Neuropharmacology 55 (2008) 704–711

Contents lists available at ScienceDirect

Neuropharmacology

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

6-OHDA-induced hemiparkinsonism and chronic L-DOPA treatment increase dopamine D1-stimulated [^{3}H]-GABA release and [^{3}H]-cAMP production in substantia nigra pars reticulata of the rat

Claudia Rangel-Barajas [a], Isaac Silva [b], Martha García-Ramírez [c], Enrique Sánchez-Lemus [a], Leonor Floran [a], Jorge Aceves [a], David Erlij [d], Benjamín Florán [a,*]

[a] Departamento de Fisiología, Biofísica y Neurociencias. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. Apartado Postal 14-740, 07000 México D.F. México
[b] Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Mexico
[c] Departamento de Fisiología, Escuela Nacional de Ciencias Biológicas, IPN, Mexico
[d] Department of Physiology, SUNY Downstate Medical Center, Brooklyn, NY 11203, USA

ARTICLE INFO

Article history:
Received 11 April 2008
Received in revised form 27 May 2008
Accepted 3 June 2008

Keywords:
L-DOPA
Basal ganglia
GABA release
cAMP
Hemiparkinsonism
Substantia nigra

ABSTRACT

It has been proposed that striatonigral GABAergic transmission in the substantia nigra reticulata (SNr) is enhanced during Parkinson's disease and subsequent L-DOPA treatment. To evaluate this proposal we determined the effects of activating dopamine D1 receptors on depolarization induced [^{3}H]-GABA release and on [^{3}H]-cAMP accumulation in slices of SNr of rats with unilateral 6-OHDA lesions with and without L-DOPA treatment. Denervation increased depolarization induced D1-stimulated [^{3}H]-GABA release, while repeated L-DOPA treatment further enhanced this response. Both also enhanced the effects of forskolin on [^{3}H]-cAMP production and [^{3}H]-GABA release, while neither modified the stimulating effects of 8-Br-cAMP on the release. These results shown that, after 6-OHDA lesions and L-DOPA treatment, cAMP signaling is enhanced. Furthermore, the results suggest that activation of sites in the signaling cascade downstream of cAMP synthesis is not required to increase release.

1. Introduction

The most commonly used treatment for Parkinson's disease is L-DOPA because it effectively controls the motor abnormalities observed in this condition. Its efficacy is, however, limited because after prolonged treatment it leads to the development of dyskinesia, an abnormal form of involuntary movements. Evidently, development of new strategies for long-term use of L-DOPA could benefit from the identification of the cellular and molecular changes produced by dopaminergic denervation and subsequent L-DOPA administration.

Changes in GABAergic transmission in the pars reticulata of the substantia nigra (SNr) in Parkinsonism could play a major role in the genesis of dyskinesia (Soghomonian et al., 1994; Mela et al., 2007). An exaggerated increase in GABA release from striatonigral terminals would inhibit neuronal discharge by GABAergic SNr neurons, which in turn would disinhibit passage of impulses through the relay nuclei of the thalamus. Such increased impulse traffic could contribute to the generation of abnormal movements (Bezard et al., 2001).

Three types of experiments are consistent with the proposal that GABA transmission in the SNr may be significantly modified during Parkinson's disease and subsequent L-DOPA treatment. First, GABA synthesizing enzyme glutamate decarboxylase (GAD) and its mRNA are enhanced by both treatments (Lloyd et al., 1975; Aceves et al., 1994). Thus, GAD-67 (Katz et al., 2005) and GAD-67 mRNA (Cenci et al., 1998) in the striatum are decreased during dopaminergic denervation, while L-DOPA administration or D1 receptor stimulation increases both the enzyme and its mRNA (Soghomonian et al., 1994; Laprade and Soghomonian, 1997). Second, evidence obtained by in vivo microdialysis shows that 6-OHDA lesions or L-DOPA treatment may increase interstitial GABA levels in the SNr. These experiments, however, have not yielded uniform results (Windels and Kiyatkin, 2006; Ochi et al., 2004; Mela et al., 2007). Finally, experiments showing that denervation and L-DOPA modify cAMP signaling pathway in the striatum (Mishra et al., 1974; Nagatsu et al., 1978; Pifl et al., 1992; Tong et al., 2003) may also be significant because striatonigral terminals have a high density of dopamine D1 receptors (Gerfen et al., 1990). In turn, D1 receptor activation

* Corresponding author. Tel.: +52 55 57473800x5137; fax: +52 55 7473754.
E-mail address: bfloran@fisio.cinvestav.mx (B. Florán).

0028-3908/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2008.06.002

stimulates cAMP production (Forn et al., 1974) and cytoplasmic cAMP is a major modulator of GABA release (Arias-Montano et al., 2007; Nava-Asbell et al., 2007).

To examine more directly the modifications of the transmission process produced by unilateral 6-OHDA lesions and repeated L-DOPA administration we determined the effects of D1 dopamine receptor activation on depolarization induced [^{3}H]-GABA release in slices of the SNr. Given the central role of adenylyl cyclase in D1 signaling we also studied whether some specific steps of the cAMP signaling cascade are modified during denervation and L-DOPA treatment.

Preliminary accounts of these findings have been published previously in abstract form (Silva et al., 2001; Rangel-Barajas et al., 2006).

2. Materials and methods

2.1. Animals

Male Wistar rats (200–250 g) were used. They were housed together (5 per cage) with water and food available ad libitum and kept under natural light cycle. All the procedures were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care Committee of the CINVESTAV.

2.2. 6-OHDA lesion

Rats were anaesthetized with chloral hydrate (300 mg/kg i.p.) and placed on a David Kopf stereotaxic frame and injected unilaterally with 6-hydroxydopamine (6-OHDA; 16 μg/1 μl of saline containing 0.1% ascorbic acid) in the medial forebrain bundle at coordinates (A −1.8, L 2.4, V −7 mm) according to the atlas of Paxinos and Watson. Rats were pre-treated with desipramine (10 mg/kg i.p. 40 min) prior the surgery in order to prevent noradrenergic neurons damage. To ensure the degree of lesion, 8 days after surgery animals were challenged with methamphetamine (10 mg/kg i.p.) and tested for circling behavior. Only rats showing 10 or more ipsilateral turns/min at 30 min after injection were included in the study (Hudson et al., 1993).

2.3. Experimental design

The sequence of 6-OHDA lesion and L-DOPA administration is diagrammatically illustrated in the Fig. 1. Sixteen days after the 6-OHDA lesion, chronic L-DOPA treatment was initiated. Two routes of administration were used. In one group, in analogy with therapeutic procedures, L-DOPA (200 mg/kg) plus carbidopa (20 mg/kg) were administered orally (Costa et al., 2006). In the other, similar to that used in many experimental studies, L-DOPA methyl ester (10 mg/kg) plus benserazide (15 mg/kg) were given intraperitoneally (Johnston et al., 2005; Mela et al., 2007). Both treatments produced similar motor effects (see Fig. 2). Oral treatment was used only for the [^{3}H]-SCH-23390 binding assays. [^{3}H]-GABA release and [^{3}H]-cAMP accumulation assays were performed in animals that were treated intraperitoneally. L-DOPA was given daily for 20 days. Everyday, immediately after L-DOPA administration, the rats were placed in automated rotameter bowls for 3 h and the number of turns was automatically recorded. After 20 days of treatment, animals were sacrificed and used for different assays.

2.4. Preparation of SNr slices

After rapid decapitation, the brain was removed and submerged in ice-cold artificial cerebrospinal fluid (aCSF), composed as follows (mM): NaCl: 118.25, KCl: 1.75, MgSO: 1, KH_2PO_4: 1.25, $NaHCO_3$: 25, CaCl: 2 and D-glucose: 10. The brain was glued to a metal cube mounted on a Petri dish filled with ice-cold aCSF, and brain slices (300 μm thick) containing the SNr were obtained with a vibroslicer (Campden Inc., Cambridge, UK). The slices were transferred to cold slides and under a stereoscopic microscope SNr was microdissected separately from control and lesioned sides.

2.5. D1 receptor binding assay

SNr slices were pooled and placed in 4 ml of 50 mM Tris–HCl buffer containing 1 mM EGTA (pH 7.4) and homogenized using 12 strokes of a hand-held homogenizer. The homogenate was brought up to 15 ml of buffer and centrifuged (25,000*g*, 10 min at 4 °C). The pellet was resuspended in 15 ml of buffer and centrifuged again. The resulting pellet (crude membrane preparation) was resuspended and aliquots were incubated during 30 min at 37 °C in 0.5 ml of incubation buffer (Tris–HCl, 50; $NaCl_2$, 120; KCl, 5; $CaCl_2$, 2; $Na_2S_2O_5$, 0.02; in mM and Pargyline 10 μM at pH 7.4), containing [^{3}H]-SCH-23390 (range 0.1–5 nM for saturation experiments). Incubations were terminated by rapid filtration under vacuum through Whatman GF/B glass fiber paper, presoaked in 0.3% polyethylenimine. Nonspecific binding was determined by incubating with 10 μM of non-labeled SCH-23390. Protein contents were determined according to Lowry and used to estimate receptor binding as fmol/mg protein.

2.6. [^{3}H]-GABA release

[^{3}H]-GABA release was determined using two methods. Dose dependence of SKF 38393 (Method 1) was determined following the normalization procedure of Frankhuyzen and Mulder (1982) (for a detailed description of the method see Floran et al., 1990). All other release determinations were carried out with methods described in detail by Nava-Asbell et al. (2007) (Method 2). For both methods, nigral slices from 10 rats were pooled and left equilibrating for 30 min in aCSF maintained at 37 °C and gassed with O_2/CO_2 (95:5 v/v); then they were incubated for 30 min within aCSF containing 80 nM [^{3}H]-GABA (95 Ci/mmol). The labeling and perfusion solutions had aminooxyacetic acid (10 μM), to prevent degradation of label by GABA transaminase. At the end of this period, label excess was removed by washing twice with ice-cold aCSF that contained 10 μM nipecotic acid to prevent recapture of [^{3}H]-GABA. Nipecotic acid was also included in all solutions used in the following steps of the experiment. For each experimental group, slices were apportioned randomly between the chambers (80 μl; 20 superfusion chambers in parallel) and superfused at a rate of 0.5 ml/min. Each chamber contained 2–3 slices. The slices were superfused with normal aCSF for 30 min before collecting fractions. Superfusates were collected in a fraction collector every 4 min. To determine the total amount of tritium remaining in the tissue at the end of the experiment, the slices were collected, treated with 1 ml of 1 M HCl and allowed to stand for 1 h before adding the scintillator.

2.7. cAMP accumulation assay

cAMP accumulation assays were performed as previously described in Alexander (1995). Synaptosomal fractions were isolated from SNr slices. The slices were homogenized in buffer (sucrose, 0.32 M; HEPES, 0.005 M, pH 7.4), and then homogenates were centrifuged at 800*g* during 10 min. The resulting supernatant was further centrifuged at 20,000*g* during 20 min. From this second centrifugation the supernatant (S1) was discarded and the pellet (P1) was resuspended and newly centrifuged at 20,000*g* during 20 min, finally supernatant was discarded and the pellet (P2) containing synaptosomes was used. The fraction was incubated with [^{3}H]-adenine (130 nM) during 1 h at 37 °C, after this period it was suspended, in Krebs–Henseleit buffer composition: $NaCl_2$, 127; KCl, 3.73; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $CaCl_2$, 1.8; HEPES, 20; Glucose, 11; and 3-isobutyl-1-metylxantine, 1; all in mM. Aliquots of 250 μl of the synaptosomes were located in tubes and the drugs to the study were added in 10 μl volume, the incubations were continued no more than 15 min and finished by adding 100 μl ice-cold trichloroacetic acid (15%) containing unlabelled ATP (2.5 mM) and cAMP (4.5 mM). After a period of 20 min on ice, the tubes were centrifuged (4000 rpm, 5 min, 4 °C), the supernatants loaded onto Dowex 50W-X4 (300 μl per column). A fraction containing [^{3}H]-ATP was eluted with 3 ml of distilled water. A second eluant obtained with 5 ml distilled water was

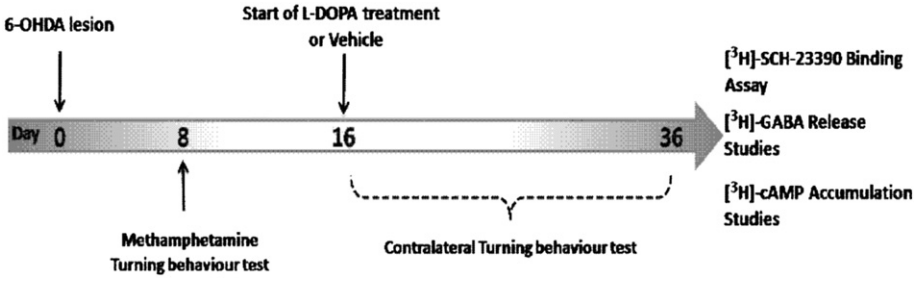

Fig. 1. Time chart showing treatment protocol. The numbers within the time arrow indicate the number of days after 6-OHDA lesion.