Neurobiology of Disease 47 (2012) 393–406

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

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

Serotonergic and dopaminergic mechanisms in graft-induced dyskinesia in a rat model of Parkinson's disease

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ARTICLE INFO

Article history:
Received 18 January 2012
Revised 23 March 2012
Accepted 31 March 2012
Available online 12 April 2012

Keywords:
Graft-induced dyskinesia
L-DOPA-induced dyskinesia
Parkinson's disease
Serotonin
Dopamine D_2 receptor
Cell transplantation

ABSTRACT

Dyskinesia seen in the *off*-state, referred as graft-induced dyskinesia (GID), has emerged as a serious complication induced by dopamine (DA) cell transplantation in parkinsonian patients. Although the mechanism underlying the appearance of GID is unknown, in a recent clinical study the partial 5-HT_{1A} agonist buspirone was found to markedly reduce GID in three grafted patients, who showed significant serotonin (5-HT) hyperinnervation in the grafted striatum in positron emission tomography scanning (Politis et al., 2010, 2011). Prompted by these findings, this study was performed to investigate the involvement of serotonin neurons in the appearance of GID in the rat 6-hydroxydopamine model.

L-DOPA-primed rats received transplants of DA neurons only, DA plus 5-HT neurons or 5-HT neurons only into the lesioned striatum. In DA cell-grafted rats, with or without 5-HT neurons, but not in 5-HT grafts, GID was observed consistently after administration of amphetamine (1.5 mg/kg, *i.p.*) indicating that grafted DA neurons are required to induce GID. Strikingly, a low dose of buspirone produced a complete suppression of GID. In addition, activation of 5-HT_{1A} and 5-HT_{1B} receptors by 8-OH-DPAT and CP 94253, known to inhibit the activity of 5-HT neurons, significantly reduced GID, whereas induction of neurotransmitter release by fenfluramine administration significantly increased GID, indicating an involvement of the 5-HT system in the modulation of GID. To investigate the involvement of the host 5-HT system in GID, the endogenous 5-HT terminals were removed by intracerebral injection of 5,7-dihydroxytryptamine, but this treatment did not affect GID expression. However, 5-HT terminal destruction suppressed the anti-GID effect of 5-HT_{1A} and 5-HT_{1B} agonists, demonstrating that the 5-HT_1 agonist combination exerted its anti-GID effect through the activation of pre-synaptic host-derived receptors. By contrast, removal of the host 5-HT innervation or pre-treatment with a 5-HT_{1A} antagonist did not abolish the anti-GID effect of buspirone, showing that its effect is independent from activation of either pre- or post-synaptic 5-HT_{1A} receptors. Since buspirone is known to also act as a DA D_2 receptor antagonist, the selective D_2 receptor antagonist eticlopride was administered to test whether blockade of D_2 receptors could account for the anti-dyskinetic effect of buspirone. In fact, eticlopride produced complete suppression of GID in grafted animals already at very low dose. Together, these results point to a critical role of both 5-HT_1 and D_2 receptors in the modulation of GID, and suggest that 5-HT neurons exert a modulatory role in the development of this side effect of neuronal transplantation.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by an excessive loss of dopamine (DA) neurons in substantia nigra (SN). L-3,4-dihydroxyphenylalanine (L-DOPA), as a precursor of DA, is widely used to increase central production of DA, and provide alleviation of motor symptoms. Although L-DOPA is very effective during the first years of administration, its long-term use can often cause unwanted motor side effects, in particular L-DOPA-induced dyskinesia (LID). Moreover, the efficacy of L-DOPA declines over-time, as the DA neurodegeneration progresses. Therefore, other approaches have been tested to alleviate parkinsonian symptoms, such as neural transplantation of DA precursor cells. Embryonic ventral mesencephalic (VM) cells have been transplanted into rodent (Bjorklund, 1992; Herman and Abrous, 1994; Winkler et al., 2000) and monkey (Redmond et al., 2008) models of PD, and in PD patients (Freed et al., 1992; Lindvall et al., 1992, 1994; Olanow et al., 2003; Piccini et al., 1999). While grafted cells are efficient in providing

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

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doi:10.1016/j.nbd.2012.03.038

restoration of motor functions in pre-clinical PD models, the clinical results have been more variable (Lindvall and Bjorklund, 2004; Olanow et al., 2009; Winkler et al., 2005). One complication that has contributed to prevent further application of the transplantation approach in PD is the appearance of *off*-drug uncontrolled movements, so-called graft-induced dyskinesia (GID) in a subset of grafted patients (Freed et al., 2001; Hagell et al., 2002; Olanow et al., 2003). Although this form of uncontrolled movements persists even after withdrawal of L-DOPA medication, GID appears to share some cellular mechanisms with LID. Thus, priming with L-DOPA is required for development of GID (Lane et al., 2009b) and animals with severe pre-operative LID carry an increased risk for the development of GID (Garcia et al., 2011b). An increasing body of evidence points to the serotonin (5-HT) system as a key player in the appearance of LID in animal models (Carta et al., 2007, 2010; Munoz et al., 2008) and patients (Bara-Jimenez et al., 2005; Olanow et al., 2004; Rylander et al., 2010). In fact, recent animal work (Carta et al., 2007; Lindgren et al., 2010; Munoz et al., 2008) has suggested that abnormal release of DA from 5-HT neurons may be responsible for the excessive swings in synaptic DA levels observed in dyskinetic PD patients after L-DOPA administration (de la Fuente-Fernandez et al., 2004). Moreover, a significant 5-HT hyperinnervation has recently been found in caudate putamen of dyskinetic PD patients (Rylander et al., 2010).

Interestingly, a marked serotonergic hyperinnervation has also been observed in grafted patients using positron emission tomography (PET) scanning (Politis et al., 2010, 2011). In addition, an elevated 5-HT/DA transporter ratio has been recently measured in the striatum of a grafted patient compared to healthy normal individuals and advanced PD patients (Politis et al., 2011). The embryonic tissue used for transplantation is, in fact, known to contain a variable number of 5-HT cells, depending on the landmarks used for dissection of the fetal tissue (Carlsson et al., 2007), and Mendez et al. (2008) have reported large numbers of 5-HT neurons in VM grafts in long-term PD patients studied post-mortem. Based on these observations, it has been proposed that 5-HT neurons may play a role in the induction of GID. In support of this idea, Politis et al. (2010, 2011) have reported that GID is almost completely suppressed after administration of the partial 5-HT_{1A} receptor agonist buspirone, raising the possibility that 5-HT_1 receptor activation can suppress GID through inhibition of 5-HT neuron activity, as already seen for LID.

This study was designed to investigate the relative involvement of dopaminergic and serotonergic mechanisms in the development of GID. Although some form of stereotyped abnormal movements (tapping stereotypy and litter retrieval/chewing) has been reported to appear in rats after grafting in absence of any drug challenge (Soderstrom et al., 2008), abnormal movements phenotypically similar to LID can only be seen after administration of amphetamine (Carlsson et al., 2006; Lane et al., 2006), which is known to evoke massive DA release from grafted DA neurons (Zetterstrom et al., 1986). These abnormal movements can be scored with the same scale as for LID (Carlsson et al., 2006; Lane et al., 2006) and are now widely used as a convenient and reproducible model of GID (Carlsson et al., 2007; Garcia et al., 2011b; Lane et al., 2008, 2009a, 2009b). In the present study, we have used this model to investigate the involvement of 5-HT neurons in the appearance of GID, and the mechanisms underlying the anti-GID effect of 5-HT_1 receptor agonists.

Materials and methods

Animals

A total of 120 adult female Sprague–Dawley rats (225–250 g at purchase, Charles River, Sweden) were used in the present study and housed on a 12 h light/dark cycle (light on 7:00–19:00) with free access to food and water. All animal works were performed in accordance with regulations set by the Ethical Committee for the use of laboratory animals at Lund University.

Drugs

All the drugs were diluted in 0.9% sterile saline and injected *s.c.* unless otherwise stated. 8-[4-[4-(2-Pyrimidinyl)-1-pipirazin yl]butyl]-8-azaspiro[4,5]decane-7,9-dione hydrochloride (Buspirone, 1 mg/kg); (±)-7-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT, 0.1 mg/kg); 5-Propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo [3,2-*b*]pyridine hydrochloride (CP 94253, 1.75 mg/kg); (*S*)-*N*-*tert*-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride (WAY-100135, 0.4 mg/kg); 3-Chloro-5-ethyl-*N*-[[(2*S*)-1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride (Eticlopride, 0.03 mg/kg); and 4-[2-(Dipropylamino)ethyl]-1,3-dihydro-2*H*-indol-2-one hydrochloride (Ropinirole, 0.2 mg/kg, *i.p.*) were purchased from Tocris Bioscience, UK. (±)-N-Ethyl-α-methyl-*m*-[trifluoromethyl]phenethylamine hydrochloride (Fenfluramine, 2 mg/kg, *i.p.*); DL-Serine 2-(2,3,4-trihydroxybenzyl)hydrazide hydrochloride (Benserazide, 10 mg/kg); 2,4,5-Trihydroxyphenethylamine hydrochloride (6-OHDA, 3.5 μg/μl free base in 0.02% L-ascorbic acid in 0.9% saline, into the medial forebrain bundle (MFB)); and 5,7-Dihydroxytryptamine creatinine sulfate salt (5,7-DHT, 5 μg/μl free base in 0.02% L-ascorbic acid in 0.9% saline, into the MFB) were purchased from Sigma-Aldrich, Sweden. L-3,4-Dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA, 6 mg/kg) and D-Amphetamine Sulphate (1.5 or 2.5 mg/kg, *i.p.*) were purchased from Research Organics, Cleveland, OH and Apoteksbolaget, Sweden, respectively.

Experimental design

All rats received injections of 6-OHDA unilaterally into the MFB (details in the following section). Three weeks after surgery animals were injected with 2.5 mg/kg of amphetamine and the rotational behavior was measured by an automated system. Only animals exhibiting at least 6 turns/min were recruited into the study. Starting a week later, L-DOPA and benserazide were injected (except Drug Naïve group) daily for 6 weeks to establish stable LID, as measured by the abnormal involuntary movement rating scale. Dyskinetic rats (total AIMs score ≥28) were evenly split into 4 groups (average AIMs score 44) and received either DA narrow, DA wide, 5-HT, or sham transplantation into lesioned striata, as described below. L-DOPA injection was resumed 2 weeks post-grafting twice weekly to maintain dyskinesia (Lee et al., 2000). In order to monitor changes in LID, AIMs were scored at 4, 8, 10, 13, 20 and 27 weeks post-grafting. Amphetamine-induced rotation was repeated at 13 and 28 weeks post-transplantation, while the cylinder test was performed at 27 weeks. Amphetamine-induced dyskinesia has been monitored with/out other drugs between 14 weeks and 43 weeks post-grafting. In order to investigate the involvement of the endogenous 5-HT system in amphetamine-induced dyskinesia, 5,7-DHT lesion of the intrinsic 5-HT system was carried out on DA narrow and DA wide groups at 37 weeks post-grafting (see below). Two weeks later, GID testing was resumed. Trans-cardial perfusion with 4% paraformaldehyde was performed at the end of the pharmacological studies.

Lesion surgery

Stereotaxic surgery was performed under general anesthesia, induced by *i.p.* injection (1.4–1.6 ml) of a 20:1 mixture of Fentanyl and Dormitor® (Apoteksbolaget, Sweden). 14 μg of 6-OHDA (3.5 μg/μl free base in 0.02% L-ascorbic acid in 0.9% sterile saline) were injected into the MFB (AP = −4.4 mm from bregma; ML = −1.2 mm from bregma; DV = −7.8 from the dura surface; Tooth bar = −2.4 mm) using a stereotaxic frame (Stoelting, Wood Dale, IL). 4 μl was injected over 4 min and the Hamilton syringe was kept in place for an additional