Role of Dopaminergic Treatment in Dopamine Receptor Down-regulation in Advanced Parkinson Disease

A Positron Emission Tomographic Study

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Background: In patients with advanced Parkinson disease (PD) who are undergoing long-term treatment with a dopaminergic medication, a down-regulation of striatal dopamine D2 receptor expression has been demonstrated and interpreted as a consequence of either the disease itself or dopaminergic drug administration.

Objective: To compare, using positron emission tomography, the striatal binding of raclopride carbon C 11, a dopamine D2 receptor ligand, in PD patients who completely discontinued dopaminergic therapy (off drug) with that in PD patients who continued receiving dopaminergic therapy (on drug) after undergoing subthalamic nucleus stimulation.

Main Outcome Measures: The positron emission tomographic data were acquired in off-stimulation and, for 12 hours, off-medication conditions. Five off-drug PD patients, 7 on-drug PD patients, and 8 healthy subjects participated.

Results: In off-drug PD patients, the putaminal raclopride C 11 binding was 24% higher than in on-drug PD patients. The same tendency was noted for the caudate nucleus, but it was not significant (P=.07). Compared with control subjects, the putaminal raclopride C 11 binding was increased by 21% in off-drug and was normal in on-drug PD patients. Compared with controls, the caudate raclopride C 11 binding was reduced by 23% in on-drug and was normal in off-drug PD patients. Further analysis using statistical parametric mapping showed a significant increase of binding bilaterally in the caudate nucleus and putamen in off-drug compared with on-drug PD patients (P=.002 at cluster level).

Conclusions: The down-regulation of dopamine D2 receptors probably relates to the long-term and intermittent administration of dopaminergic treatments rather than to disease progression. This phenomenon is reversed by the complete withdrawal of dopaminergic drugs. Furthermore, an up-regulation of putaminal dopamine D2 receptors is demonstrated in late-stage PD after dopaminergic drug withdrawal.

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Dopamine D2 receptor function and availability can be assessed in vivo using positron emission tomography (PET) and raclopride carbon C 11, a dopamine D2 receptor ligand. In drug-naive or early-stage Parkinson disease (PD) patients, most PET studies using raclopride C 11 show an increased raclopride C 11 binding in the putamen, whereas binding is usually normal in the caudate nucleus. This result has generally been interpreted as a compensatory dopaminergic receptor up-regulation. In advanced PD patients undergoing long-term dopaminergic treatment, a reduction of the striatal raclopride C 11 binding is usually observed in the caudate nucleus and less constantly in the putamen. This modification of dopaminergic D2 receptor expression may reflect either an effect of disease progression on striatal dopamine D2 receptor density or a down-regulation of these receptors induced by levodopa and other dopaminergic drugs. To our knowledge, the reversibility of decreased striatal raclopride C 11 binding in advanced PD patients, after complete dopaminergic drug withdrawal, has never been previously assessed because of the inability to completely discontinue dopaminergic medication for any significant period because of the severity of motor symptoms. With the recent advance of deep brain stimulation of the subthalamic nucleus (STN), it is possible, in some patients, to completely interrupt levodopa and dopaminergic agonists after STN stimulation. This allows for the

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investigation of the respective effects of dopaminergic drug withdrawal and disease progression on striatal dopamine D2 receptor density and function by using the noninvasive PET raclopride C 11 technique.

The present study compares the striatal raclopride C 11 binding in PD patients undergoing STN stimulation: one group who has completely discontinued dopaminergic drug therapy (off-drug patients) is compared with one group still receiving dopaminergic medications (on-drug patients). Our hypothesis is that if the reduction of striatal raclopride C 11 binding is due to long-term dopaminergic drug intake and not to disease progression, then this down-regulation of dopamine D2 receptors should be reversed after dopaminergic drug withdrawal.

**METHODS**

**PATIENTS**

Twelve severely affected right-handed PD patients were studied. The main clinical characteristics of both groups of patients are presented in Table 1. The patients fulfilled the United Kingdom Parkinson's Disease Brain Bank criteria for idiopathic PD.13 All patients had clear and sustained levodopa responsiveness, severe off states, motor fluctuations, and dyskinesias. These patients underwent bilateral surgical implantation of electrodes in the STN (model 3389; Medtronic, Minneapolis, Minn) that were connected to a telemetrically controllable pulse generator (Kineta Medtronic), as previously described.12 At the time of the PET study, patients had been treated by STN stimulation for at least 3 months. The beneficial effects of STN stimulation allowed 5 patients (3 men and 2 women) to completely discontinue all antiparkinsonian medications after STN stimulation (off-drug patients), while 7 patients (3 women and 4 men) continued to receive a long-term dopaminergic treatment, with a mean ± SD 66% ± 10% reduction of the dose (on-drug patients). In the off-drug group of patients, drug withdrawal was achieved within the first month interval between drug withdrawal and PET: mean ± SD 17 ± 6 months (range, 5–29 months). Both groups of patients were similar for age (P = .80), preoperative levodopa responsiveness (assessed by the reduction of the Unified Parkinson’s Disease Rating Scale [UPDRS] III motor score) (P = .30), disease duration (P = .70), delay between surgery and PET scanning (P = .60), stimulation variables (P = .80), and improvement of motor complications (assessed by the reduction of the UPDRS IV score) (P = .80).14

At the time of PET scanning (off medication), the stimulation reduced the UPDRS motor score by a mean ± SD of 58% ± 9% and 76% ± 6% in on-drug and off-drug PD patients, respectively. The UPDRS III motor scores in on- and off-stimulation conditions (off medication) were significantly higher in on-drug vs off-drug patients (P < .01).

All patients were off drug on the day of the PET study for at least 12 hours, and the stimulator was switched off 1 hour before scan acquisition.

**CONTROL SUBJECTS**

Eight right-handed, age-matched, healthy subjects (5 men and 3 women) participated in the study (mean ± SD age, 35.7 ± 9.7 years; range, 41-69 years). These subjects had no history of psychiatric or neurological disorders, had a normal physical examination result, and did not receive any medication. The study was performed after approval by the Lyon University Hospitals Ethics Committee. All subjects participated after signing an informed consent form according to the Declaration of Helsinki.

**PET PROTOCOL**

The PET scans were performed in 3-dimensional mode using a tomograph (Siemens CT11R+ tomograph; CT/Siemens, Knoxville, Tenn). The scanning procedure has been described elsewhere.13 One raclopride C 11 PET scan was obtained for all patients. The stimulator was switched off 1 hour before the PET session. This time frame is consistent with the latency of recurrence of most parkinsonian signs. For assessment of dopamine D2 receptor binding, 28 sequential scans were obtained over 60 minutes, starting at the time of injection of raclopride C 11 into an antecubital vein over 15 seconds with an automated infusion pump. The mean ± SD injected activity was 0.0059 ± 0.0006, 0.00064 ± 0.0007, and 0.0059 ± 0.0004 C for on-drug patients, off-drug patients, and controls, respectively.

**IMAGE ANALYSIS**

**Region of Interest Analysis**

One elliptical region of interest (ROI) (10 × 30 mm) was placed along the axis of each putamen, and one circular ROI (10 × 10 mm) was positioned on each head of the caudate nucleus. These ROIs were placed on the 5 consecutive planes. The nonspecific background activity was averaged from cerebellar elliptical ROIs drawn on 2 consecutive planes.16 The raclopride C 11 binding potential (binding_assessment/Kd) was determined from the distribution volume evaluated using a graphical approach and a tissue input function.17

**Statistical Parametric Mapping Analysis**

Image and statistical analyses were performed using an analytical software program MATLAB 5.3 (MathWorks, Natick,
STATISTICAL ANALYSIS

The main clinical characteristics of both groups of patients were compared using the Mann-Whitney nonparametric test, with significance at \( P < .05 \).

The mean raclopride C 11 binding values of on-drug PD patients were compared with those of off-drug PD patients and with those of controls using a 1-way analysis of variance. A post hoc analysis was performed using an unpaired 2-tailed \( t \) test with Bonferroni correction (\( P < .05 \)).

In addition, categorical comparisons of mean raclopride C 11 voxel-based binding values between both groups of PD patients and controls were made, applying SPM. These analyses were limited to the striatal regions using an appropriate mask. Significant differences were localized using SPM, with residual df of 10 from 12 images. The contrasts were used to derive between-condition \( z \) scores on a voxel-by-voxel basis using the general linear model. The \( z \) values were corrected for multiple comparisons implicit in SPM. In the voxel-based analysis, a corrected \( P \) value of less than .01 at the cluster level was considered significant. These voxels were localized using their coordinates in the Talairach and Tournoux brain atlas after having converted coordinates obtained from SPM software (SPM 99) in Talairach coordinates using the appropriate formula (to get from McGill [MNI]-SPM96 coordinates to Talairach 88-SPM95 coordinates: \( x' = 0.88x - 0.8; y' = 0.97y - 3.32; \) and \( z' = 0.05y + 0.88z - 0.44 \)).

RESULTS

ROI ANALYSIS

In off-drug PD patients, the mean ± SD raclopride C 11 binding values in the off STN stimulation condition were 3.4 ± 0.5 and 2.1 ± 0.4 for the putamen and caudate nucleus, respectively.

In on-drug PD patients, the mean ± SD binding values in the off STN stimulation condition were 2.6 ± 0.6 and 1.7 ± 0.2 for the putamen and caudate nucleus, respectively.

In control subjects, the mean ± SD binding values were 2.7 ± 0.3 and 2.2 ± 0.2 for the putamen and caudate nucleus, respectively.

An analysis of variance showed a highly statistically significant heterogeneity among groups for the caudate nucleus and putamen.

Off-Drug vs On-Drug PD Patients

A significant increase of raclopride C 11 binding was observed after dopaminergic drug withdrawal in the putamen (mean ± SD, 3.4 ± 0.5 vs 2.6 ± 0.6; \( P = .03 \)). The same trend was noted for the caudate nucleus (2.1 ± 0.4 vs 1.7 ± 0.2), but did not reach statistical significance (\( P = .07 \)).

PD Patients vs Controls

No difference in putamen raclopride C 11 binding was noted between control subjects and on-drug patients, while raclopride C 11 binding was higher in off-drug patients compared with on-drug PD patients and controls (\( P = .04 \)). In the caudate nucleus, on-drug PD patients had lower raclopride C 11 binding than did controls (\( P = .04 \)) while no significant difference was noted between controls and off-drug PD patients (\( P = .3 \)).

SPM ANALYSIS

The present study demonstrates that, in advanced PD patients undergoing long-term STN stimulation, only those requiring dopaminergic treatment have a reduced striatal raclopride C 11 binding compared with controls. In contrast, patients not receiving antiparkinsonian medication displayed an increased raclopride C 11 putaminal binding.

Both methods of analysis provided the same results, except for the caudate nucleus, for which the ROI analysis did not show any significant difference of raclopride C 11 binding between on- and off-drug patients, whereas the SPM analysis demonstrated a higher raclopride C 11 binding for the caudate and the putamen in off-drug patients. This may represent a higher sensitivity of this method of analysis, which is voxel based.

In advanced on-drug PD patients, the reduced raclopride C 11 binding in the caudate nucleus is in keeping with the results of most PET studies and with postmor-
term histological studies that reported normal or reduced striatal dopamine D2 receptor density. However, is this a consequence of the long-term dopaminergic treatment or of PD progression? Trying to answer this question is complex because the usual 12-hour antiparkinsonian drug withdrawal before PET data acquisition does not prevent direct competition for receptor binding between dopamine, long half-lived dopamine agonists, and raclopride C11. Thus, differentiating a direct competition effect from a progressive drug-induced modification of dopamine D2 receptor expression is difficult. In our study, taking advantage of the possibility, after STN stimulation, to discontinue dopaminergic drugs in some PD patients, we addressed the question of the long-term changes of dopamine receptors induced by dopaminergic drugs by studying the modifications of dopamine D2 receptors after prolonged medication withdrawal.

Our results support a direct effect of long-term and intermittent dopaminergic drug administration on dopaminergic D2 receptor expression and show that these effects are reversible after medication withdrawal. Indeed, the down-regulation of dopamine D2 receptors disappeared in patients in whom medications were withdrawn for months. This down-regulation may be mediated by an internalization of dopamine D1/D2 receptors, making ligands unable to access their targets.

Furthermore, in our unmedicated advanced PD patients, the putaminal raclopride C11 binding was significantly higher than in healthy subjects. Such up-regulation of putaminal dopamine D2 receptors has only been observed in de novo drug-naïve PD patients. This is usually interpreted as a compensatory mechanism. Reduced receptor competition secondary to the reduction of endogenous dopamine release in PD has also been suspected, but the compensatory hypothesis has been supported by studies using a combination of reversible (raclopride C11) and irreversible (methyl-speripone labeled with C11) dopamine receptor ligands. Our data suggest that this compensatory up-regulation persists in advanced PD patients in the absence of dopaminergic treatment. This fits well with histological data showing that hypersensitivity of dopaminergic D2 receptors occurs when 80% or more of the nigrostriatal neurons are destroyed in monkeys or rats treated with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or 6-hydroxy-dopamine and in humans with advanced PD. In monkeys treated with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, this increase of dopamine D2 receptor binding has been demonstrated in the putamen and the caudate. In the present study, we did not observe such up-regulation in the caudate. However, our data clearly showed, for the putamen and the caudate, an increase of raclopride C11 binding, but from a normal to an increased level for the putamen and from a down-regulated to a normal level for the caudate. This further supports the role of dopaminergic medications, which equally down-regulate dopamine D2 receptors in both elements of the striatum.

Finally, our results cannot be explained by differences in the clinical characteristics of our 2 groups because their disease duration, age, preoperative levodopa responsiveness, time to PET scanning after STN surgery, and motor complication improvement after STN stimulation were similar. The reasons that led one group to complete drug withdrawal while the other still required medication are, therefore, probably more related to the stimulation itself (eg, electrode placement) or to more severe disability and to nondopaminergic characteristics of our on-drug PD patients, as suggested by the higher UPDRS motor score at the time of the PET scan.

| Table 2. SPM Comparison of Raclopride C11 Binding Among the 3 Groups |
|----------------------------------------|------------------|--------|--------|--------|------------------|
| Stereotactic Coordinates (Regional Maxima) | | | | | |
| Area                      | x                | y      | z      | Corrected P Value (Cluster Level) |
| Putamen                   |                  |        |        |                                 |
| Right side                | 13               | 18     | -5     | 2.35 .002                        |
| Left side                 | -13              | 10     | -5     | 2.21 .002                        |
| Caudate nucleus           |                  |        |        |                                 |
| Right side                | 8                | 2      | 14     | 2.34 .002                        |
| Left side                 | -8               | -2     | 14     | 2.38 .002                        |
| Increase of Binding in Off-Drug Compared With On-Drug PD Patients |
| Putamen                   |                  |        |        |                                 |
| Right side                | 20               | 5      | 12     | 2.50 .001                        |
| Left side                 | -18              | -4     | 12     | 2.38 .002                        |
| Caudate nucleus           |                  |        |        |                                 |
| Right side                | 25               | 20     | 8      | 2.36 .002                        |
| Left side                 | -23              | 15     | 8      | 2.40 .002                        |
| Decrease of Binding in On-Drug Compared With Controls |

Abbreviations: PD, Parkinson disease; SPM, statistical parametric mapping.
effects of the antiparkinsonian medication on dopamine D2 receptor regulation.

In conclusion, our study suggests the following: (1) The dopaminergic D2 receptor down-regulation in late-stage PD is mainly secondary to antiparkinsonian medication and could be reversible after long-term drug withdrawal. Future longitudinal prospective studies of the striatal raclopride C 11 binding changes before and prospectively after STN surgery would be necessary to confirm and precisely delineate the timing of the normalization of dopamine D2 receptor expression, and (2) In off-drug late-stage PD patients, an up-regulation of dopaminergic D2 receptors is observed, in keeping with postmortem histological observations in those with severe dopaminergic lesions.

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