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COMMENTARY

Biological Imaging and the Molecular Basis of Dopaminergic Diseases

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ABSTRACT. The development and validation of preclinical biological probes of nigrostriatal dysfunction are part of the next frontier for battling diseases involving dopamine deficiency. In this work, the quantitative relationship relationship between radiofluorinated L-DOPA, [e.g., L-3,4-dihydroxy-6-[^{18}F]fluorophenylalanine (6-[^{18}F]fluoro-L-DOPA, FDOPA)] kinetics measured with positron emission tomography and central dopamine biochemistry is discussed. A hypothesis of a possible "non-linearity" of FDOPA kinetics with dopaminergic cell losses is presented to explain apparent discrepancies in post-mortem biochemical and histological determinations in Parkinson's disease. Similar observations have been made in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-exposed monkeys and human subjects where the FDOPA uptake constantly fell within normal values unless severe nigral damage had occurred. The limitations of FDOPA, and other biological probes, for examining the asymptomatic phase of dopaminergic diseases and the future direction of research are discussed. BIOCHEM PHARMACOL **54**;3:341–348, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. positron emission tomography; 6-[^{18}F]fluoro-L-DOPA; dopaminergic mechanisms

The central dopaminergic system has been implicated in neurodegenerative disorders involving motor function (e.g., Parkinson's disease) [1–3], in psychiatric syndromes (e.g., schizophrenia) [4], and in the effects of drugs of abuse (e.g. cocaine, *m*-amphetamine) [5, 6]. In idiopathic parkinsonism, the degree of dopaminergic deficiency corresponds with the loss of midbrain dopaminergic neurons in nuclei A8, A9, and A10, which project to the striatum [7]. Remarkably, administration of MPTP† to non-human primates [8, 9] and humans [10] leads to a progressive syndrome closely resembling idiopathic Parkinson's disease. Indeed, MPTP causes all of the cardinal signs of parkinsonism (akinesia, resting tremor, cogwheel rigidity, and postural reflex impairment) [11], and has provided an animal model for the disease [8, 9]. It has also raised many questions regarding the etiology and pathogenesis of Parkinson's disease [12].

The observation by Tetrud *et al.* [13] that the majority of MPTP-exposed subjects have remained asymptomatic, in spite of a partial striatal dopamine deficiency, has intensified the search for a practical, preclinical marker for dopamine deficiency in humans [1, 12, 14]. Parkinson's disease has been suggested to exist in several phases [15]: (i) a disease-free state in which risk factors are present; (ii) a presymptomatic phase characterized by initiation and progression of the disease; and (iii) a symptomatic phase when the disease breaks the threshold, when the degree of dopaminergic deficiency can no longer sustain normal function. If ways can be found to detect preclinical dopaminergic deficiencies, an opportunity exists to treat them and to evaluate therapies [16]. Biological imaging, in particular PET [17], offers that opportunity. Beyond this, biological imaging allows the evaluation of the molecular and cellular basis of the *in vivo* regulatory mechanisms of the central dopaminergic system.

BACKGROUND AND ANALYSIS

When 6-[^{18}F]fluoro-L-DOPA (also called FDOPA) was first introduced in 1983 [18] as a "marker" of dopamine synthesis, no one questioned the importance of this development. After all, this was the first *in vivo*; non-invasive observation of the neurotransmitter machinery at work in a living human being. Not surprisingly, a flurry of activity followed, stimulated by the remarkable specificity of FDOPA to examine changes in the presynaptic dopaminergic function in central brain structures [19–31] (Fig. 1).

Investigations in rodents [32–34], non-human primates [35–40], and humans [21, 39] have demonstrated unquestionably that accumulation of F-18 activity in the basal ganglia is related to the functional integrity of dopaminergic neurons, since that activity could be attributed to AAAD- (EC 4.1.1.28) mediated formation of FDA and its metabolites [6-[^{18}F]fluorohomovanillic acid (FHVA) and

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† *Abbreviations:* MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PET, positron emission tomography; 6-[^{18}F]fluoro-L-DOPA or FDOPA, L-3,4-dihydroxy-6-[^{18}F]fluorophenylalanine; AAAD, aromatic L-amino acid decarboxylase; and FDA, 6-[^{18}F]fluorodopamine.

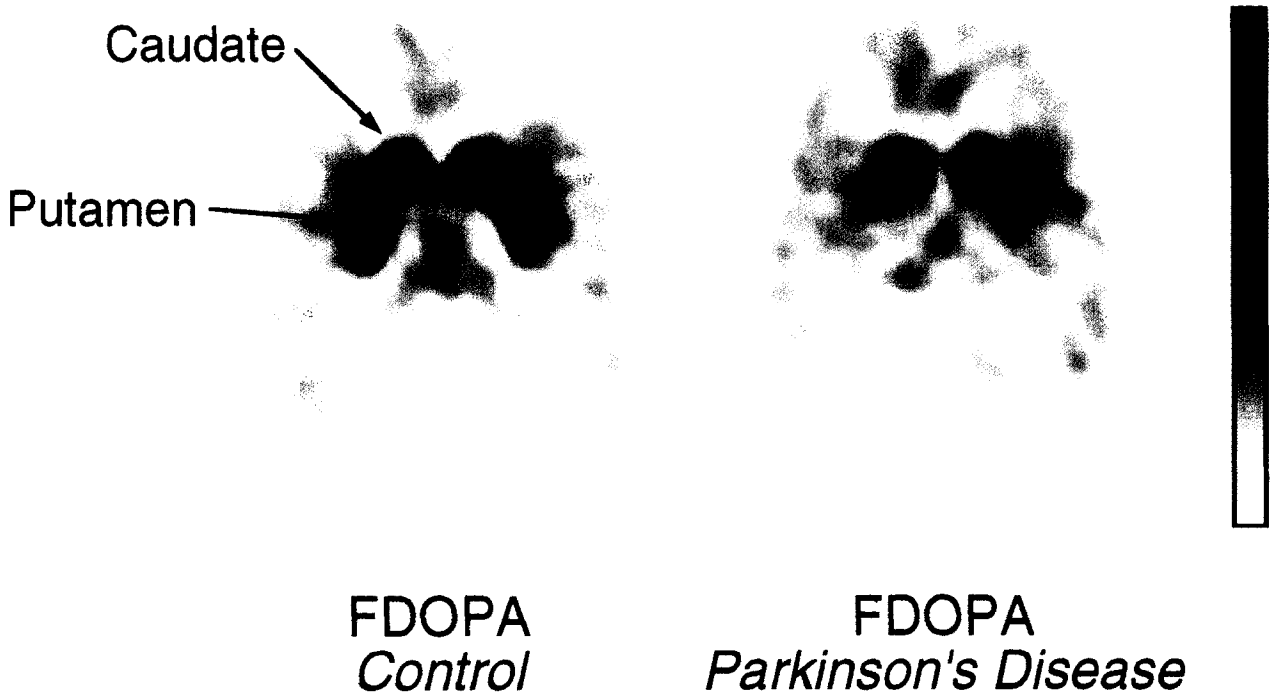

FIG. 1. Typical example of human brain PET images of FDOPA obtained 90–120 min post-injection of the radiotracer probe (top: front; bottom, back of the brain). Current tomographs permit analysis of the entire brain by obtaining 63 slices separated by 2.4 mm. Images shown are at the level of the basal ganglia. Note the significant reduced FDOPA uptake in putamen of a patient with Parkinson's disease. Less reduction is observed in the caudate nuclei, which appears more preserved.

6-[^{18}F]fluoro-3,4-dihydroxyphenylacetic acid FDOPAC)]. One important feature of FDOPA kinetics is that the tracer is rapidly converted systemically to L-3,4-dihydroxy-6-[^{18}F]fluoro-3-O-methylphenylalanine (3-OMFD) [32, 33, 41], which, in turn, crosses the blood–brain barrier and contributes to the total radioactivity measured in cerebral tissue. This complicates somewhat the interpretation of PET images, in which only the total radioactivity concentration in local tissue regions can be measured.

Various approaches have been used to quantitate FDOPA brain tissue kinetics. Early work has used the radioactivity ratio between striatum and cerebellum (or background), at 60–120 min after FDOPA injection, as a differential index between normal subjects and patients [20, 24, 37, 42]. In some studies, the background activity (cerebellum) was subtracted from the striatal activity, before striatum/cerebellum ratio determination, to minimize the effect of 3-OMFD [27]. In most studies, the striatum/cerebellum ratio of the F-18 concentration was shown to increase at a constant rate after injection [43]. Modeling analysis has been applied to show that plots of this ratio versus the normalized time [44] (Patlak's method), using either the cerebellar time–activity curve or the total blood–activity curve as the input function, are approximately linear [31, 45, 46]. The Patlak graphical method has also been formally applied to analyze FDOPA data [25], using the plasma FDOPA curve as the input function to give the uptake constant of FDOPA from plasma to striatum. In contrast to earlier analyses, this last approach explicitly accounted for the presence of 3-OMFD in plasma and in brain tissue.

Since FDOPA is an analog of L-DOPA, the reaction rate constants of FDOPA should be similar to those of L-DOPA, as shown below. Under the conditions of uniform concentration of both enzyme and substrate, the conversion of L-DOPA (S) to dopamine (P) by AAAD (E) follows the Michaelis–Menten equation.

$$V = \frac{V_{max}[S]}{K_m + [S]} = \frac{k_{cat}[E][S]}{K_m + [S]} \tag{1}$$

for a biomolecular reaction of

$$E + S \rightleftarrows ES \rightleftarrows EP \rightarrow E + P$$

where K_m is the Michaelis–Menten constant; k_{cat} is the first order constant for decomposition of ES (the turnover number and a measure of the catalytic efficiency of the enzyme); V is the reaction rate; and V_{max} is the maximum reaction rate. Under normal conditions in striatum, V was estimated experimentally in rodents by turnover-rate measurements to be around 0.3 nmol $\cdot$ g^{-1} $\cdot$ min^{-1} (for review, see Ref. 47). For a competitive substrate that is used in tracer concentrations, such as FDOPA, its concentration [S'] is low; then the reaction rate (V') would be

$$V' = \frac{V'_{max}[S']}{K'_m + [S]} = k_3[S'] \tag{2}$$

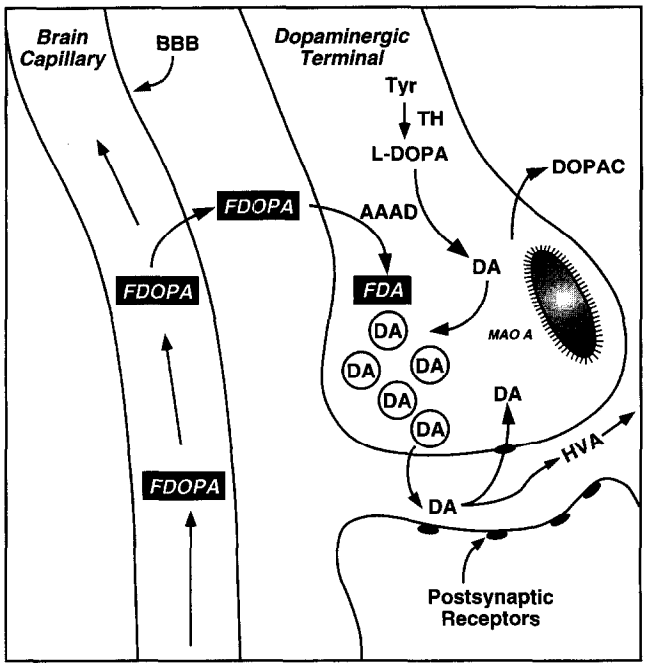

FIG. 2. Schematic illustration of the central dopaminergic nerve synapse with pre- and post-synaptic elements. A brain capillary is also depicted, indicating brain transport of FDOPA and its subsequent AAAD-mediated decarboxylation to FDA. After formation, FDA follows the dopamine (DA) pathway of vesicle storage, release, reuptake, and metabolism. Abbreviations: Tyr, tyrosine; L-DOPA, 3,4-dihydroxyphenylalanine; HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; TH, tyrosine hydroxylase; AAAD, aromatic L-amino acid decarboxylase; MAO A, intra-neuronal monoamine oxidase in the outer mitochondrial membrane.

where V'_{max} is the maximal rate for FDOPA, and K'_m the Michaelis–Menten constant of FDOPA. Assuming $V'_{max} \sim V_{max}$ and $K'_m \sim K_m$, the value of k_3 obtained for FDOPA can be used for estimating the decarboxylation rate of L-DOPA according to Equation 1, if the concentration of L-DOPA in tissue is known (see below).

The affinities of L-DOPA and FDOPA for AAAD are known to be relatively close [32, 47, 48]; therefore, the use of analog FDOPA as a biochemical probe is justified (Fig. 2). However, even though tracer kinetic modeling approaches to quantitate FDOPA transport, decarboxylation rates, and metabolite clearance were developed, permitting quantitation of the central kinetics of the radiofluorinated analog [49, 50], many questions can still be raised. A few critical ones will be addressed in the following subsections.

How Closely Does Exogenous FDOPA Uptake Represent Endogenous Dopamine Synthesis?

The most important question about the use of FDOPA with PET relates to the functional significance of FDOPA kinetics in health and disease states when no defined correlations between *exogenous* FDOPA tracer kinetics and *endogenous* dopamine synthesis had been established. Unquestionably, the specific accumulation of F-18 activity in the basal ganglia was related to the functional integrity of dopaminergic neurons, since that activity could be attributed to the AAAD-mediated formation of FDA and its metabolites [34]. But it could not be assumed, however, that such measurements would reflect endogenous dopamine synthesis and turnover, because the AAAD-mediated decarboxylation of FDOPA to FDA bypassed the major regulatory enzyme involved in endogenous dopamine synthesis, namely, tyrosine hydroxylase (TH; EC 1.14.16.2) [51]. Such a line of questioning on the use of FDOPA is valid. It has been argued that, if developed, exogenous probes for TH (the rate-limiting step in the synthesis of dopamine) would be more suitable for *in vivo* work [52].

The use of FDOPA became biochemically validated when it was demonstrated in double-labeling experiments in rats that the cerebral metabolism of FDOPA and that of [^{3}H]L-DOPA were correlated [34]. These results demonstrated that FDOPA was centrally decarboxylated by AAAD to FDA, which was mainly stored in a slow turnover rate functional pool, similar to the large endogenous dopamine pool that has a turnover rate of 0.21 hr^{-1} [53, 54]. Similar results had been obtained in rodents *in vivo* with radiolabeled tyrosine, although some controversy has existed regarding the existence of multiple pools [55, 56]. The observed correlation, albeit semiquantitative, between the kinetics and biochemistry of an exogenous probe (e.g. FDOPA) with the endogenous dopamine system was an important piece in the puzzle. Further, the observation that AAAD is modulated *in vivo* [57] by physiological and electrical stimulus [58, 59], light [60], and by pharmacological agents, such as α_2-adrenoreceptor drugs [6], indicated that neuronal regulation among dopaminergic enzymes is not limited to TH. Further, the central metabolic behavior of the exogenous probe (FDOPA) paralleled that of endogenous dopamine in all cases evaluated (e.g. pharmacological modulation and electrical stimulation) [58, 59, 61].

Very strong correlations between exogenous FDOPA kinetics and endogenous dopamine synthesis were also encountered *with severe nigrostriatal degeneration*. Biochemical data from MPTP-treated hemiparkinsonian monkeys showed that striatal concentrations of endogenous dopamine, 3,4-dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA) were reduced significantly on the side of the nigrostriatal lesion. However, decreases in DOPAC and HVA concentrations were less pronounced than that of dopamine, which dramatically increased the ratio of metabolites/dopamine. Similarly, the ratio (FHVA + FDOPAC)/FDA was elevated by more than an order of magnitude (from 0.5 in the normal to >6 in the MPTP-lesioned putamen) [36, 62].

Indeed, data in the literature suggest that dopaminergic neurons surviving nigrostriatal lesions have a much higher rate of dopamine metabolism (higher turnover rate). These observations are also supported by earlier reports indicating that dopamine synthesis and release are accelerated in neurons surviving a massive lesion of the nigrostriatal system [63, 64]. This increase in dopamine utilization with

neuronal losses indicates that comparing dopamine concentrations at a single point in time provides little information on its rate of synthesis [65]. Other factors, like dopamine release and metabolism, would affect the relationship between dopamine synthesis and dopamine levels [65].

Nevertheless, no *quantitative relationship* has yet been established between FDOPA kinetics and endogenous dopamine synthesis, in spite of progress made in the quantification of FDOPA kinetics and its correlation with the functional state of the dopaminergic system in the brain (*vide supra*). One may reason that if the power of numbers is not applied to this relationship, one would be at a clear disadvantage in interpreting specific changes in FDOPA *in vivo* kinetics as a result of disease or pharmacological interventions. This may be so. However, the proposition to establish a correlation between FDOPA kinetics and endogenous dopamine synthesis is not as obvious as it seems.

Based on the arterial plasma L-DOPA concentration (200 nM) and the uptake constant of FDOPA from plasma to tissue (K_3 = 0.01 mL · min^{-1} · g^{-1} in monkeys) [39], the exogenous utilization rate of L-DOPA is in the order of 2 pmol · g^{-1} · min^{-1}. This is far below the 0.3 nmol · g^{-1} · min^{-1} rate experimentally measured for dopamine synthesis in rodents (see above) [47]. Even using the average tissue concentration of 400 nM for L-DOPA in striatum [66] and the FDOPA decarboxylation rate constant (k_3) of 0.044/min [49], the calculated decarboxylation rate of L-DOPA in striatum is only around 17.6 pmol · g^{-1} · min^{-1} (equation 2), still only about 6% of the estimated total dopamine synthesis rate. Also, consistent with these estimates, *in vivo* FDOPA decarboxylation rates in rats were found to be significantly lower than those measured *in vitro* [47]. The above discrepancy cannot be attributed to differences in affinities between L-DOPA and FDOPA for AAAD (see above, and also Refs. 32, 47, and 48).

The realization that most AAAD studies have been performed in animals allows for a better perspective. AAAD activity in the human brain has consistently been reported to be much lower than in other species [67]. For example, the total activity of AAAD in caudate (measured *in vitro*) has been reported to be 6402 μmol · hr^{-1} · g wet $tissue^{-1}$ in the rat, 1530 in the rabbit, 2130 in the cat, and only 682 in the human [68]. These observations have prompted speculation that AAAD may be rate-limiting in monoamine synthesis in the human brain [67], and therefore FDOPA decarboxylation rate could potentially be a measure of dopamine synthesis in humans.

Is FDOPA Decarboxylation Rate-Limited?

AAAD is a soluble, mostly cytosolic enzyme [68] that in striatum is contained almost exclusively in dopaminergic terminals. A single gene codes for AAAD in both neuronal and non-neuronal tissues [69]. Essential amino acid sequences for the cofactor (pyridoxal phosphate) binding sites are also identical in several species [70]. AAAD performs a neuron-specific role as a neurotransmitter biosynthetic enzyme; this provides both anatomical and biochemical specificity to the use of FDOPA. However, as indicated above, FDOPA kinetic data are suggestive of restrictions in the decarboxylation rate of FDOPA.

One possible explanation is that under *normal conditions* FDOPA decarboxylation kinetics is independent of enzyme (AAAD) concentrations, because either the enzyme is in large excess, the substrate has limited accessibility to it, or other factors. When AAAD concentration becomes limiting (e.g. in nigrostriatal cell degeneration), the decarboxylation reaction follows second order kinetics and more closely obeys the Michaelis–Menten equation (*vide supra*). Thus, clear differences are observed in FDOPA kinetics between normal subjects and disease states.

A second possible explanation focuses on FDOPA transport restrictions limiting accessibility to the enzyme. FDOPA transport at the blood–brain barrier is mediated by the Na^+-independent neutral L-amino acid carrier [71, 72], as demonstrated by its inhibition by neutral amino acids in rodents (K_i = 177 μM; Phe) [73] and humans [43]. The biochemical and kinetic effects of carbidopa [α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl) propionic acid] on FDOPA transport have been studied in rodents, nonhuman primates, and humans [33, 39]. In rodents, it has been shown that about half of the FDOPA available for transport is decarboxylated in brain capillaries (in the absence of carbidopa) [73]. The endothelial cell lining of the cerebrovascular bed constitutes a morphological and enzymatic blood–brain barrier mechanism to neurotransmitter monoamines [74]. Due to the presence of AAAD in the brain microvessel, dopamine is formed from L-DOPA derived from the circulation [75]. The considerable monoamine oxidase activity present in endothelial cells and pericytes of the brain microvessels rapidly metabolizes dopamine (and other biogenic amines), thus preventing its entrance into the brain parenchyma [76–78].

That partial, non-specific decarboxylation of FDOPA may occur in the capillary bed (in the absence of carbidopa) is a potentially worrisome observation for FDOPA–PET studies. However, it was demonstrated that in brain capillaries, the AAAD-mediated decarboxylation of FDOPA is inhibited by carbidopa by depletion of the enzyme cofactor (pyridoxal phosphate) at the enzyme site [73]. Similarly, it was demonstrated by biochemical analyses that, in monkey brain, FDOPA decarboxylation is neuron-specific, with no evidence of non-specific decarboxylation [36, 62].

Therefore blood–brain barrier transport is an unlikely culprit. One intriguing possibility, however, to account for restrictions in FDOPA central decarboxylation rates measured *in vivo* with PET is that the exogenous substrate (FDOPA) may have limited accessibility to neuronal AAAD, either because of multiple dopamine (and enzyme) pools [55, 56] or limiting neuronal permeability. The existence of multiple dopamine pools has been the focus of heated debate in the literature, whereas little is known about L-DOPA neuronal transport.

The neutral L-amino acid carrier competitively transports

Phe, Tyr, L-DOPA, Trp, Leu, and Val at the blood–brain barrier. Leu transport is saturable, and the apparent K_m was determined to be 180 μM [79]. It is expected that similar transport systems would exist in neuronal systems (as an analogy, glucose transport from blood to brain is facilitated by GLUT1 in brain capillaries and by GLUT3 in neurons [80]). That FDOPA neuronal transport may be limiting is supported by the high-affinity (K_m = 4 μM) transport constant of Leu in rat striatal synaptosomes [81]. If similar transport characteristics hold for FDOPA (and L-DOPA), and if they share the same neutral L-amino acid carrier as expected, *in vivo* measurements of decarboxylation rate constants with PET will be affected by neuronal transport rates of the substrate.

Is FDOPA Kinetics Related to Other Endogenous Parameters (e.g. Dopaminergic Cell Number)?

We have now described a number of limitations in the interpretation of FDOPA kinetics. If no specific quantitative relationship has been established with endogenous dopamine synthesis, is it possible to establish a quantitative relationship between FDOPA kinetics and other endogenous parameters (e.g. dopaminergic cell losses)? The establishment of these relationships may be closer to reality, but careful judgment would be necessary to determine its scope. Principles of neuronal plasticity indicate that axonal branches bearing synapses would respond quickly to early signs of nigrostriatal dopaminergic degeneration as part of the compensatory mechanisms to maintain functional activity [82, 83]. If so, substantial changes in terminal number and AAAD concentration are not expected to occur during this adjustment period, and, therefore, the AAAD-dependent FDOPA kinetics would not be sensitive to these initial changes (e.g. in presymptomatic phases of nigrostriatal cell degeneration).

Thus, if this line of reasoning is correct, a non-linear relationship will exist between dopamine-cell losses in the substantia nigra and FDOPA kinetics in axonal terminals (e.g. in putamen), particularly in the presymptomatic phase of dopaminergic deficits (Fig. 3). It is conceivable that *presynaptic dopaminergic reuptake site* numbers will also be maintained initially by compensatory axonal sprouting to replace axons lost by cell death. How would dopamine concentrations in presynaptic terminals be affected by these compensatory mechanisms? In any event, when severe nigrostriatal cell losses have occurred, remaining healthy or sick neurons may not have sufficient capacity to maintain normal function. FDOPA kinetics would then change in response to changes in AAAD concentrations [84] and other factors, and concomitant changes in presynaptic dopaminergic reuptake sites will also occur [85].

The hypothesis on the possible "non-linearity" of FDOPA kinetics with dopaminergic cell losses in the nigrostriatal system may explain the apparent discrepancy observed in Parkinson's disease between dopaminergic cell losses in *post-mortem* determinations (60–86% of age-matched controls) and loss of dopamine in the nerve terminals in the striatum (90–99% loss in putamen and 58–95% loss in caudate nucleus) [4]. Other lines of evidence appear to support this idea: (a) the relationship of estimated kinetic rate constants for striatal FDOPA uptake (K_3) to the normal aging process has been the subject of conflicting reports. Despite post-mortem observations supporting age-dependent striatal dopamine innervation losses [3], the most recent evidence [86] fails to support a correlation between quantitative FDOPA/PET and age; (b) non-parallel reduction of AAAD activity and dopamine levels in *post-mortem* striatal tissue of Parkinson's patients [87]; and (c) the lack of clear correlations between behavior/symptoms and the FDOPA uptake constant, unless severe nigral damage has occurred. For example, in previous investigations our group has shown that repeated-subclinical doses of MPTP fail to induce changes in striatal FDOPA uptake constant (K_3) in *Macaca nemestrina* monkeys; similar observations were made with asymptomatic MPTP-exposed subjects (unpublished observations). In one animal with subclinical damage and 60% striatal dopamine depletion, it was also reported that only 20% reduction in FDOPA uptake was observed [88].

The above-stated hypothesis is not inconsistent with the most recent observations that decreases in FDOPA uptake in symptomatic MPTP monkeys [89] and end-stage, preterminal humans with Parkinson's disease and progressive supranuclear palsy are correlated with nigral cell losses [90]. It is likely that in intermediate and severe stages of cell de generation, FDOPA decarboxylation may already be limited by AAAD, as indicated above.

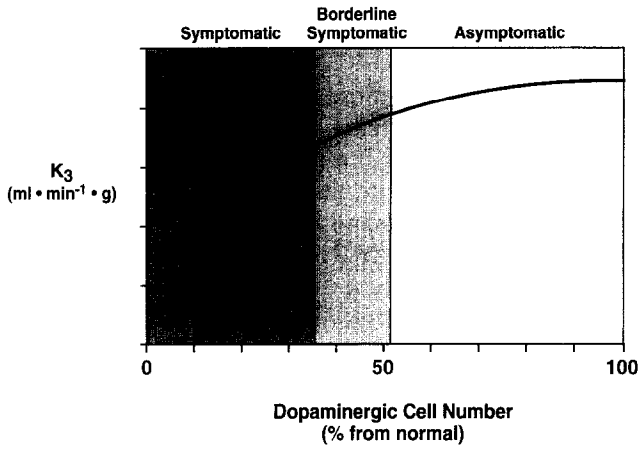

FIG. 3. Hypothetical relationship between FDOPA decarboxylation kinetic parameters (e.g. K_3) and dopaminergic cell number in substantia nigra. The sketch attempts to illustrate the insensitivity of K_3 to decreases in dopaminergic cell number in the asymptomatic phase of neurodegenerative disease (e.g. Parkinson's disease) due to axonal plasticity and other factors. The relationship between dopaminergic cell degeneration and symptoms is species dependent. In contrast to rodents, primates develop symptoms with progressive cell degeneration, but significant variations may also exist between individuals.

Future Directions

Clearly, more work is needed to address the issues raised in this commentary on the use of presynaptic dopaminergic probes in biological imaging. Well-designed and executed experiments are expected to provide valuable information to resolve these issues. The observations made with FDOPA may likely apply to all amino acid substrates transported by similar carriers and subjected to AAAD-mediated neuronal decarboxylation (e.g. 4- and 6-[^{18}F]fluoro-L-*m*-tyrosine [91]). The existence of compensatory mechanisms, like axonal sprouting upon nigrostriatal cell death in the early, asymptomatic stage of Parkinson's disease, may also hide nigral cell death in response to the use of other external probes (e.g. radioligands for presynaptic dopaminergic reuptake sites [85] and for the vesicular dopamine transporter [92, 93]). It should also be made clear that the proposed use of L-[3-^{11}C]DOPA [94] as a "natural" dopaminergic probe neither alleviates the problems encountered with the use of FDOPA nor answers any of the questions presented in this commentary.

At stake here is the existence, and future development, of *preclinical biological probes of nigrostriatal dysfunction* sensitive to the neurological features of the syndromes. Whereas FDOPA and presynaptic dopamine reuptake site ligands have been effective in the diagnosis and staging of symptomatic neurodegenerative syndromes, they have been mostly ineffective in the asymptomatic phase, as predictors of the disease. If biological probes are indeed preclinically insensitive, how can they be made sensitive? Would pharmacological interventions or motor tasks do the trick?

"The ultimate goal is to prevent the disease altogether" [1], a formidable task, indeed, but within reach considering the power of biological imaging demonstrated in the last two decades on the *in vivo*, non-invasive determination of neurotransmitter function. The development and characterization of preclinical markers are now part of the next frontier for diseases involving dopamine deficiency [15], by helping in the identification of high risk subjects and the development of prophylactic treatments [1].

This commentary is dedicated to the memory of Jose Barrio, father of J. R. B., who died of Parkinson's disease. This work was made possible by the financial support of the National Institutes of Health (RO1 NS 33356) and the Department of Energy (DE FC0387-ER60615).

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