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ScienceDirect
Nuclear Medicine and Biology 36 (2009) 11 – 16
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

# Dopamine transporter binding in rat striatum: a comparison of [*O*-methyl-$^{11}$C]β-CFT and [*N*-methyl-$^{11}$C]β-CFT

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Received 11 June 2008; received in revised form 13 August 2008; accepted 15 October 2008

**Abstract**

**Introduction:** Positron emission tomography scanning with radiolabeled phenyltropane cocaine analogs is important for quantifying the in vivo density of monoamine transporters, including the dopamine transporter (DAT). [$^{11}$C]β-CFT is useful for studying DAT as a marker of dopaminergic innervation in animal models of psychiatric and neurological disorders. [$^{11}$C]β-CFT is commonly labeled at the *N*-methyl position. However, labeling of [$^{11}$C]β-CFT at the *O*-methyl position is a simpler procedure and results in a shorter synthesis time [desirable in small-animal studies, where specific activity (SA) is crucial]. In this study, we sought to validate that the *O*-methylated form of [$^{11}$C]β-CFT provides equivalent quantitative results to that of the more commonly reported *N*-methyl form.

**Methods:** Four female Sprague–Dawley rats were scanned twice on the IndyPET II small-animal scanner, once with [*N*-methyl-$^{11}$C]β-CFT and once with [*O*-methyl-$^{11}$C]β-CFT. DAT binding potentials (BP≡$B'_{avail}/K_d$) were estimated for right and left striata with a nonlinear least-squares algorithm, using a reference region (cerebellum) as the input function.

**Results:** [*N*-Methyl-$^{11}$C]β-CFT and [*O*-methyl-$^{11}$C]β-CFT were synthesized with 40–50% radiochemical yields (HPLC purification). Radiochemical purity was >99%. SA at end of bombardment was 258±30 GBq/μmol. Average BP values for right and left striata with [*N*-methyl-$^{11}$C]β-CFT were 1.16±0.08 and 1.23±0.14, respectively. BP values for [*O*-methyl-$^{11}$C]β-CFT were 1.18±0.08 (right) and 1.22±0.16 (left). Paired *t* tests demonstrated that labeling position did not affect striatal DAT BP.

**Conclusions:** These results suggest that [*O*-methyl-$^{11}$C]β-CFT is quantitatively equivalent to [*N*-methyl-$^{11}$C]β-CFT in the rat striatum.


*Keywords:* Positron emission tomography; Dopamine transporter; [$^{11}$C]β-CFT; Striatum; Dopamine; Animal models

## 1. Introduction

Positron emission tomography (PET) scanning with radiolabeled phenyltropane cocaine analogs allows quantification of in vivo neuronal monoamine uptake sites. The most commonly employed radioligands, β-CIT [2β-carbomethoxy-3β-(4′-iodophenyl)tropane, RTI-55] [1] and β-CFT [2β-carbomethoxy-3β-(4′-fluorophenyl)tropane, WIN 35,428 (**3**)] [2], have a high affinity for the dopamine transporter (DAT) and are used as markers for striatal central dopaminergic innervation [3–10]. [$^{11}$C]β-CFT is widely used to study DAT in animal models of psychiatric and neurological disorders and in human diseases such as Parkinson's disease [11–13]. The *N*-methylated form of [$^{11}$C]β-CFT is more commonly seen in the literature, as the precursor used for radiolabeling, nor-β-CFT [2β-carbomethoxy-3β-(4′-fluorophenyl)nortropane (**5**)], is commercially available. However, it is more cost-effective to synthesize precursor de novo. We pursued the use of [*O*-methyl-$^{11}$C]β-CFT [13–15] for several reasons: (a) the synthesis of β-CFT-acid [2β-carboxylic acid-3β-(4′-fluorophenyl)tropane (**4**)] for the production of [*O*-methyl-$^{11}$C]β-CFT is simpler than that of nor-β-CFT; (b) synthesis of β-CFT-acid results in higher chemical yields than the synthesis of nor-β-CFT; and (c) labeling of [$^{11}$C]β-CFT at the *O*-methyl position results in a slightly shorter synthesis

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doi:10.1016/j.nucmedbio.2008.10.007

time, which is advantageous in small-animal PET studies where specific activity (SA) is crucial.

Our group and others have successfully labeled the cocaine congener β-CIT at both nitrogren and oxygen positions [16,17]. Lundkvist et al. [17] reported that the late-time striatum:cerebellum ratios were similar for [*N*-methyl-$^{11}$C]β-CIT and [*O*-methyl-$^{11}$C]β-CIT. As β-CIT is chemically similar to β-CFT, the semiquantitative observation of Lundkvist et al. suggests that [*N*-methyl-$^{11}$C]β-CFT and [*O*-methyl-$^{11}$C]β-CFT may produce similar estimates of DAT binding. The purpose of this study was to determine if estimates of DAT binding in rat striata with [$^{11}$C]β-CFT labeled at the *O*-methyl position were equivalent to those from [*N*-methyl-$^{11}$C]β-CFT.

## 2. Materials and methods

### 2.1. General

All commercial reagents and solvents were used without further purification. [$^{11}$C]Methyl triflate ([$^{11}$C]$CH_3OTf$) was prepared according to a literature procedure [18]. $^1$H NMR spectra were recorded on a Bruker QE 300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million ($\delta$ scale) relative to internal standard TMS ($\delta$ 0.0), and coupling constants ($J$) were reported in hertz. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical HPLC was performed using a Prodigy (Phenomenex) 5-μm C-18 column, 4.6×250 mm; 3:1:3 $CH_3CN$/MeOH/20 mM, pH 6.7, phosphate (buffer solution) mobile phase; 1.5 ml/min flow rate; and UV (254 nm) and gamma ray (NaI) flow detectors. Semipreparative HPLC was performed using a YMC-Pack ODS-A, S-5 μm, 12 nm, 10×250 mm i.d. (Waters) C-18 column; 20% EtOH/80% 20 mM $H_3PO_4$ mobile phase, 5.0 ml/min flow rate, UV (254 nm) and gamma ray (NaI) flow detectors. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation (Bedford, MA).

The overall scheme of synthesis is depicted in Fig. 1.

#### 2.1.1. Precursors and β-CFT standard syntheses

Hydrolysis of cocaine under acid conditions gave 2β-carboxylic acid-3β-hydroxytropane (**1**), which was used in the next step reaction without further purification. Compound **1** was reacted with $POCl_3$ and MeOH to provide anhydroecgonine methyl ester (**2**) in 86% yield [16,19]. $^1$H NMR ($CDCl_3$): $\delta$ 6.81 (t, 1H, $J$=3.0 Hz), 3.78 (d, 1H, $J$=5.6 Hz), 3.74 (s, 3H, $OCH_3$), 3.24 (t, 1H, $J$=5.4 Hz), 2.69–2.62 (d, br, 1H, $J$=19.8 Hz), 2.35 (s, 3H, $NCH_3$), 2.20–2.07 (m, 2H), 1.88–1.81 (dd, 1H, $J$=19.8 Hz, 4.0 Hz), 1.80–1.26 (m, 2H).

The conjugate addition of Grignard reagent 4-fluorophenylmagnesium bromide to Compound **2** after acid workup [19] formed a mixture of two phenyltropane isomers: the biologically potent β-CFT (Compound **3**) and the less biologically-active 2α-carbomethoxy-3β-(4′-fluorophenyl) tropane (α-CFT) [20] (45% and 17% yields, respectively). In addition to being an intermediate in the synthesis of precursors, β-CFT served as the standard compound. β-CFT $^1$H NMR ($CDCl_3$): $\delta$ 7.23–7.19 (m, 2H), 6.98–6.92 (m, 2H), 3.57–3.55 (m, 1H), 3.50 (s, 3H, $OCH_3$), 3.38–3.37 (d, br, 1H, $J$=3.0 Hz), 3.02–2.94 (m, 1H), 2.88–2.86 (d, br, 1H, $J$=6.0 Hz), 2.63–2.53 (m, 1H), 2.23 (s, 3H, $NCH_3$), 2.18–2.03 (m, 2H), 1.78–1.56 (m, 3H). α-CFT $^1$H NMR ($CDCl_3$): $\delta$ 7.25–7.20 (m, 2H), 6.97–6.91 (m, 2H), 3.51 (s, 3H, $OCH_3$), 3.44–3.42 (d, br, 1H, $J$=6.0 Hz), 3.28–3.26 (m, 1H), 3.12–3.09 (m, 2H), 2.42 (s, 3H, $NCH_3$), 2.14–2.08 (ddd, 1H, $J$=18.0 Hz), 1.98–1.82 (m, 3H), 1.76–1.58 (m, 2H).

Hydrolysis of β-CFT (Compound **3**) under neutral conditions (50% dioxane/$H_2O$) [21] provided precursor β-CFT-acid (Compound **4**) in 97% yield. $^1$H NMR ($CDCl_3$): $\delta$ 7.24–7.19 (m, 2H), 7.01–6.95 (m, 2H), 3.61–3.58 (m, 2H), 3.21–3.12 (m, 1H), 2.67–2.56 (m, 2H), 2.51 (s, 3H, $NCH_3$), 2.31–2.28 (m, 2H), 2.01–1.94 (m, 2H), 1.82–1.74 (m, 1H).

*N*-Desmethylation of Compound **3** with 1-chloroethyl chloroformate [19] afforded another precursor, nor-β-CFT (Compound **5**), in 75% yield. $^1$H NMR ($CDCl_3$): $\delta$ 7.18–7.15 (t, 2H, $J$=9.0 Hz), 6.97–6.94 (t, 2H, $J$=9.0 Hz), 3.78–3.59 (m, 2H), 3.40 (s, 3H, $OCH_3$), 3.24–3.21 (m, 1H), 2.68–2.39 (m, 3H), 2.26–2.20 (m, 1H), 1.98–1.95 (m, 1H), 1.80–1.58 (m, 3H).

#### 2.1.2. Radiochemistry of [O-methyl-$^{11}$C]β-CFT and [N-methyl-$^{11}$C]β-CFT

Radiosynthesis was performed in an automated, multipurpose $^{11}$C-radiosynthesis module, which allows measurement of SA during synthesis [22,23]. [$^{11}$C]$CO_2$ was produced by the $^{14}$N(p,α)$^{11}$C nuclear reaction in a small-volume (9.5 $cm^3$) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+1% $O_2$) in a Siemens radionuclide delivery system (Eclipse RDS-111). The precursor β-CFT-acid (Compound **4**, 0.1 mg) was dissolved in $CH_3CN$ (300 μl). To this solution was added 3 N NaOH (2 μl). The mixture was transferred to a small reaction vial. No-carrier-added (high SA) [$^{11}$C]$CH_3OTf$ that was produced by the gas-phase production method [18] from [$^{11}$C]$CO_2$ through [$^{11}$C]$CH_4$ and [$^{11}$C]$CH_3Br$ with silver triflate (AgOTf) column was passed into the reaction vial, which was cooled to 0°C, until radioactivity reached a maximum (~2 min) and then the reaction vial was isolated and heated at 80°C for 3 min. The contents of the reaction tube were diluted with $NaHCO_3$ (1 ml, 0.1 M) and injected onto the semipreparative HPLC column with 2 ml injection loop. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum and the final product, [*O*-methyl-$^{11}$C]β-CFT (*O*-[$^{11}$C]**3**), was formulated in saline, sterile filtered through a sterile vented Millex-GS 0.22-μm cellulose acetate membrane and collected into a sterile vial containing 8% $NaHCO_3$ solution (0.25 ml) for adjustment of pH of the product. Total radioactivity was assayed and total volume was noted for