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The anti-dyskinetic effect of dopamine receptor blockade is enhanced in parkinsonian rats following dopamine neuron transplantation

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ABSTRACT

Graft-induced dyskinesia (GID) is a serious complication induced by dopamine (DA) cell transplantation in parkinsonian patients. We have recently shown that DA D_2 receptor blockade produces striking blockade of dyskinesia induced by amphetamine in grafted 6-OHDA-lesioned rats, a model of GID.
This study was designed to investigate whether blockade of DA D_1 receptors could produce similar outcome, and to see whether the effect of these treatments in grafted rats was specific for dyskinesia induced by amphetamine, or could also influence L-DOPA-induced dyskinesia (LID). L-DOPA-primed rats received transplants of fetal DA neurons into the DA-denervated striatum. Beginning at 20 weeks after transplantation rats were subjected to pharmacological treatments with either L-DOPA (6 mg/kg) or amphetamine (1.5 mg/kg) alone, or in combination with the D_1 receptor antagonist SCH23390, the D_2 receptor antagonist eticlopride, and the 5-HT_{1A} agonist/D_2 receptor antagonist buspirone. Grafted rats developed severe GID, while LID was reduced. Both eticlopride and SCH23390 produced near-complete suppression of GID already at very low doses (0.015 and 0.1 mg/kg, respectively). Buspirone induced similar suppression at a dose as low as 0.3 mg/kg, which is far lower than the dose known to affect LID in non-grafted dyskinetic rats. In agreement with our previous results, the effect of buspirone was independent from 5-HT_{1A} receptor activation, as it was not counteracted by the selective 5-HT_{1A} antagonist WAY100635, but likely due to D_2 receptor blockade. Most interestingly, the same doses of eticlopride, SCH23390 and buspirone were found to suppress LID in grafted but not in control dyskinetic rats. Taken together, these data demonstrate that the DA cell grafts strikingly exacerbate the effect of DA D_1 and D_2 receptor blockade against both GID and LID, and suggest that the anti-GID effect of buspirone seen in patients may also be due to blockade of DA D_2 receptors.

Introduction

Administration of L-DOPA remains the most effective treatment for Parkinson's disease (PD). However, the appearance of dyskinesia during the progression of the disease has prompted researchers to investigate alternative approaches to treat this debilitating condition. A number of pre-clinical studies demonstrated that transplantation of ventral mesencephalic (VM) cells into the host striatum generates fully mature dopamine (DA) neurons and provides significant restoration of motor functions in animal models (Bjorklund, 1992; Redmond et al., 2008; Winkler et al., 2000). Based on these promising experimental results, clinical investigations have been performed in advanced PD patients using tissue from aborted foetuses (Freed et al., 1992; Lindvall et al., 1992, 1994). While open-label trials have provided promising, albeit highly variable, results, double-blind studies have been largely disappointing (Freed et al., 2001; Olanow et al., 2003). Nevertheless, some of the transplanted patients have greatly benefited from cell grafting, such that they could reduce, or even suspend, L-DOPA treatment, providing proof-of-concept that this approach can yield significant and long-lasting amelioration of motor function. Moreover, recent post-mortem and positron emission tomography (PET) studies have shown that a significant number of grafted DA cells survived in the host caudate/putamen up to sixteen years after transplantation (Li et al., 2010; Mendez et al., 2008; Politis et al., 2010, 2011). Most probably, the lack of standardization in the surgical procedures, tissue preparation and patient selection, as well as the presence or absence of post-surgery immunosuppression have contributed to the variability of the results and the negative outcome of the double-blind trials (Barker et al., 2013). In fact, a new clinical study has recently been funded by the EU FP7 program. The goal of this project is to optimize the procedure for DA cell transplantation using embryonic tissue, with

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the intent to improve the overall efficacy and reproducibility, and pave the way for future stem cell therapies (Barker et al., 2013).

One complication that has hampered further exploration of this therapeutic approach is the appearance of *off*-state dyskinesias, which are independent from L-DOPA administration, in a subset of grafted patient (Freed et al., 2001; Hagell et al., 2002; Olanow et al., 2003). Recent animal and human studies have suggested that inclusion of serotonergic neurons in the graft may contribute to the appearance of these so-called graft-induced dyskinesias (GID). In fact, the embryonic VM tissue used for transplantation is known to contain a variable number of serotonin (5-HT) neurons, depending on the landmarks used for dissection of the fetal tissue (Carlsson et al., 2007). In agreement, Mendez et al. (2008) have reported that the VM grafts contain large numbers of 5-HT neurons, as studied post-mortem. Moreover, PET imaging studies have revealed the presence of an intense 5-HT hyperinnervation in the striatum of grafted patients showing GID, and administration of buspirone, a 5-HT_{1A} receptor agonist able to dampen activity of serotonin neurons, has been shown to significantly reduce GID in these patients (Politis et al., 2010, 2011).

Abnormal movements phenotypically similar to L-DOPA-induced dyskinesia (LID) can be seen in VM grafted rats only 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). Thus, amphetamine-induced dyskinesia in grafted rats has become a widely used and reproducible model of GID (Carlsson et al., 2007; Garcia et al., 2011; Lane et al., 2008, 2009a,b). Using this model, we have recently shown that buspirone can produce suppression of GID in grafted rats, as seen in patients; however, we have demonstrated that this effect is independent from activation of 5-HT_{1A} receptors on serotonergic neurons, but conceivably due to blockade of DA D_2 receptors, for which buspirone is known to be a weak antagonist (Eison and Temple, 1986; McMillen et al., 1983; Rijnders and Slangen, 1993; Scuvee-Moreau et al., 1987). Indeed, buspirone could fully suppress GID even when the intrinsic serotonergic innervation was removed by a lesion with a selective toxin, and its effect was mimicked by the administration of a low dose of the selective DA D_2 receptor antagonist eticlopride (Shin et al., 2012).

The present study was designed to investigate whether the striking effect induced by DA receptor blockade in suppressing amphetamine-induced dyskinesia in grafted rats is restricted to DA D_2 receptors, or extends to DA D_1 receptors as well. Moreover, DA D_1 and D_2 receptor antagonists were also tested against LID in grafted and control dyskinetic rats to investigate whether the anti-dyskinetic effect was specific for dyskinesia induced by amphetamine, or also by L-DOPA, possibly revealing a general exacerbation of the anti-dyskinetic effect of DA receptor blockade induced by the graft.

Materials and methods

Animals

Adult female Sprague–Dawley rats (225–250 g at the start of the experiment, 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 Swedish legislation 1988:543 and EU-directive 2010/63.

Drugs

All the drugs were diluted in 0.9% sterile saline and injected *s.c.* unless otherwise stated. 3-Chloro-5-ethyl-*N*-[[(2*S*)-1-ethyl-2-pyrrolidinyl) methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride (Eticlopride, 0.015 and 0.03 mg/kg); (*R*)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH23390, 0.1 mg/kg, *i.p.*); 8-[4-[4-(2-Pyrimidinyl)-1-pipirazin yl]butyl]-8-azaspiro [4,5]decane-7,9-dione hydrochloride (Buspirone, 0.3 and 1 mg/kg); (*S*)-*N*-*tert*-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride (WAY-100135, 0.4 mg/kg); 4-[2-(Dipropylamino)ethyl]-1,3-dihydro-2*H*-indol-2-one hydrochloride (Ropinirole, 0.2 mg/kg, *i.p.*); and (±)-6-Chloro-2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzazepine hydrobromide (SKF81297, 0.5 mg/kg) were purchased from Tocris Bioscience, UK. DL-Serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride (Benserazide, 10 mg/kg) and 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)) were purchased from Sigma–Aldrich, Sweden. L-3,4-Dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA, 6 or 12 mg/kg) and D-Amphetamine Sulphate (1.5 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 (detailed below). 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 3 turns/min were recruited into the study (Tronci et al., 2012). Starting a week later, L-DOPA and benserazide were injected daily for 3 weeks to establish stable LID, as measured with the abnormal involuntary movement scale (AIMs). Dyskinetic rats (total AIMs score ≥30) were split into 2 groups to receive a suspension of fetal VM cells into the lesioned striatum, or saline as control. L-DOPA injection was resumed 2 weeks post-grafting twice weekly (Lee et al., 2000). From 20 weeks post-grafting pharmacological tests were carried out including LID, GID, and DA agonists-induced dyskinesia. For GID tests, grafted rats were allocated into two groups, with equal baseline AIMs score, to receive either amphetamine only, or amphetamine plus the selected compound. For LID tests, both grafted and control rats were allocated into two groups (4 subgroups in total), with equal AIMs scores, to receive either L-DOPA only, or L-DOPA plus the selected compound. A minimum of a 3-day washout was allowed between drug tests. New baselines were taken during the course of the study to make sure of the stability of the AIMs score, both for GID and LID. 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 were injected over 4 min and the Hamilton syringe was kept in place for an additional 3 min before retracted slowly. Antisedan® (0.28 mg/kg, *s.c.*, Apoteksbolaget, Sweden) was injected to reverse sedative effects of anesthetics and Temgesic® (0.04 mg/kg, *s.c.*, Apoteksbolaget, Sweden) to relieve pain after the surgery.

Cell preparation and transplantation surgery

The conventional VM cut was made from E14 rat embryonic brain, as described previously (Carlsson et al., 2007; Kirik et al., 2001; Nikkhah et al., 1994; Winkler et al., 1999). In this dissection, the caudal cut of VM was made slightly caudal of the isthmus to include the rostral part of the pontine raphe region.

Dissected tissues were incubated in Dulbecco's modified eagle medium (DMEM, Invitrogen, Sweden) containing 0.1% trypsin (Sigma–