doi:10.1093/brain/awm082 *Brain* (2007), **130**, 1819–1833

Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats

Manolo Carta,[1,2] Thomas Carlsson,[2] Deniz Kirik[2] and Anders Björklund[1]

[1]Neurobiology Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden and [2]CNS Disease Modeling Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden

Correspondence to: Manolo Carta, PhD, Wallenberg Neuroscience Center, BMC-A11, 22184 Lund, Sweden
E-mail: manolo.carta@med.lu.se

In patients with Parkinson's disease, the therapeutic efficacy of L-DOPA medication is gradually lost over time, and abnormal involuntary movements, dyskinesias, gradually emerge as a prominent side-effect in response to previously beneficial doses of the drug. Here we show that dyskinesia induced by chronic L-DOPA treatment in rats with 6-hydroxydopamine-induced lesions of the nigrostriatal dopamine pathway is critically dependent on the integrity and function of the serotonergic system. Removal of the serotonin afferents, or dampening of serotonin neuron activity by 5-HT_{1A} and 5-HT_{1B} agonist drugs, resulted in a near-complete block of the L-DOPA-induced dyskinesias, suggesting that dysregulated dopamine release from serotonin terminals is the prime trigger of dyskinesia in the rat Parkinson's disease model. In animals with complete dopamine lesions, the spared serotonin innervation was unable to sustain the therapeutic effect of L-DOPA, suggesting that dopamine released as a 'false transmitter' from serotonin terminals is detrimental rather than beneficial. The potent synergistic effect of low doses of 5-HT_{1A} and 5-HT_{1B} agonists to suppress dyskinesia, without affecting the anti-parkinsonian effect of L-DOPA in presence of spared dopamine terminals, suggests an early use of these drugs to counteract the development of dyskinesia in Parkinson's disease patients.

Keywords: Parkinson's disease; dyskinesia; serotonin; dopamine; 5-HTIA/IB agonists

Abbreviations: AIM = abnormal involuntary movement; DA = dopamine; MFB = medial forebrain bundle; PD = Parkinson's disease

Received December 5, 2006. Revised January 31, 2007. Accepted March 16, 2007. Advance Access publication April 23, 2007

In patients with Parkinson's disease (PD), the efficacy of L-DOPA therapy depends on its ability to restore dopamine (DA) neurotransmission in the denervated areas of the forebrain, above all in the striatum. Formation of DA from systemically administered L-DOPA, and its storage and release, takes place both in remaining dopaminergic axon terminals, as well as in other cellular elements within the striatal tissue, including the serotonergic afferents. The brainstem serotonin neurons are known to be to a varying degree affected by the disease process (Agid and Javoy-Agid, 1987; Hornykiewicz, 1998). Nevertheless, the serotonin innervation of the striatal complex remains relatively intact in most PD patients and may thus play a role not only in the control of motor behaviour, but also in the handling of systemic administered L-DOPA (Lavoie and Parent, 1990; Nicholson and Brotchie, 2002). The serotonin neurons have been shown to be able to convert exogenous L-DOPA to DA, and store and release DA in an activity-dependent manner (Ng *et al.*, 1970, 1971; Hollister *et al.*, 1979; Arai *et al.*, 1994, 1995, 1996; Tanaka *et al.*, 1999; Maeda *et al.*, 2005). L-DOPA-derived DA acting as a 'false transmitter' in serotonergic neurons may be particularly important in advanced stages of the disease when a major part of the nigrostriatal DA system has degenerated and the remaining DA neurons are in a compromised functional state (Miller and Abercrombie, 1999; Tanaka *et al.*, 1999; Kannari *et al.*, 2001). The integrity of the serotonin system may thus be an important factor in determining the efficacy of L-DOPA therapy in PD patients, and in particular in the development and maintenance of the most problematic side-effect of L-DOPA medication, the drug-induced dyskinesias. The ability of L-DOPA to induce dyskinesia increases over time, resulting in a gradual loss of the therapeutic window of L-DOPA medication in more advanced PD patients (Mouradian *et al.*, 1989). In a recent PET imaging study, de la Fuente-Fernandez *et al.* (2004) have shown that peak-dose dyskinesias in advanced PD patients are associated

with excessive swings in synaptic DA (and hence DA receptor occupancy) induced by oral L-DOPA administration. This raises the possibility that, in the absence of a functional striatal DA innervation, L-DOPA-derived DA is released in a non-physiological 'dysregulated' manner.

In the present study we provide evidence that DA released as a false transmitter from the residual serotonin innervation may be the source of this dysregulated DA release. The results show that L-DOPA-induced abnormal involuntary movements (AIMs) in the 6-hydroxydopamine (6-OHDA) lesion rat model, resembling peak-dose dyskinesia in L-DOPA-treated PD patients, is critically dependent on the integrity and function of the serotonin innervation, while the therapeutic effect of systemically administered L-DOPA is maintained primarily by spared DA terminals.

Material and Methods

Experimental design

A total of 250 adult female Sprague–Dawley rats weighing 225–250 g were used in the present study (B&K Universal, Stockholm, Sweden). The animals were housed under a 12 h light/12 h dark cycle with free access to water and food. All surgical procedures were performed according to the regulations set by the Ethical Committee for use of Laboratory animals at Lund University.

At the beginning of the study, all animals received a unilateral 6-OHDA lesion on the right side in either the striatum, at four sites, or in the medial forebrain bundle (MFB) at one site, in order to achieve a partial or complete lesion of the nigrostriatal pathway, respectively. Two weeks later, the rats were then screened behaviourally in the amphetamine-induced rotation test and in the cylinder test. Animals exhibiting $\geq$6 full body turns/min towards the side of DA deficiency and $\leq$20% left forepaw use in the cylinder were included in the study. In order to develop stable AIMs (equivalent to peak-dose dyskinesia seen in PD patients), the rats were treated with daily injections of 6 mg/kg L-DOPA (plus benserazide 15 mg/kg) for 3–4 weeks.

In the double lesion experiment (Fig. 1A), dyskinetic and non-dyskinetic rats were balanced, according to their dyskinesia score, into two well-matched subgroups and they further received either a bilateral intraventricular injection of 5,7-dihydroxytryptamine (5,7-DHT), in order to lesion the sertoninergic system, or vehicle. One group of drug-naïve partial-lesioned rats was subjected to the same surgical procedure. Two weeks later, daily L-DOPA injections were reintroduced and the animals were tested every second day for 2 weeks at a dose of 6 mg/kg. Finally, the dose was increased to 12 mg/kg L-DOPA and the animals were tested for another three times. Performance in the cylinder test was evaluated off- (baseline) and on L-DOPA (6 mg/kg). Two additional groups of drug-naïve rats, which had received partial and complete 6-OHDA lesions, respectively, were included in this test. Finally, 2 weeks after the last experiment, the animals were sacrificed, the striata dissected out and taken for HPLC measurements of DA and serotonin levels.

In the second part of the study (Fig. 1B), partial and complete 6-OHDA-lesioned animals were made dyskinetic by daily L-DOPA (6 mg/kg plus benserazide) as described earlier to evaluate the effect of the selective 5-HT_{1A} agonist, 8-OH-DPAT ((±)-8-hydroxy-2-dipropylaminotetralin hydrobromide; TOCRIS, Sweden), and the 5-HT_{1B} agonist, CP-94253 (TOCRIS, Sweden) on dyskinesia. The agonists were injected s.c. 5 min before L-DOPA, either alone at two different doses (8-OH-DPAT: 0.05 and 0.2 mg/kg; CP-94253: 1.0 and 2.5 mg/kg) or in combination at three different doses (8-OH-DPAT/CP94253: 0.035/0.75, 0.05/1.0 and 0.1/1.75 mg/kg). In each series of tests, the animals receiving L-DOPA plus the first dose of the agonists in the first test were injected with L-DOPA only in the subsequent test, and vice versa for the other group. By alternating the groups at each agonist dose, we could avoid any carry-over effect of the drugs on the following administration of L-DOPA. For the same reason, the animals were tested every second day in test series and at least 1 week wash-out was allowed before next agonist, or combination of agonists were tested. A new baseline (average of three tests) of L-DOPA-induced dyskinesia was obtained before each new test series. In the apomorphine experiment, the agonists were administered 15 min before apomorphine (0.025 mg/kg, s.c.). In this case a cross-over design was adopted. The animals receiving apomorphine plus the first dose of agonist on the first day, received apomorphine only 2 days later, and vice-versa for the other group at the same dose. The same design was used for each dose.

6-OHDA lesions

All 6-OHDA injections were conducted under anaesthesia induced by an injectable 20:1 mixture of Fentanyl and Dormitor (Apoteksbolaget, Sweden) using a stereotaxic frame (Stoelting, Wood Dale, IL) with an attached Hamilton syringe. The animals received 6-OHDA (Sigma-Aldrich AB, Sweden) injection into either the striatum, at four sites, for a total of 28 µg (3.5 µg/µl free base in 0.02% ascorbic acid in saline, 2 µl in each site), in order to induce a partial lesion of the nigrostriatal pathway or into the MFB (14 µg free base in 4 µl) in order to achieve a complete lesion of the nigrostriatal pathway, at the following coordinates (relative to bregma; see Paxinos and Watson, 1998): partial lesion (1) AP: +1.3 mm, ML: −2.3 mm; (2) AP: +0.4 mm, ML: −3.2 mm; (3) AP: −0.4 mm, ML: −4.2 mm; (4) AP: −1.3 mm, ML: −4.5 mm. Complete lesion: AP: −4.4 mm, ML: −1.2 mm. The dorso-ventral (DV) coordinates were calculated from the dura and were − 5.0 and − 7.8 mm for all the striatal and MFB injections, respectively. The tooth bar was set at 0.0 mm for injections into the striatum and − 2.4 mm for the injection in the MFB. Injection speed was 1.0 µl/min and the syringe was kept in place for an additional 3 min before it was slowly retracted. All injections in the striatum were performed using an attached glass capillary (outer diameter 60–80 µm) on the Hamilton syringe in order to minimize the mechanical damage of multiple injections.

5,7-DHT injections

Lesions of the serotonergic system were performed using 1–2% isofluorane anaesthesia. 5,7-DHT creatine sulphate (150 µg free base in 20 µl 0.02% ascorbate-saline; Sigma-Aldrich AB, Sweden) were infused bilaterally (10 µl/side) at the following coordinates, in respect to the bregma; AP: −0.8 mm; ML: ±1.4 mm; DV: −4.6 mm; the tooth bar was set to −3.3 mm. Injection was performed over 1 min on each side and the needle was kept in place for additional 3 min before it was retracted. The 'sham' controls received similar injection of saline. In order to protect the noradrenergic system, the catecholamine re-uptake blocker desipramine (25 mg/kg; Sigma-Aldrich AB, Sweden) was injected

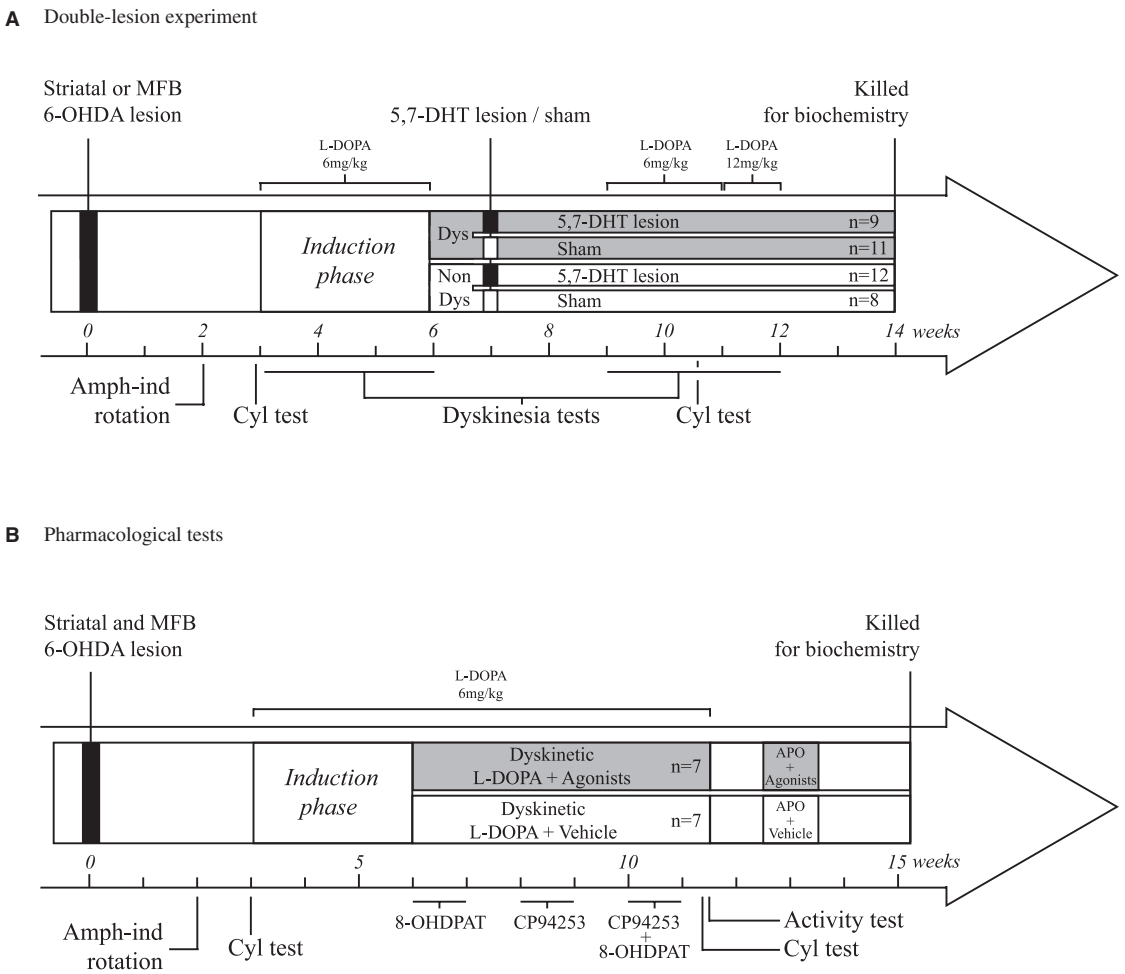

Fig. 1 Experimental design. At the beginning of the study, animals were lesioned with 6-OHDA into either the striatum or the MFB. Two and three weeks after lesion, the severity of DA depletion was screened by amphetamine-induced rotation and forelimb use at the cylinder, respectively. Only the animals exhibiting $\geq$6 full body turns/min towards the side of DA deficiency and $\leq$20% left forepaw use in the cylinder were included in the study. In order to develop stable AIMs (equivalent to peak-dose dyskinesia seen in PD patients), the rats were treated with daily injections of 6 mg/kg L-DOPA (plus benserazide 15 mg/kg) for 3–4 weeks (induction phase). In the double-lesion experiment (**A**), dyskinetic and non-dyskinetic rats were balanced, according to their dyskinesia score, into two well-matched subgroups and they further received either a bilateral intraventricular injection of 5,7-DHT sulphate (150 μg free base in 20 μl 0.02% ascorbate-saline), in order to lesion the serotonergic system or vehicle. One group of drug-naïve partial-lesioned rats was subjected to the same surgical procedure. Two weeks later, daily L-DOPA injections were reintroduced and the animals were tested every second day for 2 weeks at a dose of 6 mg/kg. Performance in the cylinder test was evaluated off- (baseline) and on- L-DOPA (6 mg/kg). Two additional groups of drug-naïve rats, that had received partial and complete 6-OHDA lesions, respectively, were included in this test. Finally, the dose was increased to 12 mg/kg L-DOPA and the animals were tested for additional three times. For the pharmacological experiments (**B**), only the dyskinetic rats were employed. One week of washout was allowed to the animals between different drug testing, and a new baseline of dyskinesia was always measured. The same rats were tested in the cylinder in order to evaluate the effect of the treatment with the combined serotonin autoreceptor agonists, 8-OHDPAT (5-HT_{1A}) and CP94253 (5-HT_{1B}), on left forelimb use. An activity test was also performed to compare the effect of L-DOPA alone with L-DOPA + agonists on general motor activation. Finally, the ability of the agonists to reduce dyskinesia induced by apomorphine (0.025 mg/kg s.c.) was tested in the striatal-lesioned rats. Two weeks after the last experiment, the animals were sacrificed, the striata dissected out and taken for HPLC measurements of DA and serotonin levels. A subset of these animals were injected with either L-DOPA at 6 mg/kg or vehicle 60 min before sacrifice in order to investigate the impact of L-DOPA treatment on striatal DA and serotonin levels.

i.p. 30 min before 5,7-DHT or saline infusion (Bjorklund *et al.*, 1975). After surgery, all rats received Temgesic (Apoteksbolaget, Sweden) as analgesic treatment and physiological saline to prevent post-surgical dehydration. They were also provided with additional bedding and palatable foods until stabilized intake of food and water were achieved. A 2-week recovery period was allowed for the animals.

HPLC measurements

All animals were killed and striata were rapidly dissected out, frozen on dry ice and stored in a −80°C freezer until analysis. At the time of the analysis, tissue was homogenized in 0.1 M perchloric acid and centrifuged at 10 000 rpm for 10 min before filtering though minispin filters for additional 3 min at 10 000 rpm. The tissue extract were then analysed by HPLC

as described earlier (Carta *et al.*, 2006) with some little modification. Briefly, 25 µl of each sample were injected by a cooled autosampler (Midas) into an ESA Coulochem III coupled with an electrochemical detector. The mobile phase (sodium acetate 5 g/l, Na_2-EDTA 30 mg/l, octane–sulphonic acid 100 mg/l, methanol 9%, pH 4.2) was delivered at a flow rate of 500 µl/min to a reverse phase C18 column (4.6 mm Ø, 150 mm length, Chrompack). The peaks were processed by the Azur Chromatographic Software (Datalys, France).

Behavioural analyses

Amphetamine-induced rotation

Amphetamine-induced rotation was performed at 2 weeks after the 6-OHDA injection to evaluate the extent of the DA lesion. Right and left full body turns were recorded over 90 min, using automated rotometer bowls (AccuScan Instrument Inc., Columbus, Ohio), following an i.p. injection of 2.5 mg/kg of D-amphetamine sulphate (Apoteksbolaget, Sweden). The data are expressed here as net full body turns per minute, where rotation towards the side of the lesion was given a positive value.

Cylinder test

Forelimb use in the cylinder test (Schallert and Tillerson, 1999) was assessed as previously described (Kirik *et al.*, 2000). Individual rats were placed in a Ø 20 cm glass cylinder, where they could move freely. Each animal was recorded on videotape for 2 min, and later scored from the tape up to a total of 20 touches by an observer blinded to the identity of the animals. At each test session, a new baseline value was obtained off-drug before injecting the animals with L-DOPA and testing them again 60 min later. Combination of 5-HT_{1A} and 5-HT_{1B} agonists (8-OH-DPAT/CP-94253: 0.1/1.75 mg/kg, dissolved in 0.02% ascorbate in saline) were given 5 min prior to L-DOPA. The data present left forepaw touches as percentage of the total number of touches. In this test, normal, unbiased rats would receive a score of 50% (indicated as a dashed line in Figs 2B, D and 3B, D).

L-DOPA and apomorphine-induced dyskinesia

In order to induce stable AIMs, L-DOPA methyl ester (6 mg/kg; Research Organics, Cleveland, Ohio) combined with the peripheral DOPA–decarboxylase inhibitor, benserazide (15 mg/kg, Sigma-Aldrich, Sweden), was dissolved in physiological saline and administered daily to each rat as an i.p. injection for 3–4 weeks. At all tests the AIMs were evaluated according to the rat dyskinesia scale described in detail previously (Lee *et al.*, 2000; Lundblad *et al.*, 2002). Briefly, the animals were placed individually in transparent plastic cages without bedding material and scored every 20 min following the injection of L-DOPA. The AIMs were further classified into four subtypes according to their topographic distribution as Forelimb (Li), Orolingual (Ol), Axial (Ax) and Locomotive (Lo) behaviours. The Forelimb and Orolingual dyskinesia are predominantly seen as hyperkinesia, while the axial dyskinesia is essentially of a dystonic type. The locomotive dyskinesia was expressed as circling movements away from the lesioned side. Enhanced manifestations of normal behaviours such as grooming, gnawing, rearing and sniffing were not included in the rating. The severity of each AIM subtype was assessed using scores from 0 to 4 (0: absent, 1: occasional, i.e. present less than 50% of the time; 2: frequent, i.e present more than 50% of the time; 3: continuous, but interrupted by strong sensory stimuli and 4: continuous, not interrupted by strong sensory stimuli).

Dyskinesias were also evaluated after apomorphine injection (0.025 mg/kg, dissolved in saline containing 0.02% ascorbic acid; Apoteksbolaget, Sweden). Here, scoring was performed every 10 min using the same rating scale as for the L-DOPA-induced dyskinesias. The data are presented as integrated scores, area under the curve in a raw data plot of total Ax + Li + Ol AIM scores.

Activity test

Locomotor activity was assessed in open-field chambers, each equipped with a 16 × 16 infrared photobeam system (dimensions 40.6 cm × 40.6 cm × 38.1 cm) using the Flex-Field Software system (San Diego Instruments, San Diego, CA). Animals were habituated for 1 h before the drugs were injected and the measurements begun.

Statistical analysis

Group comparisons were performed using either one-way factorial ANOVA or two-way repeated measures ANOVA where appropriate. *Post hoc* analysis was performed using the Student's *t*-test or the Tukey–Kramer HSD analysis. Statistical significance was set at $P < 0.01$. All values are presented in the study as mean ± SEM. All statistics in this study were performed using the JMP Statistical software version 5.0.1.2 (SAS Institute Inc., Cary, USA).

Results

The involvement of the serotonergic system in L-DOPA-induced dyskinesia, and the functional, therapeutic effect of the drug, were studied in rats with either partial lesion of the striatal DA innervation, induced by injection of 6-OHDA into the striatum, or in rats with complete DA lesion induced by injection of the toxin into the MFB. These two lesion protocols resulted in depletion of striatal DA by about 90 and >99%, respectively, while the serotonin innervation was spared (Table 1). In half of the animals in each group, the serotonin raphe projection was subsequently lesioned by an intraventricular injection of the specific serotonin neurotoxin 5,7-DHT. This design allowed us to compare the effect of removing the striatal serotonin innervation in rats with either partial or complete striatal DA lesions, and to study the relative role of the spared DA and serotonin innervations in the induction of L-DOPA-induced dyskinesia and the maintenance of the functional therapeutic effect of the drug. In the second part of the study, we made use of selective agonists of two serotonergic autoreceptors, 5-HT_{1A} and 5-HT_{1B}, known to be effective in inhibiting transmitter release from serotonin terminals, as a tool to dampen the release of L-DOPA-derived DA from the serotonin afferents in dyskinetic 6-OHDA-lesioned animals.

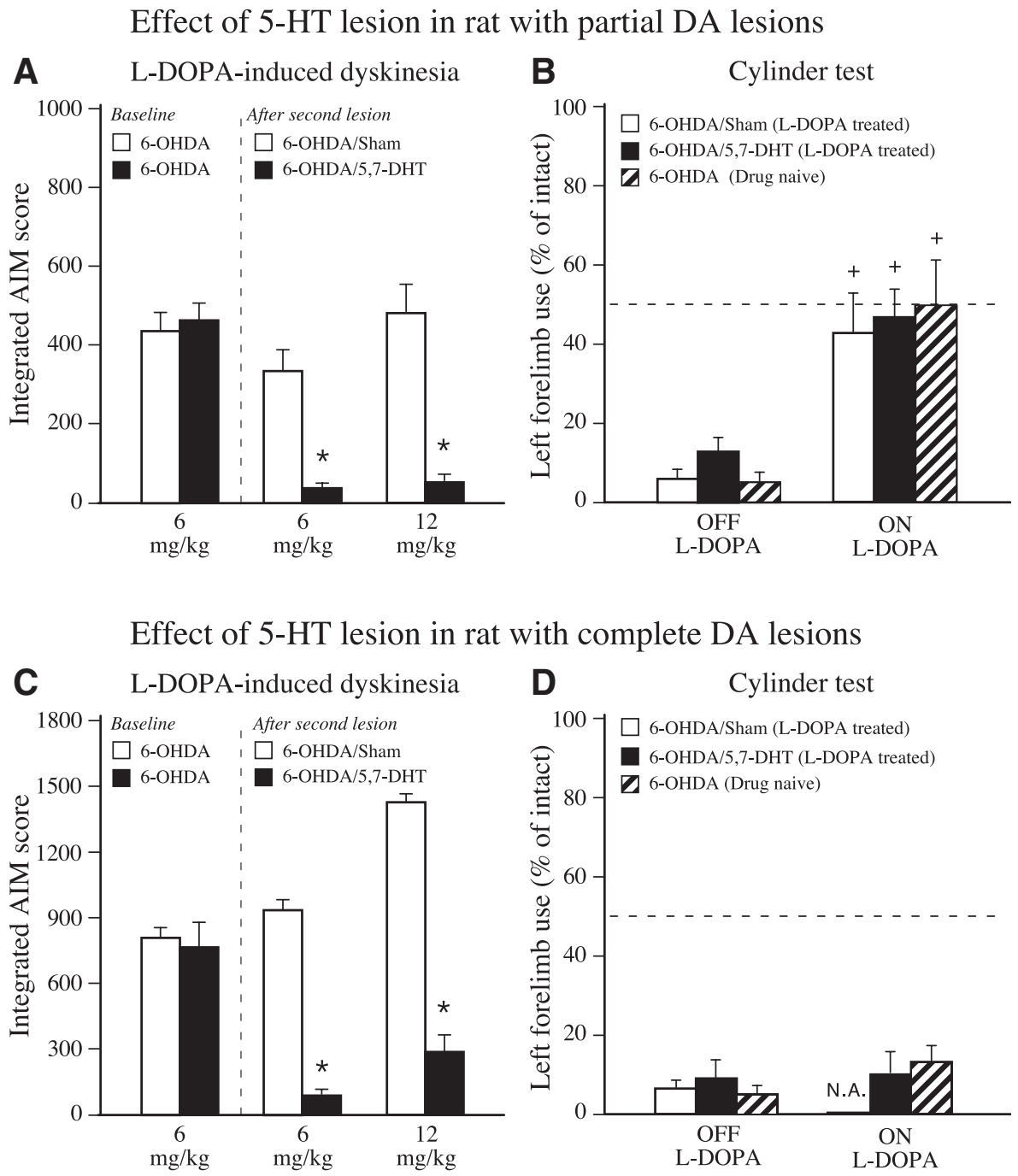

Fig. 2 Effect of combined 6-OHDA and 5,7-DHT lesion on L-DOPA-induced dyskinesia and forelimb use. Rats with either partial intrastriatal 6-OHDA lesion (**A**, **B**), or complete MFB 6-OHDA lesion (**C**, **D**) were treated with L-DOPA (6 mg/kg plus benserazide 15 mg/kg i.p.) for 3 weeks to reach a stable level of dyskinesia. At that point the dyskinetic rats were allocated into two subgroups, balanced according to their AIMs score, and subjected to either a serotonin lesion by intraventricular injection of 5,7-DHT or to a sham lesion (saline). Two weeks after surgery, daily L-DOPA treatment was resumed. Dyskinesia was significantly decreased in the lesion group compared to the sham in both partial DA-lesioned rats [**A**; repeated measures ANOVA, time × group interaction $F(2,9) = 19.94$, $P = 0.0005$, followed by Tukey–Kramer HSD *post hoc* test with alpha level set to 0.01; $n = 13$ per group at 6 mg/kg L-DOPA, $n = 6$ per group at 12 mg/kg L-DOPA] and in rats with complete DA lesions [**C**; repeated measures ANOVA, $F(2,11) = 76.38$, $P < 0.0001$, followed by Tukey–Kramer HSD *post hoc* test with alpha level set to 0.01; $n = 7$ per group]. Each bar represents average of three consecutive tests. The same animals, plus two additional groups of drug-naïve rats, were tested for forelimb use in the cylinder test. In all partial-lesioned animals, including the 5,7-DHT-lesioned ones, left forelimb use was restored to normal after L-DOPA (6 mg/kg) [**B**; repeated measures ANOVA, group effect $F(2,23) = 0.38$, $P = 0.69$; time effect $F(1,23) = 37.28$, $P < 0.0001$; time × group interaction $F(2,23) = 0.27$, $P = 0.77$; $n = 9$ for 6-OHDA/5,7-DHT, $n = 11$ for 6-OHDA/sham, $n = 6$ for the drug-naïve group], while forelimb use in the complete DA-lesioned rats was unaffected by the L-DOPA treatment [**D**; repeated measures ANOVA, group effect $F(1,19) = 0.008$, $P = 0.93$; time effect $F(1,19) = 2.28$, $P = 0.15$; time × group interaction $F(1,19) = 1.51$, $P = 0.23$; $n = 6$ for 5,7-DHT, $n = 15$ for sham]. * = significant from sham; + = significant from OFF L-DOPA.

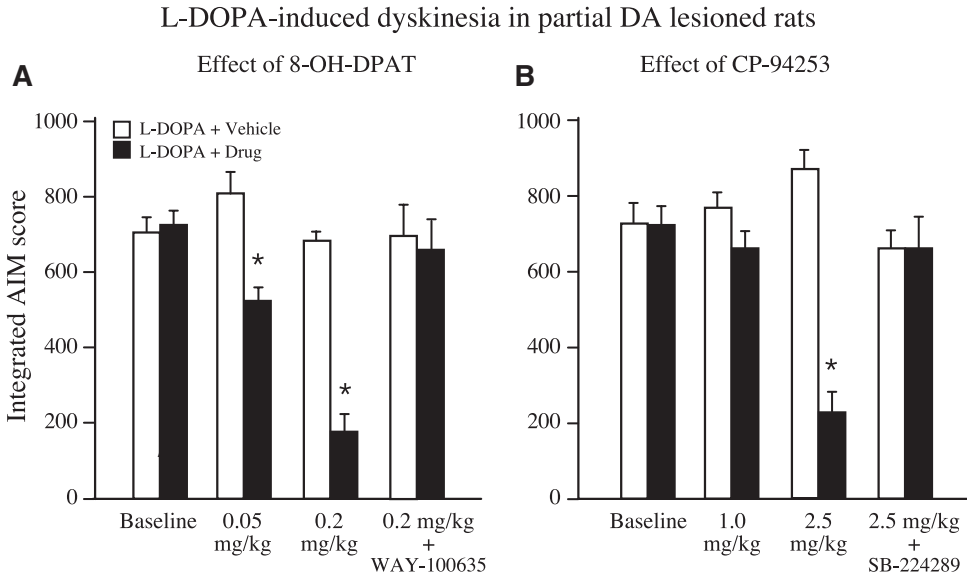

Fig. 3 Effect of 5-HT_{1A} and 5-HT_{1B} agonists on L-DOPA-induced dyskinesia in rats with partial intrastriatal 6-OHDA lesions. (**A**) Animals were primed with daily injections of L-DOPA at 6 mg/kg dose for 3 weeks. Once a stable expression of dyskinesia was achieved, dyskinetic rats were allocated into two well-matched groups. One group received the 5-HT_{1A} agonist 8-OH-DPAT at the low dose (0.05 mg/kg s.c.), while the second group received L-DOPA alone. At the second dose of the agonist (0.2 mg/kg), groups were switched to exclude any residual effect of the first administration on the result observed. Both doses significantly reduced L-DOPA-induced dyskinesia (mean ± SEM, 526 ± 34 versus 811 ± 59 in treated and control animals, respectively at the low dose; 177 ± 46 versus 686 ± 24 in treated and control rats, respectively at the higher dose). Pre-treatment with the 5-HT_{1A} antagonist WAY-100635 (0.4 mg/kg) blocked this effect (repeated measures ANOVA, time × group interaction $F(3,10) = 16.37$, $P = 0.0003$, followed by Tukey–Kramer *post hoc* test with alpha level set to 0.01; $n = 7$ per group). (**B**) One week wash-out was allowed before collecting a new baseline (average of three tests) and testing the 5-HT_{1B} agonist CP-94253 with the same procedure described earlier. Significant reduction of dyskinesia was observed only at the higher dose (2.5 mg/kg; mean ± SEM, 229 ± 56 versus 874 ± 53 in treated and control rats, respectively), and this effect was blocked by the 5-HT_{1B} antagonist SB-224289 (3 mg/kg) (repeated measures ANOVA, time × group interaction $F(3,10) = 75.68$, $P < 0.0001$; $n = 7$ per group). * = significant from vehicle.

Removal of the serotonin afferents by 5,7-DHT

In the double-lesion experiment the animals received, first, a unilateral injection of 6-OHDA into either the right striatum (four deposits of 7 µg free base; *partial DA lesion*) or the right MFB (14 µg free base in one deposit; *complete DA lesion*). Starting 4 weeks after the 6-OHDA injection, the lesioned rats were subjected to daily systemic injections of L-DOPA for 3 weeks, at a dose of 6 mg/kg (combined with 15 mg/kg of the peripheral decarboxylase blocker benserazide), which is known to be at the threshold for induction of improved motor performance in rats with intrastriatal partial 6-OHDA lesions (Winkler *et al.*, 2002). This treatment was able to induce moderate-to-severe dyskinesia in about 40% of the rats subjected to a partial intrastriatal 6-OHDA lesion, and in about 70% in rats with complete 6-OHDA lesions. Dyskinetic and non-dyskinetic rats were divided in two well-matched subgroups, balanced according to their AIM scores. The animals in each subgroup received an intraventricular injection of either 5,7-DHT, or vehicle as a sham-lesion control. The catecholamine re-uptake blocker desipramine (25 mg/kg) was given 30 min prior to the toxin injection in order to protect the noradrenaline neurons. With this treatment regimen, the lesion induced by the 5,7-DHT toxin is highly selective for serotonergic neurons (Bjorklund *et al.*, 1975). Accordingly, no differences in noradrenaline levels were found between the lesioned striata of the 5,7-DHT injected and sham-operated rats (data not shown). At the dose of 5,7-DHT used here (150 µg free base) the total striatal serotonin level was reduced by 90–95% (Table 1).

Two weeks after the second lesion daily L-DOPA treatment was reintroduced at the 6 mg/kg dose. While the vehicle-injected dyskinetic rats continued to display dyskinesia at a level similar to the pre-injection score, the dyskinetic response to the drug was almost completely abolished in double-lesioned animals, both in the partial and the complete DA-lesioned group (Fig. 2A and C). This effect was seen at L-DOPA doses of both 6 and 12 mg/kg, which are in the range of doses used clinically. None of the previously non-dyskinetic rats developed any signs of dyskinesia after the 5,7-DHT lesion (data not shown). In a separate group of non- L-DOPA-treated (non-primed) animals subjected to partial 6-OHDA lesion alone ($n = 6$), or to partial 6-OHDA lesion in combination with intraventricular 5,7-DHT injection ($n = 6$), the development of L-DOPA-induced dyskinesia, seen in the 6-OHDA/sham animals, was almost completely blocked in the double-lesioned rats (integrated AIMs score 420 ± 180 versus

Table 1 Striatal DA and serotonin tissue levels as determined by HPLC

	Dopamine (pmol/mg wet tissue)		Serotonin (pmol/mg wet tissue)	
Groups	Intact side	Lesioned side	Intact side	Lesioned side
6-OHDA/sham (partial lesion) $n = 8$	44.13 ± 1.54	5.97 ± 1.70* (13.5%)	1.55 ± 0.04	2.07 ± 0.18
6-OHDA/5,7-DHT (partial lesion) $n = 8$	44.11 ± 2.24	4.44 ± 1.12* (10.0%)	0.23 ± 0.05	0.22 ± 0.04^{+} (10.6%)
6-OHDA/sham (complete lesion) $n = 6$	41.85 ± 4.81	0.20 ± 0.09* (0.47%)	2.39 ± 0.07	2.11 ± 0.35
6-OHDA/5,7-DHT (complete lesion) $n = 6$	46.12 ± 3.67	0.28 ± 0.17* (0.60%)	0.23 ± 0.05	0.11 ± 0.01^{+} (5.21%)
6-OHDA only (partial lesion) $n = 15$	39.37 ± 2.04	3.40 ± 0.60* (8.6%)	2.13 ± 0.09	2.61 ± 0.09

Note: Striatal DA was reduced by 99.5% in the rats with complete, MFB 6-OHDA lesions, and by about 90% in rats with partial, intrastriatal 6-OHDA lesions, while the levels of serotonin were unaffected or slightly increased. Figures in brackets give DA values as percent of the intact control side, and serotonin levels as percent of those measured in the corresponding sham group.* = significant from the intact side; $^{+}$ = significant from respective sham group (unpaired *t*-test, $P < 0.01$).

80 ± 40 in 6-OHDA/sham and 6-OHDA/5,7-DHT-injected rats, respectively, after 2 weeks of L-DOPA treatment).

The ability of the systemically injected L-DOPA to restore normal motor behaviour was studied in the cylinder test (Schallert and Tillerson, 1999). In this test the ability of the rat to use its forelimbs for body-weight adjustment is scored independently for the impaired (left) and the control (right) side. Left forelimb use was severely impaired, to <20%, in both the partial and complete 6-OHDA lesion groups (Fig. 2B and D). After injection of L-DOPA (6 mg/kg), the use of the impaired forelimb was restored to almost normal level (43 ± 10%) in the rats with partial 6-OHDA lesions (Fig. 2B), while the complete-lesioned rats were severely dyskinetic and not able to perform in this test under the influence of L-DOPA (N.A. in Fig. 2D). The functional recovery seen after L-DOPA in the partial 6-OHDA-lesioned rats was retained also after 5,7-DHT injection (47 ± 7.6%). In contrast, no L-DOPA-induced functional recovery was observed in the complete DA-lesioned/5,7-DHT-injected rats, i.e. in rats with complete lesions of both the nigrostriatal DA pathway and the serotonin afferents (filled bars in Fig. 2D). The inability of the rats with complete DA lesions to improve after L-DOPA in the cylinder test is unlikely to be due to the 5,7-DHT lesion or to the L-DOPA priming (i.e. chronic L-DOPA treatment) since the same difference in L-DOPA-induced functional recovery between partial and complete 6-OHDA-lesioned rats was observed also in L-DOPA naïve, non-dyskinetic animals with an intact serotonin system (hatched bars in Fig. 2B and D).

Effect of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists on L-DOPA-induced dyskinesia

The results of the double-lesion experiment suggest that L-DOPA-derived DA, acting as a false transmitter in serotonergic neurons, plays a major role in the induction of L-DOPA-induced dyskinesia in the 6-OHDA lesion model. If so, drugs interfering with transmitter release from serotonin terminals should be able to reduce the release of L-DOPA-derived DA and, in turn, have a dampening effect on the L-DOPA-induced dyskinesia. In this experiment we made use of two selective agonists for the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, 8-OH-DPAT and CP-94253, respectively, as a tool to selectively dampen serotonin-neuron-dependent DA release. These receptors are known to be expressed as auto-receptors at the level of the soma and dendrites (5-HT$_{1A}$) and terminals (5-HT$_{1B}$) of the serotonergic neurons, where they regulate the neuronal firing and terminal serotonin release, respectively (Chalmers and Watson, 1991; Sari *et al.*, 1999; Knobelman *et al.*, 2000; Riad *et al.*, 2000; Adell *et al.*, 2001). The same receptors are also expressed as hetero-receptors in various brain areas including the prefrontal cortex (PFC) and striatum (Chalmers and Watson, 1991; Ceci *et al.*, 1994; Casanovas *et al.*, 1999; Hajos *et al.*, 1999; Sari *et al.*, 1999) (see later). In previous studies, 8-OH-DPAT and CP-94253, administered systemically, have been shown to be highly effective in reducing the firing rate of the serotonergic neurons and inhibiting the release of serotonin, both at the level of the raphe nuclei and in their forebrain projection areas, including the striatum (Sprouse and Aghajanian, 1987; Knobelman *et al.*, 2000; Adell *et al.*, 2001).

As shown in Fig. 3A and B, both drugs were able to dose-dependently decrease the expression of dyskinesia in L-DOPA primed, partial 6-OHDA-lesioned rats, by 70–75% at the highest doses tested. Pre-treatment with the selective antagonists WAY-100635 (5-HT$_{1A}$) and SB-224289 (5-HT$_{1B}$) suppressed the anti-dyskinetic effect of 8-OH-DPAT and CP-94253, respectively (Fig. 3A and B), indicating that the effect was due to a selective action of the agonists on their respective 5-HT receptor. The most striking result was obtained when the two agonists were co-administered prior to the L-DOPA dose. In this experiment 8-OH-DPAT and CP-94253 were given at sub-threshold doses (0.05 and 1.0 mg/kg, respectively), i.e. in doses which on their own produced no or only slight reductions in dyskinesia. In combination, the two drugs were able to block L-DOPA-induced dyskinesia almost completely: by 92 ± 6% in rats with partial 6-OHDA-lesions, and by 83 ± 7% in rats with complete lesions (Fig. 4A and C). At higher doses (0.1 mg/kg 8-OH-DPAT + 1.75 mg/kg

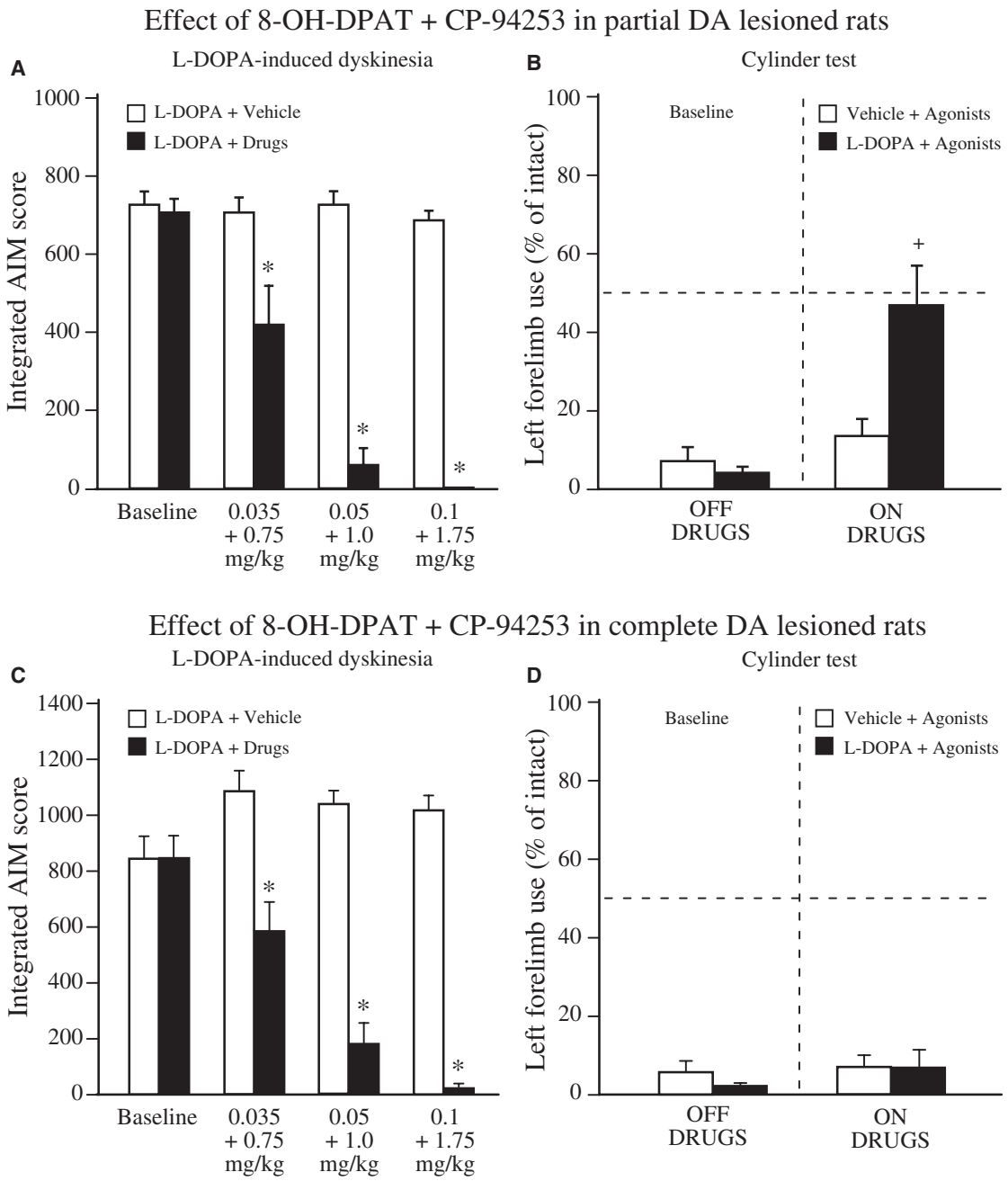

Fig. 4 Effect of 5-HT_{1A} and 5-HT_{1B} agonists in combination on L-DOPA-induced dyskinesia and forelimb use in rats with partial and complete lesion of the nigrostriatal DA pathway. When administered together, 5-HT_{1A} and 5-HT_{1B} agonists dose-dependently reduced L-DOPA-induced dyskinesia in both partial DA-lesioned rats [see **A**; mean ± SEM: 420 ± 101 versus 709 ± 38 in treated and control rats, respectively, at the first dose; 60 ± 43 versus 729 ± 36 in treated and control rats, respectively, at the second dose tested; 0 versus 686 ± 29 in treated and control rats, respectively at the highest dose tested; repeated measures ANOVA, time × group interaction $F(3,10) = 79$, $P < 0.0001$, followed by Tukey–Kramer *post hoc* test with alpha level set to 0.01; $n = 7$ per group], as well as in rats with complete DA lesions [**C**; mean ± SEM, 578 ± 100 versus 1075 ± 69 in treated and control rats, respectively at the first dose tested; 178 ± 74 versus 1028 ± 45 in treated and control rats, respectively, at the second dose tested; 20 ± 11 versus 1008 ± 52 in treated and control rats, respectively at the highest dose tested; repeated measure ANOVA, time × group interaction $F(1,14) = 108.90$, $P < 0.0001$, followed by Tukey–Kramer *post hoc* test with alpha level set to 0.01; $n = 8$ per group]. The same animals were also tested for forelimb use in the cylinder test before and 60 min after L-DOPA injection (6 mg/kg plus benserazide). L-DOPA-induced functional recovery in left forelimb use in the partial-lesioned rats [**B**; mean ± SEM, 47 ± 10 in L-DOPA/agonists treated group versus 14 ± 4 in the group treated only with agonists as control; repeated measure ANOVA, time × group interaction $F(1,12) = 8.57$, $P = 0.013$, followed by Tukey–Kramer *post hoc* test with alpha level set to 0.01; $n = 8$ per group in the L-DOPA/agonists-treated group and $n = 6$ in the group treated with only agonists], but not in the complete-lesioned group [**D**; repeated measure ANOVA, time × group interaction $F(1,14) = 0.43$, $P = 0.52$, $n = 8$ per group]. Administration of the agonists alone had no effect on the performance of the animals in either group. * = significant from vehicle; + = significant from OFF L-DOPA.

CP94253), the dyskinetic behaviour was completely suppressed, with no AIMs observed in the partial-lesioned rats and with only few episodes in the complete DA-lesioned group (Fig. 4A and C). The effect of this treatment was also tested in a small number of rats on ongoing dyskinesia. In this experiment, L-DOPA-primed rats with partial 6-OHDA lesions were monitored after a single L-DOPA injection (6 mg/kg). At the peak of L-DOPA-induced AIMs (50 min post-injection), the two agonists were administered at the 0.05 + 1.0 mg/kg doses, as earlier. The ongoing dyskinesias were completely suppressed within 10 min after the injection (data not shown, see movie in supplemental material). Importantly, the ability of L-DOPA to restore normal forelimb use in the cylinder test in the partial 6-OHDA-lesioned rats was unaffected by the combined agonist treatment (Fig. 4B), which is in line with the observations from the double-lesion experiment. The marked effect of the combined agonist treatment on dyskinesia was clearly not due to a general depression of motor behaviour. Co-administration of the agonists with L-DOPA had no negative effect on forelimb use in the cylinder test (Fig. 3B). Moreover, general locomotor activity, as assessed in an independent open-field activity test, was either normal or increased (5267 ± 581 beam-crossings over 2 h in the L-DOPA-treated rats versus 11259 ± 2348 in the L-DOPA plus agonist-treated ones; $P < 0.05$). The increased locomotor activity seen in partial 6-OHDA-lesioned rats after agonist treatment can be explained by the fact that they were no longer engaged in dyskinesia, which limited their ambulatory movement.

As also described by Iravani *et al.* (2006) with another enantiomer of 8-OH-DPAT in MPTP-lesioned marmosets, we observed signs of serotonin syndrome (i.e. flat body position) in our rats at the higher dose of 8-OH-DPAT given individually (0.2 mg/kg), which was accompanied by transitory motor depression of the animals. In contrast, such a behaviour was not observed at the combined sub-threshold doses of the two agonists, despite the more potent effect on dyskinesia. Indeed, such an event is known to be induced by activation of the post-synaptic 5-HT_{1A} receptors rather than the pre-synaptic one (Goodwin *et al.*, 1986, 1987; Yamada *et al.*, 1988; Carey *et al.*, 2004*a*, *b*, 2005), pointing to the advantage of the combined agonist treatment respect to the single drug administration.

Effect of the 5-HT_{1A} and 5-HT_{1B} receptor agonists on apomorphine-induced dyskinesia

As mentioned earlier, 5-HT_{1A} and 5-HT_{1B} receptors are expressed also as post-synaptic receptors on both cortical and striatal target neurons. Previous studies have shown that post-synaptic 5-HT_{1A} receptors can participate in the regulation of the firing of the serotonin neurons in the dorsal raphe nucleus through a feedback loop (Ceci *et al.*, 1994; Casanovas *et al.*, 1999; Hajos *et al.*, 1999). Activation of 5-HT_{1A} receptors in the PFC can also inhibit the activity of the corticostriatal glutamatergic neurons and reduce glutamate release in the striatum (Antonelli *et al.*, 2005; Mignon and Wolf, 2005). Indeed, antagonists acting on glutamatergic receptors are known to be effective in reducing dyskinesia (Blanchet *et al.*, 1998; Konitsiotis *et al.*, 2000). It seems therefore possible that the anti-dyskinetic effect of the serotonin agonists could be mediated, at least in part, by an action on the corticostriatal glutamatergic pathway.

To clarify this, we studied the effect of the two agonists, 8-OH-DPAT and CP-94253, on apomorphine-induced dyskinesia. Apomorphine is a combined D_1/D_2 receptor agonist that acts directly on the post-synaptic DA receptors, and its action is thus independent of any extrinsic L-DOPA-derived DA synthesis or release. The same group of L-DOPA primed partial 6-OHDA-lesioned rats as in the L-DOPA-induced dyskinesia experiment, above, was used in this experiment. As shown in Fig. 5A and B, 8-OH-DPAT and CP-94253, given individually, showed some effect in reducing apomorphine-induced dyskinesia at the highest doses used; however, only the effect of CP-94253 was statistically significant. When the two agonists were co-administered at doses that resulted in a near-complete suppression of L-DOPA-induced dyskinesia (Fig. 4A and C), the effect on apomorphine-induced dyskinesia was marginal and non-significant (Fig. 5C).

These data indicate that the effect of 8-OH-DPAT and CP-94253, individually, on L-DOPA-induced dyskinesia may be due to a combined effect involving both a pre-synaptic inhibition of DA release from the serotonergic terminals, as well as a post-synaptic inhibition of the corticostriatal neurons resulting in a decreased glutamatergic input to the striatum. The ability of the 5-HT_{1A} and 5-HT_{1B} agonists to act synergistically in blocking L-DOPA-induced dyskinesia at low doses, however, appear to be due, almost exclusively, to an inhibition of the release of L-DOPA-derived DA from the serotonergic terminals; in this case, in agreement with the reported higher sensitivity of the pre- versus the post-synaptic 5-HT_{1A} receptor (Sprouse and Aghajanian, 1987; Ceci *et al.*, 1994; Riad *et al.*, 2000), the effect on striatal glutamate release is likely to play a minor role.

L-DOPA-induced depletion of serotonin content

The formation of DA from L-DOPA and the ability of L-DOPA-derived DA to displace serotonin from the vesicular storage site were assessed by measuring neurotransmitter levels at the HPLC. Rats with complete 6-OHDA lesions, with or without a 5,7-DHT lesion of the serotonergic system, were employed in the experiment. The animals were subjected to an injection of either L-DOPA (6 mg/kg plus benserazide) or saline (plus

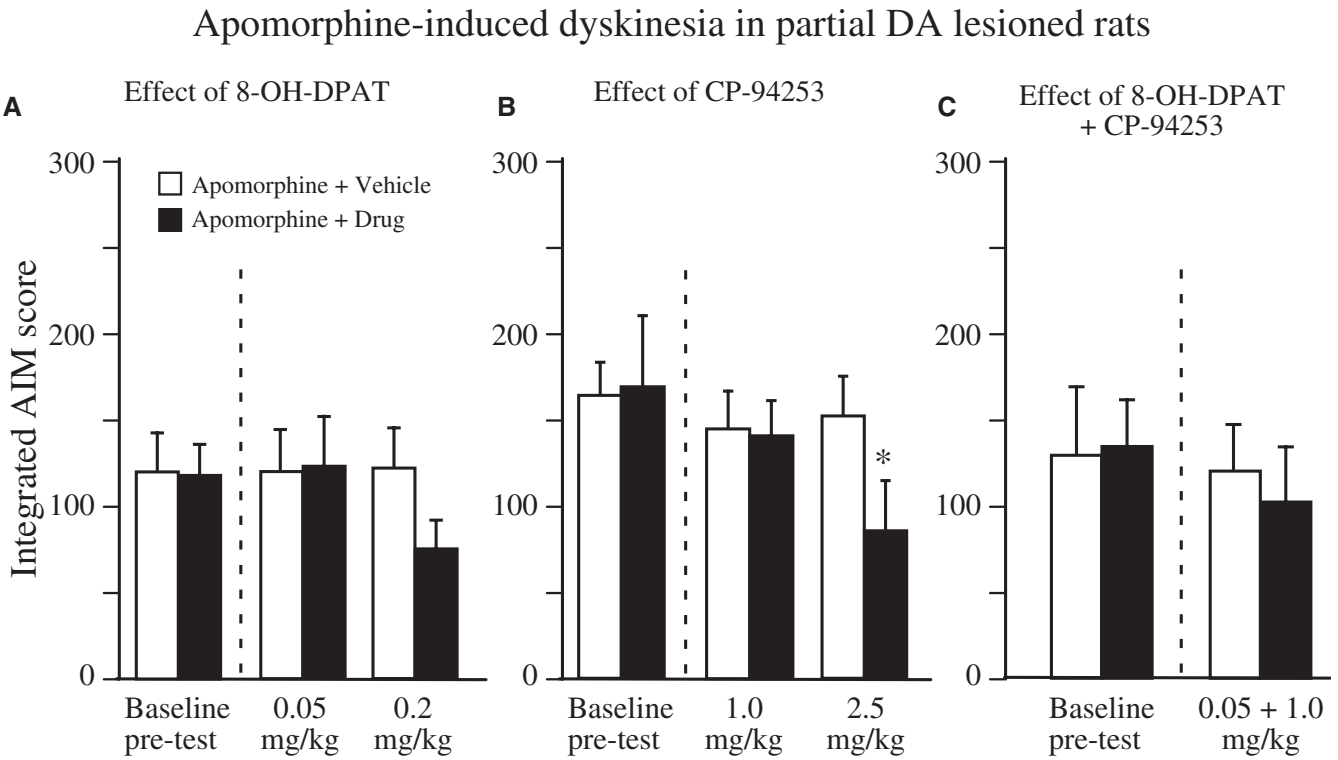

Fig. 5 Effect of 5-HT_{1A} and 5-HT_{1B} agonists, alone or in combination on apomorphine-induced dyskinesia in partial-lesioned rats. To address the contribution of post-synaptic 5-HT_{1A} and 5-HT_{1B} receptors to the anti-dyskinetic action of 8-OH-DPAT and CP-94253, the effect of the agonists on apomorphine-induced dyskinesia was also investigated. Here, a crossover design was adopted due to the bigger variability observed. Animals were balanced into two subgroups according to their AIMs score. In order to avoid possible carry-over effects, the animals were tested every second day. No differences, in any of the three experiments, were observed in repeated measures ANOVA when the two subgroups were compared (**A**–**C**). Values from the two subgroups ($n = 6$ in each) were then pooled together at each dose tested and treated as belonging to two independent groups ($n = 12$). The single drugs were effective only at the higher doses tested, but this difference was statistically significant only for CP-94253 [repeated measures ANOVA, time × group interaction for 8-OH-DPAT, $F(1,22) = 4$, $P = 0.058$; repeated measures ANOVA, time × group interaction for CP-94253, $F(1,22) = 9.97$, $P = 0.0046$; *t*-test for combination of agonists, $P = 0.55$]. Combination of the two drugs (**C**) had no effect at doses that reduced L-DOPA-induced dyskinesia by 92% (Fig. 4C). * = significant from vehicle.

benserazide; $n = 6$ in each subgroup). Sixty minutes later the animals were sacrificed, the striata rapidly removed and processed for HPLC analysis. The results are summarized in Table 2. In the 6-OHDA/sham rats, L-DOPA treatment resulted in a 48% depletion of serotonin levels on the lesioned side (2.1 ± 0.35 versus 1.1 ± 0.17 pmol/mg in vehicle and L-DOPA-treated groups, respectively $P < 0.01$). In contrast, the serotonin levels in the intact striatum were unaffected by L-DOPA treatment. Striatal DA levels in the lesioned side tended to be higher in the 6-OHDA/sham group treated with L-DOPA than in the vehicle-treated controls. However, due to the small number of animals and the variability of the 6-OHDA lesion among the animals, this difference did not reach statistical significance ($P = 0.096$).

Discussion

The results provide evidence that serotonin neurons play a central role in the induction of L-DOPA-induced dyskinesia in the rat PD model. In animals with either partial or complete lesions of the nigrostriatal DA system, dyskinesia induced by daily L-DOPA treatment was almost completely blocked when the serotonin afferents were removed. The critical role of the serotonin afferents in the induction of dyskinesia was observed not only in rats with already established dyskinesia, but also in non-dyskinetic, drug-naïve 6-OHDA-lesioned rats where the development of dyskinesia in response to repetitive, low doses of L-DOPA (6 mg/kg) was almost completely blocked when the serotonin afferents were removed. In further support, we observed that dampening of the serotonin neuron activity by 5-HT_{1A} and 5-HT_{1B} auto-receptor agonists can provide a complete blockade of L-DOPA-induced dyskinesia in L-DOPA-primed animals.

These data indicate that the dyskinetic movements induced by repetitive, low doses of L-DOPA are triggered by DA released from serotonin terminals in the DA-denervated striatum. Previous studies have shown that L-DOPA, when administered at very high doses, 50–100 mg/kg, can be decarboxylated to DA not only in dopaminergic and serotonergic neurons, but also in other

Table 2 Striatal values of DA and serotonin levels after L-DOPA injection in complete, MFB-lesioned rats

	Dopamine (pmol/mg wet tissue)		Serotonin (pmol/mg wet tissue)	
Groups	Intact side	Lesioned side	Intact side	Lesioned side
6-OHDA/5,7-DHT + vehicle ($n = 6$)	46.12 ± 3.67	0.28 ± 0.17	0.23 ± 0.05	0.11 ± 0.01*
6-OHDA/sham + vehicle ($n = 6$)	41.85 ± 4.81	0.20 ± 0.09	2.39 ± 0.07	2.11 ± 0.35
6-OHDA/5,7-DHT + L-DOPA ($n = 7$)	49.27 ± 4.57	0.38 ± 0.06	0.40 ± 0.12	0.18 ± 0.02*
L-DOPA/sham + L-DOPA ($n = 6$)	49.64 ± 2.56	0.59 ± 0.06	2.18 ± 0.13	1.10 ± 0.17*

Note: Serotonin levels were significantly different among the groups 60 min after L-DOPA injection [one-way ANOVA, $F(3,21) = 25.12$, $P < 0.0001$, followed by Tukey–Kramer HSD *post hoc* test], with a reduction of 48% in the L-DOPA/sham group compared to vehicle/sham one (mean ± SEM, 1.10 ± 0.17 versus 2.11 ± 0.35, respectively). Note that, since 5,7-DHT was injected bilaterally, the striatal serotonin levels were significantly reduced on both sides. The DA values tended to be higher in the L-DOPA treated 6-OHDA/sham animals (with an intact 5-HT system); however this difference was not statistically significant [one-way ANOVA, $F(3,21) = 2.41$, $P = 0.096$, * = significant from the 6-OHDA/sham group; $P < 0.01$]. The 6-OHDA/sham and 6-OHDA/5,7-DHT values are the same as in Table 1.

neuronal and glial elements within the striatal tissue (Melamed *et al.*, 1980; Hefti *et al.*, 1981; Mura *et al.*, 1995; Lopez-Real *et al.*, 2003). In the present study we administered L-DOPA at doses, 6–12 mg/kg, which are known to be at the threshold for induction of motor improvement in 6-OHDA-lesioned rats (Winkler *et al.*, 2002). At these dose levels, which correspond to those used clinically, storage and physiological release of L-DOPA-derived DA may take place almost exclusively from dopaminergic and serotonergic terminals. Previous studies in rats with complete 6-OHDA lesions have shown that striatal DA release after a single 50 mg/kg L-DOPA dose, as measured by microdialysis, is dependent to about 80% on the integrity of the serotonin afferents (Tanaka *et al.*, 1999), and that contraversive rotation (and striatal Fos-expression) induced by L-DOPA at a dose of 30 mg/kg (but not 100 mg/kg) are effectively blocked when the serotonin innervation is removed (Lopez *et al.*, 2001). At these higher dose levels, other sources of decarboxylation (e.g. glial cells) are likely to play a role in the conversion of L-DOPA to DA in the denervated striatum. Indeed, when the L-DOPA dose was incremented to 48 mg/kg, we observed a re-emergence of the dyskinetic behaviour in the 5,7-DHT-lesioned rats, although the magnitude of dyskinesia was lower than in controls (unpublished data). Such high doses (i.e. 50–100 mg/kg), however, are likely to far exceed those used in humans.

The presence of the aromatic amino-acid decarboxylating enzyme and the vesicular monoamine transporter-2 in serotonin neurons makes it possible for L-DOPA-derived DA to be formed, stored and released along with serotonin, thus acting as a 'false transmitter' in serotonergic terminals. The serotonin neurons, however, are unable to regulate DA release in a normal way. In dopaminergic synapses, extracellular DA concentrations are kept within a narrow physiological range through a combination of auto-receptor-mediated feedback and re-uptake via the DA transporter. Transmitter re-uptake provides an effective mechanism to eliminate excess DA from the synaptic cleft, and the D_2 auto-receptor is capable of fine-tuning release from DA terminals in response to changes in extracellular DA levels. In the absence of these auto-regulatory mechanisms, DA released from serotonin terminals is likely to generate excessive swings in extracellular DA in response to a systemic L-DOPA injection. In the model illustrated in Fig. 6, we propose that such 'dysregulated' release of L-DOPA-derived DA is the main trigger of dyskinesia in L-DOPA-primed animals, and that this is the mechanism underlying the development of dyskinesia also in non-primed, i.e. drug-naïve, animals with lesions of the nigrostriatal DA pathway.

The ability of the 5-HT_{1A} and 5-HT_{1B} receptor agonists, 8-OH-DPAT and CP-94253, to block L-DOPA-induced dyskinesia, alone or in particular when administered together at low doses, provides further support for this model. 5-HT_{1A} and 5-HT_{1B} receptors are known to be present as auto-receptors on serotonin neurons, both at the level of the cell bodies (5-HT_{1A}) and the terminals (5-HT_{1B}). Serotonin acts normally on these auto-receptors to regulate neuronal activity and serotonin release (as indicated by red arrows in Fig. 6B) (Hen, 1992; Knobelman *et al.*, 2000). Activation of the 5-HT_{1A} and 5-HT_{1B} auto-receptors thus provides a means to dampen the activity of the serotonergic neurons and, in dyskinetic animals, to reduce the release of L-DOPA-derived DA from the serotonergic terminals. Kannari *et al.* (2001) have previously shown that the increase in extracellular levels of DA seen after a single L-DOPA dose, as measured by microdialysis in 6-OHDA-lesioned rats, is significantly attenuated by pre-treatment with 8-OH-DPAT. Pre-treatment with a 5-HT_{1B} agonist (administered locally via the microdialysis probe) was in this case without effect. Here we show that the two agonists act synergistically to block L-DOPA-induced dyskinesia completely at doses that were only marginally effective when the drugs were given separately.

5-HT_{1A} receptors are known to be present also post-synaptically, on cortical neurons (Chalmers and Watson, 1991; Sari *et al.*, 1999; Riad *et al.*, 2000). Activation of these receptors by 8-OH-DPAT has been shown to inhibit both

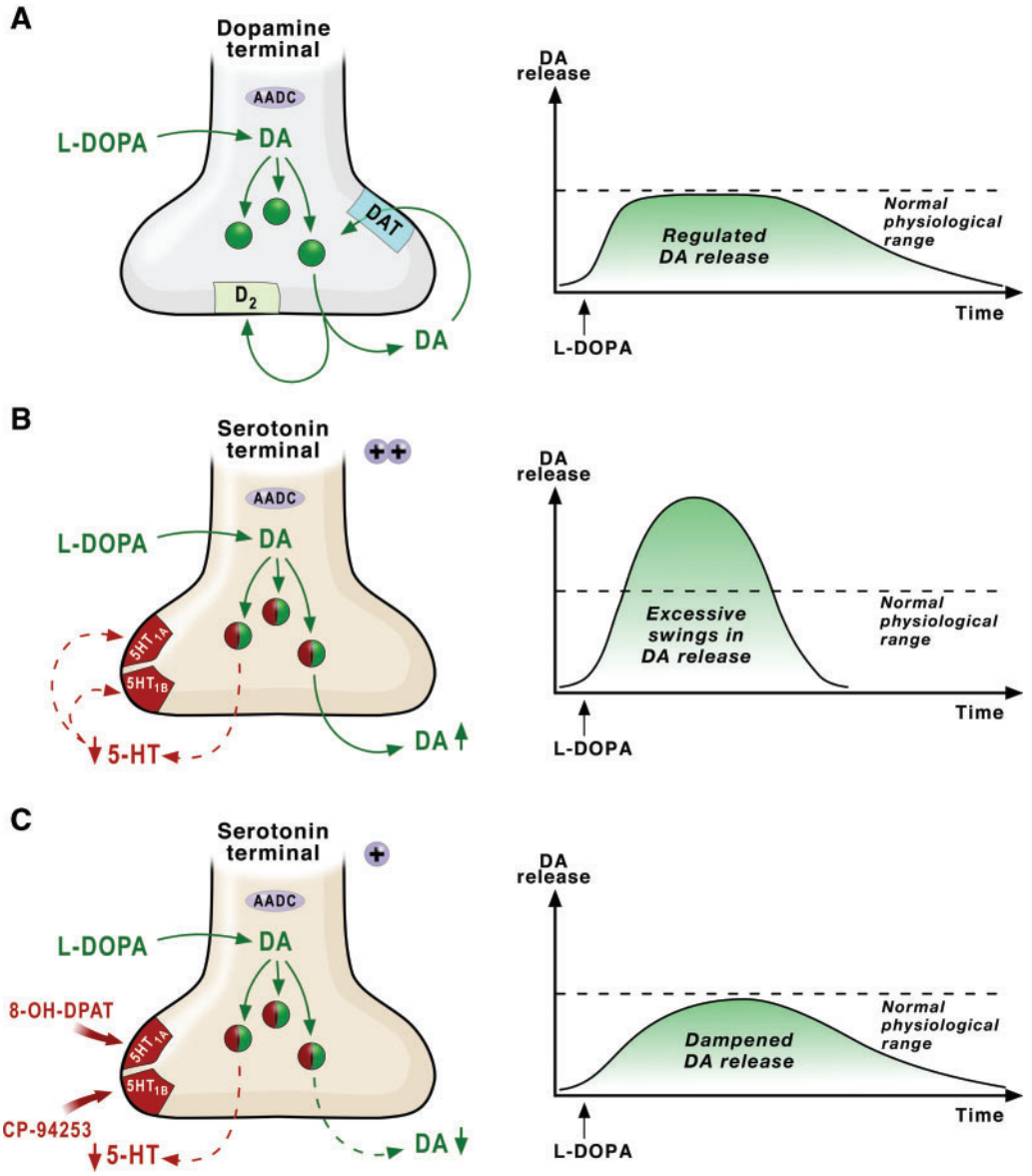

Fig. 6 Induction of L-DOPA-induced dyskinesia by dysregulated DA release from the serotonin terminals. In early stages of PD, represented in **A**, enough spared striatal DA innervation remains to mediate the therapeutic, anti-parkinsonian, effect of L-DOPA. At this stage of the disease, exogenous L-DOPA-derived DA is taken up, stored and released by striatal DA terminals. Excessive swings in extracellular DA levels are prevented by auto-regulatory feedback mediated by the DA transporter and the D_2 auto-receptors present on the dopaminergic terminals. As neurodegeneration progresses, fewer and fewer DA terminals will remain to mediate L-DOPA efficacy. At this more advanced stage, represented in **B**, the serotonin afferents will emerge as the prime site for storage and release of L-DOPA-derived DA. Due to the lack of normal auto-regulatory feedback, however, and concomitant depletion of the endogenous serotonin transmitter by DA accumulating in the storage vesicles, DA released from serotonin terminals will be poorly regulated, resulting in uncontrolled, excessive swings in DA release. The progressive loss of regulated DA release from spared DA terminals, and the gradual emergence of the serotonin afferents as the predominant source of dysregulated DA release would be therefore responsible for dyskinesia. The 5-HT_{1A} and 5-HT_{1B} receptor agonists, as represented in **C**, are proposed to act by dampening the excessive swings in L-DOPA-derived DA release from the striatal serotonergic terminals. Note that for simplicity 5-HT_{1A} receptors are positioned at the terminal level, but are indeed located at the level of the cell body and dendrites of serotonin neurons.

serotonin neuron activity (via polysynaptic feedback), as well as glutamate release from corticostriatal terminals in the striatum (Antonelli *et al.*, 2005; Mignon and Wolf, 2005). Such post-synaptic actions may thus contribute to the anti-dyskinetic effect of this drug. However, at the low doses of the combined agonists used here, dyskinesia induced by apomorphine was unaffected, which suggests that the effect on L-DOPA-induced dyskinesia was primarily due to an inhibition of DA release from the serotonin afferents (Fig. 5C).

The capacity of the serotonergic neurons to synthesize DA from L-DOPA is now well-established (Ng *et al.*, 1970, 1971; Hollister *et al.*, 1979; Arai *et al.*, 1994, 1995, 1996; Tanaka *et al.*, 1999). It has been generally assumed, however, that DA generated from L-DOPA in this way will act in consort with DA released from spared dopaminergic neurons, and thus add to the therapeutic efficacy of L-DOPA in PD patients. The present results indicate that DA released from the serotonin terminals may be detrimental rather than beneficial. In rats with partial 6-OHDA lesions, the therapeutic effect, i.e. the capacity of L-DOPA to improve forelimb use in the cylinder test, was unaffected by removal of the serotonin afferents. In these animals full recovery in forelimb use was obtained with as little as 10% of the striatal DA innervation (Table 1). In rats with complete DA lesions (striatal DA <1% of the intact side), in contrast, forelimb use remained impaired after L-DOPA, despite the presence of an intact serotonin innervation. The ability of serotonin-neuron-derived DA to improve motor function in the absence of residual DA terminals may, however, depend on the test used. In previous studies, using the so-called stepping test to monitor the rat's ability to initiate reflexive adjusting steps during passive movement, L-DOPA at the dose levels used here have been observed to induce significant albeit partial recovery also in animals with complete DA lesions (Olsson *et al.*, 1995; Chang *et al.*, 1999; Winkler *et al.*, 2002). From these results, it seems likely that serotonin-neuron-derived DA can improve some aspects of motor function in the rat PD model also in absence of any residual striatal DA innervation, but that the full anti-parkinsonian effect of L-DOPA therapy may require that some spared DA innervation is preserved.

These observations have interesting implications for the understanding of disease progression and development of dykinesia in PD patients. In the present model, the full therapeutic benefits of L-DOPA treatment will be maintained as long as there is a sufficient residual DA innervation that can provide sites for DA storage and physiologically regulated release (Fig. 6A). As DA neuron degeneration progresses, the serotonin afferents will emerge as the prime site for L-DOPA-derived DA storage and release. However, due to the lack of normal auto-regulatory feedback DA released from serotonin terminals will be poorly regulated, resulting in uncontrolled, excessive swings in DA release (Fig. 6B). In addition, in line with a previous report (Everett and Borcherding, 1970), the L-DOPA-derived DA will displace endogenous serotonin from the neuronal storage sites by about 50%, accordingly with our, HPLC data. This is likely to have additional negative consequences since serotonin released from serotonergic terminals normally exerts a negative feedback, via 5-HT_{1A} and 5-HT_{1B} auto-receptors, on neuronal impulse flow. Under this condition, the activity at the serotonin synapses is likely to be increased, which would further exacerbate the excessive swings in DA release.

The progressive loss of the therapeutic window during the course of L-DOPA treatment, and the gradual development of dyskinesia in response to previously therapeutic doses of L-DOPA (Mouradian *et al.*, 1989) is readily explained by these two parallel processes: the progressive loss of regulated DA release from spared DA terminals, and the gradual emergence of the serotonin afferents as the predominant source of dysregulated DA release. This false transmitter mechanism points to the serotonin neurons as an interesting target for anti-dyskinetic therapies (Nicholson and Brotchie, 2002; Bara-Jimenez *et al.*, 2005). The pronounced anti-dyskinetic effect of 8-OH-DPAT and CP-94253 seen here—in particular when the two agonists were administered together—indicate that drugs acting on the 5-HT_{1A} and 5-HT_{1B} auto-receptors can provide powerful pharmacological tools to dampen the activity of the serotonin neurons, and thus reduce the dyskinesia-triggering swings in extracellular DA released from serotonin terminals, as indicated in Fig. 6C. The value of this therapeutic approach, however, will depend whether or not the anti-parkinsonian effect of L-DOPA therapy will be maintained also when serotonin neuron activity is blocked. In rats with complete (>99.5%) nigrostriatal DA lesions, our results suggest that DA released from spared serotonin afferents was not sufficient to restore forelimb use in the cylinder test. Previous studies (Olsson *et al.*, 1995; Chang *et al.*, 1999; Winkler *et al.*, 2002) indicate, however, that other aspects of motor function such as single reflexive forelimb movement in the stepping test, may be at least partially ameliorated by L-DOPA also in the complete absence of DA terminals.

In MPTP-lesioned marmosets, Jackson *et al.* (2004) and Iravani *et al.* (2006) have reported that 5-HT_{1A} and 5-HT_{1B} agonists, given individually, were indeed partially effective in reducing L-DOPA-induced dyskinesias, but that this effect is accompanied by a dose-dependent worsening of motor disability. Similar observations have been made in advanced Parkinson patients with the 5-HT_{1A} agonist Sarizotan (Olanow *et al.*, 2004; Goetz *et al.*, 2006). A phase III clinical trial using this drug was recently terminated because a lack of efficacy (NCT00105521; see Merck news at website http://media.merck.de). However, Sarizotan may not be an ideal drug to target the 5-HT_{1A} auto-receptors, since it has also antagonistic properties on DA receptors that may account, at least in part, for the worsening of motor disability observed in the early trial. These reports might indicate that in primates with severe MPTP-induced DA denervation as in advanced PD patients, the anti-parkinsonian effect of L-DOPA may depend on DA released from serotonin neurons. Taken together these observations suggest that dampening of serotonin neuron activity to control or prevent dyskinesias may be most valuable in early stages of the disease when there is a residual striatal DA innervation that can maintain the therapeutic L-DOPA response. In this situation, even a complete blockade of DA release from serotonin terminals

should not have a major impact on the anti-parkinsonian efficacy of L-DOPA treatment.

In conclusion, the data provide evidence that the serotonergic system is the main inductor of L-DOPA-induced dyskinesia in the rat 6-OHDA model. Previous reports in both rats and monkeys have shown a partial reduction of dyskinesia by either 5-HT_{1A} or 5-HT_{1B} agonists. Here we show that the 5-HT_{1A} and 5-HT_{1B} agonists act synergistically to completely suppress dyskinesia at doses that were only marginally effective when administered individually. By evaluating the relative contribution of pre- and post-synaptic receptor activation, we demonstrate that the anti-dyskinetic effect of the 5-HT agonists at low doses is due to a pre-synaptic action of these drugs, i.e. dampening of the excessive swings of extracellular DA released from the serotonin neurons, pointing to the dysregulated DA release from the serotonin terminals as the unique pre-synaptic determinant of dyskinesia in the rat model. Based on these results, we propose a model of L-DOPA-induced dyskinesia, which has implications for the understanding of the development of dyskinesia in PD patients, suggesting a possible new approach for the treatment of this debilitating side effect of L-DOPA medication.

Supplementary material

Supplementary data are available at *Brain* online.

Acknowledgements

We thank Kristina Hjelm-Babeacua, Ulla Jarl, Anneli Josefsson and Bengt Mattsson for expert technical assistance. This study was supported by grants from the Michael J Fox Foundation (Community Fast Track 2005), from the Swedish Research Council (04X-3874 to AB) and from Parkinsonfonden. Manolo Carta was supported by the Parkinson's Disease Foundation (IRGP 2006).

References

Adell A, Celada P, Artigas F. The role of 5-HT1B receptors in the regulation of serotonin cell firing and release in the rat brain. J Neurochem 2001; 79: 172–82.

Agid Y, Javoy-Agid F. Biochemistry of neurotransmitters in Parkinson's disease. In: Marsden CD, Fahn Se, editors. Movement disorders 2, Vol 2. London: Butterworths; 1987. p. 166–230.

Antonelli T, Fuxe K, Tomasini MC, Bartoszyk GD, Seyfried CA, Tanganelli S, et al. Effects of sarizotan on the corticostriatal glutamate pathways. Synapse 2005; 58: 193–9.

Arai R, Karasawa N, Geffard M, Nagatsu I. L-DOPA is converted to dopamine in serotonergic fibers of the striatum of the rat: a double-labeling immunofluorescence study. Neurosci Lett 1995; 195: 195–8.

Arai R, Karasawa N, Geffard M, Nagatsu T, Nagatsu I. Immunohistochemical evidence that central serotonin neurons produce dopamine from exogenous L-DOPA in the rat, with reference to the involvement of aromatic L-amino acid decarboxylase. Brain Res 1994; 667: 295–9.

Arai R, Karasawa N, Nagatsu I. Aromatic L-amino acid decarboxylase is present in serotonergic fibers of the striatum of the rat. A double-labeling immunofluorescence study. Brain Res 1996; 706: 177–9.

Bara-Jimenez W, Bibbiani F, Morris MJ, Dimitrova T, Sherzai A, Mouradian MM, et al. Effects of serotonin 5-HT1A agonist in advanced Parkinson's disease. Mov Disord 2005; 20: 932–6.

Bjorklund A, Baumgarten HG, Rensch A. 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. J Neurochem 1975; 24: 833–5.

Blanchet PJ, Konitsiotis S, Chase TN. Amantadine reduces levodopa-induced dyskinesias in parkinsonian monkeys. Mov Disord 1998; 13: 798–802.

Carey RJ, DePalma G, Damianopoulos E, Hopkins A, Shanahan A, Muller CP, et al. Dopaminergic and serotonergic autoreceptor stimulation effects are equivalent and additive in the suppression of spontaneous and cocaine induced locomotor activity. Brain Res 2004*a*; 1019: 134–43.

Carey RJ, Depalma G, Damianopoulos E, Muller CP, Huston JP. The 5-HT1A receptor and behavioral stimulation in the rat: effects of 8-OHDPAT on spontaneous and cocaine-induced behavior. Psychopharmacology (Berl) 2004*b*; 177: 46–54.

Carey RJ, DePalma G, Damianopoulos E, Shanahan A, Muller CP, Huston JP. Evidence that the 5-HT1A autoreceptor is an important pharmacological target for the modulation of cocaine behavioral stimulant effects. Brain Res 2005; 1034: 162–71.

Carta M, Fadda F, Stancampiano R. Tryptophan-deficient diet increases the neurochemical and behavioral response to amphetamine. Brain Res 2006; 1094: 86–91.

Casanovas JM, Hervas I, Artigas F. Postsynaptic 5-HT1A receptors control 5-HT release in the rat medial prefrontal cortex. Neuroreport 1999; 10: 1441–5.

Ceci A, Baschirotto A, Borsini F. The inhibitory effect of 8-OH-DPAT on the firing activity of dorsal raphe serotoninergic neurons in rats is attenuated by lesion of the frontal cortex. Neuropharmacology 1994; 33: 709–13.

Chalmers DT, Watson SJ. Comparative anatomical distribution of 5-HT1A receptor mRNA and 5-HT1A binding in rat brain–a combined in situ hybridisation/in vitro receptor autoradiographic study. Brain Res 1991; 561: 51–60.

Chang JW, Wachtel SR, Young D, Kang UJ. Biochemical and anatomical characterization of forepaw adjusting steps in rat models of Parkinson's disease: studies on medial forebrain bundle and striatal lesions. Neuroscience 1999; 88: 617–28.

de la Fuente-Fernandez R, Schulzer M, Mak E, Calne DB, Stoessl AJ. Presynaptic mechanisms of motor fluctuations in Parkinson's disease: a probabilistic model. Brain 2004; 127: 888–99.

Everett GM, Borcherding JW. L-DOPA: effect on concentrations of dopamine, norepinephrine, and serotonin in brains of mice. Science 1970; 168: 847–50.

Goetz CG, Damier P, Hicking C, Laska E, Muller T, Olanow CW, et al. Sarizotan as a treatment for dyskinesias in Parkinson's disease: a double-blind placebo-controlled trial. Mov Disord 2006; 22: 179–86.

Goodwin GM, De Souza RJ, Green AR, Heal DJ. The pharmacology of the behavioural and hypothermic responses of rats to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). Psychopharmacology (Berl) 1987; 91: 506–11.

Goodwin GM, De Souza RJ, Wood AJ, Green AR. The enhancement by lithium of the 5-HT1A mediated serotonin syndrome produced by 8-OH-DPAT in the rat: evidence for a post-synaptic mechanism. Psychopharmacology (Berl) 1986; 90: 488–93.

Hajos M, Hajos-Korcsok E, Sharp T. Role of the medial prefrontal cortex in 5-HT1A receptor-induced inhibition of 5-HT neuronal activity in the rat. Br J Pharmacol 1999; 126: 1741–50.

Hefti F, Melamed E, Wurtman RJ. The site of dopamine formation in rat striatum after L-dopa administration. J Pharmacol Exp Ther 1981; 217: 189–97.

Hen R. Of mice and flies: commonalities among 5-HT receptors. Trends Pharmacol Sci 1992; 13: 160–5.

Hollister AS, Breese GR, Mueller RA. Role of monoamine neural systems in L-dihydroxyphenylalanine-stimulated activity. J Pharmacol Exp Ther 1979; 208: 37–43.

Hornykiewicz O. Biochemical aspects of Parkinson's disease. Neurology 1998; 51: S2–9.

Iravani MM, Tayarani-Binazir K, Chu WB, Jackson MJ, Jenner P. In MPTP treated primates, the selective 5-HT1a agonist (R)-(+)-8-hydroxy-DPAT inhibits levodopa-induced dyskinesia but only with increased motor disability. J Pharmacol Exp Ther 2006; 319: 1225–34.

Jackson MJ, Al-Barghouthy G, Pearce RK, Smith L, Hagan JJ, Jenner P. Effect of 5-HT1B/D receptor agonist and antagonist administration on motor function in haloperidol and MPTP-treated common marmosets. Pharmacol Biochem Behav 2004; 79: 391–400.

Kannari K, Yamato H, Shen H, Tomiyama M, Suda T, Matsunaga M. Activation of 5-HT(1A) but not 5-HT(1B) receptors attenuates an increase in extracellular dopamine derived from exogenously administered L-DOPA in the striatum with nigrostriatal denervation. J Neurochem 2001; 76: 1346–53.

Kirik D, Rosenblad C, Bjorklund A, Mandel RJ. Long-term rAAV-mediated gene transfer of GDNF in the rat Parkinson's model: intrastriatal but not intranigral transduction promotes functional regeneration in the lesioned nigrostriatal system. J Neurosci 2000; 20: 4686–700.

Knobelman DA, Kung HF, Lucki I. Regulation of extracellular concentrations of 5-hydroxytryptamine (5-HT) in mouse striatum by 5-HT(1A) and 5-HT(1B) receptors. J Pharmacol Exp Ther 2000; 292: 1111–7.

Konitsiotis S, Blanchet PJ, Verhagen L, Lamers E, Chase TN. AMPA receptor blockade improves levodopa-induced dyskinesia in MPTP monkeys. Neurology 2000; 54: 1589–95.

Lavoie B, Parent A. Immunohistochemical study of the serotoninergic innervation of the basal ganglia in the squirrel monkey. J Comp Neurol 1990; 299: 1–16.

Lee CS, Cenci MA, Schulzer M, Bjorklund A. Embryonic ventral mesencephalic grafts improve levodopa-induced dyskinesia in a rat model of Parkinson's disease. Brain 2000; 123 (Pt 7): 1365–79.

Lopez A, Munoz A, Guerra MJ, Labandeira-Garcia JL. Mechanisms of the effects of exogenous levodopa on the dopamine-denervated striatum. Neuroscience 2001; 103: 639–51.

Lopez-Real A, Rodriguez-Pallares J, Guerra MJ, Labandeira-Garcia JL. Localization and functional significance of striatal neurons immunoreactive to aromatic L-amino acid decarboxylase or tyrosine hydroxylase in rat Parkinsonian models. Brain Res 2003; 969: 135–46.

Lundblad M, Andersson M, Winkler C, Kirik D, Wierup N, Cenci MA. Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. Eur J Neurosci 2002; 15: 120–32.

Maeda T, Nagata K, Yoshida Y, Kannari K. Serotonergic hyperinnervation into the dopaminergic denervated striatum compensates for dopamine conversion from exogenously administered l-DOPA. Brain Res 2005; 1046: 230–3.

Melamed E, Hefti F, Wurtman RJ. Nonaminergic striatal neurons convert exogenous L-dopa to dopamine in parkinsonism. Ann Neurol 1980; 8: 558–63.

Mignon LJ, Wolf WA. 8-hydroxy-2-(di-n-propylamino)tetralin reduces striatal glutamate in an animal model of Parkinson's disease. Neuroreport 2005; 16: 699–703.

Miller DW, Abercrombie ED. Role of high-affinity dopamine uptake and impulse activity in the appearance of extracellular dopamine in striatum after administration of exogenous L-DOPA: studies in intact and 6-hydroxydopamine-treated rats. J Neurochem 1999; 72: 1516–22.

Mouradian MM, Heuser IJ, Baronti F, Fabbrini G, Juncos JL, Chase TN. Pathogenesis of dyskinesias in Parkinson's disease. Ann Neurol 1989; 25: 523–6.

Mura A, Jackson D, Manley MS, Young SJ, Groves PM. Aromatic L-amino acid decarboxylase immunoreactive cells in the rat striatum: a possible site for the conversion of exogenous L-DOPA to dopamine. Brain Res 1995; 704: 51–60.

Ng KY, Chase TN, Colburn RW, Kopin IJ. L-Dopa-induced release of cerebral monoamines. Science 1970; 170: 76–7.

Ng KY, Colburn RW, Kopin IJ. Effects of L-dopa on efflux of cerebral monoamines from synaptosomes. Nature 1971; 230: 331–2.

Nicholson SL, Brotchie JM. 5-hydroxytryptamine (5-HT, serotonin) and Parkinson's disease - opportunities for novel therapeutics to reduce the problems of levodopa therapy. Eur J Neurol 2002; 9 (Suppl 3): 1–6.

Olanow CW, Damier P, Goetz CG, Mueller T, Nutt J, Rascol O, et al. Multicenter, open-label, trial of sarizotan in Parkinson disease patients with levodopa-induced dyskinesias (the SPLENDID Study). Clin Neuropharmacol 2004; 27: 58–62.

Olsson M, Nikkhah G, Bentlage C, Bjorklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J Neurosci 1995; 15: 3863–75.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1998.

Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X, et al. Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. J Comp Neurol 2000; 417: 181–94.

Sari Y, Miquel MC, Brisorgueil MJ, Ruiz G, Doucet E, Hamon M, et al. Cellular and subcellular localization of 5-hydroxytryptamine1B receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. Neuroscience 1999; 88: 899–915.

Schallert T, Tillerson J. Intervention strategies for degeneration of dopamine neurons in parkinsonism: optimising behavioral assessment of outcome. Innovative models of CNS disease: from molecule to therapy. Clifton, NJ: Humana Press; 1999. p. 131–51.

Sprouse JS, Aghajanian GK. Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse 1987; 1: 3–9.

Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M. Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. Neuroreport 1999; 10: 631–4.

Winkler C, Kirik D, Bjorklund A, Cenci MA. L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis 2002; 10: 165–86.

Yamada J, Sugimoto Y, Horisaka K. The behavioural effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in mice. Eur J Pharmacol 1988; 154: 299–304.