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

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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-HT1A and 5-HT1B 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-HT1A and 5-HT1B 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-HT1A/1B agonists

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


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 ≥6 full body turns/min towards the side of DA deficiency and ≤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 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. 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 selective 5-HT1A agonist, 8-OH-DPAT ((±)-8-hydroxy-2-dipropylaminotetralin hydrobromide; TOCRIS, Sweden), and the 5-HT1B 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% isoflurane 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...
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 through minispin filters for additional 3 min at 10,000 rpm. The tissue extract were then analysed by HPLC measurements.
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, Na₃-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 3-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-HT₁₅ and 5-HT₁₆ 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).

**L-DOPA and apomorphine-induced dyskinesia**

In order to induce stable AIs, 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 AIs 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 AIs 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-HT₁₅ and 5-HT₁₆, 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.
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 x 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 x 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. 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.
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

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Fig. 3 Effect of 5-HT1A and 5-HT1B 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-HT1A 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 81 ± 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-HT1A 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-HT1B 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-HT1B 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.
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 Tiller, 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, nondyskinetic animals with an intact serotonin system (hatched bars in Fig. 2B and D).

Effect of 5-HT1A and 5-HT1B 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-HT1A and 5-HT1B 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-HT1A) and terminals (5-HT1B) 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-HT1A) and SB-224289 (5-HT1B) 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

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<tr>
<td>6-OHDA/sham (partial lesion) n = 8</td>
<td>6-OHDA/sham (partial lesion) n = 8</td>
<td>44.13 ± 1.54</td>
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<td>6-OHDA/5,7-DHT (partial lesion) n = 8</td>
<td>44.11 ± 2.24</td>
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<td>0.23 ± 0.05</td>
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<td>6-OHDA/sham (complete lesion) n = 6</td>
<td>6-OHDA/sham (complete lesion) n = 6</td>
<td>41.85 ± 4.81</td>
<td>0.20 ± 0.09 (0.47%)</td>
<td>2.39 ± 0.07</td>
<td>2.11 ± 0.35</td>
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<td>6-OHDA/5,7-DHT (complete lesion) n = 6</td>
<td>6-OHDA/5,7-DHT (complete lesion) n = 6</td>
<td>46.12 ± 3.67</td>
<td>0.28 ± 0.17 (0.60%)</td>
<td>0.23 ± 0.05</td>
<td>0.11 ± 0.01 (5.21%)</td>
</tr>
<tr>
<td>6-OHDA only (partial lesion) n = 15</td>
<td>6-OHDA only (partial lesion) n = 15</td>
<td>39.37 ± 2.04</td>
<td>3.40 ± 0.60 (8.6%)</td>
<td>2.13 ± 0.09</td>
<td>2.61 ± 0.09</td>
</tr>
</tbody>
</table>

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).
**Fig. 4** Effect of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> 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<sub>1A</sub> and 5-HT<sub>1B</sub> 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 $F(11,2) = 8.57, P = 0.013$, 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 $F(11,4) = 0.43, P = 0.52, n = 8$ 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.
Effect of the 5-HT₁A and 5-HT₁B receptor agonists on apomorphine-induced dyskinesia

As mentioned earlier, 5-HT₁A and 5-HT₁B receptors are expressed also as post-synaptic receptors on both cortical and striatal target neurons. Previous studies have shown that post-synaptic 5-HT₁A 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-HT₁A 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 antidynergic effect of the serotonin agonists could be mediated, at least in part, by an action on the corticostrital 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 D₁/D₂ 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 corticostrital neurons resulting in a decreased glutamatergic input to the striatum. The ability of the 5-HT₁A and 5-HT₁B 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-HT₁A 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.
benzerazide; \( n = 6 \) 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-naive 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-HT1A and 5-HT1B 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

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**Fig. 5** Effect of 5-HT1A and 5-HT1B agonists, alone or in combination on apomorphine-induced dyskinesia in partial-lesioned rats.

To address the contribution of post-synaptic 5-HT1A and 5-HT1B 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 \( \times \) group interaction for 8-OH-DPAT, \( F(1,12) = 4, P = 0.058 \); repeated measures ANOVA, time \( \times \) group interaction for CP-94253, \( F(1,12) = 9.97, P = 0.0046 \); 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.
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 increased 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 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 D2 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-naive, animals with lesions of the nigrostriatal DA pathway.

The ability of the 5-HT1A and 5-HT1B 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-HT1A and 5-HT1B receptors are known to be present as auto-receptors on serotonin neurons, both at the level of the cell bodies (5-HT1A) and the terminals (5-HT1B). Serotonin acts normally on these auto-receptors to regulate neuronal activity and serotonin release (as indicated by red arrows in Fig. 6B) (Hen, 1992; Knobelmann et al., 2000). Activation of the 5-HT1A and 5-HT1B 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-HT1B 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-HT1A receptors are known to be present also postsynaptically, 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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dopamine (pmol/mg wet tissue)</th>
<th>Serotonin (pmol/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact side</td>
<td>Lesioned side</td>
</tr>
<tr>
<td>6-OHDA/5,7-DHT + vehicle (n = 6)</td>
<td>46.12 ± 3.67</td>
<td>0.28 ± 0.17</td>
</tr>
<tr>
<td>6-OHDA/sham + vehicle (n = 6)</td>
<td>41.85 ± 4.81</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>6-OHDA/5,7-DHT + L-DOPA (n = 7)</td>
<td>49.27 ± 4.57</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>L-DOPA/sham + L-DOPA (n = 6)</td>
<td>49.64 ± 2.56</td>
<td>0.59 ± 0.06</td>
</tr>
</tbody>
</table>

*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.

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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).

**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<sub>2</sub> 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<sub>1A</sub> and 5-HT<sub>1B</sub> 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<sub>1A</sub> receptors are positioned at the terminal level, but are indeed located at the level of the cell body and dendrites of serotonin neurons.
The serotonin system in l-DOPA-induced dyskinesia

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 dyskinesia 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 (Evrard 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 of 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 inducer 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-HT1A or 5-HT1B agonists. Here we show that the 5-HT1A and 5-HT1B 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.

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