Neurobiology of Disease 32 (2008) 340–348

Contents lists available at ScienceDirect

Neurobiology of Disease

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

# Entacapone potentiates the long-duration response but does not normalize levodopa-induced molecular changes

C. Marin [a,d,*], E. Aguilar [a], G. Mengod [b,d], R. Cortés [b,d], M.C. Rodríguez-Oroz [c,d], J.A. Obeso [c,d]

[a] Laboratori de Neurologia Experimental, Àrea de Neurociències, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[b] Departament de Neuroquímica i Neurofarmacologia, Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, Barcelona, Spain
[c] Department of Neurology and Neurosurgery, Neuroscience Center, Clínica Universitaria and Medical School, University of Navarra and CIMA, Pamplona, Spain
[d] Centro de Investigación en Redes sobre Enfermedades Neurodegenerativas (CIBERNED), Spain

ARTICLE INFO

*Article history:*
Received 8 May 2008
Revised 4 July 2008
Accepted 25 July 2008
Available online 5 August 2008

*Keywords:*
Parkinson's disease
Levodopa
Entacapone
6-Hydroxydopamine
Preproenkephalin
Preprodynorphin

ABSTRACT

Coadministration of entacapone with levodopa attenuates motor complications in experimental models of Parkinson's disease. The mechanisms underlying entacapone effects are unknown. We investigated the effect of entacapone, on: long-duration response (LDR) to levodopa, levodopa-induced postsynaptic pharmacodynamic mechanisms and molecular changes in hemiparkinsonian rats. 6-Hydroxydopamine-unilaterally lesioned rats were treated with levodopa (25 mg/kg)+vehicle; levodopa+entacapone (30 mg/kg) or saline, twice daily for 22 days. The LDR and the apomorphine-induced rotations were measured. In situ hybridization was performed measuring the expression of striatal preproenkephalin, preprodynorphin and dopamine D-3 receptor mRNAs, subthalamic cytochrome oxidase mRNA and nigral glutamic acid decarboxylase mRNA. Entacapone potentiated the LDR but did not modify either the apomorphine-induced rotational behavior or the molecular changes. Our results suggest that the effects of entacapone on levodopa-induced motor response are not mediated by postsynaptic mechanisms and that administration of entacapone is not able to normalize the molecular alterations induced by levodopa in the basal ganglia.


## Introduction

Under physiological circumstances, extra-synaptic striatal dopamine (DA) levels are relatively constant and DA receptors are stimulated in a relatively continuous manner (Abercrombie et al. 1990; Floresco et al. 2003; Venton et al. 2003). In Parkinson's disease (PD), there is a loss of dopaminergic neurons and terminals and striatal DA levels are increasingly dependent on the peripheral availability of levodopa (Abercrombie et al. 1990; Miller and Abercrombie 1999; De la Fuente-Fernández et al. 2004). Therefore, in advanced PD the fluctuations in plasma levels of short-acting dopaminergic agents, such as levodopa, lead to alternating high and low levels of activation of striatal DA receptors. This "pulsatile" pattern of receptor stimulation is a function of both, disease severity (Bédard et al. 1986; Pearce et al. 1998, 2001; Jenner 2000) and the short half-life of the dopaminergic agent employed (Bédard et al. 1986; Nutt 1990; Pearce et al. 1998; Olanow and Obeso 2000; Olanow et al. 2006). Sustained evidence has been accumulated indicating that levodopa-related motor complications in PD are associated with non-physiological, intermittent or pulsatile stimulation of striatal DA receptors (Chase et al. 1989, 1994; Juncos et al. 1989; Blanchet et al. 1995; Grondin et al. 1996; Stocchi and Olanow 2004; Olanow et al. 2006; Fabbrini et al. 2007).

This concept suggests that a more continuous stimulation of DA receptors may ameliorate or even reduce the development of motor complications. The former has been shown in PD (Quinn et al. 1984; Obeso et al. 1986; Mouradian et al. 1990; Nutt 1990; Olanow et al. 2006; Nyholm 2007) and the latter in experimental parkinsonism (Bédard et al. 1986; Blanchet et al. 1995; Grondin et al. 1996; Pearce et al. 1998; Maratos et al. 2001; Jenner 2004; Smith et al. 2005; Nyholm 2007). The concept of continuous dopaminergic stimulation (CDS) is supported by a wealth of experimental data showing that the dopaminergic system exerts a dual (tonic and phasic) effect on the striatum (Grace 1991; Floresco et al. 2003). In patients with PD, clinical pharmacological studies also support the therapeutical concept of CDS (Chase et al. 1989, Obeso et al. 1994, 2000; Olanow and Obeso 2000; Olanow et al., 2006).

There is evidence suggesting that pulsatile striatal stimulation induces postsynaptic pharmacodynamic mechanisms which result in altered regulation of striatal genes and proteins, which ultimately leads to abnormal neuronal firing patterns in the striatopallidal circuitries (Herrero et al. 1995; Jolkkonen et al. 1995; Morissette et al. 1997, 1999; Olanow and Obeso 2000; Aubert et al. 2005; Gardoni et al. 2006; Bychkov et al. 2007). In more detail, chronic levodopa or dopamine agonist treatment elevates the expression of the striatal enkephalin precursor preproenkephalin (PPE), the opioid peptide expressed in the striatal neurons that project to the pars externa of the

* Corresponding author. Laboratori de Neurologia, Servei de Neurologia, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain. Fax: +34 93 2275783.
*E-mail address:* cmarin@clinic.ub.es (C. Marin).
**Available online on ScienceDirect (www.sciencedirect.com).**

0969-9961/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2008.07.019

globus pallidus (GPe), above the levels reached after DA denervation alone (Gerfen et al. 1990; Herrero et al. 1995; Henry et al. 1999; Ravenscroft et al. 2004; Chen et al. 2005). Moreover, a significant correlation has been found between levodopa-induced complications and increased striatal levels of preprodynorphin (PDyn) mRNA, the opioid peptide expressed in the striatal neurons that project directly to the output structures of the basal ganglia, in a rodent model of PD (Cenci et al. 1998; Andersson et al. 1999; Carta et al. 2002; Winkler et al. 2002; Ravenscroft et al. 2004). Levodopa-induced changes in striatal gene expression are believed to result form abnormal, intermittent stimulation of supersensitive receptors on DA-denervated striatum because PPE and PDyn mRNA changes can be normalized with long-acting DA agonists that do not induce dyskinesia (Henry et al. 1999; Calon et al. 2000) and when the same short-acting DA agonists are continuously administered (Morissette et al. 1997).

Entacapone is a potent, selective and reversible peripherally acting inhibitor of catechol-*O*-methyl transferase (COMT), the enzyme that provides the main breakdown pathway for levodopa in the blood after dopa decarboxylase. Inhibition of COMT by entacapone prolongs the half-life of levodopa (Nutt et al. 1994; Ruottinen and Rinne 1996) and this is associated with improved clinical efficacy in PD patients (Kaakkola and Wurtman 1993; Nutt et al. 1994; Ruottinen and Rinne 1996; Parkinson Study Group 1997; Rinne 1998; Poewe et al., 2002, Stocchi et al. 2004; Grandas et al. 2007; Deuschl et al. 2007; Müller et al. 2007) and in experimental models of parkinsonism (Smith et al. 2003; 2005; Marin et al. 2005, 2006). By extending the half-life of levodopa with entacapone, it is possible to deliver the drug in a way that is less pulsatile, thereby allowing the benefit of levodopa with a reduced risk for motor complications. In detail, the administration of entacapone attenuates and prevents the development of levodopa-induced motor fluctuations (Marin et al. 2005) and dyskinesias in 6-OHDA-lesioned rats (Marin et al. 2006) and in MPTP-treated monkeys (Smith et al. 2003, 2005). However, it is still unknown whether the administration of entacapone modifies the molecular changes induced by levodopa in the basal ganglia nuclei.

The motor response to levodopa is comprised of two components: the long-duration response (LDR) and the short duration response (SDR) (Muenter and Tyce 1971). The SDR is characterized by a short-lasting motor improvement typically lasting 3–4 h following a single dose of levodopa. The SDR is the basis for the clinical phenomenon of the motor fluctuation known as "wearing-off". However, the LDR is a sustained motor improvement that takes days to build up and lasts for many hours to days after levodopa discontinuation (Nutt et al. 1995; Quattrone et al. 1995; Zappia et al. 1997). A study in the *novo* patients (the ELLDOPA trial) has shown that the LDR may actually last for several weeks after cessation of levodopa treatment (Parkinson Study Group 2004). An experimental model of the LDR to levodopa has been recently described in 6-OHDA-lesioned rats (Marin et al. 2007). An improvement in forelimb akinesia that lasted for at least 2 days after levodopa discontinuation resembling the LDR to levodopa in patients, has been observed (Marin et al., 2007). The effect of entacapone on the LDR to levodopa is still unknown.

Based in the hypothesis that entacapone may provide a greater bioavailability of levodopa in the brain and thus, a more continuous stimulation of dopaminergic receptors, we investigated if entacapone administration normalizes the molecular alterations induced by levodopa treatment. We tried to define in more detail the mechanism of action by which levodopa plus entacapone administration reduces motor complications in the 6-OHDA rat model by assessing the effect of entacapone on: (i) the LDR to levodopa, (ii) the postsynaptic pharmacodynamic mechanisms induced by levodopa, and (iii) the levodopa-induced molecular changes in the basal ganglia nuclei. A better definition of putative postsynaptic mechanisms mediating the entacapone effect may be clinically relevant as they may be involved in the development or maintenance of levodopa-induced motor complications and thus, might be avoided with future treatment strategies.

## Materials and methods

### *6-OHDA lesions*

Forty-four male Sprague–Dawley rats weighing 220–240 g were housed on a 12-hour light/dark cycle with free access to food and water. Under sodium pentobarbital anaesthesia (50 mg/kg, intraperitoneal, i.p.), rats were placed in a stereotactic frame with the incisor bar positioned 4.5 mm below the interaural line. Each animal received a 6-OHDA (Sigma, Spain) injection (8 μg in 4 μl of saline with 0.02% ascorbate over 8 min) into the left medial forebrain bundle by means of a Harvard infusion pump. 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). Adequate measures were taken to minimize pain or discomfort. All animal experiments were carried out in accordance with the National Institutes of Health guide for care and use of laboratory animals and approved by the Local Government.

### *Protocol of treatments*

Animals were distributed in three groups and treated with (1) levodopa methyl ester (25 mg/kg with 6.25 mg/kg benserazide, i.p.) (Sigma, Spain) plus vehicle ($n=16$) twice a day; (2) levodopa methyl ester (25 mg/kg with 6.25 mg/kg benserazide, i.p.) plus entacapone (30 mg/kg, i.p., $n=18$) twice a day, or vehicle ($n=10$) for 22 consecutive days.

### *Experimental design*

Three sets of experiments were performed in order to investigate the effect of chronic entacapone administration on: i) levodopa-induced LDR ii) levodopa-induced postsynaptic pharmacodynamic alterations and, iii) levodopa-induced molecular changes in the basal ganglia nuclei.

In the first set, the effect of chronic entacapone on levodopa-induced LDR was evaluated. The effect of entacapone on the use of the parkinsonian limb induced by levodopa was investigated by performing the cylinder test. LDR was investigated before and after a dose test of levodopa (6 mg/kg) in 6-OHDA-lesioned rats during and until 7 days after levodopa or levodopa plus entacapone chronic treatment (Marin et al. 2007).

In the second set of experiments, we evaluated whether postsynaptic pharmacodynamic mechanisms were involved in the entacapone effect on levodopa-induced motor response. The rotational response to apomorphine (0.5 mg/kg, sc) (Sigma, Spain) was evaluated 3 days after the last treatment day in both, levodopa plus vehicle and levodopa plus entacapone, treated animals.

And finally, to investigate the effects of entacapone on levodopa-induced molecular changes in the basal ganglia nuclei, groups of animals treated following the same protocol than for the behavioral studies, were sacrificed after 3 days of washout and the in situ hybridization studies were performed.

### *Rotational screening*

For the measurement of rotational behavior, rats were placed in circular cages and tethered to an automated rotometer. The number of complete (360°) turns made during each 5-minute period was automatically recorded by a computerized system. Rats were allowed 15 min to habituate to the rotometer before drug administration. Following a three-week recovery period, rats exhibiting a vigorous rotational response (>100 total turns) to apomorphine (0.05 mg/kg, subcutaneous (sc) were selected for further study. It has been previously demonstrated that rats meeting this criterion have a greater than 95% depletion of striatal dopamine (Papa et al. 1994). Apomorphine-induced rotations (0.5 mg/kg, sc) were measured to evaluate the involvement of postsynaptic mechanisms in entacapone effects.