Evaluation of Carbon-11-Labeled 2β-Carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane as a Potential Radioligand for Imaging the Serotonin Transporter by PET

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The nortropane cocaine analogue, 2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane (ZIENT), is a high affinity, selective serotonin transporter (SERT) ligand that has shown promise as a SERT imaging agent for single photon computed tomography (SPECT) when labeled with I-123. Synthesis of the labeling precursor, radiosynthesis of [^{11}C]ZIENT, and in vivo evaluation in anesthetized and awake monkeys have been performed to determine the suitability of [^{11}C]ZIENT as a PET agent for SERT imaging.

Introduction

The neuronal uptake of serotonin from the extracellular space into the presynaptic neuron is regulated by the serotonin transporter (SERT). The SERT proteins reside in high density on the cell bodies of the medial and dorsal raphe nuclei in the brainstem and the terminals of the serotonergic neurons located mainly in the hypothalamus, thalamus, striatum, and cerebral cortex.[2−4] The SERT has attracted considerable attention in recent years because of its involvement in the pathophysiology of neuropsychiatric disorders.[5−9] The in vivo imaging of the SERT with positron emission tomography (PET) or single photon emission computed tomography (SPECT) has been a major target of neuroimaging research because of the potential use of SERT imaging agents to further understanding of neuropsychiatric disorders.

We recently reported the synthesis and in vitro characterization of the candidate SERT ligand iodine-123-labeled 2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane ([^{123}I]-ZIENT).[1] This compound possesses high affinity and selectivity for the human SERT (K_i = 0.03 nM) with affinities for the human DAT and NET 69 and 474-fold lower, respectively, than for SERT. The biodistribution and imaging studies previously performed with ZIENT labeled with iodine-123 demonstrated its suitability for visualizing the SERT in nonhuman primates. In attempts to develop a SERT radioligand possessing a positron-emitting radioisotope to take advantage of the superior temporal and spatial resolution of PET, we investigated the properties of carbon-11-labeled 2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]tropane [^{11}C]ZIET. This tropane, obtained by N-alkylation of ZIENT using [^{11}C]methyl iodide, was evaluated in vitro and in vivo to determine its affinity and selectivity for the SERT and imaging properties.[10] [^{11}C]ZIET showed high uptake in the SERT-rich regions; however, the inability of the SERT-selective ligand, citalopram, to displace the activity from the striatum may reflect the presence of a DAT component. Given that competitive binding studies previously reported in the literature demonstrated that tropanes have typically a lower selectivity for the SERT versus the DAT than their corresponding *N*-desmethyl nortropanes,[11] we have synthesized the carbon-11-labeled 2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane [^{11}C]ZIENT. The present work describes the synthesis of the precursor for [^{11}C]ZIENT, the radiolabeling conditions, and in vivo microPET imaging in cynomolgus monkeys and in vivo PET imaging in awake rhesus monkeys.

Experimental Section

General. All reagents used were obtained from commercially available sources except unlabeled ZIENT and 2β-carbomethoxy-3β-(4′-bromophenyl)nortropane which were prepared as described previously.[1,10] Solvents used in reactions were purchased from Aldrich Chemicals (Milwaukee, WI) while solvents for chromatography were obtained from VWR (West Chester, PA). ^{1}H NMR spectra were recorded on a Varian spectrometer at 400 MHz or 300 MHz and referenced to the NMR solvent (chemical shifts in ppm values, *J* values in Hz). Mass spectra were determined on a VG 70-S double focusing mass spectrometer using high-resolution electron ionization (EI) or a JEOL JMS-SX102 double focusing mass spectrometer using fast atomic bombardment (FAB). Silica gel column chromatography was performed using Merck silica gel 60 (40−63 μm particle size). Thin-layer chromatography (TLC) was performed using 250 μm layers of F-254 silica on aluminum plates obtained from Whatman (Clifton, NJ).

Chemistry. 3β-(4′-Bromophenyl)nortropane 2β-carboxylic Acid (1). A solution of 2β-carbomethoxy-3β-(4′-bromophenyl)-nortropane (1.0 g, 3.08 mmol) in H_2O/1,4-dioxane (1/1.3 mL) was refluxed for 3 days. After completion of the reaction, the solvents were removed. The residue was taken up in acetonitrile and filtered to give a white solid (0.86 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62−2.15 (m, 5 H), 2.54−2.60 (m, 1H), 2.64−2.70 (m, 1 H), 3.04−3.15 (m, 1H), 3.21 (s, 3H), 3.84−3.95 (m, 2H), 7.17 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 10.05 (br s, 1H), 11.6 (br s, 1H).

***N*-(*tert*-Butoxycarbonyl)-3β-(4′-bromophenyl)nortropane 2β-carboxylic Acid (2).** To a suspension of acid **1** (0.3 g, 9.67 mmol) in dichloromethane (20 mL) was added triethylamine (250 μL) followed by a solution of di-*tert*-butyl dicarbonate (280 mg, 1.28 mmol) in dichloromethane (6 mL). The reaction mixture was stirred at room temperature for 2 h. The volatiles were evaporated under reduced pressure, and the residue was purified by flash chromatography (dichloromethane:acetone 95:5 to 88:12) to afford a white solid (0.35 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.60−1.74 (m, 2H), 1.78−1.86 (m, 1H), 1.97−2.08 (m, 1H), 2.10−2.20 (m, 1H), 2.61−2.70 (m, 1H), 2.85−2.87 (m, 1H), 3.16−3.19 (m, 1H), 4.49−4.52 (m, 1H), 4.66−4.68 (m, 1H), 7.10 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 9.15−9.60 (br s, 1H).

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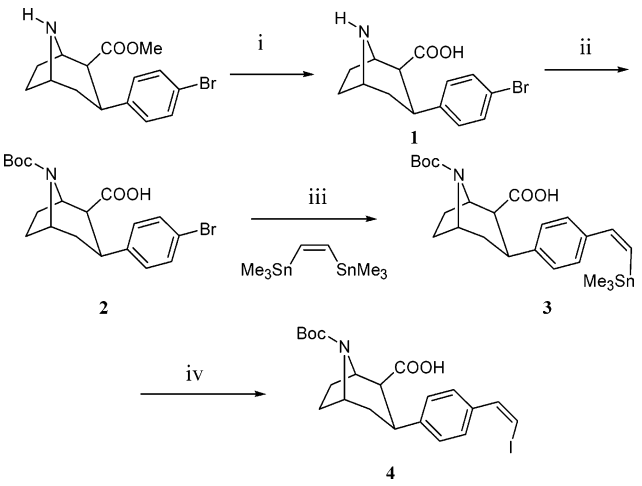
***N*-(*tert*-Butoxycarbonyl)-3β-[4′-((*Z*)-2-trimethylstannylethenyl)-phenyl]nortropane 2β-carboxylic Acid (3).** The acid **2** (0.24 g, 0.58 mmol) was dissolved in toluene (15 mL) which had been degassed by bubbling argon through for 5 min. Triethylamine (120 mg, 1.19 mmol), tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.03 mmol), and (*Z*)-1,2-trimethylstannylethylene (0.4 g, 1.0 mmol) were successively added. The reaction mixture was stirred at 100 °C for 3 h. The volatiles were evaporated under reduced pressure. The residue was purified by flash chromatography (dichloromethane:acetonitrile:triethylamine 9:1:0.1) to afford a yellow oil (0.135 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ-0.02−0.16 (m, 9H), 1.40 (s, 9H), 1.60−1.84 (m, 3H), 1.92−2.16 (m, 2H), 2.71−2.81 (m, 1H), 2.86−2.88 (m, 1H), 3.14−3.19 (m, 1H), 4.49−4.52 (m, 1H), 4.66−4.68 (m, 1H), 6.11 (d, J = 13.5 Hz, 1H), 7.14 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 13.8 Hz, 1H).

***N*-(*tert*-Butoxycarbonyl)-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane 2β-carboxylic Acid (4).** To a solution of *N*-(*tert*-butoxycarbonyl)-3β-[4′-((*Z*)-2-trimethylstannylethenyl)phenyl]nortropane 2β-carboxylic acid (**3**) (50 mg, 0.09 mmol) in dichloromethane (5 mL) at 0 °C was added dropwise iodine monochloride (1 M in dichloromethane, 0.125 mL). The reaction mixture was stirred at this temperature for 7 min and then quenched with an aqueous solution of sodium metabisulfite (5% w/w, 10 mL). The organic layer was separated, washed with water, and evaporated under reduced pressure. The residue was purified by HPLC (Waters, X-terra RP_{18} 19 × 50 mm, 28% acetonitrile in H_2O + 0.1% Et_3N, 5 mL/min, rt = 6.55 min). To the combined fractions containing the desired compound were added a few drops of acetic acid to neutralize the triethylamine. Then, the acid **4** was extracted with dichloromethane. Evaporation of the organic phase gave a thick oil (22 mg, 47%) that slowly crystallized. ^{1}H NMR (400 MHz, $CDCl_3$) δ1.31 (s, 9H), 1.63−1.72 (m, 2H), 1.78−1.85 (m, 1H), 1.98−2.04 (m, 1H), 2.08−2.16 (m, 1H), 2.64−2.71 (m, 1H), 2.88−2.91 (m, 1H), 3.19−3.25 (m, 1H), 4.48−4.50 (m, 1H), 4.65−4.67 (m, 1H), 6.49 (d, J = 8.4 Hz, 1H), 7.22−7.27 (m, 3 H), 7.57 (d, J = 8.0 Hz, 2H). HRMS (EI) calcd for $C_{21}H_{26}INO_4$: 483.09066, Found 483.09141.

Radiosynthesis of [^{11}C]ZIENT. No carrier-added [^{11}C]CO_2 was produced through the bombardment of $^{14}N_2$ gas containing 1% $^{16}O_2$ by a Siemens 11 MeV RDS 112 cyclotron at Emory University Hospital through the ^{14}N[p,α]^{11}C reaction. A GE MicroLab methyl iodide system was employed for the conversion of [^{11}C]CO_2 to [^{11}C]-CH_3I. The synthesis of [^{11}C]ZIENT was performed using the N-protected acid precursor **4**. The precursor (∼0.65 mg) dissolved in *N*,*N*-dimethylformamide (250 μL) was placed in a sealed v-vial (3.0 mL). A 2 μL portion of tetrabutylammonium hydroxide (0.33 M in H_2O) was added. The [^{11}C]CH_3I was delivered as a gas to the reaction vial and bubbled through the solution containing the precursor with ice bath cooling. After delivery of [^{11}C]CH_3I, the sealed vessel was heated at 85 °C for 2.5 min, and then 6 N hydrochloric acid (50 μL) was added. After 7 min at 85 °C, the vessel was cooled for 30 s in a water-ice bath and aqueous ammonia (30% w/w, 80 μL) was added. The reaction mix was diluted with HPLC mobile phase (300 μL) prior to injection onto the semi-prep HPLC column. HPLC purification was performed using a reverse phase C_{18} column (Waters, X-terra RP_{18} 19 × 100 mm,) at a 9 mL/min flow rate with a mobile phase consisting of 45% of water in ethanol. At a flow rate of 9 mL/min, the retention time of [^{11}C]-ZIENT was approximately 10 min, and fractions eluting at this time containing radioactivity were purified using a modified solid-phase extraction (SPE) procedure as reported earlier.[12] The eluting fractions containing product were combined with one volume of water. After stirring, the homogeneous solution was applied to a Waters C_{18} SepPak (previously activated with ethanol (10 mL) and water (10 mL)) by transferring under vacuum through a Teflon line. The eluate was directed to the waste.

The C_{18} SepPak was successively washed with saline (0.9% NaCl, 40 mL) and ethanol (0.5 mL) with the eluate directed to waste. The radiolabeled tropane was eluted with ethanol (1.5 mL) and driven to a sterile empty vial containing 3.5 mL of saline. The resulting solution was transferred under argon pressure through a Millipore filter (pore size 1.0 μm) followed by a smaller one (pore size 0.2 μm), to a 30 mL sterile vial containing 10 mL of saline. Radiochemical purity and specific activity were determined by reverse-phase analytical HPLC (NovaPak, 3.9 × 150 mm, 1.0 mL/min flow rate, mobile phase of 75:25:0.1 methanol:water:triethylamine).

Scheme 1[a]



[a] Reagents: (i) H_2O/1,4-dioxane, reflux. (ii) Boc_2O, Et_3N, DCM, rt. (iii) (*Z*)-1.2-trimethylstannylethylene, $Pd(PPh_3)_4$, Et_3N, toluene, 100 °C. (iv) ICl, DCM, rt.

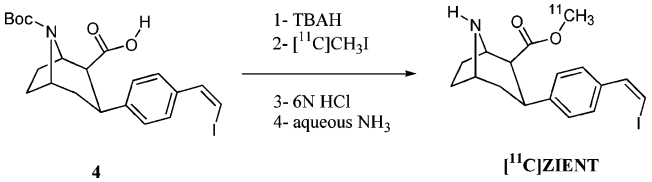
PET Studies in Anesthetized Monkeys. *Micro*PET studies were performed using adult male cynomolgus monkeys (4.5−9 kg) as reported previously.[10] Concorde *Micro*PET P4 system (Knoxville, TN). The *Micro*PET imaging studies were performed using [^{11}C]-ZIENT in cynomologus monkeys with and without injection of pharmacological doses of monoamine transporter ligands. Emission data were collected in the *Micro*PET imaging studies continuously for 125 min after injection of [^{11}C]ZIENT and then binned for analysis. The data were converted to standard uptake values (SUV). SUV values were defined as the pixel value in [(μCi/mL × weight of animal (mg)]/[(dose(μCi)]

PET Studies in Awake Monkeys. PET studies were performed in awake rhesus monkeys. These subjects received extensive behavioral training to ensure immobility throughout the neuroimaging session as described in a previous publication.[13] Quantitative brain PET images were acquired using an ECAT 921 EXACT scanner (Siemens/CTI). The PET images for each subject were co-registered with MRI. The data were converted to standard uptake values (SUV).

Results and Discussion

Chemistry. 2β-Carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane (ZIENT) was synthesized according to a procedure described previously.[1] The preparation of [^{11}C]ZIENT through an O-methylation reaction required preparation of the corresponding carboxylic acid. The obvious synthetic strategy for obtaining this precursor focused on the hydrolysis of the methyl ester. Numerous efforts to hydrolyze the ester of ZIENT were undertaken, but none of the conditions provided the required acid. The conditions that were tried included 4 N hydrochloric acid at reflux, 5 N sodium hydroxide at reflux, neutral conditions with a mix of water/1,4-dioxane at reflux, porcine liver esterase at 37 °C, and the Lewis acid boron tribromide in dichloromethane. Consequently, another strategy was developed which consisted of hydrolyzing the methyl ester earlier in the reaction sequence as depicted in Scheme 1. The 3β-(4′-bromophenyl)-nortropane 2β-carboxylic acid (**1**) was obtained by refluxing 2β-carbomethoxy-3β-(4′-bromophenyl)nortropane in a mixture of water and 1,4-dioxane. The secondary amine was selectively protected by treatment with di-*tert*-butyl dicarbonate in the

Scheme 2



presence of triethylamine to give the *N*-boc acid **2**. The vinyltin **3** was prepared via a palladium catalyzed cross coupling reaction using freshly made (*Z*)-1,2-trimethylstannylethylene and tetrakis-(triphenylphosphine)palladium(0) as catalyst in toluene. Triethylamine was added to prevent the destannylation in the presence of the carboxylic acid. The compound **3** was then converted to the labeling precursor *N*-(*tert*-butoxycarbonyl)-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane 2β-carboxylic acid (**4**) by iododestannylation using iodine monochloride in dichloromethane.

Radiosynthesis of [^{11}C]ZIENT. The radiolabeling of nortropanes with carbon-11 at the 2β-position has been previously reported.[14,15] The standard conditions involve treating the nortropane acid with [^{11}C]methyl iodide or [^{11}C]methyl triflate in the presence of a base. Although these conditions have been proved to be effective, the competitive reactivity of the two nucleophilic sites for alkylation leads to the formation of two compounds labeled either at the O-position or at the N-position. The ratios of {*O*-[^{11}C]methyl} incorporation versus {*N*-[^{11}C]-methyl} reported in the literature were ranged from 1.5 to 1.

To prevent N-alkylation, the radiosynthesis of [^{11}C]ZIENT was performed in two steps from the *N*-Boc acid **4** as shown in Scheme 2. First, the ammonium salt of the acid **4** was formed using tetrabutylammonium hydroxide (TBAH) in water as base. The choice of TBAH in water instead of the standard TBAH in methanol was dictated by the reactivity of the carboxylic acid, which could readily react with the methanol in the presence of hydrochloric acid to form undesired cold ZIENT. The salt obtained reacted with the [^{11}C]methyl iodide at 85 °C for 2 min, then the *N*-Boc group was cleaved by treatment with 6 N hydrochloric acid at 85 °C for 7 min. Finally, the hydrochloric salt formed was neutralized with concentrated aqueous ammonia before HPLC purification.

Starting with 400−500 mCi of cyclotron produced methyl iodide, typical syntheses provided 30−60 mCi (uncorrected) of [^{11}C]ZIENT in an average decay-corrected yield (dcy) of 45% (n = 10, yield calculated from [^{11}C]methyl iodide) in a total synthesis time of 45 min. Analytical HPLC demonstrated that the radiolabeled product was over 99% radiochemically pure, and the specific activity ranged from 0.3 to 0.9 Ci/μmol at time of injection (15 to 20 min after EOS).

MicroPET Anesthetized Nonhuman Primate Imaging. MicroPET imaging studies were performed with [^{11}C]ZIENT to determine its brain regional distribution, imaging properties, and in vivo selectivity for the SERT.

The time−activity curves (Figures 1 and 2) were generated from the images acquired after administration of [^{11}C]ZIENT in cynomologus monkeys by manually drawing the brain regions-of-interest (ROIs).

The microPET images showed an accumulation of radioactivity in SERT-rich brain region. The summed images collected from 0 to 125 min following tracer injection showed a clear visualization of the midbrain, putamen, caudate, thalamus, pons, and medulla. The resulting time-activity curves for [^{11}C]ZIENT binding in monkey are presented in Figure 1. The highest ratios relative to cerebellum, a brain region with low SERT density,

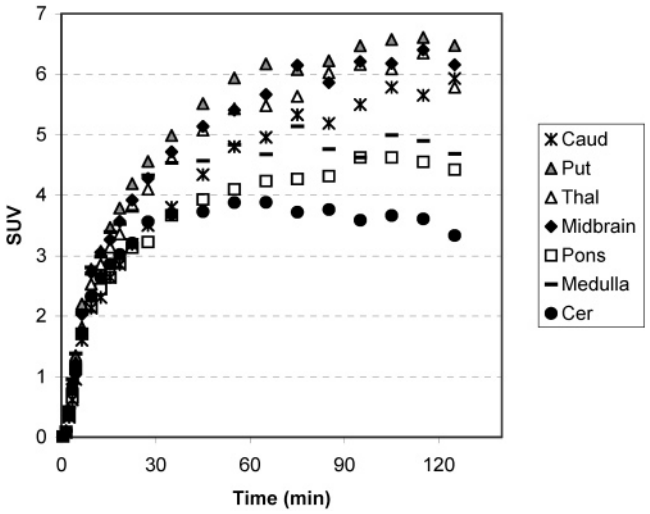


Figure 1. Time−activity curves for brain regions for [^{11}C]ZIENT in an anesthetized cynomolgus monkey. Images were acquired for a total time of 125 min.

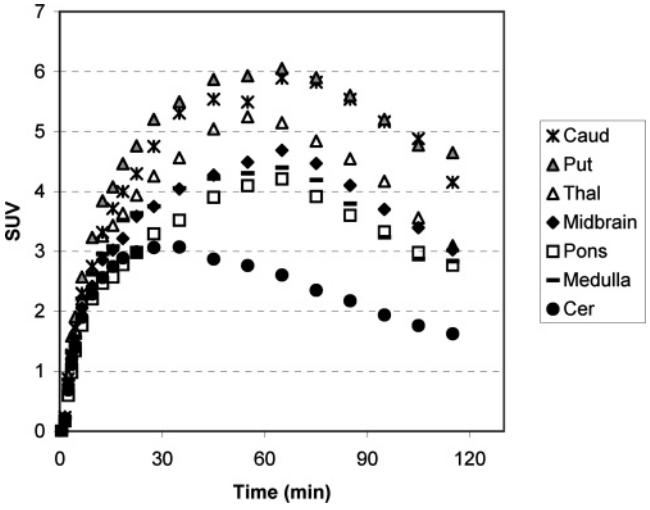


Figure 2. Time−activity curves for [^{11}C]ZIENT in an anesthetized cynomolgus monkey with citalopram administered at 60 min. Images were acquired for 115 min.

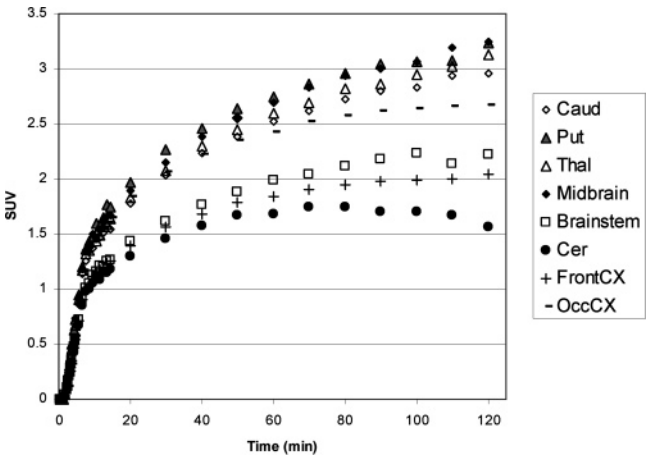


Figure 3. Study 1 time−activity curves for brain regions for [^{11}C]-ZIENT (10.53 mCi) in an awake rhesus monkey (9.65 kg). PET images were acquired for 120 min.

occurred at the end of the study (125 min) and were 1.9, 1.9, 1.8, 1.7, 1.4, and 1.3 for the putamen, midbrain, caudate, thalamus, medulla, and pons, respectively. The uptake of [^{11}C]-

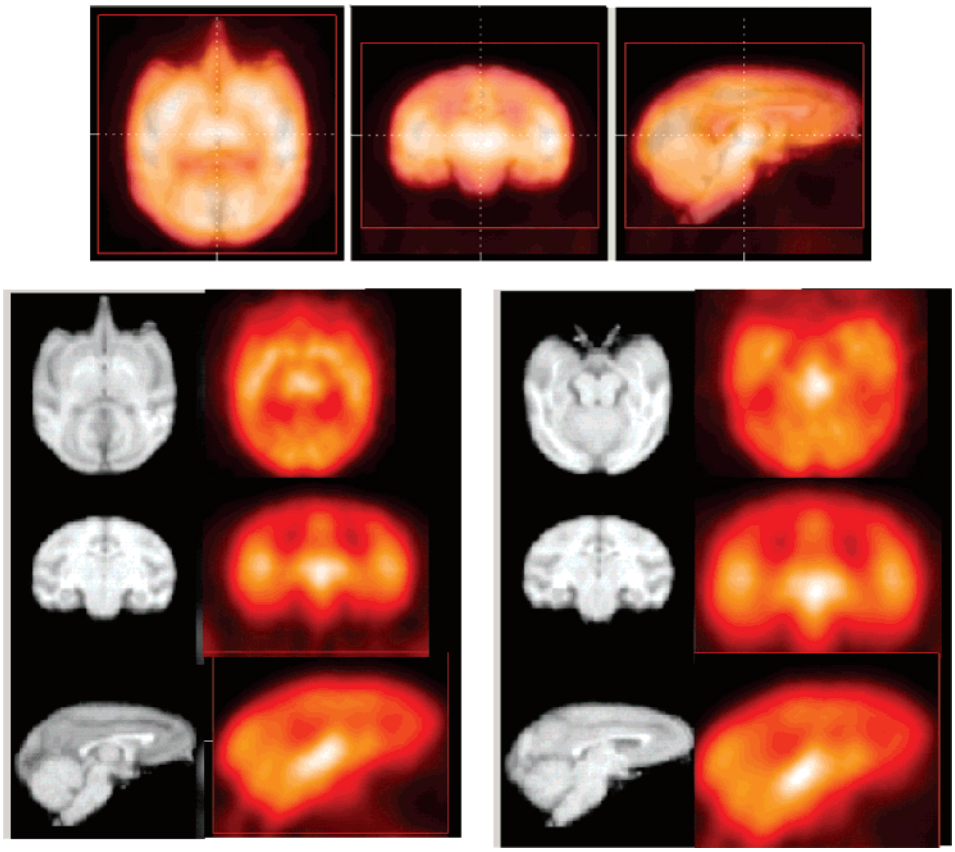


Figure 4. Transverse, coronal, sagittal PET images acquired using [^{11}C]ZIENT and coregistered with MRI. These images are the result of summed images from 0 to 120 min postinjection of 5 mCi of [^{11}C]ZIENT

ZIENT reflects the known distribution of the SERT in monkey brain[13,18] and is in agreement with its in vivo binding affinities. However, the concentration of radioactivity in the putamen and the caudate is slightly higher than the distribution previously reported for selective SERT imaging agents. This phenomena was already observed with the corresponding tropane [^{11}C]-ZIET[22] and suggested that a DAT component might be present. To assess the specificity of [^{11}C]ZIENT binding, a displacement study was performed using the selective SERT ligand citalopram. A dose of citalopram (1.5 mg/kg) was administered intravenously 60 min after injection of radiotracer and produced a decrease of [^{11}C]ZIENT binding to the SERT-rich regions (Figure 2). Due to the slow kinetics of [^{11}C]ZIENT relative to the half-life of carbon-11, the imaging session did not allow determination if radioactivity in ROIs could be reduced to cerebellar levels with administration of citalopram. This study with citalopram displacement demonstrated that a component of SERT binding is present in the ROIs evaluated but does not exclude the possibility of a component of DAT binding in the striatum.

To address the selectivity of [^{11}C]ZIENT for SERT over DAT in vivo, a displacement study was carried out using the selective DAT ligand RTI-113. A dose of RTI-113 (0.3 mg/kg) administered 60 min postinjection of [^{11}C]ZIENT led to no significant displacement from any part of the brain. It is noteworthy that the uptake of radioactive tracer during this study was higher in the midbrain and the thalamus than in the putamen reflecting the known pattern of SERT concentration in the brain. The radioactivity uptake in the putamen and caudate as well as in the regions known to have DAT density was slightly decreased following the administration of RTI-113. However, this washout was not specific of the DAT-rich regions (putamen and caudate). This decrease might be a consequence of an alteration in nonspecific binding and/or brain perfusion with the administration of RTI-113. To confirm this result, this study was repeated in a different cynomologus monkey. The time−activity curves obtained indicate that [^{11}C]ZIENT binds primarily to the SERT as no displacement was induced by injection of RTI-113.

PET Imaging in Awake Nonhuman Primates. Recent work performed using [^{18}F]FECNT, a selective DAT radioligand, demonstrated that 1% isoflurane anesthesia increases radiotracer binding to DAT-rich regions, consistent with an increase in plasma membrane expressed DAT.[16] To address the possibility of isoflurane influencing the kinetics and/or binding sites of [^{11}C]ZIENT, PET studies were performed in awake rhesus monkeys. Consequently, PET imaging studies were performed in awake monkeys to evaluate the effects of the isoflurane anesthesia. Cynomolgus and rhesus both are macaque monkeys with very similar physiology and neurochemistry. They are frequently used interchangeably in behavioral, physiological and pharmacologcial studies, and it is a reasonable assumption that tracer kinetics will be very similar in cynomolgus and rhesus monkeys.

The 921 PET scanner has a lower resolution than the *micro*PET scanner used previously, but it offers the advantage of being more sensitive and allows the acquisition for a longer period of time with carbon-11 as the radioisotope. To obtain reliable data 120 min postinjection with the *micro*PET scanner, 11 to 15 mCi of [^{11}C]ZIENT were injected and, therefore, more carbon-12 ZIENT was available to potentially occupy the SERT sites. Repeated baseline imaging studies of the brain were performed using [^{11}C]ZIENT (approximately 10 mCi) in conscious rhesus monkeys (Figure 3). The PET images were acquired for 120 min and coregistered with MRI (Figure 4) to accurately identify the regions-of-interest. Comparison of the three different time−activity curves obtained showed the

Table 1. Ratios of Uptake of Radioactivity in Various Brain Regions versus Cerebellum at 120 min after iv Injection of [^{11}C]ZIENT in Awake Monkeys

tissue	study no. 1	study no. 2	study no. 3
caudate	1.89	1.92	1.61
putamen	2.07	2.05	1.61
thalamus	2	2.25	1.67
midbrain	2.08	2.25	1.78
brainstem	1.42	1.56	1.75
frontal cortex	1.3	1.2	1.13
occipital cortex	1.71	1.54	1.21

reproducibility of these experiments. The kinetics of [^{11}C]ZIENT were not significantly decreased compared to the anesthetized monkeys studies. In both awake and anesthetized monkeys, [^{11}C]-ZIENT showed prolonged retention in every part of brain over the course of the imaging session. However, the SUVs in the putamen were not higher than in the thalamus and midbrain. The ratios of tissue uptake relative to cerebellum at 120 min postinjection reported in Table 1 were equal or higher for the thalamus and midbrain than the putamen, reflecting the expected order of SERT concentration. The difference in the rank order of uptake of radioactivity in thalamus, midbrain, and putamen in awake in comparison to anesthetized monkeys suggests that anesthesia influences the binding potential of [^{11}C]ZIENT in these regions.

Conclusion

The SERT ligand [^{11}C]ZIENT has been synthesized, radiolabeled, and evaluated in anesthetized and awake nonhuman primates. In vivo microPET imaging studies in anesthetized monkeys demonstrated that [^{11}C]ZIENT exhibited high uptake in the putamen, thalamus, midbrain, and brainstem, regions rich in SERT. In vivo PET imaging studies in awake monkeys demonstrated that [^{11}C]ZIENT consistently exhibited higher uptake in the thalamus and midbrain than putamen and caudate regions. These results suggest that anesthesia influences the binding potential of [^{11}C]ZIENT at the SERT and supports further study of the effects of anesthesia on SERT function using [^{11}C]ZIENT and PET. Finally, the high CNS SERT rich region-to-cerebellar ratios in awake nonhuman primates support the candidacy of [^{11}C]ZIENT for further study as a radioligand for in vivo quantitation of SERT sites by PET in humans.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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