Neurochemical Imaging of the Dopamine Transporter (DAT)

In the presynaptic dopaminergic neuron, the amino acid L-tyrosine is converted to dihydroxyphenylalanine (L-DOPA). Subsequently, DOPA decarboxylase or aromatic L-amino acid decarboxylase (AAADC) converts L-DOPA to dopamine. AAADC is a regulated enzyme, and its activity influences the rate of dopamine synthesis (1). Cytoplasmic dopamine is then moved into synaptic vesicles for storage and subsequent release through the vesicular monoamine transporter 2 (VMAT2). Dopamine cannot enter the brain to an appreciable degree because it is a polar molecule and the blood-brain barrier does not contain carriers for dopamine. The blood-brain barrier and brain cells do contain carrier systems for amino acids, and one of these is able to transport the dopamine precursor L-DOPA (L-amino acid transporter, LAT).

The DAT (SLC6A3 [GenBank DQ307031]) is a sodium chloride–dependent protein located on the presynaptic membrane of a dopaminergic terminal, and it regulates dopamine transmission at the synapse by removing dopamine from the synaptic cleft through active reuptake. DAT structure is characterized by 12 α-helical transmembrane domains, which consist of 20 to 24 amino acids, a large second hydrophilic extracellular loop with two to four potential glycosylation sites, and intracellular localization of both N- and C-terminals (2). It also has several putative phosphorylation sites for various kinases (for a review on DAT regulation see (3)). Phosphorylation and dephosphorylation of DAT at these sites regulate the reuptake of dopamine by controlling the cycle between transporter internalization and recruitment back to the plasma membrane and thus the number of active transporters (2). The gene that encodes the DAT protein is located on human chromosome 5, consists of 15 coding exons, and is roughly 64 kbp long (4). This gene has a variable number tandem repeat (VNTR) at the 3′ end (rs28363170), and the genetic polymorphism can affect the basal level of expression of the transporter (5).

In agreement with the main localization of DAT in dopaminergic axons, the highest levels of DAT are found in striatum (including putamen, caudate nucleus, and nucleus accumbens) and olfactory tubercle, with much lower levels in amygdala, hypothalamus, hippocampus, some thalamic nuclei, and neocortex (2). Because striatal DATs are located exclusively on dopamine-synthesizing

neurons, the measurement of striatal DAT binding is a specific marker of dopaminergic neuron density or integrity or innervation into the striatum (6). Genetic deletion of the DAT gene in mice results in a persistent extracellular hyperdopaminergic tone, which is functionally revealed as hyperactivity (for a review of animal DAT models see (7)). The lack of a reuptake mechanism produces a marked increase in functional extracellular dopamine that results in profound alteration of dopamine homeostasis (7). Numerous drugs have important pharmacological interactions with DAT (6). In general, these compounds fall into two categories: those that block dopamine transport (e.g., cocaine, methylphenidate) and those that serve as substrates for transport (e.g., dopamine, amphetamine, and 3,4-methylenedioxymethamphetamine [MDMA or "ecstasy"]) (8). Age is one of the factors influencing DAT density, and investigation of the effect of age on DAT availability has provided consistent results indicating a 6%–8% decline of DAT per decade, as shown with various SPECT or PET radioligands (9).

Because DAT distribution in the central nervous system coincides with dopaminergic innervation, DAT ligands have been developed for use in PET/SPECT neuroimaging as in vivo markers of dopaminergic systems.

The radioligands [^{99m}Tc]TRODAT, [^{123}I]β-CIT, [^{123}I]IPT, [^{123}I]FP-CIT, [^{18}F]CFT, and [^{11}C]PE2I can be used to measure dopamine active transport density in the striatum (for comprehensive reviews see (2, 9, 10)). In the early 1990s the phenyltropane analogs of cocaine [^{18}F]CFT (2β-carbomethoxy-3β-[4-fluorophenyl] tropane) and [^{11}C]CFT were shown to be highly selective for DAT (2). However, [^{18}F]CFT can be used in vivo exclusively with PET. Following the suggestion to convert CFT into an iodine-containing analog, [^{123}I]β-CIT (2β-carbomethoxy-3β-[4-iodophenyl] tropane) was developed for use with SPECT cameras (2). However, as the affinities of [^{123}I]β-CIT for DAT and serotonin transporter (SERT) are equivalent, analysis of tracer radioactivity distribution may be considered rather difficult. Another potential drawback associated with use of [^{123}I]β-CIT is that the uptake in human striatum is characterized by slow kinetics, and quantification of DAT uptake in the human striatum usually requires a delay of 24 hours between injection and imaging. In a quest to find radiotracers with higher selectivity and faster kinetics, several novel

compounds have been developed. The fluoropropyl derivative of [^{123}I]β-CIT, *N*-(-fluorpropyl-2β-carbomethoxy-3-[4-iodophenyl]) tropane, or [^{123}I]FP-CIT, has faster kinetics and allows imaging as early as 70 to 240 minutes after injection (2). Similarly, [^{123}I]*N*-(3-iodopropen-2-yl)-2β-carbomethoxy-3β-(4-chlorophenyl) tropane, or [^{123}I]IPT, can reach the maximum striatal uptake 1–2 h after injection (11). Another compound, [^{11}C/^{123}I]PE21 (*N*-[3-iodoprop-(2*E*)-enyl-2β-carboxymethoxy-3β-(4-methylphenyl) nortropane]) has been labeled recently for both PET and SPECT use and offers very good selectivity for striatal DAT (more than 30-fold for other monoamine transporters) (2, 12), allowing the evaluation of extrastriatal DAT bindings (13). The tropane derivative TRODAT-1 [ethanethiol, 2-((2(((3-(4-chlorophenyl)-8-methyl-8-azabicyclo [3, 2, 1]oct-2-yl)methyl) (2-mercaptoethyl) amino) ethyl)amino, (1R-exo-exo))-], labeled with [^{99m}Tc] is the only available technetium-labeled radioligand and has drawn interest because of its potential for routine clinical use (9); binding specifically changes in response to synaptic endogenous dopamine content (14) without high serotonin transporter affinity. Additionally, [^{99m}Tc] is readily available and relatively inexpensive, radiation exposure is low, and it offers the possibility of simultaneously using a second iodine-labeled ligand (14).

Image analysis of SPECT and PET results usually uses quantitative region of interest (ROI) ratios and/or qualitative visual grading. ROIs are placed on the whole striatum, caudate, and putamen. Striatal uptake is typically calculated in relation to a reference site with negligible DAT density (occipital or cerebellar cortex).

1. Cumming P, Gjedde A. Compartmental analysis of dopa decarboxylation in living brain from dynamic positron emission tomograms. Synapse. 1998;29(1):37-61.

2. Piccini PP. Dopamine transporter: basic aspects and neuroimaging. Mov Disord. 2003;18 Suppl 7:S3-8.

3. Schmitt KC, Reith ME. Regulation of the dopamine transporter: aspects relevant to psychostimulant drugs of abuse. Ann N Y Acad Sci. 2010;1187:316-40.

4. Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, et al. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics. 1992;14(4):1104-6.

5. Sano A, Kondoh K, Kakimoto Y, Kondo I. A 40-nucleotide repeat polymorphism in the human dopamine transporter gene. Hum Genet. 1993;91(4):405-6.

6. Meisenzahl EM, Schmitt GJ, Scheuerecker J, Moller HJ. The role of dopamine for the pathophysiology of schizophrenia. Int Rev Psychiatry. 2007;19(4):337-45.

7. Gainetdinov RR. Dopamine transporter mutant mice in experimental neuropharmacology. Naunyn Schmiedebergs Arch Pharmacol. 2008;377(4-6):301-13.

8. Williams JM, Galli A. The dopamine transporter: a vigilant border control for psychostimulant action. Handb Exp Pharmacol. 2006(175):215-32.

9. Varrone A, Halldin C. Molecular imaging of the dopamine transporter. J Nucl Med. 2010;51(9):1331-4.

10. Marshall V, Grosset D. Role of dopamine transporter imaging in routine clinical practice. Mov Disord. 2003;18(12):1415-23.

11. Schwarz J, Linke R, Kerner M, Mozley PD, Trenkwalder C, Gasser T, et al. Striatal dopamine transporter binding assessed by [I-123]IPT and single photon emission computed tomography in patients with early Parkinson's disease: implications for a preclinical diagnosis. Arch Neurol. 2000;57(2):205-8.

12. Emond P, Guilloteau D, Chalon S. PE2I: a radiopharmaceutical for in vivo exploration of the dopamine transporter. CNS Neurosci Ther. 2008;14(1):47-64.

13. Arakawa R, Ichimiya T, Ito H, Takano A, Okumura M, Takahashi H, et al. Increase in thalamic binding of [(11)C]PE2I in patients with schizophrenia: a positron emission tomography study of dopamine transporter. J Psychiatr Res. 2009;43(15):1219-23.

14. Schmitt GJ, Frodl T, Dresel S, la Fougere C, Bottlender R, Koutsouleris N, et al. Striatal dopamine transporter availability is associated with the productive psychotic state in first episode, drug-naive schizophrenic patients. Eur Arch Psychiatry Clin Neurosci. 2006;256(2):115-21.