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Glial activation is associated with L-DOPA induced dyskinesia and blocked by a nitric oxide synthase inhibitor in a rat model of Parkinson's disease

Mariza Bortolanza [a,b,1], Roberta Cavalcanti-Kiwiatkoski [b,c,1], Fernando E. Padovan-Neto [b,d], Célia Aparecida da-Silva [a,b], Miso Mitkovski [f], Rita Raisman-Vozari [e], Elaine Del-Bel [a,b,c,d,*]

[a] University of São Paulo (USP), School of Odontology of Ribeirao Preto, Department of Morphology, Physiology and Basic Pathology, Av. Café S/N, 14040-904 Ribeirão Preto, SP, Brazil
[b] USP, Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), Brazil
[c] USP, Medical School, Department of Physiology, Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, SP, Brazil
[d] USP, Department of Behavioral Neurosciences, Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, SP, Brazil
[e] Sorbonne Université UPMC UM75 INSERM U1127, CNRS UMR 7225, Institut de Cerveau et de la Moelle Epinière, Paris, France.
[f] Light Microscopy Facility Max-Planck-Institute of Experimental Medicine, Hermann-Rein-Str. 3, 37075 Göttingen, Germany

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ABSTRACT

L-3, 4-dihydroxyphenylalanine (L-DOPA) is the most effective treatment for Parkinson's disease but can induce debilitating abnormal involuntary movements (dyskinesia). Here we show that the development of L-DOPA-induced dyskinesia in the rat is accompanied by upregulation of an inflammatory cascade involving nitric oxide. Male *Wistar* rats sustained unilateral injections of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. After three weeks animals started to receive daily treatment with L-DOPA (30 mg/kg plus benserazide 7.5 mg/kg, for 21 days), combined with an inhibitor of neuronal NOS (7-nitroindazole, 7-NI, 30 mg/kg/day) or vehicle (saline-PEG 50%). All animals treated with L-DOPA and vehicle developed abnormal involuntary movements, and this effect was prevented by 7-NI. L-DOPA-treated dyskinetic animals exhibited an increased striatal and pallidal expression of glial fibrillary acidic protein (GFAP) in reactive astrocytes, an increased number of CD11b-positive microglial cells with activated morphology, and the rise of cells positive for inducible nitric oxide-synthase immunoreactivity (iNOS). All these indexes of glial activation were prevented by 7-NI co-administration. These findings provide evidence that the development of L-DOPA-induced dyskinesia in the rat is associated with activation of glial cells that promote inflammatory responses. The dramatic effect of 7-NI in preventing this glial response points to an involvement of nitric oxide. Moreover, the results suggest that the NOS inhibitor prevents dyskinesia at least in part via inhibition of glial cell activation and iNOS expression. Our observations indicate nitric oxide synthase inhibitors as a therapeutic strategy for preventing neuroinflammatory and glial components of dyskinesia pathogenesis in Parkinson's disease.

Introduction

Parkinson's disease (PD) is a chronic, progressive and disabling neurodegenerative disorder characterized by motor and psychiatric alterations (Dauer and Przedborski, 2003; Jenner, 2008, 2013). It is now evident that the pathology of PD is more extensive than previously thought (Braak et al., 2003; Ferrer, 2011; Jenner, 2008, 2013). Also, PD etiology and clinical presentation may differ greatly between individual cases (Jenner, 2013). Nonetheless, PD is invariably associated with the loss of dopaminergic neurons in the substantia nigra compacta (SNc), mitochondrial dysfunction (Dauer and Przedborski, 2003; Moore et al., 2005) and neuroinflammation (Hirsch and Hunot, 2009; McGeer and McGeer, 2008) seem to be a constant element in PD pathogenesis.

That chronic inflammation is present in the brain of PD patients was first shown by McGeer et al. (1988) through post-mortem pathological investigations. These findings were later confirmed by several authors (Banati et al., 1998; Hunot et al., 1997; Knott et al., 2000; Mogi et al., 1994a,b, 1995, 2007; for a recent review see Tansey and Goldberg, 2010). Indeed, the loss of dopaminergic neurons in PD is associated with a significant microglial reaction in the substantia nigra (Niranjan, 2014; Przedborski, 2010; Teismann et al., 2003). An ongoing neuroinflammatory process in the substantia nigra has been detected, many years after the toxic insult, in patients affected by 1-methyl-4-phenyl-

* Corresponding author at: University of Sao Paulo, School of Odontology, Department of Morphology, Physiology and Basic Pathology, Campus Ribeirão Preto, SP 14049-904, Brazil. Fax: +55 16 3633 0999.
E-mail address: eadelbel@forp.usp.br (E. Del-Bel).
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[1] Both authors contributed equally to the paper.

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1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (Langston et al., 1999) and in MPTP-lesioned non-human primates (Barcia et al., 2013). In vivo imaging studies have also confirmed the presence of neuroinflammation of the brain of PD patients (Bartels and Leenders, 2007; Gerhard et al., 2006; Ouchi et al., 2005). Moreover, it has been suggested that non-steroidal anti-inflammatory drugs may have beneficial effects in PD (Doherty, 2011; for review see Chen et al., 2005; Tomasiuk et al., 2014), although some studies have presented conflicting results (Becker et al., 2011; Driver et al., 2011). The neuropathological picture of activated glial cells found in the SN in human PD is similar to that found in experimentally induced disease models (Teismann et al., 2003).

The symptoms of PD are improved by the dopamine (DA) precursor L-DOPA (Fahn, 2008; Iravani and Jenner, 2011; Olanow et al., 2008). However, there is no evidence that L-DOPA therapy can prevent or retard the progression of neurodegeneration. Instead, chronic administration of L-DOPA often causes a development of abnormal involuntary movements (L-DOPA-induced dyskinesia) and psychiatric complications, which may be as debilitating as PD itself (for a review see Iravani et al., 2012; Jenner, 2013). Understanding the mechanisms behind these problems may lay a ground for developing alternative or adjunctive treatment to prevent them.

L-DOPA-induced dyskinesia is attributed to a disorder of DA transmission involving both pre- and post-synaptic mechanisms (Cenci, 2007). However, some recent studies have suggested that L-DOPA-induced dyskinesia may be related to a "pro-inflammatory" environment in the striatum. Thus, an increased expression of genes implicated in the inflammatory cascade has been detected in the striatum in rats affected by L-DOPA-induced dyskinesia (Hurley et al., 2014; Lortet et al., 2013; Wang et al., 2014). The pro-inflammatory environment may in turn be induced by excessive levels of neurotransmitters such as glutamate (Buck and Ferger, 2008; Picconi et al., 2002; Robelet et al., 2004) and DA (Meissner et al., 2006) that are released in the striatal extracellular fluid following the administration of L-DOPA. A role for neuroinflammation in L-DOPA-induced dyskinesia is supported by the antidyskinetic effects of anti-inflammatory treatments, such as corticosterone, observed in parkinsonian rodents (Barnum et al., 2008). Interestingly, amantadine which is the only clinical treatment for L-DOPA-induced dyskinesia in PD (Gao et al., 2003) can act on microglia in the central nervous system to inhibit their inflammatory activation (Kim et al., 2012). This and other lines of evidence suggest that, in the advanced stages of PD, L-DOPA treatment could aggravate neuroinflammation in the brain of PD patients (Cunningham et al., 2005; Gao et al., 2003). It is therefore warranted to hypothesize that the increased expression of inflammatory markers observed in human PD patients (Bartels and Leenders, 2007; Boka et al., 1994; Mogi et al., 1994a,b, 1995, 2007; Shimoji et al., 2009) could be partly depend on the effects of the treatment or its complications. Indeed, nearly all L-DOPA-treated PD patients will develop dyskinesia during the course of the disease (Jenner, 2008).

Nitric oxide (NO) has been proposed to have a role in inflammation (Coleman, 2001; Guzik et al., 2003; Tripathi et al., 2007) and in the inflammatory processes in PD (Hunot et al., 1996, 1997; L'Episcopo et al., 2010). High levels of nitric oxide synthase isoforms (NOS), both neuronal (nNOS, Eve et al., 1998) and inducible (iNOS, Hunot et al., 1996; Knott et al., 2000), were found in the SN and the striatum (Str) of post-mortem PD brain. The upregulation of nNOS was reported both in the basal ganglia (Eve et al., 1998; Gatto et al., 2000) and in circulating neutrophils of PD patients (Gatto et al., 2000). Accordingly, increased cerebrospinal fluid concentrations of nitrite (a metabolite of NO) have been found in L-DOPA-treated PD patients (Qureshi et al., 1995). An increase of nNOS levels has been described in the Str of parkinsonian rats (Czarnecka et al., 2013; Gomes et al., 2008). L-DOPA treatment of parkinsonian rats also increases the expression of nNOS mRNA in the Str and frontal cortex (Padovan-Neto et al., 2011).

Our group discovered that L-DOPA-induced dyskinesia in hemiparkinsonian rodents could be attenuated with NOS inhibitors, such as (7-nitroindazole [7-NI] or NG-nitro-L-arginine [L-NOARG]), without altering L-DOPA-induced motor improvements (Del-Bel et al., 2011; Novaretti et al., 2010; Padovan-Neto et al., 2009, 2011, 2013, 2014). Recently, results from other laboratories have corroborated our observations (Takuma et al., 2012). The precise mechanisms underlying the effects of 7-NI treatment remain to be clarified.

In this study, we have used hemiparkinsonian rats to determine whether L-DOPA-induced dyskinesia is associated with pro-inflammatory glial reactions in the Str, and whether these responses can be modulated by cotreatment with the preferential nNOS inhibitor, 7-NI. Our results show that the nNOS inhibitor prevents the development of L-DOPA-induced dyskinesia while suppressing glial components of the inflammatory cascade.

Methods

Subjects

Adult male *Wistar* rats (200–250 g) were housed in groups of five per cage in a temperature-controlled room (23 °C), under a 12 h light/dark cycle with free access to food and water. All experiments were conducted according to the principles and procedures described by the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (Institute for Laboratory Animal Research, USA) and the Guidelines of the School of Medicine (USP, Brazil), whose Animal Ethics Committee analyzed and approved the experimental procedures. All efforts were made to minimize animal suffering and number. Behavioral tests were always performed between 2:00 to 6:00 PM.

Drugs and administration routes

6-Hydroxydopamine (6-OHDA; Sigma-Aldrich, St. Louis, MO, USA) was injected in the amount of 16 μg, dissolved in 3 μL of 0.9% saline solution containing 0.05% ascorbic acid. L-DOPA (30 mg/kg) and benserazide–HCl (7.5 mg/kg) (Prolopa dispersive, Hoffman-LaRoche, Rio de Janeiro, RJ, Brazil) were given by oral gavage (Padovan-Neto et al., 2009). 7-NI (Sigma-Aldrich, St. Louis, MO, USA); was dissolved in saline-PEG 50% and administered intraperitoneally (i.p.) at the dose of 30 mg/kg, in a volume of 2 mL/kg, 30 min before L-DOPA (Kalisch et al., 1996; Padovan-Neto et al., 2009; Del-Bel et al., 2005). Desipramine hydrochloride (25 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA) was administered i.p. 30 min before injecting 6-OHDA.

6-OHDA lesion

Stereotaxic surgery parameters were implemented as previously described (Gomes and Del Bel, 2003; Gomes et al., 2008; Padovan-Neto et al., 2009). Briefly, rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (14 mg/kg, both i.p.) and then received a single injection of 6-OHDA (16 μg in 3 μL of 0.9% saline solution containing 0.05% ascorbic acid) into the right medial forebrain bundle (MFB) at the following coordinates (in mm, Paxinos and Watson, 2007): anteroposterior (AP): −4.4 from the bregma; lateral (L) 1.2; dorsoventral (DV): −8.2 from the skull. The toxin was infused at 1 μL/min, and the cannula was left in place for 3 min before withdrawal. To limit the damage in noradrenergic neurons, desipramine hydrochloride was administered 30 min before 6-OHDA microinjection. Rats were placed in clean cages on warming pads to recover from the surgery, after which they were returned to group housing.

The extent of 6-OHDA-induced injury to dopaminergic neurons was estimated three weeks after surgery using a test of apomorphine-induced rotation. Animals were challenged with an acute dose of apomorphine (0.5 mg/kg), and rotational behavior was measured for 45 min (Padovan-Neto et al., 2009). The lesion was verified histologically at the end of the behavioral studies by tyrosine hydroxylase (TH) immunohistochemistry. This confirmed that all animals in this study had