Behavioural Brain Research 263 (2014) 46–50

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Overexpression of the dopamine D3 receptor in the rat dorsal striatum induces dyskinetic behaviors

Samantha R. Cote[a], Vineet C. Chitravanshi[b], Carina Bleickardt[a], Hreday N. Sapru[b], Eldo V. Kuzhikandathil[a,*]

[a] Department of Pharmacology and Physiology, Rutgers-New Jersey Medical School, Newark, NJ 07101, USA
[b] Department of Neurological Surgery, Rutgers-New Jersey Medical School, Newark, NJ 07101, USA

HIGHLIGHTS

- AAV-2 expressing D3 receptors effectively transduces neurons in rat striatum.
- Ectopic expression of D3 receptor in rat striatum contributes to L-DOPA-induced dyskinesia.
- D3 receptor tolerance property contributes to L-DOPA-induced dyskinesia.
- Chronic L-DOPA treatment of rats overexpressing D3 receptor induces vacuous chewing.

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 11 January 2014
Accepted 15 January 2014
Available online 23 January 2014

Keywords:
Parkinson's disease
Levodopa-induced dyskinesia
Dopamine receptor agonist
Tolerance
Adeno-associated virus

ABSTRACT

L-DOPA-induced dyskinesias (LID) are motor side effects associated with treatment of Parkinson's disease (PD). The etiology of LID is not clear; however, studies have shown that the dopamine D3 receptor is upregulated in the basal ganglia of mice, rats and non-human primate models of LID. It is not known if the upregulation of D3 receptor is a cause or result of LID. In this paper we tested the hypothesis that overexpression of the dopamine D3 receptor in dorsal striatum, in the absence of dopamine depletion, will elicit LID. Replication-deficient recombinant adeno-associated virus-2 expressing the D3 receptor or enhanced green fluorescent protein (EGFP) were stereotaxically injected, unilaterally, into the dorsal striatum of adult rats. Post-hoc immunohistochemical analysis revealed that ectopic expression of the D3 receptor was limited to neurons near the injection sites in the dorsal striatum. Following a 3-week recovery period, rats were administered saline, 6 mg/kg L-DOPA, 0.1 mg/kg PD128907 or 10 mg/kg ES609, i.p., and motor behaviors scored. Rats overexpressing the D3 receptor specifically exhibited contralateral axial abnormal involuntary movements (AIMs) following administration of L-DOPA and PD128907 but not saline or the novel agonist ES609. Daily injection of 6 mg/kg L-DOPA to the rats overexpressing the D3 receptor also caused increased vacuous chewing behavior. These results suggest that overexpression of the D3 receptor in the dorsal striatum results in the acute expression of agonist-induced axial AIMs and chronic L-DOPA-induced vacuous chewing behavior. Agonists such as ES609 might provide a novel therapeutic approach to treat dyskinesia.

Parkinson's disease (PD) patients undergoing chronic L-DOPA treatment develop LID, a motor side-effect characterized by hyperkinetic movements [1–4]. In rodent and non-human primate models of LID, dorsolateral striatal D3 receptor expression is elevated specifically in dyskinetic animals [5–9]. Studies using D3 receptor-selective agonist PD128907 and D3 receptor-selective antagonists S-33,084 and nafadotride have also implicated the D3 receptor in development of LID [7,10,11]. Despite these studies, it is not clear if striatal D3 receptor overexpression contributes to LID.

The D3 receptor, but not the closely-related D2 receptor, exhibits tolerance and slow response termination (SRT) properties and we have proposed that these signaling properties might contribute to LID [12,13]. Tolerance is defined as a progressive loss of D3 receptor signaling function upon repeated agonist stimulation and SRT is defined as a slow termination of signaling

Abbreviations: PD, Parkinson's disease; LID, levodopa-induced dyskinesia; AIMs, abnormal involuntary movements; EGFP, enhanced green fluorescent protein; L-DOPA, L-3,4-dihydroxyphenylalanine; SRT, slow response termination; ANOVA, analysis of variance.

* Corresponding author at: Department of Pharmacology and Physiology, Rutgers-New Jersey Medical School, 185 South Orange Avenue, MSB, I-647, Newark, NJ 07103, USA. Tel.: +1 973 972 1157; fax: +1 973 972 4554.
E-mail address: kuzhikev@njms.rutgers.edu (E.V. Kuzhikandathil).

0166-4328/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2014.01.011

response upon removal of the agonist. These signaling properties are agonist-dependent; while traditional agonists such as dopamine, pramipexole, rotigotine, PD128907, quinpirole and 7-OH-DPAT induce tolerance and SRT, a novel class of agonists that includes ES609 and *cis*-8-OH-PBZI do not [12–15]. The aim of this study was to determine if overexpression of D3 receptor in the absence of dopamine depletion caused dyskinesia, and if D3 receptor tolerance and SRT properties are involved in dyskinetic behaviors. We hypothesized that unilateral overexpression of the D3 receptor in the dorsolateral striatum would induce dyskinetic behaviors and that treatment with the novel agonist, ES609, would alleviate these behaviors.

Female 4 month-old Sprague-Dawley rats (Charles River Labs, Wilmington, MA, USA) weighing approximately 300 g with *ad libitum* access to standard rodent chow and water were single-housed in a temperature and humidity controlled environment, with a 12 h light (on at 7 a.m.):12 h dark (off at 7 p.m.) cycle. All experiments were performed in the light phase. All procedures were approved by the Institutional Animal Care and Use Committee at Rutgers-New Jersey Medical School. The surgery and unilateral stereotaxic injections were performed as described previously [16]. The virus particles, diluted at a titer of approximately 10^9 GC (4 μl volume), were microinjected at two sites (incisor bar = −3.3 interaural line; injection site #1: AP +1.6, ML +2.2, and DV −4.8; injection site #2: AP +0.2, ML +3.2, and DV −4.4) (reference point – Bregma), at a rate of 2 μl/min. Rutgers IBS committee approved the use of recombinant AAV2 in rats.

Three weeks after the stereotaxic surgery, levodopa methyl ester (Sigma, St. Louis, MO, USA) and peripheral DOPA decarboxylase inhibitor, benserazide hydrochloride, (Sigma, St. Louis, MO, USA) were dissolved in saline and injected intraperitoneally (i.p.) at 6 mg/kg and 15 mg/kg daily for 3 weeks. R-(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-(1)benzopyrano[4,3-b]-1,4-oxazine-9-ol (PD128907; Tocris, Minneapolis, MN, USA) and 4-(2-chlorophenyl)-butan-2-amine (ES609; Asinex, Winston Salem, NC, USA) were dissolved in saline and injected subcutaneously (s.c.). Custom-generated recombinant AAV2 expressing Flag^{TM}-tagged human dopamine D3 receptor (AAV2-Flag^{TM} D3) or EGFP (AAV2-EGFP) (Vector Biolabs, Philadelphia, PA) with a titer of 1×10^{13} GC/ml were used in this study. The 3XFlag^{TM} tag (Sigma) was introduced at the N-terminus of the D3 receptor to facilitate detection of the overexpressed D3 receptor (Supplementary Fig. 1). The binding properties and signaling function of Flag^{TM}-tagged D3 receptor are indistinguishable from the non-tagged D3 receptor [12–14].

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2014.01.011.

Following a 3 week-period for surgical recovery and induction of D3 receptor expression, dyskinetic behaviors were assessed as described previously [17]. Behaviors assessed included rearing, grooming, axial AIMs, rotational AIMs, and vacuous chewing. Rearing is defined as standing on hind limbs. Grooming is defined as grooming the face and head and/or body with hands and mouth (Supplementary Videos 1 and 2). Axial AIMs are defined as abnormal or excessive lateral deviations or grooming events to the right or left. Rotational AIMs are defined as complete 360° clockwise or counterclockwise rotations. Vacuous chewing is defined as chewing or orofacial movements with no purpose (Supplementary

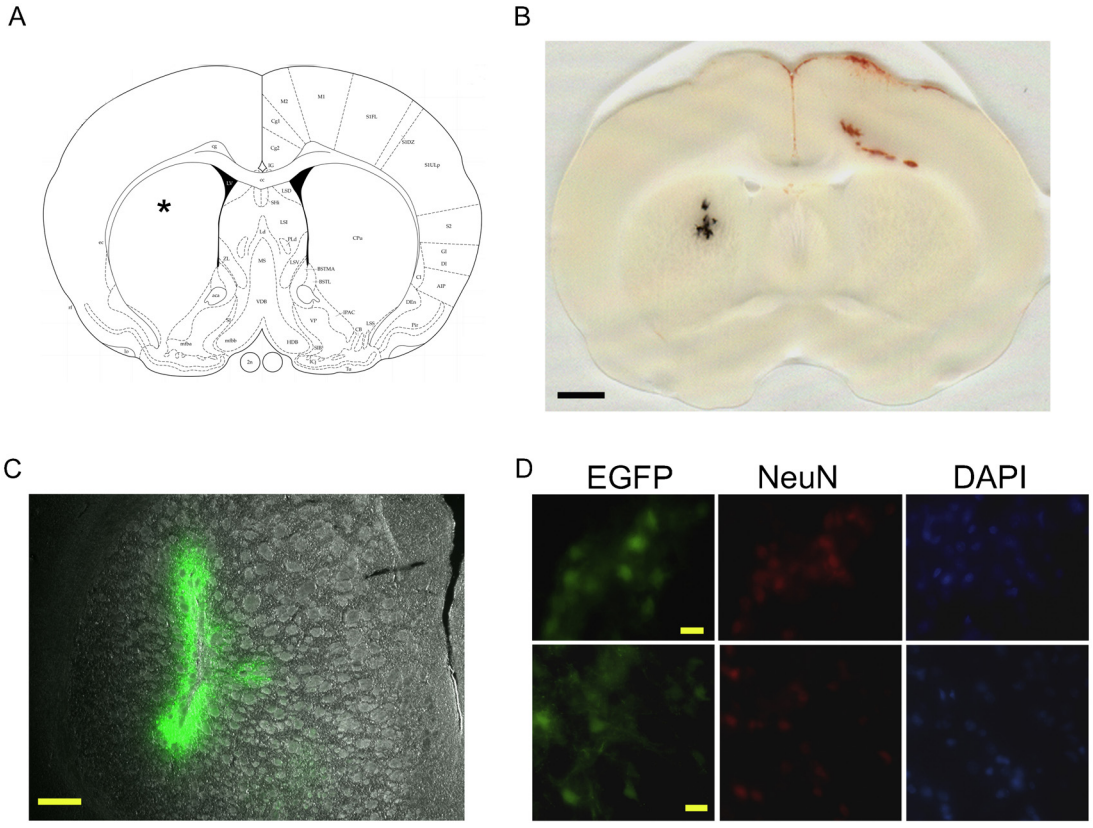

Fig. 1. (A) Schematic depiction of a coronal striatal section adapted from The Rat Brain in Stereotaxic Coordinates atlas (Paxinos and Watson). The star indicates the approximate location of the targeted stereotaxic injection sites. (B) Representative coronal striatal section from a rat injected with India ink to verify coordinates of the stereotaxic injection site. Scale bar = 2 mm. (C) Representative merged image of a coronal striatal section from a rat injected with AAV2-EGFP. Images of individual coronal sections were captured in bright field and fluorescence modes and merged using the image acquisition and analysis software. The EGFP fluorescence is limited to cells next to the injection site in the dorsal striatum. Scale bar = 300 μm. (D) AAV2-EGFP is expressed in neurons. Representative 30 μm thick coronal sections containing the striatum were stained with an antibody against the NeuN, a neuronal marker (red). DAPI (blue) stains the nucleus and EGFP (green) is the native fluorescence of the exogenously-expressed EGFP. Top and bottom panels show striatal sections from two different rats injected with AAV2-EGFP. Scale bar = 30 μm.