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Evidence for impaired presynaptic dopamine function in parkinsonian patients with motor fluctuations

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Summary. We used [^{18}F]6-fluorodopa (FD) positron emission tomography (PET) to examine the severity of nigrostriatal dopaminergic dysfunction in 67 patients with Idiopathic Parkinsonism (IP), 52 with fluctuations and 15 with a stable response to levodopa. FD uptake (Ki) was reduced by 12% in the caudate ($p = 0.08$) and by 28% in the putamen ($p = 0.0004$) of patients with fluctuations compared to those with a stable response. However, there was considerable overlap of FD Ki values between the two groups. The fluctuators had a longer symptom duration (11.6 ± 5.7 years) than the patients with a stable response to levodopa (4.3 ± 2.4 years; $p < 0.0001$) and the age of onset of symptoms was earlier in the fluctuators (43.9 ± 8.9 versus 54.1 ± 10.4; $p = 0.0004$). Similar reductions in FD Ki in the fluctuators persisted following adjustment for these variables (7.5% in the caudate and 26% in the putamen; p = n.s. and 0.007, respectively). When smaller groups ($n = 15$ each) were matched for duration of symptoms, the reduction in caudate Ki in the fluctuators was only 1.9% (p = n.s.), but there was still a 24% reduction in putamen Ki ($p = 0.05$). These findings suggest that fluctuators and non-fluctuators may differ in the severity of their nigrostriatal damage and provide modest support for the hypothesis that fluctuations may in part reflect altered "buffering" capacity of dopaminergic nerve terminals. However, the considerable overlap between groups suggests that other factors such as altered postsynaptic mechanisms and/or increased turnover of dopamine may make a substantial contribution to the development of motor fluctuations.

Keywords: Motor fluctuations, idiopathic parkinsonism, fluorodopa uptake, positron emission tomography.

Introduction

Patients with Idiopathic Parkinsonism (IP) frequently develop motor fluctuations during chronic treatment with levodopa (Marsden et al., 1982).

Although this complication has been the subject of several studies, its pathogenesis remains unclear (Chase et al., 1993; Mouradian and Chase, 1988). It is generally thought that the progressive loss of nigrostriatal terminals may be responsible for "wearing off" deterioration in IP at the end of the inter-dose interval (storage hypothesis) (Fabbrini et al., 1988). However, the correlation between the clinical severity of IP and the occurrence of "wearing off" fluctuations is poor (Kempster et al., 1989). Postsynaptic alterations have also been implicated in the pathogenesis of "wearing off" fluctuations (Bravi et al., 1994; Verhagen Metman et al., 1997), but their exact nature and role have not been elucidated. The pathogenesis of unpredictable "on-off" phenomena is even less well defined (Chase et al., 1993; Mouradian and Chase, 1988).

Positron emission tomography (PET) using [^{18}F]6-fluorodopa (FD) permits examination of the integrity of the dopaminergic nigrostriatal projection in vivo (Garnett et al., 1983a). It has been shown that FD and levodopa are handled in similar ways by the brain. Thus, after crossing the blood-brain-barrier, FD is decarboxylated to fluorodopamine (FDA) and stored in nerve terminals (Firnau et al., 1975; Garnett et al., 1978, 1983a,b), where there is no significant loss of signal during the first 2 hours of a FD PET scan (Leenders et al., 1986; Martin et al., 1989). The striatal radioactivity over this period reflects the uptake of FD as well as synthesis and storage of FDA within the nigrostriatal terminals. The striatal FD uptake constant (Ki) obtained during the first 2 hours after the administration of FD correlates linearly with the number of surviving nigral dopaminergic neurons in IP (Snow et al., 1993). FD PET is, therefore, a useful tool for exploring the possible role played by loss of dopaminergic projections in the development of motor fluctuations in patients with IP. If motor fluctuations, particularly "wearing off", are the consequence of a diminished storage capacity, the accumulation of FDA in the striatum during a 2 hour FD PET study should be lower in fluctuators than in patients with a stable response to levodopa. We undertook studies to confirm or refute this prediction.

Methods

Subjects

Sixty-seven patients (41 men and 26 women) with clinically definite IP (Calne et al., 1992) as determined by a neurologist experienced in the assessment and management of movement disorders were studied. All patients were on levodopa with a decarboxylase inhibitor. The mean ± SD daily levodopa dose at the time of PET was 504 ± 325 mg in the stable patients; 785 ± 353 mg in the fluctuators ($P < 0.01$). The presence of fluctuations was determined based on a history of consistent end-of-dose deterioration 2 to 3 hours after taking each dose of levodopa ("wearing off" fluctuations). Despite multiple treatment re-adjustments, all fluctuators were experiencing "wearing off" fluctuations at the time of the study. As this study was not aimed at correlating PET measurements with the severity of motor fluctuations, repeated, sequential motor examinations following a single dose of levodopa were not performed. Eighteen patients (1 stable and 17 fluctuators) were taking bromocriptine, 19 patients (all fluctuators) were taking pergolide, 26 patients (3 stable and 23 fluctuators) were taking selegiline, 10 patients (1 stable and 9 fluctuators) were taking amantadine and 4 fluctuators were taking an anticholinergic at the time of the PET study. Clinical evaluations were performed on the morning of the PET scan by one

of two experienced evaluators, between 8 a.m. and 10 a.m., after at least 12 hours off medication (18 hours for controlled release levodopa/carbidopa) using the Modified Columbia Scale (MCS) with a maximum score of 92 (vegetative scores, sialorrhea and seborrhea not included) (Duvoisin, 1971).

Positron emission tomography (PET)

All PET studies were performed using an ECAT 953B/31 tomograph in 2D mode. The details of the protocol have been described elsewhere (Vingerhoets et al., 1994). The subject's head was restrained by a molded thermo-plastic face mask maintaining the orbito-meatal line parallel to the image plane. A 10 min. transmission scan was performed using three ^{68}Ge rotating rod sources, for later use in attenuation correction. Subjects received an injection of 5 mCi (185 MBq) of FD one hour after premedication with 150–200 mg of carbidopa. Twelve sequential emission scans, each lasting 10 minutes, were performed starting at the midpoint of the FD injection. Activity collected from 60 to 120 minutes after FD administration was summed to produce an integral image. On this image, regions of interest (ROIs) were established. A small circular ROI (diameter = 8.8 mm) was positioned by inspection on each caudate nucleus and three circular ROIs (diameter = 8.8 mm) were placed without overlap along the axis of each putamen. ROI position was adjusted on the integral image to maximize the average activity. Three circular background ROIs (diameter = 19.4 mm) were placed on the parieto-occipital cortex. This was repeated for the five slices where the caudate and putamen were most clearly seen, and replicated over the 12 time frames. The tissue data were analyzed using the graphical method described by Patlak and Blasberg (Martin et al., 1989; Patlak and Blasberg, 1985) on the values from 20 to 120 minutes, using a cortical input function (Brooks et al., 1990). Twenty normal subjects (10 men, and 10 women; mean age ± SD, 51.45 ± 14.46 years) were used as controls. All the subjects (patients and controls) gave written informed consent. The study was approved by the U.B.C. Ethics Committee.

Statistical analysis

Clinical scores for severity of parkinsonism and PET measurements (Ki for putamen and caudate) were compared by 2-tailed, unpaired t-tests. Additionally, following confirmation of appropriate data distribution, comparisons were made using analysis of covariance (ANCOVA) to adjust for age of onset using a linear model and for symptom duration using both linear and non-linear models. Although the progression of IP is thought to follow a curvilinear pattern over the long term (Lee et al., 1994), linear models are appropriate for the relatively short period analyzed and for all variables, provided a better fit to the data. Furthermore, for all variables, the estimated slopes against duration were the same in both patient groups. Data are summarized as means ± standard deviations (SD).

Results

Fifty-two (33 men and 19 women, age 55.5 ± 8.4 years) of the 67 patients had "wearing off" fluctuations and 15 (8 men and 7 women, age 58.3 ± 11.0 years) had a stable response to levodopa. The fluctuators had longer duration of symptoms (11.6 ± 5.7 years) than the patients with a stable response to levodopa (4.3 ± 2.4 years; $p < 0.0001$) and the age of onset of symptoms was earlier in the fluctuators (43.9 ± 8.9 years vs. 54.1 ± 10.4 years; $p = 0.0004$). The severity of parkinsonism as assessed by the MCS in the "off" state was 38.7 ± 14.1 in the fluctuators and 18.7 ± 10.5 in those with stable response to levodopa ($p < 0.0001$). Although the magnitude of the difference between the two groups was somewhat less marked following ANCOVA to adjust for

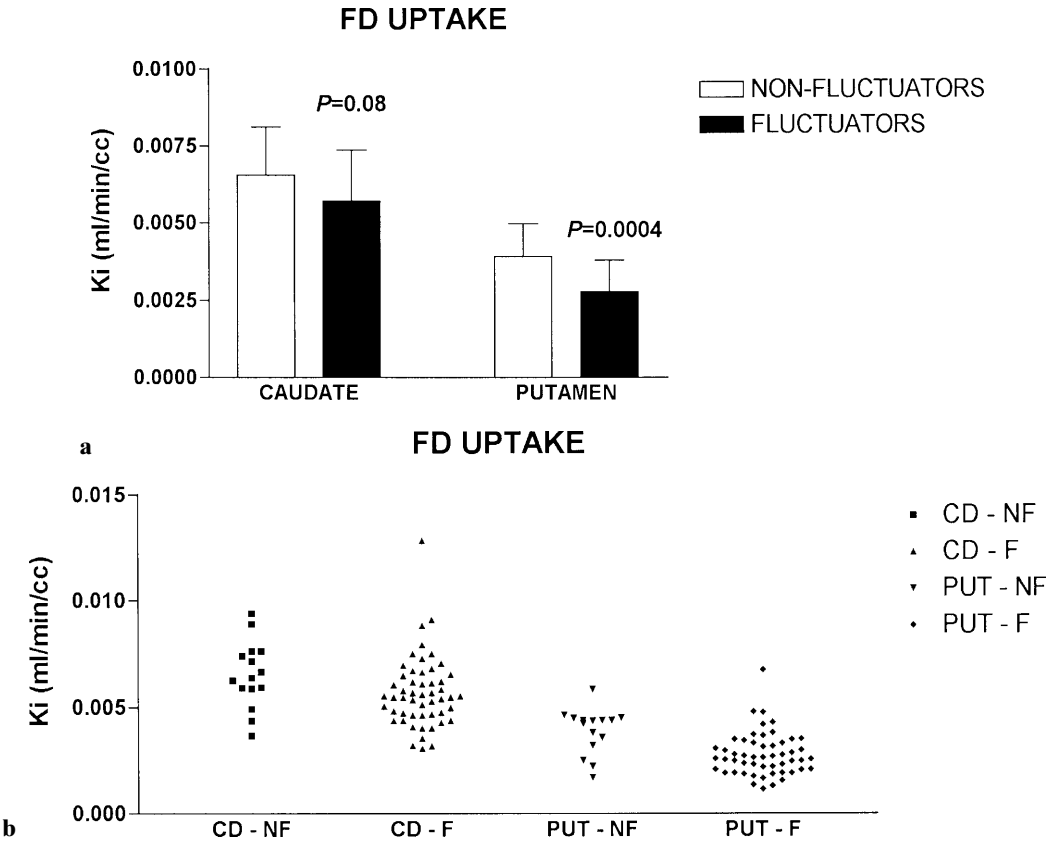

Fig. 1. **a** Mean ± SD uptake constants (Ki) determined using a tissue input function in the caudate nucleus and putamen of IP patients with a stable response to levodopa (n = 15) and those with dose-related fluctuations in motor function (n = 52). Age-matched normal values are 0.0095 ± 0.0001 in the caudate and 0.0084 ± 0.0008 in the putamen. **b** Scatterplot showing individual values for caudate and putamen Ki in IP patients with a stable response to levodopa and those with fluctuations. Note that although the two patient groups are different, there is considerable overlap between them

age of onset and symptom duration, the difference was still significant (p = 0.003).

FD Ki was reduced by 12% in the caudate of fluctuators compared to patients with a stable response to levodopa (0.0057 ± 0.0017 vs. 0.0065 ± 0.0016; p = 0.08) and by 28% in the putamen (0.0028 ± 0.0010 vs. 0.0039 ± 0.0011; p = 0.0004) (Fig. 1a). However, there was considerable overlap in the values between the two patient groups (Fig. 1b). Thus, for example, 92% of fluctuators had putamen Ki values within the range of two standard deviations of the mean putamen Ki of the stable group.

Because the two patient groups were not matched for symptom duration or age of onset of symptoms, and because both of these factors were found to affect FD Ki, a between group comparison was repeated using analysis of covariance (ANCOVA) to adjust for these variables. The adjusted values for Ki revealed a 7.5% reduction in the caudate (p = n.s.) and a 26% reduction in

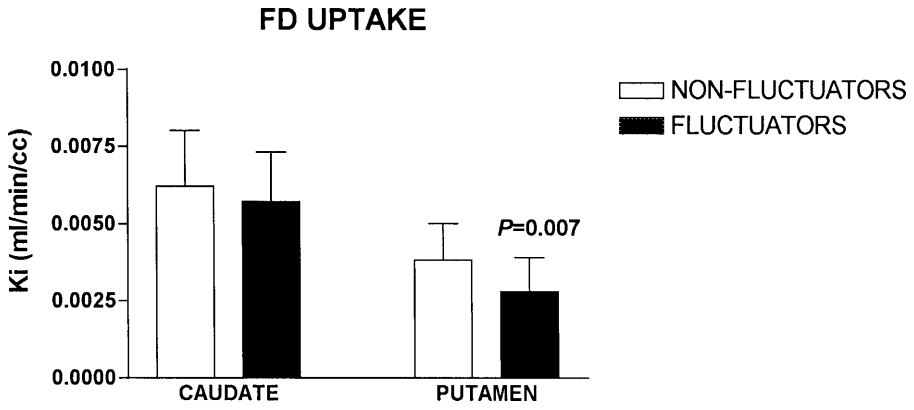

Fig. 2. Mean ± SD values for FD Ki adjusted for symptom duration and age of onset of symptoms using a linear ANCOVA. The differences are similar to those seen in Fig. 1a

the putamen ($p = 0.007$) (Fig. 2). Although the progression of IP may be non-linear, non-linear regression did not provide a better fit to the data and the slopes of Ki against either duration or age of onset of symptoms were not different between the fluctuating and non-fluctuating patients.

An alternate approach to this problem is to simply match the two patient groups for duration of illness. We did this selecting a subgroup of 15 patients with stable response to levodopa (8 men and 7 women; age 58.3 ± 11.0 years; duration 4.3 ± 2.4 years) and 15 patients with fluctuations (7 men and 8 women; age 53.9 ± 8.9; duration 5.3 ± 1.7 years, p = n.s.). The age of onset was not significantly different between the two groups (54.1 ± 10.4 years in the non-fluctuators, 48.6 ± 8.4 years in the fluctuators). Comparing these two groups, there was a 1.9% reduction in caudate Ki in the fluctuators (0.0064 ± 0.0023) compared to the patients with stable response (0.0065 ± 0.0016; p = n.s.) and a 24% reduction in putamen Ki (0.0030 ± 0.0014 vs. 0.0039 ± 0.0011; $p = 0.0534$). This was associated with increased motor disability as measured by the MCS (32.0 ± 11.6 in fluctuators vs. 18.7 ± 10.5 in the stable patients; $p < 0.003$).

Discussion

The main objective of our study was to determine whether motor fluctuations in IP depend on the severity of the nigrostriatal lesion (storage hypothesis). We found that motor fluctuations were associated with more severe parkinsonism, as measured by FD PET or by the MCS.

One potential confound in this study is the difference in duration of symptoms between the two patient groups. Patients with motor fluctuations are likely to have illness of longer duration than those with a stable response to levodopa. Thus, the lower FD Ki in the fluctuators may simply reflect increased severity of IP due to longer illness duration. We approached this problem in two ways. First, ANCOVA was performed to adjust for the effects of symptom duration [as well as age of onset, which was lower in the fluctuators, as reported by others (Golbe, 1991; Quinn et al., 1987) and independently affected Ki]. The differences in Ki and MCS between stable and

fluctuating patients persisted following adjustment for symptom duration and age of onset, both of which differed between the two groups. While the progression of IP is likely to be non-linear, measurements made over a relatively short time frame may best be described by linear models, as was indeed the case here, where ANCOVA adjusting for duration was performed using both linear and quadratic functions. Linear functions provided the best fit for the ANCOVA. Furthermore, the slopes of the functions describing Ki against duration were not different between the two patient groups.

A second approach was also taken, in which we attempted to match the two groups for symptom duration by selecting a subgroup of the entire population. The lower number of subjects (30 instead of 67) did result in some loss of statistical power; nevertheless, the between-group differences in putamen Ki and in MCS persisted and were of comparable magnitude. In addition to the attendant loss of power, attempts to match samples for factors such as duration may be associated with other difficulties in interpretation. For instance, it could be argued that patients with fluctuations after a short illness duration might have a more aggressive form of IP than those with a stable response to levodopa after the same duration of illness. In that case, differences between groups might reflect variation in the expression of IP rather than providing any insight into the basis for the fluctuations. Such considerations will apply to other studies on IP and it may be worth taking both approaches (matching and ANCOVA-derived adjustments) to this problem. In PET studies examining other parameters such as receptor density, it may be worth matching for FD Ki or some other measure of presynaptic function, such as vesicular monoamine transporter binding, as a marker of IP severity.

It has previously been shown that the number of dopaminergic neurons in the substantia nigra correlates with both clinical scores of parkinsonism (particularly bradykinesia) (Rinne et al., 1989), as well as striatal and putaminal Ki values measured in a 2 hour FD PET scan (Snow et al., 1993). Thus, our FD PET findings are compatible with the view that motor fluctuations depend at least in part on the severity of the dopaminergic nigrostriatal deficit and are in basic agreement with the findings of Leenders et al. (1986). As the capacity to decarboxylate FD is not considered to be a limiting factor even in advanced IP (Lloyd and Hornykiewicz, 1970; Nagatsu et al., 1993), these authors suggested that fluctuators have an inability to retain FDA compared to non-fluctuators (Leenders et al., 1986).

However, our data revealed considerable overlap of FD Ki between the fluctuators and those patients with a stable response to levodopa. Thus, it seems clear that factors other than reduced ability to retain FDA must contribute to motor complications. The same conclusion was drawn by Kempster et al. (1989), based on their clinico-pathological study. Two alternative working hypotheses should be considered. Motor fluctuations in IP may be related to post-synaptic changes and/or increased turnover of dopamine in the nigrostriatal system.

There is considerable evidence based on animal studies that repeated pulsatile administration of levodopa to animals with lesions of the nigrostriatal dopamine projection may result in altered expression of neu-

rotransmitters downstream to striatal dopamine receptors (without affecting concentrations of dopamine receptors themselves). Such treatment is associated with the development of behavioural sensitization (see Chase et al., 1993 for review). These downstream changes may correlate with the emergence of fluctuations in motor function. PET studies of dopamine receptor function in IP have failed to find any significant association between the levels of D1 or D2 binding and the presence of fluctuations or dyskinesias (de la Fuente-Fernández et al., 1997; Kishore et al., 1997; Turjanski et al., 1997).

Another possible explanation for fluctuations in motor function might be increased dopamine turnover due to an increase in either enzymatic degradation of FDA or increased reupatke via the dopamine transporter. Although FD uptake is linear over the first 90–120 minutes following tracer administration, longer scan times reveal loss of trapped radioactivity (Holden et al., 1997) and the ratio of the rate constant describing this loss (k_{loss}) to Ki correlates with traditional neurochemical estimates of DA turnover (Doudet et al., 1998). This might be a particular problem in interpreting the earlier results of Leenders et al. (1986), who used longer scan times – thus their measurements reflect a combination of Ki and k_{loss}. Studies examining both parameters may be helpful to address this possibility.

In conclusion, our clinical and FD PET results suggest that while the severity of nigrostriatal damage may be related to the development of motor fluctuations in IP, this is unlikely to be a sufficient explanation. We conclude that levodopa-related motor oscillations could additionally be due to postsynaptic functional changes, or may reflect accelerated turnover of dopamine in the nigrostriatal system.

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