Brain dopamine metabolism in patients with Parkinson’s disease measured with positron emission tomography

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SUMMARY L-[18F] fluorodopa was administered in trace amounts intravenously to healthy control subjects and to patients with Parkinson’s disease. Striatal uptake of radioactivity was measured using positron emission tomography. The capacity of the striatum to retain tracer was severely impaired in patients compared to controls. This may reflect a reduction of striatal dopamine storage in Parkinson’s disease. Patients showing the “on/off” phenomenon had an even greater decrease of striatal storage capacity.

Positron emission tomography (PET) is a scanning technique which allows measurement in absolute units of the regional concentration of positron emitting isotopes in the brain or other organs.1 When these isotopes are attached to suitable tracers and inhaled or injected intravenously it is possible to explore their fate by measuring the uptake of radioactivity over time in an organ such as the brain. Recently a method has been developed to label an analogue of levodopa (6-L-fluorodopa) with the positron emitting isotope fluorine-18.2 This tracer can be used to study regional dopamine metabolism in brain in vivo by measuring tomographically the accumulated radioactivity with PET.3–5

We have applied this method to a group of healthy control subjects and to patients with Parkinson’s disease. Because of severe loss of nigrostriatal dopaminergic neurons that characterises Parkinson’s disease, the dopamine content in striatum of these patients is markedly diminished.6 The aim of the present study was to investigate storage capacity for dopamine in striatum of healthy individuals and subjects with Parkinson’s disease.

Methods

Tracer
The isotope fluorine-18 (half life 110 min) was produced in

the MRC cyclotron at the Hammersmith Hospital, London. Labelled L-[18F] fluorodopa was prepared according to the technique of Firnau et al.2 (but without the final stage of separation of isomers). Therefore a mixture of 2-, 5- and 6-L-[18F] fluorodopa was used. The relative isomeric proportions were 35, 5 and 60% respectively. The radioactivity, 2–6 mCi, was associated with 8–10 mg L-fluorodopa. The estimated mean specific activity was 103.0 ± 22.9 mCi/mmol. This mixture was injected intravenously in a volume of 10 ml over two minutes using a constant infusion Harvard pump.

Construction of arterial curve
A Teflon (gauge 21) cannula was inserted into one radial artery, and 3 ml blood samples were taken at 20 second intervals during the first three minutes following tracer injection, and then every 30 to 60 seconds for a further seven minutes. Arterial sampling times were then gradually spaced out via 5 and 10, to 20 minute intervals. Usually a total of 25 samples were taken. The samples were spun and the concentration of isotopes in plasma was measured in a well-counter cross calibrated with the tomograph.

Scanning technique
The positron emission tomograph used was the ECAT-II (EG & G Ortec). This is a whole body single slice machine with a spatial resolution of 17 mm x 17 mm FWHM (full width half maximum) and a slice thickness of 16 mm (FWHM).7 Serial scans were started in most subjects about one hour after the tracer had been given. Some subjects were scanned from the moment at which tracer was injected. Owing to the slow uptake of the tracer by brain tissue, the relatively small volume of the striatum and the relatively low sensitivity of our scanner, 10 minute scans were needed to obtain sufficient counts to reconstruct one tomographic image. Only one cross-section was scanned (5 cm above and
parallel to the orbito-meatal line) at the level of the body of the striatum. The same transaxial tomographic plane was measured in a sequence of consecutive 10 minute scans for approximately two hours. A transmission scan, using an external ring source (Germanium 68/Gallium 68), was used to correct the measured emission data for tissue attenuation. After data collection the images were reconstructed using standard computer processing for all of the 10 minute scans (fig 1a). The picture element (pixel) response within each reconstructed tomographic image relates directly to the regional concentration of fluorine-18 in the tissue examined, and was corrected for physical decay from the time of injection. This procedure allowed us to follow the changes in concentration of radioactivity over time in the striatal region and in regions of the surrounding brain.

Data analysis
The tissue concentration of fluorine-18 in both striata and surrounding brain were obtained from regions of interest (ROIs) defined on the images of the emission scans. An example is given in fig 1b. The left and right striatal ROIs were obtained by summing all sequential images and determining a circular area (49 pixels; each pixel is 2.5 mm × 2.5 mm) containing the maximum concentration of isotope. Striatal ROIs thus determined were placed on each 10 minute scan separately to obtain the time course of radioactivity. The average value from left and right striatum in any one slice was taken as the "striatal value". The "surrounding brain value" was obtained as follows. A large ROI was placed on the image of the transmission scan just inside the rim representing the junction of skull and brain. This large ROI was then used to determine the outer border of the brain in each 10 minute emission scan. Two oval ROIs (radii 8 and 9, totalling 223 pixels) which completely encircled the two centrally located striatal regions with high activity were then subtracted from this large ROI to obtain the "surrounding brain value" (fig 1b). From the "striatal" and "surrounding brain" values a ratio was obtained for each 10 minute scan.

Patients and normal controls
Six healthy volunteers and 12 patients with Parkinson's disease were studied (table 1). The patients were divided into two groups. The first group comprised seven patients who had had the disease for only a relatively short time ("early" patients). Three of these patients were on regular treatment with a stable and sustained therapeutic response: their medication was stopped one day before the PET scan. The other four patients in this group had not been treated. The second group comprised five patients who had had the disease for longer, were more severely disabled, and whose response to levodopa treatment fluctuated ("on/off" patients). All of the second group were taking levodopa, but their response to treatment was variable. Throughout each day they had
Table 1  Clinical and scan data of normal controls and patients with Parkinson's disease

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Levodopa dosage (mg)</th>
<th>Disease duration (yr)</th>
<th>&quot;On/Off&quot;</th>
<th>Total disability score*</th>
<th>Usual drugs (daily dose)†</th>
<th>Time off drugs before scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>42</td>
<td>2.15</td>
<td>1</td>
<td></td>
<td>400</td>
<td>Levodopa (500 mg)</td>
<td>24 hrs</td>
</tr>
<tr>
<td>M</td>
<td>52</td>
<td>3.48</td>
<td>2</td>
<td></td>
<td>530</td>
<td>Levodopa (600 mg)</td>
<td>24 hrs</td>
</tr>
<tr>
<td>F</td>
<td>65</td>
<td>3.08</td>
<td>3</td>
<td></td>
<td>650</td>
<td>Nil</td>
<td>24 hrs</td>
</tr>
<tr>
<td>M</td>
<td>30</td>
<td>6.10</td>
<td>4</td>
<td></td>
<td>780</td>
<td>Nil</td>
<td>24 hrs</td>
</tr>
<tr>
<td>F</td>
<td>64</td>
<td>11.05</td>
<td>5</td>
<td></td>
<td>710</td>
<td>Nil</td>
<td>24 hrs</td>
</tr>
<tr>
<td>M</td>
<td>23</td>
<td>7.79</td>
<td>6</td>
<td></td>
<td>850</td>
<td>Nil</td>
<td>24 hrs</td>
</tr>
<tr>
<td>Mean</td>
<td>42.7</td>
<td>5.61</td>
<td>10</td>
<td></td>
<td>543</td>
<td>Nil</td>
<td>24 hrs</td>
</tr>
<tr>
<td>± SD</td>
<td>±18.0</td>
<td>±3.39</td>
<td></td>
<td></td>
<td>±2.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group I:

| M   | 71       | 3.44                 | 1                     |          | 400                    | Levodopa (500 mg)          | 24 hrs                    |
| M   | 62       | 5.50                 | 5                     |          | 530                    | Levodopa (600 mg)          | 24 hrs                    |
| M   | 64       | 5.66                 | 6                     |          | 450                    | Levodopa (600 mg)          | 24 hrs                    |
| M   | 32       | 4.83                 | 2.5                   |          | 255                    | Nil                        | 24 hrs                    |
| M   | 46       | 5.99                 | 3                     |          | 255                    | Nil                        | 24 hrs                    |
| M   | 76       | 3.82                 | 3                     |          | 710                    | Nil                        | 24 hrs                    |
| F   | 74       | 6.92                 | 2                     |          | 850                    | Nil                        | 24 hrs                    |
| Mean| 60.7     | 5.49                 | 3.2                   |          | 493                    | Nil                        | 24 hrs                    |
| ± SD| ±16.2    | ±2.02                | ±1.7                  |          | ±224                   | Nil                        | 24 hrs                    |

Group II:

| M   | 68       | 10                   | +                     |          | 1245                   | Levodopa (500 mg)          | 12 hrs                    |
| M   | 61       | 9.00                 | 10                    | +        | 735                    | Levodopa (500 mg), pergolide (20 mg) | 12 hrs |
| M   | 47       | 7.06                 | 8                     | +        | 750                    | Levodopa (600 mg)          | 12 hrs                    |
| M   | 41       | 5.82                 | 12                    | +        | 450                    | Levodopa (400 mg)          | 24 hrs                    |
| M   | 42       | 5.35                 | 7                     | +        | 1845                   | Levodopa (900 mg)          | 24 hrs                    |
| Mean| 51.8     | 6.81                 | 9.4                   |          | 1005                   | Nil                        | 24 hrs                    |
| ± SD| ±12.1    | ±63                  | ±2.0                  |          | ±550                   | Nil                        | 24 hrs                    |

*Maximum disability score on NYU scale is 2000.
†Levodopa dosage taken in combination with peripheral decarboxylase inhibitor.
§Group I patients comprise those with early disease or showing sustained response to levodopa therapy.
Group II comprises "on/off" patients.

periods of mobility (often with dyskinesias) alternating with periods of immobility. Such swings from mobility to immobility generally were related to the time at which levodopa was taken. Drugs were stopped the night before the PET scan in three "on/off" patients, but two could only tolerate a few hours without treatment. Each control subject and patient received 75 mg carbidopa orally about 30 minutes before the tracer was injected. Just before the patients were scanned they were examined and rated according to the New York University disability scale. The two groups of patients did not differ in respect of age or administered dose of tracer (table 1). However, patients in group II had suffered from the disease three times as long as those in group I (p < 0.001) and were more disabled (p < 0.05).

Written informed consent was obtained from each patient and healthy control. The project was approved by the Research Ethics Committee of the Hammersmith Hospital and the Maudsley Hospital, and permission for use of the isotope was obtained from the UK Administration of Radioactive Substances Advisory Committee.

Results

Figure 2 illustrates the time course of activity in striatum and surrounding brain in a control subject and an "on/off" patient. From 70 minutes onwards striatal (A), surrounding brain (B) and arterial plasma (C) activity at 10 min intervals after L-[18F] fluorodopa administration in a control subject and a patient with Parkinson's disease, expressed as a percentage of the arterial plasma peak activity. For clarity the striatal and surrounding brain values have been multiplied by a factor of 10.
Fig 3  Left hand panels (a, b, c) show sequences of 10 min scans from 70 to 170 min. (a) a normal control; the contrast between striatal uptake of L-$^{18}$F fluorodopa and that of surrounding brain increases constantly with time. (b) a patient with "early" Parkinson's disease; the contrast between uptake of the tracer in striatum and surrounding brain also is obvious, although the images are noisier owing to lower count rates, and there is little increase in contrast over time. (c) a case of Parkinson's disease showing the "on/off" phenomenon; the contrast between striatum and surrounding brain is hardly visible and the image reconstruction is very noisy owing to the low count rates. Right hand panel shows the ratios of activity in striatum to that in surrounding brain, for (A) normal subjects ($n = 6$) (top), (B) patients with "early" Parkinson's disease ($n = 7$) (middle), and (C) Parkinsonian patients with the "on/off" phenomenon ($n = 5$) (bottom).
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The ratio of activity in striatum compared to surrounding brain increased steadily over time in the normal subjects (fig 3a) for at least 120 min after injection of the tracer. Thereafter the ratio continued to increase, but the overall absolute count rates were extremely low by then, because of physical decay of the isotope, and only two subjects were studied at this time.

In contrast all Parkinsonian patients showed a lower ratio of activity for each time point after administration of the tracer (fig 3b, c). The variability of

Table 2 Average ratios of [L-18F] Fluorodopa uptake in striatum versus surrounding brain from 100 to 200 min after administration in patients with Parkinson’s disease

<table>
<thead>
<tr>
<th>Stable response patients</th>
<th>&quot;On/Off&quot; patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.58</td>
<td>1.35</td>
</tr>
<tr>
<td>1.49</td>
<td>1.29</td>
</tr>
<tr>
<td>1.43</td>
<td>1.22</td>
</tr>
<tr>
<td>1.47</td>
<td>1.42</td>
</tr>
<tr>
<td>1.38</td>
<td>1.33</td>
</tr>
<tr>
<td>1.61</td>
<td>1.34</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.47 ± 0.10 ± 0.07</td>
</tr>
</tbody>
</table>

p < 0.02* *Student’s t test.

Fig 4 The average ratios of radioactivity (striatum versus surrounding brain), plotted at intervals of 20 min, are shown for (A) normal subjects (n = 6), (B) patients with "early" Parkinson’s disease (n = 7), and (C) Parkinsonian patients with the "on/off" phenomenon (n = 5). Means ± SD are shown.

Fig 5 The mean ratio of radioactivity (striatum versus surrounding brain) for each patient between 100 and 200 min after administration of L-[18F] fluorodopa. The bars indicate means and standard deviations. The "on/off" group of patients differed significantly from the group of patients with a stable response to treatment. Two sample Student’s t test (v = 10; t = 2.84; p < 0.02).
this ratio in the patient group was considerably larger
than in the control subjects. This may have reflected
the lower overall absolute activity in the striatum of
patients, particularly towards the later part of the
study, when the absolute count rates of isotope
became very low.

Averaged ratios at 20 minute intervals for each
group of subjects are shown in fig 4. The average ratio
in the control subjects progressively increased
throughout the period of study. In contrast, in the
patient groups the average ratio plateaued about 100
min after isotope administration. In the group of
patients with “early” disease, the ratios were not
different whether the patients were treated or not. The
ratios for patients with the “on/off” phenomenon
were consistently lower than those for patients with
“early” disease.

For each patient the average ratio (of all available
ratios between 100 and 200 minutes after adminis-
tration of the tracer) was calculated (table 2). “On/off”
patients had a lower mean ratio compared with “early” patients (fig 5).

Discussion

This study confirms previous reports3–5 that
accumulation of radioactivity after administration of
fluorine-18 labelled levodopa takes place preferen-
tially in the striatum of healthy controls and in
patients with Parkinson’s disease. By observing the
time course of this uptake, we found a striking
difference between controls and patients.

PET can only determine the concentration of posi-
tron emitting isotopes in a certain volume of tissue.
The detected radioactivity (in this study fluorine-18)
may arise from L-[18F] fluorodopa itself or one of its
metabolic products, notably fluorinated dopamine
(DA), homovanillic acid (HVA), 3,4-dihydroxy
phenylacetic acid (DOPAC) or 3-methoxy,
4-hydroxy-phenylalanine (3-OM-dopa). The accumu-
lation of the isotope is greatest in striatum, which has
the highest concentration of native dopamine and
related anabolic and catabolic enzymes. Fluorodopa
has been shown to behave biochemically like
levodopa and fluorodopamine is stored in the stri-
atum.9–11 Further, in primates much of the striatal
radioactivity measured an hour after injection of
L-[18F] fluorodopa is due to [18F]-fluorodopamine.

Horne et al12 have shown in rats pretreated with
carbidopa that L-[14C] dopa accumulated in striatal
tissue mainly in the form of dopamine; dopa, HVA
and DOPAC together constituted a small fraction of
tissue radioactivity from 1 to 4 hours after intra-
venous administration. 3-OM-dopa rose slowly but
steadily in arterial plasma, was capable of passing the
blood brain barrier, and accounted for a considerable
fraction of brain tissue activity. However, 3-OM-dopa was distributed uniformly throughout
the brain. Furthermore, lesions of substantia nigra
markedly reduced striatal activity following adminis-
tration of L-[14C] dopa. Hefti et al13 reported that
lesioning of substantia nigra and medial forebrain
bundle in rats reduced dopamine concentration by
95% compared to the unlesioned contralateral side,
and also demonstrated that dopamine formation
from exogenous levodopa in striatum occurs mainly,
but not exclusively, within dopaminergic nerve ter-
minals. The clinical effects of levodopa administered in
pharmacological doses are exerted only after it is
decarboxylated to dopamine within the striatum.14
Reserpine depletes intraneuronal vesicular storage
sites of dopamine and other monoamines, and pre-
treatment of rats with reserpine decreased striatal
activity after L-[14C] dopa administration by 48% at
two hours.15 This reserpine effect has also been dem-
onstrated in monkey brain after L-[18F] fluorodopa.3

On the basis of these animal experiments we believe
that the initial activity seen in the human striatum is
an indication of its capacity to convert L-[18F]
fluorodopa to L-[18F] fluorodopamine. The absolute
concentration of tracer in striatum of normal human
controls reached a plateau between 30 and 45 min,
and thereafter decreased slightly.16 The ratio of activ-
ity between striatum and surrounding brain con-
tinued to rise steadily from 0–4 hours after injection
of the tracer in normal controls. This was due to a
greater decrease of activity in surrounding brain
rather than to an increase of activity in striatum. Our
study therefore indicates that activity derived from
L-[18F] fluorodopamine is retained in human striatum for
up to four hours after injection. This suggests that the
activity seen in striatum throughout the major part of
the period after injection of L-[18F] fluorodopa repre-
sents stored L-[18F] fluorodopamine.

In contrast to normal subjects, patients with Par-
kinson’s disease showed a different time course of stri-
tatal activity after injection of labelled L-fluorodopa.
The ratio of striatal to surrounding brain activity failed to rise after about 100 min from the injection,
indicating that the net accumulation of activity within
the striatal and surrounding brain tissue occurred at
the same rate. The absolute of Parkinsonian patients
to selectively retain the tracer in the striatum
suggests inability to store L-fluorodopamine, due to
loss of nigrostriatal dopamine terminals.

Patients with longstanding disease and fluctuating
“on/off” clinical response to levodopa treatment had
a significantly lower “storage capacity” compared
with “early” patients, either untreated or showing
sustained clinical response. The ratio of activity
between striatum and surrounding brain from about
100 min onwards was consistently lower in the youn-
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