Visualizing Vesicular Dopamine Dynamics in Parkinson’s Disease

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KEY WORDS PET; tetrabenazine; VMAT2

ABSTRACT It has been suggested that dopamine derived from exogenous levodopa may not follow vesicular dynamics in Parkinson’s disease (PD). Using a novel PET method based on the sensitivity of [11C]-dihydrotetrabenazine (DTBZ) binding to changes in vesicular dopamine levels, we show here that striatal [11C]-DTBZ binding decreases after levodopa administration in advanced PD, likely reflecting an increase in vesicular dopamine levels. Endogenous dopamine and exogenously derived dopamine seem to follow the same vesicular dynamics. Synapse 63:713–716, 2009. © 2009 Wiley-Liss, Inc.

INTRODUCTION

Levodopa remains the main treatment of Parkinson’s disease (PD) (de la Fuente-Fernández et al., 2004a). However, relatively little is still known about the dopamine dynamics in the parkinsonian brain. In particular, there is controversy as to whether dopamine derived from exogenous levodopa is incorporated into vesicles through the vesicular monoamine transporter type 2 (VMAT2) in advanced PD (Carta et al., 2007; Lopez et al., 2001; Melamed et al., 1980). Some animal studies suggest that other cell types, particularly serotonergic terminals, might contribute substantially to the synthesis, storage, and release of dopamine in severe PD cases (Carta et al., 2007; Lopez et al., 2001). Using a novel positron emission tomography (PET) method with repeated [11C]-dihydrotetrabenazine (DTBZ) measurements, we show here in vivo evidence that levodopa-derived dopamine is subject to vesicular dynamics in PD. Because tetrabenazine binds to the intravesicular site of VMAT2 (Liu and Edwards, 1997), the method is based on the sensitivity of [11C]-DTBZ binding to changes in vesicular dopamine levels. Such sensitivity most likely reflects a competition between [11C]-DTBZ and intravesicular dopamine for VMAT2 sites. We have previously shown that this competition might be amenable to in vivo detection by PET in humans (de la Fuente-Fernández et al., 2003), an observation that has recently been replicated in animal experiments (Kilbourn et al., 2008; Tong et al., 2008). In keeping with a mathematical model of striatal dopamine dynamics in PD (de la Fuente-Fernández et al., 2004a), we predicted that vesicular dopamine levels should increase after levodopa administration, and should therefore be associated with decreased [11C]-DTBZ binding. It should be noted that [11C]-DTBZ binding has previously been considered to be a relatively stable marker of dopamine neuron integrity (Vander Borght et al., 1995).

SUBJECTS AND METHODS

Subjects

Six subjects with moderate to severe PD were included in the study. All subjects (all male; age, mean ± SD, 52.67 ± 3.72 years) were on chronic treatment with levodopa. There were three subjects with stable response to chronic treatment with levodopa, and three subjects with motor complications (fluctuations and dyskinesias). Further details are provided in Table I.

Methods

Each subject underwent 3 [11C]-DTBZ PET studies on the same day: baseline (after at least 14-h off med-
results
Individual PET measurements are provided in Table II. As predicted (de la Fuente-Fernández et al., 2004a), we found statistically significant reductions in striatal [11C]-DTBZ BPND in relation to levodopa administration (P = 12.15; df = 2, 8; P = 0.0038). The [11C]-DTBZ BPND decreased 30 min after levodopa administration by some 14% in both caudate (mean, 14.4%; range, 3.3–27.3%) and putamen (mean, 14.1%; range, 5–21.9%), and showed a trend to recover to baseline values 4 h later (Fig. 2). Virtually identical results were obtained when the [11C]-DTBZ BPND were log transformed (P = 0.0035), and very similar patterns of levodopa-induced changes in [11C]-DTBZ binding were observed in the caudate nucleus and putamen (region × time interaction term, P = 0.27 and P = 0.99 for raw and log transformed data, respectively). Significant reductions in [11C]-DTBZ BPND were also found when each region was analyzed independently (for caudate, P = 0.015; for putamen, P = 0.0060; log transformed data gave very similar results: P = 0.012 and P = 0.0071, respectively). These results clearly suggest that exogenous levodopa is converted into dopamine and incorporated into presynaptic monoamine vesicles. Remarkably, the 4-h [11C]-DTBZ BPND values were still below baseline values by some 8% in both caudate and putamen, which might suggest that a substantial amount of dopamine derived from exogenous levodopa is still present within striatal vesicles long after levodopa administration. As expected, subjects with more advanced parkinsonism had lower [11C]-DTBZ BPND values than subjects with milder disease severity (fluctuators vs. stable responders, P = 0.022). Thus, for example, baseline [11C]-DTBZ BPND values were substantially higher in stable responders: 35.4% higher in caudate (P = 0.027) and 33.4% higher in putamen (P = 0.033). However, there were no between-group differences in the overall pattern of changes in [11C]-DTBZ binding over the 4-h study (motor fluctuations × time interaction term, P = 0.53 and P = 0.77 for raw and log transformed data, respectively). This observation suggests that presynaptic vesicles govern dopamine dynamics across a wide range of disease severity.

Discussion
Although we had previously shown that [11C]-DTBZ PET can detect changes in the intravesicular concentration of dopamine in humans (de la Fuente-Fernández et al., 2003), this study is the first in vivo demon-

**Table I. Clinical characteristics of Parkinson's disease subjects**

<table>
<thead>
<tr>
<th></th>
<th>Stable responders</th>
<th>Fluctuators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>3/0</td>
<td>3/0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.67 ± 2.31</td>
<td>49.67 ± 1.53</td>
</tr>
<tr>
<td>Duration of Parkinson's disease (years)</td>
<td>4.00 ± 2.90</td>
<td>9.33 ± 6.51</td>
</tr>
<tr>
<td>UPDRS-OFF</td>
<td>25.00 ± 2.00</td>
<td>38.00 ± 17.06</td>
</tr>
<tr>
<td>Equivalent levodopa dose (mg/day)</td>
<td>458.33 ± 212.62</td>
<td>740.00 ± 17.52</td>
</tr>
<tr>
<td>LD withdrawal time to LD challenge (h)</td>
<td>18.67 ± 2.52</td>
<td>18.83 ± 2.31</td>
</tr>
</tbody>
</table>

Age was significantly different between groups (P = 0.020). LD = levodopa. Dopaminomimetic treatment is given in equivalents of standard-release levodopa/carbidopa.

**Table II. PET raw data: [11C]-DTBZ BPND**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fluctuator</th>
<th>Caudate (baseline)</th>
<th>Caudate (0.5 h)</th>
<th>Caudate (4 h)</th>
<th>Putamen (baseline)</th>
<th>Putamen (0.5 h)</th>
<th>Putamen (4 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>0.492</td>
<td>0.394</td>
<td>0.486</td>
<td>0.235</td>
<td>0.184</td>
<td>0.225</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>0.650</td>
<td>0.628</td>
<td>0.687</td>
<td>0.360</td>
<td>0.332</td>
<td>0.326</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>0.582</td>
<td>0.423</td>
<td>0.478</td>
<td>0.298</td>
<td>0.248</td>
<td>0.243</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>0.672</td>
<td>0.596</td>
<td>0.603</td>
<td>0.360</td>
<td>0.282</td>
<td>0.338</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>0.360</td>
<td>0.323</td>
<td>0.328</td>
<td>0.185</td>
<td>0.175</td>
<td>0.177</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>0.378</td>
<td>0.325</td>
<td>0.331</td>
<td>0.259</td>
<td>0.230</td>
<td>0.247</td>
</tr>
</tbody>
</table>
stration that dopamine derived from exogenous levodopa is incorporated into monoamine vesicles in the human parkinsonian brain. The study shows that [$^{11}$C]-DTBZ is sensitive to intravesicular dopamine levels. As predicted by a mathematical model of dopamine dynamics (see Fig. 2 in de la Fuente-Fernández et al., 2004a), this observation suggests that [$^{11}$C]-DTBZ binding may underestimate the severity of the damage to the nigrostriatal nerve terminal in PD. This could be particularly problematic in studies involving levodopa-treated subjects, when comparing fluctuators and stable responders.

It should be noted that several cell types, including serotonin neurons, have the capability of converting levodopa into dopamine (Cooper et al., 2003; Liu and Edwards, 1997). In addition, VMAT2 is involved in the vesicular trapping of not only dopamine but also noradrenaline and serotonin (Cooper et al., 2003). Hence, it could be argued that our PET results may not be specific for the nigrostriatal dopamine system. In other words, it is conceptually possible that the levodopa-induced changes in [$^{11}$C]-DTBZ binding that we observed could be partly related to the incorporation of exogenously derived dopamine into noradrenergic or serotonergic terminals projecting to the striatum. Indeed, some animal experiments have suggested that serotonin projections may contribute significantly to the vesicular storage and release of dopamine in the dopamine-denervated striatum (Carta et al., 2007; Lopez et al., 2001). However, we found a similar pattern of levodopa-induced vesicular dopamine changes (both at 0.5 and 4 h) in regions with different degrees of PD pathology (caudate vs. putamen), and also in patients with different disease severity (stable responders vs. fluctuators). Moreover, the vesicular dopamine pattern described here clearly seems to mirror levodopa-induced changes in synaptic dopamine levels. In fact, we found virtually identical percentage changes in synaptic dopamine levels in patients with the same degree of disease severity (de la Fuente-Fernández et al., 2001, 2004b). These observations suggest that, unless serotonin and dopamine terminals share the same pattern of release/reuptake, serotonin terminals may not contribute substantially to modulate the dynamics of striatal vesicular dopamine in moderately severe PD. In other words, exogenously derived dopamine is likely to follow the same vesicular dynamics as endogenous dopamine in the parkinsonian brain. In keeping with this notion, it has recently been shown in a preliminary study that levodopa administration normalizes [$^{11}$C]-DTBZ BPND values in rodents with pharmacologically induced striatal dopamine depletion (Kilbourn et al., 2008).

The dynamic PET method here described opens a new avenue for research. In particular, the method has important applications to the in vivo study of vesicular dopamine dynamics in PD subjects receiving dopamine cell transplant.

**ACKNOWLEDGMENTS**

RFF is the recipient of the James A. Moore Chair in Parkinson’s Research. The authors thank Edwin Mak for statistical assistance.

**REFERENCES**


