed at testing whether FIN may prevent LID development when administered 3 weeks prior to L-DOPA, or when initiated at the same time of L-DOPA treatment. Thus, a set of 6-OHDA lesioned male rats were subjected to FIN (30–60 mg/kg, IP) or VEH injection for 3 weeks before starting L-DOPA/benserazide (6/6 mg/kg, SC) treatment; thereafter, co-treatment with FIN or VEH plus L-DOPA/benserazide was carried on for 24 additional days. A second group of animals received the same treatment as above, but FIN administration was initiated at the same time of L-DOPA. In both the experimental groups (pre- and co-treated groups), stepping test was conducted at the first and last day of L-DOPA administration, while AIMs were evaluated from day 2 until day 25, every third day.

In order to unravel whether the anti LID properties of FIN might be ascribed to D_1- and/or D_2/D_3 receptor pathway modulation, we investigated the acute effects of FIN (60 mg/kg) on dyskinesias induced by the selective and non-selective dopaminergic agonists apomorphine (0.1 mg/kg, SC), SKF-82958 (0.1 mg/kg, SC), ropinirole (0.2 mg/kg, SC). Animals were primed with L-DOPA for three weeks, and a baseline AIMs score was taken with each dopaminergic agonist; therefore, the day of the test, FIN or its VEH were administered 40 min prior to apomorphine, SKF, ropinirole, and AIMs were evaluated as long as dyskinetic movements were observed. One week wash-out period was allowed between each compound. The doses of the agonists were chosen based on preliminary studies, as to be sufficient to induce significant dyskinesia in our rats.

As 5AR is differently modulated by androgens in male and female rats, (Sánchez et al., 2006), the final set of experiment was aimed at