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Preparation and Biological Evaluation of Iodine-125-IACFT: A Selective SPECT Agent for Imaging Dopamine Transporter Sites

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Parkinson's disease is a progressive neurodegenerative disorder that is associated with the loss of nerve terminals from specific brain areas, particularly in the caudate and putamen, which contains the highest concentrations of dopamine transporter sites. Previously, we synthesized and evaluated a series of ^{11}C-labeled 2β-carbomethoxy-3β-aryltropane (WIN 35,428; CFT) derivatives as markers for the dopamine transporter system. These ligands have high affinity and specificity for dopamine transporter sites in vitro and in vivo in laboratory animals. The goal of this study was the preparation and preliminary biological characterization of two new ligands based on the structure of WIN 35,428, the E and Z isomers of N-iodoallyl-2β-carbomethoxy-3β-(4-fluorophenyl)tropane (E and Z IACFT). **Methods:** E and Z IACFT were synthesized and radiolabeled with ^{125}I. The ligands were characterized by in vitro assays of binding to dopamine and serotonin transporters and by autoradiography. **Results:** Iodine-125-IACFT was prepared in >60% radiochemical yield, and >98% radiochemical purity. Specific activity was 1500 Ci/mmole. In vitro, E-IACFT showed higher affinity for dopamine transporter sites than WIN 35,428 (6.6 versus 11 n*M*) and better selectivity than RTI-55. The Z isomer was found to have much lower affinity. One hour after an intravenous injection of ^{125}I IACFT in monkeys, ex vivo autoradiographs of the brain revealed high concentrations of tracer in dopamine rich regions such as the caudate-putamen. The striatum-to-cerebellum, striatum-to-cortex and striatum-to-thalamus ratios were 10.8, 7.2 and 8.3. **Conclusion:** These result suggest that radiolabeled E-IACFT may be a useful radioligand for SPECT imaging of dopamine transporter sites. IACFT could prove to be extremely useful for the noninvasive evaluation of patients with early Parkinson's disease.

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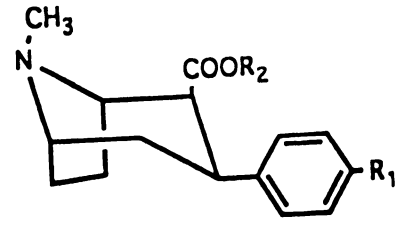
Cocaine is a stimulant and powerful reinforcer that binds to specific recognition sites associated with monoamine transporters (*1,2*). Its primary mechanism of action is ascribed to its ability to bind potently to the dopamine transporter (*3–5*). In 1973, Clarke et al. reported that replacement of the 3-benzoyloxy group in cocaine with a phenyl moiety increases potency by tenfold (*6*). Since then, many cocaine analogs have been prepared and evaluated. (*7–11*) The results of these studies have led to a better understanding of the structure-activity relationship between cocaine analogs and the DA transporter site, however, many questions still remain unanswered.

The cocaine analog, 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane (WIN 35,428; CFT) (Fig. 1:1a), (*6*) has proven to be an important probe for studying these cocaine binding sites in the striatum (*12–18*). Initial studies have revealed that the binding sites for ^{3}H-WIN 35,428 are identical to those of cocaine which are associated with the dopamine (DA) transporter (*19–22*). Tritiated WIN 35,428 binding has been shown to be decreased in postmortem striatal tissue of patients with Parkinson's disease, with a strong correlation between ligand binding and dopaminergic neuron density (*23*). Using a primate model of Parkinson's disease, we demonstrated that when WIN 35,428 is labeled with ^{11}C, disease progression can be monitored noninvasively in vivo by PET (*13*). Recently, these findings were validated in human subjects (*14*).

Although PET with ^{11}C-WIN 35,428 is a useful method for the noninvasive quantification of the density of dopamine terminals, the expense and complexity of this technique limits general applicability. Clearly, a ligand suitable for SPECT would be of significant clinical value. Recently, 2β-carbomethoxy-3β-(4-iodophenyl)tropane (RTI-55, Fig. 1:1b) was synthesized and radiolabeled with ^{125}I (*23*). This compound has



COMPOUND	R_1	R_2
1a WIN 35,428; CFT	4-F	CH_3
1b RTI-55; ß-CIT	4-I	CH_3
1c IPCFT	4-I	$CH(CH_3)_2$
1d CCT	4-Cl	CH_3
1e CPT	4-H	CH_3
1f DCCT	3,4-Cl_2	CH_3

FIGURE 1. Structural formulas for cocaine analogs. 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (1a, WIN 35,428; CFT), 2β-carbomethoxy-3β-(4-iodophenyl)tropane (1b, RTI-55), 2β-carboisopropyloxy-3β-(4-fluorophenyl)-tropane (1c, IPCFT), 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (1d, CCT), 2β-carbomethoxy-3β-benzyltropane (1e, CPT) and 2β-carbomethoxy-3β-(3,4-dichlorophenyl)tropane (1f, CDCT).

tenfold higher affinity than WIN 35,428 for cocaine binding sites in the striatum of cynomologous monkeys (IC_{50} = 1.08 versus 11.0 n*M*) (*1*). Autoradiography of radiolabeled RTI-55 in monkey brain has revealed high levels of accumulation in dopaminergic nerve terminals (*22*). In a recent comparative autoradiographic study with ^{3}H-WIN 35,428 and ^{125}I-RTI-55, Kaufman and Madras (*24*) demonstrated that although both ligands concentrate in dopamine rich regions of the brain, significant concentrations of ^{125}I-RTI-55 were also detected in serotonin-rich regions such as the cerebral cortex and thalamus (*24*). This reduction in selectivity is similar to the pattern observed with other aromatic ring substituted congeners such as 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (CCT = Fig. 1:1d), 2β-carbomethoxy-3β-benzyltropane (CPT = Fig. 1:1e) and 2β-carbomethoxy-3β-(3,4-dichlorophenyl)tropane (CDCT = Fig. 1:1f) (*11*).

The lack of selectivity of ^{125}I-RTI-55 makes it suboptimal for SPECT studies of dopamine transporter sites. Our search for a superior imaging agent for SPECT studies was influenced by the fact that introduction of iodine on the aromatic nucleus leads to loss of selectivity (*11,30,34*). Chemical stabilization of iodine can be achieved by attachment to a carbon-carbon double bond. Furthermore, allylic substitution on the nitrogen of nor WIN 35,428 had been reported to result in only minor reductions in binding potency (*19*). We therefore focused our attention on the synthesis of an N-iodoallyl derivative of nor WIN 35,428 and developed a method for preparing the E and Z isomers of ^{125}I-2β-carbomethoxy-3β-(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane (E- and Z-IACFT) as radioiodinated probes for SPECT. Very recently Goodman et al. have reported the synthesis of the 4-chloro analog of IACFT (*25*).

In this report, we describe the synthesis, radiolabeling and receptor binding properties of E- and Z-IACFT (Fig. 2:6a, 6b). The receptor affinity and selectivity of these ligands for dopamine and serotonin sites was evaluated by competitive binding assays with ^{3}H-WIN 35,428 and ^{3}H-citalopram (a high affinity and selective ligand for serotonin transporter sites) (*26*). In addition, the regional distribution of ^{125}I-E-IACFT was determined by autoradiography of squirrel monkey brains.

MATERIALS AND METHODS

Iodine-125 was purchased from New England Nuclear DuPont. Other reagents and solvents were obtained from standard commercial sources. The ^{1}H NMR spectra were recorded on a Varian XL400 spectrometer. All chemical shifts are reported in ppm downfield from TMS which was used as an internal standard. Melting points were measured with a Gallenkamp apparatus and are reported uncorrected. Thin-layer chromatography (TLC) was performed with Baker Si 250F plates. Visualization was accomplished with either iodine vapor, UV exposure, or treatment with phosphomolybdic acid (PMA). High pressure liquid chromatography (HPLC) was performed with a Brownlee RP300 C-8 column (Aquapore octyl, 300A7u, 220 × 4.6 mm). Preparative TLC was performed on Analtech uniplates; silica gel GF (2000 mm). Flash chromatography was performed with Baker silica gel (40μ). Elemental analyses were performed by Atlantic Microlab, Atlanta, GA and a Beckman 1801 scintillation counter was used for scintillation spectrometry.

Chemistry

E and Z-IACFT were prepared as outlined in Figure 2. Propargyl alcohol was treated with tri-n-butyl tin hydride in the presence of 2,2′,-azo bis(2-methylpropionitrile) (AIBN) to give, after column chromatography over silica gel, the E and Z isomers of 3-tri-n-butylstannyl-2-propen-1-ol in 51% (Fig. 2:2a) and 23% (Fig. 2:2b) yields. Each of the alcohols was then reacted with triphenylphosphine in carbon tetrachloride to obtain the E (Fig. 2:3a) and Z (Fig. 2:3b) isomers of 1-tri-n-butylstannyl-3-chloro-1-propene (80% yields). Reaction of the pure tri-n-butylstannyl allyl chlorides with 2β-carbomethoxy-3β-(4-fluorophenyl)nortropane, (Fig. 2:4), in the presence of KF-celite gave the desired 2β-carbomethoxy-3β-(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane (Fig. 2:5a, 5b), in 71 and 88% yield

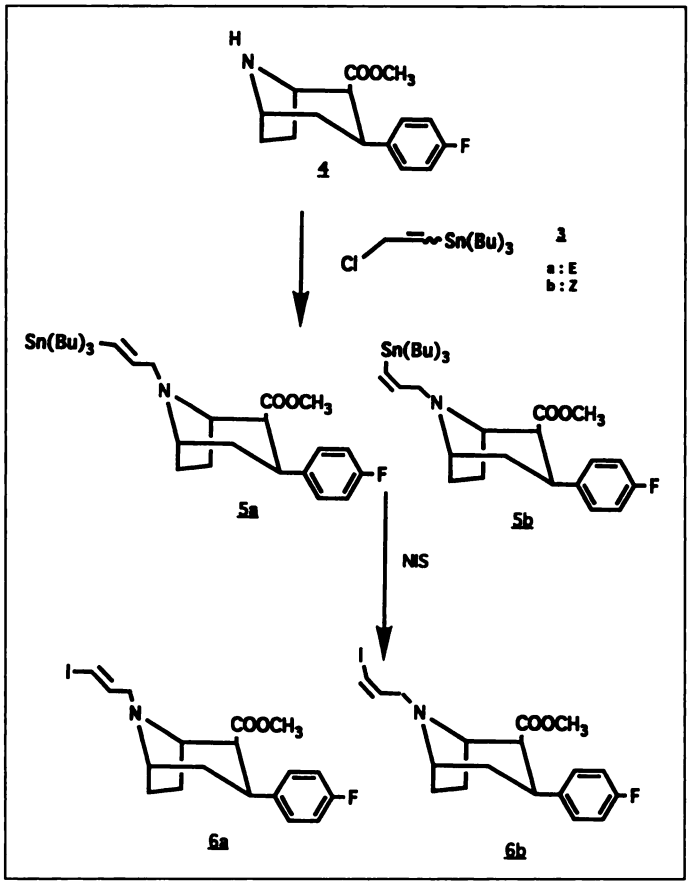


FIGURE 2. Reaction sequences for the synthesis of 1E-(tri-n-butylstannyl)-3-chloro-1-propene and ^{125}I-2β-Carbomethoxy-3β-(4-fluorophenyl)-N-[1-(E)-iodoprop-1-en-3-yl]nortropane.

respectively. The E and Z-isomers (Fig. 2:5a, 5b) were then treated with N-iodosuccinimide to give the E and Z-isomers of 2β-carbomethoxy- 3β-(4-fluorophenyl)-N-[1-(E)-iodoprop-1-en-3-yl]nortropanes (Fig. 2:6a, 6b) in 87% and 83% yields, respectively. Further details of the synthetic procedures are included in the Apendix.

Radiolabeling

Sodium ^{125}I (118 μCi) and NaOH (0.1 *M*) were combined in a 5 ml reacti-vial and the water was removed under azeotropic conditions using acetonitrile (4 × 0.5 ml) and nitrogen gas. The following solutions were then added: 2β-Carbomethoxy-3β-(4-fluorophenyl)-N-[1-(E and Z)-tri-n-butylstannylprop-1-en-3-yl]-1αH-,5αH-nortropane (100 μg) in chloroform (50 μl), 1 *M* acetic acid in chloroform (6 μl) followed by 1 *M* t-butyl peroxide in chloroform (6 μl). The mixture was heated at 60°C for 30 min and the reaction was quenching by addition of 10 ml of saturated $NaHSO_3$ and 10 ml of saturated Na_2CO_3. The mixture was then extracted with methylene chloride (3 × 0.5 ml) and the organic extracts were combined, applied to a silica gel column (70 mg) and eluted with methylene chloride. The fractions containing the desired iodoallyl product were combined and the solvent was removed with a stream of N_2. Using this procedure the radiochemical yield of ^{125}I-E-IACFT was 77 μCi (65%, TLC (10% Et_3N/hexane) − R_f = 0.27; HPLC (60% MeOH, 40% 0.2 N KH_2PO_4, flow rate 1 ml/min.) − R_t = 4.53 min. The specific activity of the final product was 1500 Ci/mmole and radiochemical purity was >98%. Iodine-125-Z-IACFT was prepared using the same procedure.

In Vitro Assays of Receptor Affinity and Selectivity

The receptor affinity and selectivity of E- and Z-IACFT were evaluated by competitive binding assays. Tritiated WIN 35,428 and ^3H-citalopram were used to evaluate dopamine and serotonin binding sites in monkey caudate-putamen membrane preparations as previously described by Madras et al. (*19*).

To determine the affinity and selectivity for binding to dopamine transporter sites, serial dilutions of the test ligands in assay buffer (50 m*M* Tris HCl, 100 m*M* NaCl) were incubated with caudate-putamen membranes (1 mg/ml wet weight) and ^3H-WIN 35,428 (0.3-10 n*M*) for 60 min at 4°C. The incubation was terminated by vacuum filtration on glass fiber filters (Whatman GF/B) and bound radioactivity was measured by liquid scintillation spectroscopy at 50% counting efficiency. All assays were performed in triplicate and each experiment was repeated at least twice using tissue from different animals. Unlabeled (−)-cocaine (30 m*M*) was used to measure nonspecific binding.

To assay binding to the serotonin transporter, serial dilutions of the test ligands in assay buffer (50 m*M* Tris HCl, 100 m*M* NaCl) were incubated with caudate-putamen membranes (1 mg/ml wet weight) and ^3H-citalopram (1 n*M*) for 2 hr at 4°C. The incubation was terminated by vacuum filtration on glass fiber filters (Whatman GF/B) and bound radioactivity was measured by liquid scintillation spectroscopy at 50% counting efficiency. All assays were performed in triplicate and each experiment was repeated at least twice using tissue from different animals. Unlabeled fluoxetine (1 μ*M*) was used to measure nonspecific binding.

Autoradiography

The brain distribution of ^{125}I-E-IACFT was determined by autoradiography following intravenous injection. Two adult squirrel monkeys, weighing 800–970 g, were anesthetized with ketamine and intravenous catheters were implanted in the saphenous vein. Each animal received 400 μCi (4 nmole) of ^{125}I-E-IACFT followed by a saline flush (1 ml) via the catheter. After 60 min, the animals were killed with an excess of sodium pentobarbital.

The brains were removed within 10 min of death, sliced into 0.5–1 cm coronal sections, frozen by immersion in isopentane, chilled with dry ice and stored at −85°C. Prior to final sectioning, the tissue was placed in a cryostat (Reichert-Jung) and allowed to equilibrate to the cryostat temperature of −18°C. Tissue sections were mounted on cryostat chucks, sectioned coronally (20 μ*M*), thaw-mounted onto gelatin-coated glass slides, and dried rapidly with a stream of cool air. Tissue sections from 11 or more anterior-to-posterior levels in triplicate were prepared. The slide-mounted tissue sections were placed in x-ray cassettes, apposed to ^3H Ultrafilm for 2–5 wk at −85°C. The x-ray films were developed and binding densities were determined using a microcomputer based image analysis system as previously described (*20,21,33*).

TABLE 1
Inhibition of Binding of Tritiated WIN 35,428 to Dopamine Transporters and Tritiated Citalopram to Serotonin Transporters by WIN 35,428 Derivatives in Caudate-Putamen Membranes of Cynomolgus Monkey

Compound	IC50 (n*M*) DA	IC50 (n*M*) 5-HT	Selectivity DA:5-HT
CFT*	11.0 ± 1.0	160 ± 20	15
CCT†	1.40 ± 0.04	5.87 ± 2.8	4
CDCT‡	1.09 ± 0.02	2.47 ± 0.14	2
CIT (RTI-55)§	1.08 ± 0.06	2.53 ± 0.02	2
CPT¶	65 ± 12	–	–
E-IACFT**	6.62 ± 0.78	182 ± 41.8	25
Z-IACFT‡‡	80	–	–

*2β-carbomethoxy-3β-(4-fluorophenyl)tropane; †2β-carbomethoxy-3β-(4-chlorophenyl)tropane; ‡2β-carbomethoxy-3β-(3,4-dichlorophenyl)tropane; §2β-carbomethoxy-3β-(4-iodophenyl)tropane; ¶2β-carbomethoxy-3β-phenyltropane; **E-2β-Carbomethoxy-3β-(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane; ‡‡Z-2β-Carbomethoxy-3β-(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane.

RESULTS

Chemistry

The stannyl precursors for ^{125}I-E- and Z-2β-carbomethoxy-3β-(4-fluorophenyl)-N-[1-(E)-iodoprop-1-en-3-yl]nortropane

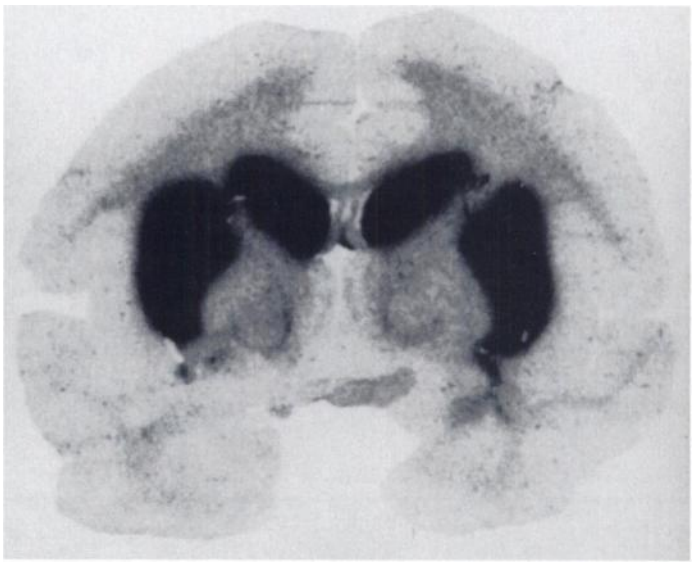


FIGURE 3. Representative autoradiogram of squirrel monkey brain at the caudate-putamen level. The animal was injected intravenously with 400 μCi (4 nmole) of ^{125}I-E-IACFT and killed 1 hr later. Triplicate coronal sections were mounted on x-ray film and incubated for 2–5 wk. The x-ray films were developed and binding densities were determined using a microcomputer based image analysis system.

TABLE 2
Regional Concentrations (%ID/gram) for Tritiated WIN 35,428, Iodine-125-RTI-55 and Iodine-125-IACFT in Monkey Brain*

Region	^{3}H-CFT[a]	^{125}I-RTI-55[b]	^{125}I-IACFT[c]
Striatum	6.67	3.38	1.35
Cortex	2.78	1.78	0.19
Cerebellum	2.06	1.30	0.13
Thalamus	2.16	1.88	0.16

*[a]2β-carbomethoxy-3β-(4-fluorophenyl)tropane, [b]2β-carbomethoxy-3β-(4-iodophenyl)tropane, [c]E-2β-Carbomethoxy-3β-(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane.

(IACFT) were prepared in excellent yield and chemical purity as outlined in the appendix. Radioiodinations of the E- and Z-isomers were performed using t-butyl peroxide and acetic acid in chloroform. After purification by silica gel chromatography, the radioiodinated compounds were obtained in high radiochemical yields (50%) and high specific activity (1500 Ci/mmole).

Receptor Affinity and Selectivity Measurements

The measured affinities of E- and Z-IACFT for the dopamine and serotonin transporter sites expressed as the IC_{50}'s for inhibition of ^{3}H-WIN 35,428 (dopamone sites) and ^{3}H-citalopram (serotonin sites) binding are given in Table 1. For comparison, the affinities of WIN 35,428 (Fig. 1:1a), CCT (Fig. 1:1d), CPT (Fig. 1:1e), CDCT (Fig. 1:1f) and RTI-55 (Fig. 1:1b) have been reproduced here. These results indicate that the trans isomer of IACFT has much higher affinity for the dopamine transporter than the cis isomer (IC_{50}: 6.62 versus 80 n*M*). The low binding affinity of E-IACFT for the serotonin transporter (IC_{50} = 182 n*M*) demonstrates that this ligand has very high selectivity for the dopamine transporter (dopamine/serotonin = 25). The IC_{50} of the E-isomer of IACFT is of intermediate potency between WIN 35,428 and RTI-55, however, its selectivity for dopamine transporter sites is much higher than either WIN 35,428 (DA/5-HT = 15) or RTI-55 (DA/5-HT = 2). The Z-isomer of IACFT has lower affinity than any of the other ligands.

Autoradiography

Figure 3 shows a representative autoradiogram of a monkey brain at the caudate-putamen level. From this image, it is clear that the caudate and putamen accumulated extremely high concentrations of radioactivity. Lower levels of radioactivity were detected in the cerebral cortex and the pons, and very low concentrations of radioactivity were measured in the thalamus, hypothalamus or cerebellum. Regional concentrations and concentration ratios (striatum/cortex, striatum/thalamus and striatum/cerebellum) are summarized in Tables 2 and 3. For comparison, the corresponding ratios for ^{3}H-WIN 35,428 and ^{125}I-RTI-55 are also presented. From these data, it is clear that differentiation of dopamine terminal rich brain areas is best with ^{125}I-E-IACFT.

TABLE 3
Concentration Ratios for Tritiated WIN 35,428, Iodine-125-RTI-55 and Iodine-125-IACFT in Monkey Brain

Region	^{3}H-CFT*	^{125}I-RTI-55†	^{125}I-IACFT‡
Striatum/Cortex	2.4	1.9	7.2
Striatum/Cerebellum	3.2	2.6	10.8
Striatum/Thalamus	3.1	1.8	8.3

*2β-carbomethoxy-3β-(4-fluorophenyl)tropane; †2β-carbomethoxy-3β-(4-iodophenyl)tropane; ‡E-2β-Carbomethoxy-3β-(4-fluorophenyl)-N-(1-iodoprop-1-en-3-yl)nortropane.

DISCUSSION

Table 1 summarizes the IC_{50}'s of E and Z-IACFT (Fig. 2:6a, 6b) and other cocaine analogs for inhibition of ^{3}H-WIN 35,428 binding to dopamine transporters and inhibition of ^{3}H-citalopram binding to serotonin transporters. The binding affinity of E-IACFT for the DA transporter is greater than WIN 35,428 and CPT but less than CCT, CDCT and RTI-55 (Fig. 3). This result is consistent with previous reports (*11*). Of greater significance, however, E-IACFT binding to 5-HT sites is ~30 fold less than the other analogs; resulting in a DA:5HT selectivity of ~25 to 1. The order of binding affinity is RTI-55 ≅ CDCT > CCT > IACFT > WIN 35,428 > CPT and the order of selectivity is E-IACFT > WIN 35,428 > CCT > CDCT ≅ RTI-55. This inverse relationship between binding potency for the DA transporter and selectivity is supported by our studies with MPTP-treated monkeys (*27*). In these investigations, we observed that, although symptomatic Parkinsonian MPTP-treated monkeys did not have significant levels of caudate-putamen activity when imaged with ^{11}C-CCT or ^{11}C-CDCT (due to >95% decrease in dopamine terminals), significant levels of accumulation were detected in serotonin-rich regions such as the cerebral cortex and thalamus. This accumulation of radiopharmaceutical cannot be ascribed to dopaminergic neurons.

As the results in Table 2 indicate, absolute striatal accumulation of IACFT is less than RTI-55 at 1 hr after injection. The observation, however, that the concentration ratios for IACFT are considerably higher suggests that it may be possible to acquire diagnostic quality images within 1 hr after injection of IACFT. This is in marked contrast to RTI-55 for which a delay of 24 hr between injection and imaging is required to obtain adequate definition of the striatum. This early specific localization inconjunction with extremely high selectivity suggests that IACFT may become an important radiopharmaceutical for clinical SPECT imaging of DA transporter sites.

Interestingly, the Z-isomer of IACFT has the lowest binding affinity (IC_{50} = 80 nmol) for the DA transporter of all the cocaine analogs in this series. Due to this low affinity, the compound was not investigated further. This dramatic decrease in affinity indicates that the stereochemistry of N-substituents has significant effects on binding. Clearly, the binding interactions at DA transporter sites are not fully understood and it has been suggested that there may be more than one type of DA transporter, particularly in the caudate nucleus (*28,29*).

Recently, it has been reported that replacement of the methyl group in the 2-carbomethoxy functionality with an isopropyl moiety reduces 5-HT binding and thereby increases selectivity for DA transporter sites (>200:1) (*30*). In that study, the isopropyl ester analogs of RTI-55 and CCT were prepared, and although their affinities were considerably enhanced, the order of selectivity remained the same when compared to the methyl esters; the isopropyl ester analog of WIN 35,428 was not reported. It would be interesting to determine if introduction of an isopropyl ester effects the nonspecific binding of IACFT. Recently, Goodman et al. (*25*) reported the in vitro and in vivo characteristics of the E and Z isomers of iodo allyl CCT. Although in vitro binding studies with rat striatal membranes showed a lower selectivity for the E isomer (dopamine/serotonin = 6.5/3.2 = 2.02), biodistribution studies in rats revealed a striatum-to-cerebellum ratio of 15.97. In addition to these compounds, other cocaine analogs have been shown to have

high levels of selective binding to the caudate and putamen (*15*). Unfortunately, due to differences in assay techniques and in vivo models, it is difficult to compare the results of different studies. In the future, it will be important to evaluate these ligands in the same animal species under identical conditions and compare their abilities to measure the progression of neuron fiber degeneration.

CONCLUSION

Our studies indicate that E - IACFT has higher affinity for the dopamine transporter sites than WIN 35,428 and better selectivity than most other cocaine congeners. Other ligands such as the GBR series, RTI-55 (Fig. 1:1b) and CCT (Fig. 1:1d) have higher affinity for the DA transporter, however, since binding to other sites (serotonin) is also higher these compounds are less useful for imaging specific transporter sites. Although additional comparative studies with ligands such as isopropyl analogs and iodo allyl CCT (*15,25,30*) are required to identify the radiopharmaceutical of choice for clinical imaging, the results presented here demonstrate that E-IACFT is an excellent candidate. Autoradiography with E-IACFT confirms its suitability as a radiopharmaceutical for the noninvasive evaluation of patients with early Parkinson's disease.

APPENDIX

Chemical Synthesis and Characterization of the Precursor Used for Preparing Iodine-125-Labeled IACFT

1-(E and Z)-(tri-n-butylstannyl)-3-chloroprop-1-ene (Fig. 2:3a, 3b). These compounds were prepared by procedures similar to those reported by Goodman et al. (*25*). Propargyl alcohol (5.5 ml, 94 mmole) was added dropwise to a mixture of tri-n-butyltin hydride (35 ml, 130 mmole) and 2,2',-azobis(2-methylpropionitrile) (AIBN) (1.6 g, 9.7 mmole) at 80°C. After heating at 80°C for 2 hr, the reaction mixture was purified by chromatography on silica gel (5% EtOAc/hexane) to yield the E and Z-isomer of 3-Tri-n-butylstannyl-2-propen-1-ol as clear oils; E-isomer 16.7 g (51%), R_f0. 0.2 (5%EtOAc/hexanes; 2 elutions), Z-isomer, 7.5 g (23%), R_f0. 0.28 (5%EtOAc/hexanes; 2 elutions).

The E- and Z-alcohols (12.8 g, 37 mmole) were combined with triphenylphosphine (9.7 g, 37 mmole) and CCl_4 (100 ml) and heated at reflux for 16 hr. Excess CCl_4 was removed and the residue was purified by chromatography on silica gel (hexane) to give the tri-n-butylstannyl allyl chlorides as clear oils in 80% yield.

Figure 2:3a. E-Isomer: R_f0. 0.52 (hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85–0.92 (m, 15H, $SnBu_3$), 1.29 (m, 6H, $SnBu_3$), 1.47 (m, 6H, $SnBu_3$), 4.06 (d, 2H, CH_2Cl, J = 6.12 Hz), 6.05 (dt, CH_2CH = CHSn), J = 6.15, 18.6 Hz), 6.29 (d, 1H, CH_2CH = CHSn, J = 18.5 Hz).

Figure 2:3b. Z-Isomer: R_f0. 0.52 (hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85–0.92 (m, 15H, $SnBu_3$), 1.31 (m, 6H, $SnBu_3$), 1.49 (m, 6H, $SnBu_3$), 3.97 (d, 2H, CH_2Cl, J = 7.35 Hz), 6.17 (d, CH_2CH = CHSn, J = 12.5 Hz), 6.64 (dt, 1H, CH_2CH = CHSn, J = 12.5, 7.35 Hz).

Assignment of the E and Z geometry was confirmed by ^{1}H NMR spectroscopy. For each isomer, the proton geminal to the tin was split into a doublet. In the E isomer, (Fig. 2:3a) this doublet had a coupling constant of 18.5 Hz, while for the Z isomer, (Fig. 2:3b) this coupling was 12.5 Hz. Also, the doublet of triplets assigned to the vinyl proton β to the tin in the Z isomer was shifted downfield relative to that in the E isomer (δ 6.64 versus δ 6.05).

2β-Carbomethoxy-3β-(4-fluorophenyl)-N-[1-(E and Z)-tri-n-butylstannylprop-1-en-3-yl]-1αH-,5αH-nortropane (Fig. 2:5a, 5b). 2β-Carbomethoxy-3β-(4-fluorophenyl)-1αH,5αH-nortropane, (Fig. 2:4), was synthesized as described previously (*11*). Briefly, tri-n-butylstannyl allyl chloride (102 mg, 0.39 mmole), Figure 2:3a, (143 mg, 0.39 mmole), KF-celite (50%; 226 mg, 1.9 mmole) and CH_3CN (10 ml) were combined and heated at 70°C for 17 hr. The reaction mixture was diluted with 40 ml of ether and filtered through a celite pad. The filtrate was concentrated to dryness and the residue was chromatographed on silica gel (2% Et_3N/hexane) to yield 163 mg (71%) of 2β-carbomethoxy-3β-(4-fluorophenyl)-N-[1-(E)-tri-n-butylstannylprop-1-en- 3-yl]nortropane Figure 2:5a as a low melting solid. 2β-Carbomethoxy-3β-(4-fluorophenyl)-N-[3-(Z)-tri-n-butylstannylprop-1-en-13-yl]-1αH-,5αH-nortropane, Figure 2:5b was prepared in a similar manner (88%).

Figure 2:5a. Mp: 30.0–30.5°C; R_f – 0. 48 (Et_3N:hexanes: 1:9); HPLC (60% MeOH, 40% 0.2 N KH_2PO_4, flow rate 1 ml/min.) – R_t = 8.03 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (m, 15H, $SnBu_3$), 1.28 (m, 6H, $SnBu_3$), 1.45 (m, 6H, $SnBu_3$), 1.65 (m, 3H), 2.06 (m, 2H, H-6,7), 2.58 (ddd, 1H, H-4), 2.84 (m, 2H, H-2, NCH), 2.98 (dt, 1H, H-3), 3.12 (dd, 1H, NCH, J = 4.0, 13.2 Hz), 3.39 (m, 1H, H-5), 3.47 (s, 3H, OCH_3), 3.66 (m, 1H, H-1), 5.80 (ddt, 1H, CH_2CH = CHSn, J = 4.25, 6.61, 19.0), 6.00 (d, 1H, CH_2CH = CHSn, J = 19.0 Hz), 6.93 (t, 2H, ArH, J = 8.7 Hz), 7.20 (dd, 2H, ArH, J = 8.7, 14.0 Hz). Anal. Calcd. for $C_{30}H_{48}NO_2FSn$: C, 60.82, H, 8.17, N, 2.36; Found, C, 60.94, H 8.18, N, 2.32.

Figure 2:5b. R_f – 0. 0.48 (10%Et_3N/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (m, 15H, $SnBu_3$), 1.30 (m, 6H, $SnBu_3$), 1.50 (m, 6H, $SnBu_3$), 1.65 (m, 3H), 2.05 (m, 2H, H-6,7), 2.60 (ddd, 1H, H-4, J = 7.8, 12.6 Hz), 2.80 (m, 1H, NCH), 2.90 (m, 1H, H-2), 3.01 (dt, 1H, H-3), 3.09 (dd, 1H, NCH, J = 4.0, 13.2 Hz), 3.42 (m, 1H, H-5), 3.50 (s, 3H, OCH_3), 3.69 (m, 1H, H-1), 5.93 (d, 1H, CH_2CH = CHSn, J = 7.5 Hz), 6.45 (m, 1H, CH_2CH = CHSn), 6.95 (t, 2H, ArH), 7.23 (dd, 2H, ArH).

2β-Carbomethoxy-3β-(4-fluorophenyl)-N-[1-(E and Z)-iodoprop-1-en-3-yl]nortropane, (Fig. 2:6a, 6b) (IACFT). 2β-Carbomethoxy-3β-(4-fluorophenyl)-N-[3-(E)-tri-n-butylstannylprop-1-en-3-yl]nortropane, Fig. 2:5a, (161 mg, 0.27 mmole) in THF (10 ml) was degassed by bubbling N_2 for 5 min. N-iodosuccinimide (61.8 mg, 0.275 mmole) was added and the mixture was maintained at room temperature for 15 min. The THF was removed and the residue was chromatographed on silica gel (2% NEt_3/hexane) to yield 102 mg (87%) of 2β-carbomethoxy-3β-(4-fluorophenyl)-N-[1-(E)-iodoprop-1-en-3-yl]nortropane, Fig. 2:6a, as a white solid. 2β-Carbomethoxy-3β-(4-fluorophenyl)-N-[1-(Z)-iodoprop-1-en-3-yl]-nortropane, Fig. 2:6b, was prepared in a similar manner (83%).

Figure 2:6a. Mp: 115–116°C; R_f – 0. 0.27 (10%Et_3N/hexane); HPLC (60% MeOH, 40% 0.2 N KH_2PO_4, flow rate 1 ml/min.) – R_t = 4.53 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.6–1.8 (m, 3H), 2.0 (m, 2H, H-6,7), 2.56 (ddd, 1H, H-4), 2.8–3.0 (m, 4H), 3.39 (m, 1H, H-5), 3.51 (s, 3H, OCH_3), 3.63 (m, 1H, H-1), 6.18 (dd, 1H, ICH = CH, J = 14.5, 1.5 Hz), 6.46 (ddd, 1H, ICH = CH, J = 14.5, 9.8, 5.2 Hz), 6.94 (t, 2H, ArH), 7.20 (dd, 2H, ArH). Anal. Calcd. for $C_{18}H_{21}NO_2IF$: C, 50.36, H, 4.93, N,3.26, I, 29.56. Found, C, 50.46, H, 4.98, N,3.22, I, 29.64.

Figure 2:6b. Mp: 112–114°C; R_f – 0. 29 (Et_3N:hexanes:1: 9); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.6–1.9 (m, 3H), 2.1–2.3 (m, 2H), 2.57 (ddd, 1H, H-4, J = 2.7, 12.5 Hz), 2.8–3.1 (m, 4H), 3.43 (m, 1H, H-5), 3.49 (s, 3H, OCH_3), 3.63 (m, 1H, H-1), 6.20 (m, 1H, ICH = CH), 6.30 (d, 1H, ICH = CH, J = 7.5 Hz), 6.95 (t, 2H, ArH, J = 8.7 Hz), 7.20 (dd, 2H, ArH, J = 8.7, 13.3 Hz). Anal. Calcd. for $C_{18}H_{21}NO_2IF$: C, 50.36, H, 4.93, N,3.26, I, 29.56. Found, C, 50.48, H, 4.95, N,3.20, I, 29.52.

The stereochemical integrity at C2 and C3 remained intact as confirmed by the multiplicity observed by ^{1}H-NMR spectroscopy (400 MHz). Also, the vinyl proton doublet of the E-isomer appeared at 6.18 ppm and had the characteristic trans coupling constant (J = 14.5 Hz), while the doublet of the Z-isomer appeared at 6.30 ppm and had the characteristic cis coupling constant (J = 7.5 Hz).

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