Neurobiology of Disease 54 (2013) 339–348

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

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

Persistent activation of the D1R/Shp-2/Erk1/2 pathway in L-DOPA-induced dyskinesia in the 6-hydroxy-dopamine rat model of Parkinson's disease

Chiara Fiorentini [a,*,1], Paola Savoia [a,1], Daria Savoldi [a], Alessandro Barbon [b], Cristina Missale [c]

[a] Division of Pharmacology, Department of Biomedical Sciences and Biotechnology and National Institute of Neuroscience, Italy
[b] Division of Biology and Genetics, Department of Biomedical Sciences and Biotechnology and National Institute of Neuroscience, Italy
[c] "Camillo Golgi" Woman Health Centre, University of Brescia, Viale Europa 11, 25123 Brescia, Italy

ARTICLE INFO

Article history:
Received 8 August 2012
Revised 17 December 2012
Accepted 4 January 2013
Available online 14 January 2013

Keywords:
Parkinson's disease
L-DOPA-induced dyskinesia
Caudate-putamen
Dopamine D1 receptors
Shp-2

ABSTRACT

Prolonged L-3,4-dihydroxyphenylalanine (L-DOPA) administration, the gold standard therapy for Parkinson's disease (PD) is associated with serious motor complications, known as L-DOPA-induced dyskinesia (LID). One of the major molecular changes associated with LID is the increased activity of the extracellular signal-regulated kinases 1/2 (Erk1/2) signaling in the medium spiny neurons of the striatum induced by malfunctioning in the dopamine D1 receptor (D1R)-mediated transmission.

We have previously established that in the striatum, activation of Shp-2, an intracellular tyrosine phosphatase associated with the D1R, is a requisite for the D1R to activate Erk1/2.

In this study, we investigated the role of striatal D1R/Shp-2 complex in the molecular event underlying LID in the 6-OHDA-lesioned rat model of PD.

We found that in hemiparkinsonian rats experiencing LID, the physiological interaction between D1R and Shp-2 in the striatum was preserved. In these animals, the chronic activation of D1R either by L-DOPA or by the selective D1R agonist SKF 38393 induced both dyskinesia and Shp-2/Erk1/2 activation. These effects were prevented by the selective D1R-antagonist SCH23390 suggesting the involvement of striatal D1R/Shp-2 complex, via Erk1/2 activation, in the molecular events underlying LID. Interestingly, we found that D1R-mediated Shp-2-Erk1/2 activation was persistently detected in the striatum of dyskinetic rats during L-DOPA washout, with a close correlation between LID severity and the extent of long term activation of both Shp-2 and Erk1/2.

Taken together, our data show that in hemiparkinsonian rats developing dyskinesia, the aberrant phosphorylation of Shp-2 by D1R activation, represents an upstream molecular event leading to the persistent phosphorylation of Erk1/2 and therefore a novel therapeutic target to counteract LID development and maintenance during L-DOPA therapy.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder with serious motor impairments caused by the selective degeneration of dopaminergic neurons of the substantia nigra pars compacta, leading to the loss of dopamine (DA) input to the dorsal striatum. Striatal DA replacement, obtained by L-3,4-dihydroxyphenylalanine (L-DOPA) administration, represents the most effective therapy currently used in PD. The loss of nigrostriatal input, however, leads to dramatic changes in the response of striatal medium spiny neurons (MSNs) to dopaminergic drugs, including L-DOPA. As a result, the majority of patients develop severe motor complications, known as L-DOPA-induced dyskinesias (LID), during chronic therapy (Barroso-Chinea and Bezard, 2010) that vanish the beneficial effects of L-DOPA. Despite the severity and the clinical relevance of these side effects, the molecular and cellular mechanisms of LID are far from clear, although important advances have been made in recent years. Among different mechanisms, several lines of evidence point to aberrant D1 receptor (D1R) signaling as a central event in the development of LID (Gerfen et al., 2002). In particular, increased D1R coupling to $G_{alphaolf}$ proteins (Aubert et al., 2005; Corvol et al., 2004; Herve et al., 1993) and adenylyl cyclase (AC) 5/6 (Rangel-Barajas et al., 2011), resulting in exaggerated cyclic AMP formation and overactivation of the cAMP-dependent protein kinase A (PKA) (Corvol et al., 2001; Picconi et al., 2003; Santini et al., 2007; Zhuang et al., 2000), has been demonstrated. These events result in the aberrant phosphorylation of downstream DA- and cAMP-regulated phosphorprotein 32 kDa (DARPP-32), leading to loss of depotentiation at cortico-striatal synapses (Picconi et al., 2003) and intensifying D1R-cAMP-mediated activation of

Abbreviations: NMDAR, N-methyl-D-aspartate receptor; ITIM, immunoreceptor tyrosine-based inhibitory motif; STEP, striatal-enriched protein tyrosine phosphatase; MEK, mitogen-activated protein kinase kinase 1; MSK-1, mitogen and stress-activated kinase-1; TH, tyrosine hydroxylase; Shp-2, Src homology 2 domain (SH-2)-containing tyrosine phosphatise; CREB, cAMP response element-binding.

* Corresponding author at: Department of Biomedical Sciences and Biotechnology, University of Brescia, Viale Europa 11, 25123 Brescia, Italy. Fax: +39 0303717529.
E-mail address: chiara.fiorentini@med.unibs.it (C. Fiorentini).

Available online on ScienceDirect (www.sciencedirect.com).

[1] These authors equally contributed to this work.

0969-9961/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nbd.2013.01.005

the extracellular signal-regulated kinases 1 and 2 (Erk1/2) (Greengard, 2001), the two major effectors involved in the modulation of synaptic plasticity. Exaggerated phosphorylation of striatal Erk1/2 in D1R-expressing MSNs, has been associated to the development of LID in different animal models of PD (Darmopil et al., 2009; Pavon et al., 2006; Santini et al., 2007, 2009; Westin et al., 2007), even Erk1/2 phosphorylation is not activated by striatal D1R under normal condition (Gerfen et al., 2002). Over-activation of Erk1/2 and its downstream nuclear effectors, such as the mitogen and stress-activated kinase-1 (MSK-1) and histone H3 (Darmopil et al., 2009; Santini et al., 2009; Westin et al., 2007), have been in fact proposed to lead to transcriptional responses likely related to the development and expression of dyskinesia. In particular, a transient activation of the Erk1/2/MSK-1/histone 3 cascade has been detected in MSNs of the direct pathway during L-DOPA administration (Santini et al., 2009; Westin et al., 2007). The central role of the D1R-Erk1/2 cascade in LID development was further supported by the observation that, in several animal models, interfering with intracellular effectors mediating Erk1/2 activation resulted in amelioration of dyskinetic symptoms. For example, intrastriatal PKA inhibition by Rp-cAMP (Ba et al., 2011; Lebel et al., 2010; Oh et al., 1997), administration of the MEK inhibitor SL327 (Santini et al., 2007) or of statin, that inhibits the Ras-Erk cascade (Schuster et al., 2008), reduced the ability of chronic L-DOPA to generate dyskinesia in 6-OHDA rat model of PD.

Identifying novel antidyskinetic strategies is an urgent need that necessarily imply a deep understanding of the neuronal mechanisms mediating these motor dysfunctions. In this context, a crucial issue is to identify the intracellular effectors involved in D1R-mediated Erk 1/2 activation and to define whether aberrant activation of Erk1/2 is persistent after L-DOPA withdrawal so that, by a long lasting modification of synaptic plasticity (Picconi et al., 2003), may mediate maladaptive processes responsible for dyskinesia. We have recently identified a new mechanism for D1R-mediated activation of Erk1/2 in striatal MSNs involving the tyrosine phosphatase Shp-2. In particular, in the striatum, Shp-2 directly interacts with the D1R and is activated by D1R stimulation and this activation is essential for the D1R to transduce Erk1/2 signaling (Fiorentini et al., 2011). We have also shown that phosphorylation of both Shp-2 and Erk1/2 by the D1R requires the cAMP/PKA pathway and involves the tyrosine kinase Src (Fiorentini et al., 2011). In the striatum D1R-mediated PKA activation thus promotes phosphorylation of two effectors, D1R-associated Shp-2 and DARPP-32, that synergistically act to trigger and sustain Erk1/2 activation. Our data suggested that activated Shp-2 initiates the Erk1/2 signaling cascade, since its inactivation completely abrogated this signaling pathway (Fiorentini et al., 2011). It is thus crucial to define the role of the D1R/Shp-2 complex in the development of LID since it could represent a promising drug target.

The aim of this study was to investigate the role of the D1R/Shp-2 complex in L-DOPA-induced aberrant activation of Erk1/2 signaling and development of dyskinesia in the 6-OHDA rat model of PD.

Materials and methods

Animals. Male Wistar Han rats (Charles River Laboratories, Italy) (275–300 g) were housed under a 12-hour light–dark cycle, with ad libitum access to food and water.

Drugs. 6-Hydroxydopamine hydrochloride (6-OHDA), L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA), apomorphine hydrochloride, benserazide hydrochloride, SKF 38393 hydrochloride, SCH 23390 hydrochloride were purchased from Sigma-Aldrich (Milano, Italy).

Antibodies. Anti-Shp-2, phospho-Erk1/2, Erk1/2, Src primary antibodies were from Santa Cruz Biotechnologies (Heidelberg, Germany); anti-phospho-Shp-2 antibody was from Cell Signaling (Euroclone, Milano, Italy); the rat monoclonal anti-D1R antibody (clone 1-1-F11-S.E6) and the monoclonal anti-tubulin antibody were from Sigma-Aldrich. The rabbit anti-D1R antibody was from Alomone (Israel). The horseradish peroxidase-conjugated anti-mouse and anti-rabbit secondary antibodies were from Santa Cruz Biotechnologies.

6-Hydroxydopamine (6-OHDA) lesion

Experiments were performed according to the European Community Council Directive, November 1986 (86/609/EEC). Male Wistar rats (290–300 g; n = 98) were treated with carprofen (5 mg/kg) and deeply anesthetized with tiletamine/zolazepam (80 mg/kg). They were mounted on a stereotaxic frame (Stoelting, Wood Dale, IL, USA) and injected with 12 μg of 6-OHDA dissolved in 4 μl of saline containing 0.2% ascorbic acid at the rate of 0.38 μl/min into the left medial forebrain bundle (from bregma: AP, -2.2; L, + 1.5; DV, -7.9; tooth bar, -3.3) according to the atlas of Paxinos and Watson (1986). Sham-operated rats ($n = 7$) were injected with saline. Two weeks after surgery, limb-use asymmetry was evaluated as an index of akinesia with the cylinder test (Fiorentini et al., 2006; Schallert et al., 2000). The extent of the lesion was also evaluated by measuring contralateral turning behavior induced by a low dose of apomorphine (0.05 mg/kg s.c.). Only rats showing more than 200 turns contralateral to the lesion in 40 min, corresponding to greater than 95% depletion of striatal dopamine (Fiorentini et al., 2006; Papa et al., 1994), were included in the study ($n = 82$) (Fig. 1).

Drug treatment and behavioural testing

Three weeks after lesion, rats were treated with either saline ($n = 7$) or L-DOPA (8 mg/kg i.p.) combined with benserazide (7.5 mg/kg, i.p.) for 21 days ($n = 32$) (Cenci et al., 1998; Fiorentini et al., 2006; Lundblad et al., 2002). Drugs were dissolved in physiological saline immediately prior to use. L-DOPA-induced abnormal involuntary movements (AIMs) were recorded twice a week in individual animals for a total of six observations during the three week treatment, according to Cenci et al. (1998), and classified as axial, limb, orolingual and locomotor. Each of these symptoms was scored on a validated severity scale from 0 to 4 (Cenci et al., 1998; Fiorentini et al., 2006; Lundblad et al., 2002). In each test session, animals were individually recorded for 1 min every 20 min from 20 to 180 min after L-DOPA administration, for a total of nine observations. The AIMs score represents the sum of these observations. The theoretical maximum score that can be accumulated by one animal in one testing session was 144 (maximum score per observation point = 16; number of observation points per session = 9). Statistical significance of the data was determined by repeated measures analysis of variance (ANOVA), followed by *post-hoc* Bonferroni's test. Animals were killed by decapitation after 21 days of L-DOPA treatment, either 45 min ($n = 11$) or 24 h ($n = 21$) after the last L-DOPA injection. The striata were rapidly dissected, frozen in liquid nitrogen and stored at −80 °C.

In another set of experiments, lesioned rats were injected with L-DOPA (8 mg/kg, i.p.) plus benserazide (7.5 mg/kg, i.p.) in the absence ($n = 10$) or in the presence of the selective D1R antagonist SCH 23390 (0.25 mg/kg, i.p.) ($n = 12$), given 30 min before L-DOPA administration; a group of hemiparkinsonian rats ($n = 11$) was also injected with the D1R agonist SKF 38393 (1.5 mg/kg s.c.), in parallel with a group treated with L-DOPA (8 mg/kg, i.p.) plus benserazide (7.5 mg/kg, i.p.) ($n = 10$) for 21 days and tested for AIMs, as previously described. Animals were killed by decapitation 45 min or 24 h after the last drug injection and the striata were rapidly dissected, frozen in liquid nitrogen and stored at −80 °C (Fig. 1).

Western blot (WB)

Tissues were homogenized with a glass–glass homogenizer in ice-cold Tris/EDTA buffer (10 mM Tris–HCl, 5 mM EDTA, pH 7.4) containing 2 mM Na_3VO_4, 10 mM NaF and a complete set of protease inhibitors (Roche, Milano, Italy) and centrifuged at 700 ×*g* at 4 °C. The resulting