Neurobiology of Disease 36 (2009) 213–222

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

# *Nur77* mRNA levels and L-Dopa-induced dyskinesias in MPTP monkeys treated with docosahexaenoic acid

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ARTICLE INFO

*Article history:*
Received 26 May 2009
Revised 10 July 2009
Accepted 17 July 2009
Available online 25 July 2009

*Keywords:*
NGFI-B
NR4A1
DHA
Retinoid X receptor (RXR)
MPTP-treated monkey
Parkinson's disease
L-Dopa-induced dyskinesia (LID)
Substance P
Basal ganglia
Caudate nucleus
Putamen
Striatum

ABSTRACT

We have previously shown that docosahexaenoic acid (DHA) significantly reduced L-Dopa-induced dyskinesia (LID) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkeys (Samadi et al., Ann. Neurol. 59:282–288, 2006). In the present study, we measured for the first time mRNA levels of *Nur77*, an orphan nuclear receptor that participates to adaptive and/or aberrant dopamine-related behaviors, and retinoid X receptor γ1 (RXRγ1), a putative brain receptor for DHA and transcriptional partner of *Nur77*, in MPTP monkeys treated with L-Dopa and DHA. The RXRγ1 mRNA is strongly expressed in monkey caudate nucleus and putamen, but no change in levels of RXRγ1 was observed following MPTP and L-Dopa treatments. On the other hand, denervation reduced *Nur77* mRNA levels, whereas chronic L-Dopa treatment strongly induced *Nur77* transcripts. These modulations are taking place in substance P positive cells and are associated with both caudate-putamen matrix and striosome compartments. Interestingly, combination of L-Dopa with DHA further increases *Nur77* mRNA levels in the anterior caudate-putamen, and mainly in striosomes. This is accompanied by a significant inverse correlation between *Nur77* mRNA levels and dyskinetic scores. Taken together, our results show that *Nur77* expression is modulated following dopamine denervation and chronic L-Dopa therapy in a non-human primate model of Parkinson's disease, and suggest that strong modulation of *Nur77* expression might be linked to a reduced risk to develop LIDs.


## Introduction

Levodopa (L-Dopa) therapy is the most common treatment for Parkinson's disease (PD). However, in a large proportion of individuals, therapy is hampered by the development of motor complications such as fluctuations, shortening of the motor response (also called wearing off) and dyskinesias. L-Dopa-induced dyskinesia (LID) is an important motor complication of chronic L-Dopa administration, with a prevalence ranging from 45 to 85% (Brotchie et al., 2005). The development of LIDs over time is a complex process that remains only partially understood, for review see Brotchie et al. (2005), Calabresi et al. (2008), Cenci (2007). Importantly, once the brain is primed to elicit dyskinesias, it is difficult to treat parkinsonian symptoms without inducing dyskinesias. LIDs are also extremely difficult to reduce or reverse once they have appeared. In fact, LID can become so severely disabling as to negate any clinical benefit from dopaminergic therapy in advanced PD patients.

Non human primates intoxicated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin develop a parkinsonian-like syndrome that is very similar to motor symptoms associated with PD in humans. This model also allows insights about molecular changes taking place subsequently to dopamine neuron loss and further treatment. In addition, this parkinsonian primate model has been proven to mimic particularly well both the initial characteristic of L-Dopa behavioral response and the development of long-term motor complications, such as LID (Calon et al., 2000).

LIDs can be assimilated to a form of pathological learning or plasticity (Bédard et al., 1999; Cenci, 2007). Their delayed appearance and persistence after treatment cessation strongly suggest that long-term and possibly permanent basal ganglia circuitry alterations are involved. Therefore, transcription factors, which regulate gene expression, are likely to be involved in these molecular processes. Indeed, chronic Fos proteins of the ΔFosB family of transcription factors in the caudate-putamen, which, when coupled with Jun-D, form AP-1 complexes that can modulate the expression of several genes associated with the generation of LIDs in rodent and primate

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doi:10.1016/j.nbd.2009.07.017

models of PD (Andersson et al., 1999; Doucet et al., 1996; Pavon et al., 2006). In recent years, we have shown that an orphan transcription factor of the nuclear receptor family, namely *Nur77* (also known as Nerve-Growth Factor Inducible gene B (NGFI-B) or NR4A1) might also be associated with movement disorders in rodent models of PD (Sgambato-Faure et al., 2005; St-Hilaire et al., 2006, 2005, 2003; van den Munckhof et al., 2006).

*Nur77* is a member of the *Nur* family, which also includes *Nurr1* and *Nor-1*. Basal levels of *Nur77* are found in most of dopaminoceptive structures such as the striatum, nucleus accumbens, olfactory tubercle and prefrontal cortex (Xiao et al., 1996; Zetterström et al., 1996b). Its expression is strongly modulated after manipulation of dopamine neurotransmission, for review see Lévesque and Rouillard (2007). Unilateral denervation induced by local injection of 6-hydroxydopamine (6-OHDA) in rats produces a complex regulation of *Nur77* in the striatum (St-Hilaire et al., 2005, 2003). The expression of *Nur77* transcripts is selectively up-regulated in enkephalin (ENK)-containing cells of the indirect striatal output pathway, whereas *Nur77* expression is reduced in dynorphin (DYN)-positive cells of the direct output pathway in the denervated striatum (St-Hilaire et al., 2005, 2003). Interestingly, subsequent chronic L-DOPA treatment further reduced *Nur77* expression in DYN-positive cells in specific striatal areas, whereas it strongly increased *Nur77* mRNA levels in this same cell subpopulation in the non-denervated striatum (St-Hilaire et al., 2005, 2003).

*Nur77* can exert its transcriptional activity as a monomer, homodimer or heterodimer with retinoid X receptors (RXR) (Forman et al., 1995; Maira et al., 1999; Zetterström et al., 1996a). Double *in situ* hybridization labeling indicated that the typical antipsychotic haloperidol strongly increased the co-localization of *Nur77* and RXRγ1 isoform in striatal cells (Ethier et al., 2004a). Accordingly, RXR ligands can modulate biochemical and behavioral responses associated with antipsychotic drug administration (Ethier et al., 2004a,b). For example, haloperidol-induced vacuous chewing movements in mice, which resemble tardive dyskinesias in humans, were exacerbated in animals treated with a synthetic RXR antagonist (HX531), whereas administration of the polyunsaturated fatty acid docosahexaenoic acid (DHA), an endogenous RXR agonist in brain (Mata de Urquiza et al., 2000), significantly reduced haloperidol-induced oro-facial dyskinesias (Ethier et al., 2004b). Interestingly, effects of the RXR agonist and antagonist were abolished in *Nur77* knockout mice, indicating that *Nur77* is necessary for the activity of these RXR compounds (Ethier et al., 2004b).

Since tardive dyskinesias induced by chronic dopamine receptor blockade with conventional antipsychotic drugs and LIDs may share common biological substrates, we hypothesized that DHA may also reduce LIDs in MPTP monkeys. We previously reported the behavioral data of concomitant administration of DHA with L-Dopa (Samadi et al., 2006). This study showed that DHA significantly reduced LID scores in MPTP-treated monkeys without altering the anti-parkinsonian activity of L-Dopa (Samadi et al., 2006). In the present study, we report, for the first time, the expression of *Nur77* and RXRγ1 in non-human primate brains. The data suggest that strong modulation of *Nur77* expression might be linked to a reduced risk to develop LIDs.

## Materials and methods

### *Animals and treatments*

Handling of primates was performed in accordance to the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures, including means to minimize discomfort, were reviewed and approved by the Institutional Animal Care Committee of Laval University. Cynomolgus (*Macaca Fascicularis*) ovariectomized female monkeys weighing 3–5 kg were used in the present study. Animals were housed separately in individual observation cages in a temperature-controlled room and exposed to a 12-hour light/dark cycle. They were fed once daily in the afternoon, and water was provided *ad libitum*. The number of animals was kept to a minimum, and all efforts were made to avoid animal suffering. Five animals were used as healthy controls, while the other 15 received the neurotoxin MPTP (Sigma-Aldrich Canada, Oakville, Ontario) dissolved in sterile water and injected continuously at 0.5 mg/24 h using Alzet minipumps (Alzet Inc. Cupertino, CA, USA). In general, one month was needed to induce a stable parkinsonian syndrome. The cumulative dose to achieve this goal was in average 18.1 ± 3.8 and 13.9 ± 2.5 mg for L-Dopa and L-Dopa plus DHA groups, respectively (Samadi et al., 2006). The animals were scored several times a week with a disability scale, as previously described (Bélanger et al., 2003). This study was undertaken several months after a stable parkinsonian syndrome had developed (the time after MPTP was 149 ± 63 and 190 ± 42 days for L-Dopa and L-Dopa + DHA groups, respectively) (Samadi et al., 2006). Ten *de novo* MPTP intoxicated animals were treated with L-Dopa, five received L-Dopa alone, while the others received L-Dopa plus DHA. We used a fixed high daily oral dose of 100/25 mg of L-Dopa/benserazide (Sigma-Aldrich Canada, Oakville, Ontario). For the L-Dopa plus DHA group, MPTP-treated monkeys were first exposed to DHA (100 mg/kg, p.o., in a volume of 20–25 ml according to the weight of the animal) for 3 days before L-Dopa therapy was introduced. Then, combined oral administration of L-Dopa and DHA was performed on a daily basis for 1 month. Locomotor activity, as well as parkinsonian and dyskinetic scores of these animals have been previously reported (Samadi et al., 2006). All animals were sacrificed by an overdose of pentobarbital 4 h after their last L-Dopa dose.

### *Tissue preparation*

Brains were rapidly removed and stored, as previously described (Morissette et al., 2006). Briefly, they were placed in isopentane cooled in dry ice (−40 °C) and kept frozen at −80 °C. Hemisected brains were cut into coronal sections of 12 μm on a cryostat (−20 °C). The slices were thaw-mounted onto SuperFrostPlus (Fisher Scientific Ltd, Nepean, ON, Canada) 75 × 50 mm slides and stored at −80 °C until use.

### *Dopamine concentrations and [$^{125}$I]RTI-121 autoradiography*

Pieces of coronal brain sections were homogenized in 250 μl of 0.1 M $HClO_4$ at 4 °C. The homogenate was centrifuged at 10,000 × g for 20 min. The supernatants were kept at −80 °C. The pellets were dissolved in 100 μl of 0.1 M NaOH for assays of protein content. The concentration of dopamine was measured by high-performance liquid chromatography with electrochemical detection (Morissette et al., 2006). Extent of denervation was also evaluated by measuring the dopamine transporter (DAT) with [$^{125}$I]RTI-121 (3β-(4-I-iodophenyl) tropane-2β-carboxylic acid isopropyl ester, 2200 Ci/mmol; Mandel, Boston, MA, USA) binding autoradiography. Specific binding was measured using 25 pM of [$^{125}$I]RTI-121. Non-specific binding was determined by adding 100 nM of Mazindol (Sandoz Pharmaceuticals, Dorval, Qc, Canada) to the incubation buffer (Morissette et al., 2006).

### *Complementary RNA probe preparation and synthesis*

In order to label *Nur77* mRNA in monkey brain tissues, we have produced a complementary RNA (cRNA) probe from total RNA of human caudate-putamen tissues. The cRNA probe for *Nur77* stems from an 814 bp (nucleotides 19 to 832) fragment of the full-length human cDNA (GeneBank accession no: NM002135) subcloned into pCRII/TOPO and linearized with Bam HI. The RXRγ1 cRNA probe was generated from a 320 bp fragment of the rat full-length RXRγ1 cDNA contained in the pBS-SK$^+$ vector and linearized with EcoRI. Both