Drugs of the Future 2001, 26(3): 271-279

CCC: 0377-8282/2001

Review Article

Dopamine transporter imaging as an *in vivo* marker of dopaminergic neurons

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Introduction

In recent decades much progress has been made on the imaging of the dopaminergic neurotransmission system (1-3). Especially, the development of radiotracers for imaging of dopamine transporters has been successful. Loss of dopamine transporters has been implicated in diseases characterised by degeneration of dopaminergic cells, *e.g.* Parkinson's disease. Since dopamine transporters are positioned exclusively on the membrane of dopaminergic neurons, imaging of these transporters can be used as an *in vivo* marker of the density of dopaminergic cells. Due to these developments, we are now in the area that dopamine transporter imaging is becoming a clinical tool to visualize and quantify dopaminergic neurons in routine medicine. This development is particularly important since properly diagnosis of individual patients suffering from different forms of parkinsonism can be difficult at times.

Detection or exclusion of nigrostriatal dopaminergic degeneration in parkinsonian patients is not only of interest for routine clinical studies (2, 3). Dopamine transporter studies can also be of interest in scientific studies: a) to evaluate the availability of dopamine transporters *in vivo* when studying the pathophysiology of neuropsychiatric diseases, b) to study neurotoxic effects of drugs on dopaminergic neurons and c) to determine the occupancy of these transporters by drugs (4-7).

In this review, the fascinating developments in the field of dopamine transporter imaging will be described and its role in detecting dopaminergic degeneration in the most common parkinsonian syndromes.

The dopaminergic neurotransmission system

In the human central nervous system communication between neurons is maintained by their ability to transduce signals to one another. Signal transduction, by the release of chemicals known as neurotransmitters, is thought to be an important component of cellular communication.

The neurotransmitter dopamine belongs to the category of monoamine neurotransmitters. In the late 1950s, dopamine was recognized as a neurotransmitter in the central nervous system by the recent Nobel Prize winner Carlson (8). This finding provided the incentive for studying the dopaminergic neurotransmission system intensively. Dopamine has been shown to occur in high concentrations in the basal ganglia, in some limbic and cortical areas and in the hypothalamus of the human brain (9). Dopamine neurons reside predominantly in the mesencephalon in three neuronal groups: the substantia nigra, the retrobulbar area and the ventral tegmental area. Dopamine neurons from the substantia nigra pars compacta project primarily to the dorsal striatum and are mainly concerned with initiation and execution of movements. Those from the ventral tegmental area project predominantly to limbic and limbic-connected areas (for example the nucleus accumbens, amygdala, orbital and cingulate cortices) and are involved with reinforcement, motivation, mood and the organization of thought.

Dopamine neurons from the retrobulbar area project to the hypothalamus where they regulate hormone secretion from the pituitary. Recent studies have revealed that the dopaminergic projections from the substantia nigra and the ventral tegmental area are just part of the neuronal elements integrated in extensive, strictly topographically organized, basal ganglia-thalamocortical circuits which are intimately involved in the regulation of motor activity, cognitive and complex behavioural processes (10, 11). The estimated number of dopaminergic neurons in the substantia nigra of healthy humans is 550,000 (12), which is less than 1 in every 10^5 neurons in the mammalian brain.

Imaging of the dopaminergic system

The ultimate goal in neuroscience is to understand how various elements of the brain function in the living human being, in physiological as well as in pathophysiological conditions. In the last decades, the introduction of imaging techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) have dramatically increased knowledge of the dopaminergic neurotransmission system. It was in the late 1970s that Garnett et al. (13, 14) reported on the successful application of 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine ([^{18}F]DOPA) for PET studies of dopaminergic neurons. From then on, the ability of [^{18}F]DOPA PET to demonstrate loss of dopaminergic cells, for example in patients with Parkinson's disease, has repeatedly been reproduced (15, 16). [^{18}F]DOPA PET provides a measure of the structural as well as the biochemical integrity of the dopaminergic neurons: the uptake rate constant of [^{18}F]DOPA is determined by the transfer of DOPA across the blood-brain barrier, its decarboxylation to fluorodopamine by l-aromatic acid decarboxylase and its retention in nerve terminals.

Another approach to the visualization of dopaminergic neurons is the use of radiotracers for the dopamine transporter. Since the late 1980s, studies have shown the possibility to quantify dopaminergic neurons *in vivo* by means of PET or SPECT using the dopamine transporter as a neurochemical marker (17-24).

The dopamine transporter

Dopamine is synthesized in dopaminergic neurons where it is stored in vesicles that protect it from oxidation by monoamine oxidase. In response to an action potential, dopamine is released into the synaptic cleft and interacts with receptors. To terminate the interaction with receptors, the extracellular dopamine is actively pumped back into the dopaminergic terminal by the dopamine transporter. The dopamine transporter, or reuptake site, is presumably a unique constituent of dopaminergic nerve terminals. Recent studies have shown that dopamine transporter immunoreactivity exists only in axons and axon terminals of dopaminergic neurons. In the human brain, no dopamine transporter immunoreactivity was demonstrated in glia or nondopaminergic neurons (25, 26).

The dopamine transporter was identified more than 25 years ago in *in vitro* uptake studies with striatal tissue (27). Many properties of the transporter have since then been elucidated. The use of molecular biology techniques in the 1980s and 1990s has allowed the cloning and characterization of the main molecular components involved in synaptic transmission, including the dopamine transporter. The reuptake of dopamine via the transporter is temperature-dependent and is inhibited by a variety of drugs, including amphetamine and cocaine (28). The dopamine transporter is also an important means of releasing dopamine under pharmacological and, perhaps, physiological conditions (29).

The dopamine transporter has been cloned from rat, bovine and human brain (30-33). Studies with various exo- and endonucleases indicate that the dopamine transporter is a *N*-linked glycoprotein containing *N*-acetylglucosamine and terminal sialic acid residues (29). The dopamine transporter is a member of a family of substrate-specific, high-affinity, sodium-dependent membrane transporters (34). The deduced primary amino acid sequences and predicted secondary structure of the dopamine transporters are highly homologous with other monoamine transporters. The most highly conserved sequences are thought to encompass the 12 hydrophobic (membrane spanning) domains (29). A single human dopamine transporter gene has been localized at the distal end of chromosome 5 (5p15.3). The dopamine transporter gene spans over 64 kb, consisting of 15 exons separated by 14 introns (35). Human cDNA encoding the dopamine transporter has been shown to possess a 40-nucleotide repeat element in the 3' untranslated region (locus: SLC6A3) (35, 36). At SLC6A3, the variable number of tandem repeats (VNTR) polymorphism in the 3' untranslated region has repeat numbers ranging from 3 to 11.

SPECT and PET tracers for the dopamine transporter

Either a γ-emitting (for example ^{123}I or ^{99m}Tc) or a positron-emitting (for example ^{18}F or ^{11}C) dopamine transporter binding radiopharmaceutical would be a potential radioactive molecule for imaging of dopaminergic neurons using SPECT or PET, respectively. In SPECT, the gamma photon emissions of the radionuclide are detected, whereas in PET, the annihilation radiation produced by a positron-emitting radionuclide is detected. Compared with SPECT, PET gives higher resolution images and more adequate quantification. The radioisotopes suitable for PET have short half-lives (for example 110 min for ^{18}F). Therefore, an on-site or locally situated cyclotron is needed for the production of the isotopes together with special facilities for the synthesis of the PET radiopharmaceuticals. In addition, PET instrumentation is

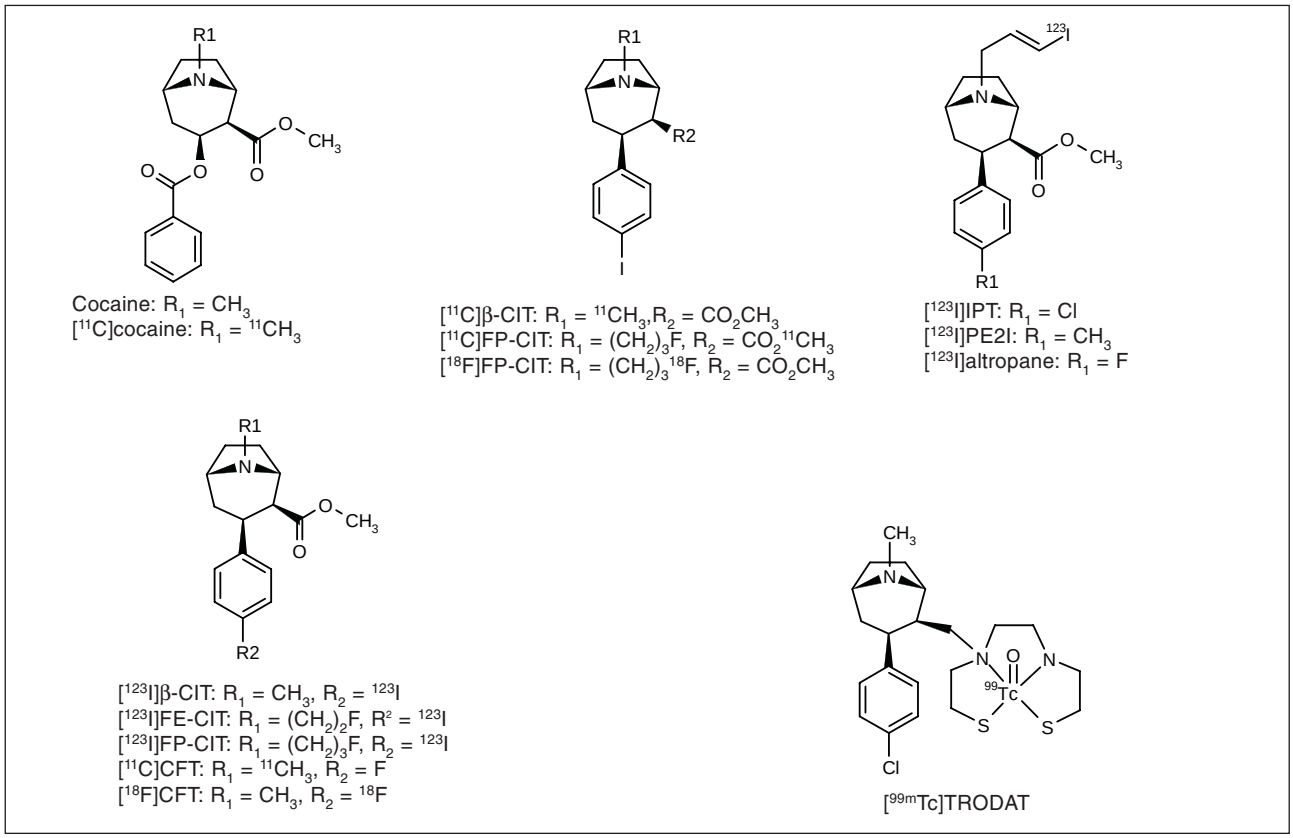

Fig. 1. Structural formulas of cocaine, [^{11}C]cocaine, [^{11}C]CFT, [^{18}F]CFT, [^{123}I]β-CIT, [^{11}C]β-CIT; [^{123}I]FE-CIT, [^{123}I]FP-CIT, [^{18}F]FP-CIT, [^{11}C]FP-CIT, [^{123}I]IPT, [^{123}I]PE2I, [^{123}I]altropane and [^{99m}Tc]TRODAT.

expensive. Consequently, PET studies are restricted to a few specialized centers. SPECT is available in almost every department of nuclear medicine. The SPECT imaging instrumentation is less expensive than the PET instrumentation. The relatively long half-lives of SPECT radiopharmaceuticals lead to simple logistics and low related costs in production and distribution. In addition, the long half-lives frequently offer the possibility to perform studies at the moment that transient equilibrium binding conditions have been established. In routine clinical practice, SPECT is practical and appropriate.

SPECT and PET tracers for the dopamine transporter can be broadly classified as either cocaine-like or noncocaine-like. Generally, cocaine and its analogs label the high- and low-affinity sites on the dopamine transporter, whereas noncocaine analogs only label the high-affinity site. Figure 1 shows the structure of cocaine and the most important cocaine-like SPECT and PET ligands for the dopamine transporter which have been examined *in vivo* in human subjects during recent years (18-23, 37-43).

The benzoyltropane cocaine is one of the most powerful stimulants of the central nervous system. Cocaine preferentially binds to dopamine transporters, with lesser interactions with monoamine transporters for norepinephrine and serotonin. Amino acids (especially aspartate) in transmembrane domain 1 of the dopamine transporter, as well as serines in transmembrane domain 7, are considered to play a role in cocaine binding (29, 44).

Cocaine is rapidly inactivated metabolically by hydrolysis of the 3β-benzoyl ester. Recently, a series of cocaine analogs have been developed in which the aromatic ring is attached directly to the tropane system, thus avoiding hydrolysis of the ester (38, 45, 46). Several of these ^{123}I-, ^{99m}Tc-, ^{11}C- or ^{18}F-labeled phenyltropanes are listed in Figure 1. These radiotracers differ with respect to their affinities and selectivities for the dopamine transporter, their specific to nonspecific uptake ratios and to their biokinetics.

Preclinical studies

For the evaluation of the ability of radiotracers to bind to the dopamine transporter, characterization studies have been performed. Most of the analogs in Figure 1 label dopamine transporters nonselectively. Apart from showing affinity for the dopamine transporter most of them also bind to serotonin and norepinephrine transporters (24, 47-51).

Although these radiotracers are not selective for the dopamine transporter, they show great potential for imaging of nigrostriatal dopaminergic neurons, since the vast

majority of monoaminergic transporters in the striatum are dopaminergic.

For imaging studies, only tracer amounts of the radioligand (< 5 nmol) will be injected. These amounts only occupy a very small percentage of the human dopamine transporters. Therefore, no pharmacological effects can be expected and have not been reported.

Clinical studies

Studies in human controls

Natural ageing is associated with a decline in dopaminergic neurons. Postmortem studies estimated a fallout of dopaminergic cells with advancing age in the substantia nigra pars compacta at a rate of 5-7% per decade (52). Age-associated reductions in striatal dopamine transporters have been reported in *in vivo* studies by both PET and SPECT imaging. PET and SPECT studies estimated an age-associated loss of 3-8% per decade (53-57) (Fig. 2). A recent [^{123}I]FP-CIT SPECT study also showed that the density of striatal dopamine transporters was slightly but significantly higher in healthy females than males (57).

Scintigraphic studies have shown a large variability in the density of striatal dopamine transporters in healthy volunteers. This variability could not be explained completely by ageing. Interestingly, recent studies using [^{123}I]β-CIT SPECT showed that genetic variation at the SCL6A3 3' VNTR polymorphism influences dopamine transporter binding in healthy controls. Two studies showed that subjects homozygous for the 10-repeat allele at the SCL6A3 locus demonstrated significantly lower striatal dopamine transporter binding than carriers of the 9-repeat allele (58, 59). Allelic variation at this locus has been found to be associated with several psychiatric disorders (60).

Studies in common forms of parkinsonism

1) Parkinson's disease

The major cause of parkinsonism is Parkinson's disease, accounting for approximately 60-85% of all cases (61, 62). Nigral dopaminergic projections to the striatum are targeted in Parkinson's disease, especially those to the putamen, while those to the caudate nucleus are relatively spared (63, 64). Degeneration of the nigrostriatal dopaminergic projection leads to loss of dopamine and dopamine transporters in the striatum (63-65). This lack of innervation to the striatum is believed to be responsible for the deficits in motor function, such as tremor, rigidity, bradykinesia (slowness of movement), hypokinesia (reduced movement), akinesia (loss of movement) and postural abnormalities, observed in patients with Parkinson's disease (63, 64, 66, 67).

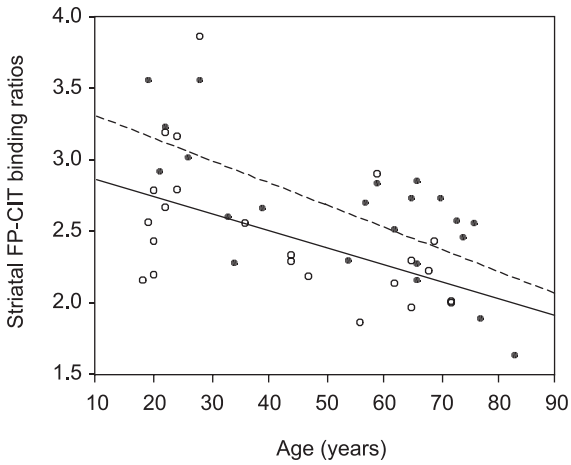

Fig. 2. Specific to nonspecific striatal [^{123}I]FP-CIT binding ratios (representing dopamine transporter density in the striatum) *versus* age in 45 healthy volunteers. Closed circles represent females (dotted line), open circles represent males (unbroken line).

Until now, the antemortem diagnosis of Parkinson's disease has been based on clinical observations, whereas the postmortem neuropathological examination of the brain with demonstration of loss of dopaminergic neurons in the substantia nigra and Lewy bodies is generally considered to be the gold standard (61). The correlation between a clinical diagnosis and the postmortem findings is relatively poor. About 24% of patients with a clinical diagnosis of Parkinson's disease were found to have a neuropathological diagnosis other than Parkinson's disease (62).

2) Imaging of presynaptic nigrostriatal dopaminergic neurons

Until recently, most research on the dopaminergic deficit in Parkinson's disease was performed with [^{18}F]DOPA PET scanning (15, 16, 54, 68-70). In agreement with results from necropsy studies, these studies showed a more pronounced reduction of striatal uptake in the putamen than in the caudate nucleus. Moreover, striatal uptake of the radiotracer was asymmetric (in nearly all patients with Parkinson's disease symptomatology starts unilaterally) and correlated with disease severity.

The results of SPECT and PET studies using tracers for the dopamine transporter are consistent with the results of [^{18}F]DOPA PET studies. These studies also showed a more pronounced reduction of striatal binding in the putamen than in the caudate nucleus, asymmetric loss of striatal dopamine transporters and a correlation with disease severity (21, 37, 41, 71, 72).

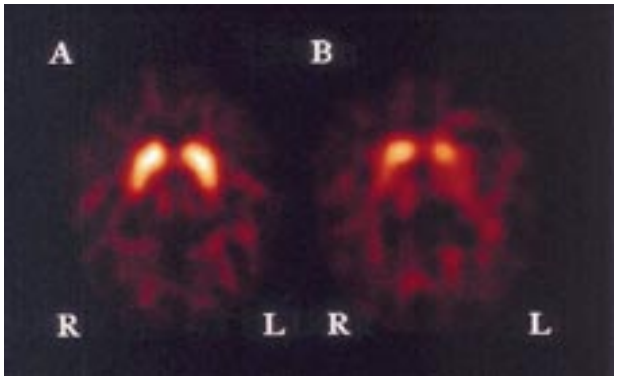

Fig. 3. [^{123}I]FP-CIT SPECT images obtained 3 h after injection of the radiotracer of (A) 65-year old healthy woman and (B) 59-year old female patient with hemi-Parkinson's disease (Hoehn and Yahr stage I). Transversal slices from brain at the level of the striatum (approximately 3 cm above orbitomeatal line) (L = left side; R = right side). In both images, level of activity is color coded from low (black) to high (white) and scaled to maximum in the slice of the healthy woman. Note the lower striatal (especially in the putamen) and asymmetrical activity in the patient compared to the healthy volunteer.

3) Imaging of nigrostriatal pathway in early Parkinson's disease

For clinical practice, results obtained in patients at an early phase of Parkinson's disease are of special interest. SPECT imaging with ^{123}I-labeled cocaine analogs (45) such as FP-CIT, β-CIT, IPT and altropane or ^{99m}Tc-labeled TRODAT showed a dramatic loss of striatal dopamine transporters in patients with Parkinson's disease with high signal to noise ratios (23, 41, 54, 71-73). Of these cocaine analogs for SPECT imaging, most experience has been gained with the tracers [^{123}I]FP-CIT and [^{123}I]β-CIT. Importantly, both [^{123}I]FP-CIT and [^{123}I]β-CIT SPECT studies showed loss of striatal dopamine transporters in patients with early Parkinson's disease (72, 74, 75) (Fig. 3). Moreover, these techniques were able to show bilateral loss of striatal dopamine transporters in patients with hemi-Parkinson's disease (72, 74, 75). Furthermore, both the [^{123}I]FP-CIT and [^{123}I]β-CIT SPECT techniques were able to discriminate completely groups of patients with Parkinson's disease from groups of healthy controls (71, 75). [^{123}I]FP-CIT was very recently registered for the European market as DaTSCAN.

PET in Parkinson's disease, using the ^{11}C- or ^{18}F-labeled cocaine analog β-CFT (or WIN 35,428) as a radiotracer for the dopamine transporter, discriminated patients with early Parkinson's disease from controls (37, 76). A recent study showed that not only the striatal but also the orbitofrontal and amygdalar presynaptic dopaminergic neurons were altered in patients with early Parkinson's disease (77). In addition, the authors showed that the putaminal and orbitofrontal β-CFT binding levels were correlated positively with motor and mental scores, respectively, of the Unified Parkinson's Disease Rating Scale.

The fast kinetics of FP-CIT offers the potential to be a useful radiotracer not only for SPECT imaging of the dopamine transporter but also for PET imaging (42, 46, 51). Interestingly, the results of recent imaging studies showed that [^{11}C]FP-CIT and [^{18}F]FP-CIT binding to striatal dopamine transporters reaches binding pseudo-equilibrium within the time course of a typical PET experiment (42). Moreover, the signal to noise ratios were very high. A recent [^{18}F]FP-CIT PET study showed a significant reduction in striatal dopamine transporter binding in patients with *de novo* Parkinson's disease (55).

4) Multiple system atrophy

Multiple system atrophy is a neurodegenerative disease that may account for up to 10% of patients with parkinsonism (78). It may present with any combination of extrapyramidal, pyramidal, autonomic and cerebellar features and is often poorly responsive to dopaminergic medication (78). Multiple system atrophy is neuropathologically characterized by neuronal degeneration and gliosis in the caudate and putamen, globus pallidus, brain stem, cerebellum and spinal cord (79). As in Parkinson's disease, degeneration of the nigrostriatal dopaminergic pathway has been reported (80).

Brooks and coworkers (16) have shown with [^{18}F]DOPA PET that patients with multiple system atrophy may present with relatively more impairment of the nigrostriatal dopaminergic projections to the caudate nucleus than patients with Parkinson's disease. Consequently, they suggested that putamen to caudate nucleus ratios could be used in the differential diagnosis among different forms of parkinsonism. However, the results from more recent [^{18}F]DOPA PET studies (81, 82) and from recent [^{123}I]β-CIT SPECT and [^{123}I]FP-CIT studies (83, 84) showed that the pattern of presynaptic dopaminergic degeneration is comparable in patients with multiple system atrophy and patients with Parkinson's disease. Therefore, it is not clear whether it is possible to make a distinction in individual cases on the basis of the results of imaging studies of the nigrostriatal pathway alone.

5) Progressive supranuclear palsy

Progressive supranuclear palsy is another akinetic-rigid syndrome characterized by an increased axial tone, bulbar palsy, rigidity of the extensors of the neck and supranuclear palsy. Neuropathologically, degeneration is found in the basal ganglia and the brain stem nuclei without Lewy bodies but, unlike in multiple system atrophy, with neurofibrillary tangles. Degeneration of the nigrostriatal dopaminergic pathway has been reported in progressive supranuclear palsy. In contrast to findings in Parkinson's disease, a similar degree of depletion of

dopamine was found in the caudate nucleus and putamen (85). In agreement with this postmortem finding, striatal [^{18}F]DOPA uptake is reduced in patients with progressive supranuclear palsy (16), putamen and caudate nucleus tracer uptake being similarly affected. This finding contrasted with findings in patients with Parkinson's disease, in whom caudate uptake is relatively spared (16). However, more recent [^{123}I]β-CIT SPECT and [^{11}C]CFT PET studies in patients with progressive supranuclear palsy on the patterns of striatal dopamine transporter loss revealed conflicting results. Brücke *et al*. (83) and Benamer *et al*. (84) showed that the pattern of loss of striatal dopamine transporters in patients with progressive supranuclear palsy is comparable to the pattern in Parkinson's disease. In contrast to this finding, Messa *et al*. (86) and Ilgin *et al*. (87) showed a relatively uniform degree of dopamine transporter loss in the caudate and putamen in progressive supranuclear palsy. Although it is possible that a pattern different from Parkinson's disease may be found in a sample of patients with supranuclear palsy, it does not seem possible to make a distinction in individual cases on the basis of the results of imaging studies of the striatal dopamine transporter density alone (83).

6) Essential tremor

Classically, patients with essential tremor present with a postural tremor of approximately 7 Hz with or without a kinetic tremor, involving hands or forearms. Parkinson's disease is clinically characterized by resting tremor, rigidity, bradykinesia and postural instability. However, in addition to a rest tremor, a postural tremor similar to that in patients with essential tremor may occur in patients with Parkinson's disease (88). On the other hand, the elderly patient with essential tremor may also have rest tremor and mild parkinsonian features as well. Consequently, this may occasionally lead to difficulties in distinguishing patients with essential tremor from patients with Parkinson's disease.

SPECT and PET studies have shown that there is no loss of nigrostriatal dopaminergic neurons in patients with classical essential tremor (84, 89). For example, Lee *et al*. (90) showed no loss of dopamine transporters in patients with isolated postural tremor using [^{123}I]IPT SPECT. However, in patients without parkinsonism, in whom rest tremor developed after the onset of postural tremor, they showed mild loss of striatal dopamine transporters. Consequently, SPECT or PET may at least be of value to discriminate classic essential tremor from Parkinson's disease.

Conclusions

Dopamine transporter imaging by either SPECT or PET has enhanced the possibility to study dopaminergic systems in the living human brain. This will be of interest not only in scientific studies aimed at studying the dopamine transporter availability in different diseases and disorders but also in other conditions, *e.g.*, occupancy of dopamine transporters by drugs. The results of several studies already indicated that imaging of the dopamine transporter is a very sensitive means of detecting disturbances of the nigrostriatal dopaminergic pathway in patients characterized with degeneration of dopaminergic neurons, such as Parkinson's disease. Therefore, imaging of the nigrostriatal pathway may become an important diagnostic tool in clinical practice.

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