VMAT2 Binding Is Elevated in Dopa-Responsive Dystonia: Visualizing Empty Vesicles by PET

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ABSTRACT Dopa-responsive dystonia (DRD) is a lifelong disorder in which dopamine deficiency is not associated with neuronal loss and therefore it is an ideal human model for investigating the compensatory changes that occur in response to this biochemical abnormality. Using positron emission tomography (PET), we examined the (±)-α-[^{11}C]dihydrotetrabenazine ([^{11}C]DTBZ) binding potential of untreated DRD patients and normal controls. Two other PET markers of presynaptic nigrostriatal function, *d-threo*-[^{11}C]methylphenidate ([^{11}C]MP) and 6-[^{18}F]fluoro-L-dopa ([^{18}F]-dopa), and [^{11}C]raclopride were also used in the study. We found increased [^{11}C]DTBZ binding potential in the striatum of DRD patients. By contrast, no significant changes were detected in either [^{11}C]MP binding potential or [^{18}F]-dopa uptake rate constant. In addition, we found evidence for increased dopamine turnover in one DRD patient by examining changes in [^{11}C]raclopride binding potential in relation to levodopa treatment. We propose that the increase in [^{11}C]DTBZ binding likely reflects the dramatic decrease in the intravesicular concentration of dopamine that occurs in DRD; upregulation of vesicular monoamine transporter type 2 (VMAT2) expression may also contribute. Our findings suggest that the striatal expression of VMAT2 (as estimated by [^{11}C]DTBZ binding) is not coregulated with dopamine synthesis. This is in keeping with a role for VMAT2 in other cellular processes (i.e., sequestration and release from the cell of potential toxic products), in addition to its importance for the quantal release of monoamines. **Synapse 49:20–28, 2003.**

INTRODUCTION

Endogenous dopamine is synthesized from tyrosine and then stored in presynaptic vesicles until depolarization-induced release into the synaptic cleft (Cooper et al., 1996). In the central nervous system the vesicular packaging of dopamine is carried out by the vesicular monoamine transporter type 2 (VMAT2) (Liu and Edwards, 1997). Although VMAT2 is not selective for any particular monoamine, in the striatum most VMAT2 sites are located in nigrostriatal dopaminergic terminals. Binding to striatal VMAT2 sites can be studied in vivo by positron emission tomography (PET) using (±)-α-[^{11}C]dihydrotetrabenazine ([^{11}C]DTBZ) (Frey et al., 1996).

It has long been assumed that the primary function of vesicles is neurotransmitter storage in order to 1) prevent the potential cytotoxic effect of high cytoplasmic levels of neurotransmitters (and also to prevent neurotransmitter metabolism in the cytoplasm), and 2) guarantee quantal release of neurotransmitters (Cooper et al., 1996; Liu and Edwards, 1997). This theory predicts a linkage between dopamine synthesis and

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TABLE I. Clinical characteristics and GTP-CH I gene abnormalities of patients with dopa-responsive dystonia

	Gender	Age (yr)	MCS	Dystonia	GTP-CH I gene abnormality
Family A*					
Patient 1	F	58	14	Foot dystonia	T614A (Val 205 Glu)
Patient 2	F	45	12	None	T614A (Val 205 Glu)
Patient 3	M	18	10	Writer's cramp	T614A (Val 205 Glu)
Family B**					
Patient 1	F	25	2	Foot dystonia	Deletion involving exon 3
Patient 2	F	25	0	Foot dystonia	Deletion involving exon 3

*The proband having the same missense mutation was reported previously (as an apparently sporadic patient at that time) (Furukawa et al., 1999).
**The large genomic deletion, which cannot be detected by conventional genomic DNA sequencing of the GTP-CH I gene, was reported previously (Furukawa et al., 2000). GTP-CH I = GTP cyclohydrolase I.
MCS = Modified Columbia Scale (motor score for parkinsonism; dystonia not included).

vesicular biogenesis (also, between dopamine synthesis and VMAT2 expression). Some authors, however, have suggested that vesicular neurotransmitter transporters (and in particular VMAT2) may have evolved from an ancient detoxification system (Schuldiner, 1994; Liu and Edwards, 1997). If, in an evolutionary scale, the primary function of vesicles is indeed to protect the cell from the action of toxins present in the cytoplasm, there should be no connection between dopamine synthesis and VMAT2 expression.

Dopa-responsive dystonia (DRD) is a human disorder that represents a biochemically pure state of dopamine deficiency (Rajput et al., 1994). DRD is an autosomal dominant disorder caused by mutations in the gene for guanosine triphosphate (GTP)-cyclohydrolase I (GTP-CH I) (Ichinose et al., 1994), which is the rate-limiting enzyme in the synthesis of tetrahydrobiopterin (an essential cofactor for tyrosine hydroxylase). Tyrosine hydroxylase, which catalyzes the conversion of tyrosine into levodopa, is in turn the rate-limiting enzyme for dopamine synthesis (Cooper et al., 1996). Thus, we can in many ways consider DRD as equivalent to a heterozygote knockout model of dopamine deficiency. Clinically, most DRD patients present with dystonia and variable degrees of parkinsonism and have an excellent and sustained response to low doses of levodopa (Hwang et al., 2001; Nutt and Nygaard, 2001).

Because drug treatment could confound the results of PET (Lee et al., 2000), we studied only levodopa-naïve DRD patients in order to examine in vivo the expression of VMAT2 sites in nigrostriatal dopaminergic terminals. According to our hypothesis that dopamine synthesis and VMAT2 expression are not coregulated, we predicted that [^{11}C]DTBZ binding should not be downregulated in DRD. We also analyzed two other markers of presynaptic dopaminergic function: 1) *d-threo*-[^{11}C]methylphenidate ([^{11}C]MP), which binds to the plasma membrane dopamine transporter (DAT), and 2) 6-[^{18}F]fluoro-L-dopa ([^{18}F]-dopa), whose uptake rate constant is an index of both the activity of the enzyme aromatic L-amino acid decarboxylase (AAAD; dopa decarboxylase), and the storage capacity of the nigrostriatal dopamine system (Lee et al., 2000). AAAD, which catalyzes the conversion of levodopa into dopamine, is not considered to be a rate-limiting enzyme (Cooper et al., 1996) and is not thought to be affected in DRD (Snow et al., 1993).

MATERIALS AND METHODS

Subjects

We recruited five levodopa-naïve DRD patients (one male, four female; age, 34.20 ± 16.69 years; range, 18–58 years) from two different families (Table I). The clinical diagnosis of DRD was confirmed by genetic analysis. In Family A, the GTP-CH I gene mutation was in exon 5 (a heterozygous T-to-A change at nucleotide position 614) and predicted a Val 205 Glu substitution (Furukawa et al., 1999). In Family B, there was a large heterozygous deletion involving exon 3 of the GTP-CH I gene (Furukawa et al., 2000). Further details of the GTP-CH I gene abnormalities in these two families can be found elsewhere (Furukawa et al., 1999, 2000).

All five DRD patients and 21 normal volunteers (12 male, 9 female) of similar age (46.62 ± 11.79 years; range, 27–61 years) were subject to the same PET protocol using three presynaptic dopaminergic ligands (as described below). In addition, one DRD patient (Patient 1 of Family A; Table I; female, 58 years of age) underwent [^{11}C]raclopride scans, whose results were compared to another group of age-matched normal controls (n = 5; one male, four female; age, 57 ± 11.96 years) and to four patients (three male, one female; age, 66.25 ± 9.98 years) with clinically definite Parkinson's disease who had similar symptom severity and a stable response to chronic levodopa treatment (de la Fuente-Fernández et al., 2001b). All subjects (patients and controls) underwent clinical assessments, which included motor scoring according to a Modified Columbia Scale (MCS) (Duvoisin, 1971). The study was approved by the University of British Columbia Ethics Committee and the Institutional Review Board of the Centre for Addiction and Mental Health.

PET protocols

All PET scans were performed in three-dimensional mode using an ECAT 953B/31 tomograph (CTI/Siemens, Knoxville, TN, USA). All five DRD patients and

21 normal controls (see above) underwent three consecutive PET scans on the same day in the following order: 1) [^{11}C]DTBZ; 2) [^{11}C]MP; and 3) [^{18}F]-dopa. Scans were separated by a 2.5-h interval to allow for radioactive decay. Using a Harvard infusion pump, we intravenously injected over 60 sec 237 MBq of [^{11}C]DTBZ, 185 MBq of [^{11}C]MP, or 75–130 MBq of [^{18}F]-dopa for each scan. Subjects were pretreated with 200 mg of carbidopa 1 h before the injection of [^{18}F]-dopa.

Patient 1 of Family A (Table I) also underwent four [^{11}C]raclopride scans: one at baseline (i.e., before starting levodopa treatment) and three after 6 months on chronic levodopa treatment. At 6 months the three scans were performed on the same day according to the following protocol: first scan (second baseline), 18 h after withdrawal of medication; the second scan, 1 h after oral administration of standard-release 250/25 mg of levodopa/carbidopa; and the third scan, 4 h after levodopa administration. These three scans were separated by a 2.5-h interval to allow for tracer decay. This patient's baseline [^{11}C]raclopride results were compared to those of five age-matched normal controls, and her response to levodopa (change in [^{11}C]raclopride binding potential) was compared with that of four patients with Parkinson's disease of almost identical clinical severity (see above). Patients were pretreated with domperidone (a peripherally selective dopamine D_2 receptor antagonist) to prevent peripheral side effects of levodopa. For each [^{11}C]raclopride scan, subjects received an intravenous injection of 185 MBq of [^{11}C]raclopride (specific activity >1,000 Ci/mmol).

PET data analysis

Details of PET data analyses for [^{11}C]DTBZ, [^{11}C]MP, [^{18}F]-dopa, and [^{11}C]raclopride can be found elsewhere (Lee et al., 2000; de la Fuente-Fernández et al., 2001b). Briefly, one circular region of interest (ROI) of 61.2 mm^2 was positioned on the head of each caudate nucleus (Caud) 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 (P1 = rostral putamen, P2 = intermediate putamen, and P3 = caudal putamen) and were similarly adjusted. Background activity was calculated using three circular ROIs (296 mm^2) on the occipital cortex on each side ([^{11}C]DTBZ, [^{11}C]MP, and [^{18}F]-dopa) or a single elliptical ROI (2,107 mm^2) on the cerebellum ([^{11}C]raclopride). For [^{11}C]DTBZ, [^{11}C]MP, and [^{11}C]raclopride we determined the binding potential ($BP = B_{max}/K_d$, where B_{max} represents binding concentration and K_d is the apparent dissociation constant) using a graphical approach and a tissue input function as described elsewhere (Logan et al., 1996). For sites at which dopamine binds competitively, it can be shown that K_d (apparent) = $K_d \{1 + [DA]/K_{DA}\}$, where K_{DA} is the affinity of dopamine for the receptor/transporter, and [DA] represents the concentration of dopamine (de la Fuente-Fernández et al., 2001a). The [^{18}F]-dopa data were analyzed with a graphical method (Patlak and Blasberg, 1985) using the radioactivity time course of the occipital cortex as input function. This method gives a [^{18}F]-dopa uptake rate constant (K_{occ}).

TABLE II. Positron emission tomography measurements in patients with dopa-responsive dystonia (DRD) and normal volunteers

	DRD	Controls
[^{11}C]DTBZ		
Caudate	1.205 ± 0.138	0.953 ± 0.093
Putamen	1.182 ± 0.134	0.979 ± 0.097
[^{11}C]MP		
Caudate	1.854 ± 0.283	1.551 ± 0.231
Putamen	1.658 ± 0.278	1.413 ± 0.233
[^{18}F]-dopa		
Caudate	0.0107 ± 0.0013	0.0112 ± 0.0009
Putamen	0.0095 ± 0.0012	0.0102 ± 0.0012

[^{11}C]DTBZ = (±)-α-[^{11}C]dihydrotetrabenazine; [^{11}C]MP = *d-threo*-[^{11}C]methylphenidate; [^{18}F]-dopa = 6-[^{18}F]fluoro-L-dopa.

Statistical analyses

Caudate and putamen PET data were compared between DRD patients and normal controls by *t*-tests; analysis of covariance (ANCOVA) (Altman, 1991) was used to adjust for age differences where appropriate (Kish et al., 1995; Frey et al., 1996; Wang et al., 1997; Lee et al., 2000). Repeated measures analysis of variance (Altman, 1991), without (ANOVA) and with (ANCOVA) adjustment for age differences, was used to analyze PET measurements in the different striatal subregions (Caud, P1, P2, and P3). All PET values represent the mean of both sides of the striatum for each particular subregion. Statistical significance was set at two-tailed *P*-values less than 0.05.

RESULTS

Clinical characteristics, genetic mutations, and PET measurements of DRD patients are summarized in Tables I and II. As compared to normal controls, we found an increase in [^{11}C]DTBZ binding potential values in both the caudate nucleus ($P < 0.0001$) and the putamen ($P < 0.001$) (Table II). These between-group differences were still highly significant after adjusting for age ($P < 0.001$ for the caudate nucleus and $P < 0.005$ for the putamen). Subregional differences in striatal [^{11}C]DTBZ binding were also highly significant ($P < 0.0001$), with DRD patients showing higher binding potential values in each striatal subregion ($P < 0.001$; $P < 0.005$ after adjusting for age) (Fig. 1). There was a trend for an increasing rostrocaudal gradient in binding potential, except for the most caudal part of the putamen (P3) (Fig. 1). This striatal subregion (P3) is often subject to partial volume effects (Kessler et al., 1984).

Although [^{11}C]MP binding potential values were also higher in DRD patients compared with controls ($P = 0.018$ for the caudate nucleus; $P = 0.053$ for the puta-

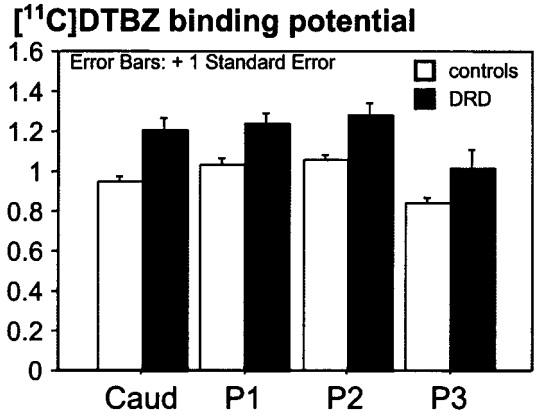

Fig. 1. [^{11}C]DTBZ binding potential (mean ± SEM) in the striatum of patients with dopa-responsive dystonia (DRD; solid bars) and normal controls (open bars). Regions of interest (ROIs) are on the head of the caudate nucleus (Caud) and on the putamen, from rostral (P1) to caudal (P3). DRD patients had increased binding potential values in each striatal subregion ($P < 0.005$). [^{11}C]DTBZ = (±)-α-[^{11}C]dihydrotetrabenazine.

men) (Table II; Fig. 2A), these differences were no longer statistically significant after adjusting for age differences between the two groups ($P = 0.13$ for the caudate nucleus; $P = 0.48$ for the putamen) (Fig. 2B). There were subregional differences in striatal [^{11}C]MP binding ($P < 0.0001$) according to a decreasing rostrocaudal gradient, but there were no significant age-adjusted differences between DRD patients and control subjects ($P = 0.36$) (Fig. 2B).

The [^{18}F]-dopa uptake rate constant, an index of the activity of AAAD ([^{18}F]-dopa → [^{18}F]-dopamine) and the subsequent storage of [^{18}F]-dopamine in synaptic vesicles (Lee et al., 2000) was found to be within the normal range in our DRD patients ($P = 0.30$ for the caudate nucleus; $P = 0.31$ for the putamen) (Table II; Fig. 2C). This confirms that the activity of AAAD is essentially normal in this disorder, in keeping with our previous observations (Snow et al., 1993).

In order to assess whether the turnover of dopamine might be affected by dopamine deficiency, we examined the displacement of [^{11}C]raclopride binding following levodopa administration in one of our DRD patients (Patient 1 of Family A; Table I). This paradigm is based on the ability of dopamine to compete with [^{11}C]raclopride for D_2/D_3 dopamine receptors (de la Fuente-Fernández et al., 2001b). Thus, the difference between baseline and post-levodopa [^{11}C]raclopride binding potentials is an estimate of levodopa-induced release of dopamine.

In keeping with our previous observations (Kishore et al., 1998), the baseline [^{11}C]raclopride binding potential was above the upper limit of age-matched control values in both the caudate nucleus (patient, 2.705; controls, 2.403 ± 0.139, range 2.237–2.585) and the putamen (patient, 2.793; controls, 2.431 ± 0.209, range 2.116–2.658). This increase most likely reflects a com-

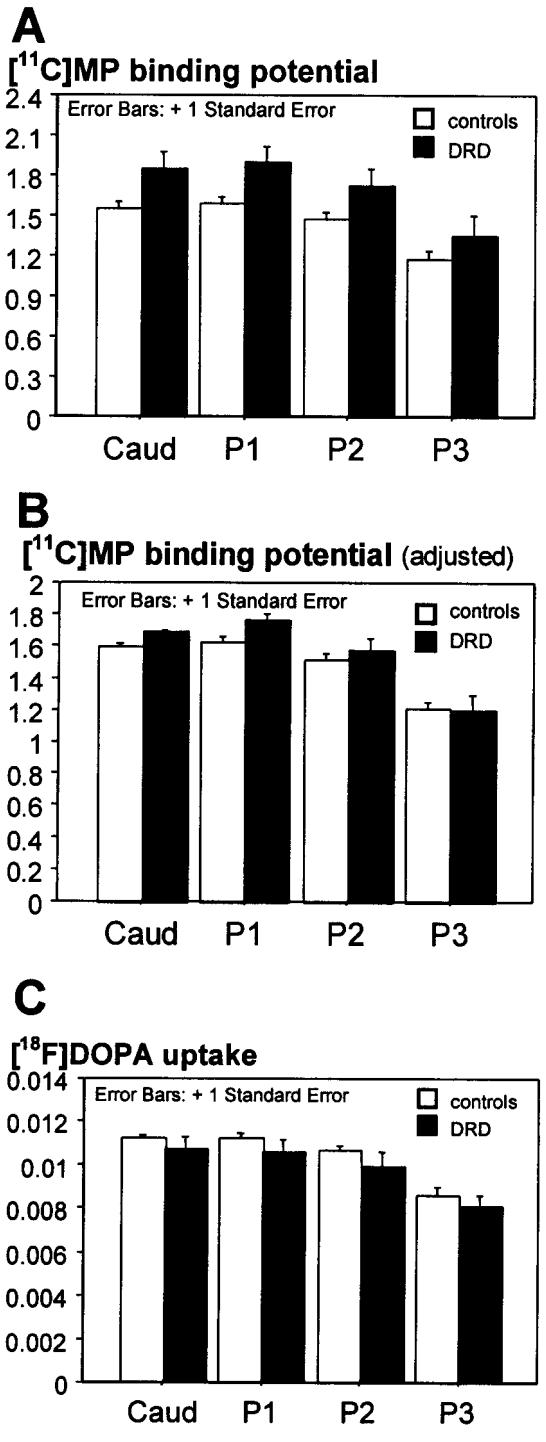

Fig. 2. [^{11}C]MP binding potential (**A,B**) and [^{18}F]-dopa uptake rate constant (K_{occ}) (**C**) (mean ± SEM) in the striatum of patients with dopa-responsive dystonia (DRD; solid bars) and normal controls (open bars). ROIs are on the head of the caudate nucleus (Caud) and on the putamen, from rostral (P1) to caudal (P3). [^{11}C]MP binding potential values were higher in DRD patients than in controls (**A**). However, there was a strong age effect on [^{11}C]MP binding potential ($P < 0.0001$ for both caudate and putamen). No statistically significant differences were detected after adjusting for age differences between groups by ANCOVA (**B**) (for the caudate nucleus, $P = 0.13$; for the putamen, $P = 0.48$). DRD [^{18}F]-dopa uptake (K_{occ}) values (**C**) were within the normal range both in the caudate nucleus ($P = 0.30$) and the putamen ($P = 0.31$). [^{11}C]MP = *d-threo*-[^{11}C]methylphenidate; [^{18}F]-dopa = 6-[^{18}F]fluoro-L-dopa.

TABLE III. Levodopa-related changes in [^{11}C]raclopride binding potential in one patient with dopa-responsive dystonia

Striatal subregion	Baseline 1 (B1)	Baseline 2 (B2)	% change 1 (B1 vs. B2)	4 h after LD	% change 2 (B2 vs. 4 h-LD)
Caudate (Caud)	2.705	2.402	11.20	2.046	14.80
Putamen					
Rostral (P1)	3.059	2.836	7.27	2.399	15.41
Intermediate (P2)	3.052	2.794	8.47	2.293	17.92
Caudal (P3)	2.267	2.129	6.08	1.709	19.72

Baseline 1 = before starting treatment with levodopa/carbidopa (375 mg/day).
Baseline 2 = 18 h after withdrawal of levodopa (6 months after Baseline 1). 4 h-LD = 4 h after oral administration of 250/25 mg of levodopa/carbidopa (on the same day as Baseline 2).

bination of synaptic dopamine depletion and dopamine receptor upregulation. Striatal changes in [^{11}C]raclopride binding potential between the first (before starting levodopa treatment) and second (6 months later, 18 h after withdrawal of medication) baseline scans were found to follow a decreasing rostrocaudal gradient (11% change in the caudate nucleus; 7% change in the putamen) (Table III).

DISCUSSION

In contrast to Parkinson's disease, where dopamine deficiency is associated with cell loss, DRD offers an excellent opportunity to study compensatory changes that may occur in response to pure dopamine deficiency. We examined whether dopamine synthesis and VMAT2 expression are coregulated. If vesicular biogenesis were linked to neurotransmitter synthesis, VMAT2 expression (and thus [^{11}C]DTBZ binding) should be reduced in DRD. However, we found increased [^{11}C]DTBZ binding potential in DRD patients. This may be related to decreased competition for [^{11}C]DTBZ binding from intravesicular dopamine (i.e., a decrease in K_d [apparent]), elevated cell activity leading to an increase in B_{max}, or a combination of the two. We conclude that dopamine synthesis and VMAT2 expression are independent processes. It should be emphasized that the term VMAT2 expression refers to the density of VMAT2 binding sites (as estimated by [^{11}C]DTBZ binding potential), and not to VMAT2 function.

Dopamine synthesis enzymes in DRD

Apart from reduced activity in GTP-CH I (Ichinose et al., 1994), the genetic defect of DRD also leads to decreases in both the activity and protein level of tyrosine hydroxylase in nigrostriatal terminals (Rajput et al., 1994; Furukawa et al., 1999). On the other hand, the protein level of AAAD (dopa decarboxylase) in treated DRD was reported to be normal on two postmortem examinations (Furukawa et al., 1999). There are abundant experimental data suggesting that AAAD activity is reduced by levodopa treatment (Hadjiconstantinou et al., 1993). However, in keeping with our previous observations (Snow et al., 1993), here we have confirmed in vivo by PET that the decarboxylation of [^{18}F]-dopa to [^{18}F]-dopamine is neither impaired nor increased in levodopa-naïve DRD patients. The same should apply to the synthesis of dopamine from exogenous levodopa. Our results suggest that a biochemically pure dopamine deficiency state may not lead to significant compensatory AAAD upregulation, in contrast to what occurs in the presence of denervation or following treatment with dopamine antagonists (Hadjiconstantinou et al., 1993; Lee et al., 2000). In such circumstances (e.g., Parkinson's disease) AAAD may even become a rate-limiting enzyme (Neff and Hadjiconstantinou, 1995).

Dopamine levels and dopamine turnover in DRD

Two autopsy reports on DRD have demonstrated that striatal dopamine levels are greatly reduced in this condition (Rajput et al., 1994; Furukawa et al., 1999). Indeed, the degree of striatal dopamine deficiency in DRD seems to be comparable in magnitude to that found in patients with moderately severe Parkinson's disease (Rajput et al., 1994). In addition, dopamine deficiency in DRD was found to follow a rostrocaudal gradient, so that the caudal putamen is the most depleted striatal subregion while the caudate nucleus is least affected. There was also some evidence for increased dopamine turnover. Thus, the ratio between homovanillic acid and dopamine was increased, particularly in the caudal subregions of the putamen (Rajput et al., 1994; Furukawa et al., 1999). These postmortem studies, however, were subject to the caveat that both DRD cases had been chronically treated with levodopa. A clear example of the potential effect of treatment can be found by comparing dopamine levels between Case 1 and Case 2 in Furukawa et al. (1999).

Although we do not have any tool to measure directly by PET striatal dopamine levels in vivo, we found evidence for increased dopamine turnover in one DRD patient by examining changes in [^{11}C]raclopride binding potential in relation to levodopa treatment. The change in baseline [^{11}C]raclopride binding following introduction of levodopa therapy was greater in the caudate nucleus than in the posterior putamen. This is compatible with the notion that levodopa has a more prolonged effect secondary to more sustained medication-induced dopamine release in subregions with lower dopamine turnover (e.g., the caudate nucleus)

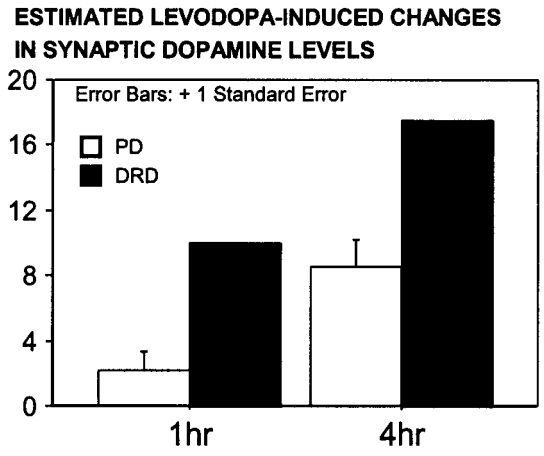

Fig. 3. Estimated levodopa-induced changes in the synaptic level of dopamine in a 58-year-old patient with dopa-responsive dystonia (DRD; solid bars) and four patients with Parkinson's disease (PD; open bars). These PD patients, who have been described previously (de la Fuente-Fernández et al., 2001b), were long-term stable responders and had motor scores almost identical to the DRD patient. Values are expressed as percent reduction from baseline of putamen [^{11}C]raclopride binding potential at both 1 h and 4 h after levodopa administration (250/25 mg levodopa/carbidopa). As expected, the estimated increase in the synaptic level of dopamine was still present 4 h after levodopa administration (i.e., stable responder pattern). However, the estimated release of dopamine at 1 h after levodopa administration was 5 times higher in the DRD patient. This suggests increased dopamine turnover.

than in those with higher turnover of dopamine (e.g., the putamen). Also in keeping with this concept, the acute administration of oral levodopa led to an increasing rostrocaudal gradient in striatal [^{11}C]raclopride binding changes (i.e., greater changes in subregions with higher dopamine turnover; 15% in the caudate nucleus; 18% in the putamen) (Table III). The changes induced by the acute levodopa test were of the same magnitude as those reported in normal volunteers after amphetamine administration (Breier et al., 1997), and substantially greater than those obtained after oral levodopa administration in patients with Parkinson's disease of almost identical clinical severity (MCS, 14 vs. 14.25 ± 8.30) who had a stable response to levodopa maintained over the years (Fig. 3) (de la Fuente-Fernández et al., 2001b).

Vesicular and plasma membrane dopamine transporters in DRD

One of the two postmortem studies on DRD mentioned earlier reported normal [^{3}H]DTBZ and [^{3}H]WIN 35428 (DAT) binding in two DRD patients (Furukawa et al., 1999). However, as already noted, those two DRD patients had received chronic levodopa treatment, which could have confounded the results. Indeed, there are several reports indicating drug-induced regulatory DAT changes (Gordon et al., 1996; Wilson and Kish, 1996). Although the synthesis of VMAT2 might not be regulated by dopaminergic drug treatments (Vander Borght et al., 1995; Wilson and Kish, 1996), levodopa-induced changes in intravesicular dopamine levels could, as described below, influence the [^{11}C]DTBZ binding potential.

The finding of increased [^{11}C]DTBZ binding in DRD suggests first that VMAT2 expression is not coregulated with the production of monoamine synthesizing enzymes or with dopamine synthesis. This notion is in keeping with previous experimental work (Scherman and Weber, 1987). As the relation between the number of VMAT2 sites and the number of vesicles appears to be stable (1–3 VMAT2 sites per vesicle) (Scherman and Boschi, 1988; Liu and Edwards, 1997), we can extend our argument to suggest that the production of vesicles is not linked to dopamine synthesis.

There is no definite explanation for the observed increase in [^{11}C]DTBZ binding in DRD. Two factors, however, may be at play: 1) low intravesicular dopamine levels, and 2) increased cell firing. To explore the first possibility, we attempted to estimate the expected change in [^{11}C]DTBZ binding resulting from dopamine depletion. Using formulations previously described for competitive displacement (de la Fuente-Fernández et al., 2001a), and assuming no change in B_{max}, the ratio between the [^{11}C]DTBZ binding potential (BP) of DRD patients and that of normal controls is: $BP_{DRD}/BP_{CONTROLS} = \{K_{DA} + [DA]_{CONTROLS}\}/\{K_{DA} + [DA]_{DRD}\}$, where K_{DA} is the dopamine kinetic parameter for [^{11}C]DTBZ displacement and [DA] represents the concentration of dopamine. Although dopamine K_m for VMAT2 is ~1 μM (Liu and Edwards, 1997), it has repeatedly been shown that transport substrates block [^{3}H]DTBZ binding only at concentrations more than 100-fold higher than their apparent K_m (Henry and Scherman, 1989; Schuldiner, 1994). Whereas reserpine binds to the cytoplasmically oriented substrate-recognition site of VMAT2, tetrabenazine may bind to its lumenally oriented conformation, which has a very low affinity for the substrate (Henry and Scherman, 1989; Liu and Edwards, 1997; Schuldiner, 1994). While cytoplasmic dopamine levels are too low to compete with [^{11}C]DTBZ for VMAT2 binding (Liu and Edwards, 1997), vesicles can accumulate large amounts of dopamine under normal circumstances (Henry and Scherman, 1989; Pothos et al., 2000). Indeed, it has been shown that neurotransmitters can reach an intravesicular concentration more than 1,000-fold greater than that in the cytoplasm (Schuldiner, 1994). Thus, normal levels of intravesicular dopamine could compete for [^{11}C]DTBZ binding. Experimental estimates are compatible with K_{DA} values for [^{3}H]DTBZ displacement three to four times higher than intravesicular [DA] (Henry and Scherman, 1989). Hence, substituting our values for the ratio of [^{11}C]DTBZ binding potential between DRD patients and normal controls (e.g., 1.236 for the total striatum) in the equation above, we estimated some 80–95% loss in intravesicular dopamine in the DRD patients for any value of K_{DA} three to four

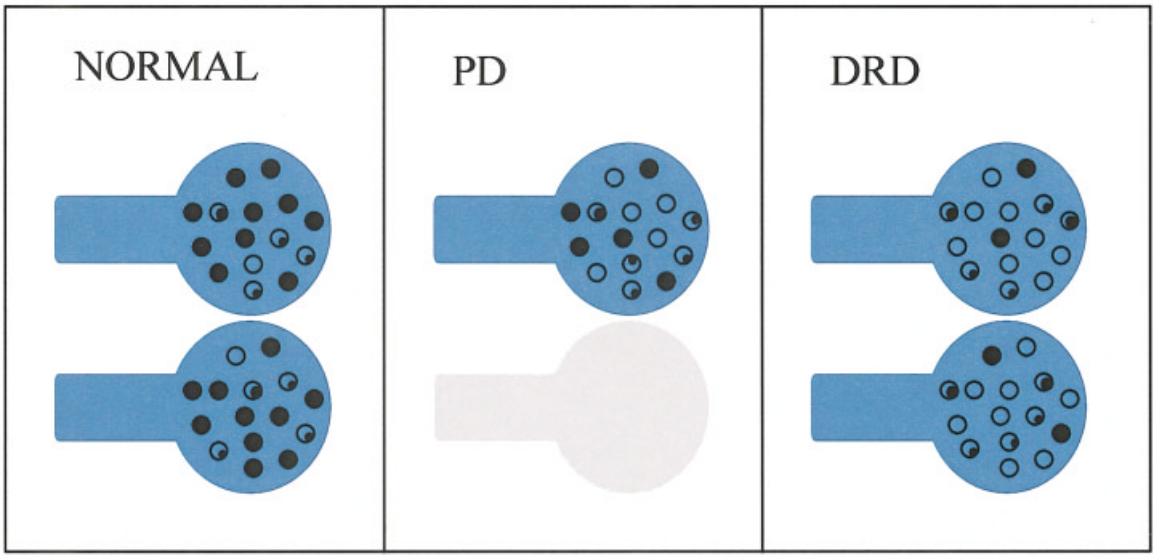

Fig. 4. Nigrostriatal dopaminergic terminals in normal controls, patients with Parkinson's disease (PD), and patients with dopa-responsive dystonia (DRD). In DRD, most vesicles (circles) contain low levels of dopamine (solid symbols). In PD, the average intravesicular concentration of dopamine in surviving terminals may also be reduced — although to a lesser extent — if the loss of dopamine resulting from increased dopamine turnover exceeds the rate of synthesis of endogenous dopamine. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

times greater than the intravesicular [DA]. This figure would be different if there were between-group differences in B_{max} for [^{11}C]DTBZ.

Although the dopamine content in vesicles cannot be directly measured by PET, our modeling of [^{11}C]DTBZ data in DRD patients and controls supports the notion that the dramatic loss of intravesicular dopamine that occurs in DRD may lead to increased [^{11}C]DTBZ binding potential compared to controls (Fig. 4). However, animal experiments have shown that chronic depolarization can induce VMAT expression (Desnos et al., 1992) at the transcriptional level (Krejci et al., 1993). Whether this also applies to human VMAT2 is unknown. Interestingly, we found evidence for elevated dopamine turnover in DRD, which is likely related to an increase in cell firing. We conclude that the increase in [^{11}C]DTBZ binding potential found in DRD could result from the combined effects of a dramatic decrease in intravesicular dopamine levels (loss of dopamine competition for VMAT2) and possibly an increase in neuronal firing (increase in B_{max}).

It is intriguing that the [^{11}C]MP binding potential is not significantly altered in levodopa-naïve (present study) or treated DRD patients (Jeon et al., 1998). One might expect that DAT should be downregulated in DRD, in order to increase synaptic dopamine levels. Such a compensatory mechanism appears to occur in Parkinson's disease (Wilson et al., 1996; Lee et al., 2000), although it is also possible that the apparent DAT downregulation observed in this neurodegenerative disorder may simply reflect preferential damage to the plasma membrane. Our results in DRD favor the notion that [^{11}C]MP binding is relatively insensitive to dopamine depletion (Gatley et al., 1995). Thus, using an approach similar to that described above for [^{11}C]DTBZ, as well as recent experimental estimates of synaptic dopamine concentration ([DA], 50 nM) (Ross, 1991), and K_{DA} for DAT (150 nM) (Krueger, 1990), our normal striatal [^{11}C]MP binding potential value of 1.482 predicts that, for a 90% loss of synaptic dopamine (i.e., [DA] = 5 nM), the striatal [^{11}C]MP binding potential value in DRD should be 1.912. Our striatal values for the ratio of [^{11}C]MP binding potential between DRD patients and normal controls (1.185 before, and 1.077 after adjusting for age) would be compatible with some 30–60% loss in the synaptic concentration of dopamine. Naturally, the combination of the opposite effects of synaptic dopamine depletion and partial DAT downregulation may also explain normal values of [^{11}C]MP binding potential in levodopa-naïve DRD patients.

Vesicular neurotransmitter transporters as ancient toxin-extruding systems

Our observations indicate that the expression of VMAT2 is not coregulated with either the activity of dopamine-synthesizing enzymes or the striatal levels of dopamine. Our findings support other evidence which suggests that the vesicular monoamine transporters (VMAT1 and VMAT2), as well as other neurotransmitter transporters (Reimer et al., 1998; Bellocchio et al., 2000), may have evolved from ancient detoxification systems aimed at sequestering a great variety of substances (both endogenously and exogenously derived) with potential cell toxicity (Schuldiner, 1994; Liu and Edwards, 1997). Thus, VMAT2 appears to be part of a superfamily of toxin-extruding

transporters (Schuldiner, 1994; Schuldiner et al., 1995). From an evolutionary perspective, cells could have taken advantage of this system to regulate neurotransmitter release. This hypothesis predicts that VMAT2 expression could, in its own right, play a crucial role in the pathogenesis of Parkinson's disease. Interestingly, neuronal stem cells express VMAT2 (Xu and Emson, 1997). By extension, this hypothesis predicts that synaptic vesicles may be primarily involved in the removal of toxic substances from the cytoplasm and their eventual release from the cell by exocytosis.

CONCLUSION

Our findings suggest that striatal dopamine deficiency in DRD is not associated with any significant regulatory change in AAAD, DAT, or VMAT2. The lack of coregulation between dopamine synthesis and VMAT2 supports the notion that VMAT2 (and synaptic vesicles) could have evolved from ancient toxin-extruding systems. [^{11}C]DTBZ PET may prove to be a useful tool to detect dynamic changes in vesicular dopamine levels.

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