Neuropharmacology 58 (2010) 528–536

Contents lists available at ScienceDirect

Neuropharmacology

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

The selective D_3 receptor antagonist, S33084, improves parkinsonian-like motor dysfunction but does not affect L-DOPA-induced dyskinesia in 6-hydroxydopamine hemi-lesioned rats

Flora Mela [a,b], Mark J. Millan [c], Mauricette Brocco [c], Michele Morari [a,b,*]

[a] Department of Experimental and Clinical Medicine, Section of Pharmacology, University of Ferrara, via Fossato di Mortara 17-19, 44100 Ferrara, Italy
[b] Neuroscience Center and National Institute of Neuroscience, University of Ferrara, Ferrara, Italy
[c] Psychopharmacology Department, Institut de Recherches Servier, Croissy-sur-Seine, France

ARTICLE INFO

Article history:
Received 4 May 2009
Received in revised form
10 July 2009
Accepted 25 August 2009

Keywords:
D_3 receptor
Drag test
Dyskinesia
L-DOPA
Parkinson's disease
S33084

ABSTRACT

Despite evidence linking dopamine D_3 receptors to the etiology of Parkinson's disease and L-DOPA-induced dyskinesia, the potential therapeutic utility of D_3 receptor ligands remains unclear. In the present study, we investigated whether the selective D_3 receptor antagonist, S33084, affects development and expression of abnormal involuntary movements (AIMs), a behavioural correlate of dyskinesia, in rats hemi-lesioned with 6-hydroxydopamine and chronically treated with L-DOPA. The ability of S33084, alone or in combination with L-DOPA, to attenuate 6-hydroxydopamine induced motor deficits was also investigated employing a battery of behavioural tests. Acute administration of S33084 (0.64 mg/kg, s.c.) did not attenuate the induction of AIMs in dyskinetic rats upon challenge with L-DOPA (6 mg/kg, s.c.). Moreover, S33084 (0.64 mg/kg) did not prevent the development of AIMs affecting axial, limb and orolingual muscles when chronically administered together with L-DOPA (6 mg/kg for 21 days). However, both acute and chronic administration of S33084 enhanced L-DOPA-induced contralateral turning, suggesting potential antiparkinsonian properties. Furthermore, S33084 (0.01–0.64 mg/kg) dose-dependently attenuated parkinsonian disabilities, including bradykinesia, in drag and rotarod tests, although, in these procedures, the combination of S33084 with L-DOPA did not produce synergistic effect. It is concluded that sustained D_3 receptor blockade does not blunt L-DOPA-induced dyskinesia in hemiparkinsonian rats. However, D_3 receptor antagonism may be associated with antiparkinsonian properties. The clinical relevance of these observations will be of interest to explore further.

1. Introduction

Levodopa (L-DOPA) therapy of Parkinson's disease (PD) is associated with the development of dyskinesia, motor complications that reflect a complex pattern of changes at the level of dopaminergic nerve terminals and other classes of neuron within the striatum and other structures of the basal ganglia (Obeso et al., 2000; Cenci, 2007). Since the identification of D_3 receptors (Sokoloff et al., 1990), growing evidence has linked them to the etiology and management of PD and L-DOPA-induced dyskinesia (LID), although the therapeutic potential of D_3 receptor ligands remains uncertain (Sokoloff et al., 2006; Joyce and Millan, 2007).

Behavioural sensitization to L-DOPA was accompanied by induction of D_3 receptor gene expression and binding in the denervated striatum of 6-hydroxydopamine (6-OHDA) hemi-lesioned rats (Bordet et al., 1997; Van Kampen and Stoessl, 2003; Visanji et al., 2009a). Consistently, a linear relationship between striatal D_3 receptor binding and dyskinesia was found in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated macaques (Bezard et al., 2003). Systemic administration of the mildly-selective D_3 receptor antagonist, nafadotride (Bordet et al., 1997), or striatal injection of D_3-directed antisense nucleotides (Van Kampen and Stoessl, 2003), attenuated the rotational response to DA agonists in L-DOPA-primed 6-OHDA hemi-lesioned rats suggesting that D_3 receptors contribute to dyskinesia expression. Accordingly, nafadotride attenuated the appearance of abnormal involuntary movements (AIMs) in primed 6-OHDA rats (Monville et al., 2005). However, the D_3 selectivity of nafadotride *in vivo* has been repeatedly questioned (Levant and Vansell, 1997; Audinot et al., 1998). Indeed, the selective D_3 receptor antagonist S33084 (Millan et al., 2000a,b) did not attenuate LID

* Corresponding author at: Department of Experimental and Clinical Medicine, Section of Pharmacology, University of Ferrara, via Fossato di Mortara 17-19, 44100 Ferrara, Italy. Tel.: +39 0532 455210; fax: +39 0532 455205.
E-mail address: m.morari@unife.it (M. Morari).

0028-3908/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.08.017

in MPTP-treated marmosets (Silverdale et al., 2004). Moreover, in MPTP-treated macaques, the selective D_3 receptor partial agonist, BP897, but not nafadotride, attenuated LID without affecting the therapeutic effect of L-DOPA, suggesting that low efficacy stimulation rather than blockade of D_3 receptors could be beneficial in treating LID once established (Bezard et al., 2003). Designed to evaluate the contribution of D_3 receptors to LID expression, these studies did not address the question of whether D_3 receptors influence the *development* of brain sensitization to L-DOPA (priming). Very recently, chronic administration of S33084 in combination with L-DOPA was reported to blunt the development of LID in MPTP-treated marmosets (Visanji et al., 2009a).

The possibility that D_3 receptor antagonists exert anti-parkinsonian properties *per se* has also been investigated. S33084 enhanced the antiparkinsonian action of ropinirole and prolonged that of L-DOPA in MPTP-treated marmosets (Silverdale et al., 2004), an effect replicated by another D_3 receptor antagonist, S33138 (Millan et al., 2008). In addition, S33084 itself relieved MPTP-induced motor impairment (Silverdale et al., 2004), overall suggesting that D_3 receptor antagonism may exert a beneficial influence upon parkinsonian disabilities.

The first aim of this present study was to investigate whether D_3 receptors participate in the expression and development of AIMs in the 6-OHDA hemi-lesioned rat model of LID (Cenci et al., 1998). S33084 was acutely combined with an L-DOPA challenge in primed, dyskinetic rats, or chronically co-administered with L-DOPA for 21 days. The effect of S33084 on priming was also tested. Since a role for D_3 receptors in turning behaviour emerged from the chronic study, the ability of S33084 to enhance L-DOPA-induced turning behaviour was investigated in L-DOPA-naïve 6-OHDA lesioned rats. Finally, to unravel the contribution of D_3 receptors to parkinsonism, the effect of S33084 on 6-OHDA-induced akinesia/bradykinesia was investigated using a battery of behavioural tests.

2. Methods

2.1. Subjects

The study was performed in male Sprague–Dawley (Harlan Italy; S. Pietro al. Natisone, Italy) and Wistar (Charles River, L'Arbresle, France) rats. The animals were housed under a 12-h light/dark cycle with water and food ad libitum. Adequate measures were taken to minimize animal pain or discomfort, and to reduce the number of animals used. This study was compliant with the European Council Directive of 24 November 1986 (86/609/EEC), conformed to the French National Committee (décret 87/848) for the care and use of laboratory animals, and approved by the Ethical Committee of the University of Ferrara and Italian Ministry of Health (licence n. 71-2004-B).

2.2. Experimental design

2.2.1. Experiment #1: acute influence of S33084 on AIMs expression in dyskinetic rats

Twelve 6-OHDA hemi-lesioned rats were treated for 21 days with a low dose of L-DOPA (6 mg/kg) in combination with benserazide (15 mg/kg). During this period, AIMs recording was performed twice a week in order pre-select animals showing appreciable AIMs scores. At the end of L-DOPA chronic treatment, AIMs rating was performed in dyskinetic rats following acute L-DOPA challenge, and in the absence or presence of S33084 (0.64 mg/kg; s.c.). This dose is known to robustly and selectively block D_3 receptors (Millan et al., 2000a,b). Rats were observed for 3 h after injection by an experimentally blinded investigator.

2.2.2. Experiment #2: chronic influence of S33084 on AIMs development and L-DOPA priming

Twenty-four rats with unilateral 6-OHDA lesions were allotted to 4 treatment groups which received daily injections of (i) S33084 (0.64 mg/kg, s.c.), (ii) L-DOPA (6 mg/kg plus 15 mg/kg benserazide, s.c.); (iii) S33084 plus L-DOPA, or (iv) vehicle (consisting of 15 mg/kg benserazide dissolved in the S33084 vehicle). This dose of S33084 produced full occupancy of D_3 receptors without any sign of D_2 receptor blockade (Millan et al., 2000a). These treatments were given for 21 days. During this period, AIMs ratings were performed 2–3 times a week. At the end of chronic treatment, all animals were given a 24-hr drug washout period and then exposed to a challenge dose of L-DOPA (6 mg/kg plus 15 mg/kg benserazide, s.c.), followed by a 3-hr AIM rating session.

2.2.3. Experiment #3: acute influence of S33084 on L-DOPA-induced contralateral rotations

Experimental sessions were performed on 6-OHDA hemi-lesioned rats once a week with an ABACADA design: "A" corresponds to an L-DOPA (10 mg/kg, i.p.) control session and "B, C, and D" to test sessions with variable drug treatment. In L-DOPA-control sessions, rats were administered with vehicle 20 min before injection of benserazide (2.5 mg/kg, i.p.), and 30 min before L-DOPA (10 mg/kg, i.p.). Rotation was measured over 1 h immediately after the L-DOPA injection. In test sessions, vehicle or S33084 (0.04, 0.16 or 0.64 mg/kg, s.c.) were injected 20 min prior to benserazide (0.64, 1.25 or 2.5 mg/kg, i.p.) or vehicle, and 30 min prior to L-DOPA (2.5, 5 or 10 mg/kg, i.p., respectively) or vehicle. Rotation was likewise measured for 1 h.

2.2.4. Experiment #4: acute influence of S33084 on 6-OHDA-induced akinesia/bradykinesia

Forty-five 6-OHDA hemi-lesioned rats were allotted to five treatment groups which received acute injections of vehicle (i) or S33084 at 0.01 mg/kg (ii); 0.04 mg/kg (iii); 0.16 mg/kg (iv) or 0.64 mg/kg (v). Motor activity was evaluated by the bar, drag and rotarod tests. The experiment consisted of three consecutive sessions carried out before (control session, "off-drug") and after (15 and 75 min) vehicle or drug administration. The bar test was always the first test to be performed, the rotarod being the last. Drugs and vehicle were administered s.c.

2.2.5. Experiment #5: interaction between S33084 and L-DOPA on 6-OHDA-induced akinesia/bradykinesia

Forty 6-OHDA hemi-lesioned rats were allotted to five treatment groups which received: (i) vehicle; (ii) L-DOPA (0.1 mg/kg plus 15 mg/kg benserazide, s.c.); (iii) L-DOPA (1 mg/kg plus 15 mg/kg benserazide, s.c.); (iv) S33084 (0.01 mg/kg) + L-DOPA (0.1 mg/kg); (v) S33084 (0.04 mg/kg) + L-DOPA (1 mg/kg). Motor activity was evaluated as described in Experiment #4.

2.3. Lesion surgery and behavioural screening

Unilateral 6-OHDA lesion of the right ascending DA bundle was performed in male Sprague–Dawley rats (150 g) according to our standard procedure for Experiments #1, 2 and 4 (Marti et al., 2005). Briefly, 8 μg of 6-OHDA (in 4 μl of saline containing 0.02% ascorbic-acid) were stereotaxically injected according to the following coordinates from bregma: anteroposterior (AP) −4.4, mediolateral (ML) −1.2, dorsoventral (DV) −7.8 below dura (Paxinos and Watson, 1982). Forelimb akinesia (cylinder test) and turning behaviour tests were performed 2–3 weeks post-lesion to select fully-lesioned rats. All rats enrolled in the study showed (i) <30% contralateral paw usage in the cylinder test and (ii) >7 ipsilateral full turns/min in response to amphetamine (5 mg/kg i.p.; 90 min testing). Previous studies have shown that this behaviours is associated with >90% depletion of DA terminals (Marti et al., 2007). Cylinder-test scores and amphetamine-induced rotations were then used as balancing criteria for the allocation of animals to different experimental groups in all experiments. Immunohistochemical verification of the lesion extent was carried out in all the animals included in Experiments #1 and 2 using an antibody against tyrosine hydroxylase (TH) as described by Marti et al. (2007). All the animals showed a virtually complete disappearance of TH-positive cell bodies and fibres in the substantia nigra (SN) compacta (SNc) on the side ipsilateral to the 6-OHDA lesion.

In Experiment #3, unilateral lesion of SNc was induced in male Wistar rats (300–330 g) by injecting 6-OHDA (as above) at the following coordinates: AP + 3.4, ML ± 2.2 and DV − 2.2 (Paxinos and Watson, 1982). Animals were allowed 3 weeks recovery prior to testing. Rotations were monitored automatically in rats coupled to a harness connected to a Rotacount 8 (Columbus Instruments, Columbus, OH) apparatus. Only animals which showed a pronounced response (>150 contralateral rotations/hour) to apomorphine (0.04 mg/kg, s.c.; two sessions one week apart) and subsequently to L-DOPA (10 mg/kg, i.p.; two further sessions, one week apart) were selected for the study.

2.3.1. Cylinder test

The cylinder test (Schallert et al., 2000; modified by Lundblad et al., 2002), evaluates spontaneous forelimb use for weight-shifting during vertical exploration. Briefly, each animal was placed in a glass cylinder (21 cm diameter and 34 cm height) and videotaped for 3 min. The number of wall contacts performed independently with the left and the right forepaw were counted up to a total of 20 wall contacts per rat per session. Only wall contacts where the animal supported body-weight on the paw with extended digits were counted. The data are presented as contralateral (left) paw use (i.e. percentage of left-paw wall contacts over the total number of wall contacts).

2.3.2. Behavioural studies

A battery of previously validated behavioural tests, the bar, drag and rotarod tests (Marti et al., 2005), was used to investigate motor effects of S33084.