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From unilateral to bilateral parkinsonism: Effects of lateralization on dyskinesias and associated molecular mechanisms

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ABSTRACT

The mechanisms underlying lateralization and progression of motor symptoms from unilateral to bilateral in Parkinson's disease (PD) remain to be elucidated. In addition, the molecular mechanisms involved in levodopa-induced dyskinesias (LIDs) depending on lateralization and disease progression from unilaterally to bilateral have not been described yet.

We investigated motor symptoms, LIDs and associated striatal molecular markers expression after unilateral left or right, and after a sequential bilateral 6-hydroxydopamine (6-OHDA)-induced nigrostriatal lesions in rats.

Sequentially bilateral lesioned animals showed a bilateral increase in striatal preproenkephalin (PPE) mRNA without changes in pre-prodynorphin (PDyn) mRNA expression. The increase in dyskinesias when parkinsonism becomes bilateral was mostly due to an increase in orolingual dyskinesias associated to a increase in PDyn mRNA expression. Right lesion induces, or facilitates when first-done, a greater level of LIDs and an increase in striatal PPE and PDyn mRNAs in the second lesioned side.

We describe a new striatal molecular pattern that appears when parkinsonism becomes bilateral and the relevance of the lateralization for the development of LIDs.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that usually has a unilateral motor onset, either the right or left side, becoming bilateral as disease progresses (Lang and Lozano, 1998). This clinical presentation is mostly due to the asymmetrical degeneration of the *pars* compacta of the substantia nigra (SNc) being the mechanisms involved in this asymmetry and in the progression from unilateral to bilateral still unknown. Presently, there is no valid explanation as to why in PD, dopaminergic (DAergic) neurons in one side of the SNc are more vulnerable than their contralateral counterparts and are damage earlier and more severely in the curse of the illness (Riederer and Sian-Hülsmann, 2012).

Since PD symptoms only appear after a very large loss of nigral neurons (40% or more) and striatal dopamine (DA) depletion (>70%), it has been considered that powerful compensatory mechanisms operate to maintain original nigral physiological functions despite such cell losses in the early stages of the disease. It has been reported that several ipsilateral DAergic compensatory mechanisms counteract DAergic denervation, such as, increased synthesis and release of DA from surviving neurons, enhanced responsiveness (supersensitivity) of the striatal postsynaptic DAergic receptors, and compensatory regulation of striatal neuropeptide expression, as established in experimental and in human PD (Ungerstedt, 1971a; Meissner et al., 2003; Boulet et al., 2008; Brotchie and Fitzer-Attas, 2009).

In addition, the asymmetrical pathological changes in the parkinsonian SNc may, in turn, lead to further asymmetrical dysfunction of neural circuits in the basal ganglia (Clower et al., 2005; Capper-Loup et al., 2009). Thus, secondary adaptive mechanisms of the less-affected side could potentially compensate for parkinsonian symptoms (Capper-Loup et al., 2013). Whether compensatory mechanisms of the less-affected side also play a role

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is largely unknown, although a number of studies have demonstrated bilateral impairments in DA turnover, neurophysiologic and adaptive striatal gene expression changes after unilateral lesion suggesting a functional and, at least in part, compensatory interdependence of the two nigrostriatal system (Chéramy et al., 1983; Perier et al., 2000; González-Hernández et al., 2004; Breit et al., 2008; Capper-Loup et al., 2013).

Levodopa continues to be the main symptomatic therapy for clinical features of PD. However, prolonged use leads to motor complications, including levodopa-induced dyskinesias (LIDs) (Nutt, 2001; Stocchi et al., 2008). The relationship between the body region in which dyskinesias first appear and the body region first affected by parkinsonian motor symptoms has been investigated. Some studies (Grandas et al., 1999; Androulidakis et al., 2007; Colosimo et al., 2010), but not others (Fabbrini et al., 2009), reported that in most patients dyskinesias appears firstly in the same side where motor symptoms started.

LIDs have been associated with plastic changes on postsynaptic neuronal targets in the striatum such as abnormal trafficking of DA D1 receptor (Guigoni et al., 2007; Berthet et al., 2009), of glutamate receptor subunits (Gardoni et al., 2006; Silverdale et al., 2010), and an increased expression of preprodynorphin mRNA (PDyn) (Cenci et al., 1998; Marin et al., 2009, 2014). However, the molecular mechanisms involved in levodopa-induced dyskinesias depending on lateralization and disease progression from unilaterally to bilateral have not been described yet.

The present study was performed to investigate and compare motor symptoms, dyskinesia levels and associated striatal molecular markers expression in the experimental model of parkinsonism obtained after unilateral left (L) or right (R) nigrostriatal lesion. In addition, the consequences of sequential bilateral L plus R (L + R) or R plus L (R + L) 6-OHDA-induced nigrostriatal lesions in dyskinesias and molecular markers have been also investigated.

2. Material and methods

2.1. 6-OHDA lesions and treatment protocols

Male Sprague–Dawley rats (Charles River, France) weighing 220–240 g were housed on a 12 h light/dark cycle with free access to food and water. Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), rats were placed in a stereotactic frame with the incisor bar positioned 4.5 mm below the interaural line. Animals were unilaterally or sequential bilaterally lesioned with 6-OHDA (8 μg in 4 μl of saline with 0.02% ascorbate over 8 min) or vehicle into the medial forebrain bundle (MFB) by means of a Harvard infusion and distributed in the following groups: 1) left MFB-lesion (L-lesion, n = 12), 2) left MFB-sham (L-sham, n = 8), 3) right MFB-lesion (R-lesion, n = 11), 4) right MFB sham (R-sham, n = 8), 5) left MFB lesion and three weeks later right MFB lesion (L + R-lesion, n = 15), 6) right MFB lesion and three weeks later left MFB lesion (R + L-lesion, n = 11); 7) left MFB sham and three weeks later right MFB sham (L + R-sham, n = 8). Stereotactic injections were placed 4.0 mm anterior to the interaural line, 1.3 mm lateral to the midline and 8.4 mm ventral to the surface of the skull, according to the atlas of Paxinos and Watson (1986). After surgery, some of sequentially bilateral lesioned animals unable to feed themselves were hand-feed, 2 times/day, with nutritional supplement drink and subcutaneous glucose serum until complete recovery. None of the unilateral lesioned rats died during the study. Mortality rate in sequentially bilateral lesioned animals was approximately 13% (Eskow et al., 2012).

Three weeks after last 6-OHDA or sham lesion, unilaterally and sequentially bilateral-lesioned animals were treated with levodopa methyl ester (6 mg/kg with 15 mg/kg benserazide, i.p.) or saline twice daily for 22 consecutive days.

All animal experiments were carried out following European (2010/63/UE) and Spanish (RD 53/2013) regulations for the care and use of laboratory animals and approved by the local Government.

2.2. Forelimb akinesia-cylinder test

Rats were placed individually in a circular cylinder and the number of supporting wall contacts that were carried out with each forelimb was counted for a period of 5 min (Schallert et al., 2000; Marin et al., 2014). No habituation to the cylinder prior to testing was allowed. Forelimb akinesia was evaluated before and after 6-OHDA lesion in unilaterally sham and 6-OHDA-lesioned rats.

2.3. Spontaneous locomotor activity

Spontaneous locomotor activity was analyzed with a video-tracking system using behavioral analysis software Ethovision (Ethovision 3.0, Noldus, Wageningen, NL). Each animal was placed into home cages under a video camera and motor behavior was recorded for 1 h. The total distance (total distance traveled by the animal during 1 h observation time, in cm) and the spent time (in seconds) were measured. Spontaneous locomotor activity was evaluated before and after 6-OHDA lesion in sequentially bilateral-sham and 6-OHDA-lesioned rats.

2.4. Abnormal involuntary movements (AIMs) rating

Abnormal involuntary movements (AIMs) were scored by an experimenter unaware of the treatment administered (Cenci et al., 1998; Marin et al., 2009, 2014). Rats were individually placed in transparent cages and observed every 20th min, from 20 min before to 120 min after the drug injection (monitoring periods of 1 min).

As previously reported, three subtypes of AIMs were classified according to their topographic distribution as: axial (right or left), limb (left for unilateral lesions; right and left for sequentially bilateral lesions) and orolingual AIMs (Cenci et al., 1998; Paillé et al., 2007; Marin et al., 2009, 2014). Each of these subtypes was scored on a scale from 0 to 4: 0 = absent; 1 = occasional, i.e., present during less than 50% of the observation time; 2 = frequent, i.e., present during more than 50% of the observation time; 3 = continuous but interrupted by strong sensory stimuli; 4 = continuous not interrupted by strong sensory stimuli. Right and left axial and orolingual dyskinesias were evaluated at the same time. For the limb dyskinesias, right and left sides were separately scored. AIMs were evaluated the first and last day of treatments.

2.5. Tissue collection

Three days after the last drug administration rats were sacrificed under an overdose of pentobarbital anesthesia. Brains were quickly removed, frozen on dry ice and kept at −80 °C. Coronal 14 μm thick sections were collected through the striatum and nigra onto APTS (3-amino-propyltriethoxysilane) coated slides, and kept at −40 °C until used.

2.6. Dopamine transporter (DAT) and tyrosine hydroxylase (TH) immunohistochemistry

Striatal and nigral sections from all animals were processed for immunohistochemistry according to a standard peroxidase-based method (Marin et al., 2009, 2014). Briefly, sections were thawed and dried at room temperature, fixed with acetone and immersed in 0.3% hydrogen peroxide in phosphate buffer saline for 10 min. Sections were incubated with horse serum with 0.1% Triton X-100 for 20 min and incubated overnight at 4 °C with mouse anti-dopamine transporter (DAT, striatum) (#sc-1433, Santa Cruz Biotechnology, Inc. USA) and with anti-tyrosine hydroxylase (TH, substantia nigra) (#MAB5280, Merck Millipore, Darmstadt, Germany) monoclonal antibodies (1:500 and 1:5000, respectively). Sections were rinsed and ImmunoPure Ultra-Sensitive ABC Peroxidase staining kit was used to carry out the ABC staining method. By so doing, sections were incubated with biotinylated horse anti-mouse Ig-G for 30 min, followed by avidin-biotinylated peroxidase complex for 30 min and 3-3′-diaminobenzidine and 0.01% hydrogen peroxide for 15 min. Slides were washed, dehydrated in ascending alcohol concentrations, cleared in xylene and coverslipped in DPX-EXLI mounting medium.

2.7. In situ hybridization histochemistry

The oligonucleotides used were complementary to the following base sequences (GeneBank accession number in brackets): preproenkephalin (PPE) bases 513–542 (K02807), and preprodynorphin (PDyn) bases 607–654 (NM 019374). They were Q1 synthesized and HPLC purified by Amersham Pharmacia Biotech (UK) and by Isogen Bioscience BV (Maarssen, The Netherlands). The oligonucleotides were labeled at their 3′-end by using [α-^{33}P]dATP (3000 Ci/mmol) and terminal deoxynucleotidyl-transferase (TdT, Oncogene Research Products, San Diego, CA, USA), and purified using QIAquick Nucleotide Removal Kit (QIAGEN GmbH, Hilden, Germany) (Marin et al., 2009, 2014).

For *in situ* hybridization, frozen striatal tissue sections were brought to room temperature, air-dried, and fixed for 20 min in 4% paraformaldehyde (1x PBS: 2.6 mM KCl, 1.4 mM KH_2PO_4, 136 mM NaCl, 8 mM Na_2HPO_4), washed once in 3x PBS, twice in 1x PBS, 5 min each, and incubated in a freshly prepared solution of predigested pronase at a final concentration of 24 U/ml in 50 mM Tris-HCl, pH 7.5, 5 mM EDTA. The enzymatic activity was stopped by immersion for 30 s in 2 mg/ml glycine in 1x PBS. Tissues were finally rinsed in 1x PBS and dehydrated through a graded series of ethanol. For hybridization, radioactively-labeled probes were diluted to a final concentration of 10^7 cpm/ml in a solution containing 50% formamide, 4x SSC (1x SSC: 150 mM NaCl, 15 mM sodium citrate), 1x Denhardt's solution (0.02% Ficoll, 0.02% polyvinypyrrolidone, 0.02% bovine serum albumin), 10% dextran sulfate, 1% sarkosyl, 20 mM phosphate buffer pH 7.0, 250 mg/ml yeast tRNA and 500 mg/ml salmon sperm DNA. Tissues were covered with 100 μl of the hybridization solution and overlaid with Nescofilm (Bando Chemical, Kobe, Japan) coverslips to prevent

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