

Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias

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Summary

Peak-dose dyskinesias are abnormal movements that usually occur 1 h after oral administration of levodopa, and often complicate chronic treatment of Parkinson's disease. We investigated by PET with [¹¹C]raclopride whether Parkinson's disease progression modifies the striatal changes in synaptic dopamine levels induced by levodopa administration, and whether this modification, if present, could have an impact on the emergence of dyskinesias. We found that, 1 h after oral administration of standard-release 250/25 mg of levodopa/carbidopa, levodopa-induced increases in synaptic dopamine levels (as estimated by striatal changes in [¹¹C]raclopride binding potential) correlated positively with duration of Parkinson's disease symptoms (for the caudate nucleus, $r = 0.79$, $P < 0.001$; for the putamen, $r = 0.88$, $P < 0.0001$). Patients with peak-dose dyskinesias had larger 1-h

increases in synaptic dopamine levels than stable responders, but there were no between-group differences in [¹¹C]raclopride binding 4 h post-levodopa. The corresponding (time \times group) interaction term in the repeated measures analysis of covariance was significant, even after adjusting for between-group differences in duration of Parkinson's disease symptoms (for the caudate nucleus, $P = 0.030$; for the putamen, $P = 0.021$). Our results indicate that, at the synaptic level, an identical dose of levodopa induces increasingly larger 1-h changes in dopamine levels as Parkinson's disease progresses. Large levodopa-induced increases in synaptic dopamine concentration can lead to dramatic changes in receptor occupancy, which may be responsible for the emergence of peak-dose dyskinesias in Parkinson's disease.

Keywords: dopamine release; dyskinesias; Parkinson's disease; PET; raclopride

Abbreviations: BP = binding potential; Caud = caudate nucleus; MCS = modified Columbia Scale; P1, P2 and P3 = rostral to caudal putamen; RAC = [¹¹C]raclopride; ROI = region of interest

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Introduction

Motor fluctuations and dyskinesias often complicate chronic levodopa treatment in Parkinson's disease (Marsden *et al.*, 1982; Kumar *et al.*, 2003). The mechanisms contributing to the emergence of these motor complications are not yet fully understood. Recent evidence suggests that increased dopamine turnover plays a major role in the pathogenesis of motor fluctuations (de la Fuente-Fernández *et al.*, 2001b). Little is known about the pathogenesis of dyskinesias (Kumar *et al.*, 2003). The observation that dyskinesias occur not only with levodopa

therapy, but also while on treatment with direct dopamine agonists, points to post-synaptic mechanisms. However, PET studies have failed to detect abnormalities in dopamine receptors (Kishore *et al.*, 1997; Turjanski *et al.*, 1997). In contrast, several studies have confirmed downstream (post-receptor) changes (Chase *et al.*, 1993). Whether these alterations are causative of dyskinesias remains speculative.

We suggested that levodopa-related peak-dose dyskinesias might reflect, at least in part, relative increases in the levels of

dopamine in the synapse (de la Fuente-Fernández *et al.*, 2001b). In other words, dyskinesias would be related to dramatic elevations in synaptic dopamine levels (and, consequently, dramatic changes in the degree of receptor stimulation) induced by levodopa administration. This notion is supported by the observation that peak-dose dyskinesias are dose sensitive (i.e. a reduction in the dose of levodopa leads to a decrease in the severity of these abnormal movements). Based on our previous PET findings (de la Fuente-Fernández *et al.*, 2001b) and our recently developed mathematical model (de la Fuente-Fernández *et al.*, 2004), we hypothesize here that dopamine release (and dopamine turnover) may augment with Parkinson's disease progression as a means to compensate for increasing synaptic dopamine deficiency. Consequently, the transient elevations in synaptic dopamine levels induced by each levodopa dose would increase with duration of Parkinson's disease. Our model may explain the association between dyskinesias and motor fluctuations. Elevated dopamine turnover with Parkinson's disease progression would lead to increasingly greater swings in synaptic dopamine levels after levodopa administration; in addition, the duration of effect of each dose of levodopa would shorten as the re-uptake capacity decreased with Parkinson's disease severity (and intrasynaptic dopamine is subject to enzymatic degradation). Our mathematical (probabilistic) model has recently received strong support from the observation that synaptic vesicles are mostly released in a random manner (Rizzoli and Betz, 2004).

To test the hypothesis that dyskinesias may be related to dramatic changes in synaptic dopamine levels, we used PET with [¹¹C]raclopride (RAC) as described elsewhere (Tedroff *et al.*, 1996; de la Fuente-Fernández *et al.*, 2001b). This method allowed us to examine dynamic changes in the synaptic concentration of dopamine after oral administration of a single dose of levodopa. It should be emphasized that the model does not imply that peak-dose dyskinesias are associated with excessive (i.e. higher than normal) synaptic dopamine levels (de la Fuente-Fernández *et al.*, 2004). Indeed, dyskinesics often have residual (ON) parkinsonism (R. de la Fuente-Fernández *et al.*, unpublished observation), which suggests that the total concentration of synaptic dopamine (i.e. endogenous dopamine plus dopamine derived from exogenous levodopa) is below normal values. Nor does the model imply that the total levels of synaptic dopamine are larger in dyskinesics than in non-dyskinesics. Only the changes induced by levodopa administration may be greater in the dyskinesic group.

Material and methods

Subjects

We studied 16 patients with clinical criteria for definite Parkinson's disease (Calne *et al.*, 1992). All patients, recruited from our Movement Disorder Clinic, were on chronic levodopa treatment. All volunteered for the study, and there was no attempt to select patients based on age, in order to avoid potential confounding effects (Golbe,

Table 1 Clinical characteristics of Parkinson's disease patients

	Stable responders (n = 8)	Dyskinesics (n = 8)
Male/female	5/3	5/3
Age (years)	64.38 ± 11.21	66.63 ± 10.21
Duration of Parkinson's disease (years)	5.50 ± 2.63	10.38 ± 4.69*
MCS-OFF	19.13 ± 6.94	37.63 ± 12.60**
Equivalent levodopa [†] dose (mg/day)	451.25 ± 162.61	715.63 ± 248.90*

* $P < 0.05$; ** $P < 0.01$. [†]Dopaminomimetic treatment is given in equivalents of standard-release levodopa/carbidopa. MCS-OFF = MCS score during the off state.

1991). There were eight patients with peak-dose dyskinesias (five of whom also had motor fluctuations) and eight stable responders (i.e. patients without motor fluctuations or dyskinesias). Patients were clinically classified as dyskinesics or stable responders by experts in movement disorders (D.B.C., A.J.S.). As our PET method did not allow us to study the dynamics of biphasic dyskinesias, we focused on peak-dose dyskinesias. Clinical characteristics of patients are summarized in Table 1. Quantitative measurements based on the modified Columbia Scale (MSC) (Duvoisin, 1971) and RAC PET scans were performed at baseline ('off' state; 12–18 h after withdrawal of medications). As argued elsewhere (de la Fuente-Fernández *et al.*, 2001c), scoring of motor function was not performed during the scans, in order to avoid the confounding effect of movement in our PET measurements. In addition, as dyskinesias seem to behave mostly in an 'all-or-none' fashion (Verhagen Metman *et al.*, 1997), we did not predict any correlation between severity of dyskinesias and PET changes, and consequently no attempt was made to quantify the movements. All subjects gave written informed consent. The study was approved by the UBC Ethics Committee.

PET protocol

The protocol has been described in detail elsewhere (de la Fuente-Fernández *et al.*, 2001b). Briefly, all patients underwent three RAC PET scans on the same day: at baseline, 1 h after oral administration of 250/25 mg of standard-release levodopa/carbidopa and 4 h after levodopa. All PET scans were performed in three-dimensional mode using an ECAT 953B/31 tomograph (Siemens Canada, CTI, Knoxville, TN, USA). After a transmission scan performed with an external ⁶⁸Ge source, 16 sequential emission scans were obtained over 60 min, starting at the time of injection of 5 mCi of [¹¹C]raclopride (specific activity >1000 Ci/mmol at ligand injection). From the emission data (30–60 min) we obtained an integrated image with 31 planes (each 3.37-mm thick) for each subject. One circular region of interest (ROI) of 61.2 mm² was placed on the head of each caudate nucleus (Caud), and three ROIs of the same size were positioned to cover each putamen (from rostral to caudal putamen: P1, P2 and P3); all ROIs were adjusted to maximize the average ROI activity. The background activity was obtained from a single elliptical ROI (2107 mm²) on the cerebellum. The binding potential (BP = B_{max}/K_d) was determined using a graphical approach and a tissue input function (Logan *et al.*, 1996).

Statistical analysis

The relationship between levodopa-induced RAC BP changes and duration of Parkinson's disease symptoms was studied by regression analysis (Altman, 1991). Repeated measures analyses of variance (ANOVA) and covariance (ANCOVA) were used to compare changes in RAC BP between dyskinesics and stable responders (adjusting for differences in baseline RAC BP values and Parkinson's disease duration, as appropriate) (Altman, 1991). Using ANOVA and ANCOVA, we analysed: (i) changes occurring over time (i.e. RAC BP values obtained 1 h and 4 h after levodopa administration) for each major striatal structure (i.e. caudate and putamen); and (ii) topographical changes (i.e. RAC BP changes occurring in the caudate nucleus and subregions of the putamen) at 1 h and at 4 h after levodopa. Since the activity in the most posterolateral region of the putamen (P3) is often confounded by partial volume effects (Kessler *et al.*, 1984; de la Fuente-Fernández *et al.*, 2003), only Caud, P1 and P2 were considered in comparing topographical changes between dyskinesics and stable responders. Data are given as mean \pm standard deviation unless otherwise stated.

Results

Clinical results

There was a positive correlation between the severity of parkinsonism (MCS score) and the daily equivalent levodopa dose ($r = 0.53$, $P = 0.035$), which was still present when the patient with 21 years' duration of Parkinson's disease symptoms (see below) was excluded from the analysis ($r = 0.53$, $P = 0.043$). Also, MCS scores correlated positively with levodopa-induced changes in RAC BP at 1 h (for the caudate nucleus, $P = 0.039$; for the putamen, $P = 0.069$), but not with 4-h RAC BP changes (for the caudate nucleus, $P = 0.91$; for the putamen, $P = 0.62$). Compared with stable responders, dyskinesics had longer symptom duration, more severe parkinsonism (MCS score) and were receiving higher doses of dopaminomimetic treatment (Table 1). There were no between-group differences in age at onset of Parkinson's

disease symptoms (dyskinetics, 56.25 ± 8.92 years; stable responders, 58.88 ± 12.15 years; $P = 0.63$).

PET results

RAC BP was reduced by a mean of 11% in the caudate nucleus and 13% in the putamen 1 h following levodopa administration (Table 2, Fig. 1). Levodopa-induced changes in RAC BP at 1 h correlated positively with duration of Parkinson's disease symptoms in both the caudate nucleus ($r = 0.79$, $P < 0.001$) and the putamen ($r = 0.88$, $P < 0.0001$) (Fig. 2). Multiple regression analyses of 1-h BP values on both Parkinson's disease duration and baseline BP (to adjust for baseline BP differences) gave virtually identical levels of significance. The inclusion of age as a covariate in the multiple regression analysis did not alter the results (for the caudate nucleus, $P < 0.001$; for the putamen, $P < 0.0001$). Similarly, correlation results were still significant when the patient with the longest duration of symptoms (21 years) was removed from the analysis (for the caudate nucleus, $P = 0.039$; for the putamen, $P = 0.0045$) (Fig. 2). Indeed, the latter

Table 2 Striatal RAC BP

	Stable responders ($n = 8$)	Dyskinetics ($n = 8$)
Caudate		
Baseline	2.08 ± 0.32	2.01 ± 0.26
1 h after levodopa*	1.94 ± 0.33	1.71 ± 0.37
4 h after levodopa*	1.89 ± 0.33	1.85 ± 0.17
Putamen		
Baseline	2.39 ± 0.40	2.32 ± 0.38
1 h after levodopa*	2.18 ± 0.42	1.92 ± 0.43
4 h after levodopa*	2.20 ± 0.37	2.14 ± 0.23

*250/25 mg of standard-release levodopa/carbidopa.

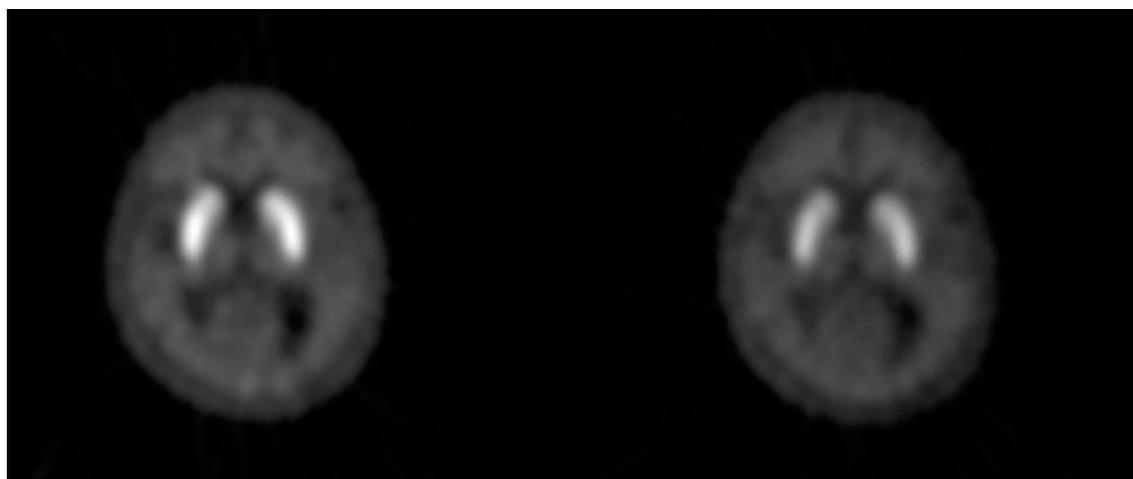


Fig. 1 RAC PET scans from a dyskinetic Parkinson's disease subject taken at baseline (left panel) and 1 h following oral administration of levodopa/carbidopa 250/25 mg (right panel). Scans represent the activity summed over 30–60 min following tracer administration, over the five axial slices in which the striatum was best visualized, and are scaled to the same maximum.

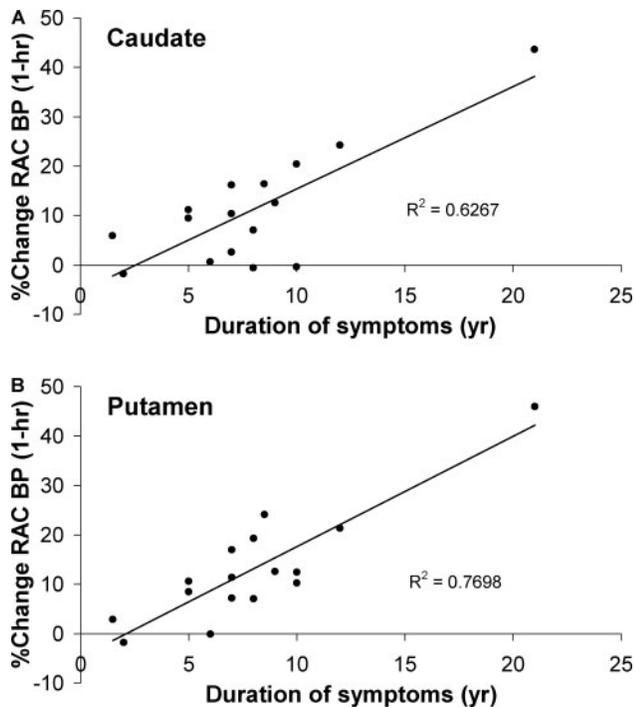


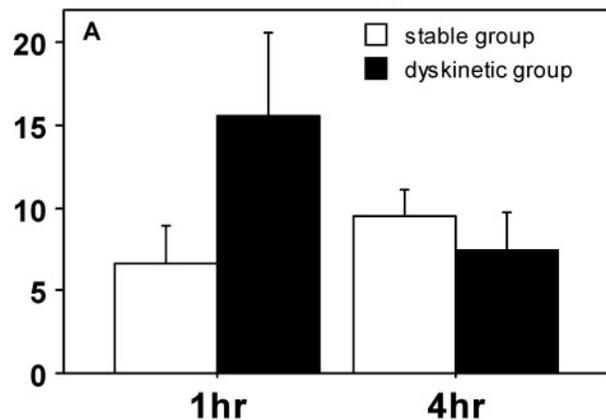
Fig. 2 Regression of percentage change in RAC BP (1 h after levodopa administration) on duration of Parkinson's disease symptoms (in years). This change in RAC BP gives an estimate of the amount of dopamine released into the synapse after levodopa administration (de la Fuente-Fernández *et al.*, 2001b). Positive correlations were found for both (A) the caudate nucleus ($r = 0.79$, $P < 0.001$), and (B) the putamen ($r = 0.88$, $P < 0.0001$). Significant correlations were still present when the patient with the longest duration of symptoms (21 years) was excluded from the analysis (e.g. for putamen: $r = 0.69$, $P = 0.0044$).

analysis gave an equation that predicted a 36% change in putamen RAC BP at 21 years of duration of Parkinson's disease symptoms (close to the 46% change observed in that patient). In contrast to the 1-h results, there was no correlation between levodopa-induced RAC BP changes 4 h after levodopa administration and Parkinson's disease duration for either the caudate nucleus ($P = 0.30$) or the putamen ($P = 0.98$). While the 1-h levodopa-induced RAC BP changes in caudate were highly correlated with those in putamen ($r = 0.89$, $P < 0.0001$), this correlation decreased 4 h after levodopa administration ($r = 0.47$, $P = 0.067$).

Although all dyskinesics and one stable responder showed peak-dose dyskinesias after levodopa administration, only two patients (both classified as dyskinesics) presented with mild dyskinesias while lying on the scan table for the 1-h PET scan. At the 4-h scan, none had abnormal movements. Striatal RAC BP values for stable responders and dyskinesics are summarized in Table 2. At 1 h after levodopa administration, the RAC BP had decreased by 16% in the caudate nucleus and by 17% in the putamen in the dyskinetic group, compared with 7% and 9%, respectively, in the stable group (Fig. 3). In contrast, similar decreases in RAC BP were observed for the two groups at the 4-h scan: dyskinesics, 7% in the caudate

Caudate: Change in RAC BP (%)

Error Bars: ± 1 Standard Error(s)



Putamen: Change in RAC BP (%)

Error Bars: ± 1 Standard Error(s)

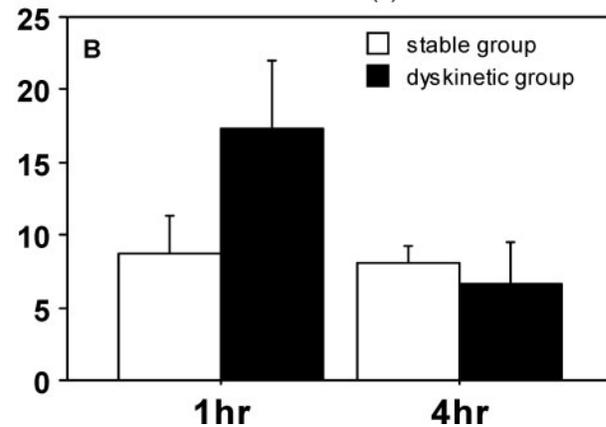


Fig. 3 Dynamic changes in RAC BP for (A) the caudate nucleus and (B) the putamen. Again, changes in RAC BP represent estimates of levodopa-induced changes in synaptic dopamine levels. The estimated increase in the synaptic levels of dopamine 1 h after levodopa administration was two times higher in patients with peak-dose dyskinesias (which usually occur 1 h after levodopa) ($n = 8$) than in stable responders ($n = 8$). At 4 h, there were no significant between-group differences. Therefore, dyskinesics and stable responders had a different pattern of response to levodopa, as reflected by an interaction term (time \times group) in the repeated measures ANCOVA analysis (see text).

nucleus and 7% in the putamen; stable responders, 9% and 8%, respectively (Fig. 3). The 'stable' patient who presented with dyskinesias after administration of 250/25 mg of levodopa/carbidopa had RAC BP changes similar to those of the dyskinetic group (at 1 h, 16% in the caudate nucleus and 24% in the putamen; at 4 h, 2% and 7%, respectively). This patient (a 79-year-old woman) was receiving a daily dose of levodopa of only 375 mg because of hallucinations. Hence, this case had clearly been misclassified as a stable responder, and was excluded from the analysis for between-group comparisons. The repeated measures ANCOVA analysis

(adjusting for between-group differences in both baseline BP values and Parkinson's disease duration) gave a significant interaction term (time \times group) for both caudate nucleus ($P = 0.030$) and putamen ($P = 0.021$) (when the misclassified patient was included in the analysis, the corresponding P -values were $P = 0.050$ and $P = 0.074$, respectively; see Fig. 3). The repeated measures ANCOVA analysis for baseline, 1-h and 4-h RAC BP values (adjusting for between-group differences in Parkinson's disease duration) gave virtually identical interaction terms (for caudate, $P = 0.037$; for putamen, $P = 0.028$). Remarkably, this analysis showed that the different pattern of response to levodopa between dyskinetics and stable responders was mostly due to changes in RAC BP from baseline to 1 h after levodopa administration, with larger decreases in RAC BP in the dyskinetic group (for the caudate nucleus, $P = 0.0013$; for the putamen, $P < 0.001$); changes in RAC BP from 1 h to 4 h played only a minor role (e.g. $P = 0.097$ for putamen).

At 1 h after levodopa administration, repeated measures ANCOVA (adjusting for region-specific baseline values) of the three analysed striatal subregions (Caud, P1 and P2) showed a lower RAC BP in the dyskinetic group compared with the stable group ($P = 0.048$); the interaction term (region \times group) was also statistically significant ($P = 0.038$). The same pattern was obtained after adjusting for duration of Parkinson's disease symptoms. Adjusted levodopa-induced changes in striatal RAC BP showed a greater rostro-caudal gradient in the dyskinetic group than in the stable group (with larger changes in P2, the most denervated subregion, than in P1 and Caud). Analysis of the 4-h results showed neither topographical differences between the two groups ($P = 0.72$) nor a significant interaction term ($P = 0.41$).

Discussion

We found that the striatal levodopa-induced changes in RAC BP obtained 1 h after levodopa administration correlated positively with duration of Parkinson's disease symptoms (and with severity of parkinsonism as estimated by MCS scores). As the PET method used here relies on the degree of competition between dopamine—not levodopa—and raclopride for dopamine D2/D3 receptors (de la Fuente-Fernández *et al.*, 2001b), our observations suggest that an identical dose of levodopa induces increasingly larger changes in synaptic dopamine levels as Parkinson's disease progresses. As discussed elsewhere (de la Fuente-Fernández *et al.*, 2001b), other interpretations, e.g. changes in high-affinity agonist state, would not explain the overall pattern of changes (see below). In contrast to the 1-h results, there was no correlation between the changes induced by levodopa in striatal RAC BP 4 h after levodopa administration and duration of Parkinson's disease symptoms. In addition, we found a significant interaction term between levodopa-induced changes and group (i.e. dyskinetics versus stable responders), which was independent of between-group differences in Parkinson's disease

duration. This interaction indicates that the two groups responded differently to levodopa. Thus, while Parkinson's disease patients with dyskinesias had more profound levodopa-induced reductions in striatal RAC BP (i.e. larger increases in synaptic dopamine levels) than stable responders 1 h after levodopa administration, there were no between-group differences at 4 h. Taken together, these observations suggest that peak-dose dyskinesias are likely associated with dramatic increases in synaptic dopamine levels induced by levodopa (our 1-h results). Although not measured by PET, we would expect the absolute levels of synaptic dopamine (i.e. the sum of endogenously and exogenously derived dopamine) to be larger in stable responders than in dyskinetics (de la Fuente-Fernández *et al.*, 2004). However, our 1-h PET results clearly suggest that the fraction of synaptic dopamine derived from exogenous levodopa is higher in the dyskinetic group. Our observations are in keeping with recent *post mortem* studies, which have shown that dopamine is mostly metabolized extraneuronally (i.e. following its release) in patients with dyskinesias (Rajput *et al.*, 2004). It should be noted that while we have focused on peak-dose dyskinesias for methodological reasons, our mathematical model predicts that biphasic dyskinesias may have a similar mechanism of pathogenesis: oscillations in synaptic dopamine levels (de la Fuente-Fernández *et al.*, 2004). Indeed, it has been suggested that biphasic and peak-dose dyskinesias may occur as a continuum (Vidailhet *et al.*, 1994).

In a previous study, we found that putamen RAC BP returns to baseline values 4 h after levodopa administration in stable patients who ended up developing early motor fluctuations in the follow-up (de la Fuente-Fernández *et al.*, 2001b). Although dyskinetics showed a similar overall pattern of levodopa-induced changes in the present study, they did not reach baseline values at the 4-h scan (despite the fact that some had both dyskinesias and fluctuations). We argue that some dyskinetics may have a relatively preserved dopamine re-uptake system, which explains why they are still able to maintain antiparkinsonian levels of dopamine even 4 h after levodopa administration. Only when this re-uptake mechanism fails would motor fluctuations appear. It should also be noted that the stable group most likely included patients who will ultimately develop early motor fluctuations (as discussed in de la Fuente-Fernández *et al.*, 2001b). This may explain the lack of between-group differences in the 4-h PET results. In addition, we know that some dopamine receptors do not recover immediately after dopamine stimulation (de la Fuente-Fernández *et al.*, 2001b). Whether this reflects receptor internalization (Sun *et al.*, 2003) remains unclear, but it may affect the 4-h results (particularly in those patients who had the largest changes at 1 h after levodopa administration, i.e. the dyskinetic group). As mentioned above, although dyskinetics and stable responders had similar levodopa-induced changes in RAC BP at the 4-h scan, this does not mean that the absolute synaptic dopamine levels were also similar. In fact, at 4 h after levodopa administration, synaptic dopamine levels are presumably

higher in the stable group than in the dyskinetic group (which included patients with motor fluctuations).

Motor function is expected to depend on the synaptic concentration of dopamine in the nigrostriatal system. Parkinson's disease pathology is invariably associated with loss of dopamine terminals, and decreased synaptic levels of dopamine. Increased dopamine release is among several mechanisms that are set in motion in order to compensate for dopamine deficiency in Parkinson's disease (Calne and Zigmond, 1991). There is evidence to suggest that such a compensatory mechanism may depend in part on certain individual characteristics (i.e. dopamine turnover seems to be higher in younger Parkinson's disease patients) (de la Fuente-Fernández *et al.*, 2001*b*). Indeed, this might explain why motor complications (fluctuations and dyskinesias) are more prevalent in younger patients (Golbe, 1991; de la Fuente-Fernández *et al.*, 2001*b*). Nonetheless, the progressive loss of dopamine terminals as Parkinson's disease progresses must also lead to a reduction in the synaptic concentration of (endogenous) dopamine in an age-independent manner. This would make the system increasingly dependent on exogenous sources of dopamine in order to maintain optimal levels of dopamine in the synapse. In keeping with our mathematical model (de la Fuente-Fernández *et al.*, 2004), our PET findings suggest that increased dopamine turnover is at the heart of motor fluctuations and dyskinesias. Specifically, we propose that peak-dose dyskinesias are related to the dramatic swings in synaptic dopamine levels obtained after levodopa administration. The resulting variations in dopamine receptor occupancy may lead to downstream changes (Gerfen *et al.*, 1990), which could also play a role in the pathogenesis of dyskinesias. Using entirely different methodology, we have found independent evidence for increased dopamine turnover with disease progression (Sossi *et al.*, 2004).

Naturally, any plausible model about the pathogenesis of dyskinesias in Parkinson's disease must explain a number of well-defined clinical observations as well as experimental data. Our model explains the following clinical observations: (i) decreasing the dose of levodopa (and, consequently, levodopa-derived dopamine levels) often alleviates dyskinesias, although sometimes precipitates the emergence of 'wearing-off' fluctuations (Nutt, 1990); (ii) in patients with asymmetric parkinsonism, dyskinesias are more severe on the body side corresponding to the more affected striatum (where levodopa-induced changes in synaptic dopamine levels must be greater) (Horstink *et al.*, 1990); and (iii) the incidence of dyskinesias might be lower in patients on treatment with direct dopamine agonists than in those on levodopa therapy (Rascol *et al.*, 2000); the long-lasting effect of dopamine agonists may prevent the occurrence of dramatic changes in the receptor occupancy level.

As to the experimental evidence, Mouradian and colleagues elegantly showed that the dose of levodopa necessary to evoke dyskinesias is much higher in stable responders than in fluctuators (Mouradian *et al.*, 1989). However, at the synaptic level, both doses may well be equivalent. Thus, we have

shown that an identical dose of levodopa induces more profound changes in synaptic dopamine levels in dyskinetics than in stable responders. We argue that the degree of synaptic dopamine deficiency in stable responders must be less severe than that in the dyskinetic group. Hence, in order to re-establish antiparkinsonian levels of dopamine in the synapse, dyskinetics would have to release a greater fraction of dopamine derived from exogenously administered levodopa than stable responders (who would store dopamine in presynaptic vesicles for longer periods of time). Mouradian and colleagues also reported that the threshold levodopa dose for the antiparkinsonian effect remains constant with Parkinson's disease progression (Mouradian *et al.*, 1989). Again, this may not be the case at the synaptic level. Although these authors reported similar threshold levodopa doses in stable responders and fluctuators (Mouradian *et al.*, 1989), the corresponding levodopa-induced changes in synaptic dopamine levels are most likely different between the two groups (i.e. larger in the fluctuator group, the more severely affected group). Also in keeping with our model is the observation that while increasing the dose of levodopa may prolong its antiparkinsonian effect (Nutt, 1990; Nutt and Holford, 1996), it does not modify to any major extent either the quality of the ON (i.e. antiparkinsonian effect) (Nutt and Holford, 1996) or the severity of dyskinesias (Verhagen Metman *et al.*, 1997), if present. While the dopamine turnover will determine the initial amount of dopamine released after levodopa administration (and, consequently, the quality of the ON and the severity of dyskinesias), the fraction of dopamine that is stored (and, consequently, the duration of benefit following each dose of levodopa) may increase with larger doses of levodopa whenever the storage capacity of the system is not saturated.

Dopamine turnover is a homeostatic mechanism set in motion to maintain optimal synaptic dopamine levels (de la Fuente-Fernández *et al.*, 2001*b*, 2004). Several factors seem to help regulate this compensatory mechanism (Cooper *et al.*, 1996). For example, down-regulation of dopamine autoreceptors is expected to lead to increased dopamine turnover. Interestingly, there is some indication that dopamine autoreceptor function may be decreased in Parkinson's disease patients with dyskinesias (de la Fuente-Fernández *et al.*, 2001*a*). Glutamatergic activity is also likely to play a role in dopamine release (and turnover) (Grace, 1991). Indeed, drugs with NMDA (*N*-methyl-D-aspartate) receptor antagonist properties may alleviate levodopa-related dyskinesias (Papa and Chase, 1996), although this may reflect effects elsewhere in the basal ganglia circuitry. As mentioned above, dopamine turnover is expected to increase as Parkinson's disease progresses, although a ceiling effect might exist. Remarkably, our PET results suggest that patients with advanced parkinsonism (up to 21 years of symptom duration) can still synthesize large amounts of dopamine from exogenous levodopa. It is clear that a major drawback of this homeostatic mechanism is that any increase in dopamine turnover, while helping to maintain transiently (quasi)normal synaptic dopamine levels, may also

lead to a reduction in the duration of the antiparkinsonian effect of each dose of levodopa (de la Fuente-Fernández *et al.*, 2001b, 2004). This is due to the fact that the parkinsonian brain has a greatly compromised dopamine re-uptake capacity related to the loss of dopamine terminals. Hence, the higher the release rate of dopamine, the greater the proportion of released dopamine that will be metabolized (and lost) per unit of time. In the absence of dopamine terminal loss, increased turnover by itself may not lead to motor fluctuations. Dopa-responsive dystonia, a disorder with compensatory increased dopamine release but intact re-uptake capacity (de la Fuente-Fernández *et al.*, 2003), provides internal validity to the model (de la Fuente-Fernández *et al.*, 2004). While dopa-responsive dystonia patients do not develop levodopa-related motor fluctuations because of the preserved re-uptake capacity of the nigrostriatal system, they can present with (usually mild) dyskinesias, which are easily controlled by reduction of the levodopa dose (de la Fuente-Fernández, 1999; Hwang *et al.*, 2001).

In keeping with our previous observations (de la Fuente-Fernández *et al.*, 2001b), our present findings suggest that levodopa treatment by itself is not the cause of either 'wearing-off' fluctuations or peak-dose dyskinesias. Levodopa usually has greater efficacy and fewer side-effects (excluding these motor complications) compared with synthetic dopamine agonists; therefore, we conclude that in general levodopa is optimal initial treatment of Parkinson's disease. Introduction of synthetic direct agonists and reduction in levodopa are rational approaches to therapy when motor complications become problematic.

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