

SPECT Imaging of Dopamine Transporter Sites in Normal and MPTP-Treated Rhesus Monkeys

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Parkinson's disease is characterized by degeneration of dopamine (DA) neurons and their terminals. Since these neurons contain dopamine transporters (DAT), radioligands that bind to these sites are promising radiopharmaceuticals for diagnosis and therapeutic monitoring of disease progression. We evaluated [^{123}I]-2 β -carbomethoxy-3 β -(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane ([^{123}I]IACFT) for SPECT imaging in an MPTP model of parkinsonism. **Methods:** Three rhesus monkeys were imaged before and at 1 and 2 mo after treatment with MPTP. The SPECT results were correlated with motor behavior and PET imaging with [^{11}C]-2 β -carbomethoxy-3 β -aryltropane ([^{11}C] - CFT). Also, biodistribution was measured by planar imaging. **Results:** In normal animals, striatal accumulation of radioactivity was rapid and peaked within 30 min. Striatal accumulation of [^{123}I]IACFT was nearly completely displaceable with unlabeled CFT (1 mg/kg) but was not affected by a similar dose of the serotonin (5-HT) transport inhibitor, citalopram. The striatal to cerebellar ratio measured at 30 min. after injection of [^{123}I]IACFT was significantly higher ($p < 0.01$) than with [^{11}C]CFT; ~6:1 versus ~2.5:1. After MPTP treatment this ratio decreased to 1.02:1 with IACFT and 1.23:1 with [^{11}C]CFT. Blood clearance of [^{123}I]IACFT was rapid with a terminal $t_{1/2}$ of ~30 min. HPLC of plasma samples demonstrated that the concentration of intact ligand decreases rapidly, approaching zero by 60 min. Low levels of accumulation were measured in extracranial tissues. **Conclusion:** These results demonstrate that [^{123}I]IACFT is an excellent SPECT ligand for dopamine transporter sites that combines the critical characteristics of: (a) high striatal to cerebellar ratios, (b) high selectivity for dopamine versus 5-HT transporter sites, (c) convenient preparation at high-specific activity and radiochemical purity and (d) a striatal localization rate that is well matched to the physical $t_{1/2}$ of ^{123}I .

Key Words: Parkinson's disease; dopamine transporters; iodine-123-IACFT; carbon-11-CFT; MPTP

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Parkinson's disease is a progressive neurodegenerative disorder that is characterized clinically by tremor at rest, rigidity, bradykinesia and postural instability. Pathologically, the condition is associated with degeneration of dopamine (DA) neurons in the substantia nigra and their terminal fields in the caudate-putamen. Despite this marked depletion in striatal dopamine neurons (1-4), most conventional imaging procedures that have been used to study patients with Parkinson's disease fail to detect decreases in dopamine innervation to the striatum before the $\geq 80\%$ reduction that is associated with clinical symptoms (5,6). In contrast, recent PET studies with [^{18}F]-6-fluoro DOPA and [^{11}C]-2 β -carbomethoxy-3 β -aryltropane (CFT), have dem-

onstrated that early asymptomatic disease can be detected at 50%-60% loss (7).

Although [^{18}F]-6-fluoro DOPA has been the most frequently used radiopharmaceutical for noninvasive measurements of dopamine neuron loss, drugs targeted to the dopamine transporter (DAT) are increasingly recognized as more accurate markers. Specifically, loss of dopamine transporter sites appear to correspond anatomically and quantitatively with loss of dopamine neurons (8,9). Most dopamine transporter ligands are cocaine analogs. Compared with cocaine, these compounds have higher affinity for dopamine transporter sites and more favorable pharmacokinetic properties (due to slower metabolism) (2,3,10,11). Recently, several cocaine analogs have been shown to be useful PET and SPECT ligands; [^{11}C]CFT (WIN 35,428) (12-15), [^{11}C] β -CIT and [^{123}I]- β -CIT (16-19) and [^{11}C]CPT (WIN 35,065) (20).

The cocaine analog, 2 β -carbomethoxy-3 β -(4-fluorophenyl)-tropane (CFT), has proven to be an important probe for studying cocaine transporter sites in the striatum (9-16,21,22). Early studies revealed that binding sites for [^3H]CFT are identical to those of cocaine which are associated with the dopamine transporter (9,10,14,23-25). Moreover, [^3H]CFT binding is decreased in postmortem striatal tissue of patients with Parkinson's disease and there is an excellent correlation between ligand binding and dopamine neuron density (3). Using a primate model of Parkinson's disease, we demonstrated that when CFT is labeled with ^{11}C , disease progression can be monitored noninvasively by PET (13). Recently, these findings were validated in human subjects (15).

PET with [^{11}C]CFT is a useful method for noninvasive quantification of the density of dopamine terminals and clinical monitoring of patients with movement disorders, but the expense and complexity of this technique currently limits general applicability. Clearly, a ligand suitable for SPECT would be of significant clinical value. Recently, 1 β -carbomethoxy-3 β -(4-iodophenyl)tropane (RTI-55) was synthesized and radio-iodinated (19). This compound has a 10-fold higher affinity than CFT for cocaine binding sites in the striatum of cynomolgous monkeys ($\text{IC}_{50} = 1.08$ versus 11 nmole) (12) and autoradiography of radiolabeled RTI-55 has revealed high levels of accumulation in dopamine nerve terminals of monkey brain (2). However, in a recent comparative autoradiographic study with [^3H]CFT and [^{125}I]RTI-55, it was demonstrated that significant concentrations of [^{125}I]RTI-55 also accumulate in serotonin-rich regions (25), although both ligands concentrate in dopamine-rich regions of the brain. This low selectivity is similar to the pattern observed with other aromatic ring substituted congeners, such as 2 β -carbomethoxy-3 β -(4-chlorophenyl)tropane (CCT) (12). In addition, a delay of 24 hr between injection and imaging is required for proper quantitation of dopamine transporter sites in the striatum (26).

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These properties of [¹²³I]RTI-55 make it suboptimal for SPECT studies of dopamine transporter sites. Since modifications of the ring nitrogen of the tropane moiety are known to preserve selectivity without significant reductions in affinity, these modifications might yield more specific ligands. In this context, we developed a method for preparing the E and Z isomers of [¹²³I]-2β-carbomethoxy-3β-(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropine (E- and Z-IACFT) as radioiodinated probes for SPECT (27). Based on comparative *in vitro* binding studies with [³H]CFT and [³H]citalopram (a high affinity and selective ligand for serotonin transporter sites (28)), this ligand was shown to have very high affinity (IC₅₀ = 6.62 ± 0.78 nmole) and selectivity (DA/5-HT = 25:1) for dopamine transporter sites. In addition, autoradiography in squirrel monkeys demonstrated extremely high levels of accumulation in the striatum and minimal activity in other brain regions (27).

In this study, we evaluated the SPECT imaging characteristics and receptor selectivity and specificity of [¹²³I]IACFT in normal and MPTP-treated rhesus monkeys. In addition, biodistribution was measured by gamma camera imaging.

MATERIALS AND METHODS

Materials

Sodium [¹²³I]iodide was purchased from Nordion, Canada. All chemical reagents and solvents were of the highest commercial grade available and were used without further purification. CFT and citalopram were obtained from Research Biochemicals International, Natick, MA.

Radiopharmaceutical Preparation

Removal of base and/or Na⁺ ions from stock solutions of [¹²³I]NaI was performed by chromatography on a strong cation exchanger (Sep-Pak, sulfonic acid on silica gel, Baker). The acidic eluate (pH 4–6) was collected in polypropylene microfuge tubes and stable iodide was added to yield a final specific activity of approximately 1 mCi of [¹²³I]/25 ng of unlabeled iodide (~5000 mCi/μmole). The tributyl Sn-precursor of IACFT (0.5 mg/50 μl in ethyl acetate) was then added, followed immediately by 10 μl of tert-BuCOOH. The reaction mixture was incubated at room temperature for 20 min and iodination yield was measured by radio-HPLC using a C18 reverse-phase column (RP300, 7 μ, 4.6 × 220-mm cartridge) eluted with methanol:phosphate (60:40, pH 7.2–7.3) at a flow rate 2 ml/min. Purification of the final product was performed using a semipreparative HPLC column eluted with the same solvent as for analysis. UV absorption was monitored at 254 nm with a flow-through spectrophotometer and radioactivity was monitored using a NaI probe interfaced to a scaler/rate meter. The outputs from both detectors were recorded and analyzed using a dedicated HPLC controller/integrator. Radiochemical purity of the final product was greater than 98%. Further details for the preparation of [¹²³I]IACFT and the methods for preparing [¹¹C]CFT have been reported elsewhere (29,30).

Animal Model

The neurotoxin MPTP (N-methyl-1,2,3,6-tetrahydropyridine), when administered to monkeys, produces a spectrum of motor, cognitive, biochemical and morphological changes that is not replicated in rodents (31,32,33). MPTP-treated monkeys develop neurological deficits (resting tremor, rigidity, akinesia, postural abnormalities), morphological changes (cell loss in the substantia nigra, ventral tegmental area, retrorubal fields) and biochemical changes (severe depletion of dopamine and decreases in norepinephrine and serotonin) that closely parallel idiopathic Parkinson's disease and postencephalitic parkinsonism (33).

Three rhesus monkeys (*Macaca mulatta*), weighing approximately 7 kg each, were treated with MPTP, 3–5 doses of 0.6 mg/kg

administered over 10 days. Treatment was performed under ketamine anesthesia (15 mg/kg). This dose of MPTP has previously been used to produce parkinsonism within 2–3 wk and depletion of [³H]CFT or [¹¹C]CFT binding sites by 1 mo. Animals treated in this manner were expected to show an inverse relationship between [¹²³I]IACFT binding and motor dysfunction. The marked depletion of dopamine terminals produced by this treatment should provide definitive evidence of the selectivity and sensitivity of [¹²³I]IACFT to detect full depletion of the nerve terminals. Each animal was imaged at baseline and at 4 and 8 wk after treatment.

Motor function was determined before imaging by videotaping each monkey in an observational cage and rating the tapes with a composite scale developed to measure signs of parkinsonism. The scale includes components that assess general level of activity, bradykinesia, stooped posture and tremor (34) and assessments of the type and body region of symptoms associated with parkinsonism that include rigidity, stooping, arm reaching and freezing (35).

Biodistribution of Iodine-123-IACFT in Normal Monkeys

Two normal rhesus monkeys weighing approximately 7 kg each were anesthetized with ketamine/xylazine (15.0 and 1.5 mg/kg) and positioned supine on the imaging bed of a double-headed gamma camera equipped with a low-energy high-resolution collimator. A 15% window was centered on the 159-keV photopeak of ¹²³I.

The monkeys were injected with approximately 5 mCi [¹²³I]-IACFT and serial simultaneous anterior and posterior whole-body images were acquired immediately after injection and at 15 and 45 min and 1, 3, 4, 24 and 48 hr later. On the first day, the images were acquired at a rate of 20 cm/min. The subsequent images were acquired at a rate of 5 cm/min. Arterial blood samples were collected at 15 and 30 sec and 1, 5 and 30 min and 1, 3 and 4 hr. Venous samples were collected at 24 and 48 hr. Phantoms prepared by adding ¹²³I to 500 ml saline infusion bags were also imaged.

ROIs were drawn over the whole body, brain, heart, lung, liver, stomach, spleen, kidney, intestine, bone, muscle and bone. Radioactivity cpm/pixel was calculated from the geometric means. Using the results of the phantom studies, these data were converted to percent injected dose * kg/g.

SPECT Imaging with Iodine-123-IACFT

Rhesus monkeys weighing approximately 7 kg were anesthetized with ketamine/xylazine (15.0 and 1.5 mg/kg) and positioned prone on the imaging bed of a single-headed rotating SPECT camera equipped with a low-energy high-resolution collimator. The heads of the animals were immobilized with a custom-fabricated head holder. A 15% window was centered on the 159-keV photopeak of ¹²³I.

The monkeys were injected with 5 to 7 mCi [¹²³I]IACFT and serial SPECT studies were acquired at between 5 and 90 min. Sixty-four 45-sec images were acquired over 360° using a 128 × 128 digital matrix. The images were reconstructed with a standard filtered back projection algorithm using a Butterworth filter (cut-off = 0.7, order = 7). Reconstructions were performed with a Siemens ICON computer. ROIs with a fixed number of pixels were drawn over the striatum and cerebellum. Six studies were performed in normal animals and three of the animals were subsequently treated with MPTP.

Selectivity of Iodine-123-IACFT for Dopamine Transporter Sites in Monkey Brain

For these studies, the animals were positioned supine on the imaging table of a gamma camera with the camera head parallel to and touching the lateral aspect of the skull. The animals were injected with 5 mCi [¹²³I]IACFT and serial 1.0-min planar images were acquired for 80 min. After acquisition of the 40th image, the

animals were injected with a receptor-blocking doses of CFT (1.0 mg/kg), citalopram [(2.0 mg/kg, (36)] or saline. Two monkeys were used in these studies and the treatments were separated by at least 2 wk. In parallel with the imaging, serial arterial blood samples were collected at 15 and 30 sec and 1, 5, 10, 30, 40 and 80 min after injection.

ROIs with a fixed number of pixels were drawn over the striatum and cerebellum. The difference between striatal and cerebellar activity (specific binding) was calculated for each image and was plotted as a function of time.

SPECT and PET Imaging in MPTP-Treated Monkeys

Three male rhesus monkeys were imaged before and at 1 and 2 mo after treatment with MPTP. The SPECT results were correlated with scores of motor behavior and PET imaging with [¹¹C]CFT. SPECT imaging was performed using the methods described above and PET imaging was performed by previously described methods (13). PET imaging was performed 48 hr before the SPECT studies.

Analysis of Iodine-123-IACFT Metabolites in Blood

The metabolism of [¹²³I]IACFT was studied as a function of time after intravenous administration. Arterial blood samples collected in the imaging studies were centrifuged (5000 × g for 5 min.) and total radioactivity was measured in aliquots of the plasma. The remainder of the plasma was analyzed by chromatography on C18 Sep-Paks that were activated with 10 ml of methanol and washed with 10 ml of phosphate buffer (pH 7.4). Twenty microliters of each plasma sample was diluted with 5 ml phosphate buffer (pH 7.4) and applied to C18 Sep-Paks. The cartridges were washed with two additional 5-ml volumes of buffer, followed by 5 ml methanol. Iodine-123 radioactivity was measured in each fraction, including the cartridge. With this chromatography system, intact [¹²³I]IACFT elutes in the methanol fraction (as confirmed by HPLC) and a small percentage is retained on the cartridge. The fraction of total radioactivity present as intact [¹²³I]IACFT at each time was used to calculate time-activity curves for unmetabolized ligand.

Statistical Analysis

The striatal-to-cerebellar ratios for [¹²³I]IACFT and [¹¹C]CFT in normal and MPTP-treated monkeys were analyzed by two-way ANOVA using a linear model in which radiopharmaceutical and drug treatment were the classification variables: Ratio = Radiopharmaceutical + Treatment + Radiopharmaceutical × Treatment. Posthoc comparisons were performed using Duncan's new multiple range test (37). The blood time-activity curves were analyzed by nonlinear least squares using a tri-exponential function. All results were expressed as mean ± s.e.m.

RESULTS

Biodistribution

Figure 1 shows representative anterior whole-body images acquired immediately after the monkey was injected with [¹²³I]IACFT and at 1, 4 and 24 hr later. In the immediate image, there was accumulation of tracer in brain, lung, myocardium, liver and kidneys. Relative photopenia was seen in the region of the stomach. In the later images, the intensity of accumulation in these regions decreased markedly. In these images, there was faint cardiac, brain and pulmonary activity. The striatum was not well visualized due to the positioning of the animal. Diffuse abdominal activity was observed, but there was no definite evidence of focal uptake in the bowel. Between 1 and 24 hr, there was marked accumulation of radioactivity in the stomach and thyroid, most probably secondary to dehalogenation and release of free iodine. Significant bladder activity was seen in the delayed images.

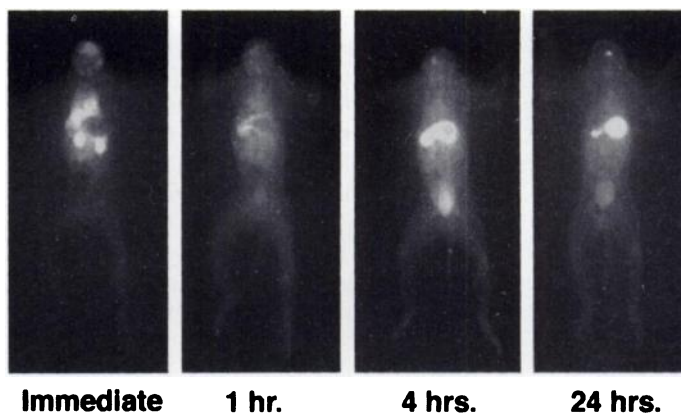


FIGURE 1. Anterior whole-body images of a monkey acquired immediately after injection of [¹²³I]IACFT and at 1, 4 and 24 hr later.

Figure 2 summarizes the quantitative biodistribution data determined by ROI analysis. In the brain, heart, lung, liver, spleen and kidney, there is a monotonic decrease in concentration of radioactivity with time. In the stomach, gastrointestinal tract, muscle, thyroid and bone, there is an initial rapid increase in concentration followed by a slower decline.

SPECT Imaging with Iodine-123-IACFT

Figure 3 shows transaxial, sagittal and coronal images of the brain of a monkey acquired at 30 min after injection. From these data, it is clear that there is a high concentration of radiopharmaceutical in the striatum with minimal accumulation in other areas of the brain. In particular, lack of accumulation in the thalamus, hypothalamus or midbrain, regions that are rich in 5-HT transporters, supports the specificity of this tracer for dopamine transporter sites. ROI analysis yielded striatal to cerebellar ratios of approximately 6 to 1 (range: 4.51–7.23).

Selectivity of Iodine-123-IACFT for Dopamine Transporter Sites in Monkey Brain

Lateral planar images of monkey brain showed significant accumulation of [¹²³I]IACFT in the striatum. In the early images there was diffuse accumulation of radioactivity throughout the brain. Over the first several minutes after injection, accumulation of tracer in the striatum intensified and the level of radioactivity in all other structures decreased. By 30 min after injection there was excellent contrast between striatum and the rest of the brain. After injection of a receptor saturating dose of unlabeled CFT, striatal accumulation of radioactivity decreased and by 60 min after injection, there was no evidence of focal accumulation in the striatum. In contrast, injection of a displacement dose of citalopram had no effect on the images. Figure 4 illustrates the quantitative relationship between specific binding and time. When a receptor-saturating dose of CFT was injected at 40 min after injection of [¹²³I]IACFT, specific binding was completely displaced. Similarly when the same dose of CFT was co-injected with [¹²³I]IACFT, no specific binding was detected (data not shown). In contrast, injection of citalopram had no effect on specific binding. These data demonstrate that specific binding is rapid and displacement is complete. The observation that treatment with citalopram was identical to saline controls (Fig. 4) supports the selectivity of [¹²³I]IACFT for dopamine transporter sites.

SPECT and PET Imaging of MPTP-Treated Monkeys

Before baseline imaging, all of the test animals had motor function ratings of 1–2 (1 = normal, 4 = maximal motor impairment). At 1 mo after MPTP treatment, the animals developed rigidity, dyskinesias, stereotypies, dystonias, stoop-

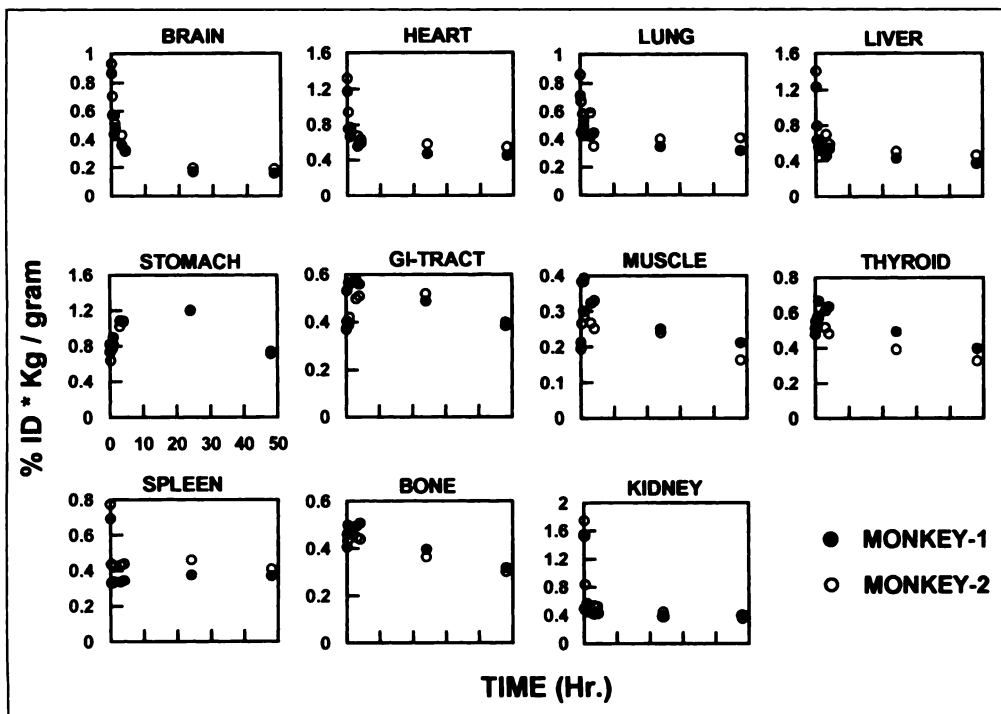


FIGURE 2. Biodistribution of [^{123}I]IACFT in two rhesus monkeys determined by gamma camera imaging immediately after injection and at 1, 4, 24 and 48 hr later. The results are expressed as percent injected dose \times kg/g.

ing posture, arm reaching, freezing and resting tremor and were rated at between 3 and 4. These symptoms and the scale scores remained unchanged 2 mo after treatment.

Figure 5 shows representative (midstriatal) transaxial, sagittal and coronal SPECT images of the brain of a rhesus monkey at baseline and 1 mo after MPTP treatment. These data demonstrate excellent striatal visualization in the baseline images. After MPTP treatment, the level of accumulation decreased markedly and the striatum could not be differentiated from surrounding structures. In addition, there was also a moderate decrease in [^{123}I]IACFT accumulation in the cerebral cortex of the MPTP-treated animals. This finding is consistent with previous observations of widespread losses of all three monoamine systems in the cerebral cortex after MPTP treatment (38). In some of the studies, tracer accumulation was observed in the thyroid and nasal region (Fig. 5), probably due to dehalogenation and uptake of free iodide.

In the baseline [^{11}C]CFT PET images the striatum was also well visualized, however, a low level of accumulation persisted after MPTP treatment (data not shown). Figure 6 summarizes the striatum-to-cerebellar ratios for all of the animals that were studied. ANOVA demonstrated significant main effects of

radiopharmaceutical ($p < 0.001$), MPTP treatment $p < 0.001$ and radiopharmaceutical by treatment interaction ($p < 0.001$). At baseline, the striatum-to-cerebellar ratio was significantly greater with [^{123}I]IACFT ($p < 0.001$). For both agents, the ratio decreased significantly after MPTP treatment ($p < 0.001$). After treatment, the ratio was significantly higher with [^{11}C]CFT ($p < 0.005$).

Metabolite Measurements

Radioactivity profiles of chromatographic fractions of plasma collected at 1, 5, 15 and 60 min after injection of [^{123}I]IACFT indicated that during the first minute after injection, nearly all of the radioactivity co-elutes with authentic [^{123}I]IACFT. At the later times, the concentration of radiolabeled metabolites increases and by 60 min after injection almost no intact [^{123}I]IACFT remains. Figure 7 shows the time dependence of: total plasma radioactivity (upper panel), the fraction of total plasma radioactivity that is present as intact [^{123}I]IACFT (middle panel) and the concentration of intact [^{123}I]IACFT in plasma (lower panel). These data demonstrate that the concentration of intact ligand decreases rapidly and approaches zero by 60 min after injection.

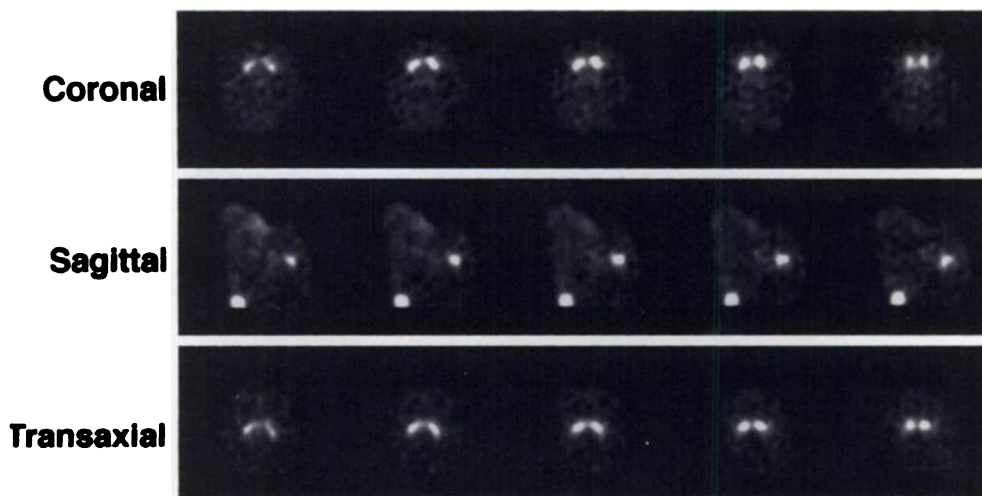


FIGURE 3. Transaxial, sagittal and coronal SPECT images of the brain of a normal rhesus monkey acquired at 30 min after injection of 7 mCi [^{123}I]IACFT.

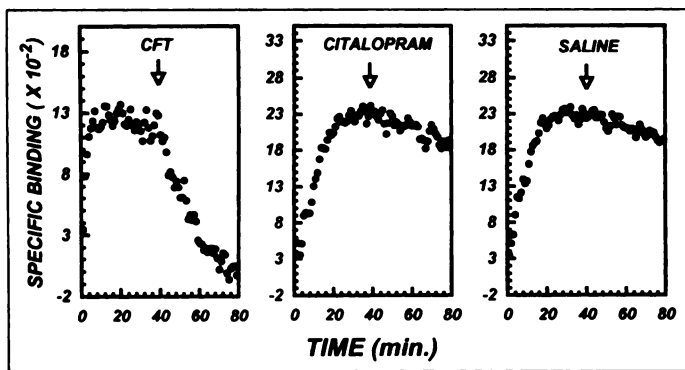


FIGURE 4. Quantitative relationship between specific binding (striatal minus cerebellar activity) and time. Unlabeled CFT (left panel), citalopram (middle panel) or saline (right panel) was injected at 40 min after radiopharmaceutical administration. These data illustrate that binding to dopamine transporter sites is rapid and selective.

DISCUSSION

As our population ages, the incidence of neuro-degenerative disorders such as Parkinson's disease will increase, and the need for sensitive and specific methods of early diagnosis will grow. Furthermore, as effective methods for treating Parkinson's disease are developed, this need will become more acute (39,40). Unfortunately, anatomic imaging techniques such as CT and MRI are ineffective for detecting early striatal abnormalities in these patients. Furthermore, since dopamine neurons contribute a small fraction to the overall metabolism of the striatum, PET tracers of glucose utilization and SPECT tracers of blood flow are of limited value for disease monitoring (5,6). Currently, the most-accepted imaging method for diagnosis and therapeutic monitoring of patients with Parkinson's disease is PET with [¹⁸F]-6-fluoro DOPA (41,42). However, this radiopharmaceutical has relatively low uptake in the striatum (striatum-to-cerebellar ratio ~2:1) and is of limited value for detecting early disease. Recently, the cocaine analog, [¹¹C]CFT, was introduced for PET imaging (13,15). This agent is highly selective for dopamine transporter sites (DA/5-HT ~ 12:1), has high accumulation in the striatum and has very low uptake in the rest of the brain.

Due to the lack of general availability of PET facilities, the

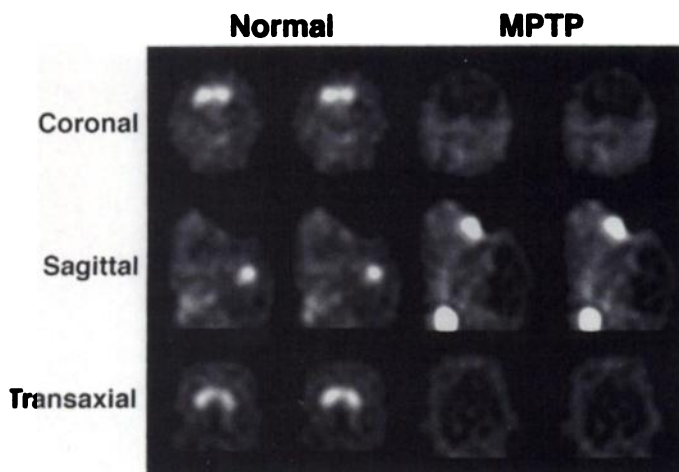


FIGURE 5. Representative (midstriatal) transaxial, sagittal and coronal SPECT images of the brain of a rhesus monkey before and after MPTP treatment. The images were acquired at 30 min after injection of 7 mCi [¹²³I]IACFT. Pre- and post-treatment images were normalized to a common gray scale. In the post-treatment sagittal images, accumulation of radioactivity is seen in the thyroid and nasal region. This is probably due to dehalogenation and uptake of free iodide.

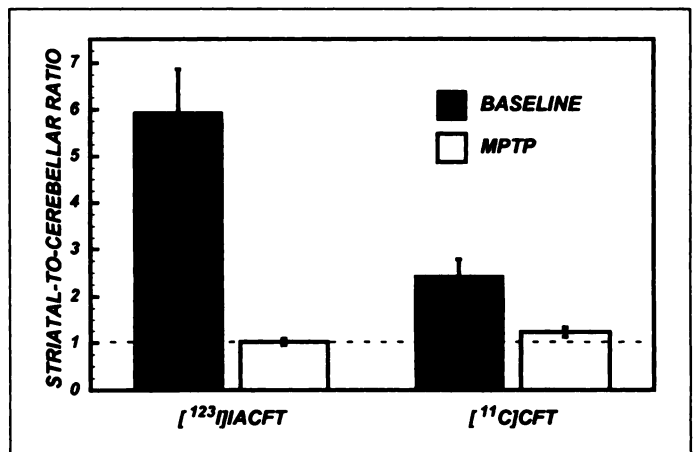


FIGURE 6. Average striatal-to-cerebellar ratios for [¹²³I]IACFT and [¹¹C]CFT at baseline and after MPTP treatment. Each value is the mean \pm s.e.m. for six studies.

SPECT ligand, [¹²³I]RTI-55 was introduced as a single-photon alternative for imaging dopamine transporter sites (43-46). Unfortunately, this ligand has relatively low in vitro and in vivo selectivity for dopamine transporter sites (DA/5-HT ~ 2:1) (27). Also its rate of localization in the striatum is poorly matched with the 13-hr half-life of [¹²³I], since effective quantification of dopamine transporter sites requires imaging at between 24 and 48 hr after injection (43).

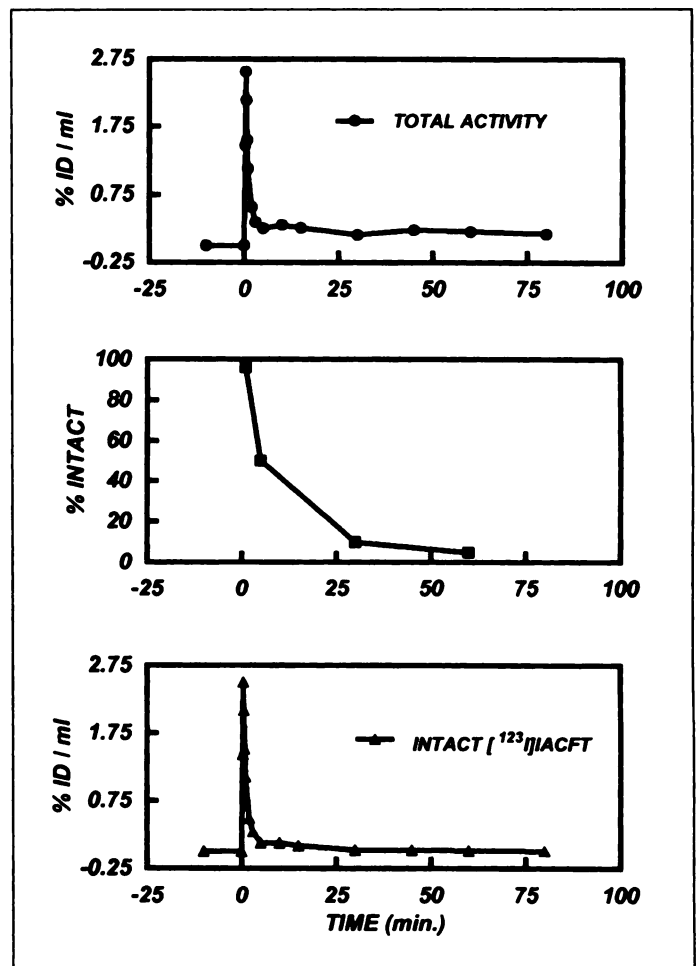


FIGURE 7. Time dependence of total plasma radioactivity (upper panel). Time dependence of the fraction of total plasma radioactivity that is present as intact [¹²³I]IACFT (middle panel). Metabolite-corrected time-activity curve (lower panel).

In contrast to [¹²³I]RTI-55, [¹²³I]IACFT has higher in vitro selectivity for dopamine transporter sites (DA/5-HT ~ 28:1). This study demonstrates that, although binding was totally displaceable with CFT, injection of a receptor-saturating dose of citalopram had a minimal effect on specific binding. Furthermore, there was no evidence of [¹²³I]IACFT accumulation in 5-HT transporter-rich regions such as the thalamus, hypothalamus and midbrain. Although it was previously demonstrated that striatal accumulation of [¹²³I]RTI-55 is similarly unaffected by citalopram, this tracer has significant accumulation 5-HT transporter rich regions (36). The observation that MPTP totally abolished striatal binding of [¹²³I]IACFT as indicated by a post-treatment striatal-to-cerebellar ratio of ~1:1 compared with a value of ~1.23:1 for [¹¹C]CFT supports this selectivity. This residual accumulation of [¹¹C]CFT could be due to association with 5-HT transporters (47), however, the superior resolution and sensitivity of PET versus SPECT might represent an alternative explanation.

Previously, Goodman et al. (48) reported the synthesis and characterization of a close structural analog of [¹²³I] IACFT, (N-(E)-3-[¹²⁵I] iodopropen-2-yl)-2β-carbomethoxy-3β-(4-chlorophenyl) tropane, [¹²⁵I] IPT) and demonstrated its potential as a dopamine transporter imaging agents. Recently, these investigators radiolabeled this compound with [¹²³I] and obtained excellent SPECT images of the striatum of normal monkeys (49). In general, [¹²³I] IACFT and [¹²³I] IPT have very similar imaging properties. However, since the selectivity of IPT is lower (DA/5-HT ~ 7:1), [¹²³I] IACFT appears to be more specific probe for dopamine transporter sites.

The observation that the striatum-to-cerebellar ratio measured with [¹²³I]IACFT decreases from ~6:1 in normal monkeys to ~1:1 in severely motor-impaired animals suggests that the dynamic range for detecting small changes in dopamine transporter concentration is much greater than with either [¹⁸F]-6-fluorodopa or [¹¹C]CFT. Furthermore, the results of the displacement studies indicate that it may be possible to use stepwise displacement of [¹²³I]IACFT for studying the receptor-binding characteristics of other dopamine transporter ligands (50).

Although the striatum-to-cerebellar ratio is a straightforward imaging index of dopaminergic function, previous studies have indicated that this parameter is a relatively poor measure of the concentration of receptor binding sites (51). Clearly, analyses based on kinetic modeling are more appropriate. Due to limitations in sensitivity of gamma cameras and the long half-lives of the tracers, dynamic studies with multiple radioligand injections are not convenient for quantitative analysis of ligand-receptor interaction with SPECT. However, the situation is more favorable when equilibrium methods are used. For example, compartmental modeling was recently used to analyze the binding of [¹²³I]iodobenzofuran to dopamine D2 receptors in normal humans (51). When ligand-receptor dissociation is slow, the long half-life of [¹²³I] can prove to be an advantage. Under these circumstances, successive displacement or primed constant infusion methods can be used to study receptor binding (50,52). Based on the pharmacokinetics of [¹²³I]IACFT, it should be possible to achieve equilibrium within 1.5 hr of infusion after a priming dose. With this technique, only a single SPECT acquisition and a venous blood sample are required for analysis.

CONCLUSION

This study demonstrates that [¹²³I]IACFT is an excellent SPECT ligand for dopamine transporter sites that combines the important characteristics of: (a) high striatal to cerebellar ratios,

(b) high selectivity for dopamine versus 5-HT transporter sites, (c) convenient preparation in high-specific activity and radiochemical purity and (d) striatal localization rate is well matched to the physical half-life of [¹²³I]. If these observations can be extended to human imaging, [¹²³I]IACFT could become a very useful radiopharmaceutical for the clinical evaluation and therapeutic monitoring of patients with Parkinson's disease and other neurological diseases.

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