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The effect of subchronic, intermittent L-DOPA treatment on neuronal nitric oxide synthase and soluble guanylyl cyclase expression and activity in the striatum and midbrain of normal and MPTP-treated mice

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Abstract

We have investigated the effects of low (10 mg/kg) and high (100 mg/kg) doses of L-DOPA on the expression and activity of neuronal nitric oxide synthase (nNOS) and guanylyl cyclase (GC) in the striatum and midbrain of mice. L-DOPA was administered subchronically for 11 days (beginning 3 days after last MPTP/NaCl injection) or for 14 days (with dosing started immediately following the last MPTP/NaCl injection). Adult mice received three intraperitoneal (i.p.) injections of physiological saline or MPTP at 2 h intervals (total dose of 40 mg/kg). Normal and MPTP-injected mice were treated twice a day for 11 or 14 days with low (10/2.5 mg/kg bw) or high (100/25 mg/kg bw) doses of L-DOPA/benserazide.

The present study indicates that several days of treatment with L-DOPA does not affect MPTP-activation of the nNOS/sGC/cGMP pathway or the neurodegenerative processes that occur in the striatum and midbrain of mice. In normal mice, L-DOPA upregulates the expression and activity of nNOS and GC to levels found in MPTP-injected mice. Due to upregulation of nNOS and GC, cGMP levels in the mouse striatum and midbrain are also elevated, however, significantly lower in mice administrated with low dose of L-DOPA. In both investigated brain regions of normal mice cGMP-dependent PDEs activities were elevated after low dose administration of L-DOPA, but no change in PDEs activities has been detected in MPTP and high L-DOPA-injected mice as compared to control values.

The enhancement of nNOS mRNA and GCβ1 mRNA levels were generated by both doses of L-DOPA, given in a time-dependent fashion. L-DOPA-injected for 11 or 14 days caused a decrease in TH protein levels in the striatum and midbrain, respectively; this result was noted irrespective of dose. L-DOPA therapy did not prevent the MPTP-induced decrease in TH protein levels in either investigated brain region.

Keywords: Nitric oxide synthase; Guanylyl cyclase; cGMP; Tyrosine hydroxylase (TH); L-DOPA; MPTP model of PD; L-DOPA treatment; Phosphodiesterases activities

1. Introduction

A key pathologic feature of Parkinson disease (PD) is degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the ventral midbrain. In addition, loss of these dopaminergic axonal projections and nerve terminals within the striatum results in the depletion of striatal dopamine (DA) and its metabolites. This was recognized as the main factor leading to the suppression of nigrostriatal dopaminergic neurotransmission (Hornykiewicz, 1966, 2006; Agid, 1991; Przedborski et al., 1996; Zhang et al., 2000).

Levodopa (L-DOPA) remains as the most effective pharmacological treatment for PD. After crossing the blood brain barrier (BBB), L-DOPA is rapidly catabolized by L-amino

Abbreviations: AA, arachidonic acid; AADC, L-amino acid decarboxylase; BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; DA, dopamine; DA-ergic, dopaminergic; DAT, dopamine transporter; cGMP, guanosine 3′,5′-cyclic monophosphate; GC, guanylyl cyclase; GCβ1, guanylyl cyclase β1 subunit; IBMX, isobuthyl-1-methylxanthine; iNOS, inducible nitric oxide synthase; i.p., intraperitoneal; L-DOPA, L-3,4-dihydroxyphenylalanine; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; 7-NI, 7-nitroindazole; $ONOO^-$, peroxynitrite; PD, Parkinson disease; PDEs, phosphodiesterase; sGC, soluble guanylyl cyclase; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase

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acid decarboxylase (AADC) to dopamine. Most striatal AADC is located within nigrostriatal dopaminergic nerve terminals (Lloyd et al., 1975; Lopez-Real et al., 2003). Therefore, a severe depletion of DA in the nigrostriatal system, as is the case in PD, may be remedied by exogenous administration of L-DOPA (Maeda et al., 1999). Although this is effective in treating PD, controversy over the toxicity of L-DOPA remains. There is a substantial amount of evidence that supports the toxicity of L-DOPA in vitro (Jenner and Brin, 1998; Koshimura et al., 2000). However, in vivo studies have failed to demonstrate the neurotoxicity of L-DOPA on nigral cells in humans (Gwinn-Hardy et al., 1999; Kingsbury et al., 1999; Olanow et al., 2004), rats, and mice (Jenner and Brin, 1998), despite the oxidative stress caused by its metabolization or autoxidation (Huie and Padjama, 1993; Basma et al., 1995; Darley-Usmar and Hallibel, 1996). In this regard, L-DOPA may serve as a source of free radicals which lead to neurodegeneration within the central nervous system (CNS) (Coyle and Puttfarcken, 1993), and may even exacerbate PD (Fahn and Cohen, 1992). Furthermore, chronic L-DOPA treatment is associated with motor fluctuations, dyskinesia, and psychiatric symptoms in patients severely affected by PD (Mizuno et al., 1994; Obeso et al., 2000; Rinne et al., 1998; Parkinsonian Study Group, 2000). A combination of striatal denervation and chronic administration of L-DOPA seems to be key for the development of L-DOPA-induced dyskinesia, both in patients with PD (Nutt, 1990), and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioned non-human primates (Schneider, 1989; Boyce et al., 1990).

Nitric oxide (NO), which is known to be synthesized in the brain by nitric oxide synthase (NOS), is involved in a wide range of physiological roles within the CNS. One such role is the regulation of striatal dopamine neurotransmission (West et al., 2002). It was found that the in vivo administration of NO donor drugs increased the extracellular level of dopamine in the striatum (West and Galloway, 1998; Trabace and Kendriek, 2000; Serra et al., 2001). This suggests that NO-induced elevation of striatal dopamine may serve a protective role in PD. Also, data suggests that chronic L-DOPA administration is not toxic for the remaining DA-ergic neurons in rats with partial lesions of the nigrostriatal pathway and may even promote the recovery of such neurons (Murer et al., 1998; Camp et al., 2000).

In contrast, several lines of evidence support the view that NO may be involved in the neurodegeneration and death of SNpc dopaminergic neurons (Przedborski et al., 1996; Dehmer et al., 2000; Zhang et al., 2000). Recent experiments have shown that treatment of mice with MPTP induced the mRNA expression of neuronal NOS (nNOS) and guanylyl cyclase β1 subunit (GCβ1), which led to elevation in their protein levels and activity within the striatum and SNpc. This effect was accompanied by a marked enhancement of cGMP formation (Chalimoniuk et al., 2004a,b, 2006). Administration of 7-nitroindazole (7-NI), a nNOS inhibitor, was able to decrease the MPTP-induced elevations in cGMP levels and partially prevent the death of DA-ergic neurons. This points to the NO/GC/cGMP pathway as a likely regulatory mechanism involved in pathogenesis of parkinsonism (Chalimoniuk et al., 2006).

There is evidence that the cerebrospinal fluid (CSF) concentration of nitrate and nitrite, both NO metabolites, is elevated in PD patients receiving dopamine replacement or dopamine receptor agonist therapy (Molina et al., 1996). Furthermore, L-DOPA therapy can cause a marked rise of cGMP levels in the cerebellum and CSF (Gumulka et al., 1976; Navarro et al., 1998). This findings agree with the fact that the serum level of cGMP is significantly elevated in patients with PD receiving L-DOPA therapy lasting several years (Chalimoniuk and Stepien, 2004). In contrast, many other studies did not show evidence of systemic changes caused by administration of L-DOPA (Belmaker et al., 1978; Kuiper et al., 1994; Qureshi et al., 1996).

It was previously demonstrated by means of microdialysis technique that L-DOPA induced striatal NO production in freely mobile mice (Itokawa et al., 2006). Study performed with dopamine agonist suggests that activation of D2 receptors, which are also located in nigrostriatal pathway (Josheph et al., 1978) may be involved in NO production (Melis et al., 1996). Because regulation of the NO/sGC/cGMP pathway is of importance in nigrostriatal intracellular signaling in PD, we attempted to elucidate the role of L-DOPA treatment in this pathway. Therefore, in the present study we analyzed nNOS and GCβ1 mRNA and protein levels, as well as the concentration of cGMP, in normal and MPTP-injected mice which received twice daily treatments with L-DOPA in low (10 mg/kg bw) or high (100 mg/kg bw) doses, lasting either 11 or 14 days. The expression of mRNA and/or protein levels of nNOS and GCβ1 were measured in the striatum and midbrain of the mice; cGMP levels and PDE activities were estimated immunobiochemically. The extent of nigrostriatal lesions achieved by MPTP administration was indicated by tyrosine hydroxylase (TH) protein levels. Synaptosomes prepared from the striatum served as an experimental tool for the measurement of dopamine uptake in all experimental conditions.

2. Materials and methods

2.1. Materials

Rabbit polyclonal anti-tyrosine hydroxylase (TH) and anti-nNOS antibodies were purchased from Biocom International (Temecula, CA, USA). The anti-GCβ1 antibodies, anti-rabbit IgG, and anti-mouse conjugated with horseradish peroxidase antibodies were purchased from Sigma–Aldrich (St. Louis, MO, USA). Mouse anti-actin antibodies were purchased from MP Biomedicals Inc. (Aurora, OH, USA).

Nitrocellulose membranes were obtained from Bio-RAD (Wien, Austria). The RT kit was obtained from Promega (Medison, WI, USA), and the Master Mix was obtained from Qiagen GmbH (Hilden, Germany). The cyclic GMP kit and ECL kit were obtained from GH Healthcare Biosciences (Piscataway, NJ, USA). Protease inhibitors were from Roche Applied Science (Indianapolis, IN, USA). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), L-3,4-dihydroxyphenylalanine (L-DOPA), benserazide, TRI-reagent, 1-isobuthyl-1-methylxantine (IBMX), Zaprinast and BAY 607550 and all other reagents were purchased from Sigma–Aldrich.

2.2. Animals

Eight-week-old C57 BL/6 mice (20–25 g) from the animal facility within the Polish Academy of Sciences, Medical Research Center were used. All procedures involving mouse care and experimentation were carried out in