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Short Communication

Visualizing Vesicular Dopamine Dynamics in Parkinson's Disease

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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 [^{11}C]-dihydrotetrabenazine (DTBZ) binding to changes in vesicular dopamine levels, we show here that striatal [^{11}C]-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.**

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 [^{11}C]-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 [^{11}C]-DTBZ binding to changes in vesicular dopamine levels. Such sensitivity most likely reflects a competition between [^{11}C]-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 [^{11}C]-DTBZ binding. It should be noted that [^{11}C]-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 [^{11}C]-DTBZ PET studies on the same day: baseline (after at least 14-h off med-

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ication), 30 min after oral administration of standard-release 250/25 mg of levodopa/carbidopa, and 4 h after levodopa. The levodopa challenge was performed between 16 and 21.5 h after withdrawal of medications (Table I). All PET scans were performed in three-dimensional mode using an ECAT 953B/31 tomograph (CTI/Siemens, Knoxville, TN). Using a Harvard infusion pump, we injected i.v. over 60 s 185 MBq of [^{11}C]-DTBZ for each scan. Details of PET data analysis for [^{11}C]-DTBZ are reported elsewhere (de la Fuente-Fernández et al., 2003). Briefly, one circular region of interest (ROI) of 61.2 mm^2 was positioned on the head of each caudate nucleus and adjusted to maximize the average ROI activity. Three circular ROIs of 61.2 mm^2 were placed without overlap along the axis of each putamen. Background activity was calculated using three circular ROIs (296 mm^2) on the occipital cortex on each side. The [^{11}C]-DTBZ binding potential (BP) ($BP_{ND} = B_{max}/K_d$) was determined using the tissue input Logan graphical method. [^{11}C]-DTBZ BP_{ND} data values were analyzed using random effects repeated measures analysis of covariance. As a first approach, three factors were considered: region (caudate and putamen), time (baseline, 0.5 and 4 h PET measurements), and motor fluctuations (yes/no). Then, the same analysis was repeated independently for caudate and putamen.

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 [^{11}C]-DTBZ BP_{ND} in relation to levodopa administration (F = 12.15; df = 2, 8; P = 0.0038). The [^{11}C]-DTBZ BP_{ND} decreased 30 min after levodopa administration by some 14% (Fig. 1) 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 [^{11}C]-DTBZ BP_{ND} were log transformed (P = 0.0035), and very similar patterns of levodopa-induced changes in [^{11}C]-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 [^{11}C]-DTBZ BP_{ND} 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 [^{11}C]-DTBZ BP_{ND} 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 [^{11}C]-DTBZ BP_{ND} values than subjects with milder disease severity (fluctuators vs. stable responders, P = 0.022). Thus, for example, baseline [^{11}C]-DTBZ BP_{ND} 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 [^{11}C]-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.

TABLE I. Clinical characteristics of Parkinson's disease subjects

	Stable responders	Fluctuators
Male/female	3/0	3/0
Age (years)	55.67 ± 2.31	49.67 ± 1.53
Duration of Parkinson's disease (years)	4.00 ± 2.00	9.33 ± 6.51
UPDRS-OFF	25.00 ± 2.00	38.00 ± 17.06
Equivalent levodopa dose (mg/day)	458.33 ± 212.62	740.00 ± 17.32
LD withdrawal time to LD challenge (h)	18.67 ± 2.52	18.83 ± 2.31

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

DISCUSSION

Although we had previously shown that [^{11}C]-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 II. PET raw data: [^{11}C]-DTBZ BP_{ND}

Subject	Fluctuator	Caudate (baseline)	Caudate (0.5 h)	Caudate (4 h)	Putamen (baseline)	Putamen (0.5 h)	Putamen (4 h)
1	Yes	0.492	0.394	0.486	0.235	0.184	0.225
2	No	0.650	0.628	0.667	0.360	0.332	0.326
3	No	0.582	0.423	0.478	0.298	0.248	0.243
4	No	0.672	0.596	0.603	0.360	0.282	0.338
5	Yes	0.360	0.323	0.328	0.185	0.175	0.177
6	Yes	0.378	0.325	0.331	0.259	0.230	0.247

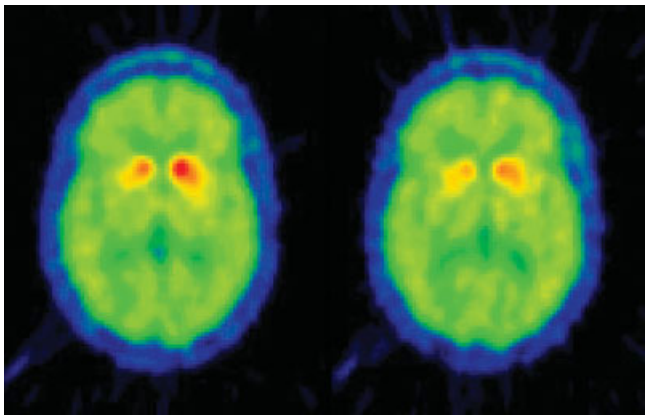

Fig. 1. Striatal [^{11}C]-DTBZ binding at baseline (left) and 0.5 h after levodopa administration (right) in one Parkinson's subject.

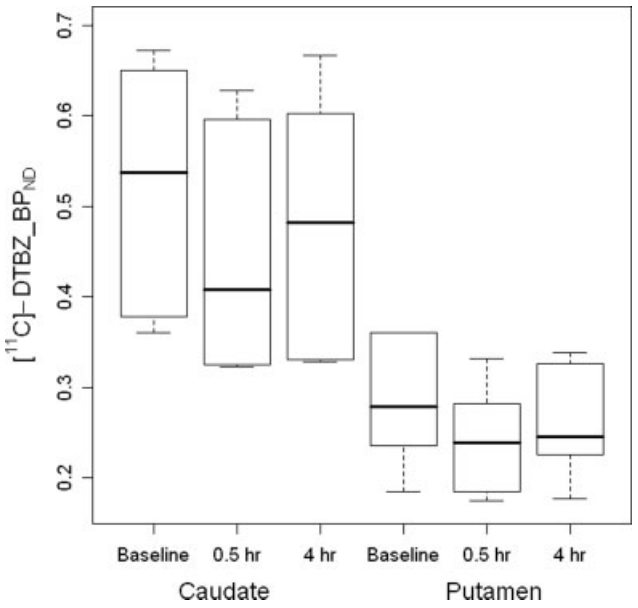

Fig. 2. Box plots of [^{11}C]-DTBZ binding before (baseline) and after (0.5 and 4 h) oral administration of levodopa (250/25 mg levodopa/carbidopa). Taking region (caudate and putamen) as a factor, there were statistically significant differences among the three measurements (F = 12.15; df = 2, 8; P = 0.0038), which likely reflects increased vesicular dopamine levels after levodopa administration. Virtually identical results were obtained when caudate and putamen [^{11}C]-DTBZ BP_{ND} values were log transformed (P = 0.0035). The horizontal solid line within each box represents the median; error bars encompass all data points.

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 norepinephrine 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 BP_{ND} 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.

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REFERENCES

Carta M, Carlsson T, Kirik D, Björklund A. 2007. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesias in parkinsonian rats. Brain 130:1819–1833.

Cooper JR, Bloom FE, Roth RH. 2003. The biochemical basis of neuropharmacology. Oxford: Oxford University Press.

de la Fuente-Fernández R, Lu JQ, Sossi V, Jivan S, Schulzer M, Holden JE, Lee CS, Ruth TJ, Calne DB, Stoessl AJ. 2001. Biochemical variations in the synaptic level of dopamine precede motor fluctuations in Parkinson's disease: PET evidence of increased dopamine turnover. Ann Neurol 49:298–303.

de la Fuente-Fernández R, Furtado S, Guttman M, Furukawa Y, Lee CS, Calne DB, Ruth TJ, Stoessl AJ. 2003. VMAT2 binding is elevated in dopa-responsive dystonia: Visualizing empty vesicles by PET. Synapse 49:20–28.

de la Fuente-Fernández R, Schulzer M, Mak E, Calne DB, Stoessl AJ. 2004a. Presynaptic mechanisms of motor fluctuations in Parkinson's disease: A probabilistic model. Brain 127: 888–899.

de la Fuente-Fernández R, Sossi V, Huang Z, Furtado S, Lu JQ, Calne DB, Ruth TJ, Stoessl AJ. 2004b. Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: Implications for dyskinesias. Brain 127: 2747–2754.

Kilbourn MR, Butch ER, Desmond T, Sherman P, Frey KA. 2008. Dopamine depletion increases in vivo [^{11}C]DTBZ binding in awake rat brain. Neuroimage 41(Suppl 2):T54 (Abstract).

Liu Y, Edwards RH. 1997. The role of vesicular transport proteins in synaptic transmission and neural degeneration. Annu Rev Neurosci 20:125–156.

Lopez A, Muñoz A, Guerra MJ, Labandeira-Garcia JL. 2001. Mechanisms of the effects of exogenous levodopa on the dopamine-denervated striatum. Neuroscience 103:639–651.

Melamed E, Hefti F, Liebman J, Schlosberg AJ, Wurtman RJ. 1980. Serotonergic neurones are not involved in action of L-dopa in Parkinson's disease. Nature 283:772–774.

Tong J, Wilson AA, Boileau I, Houle S, Kish SJ. 2008. Dopamine modulating drugs influence striatal [^{11}C]DTBZ binding in rats: VMAT2 binding is sensitive to changes in vesicular dopamine concentration. Synapse 62:873–876.

Vander Borght T, Kilbourn M, Desmond T, Kuhl D, Frey K. 1995. The vesicular monoamine transporter is not regulated by dopaminergic drug treatments. Eur J Pharmacol 294:577–583.