Synthesis of a Radiotracer for Studying Dopamine Uptake Sites *In Vivo* Using PET: 2β-Carbomethoxy-3β-(4-fluorophenyl)-[N-^{11}C-methyl]tropane ([^{11}C]CFT or [^{11}C]WIN-35,428)

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Summary

2β-Carbomethoxy-3β-(4-fluorophenyl)-[N-^{11}C-methyl]tropane, a potent inhibitor of dopamine transport, was prepared by N-methylation of the appropriate nor-methyl precursor in DMF with [^{11}C]iodomethane. After derivatization of unreacted precursor with a long chain acyl halide, the radiotracer was purified using reversed phase semipreparative HPLC. The average specific activity was 3065 mCi/μmole (calculated at the end-of-synthesis; EOS). The average time of synthesis including formulation was approximately 21 minutes.

Key Words: radiotracer, synthesis, dopamine uptake sites, carbon-11, positron emission tomography

Introduction

The dopamine uptake site has been implicated in neurological disease, psychiatric disorders, and drug abuse liability. *In vitro* binding experiments have

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revealed a significant reduction in dopamine uptake sites in striata from patients with Parkinson's disease as compared to normal controls (1-3). Similar *in vitro* binding studies in patients with Huntington's disease (4) and schizophrenia (5) have shown an increased density of these sites.

The dopamine uptake site also plays a role in cocaine's reinforcing properties. The self-administration potency of various analogs of cocaine has been correlated with their binding to dopamine uptake sites (6). A similar correlation between the dopamine uptake site binding affinity and the production of cocaine-like behavioral effects has been observed (7, 8).

During the past decade, a variety of ligands have been used *in vivo* with positron emission tomography (PET) to study the dopamine uptake site. One of the first studies in primates used nomifensine, a dopamine uptake inhibitor, labeled with ^{11}C (9, 10). Nomifensine is not however an ideal ligand due to its poor selectivity between adrenergic and dopaminergic uptake sites (11). Fowler and colleagues mapped the dopamine uptake site (the cocaine binding site) using [^{11}C]cocaine and PET (12).

A series of aryl-1,4-dialkyl(en)ylpiperazines were found to have significant affinity for the dopamine uptake site (13). One member of this series, GBR 13119, has been radiolabeled with ^{18}F. [^{18}F]GBR 13119 displayed high affinity and selectivity for dopamine uptake sites in rodents and primates (14 - 16). Another member of the series, [^{3}H]GBR 12935, labeled the dopamine uptake site (17), although its binding profile differed from that exhibited by [^{3}H]cocaine binding. [^{3}H]GBR 12935 labeled a single component in striatal tissue whereas [^{3}H]cocaine and its analogs labeled multiple components (18).

Clarke (19) developed an active series of congeners of cocaine that exhibit high affinity for the dopamine uptake site. The majority of these compounds possessed higher affinity for the dopamine uptake site than cocaine itself. Structure-affinity relationships for this series are well established (20, 21). The *in vitro* and *in vivo* binding profiles for several unlabeled and tritiated ligands have been reported (22 - 26). Recently, an iodinated congener of cocaine labeled with ^{123}I and studied with single photon emission computed tomography (SPECT) in primates was used to characterize the dopaminergic presynaptic system (27, 28). The ready availability of high specific activity [^{11}C]iodomethane and the presence of an N-methyl group on the tropane moiety make these compounds attractive candidates as radiotracers for studying the dopamine uptake site using PET. This report describes the radiochemical synthesis, purification, and quality control of a potent member of this series of cocaine congeners, 2β-carbomethoxy-3β-(4-fluorophenyl)-[N-^{11}C-methyl]tropane ([^{11}C]CFT; [^{11}C]WIN-35,428; Figure 1) as a radiotracer for studying the dopamine uptake site using PET.

Discussion

[^{11}C]CFT was synthesized by N-methylation of the corresponding free base

with ^{11}C-iodomethane (see Figure 1). The synthesis, semipreparative HPLC, and formulation was completed in an average time of 21 minutes with an average radiochemical yield of 20.6 % based on $^{11}CH_3I$ (not corrected for decay).

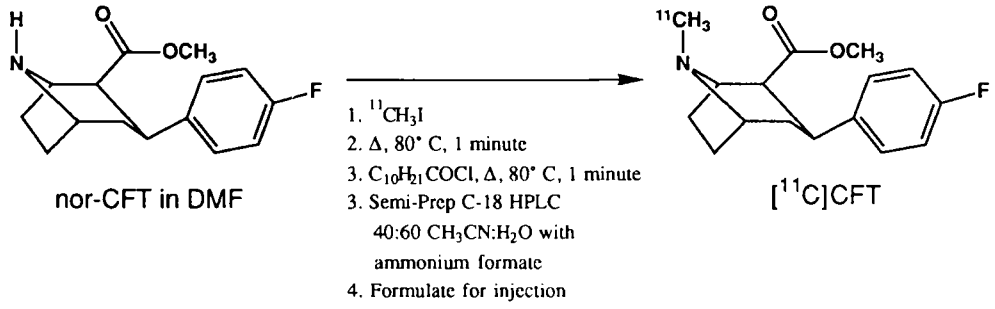

Figure 1.

In our hands, a variety of reversed phase chromatographic separation techniques would not separate [^{11}C]CFT from nor-CFT. To facilitate the chromatographic separation of nor-CFT and [^{11}C]CFT, decanoyl chloride was added to the reaction mixture (with excess triethylamine) to derivatize the secondary amine precursor, nor-CFT (30). On semipreparative chromatography, the [^{11}C]CFT (R_t = 8.7 minutes, k' = 5.2) was easily purified from the reaction mixture; the long chain acylated CFT eluted well after the desired product (R_t > 16 minutes).

Chemical and radiochemical purity was confirmed by analytical reversed phase HPLC. A single radioactive peak eluted at the same time as an authentic sample of the product. The area of the uv absorbance peak corresponding to carrier product was used to determine the amount of carrier present. Using the measured radioactivity and mass in the aliquot, the average specific activity was determined to be 3065 mCi/μmole at the end of synthesis. This corresponds to an average specific activity at the end of the bombardment of over 6000 mCi/μmole. Using an analytical mobile phase containing ammonium chloride permitted the use of a low wavelength (214 nm) detector for determining the specific activity; however, the sample retention times varied considerably depending upon the injection volume due to the lack of buffering capacity of this mobile phase. It was therefore important to co-inject an authentic sample of CFT with the final product to confirm its identity.

One major source of carrier in the final product comes from the solution of reducing agent. Although it is stored and handled under inert atmosphere, a small amount of carrier carbon dioxide may be present during the commercial manufacturing process or introduced during the transfer of the reagent to the reaction vessel. Although the target is evacuated and purged just prior to its use, one cannot rule out the presence of a trace amount of carrier carbon dioxide in the target gas.

Another possible source of carrier comes from trace amounts of CFT present in the nor-precursor itself which is prepared by demethylation of the parent

compound (19). Multiple recrystallizations and careful quality control via several chemical analyses (specifically, proton magnetic resonance spectroscopy and analytical HPLC) are extremely important to assure that the precursor is as pure and free from starting material as possible.

Sterility and apyrogenicity testing were performed using standard procedures. Samples of both radiolabeled product prepared according to the procedure described were determined to be sterile and pyrogen-free in all cases.

[^{11}C]CFT of high specific activity can be rapidly synthesized in reasonable radiochemical yields from the appropriate precursor and [^{11}C]iodomethane. A sufficient amount of the radiotracer can be prepared to allow its use for investigating regional distributions and concentrations of dopamine uptake sites in the brain *in vivo* with PET.

Experimental

Chemicals were analytical grade and were obtained commercially. Nor-CFT is commercially available from Research Biochemicals Inc. Purification and analyses of radioactive mixtures were performed with two Waters 590EF HPLC pumps, in-line uv detectors at two wavelengths (214 and 254 nm), and a single two inch NaI crystal radioactive detector. Semipreparative reversed phase HPLC was performed on an Alltech Econosil C_{18} (250 mm x 10 mm) column with CH_3CN/H_2O (30/70) containing 0.1 N NH_4HCO_2 at 7 mL/minute. Analytical HPLC was performed on an Alltech Econosil C_{18} (250 mm x 4.4 mm) column with 30/70 (CH_3CN/H_2O) containing 0.05 M NH_4Cl at 4 mL/minute. Peak areas were measured using two Hewlett-Packard 3390A recording integrators. Radioactivity measurements were made with a dose calibrator (Capintec CRC-12R).

Radiosynthesis of 2β - Carbomethoxy - 3β - (4 - fluorophenyl) - [N - ^{11}C-methyl]tropane, [^{11}C]CFT

[^{11}C]Iodomethane, produced as previously described (31), was swept by a stream of argon gas into a cooled (-78 °C) solution of nor-CFT (1 mg, 3.8 μmole) in DMF (200 μL). Following the trapping of [^{11}C]iodomethane, the solution was heated at 80 °C for 2 minutes. Triethylamine (5 μL, 36 μmole) and decanoyl chloride (2 μL, 9 μmole) was added and the reaction mixture was heated for an additional minute. HPLC semipreparative mobile phase (200 μL) was added to the reaction mixture and this mixture was applied to the semipreparative HPLC column. The desired product (retention time = 8.7 minutes; k' = 5.2) was collected and evaporated to dryness under reduced pressure. The residue was taken up in 7 mL of sterile saline. The resulting solution was passed through a 0.22 μm Millipore filter into a sterile, pyrogen free bottle and aqueous sodium bicarbonate (3 mL, 8.4%) was added.

The radiochemical purity and specific activity of the final solution were determined by analytical HPLC. Co-injection of the product and an authentic sample of CFT (retention time = 1.7 minutes; k' = 1.8) verified the identity of the product.

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