[^{123}I]-2β-Carbomethoxy-3β-(4-iodophenyl)tropane: High-Affinity SPECT Radiotracer of Monoamine Reuptake Sites in Brain[1]

The potent cocaine congener 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (**2**; CFT or WIN 35,428)[2,3] when tritiated[4,5] or labeled with $^{11}CH_3$[6] was found to be superior to [^{3}H]cocaine or [^{11}C]cocaine[7] **1** as a radioligand probe for cocaine receptors in terms of higher affinity and larger residence time on the dopamine-reuptake site. For further development of analogues suitable for PET and SPECT imaging, we have synthesized and characterized 2β-carbomethoxy-3β-(4-iodophenyl)tropane (**3a**; designated as β-CIT in analogy to CFT, and recently reported by Boja et al.[8] under the code RTI-55), its corresponding N-demethylated derivative (designated as *nor*-CIT) **4**, and the $C_{2\alpha}$ isomer **3b** (Figure 1).

Ecgonidine methyl ester (**5**) was prepared from cocaine by the procedure of Clarke et al.[2] Treatment of **5** with phenylmagnesium bromide and subsequent workup with trifluoroacetic acid at low temperature gave a mixture of C_2 epimers, **6** (45%) and **7** (31%), which were separated by flash chromatography (silica; CH_2Cl_2/CH_3OH, 25:1).[5] Direct iodination of **6** with $I_2/HNO_3/H_2SO_4$ gave the para-substituted compound **3a** (β-CIT) as an oil: 62%; $[\alpha]^{25}_D$ -2.0° (c = 0.85, $CHCl_3$). D-Tartrate salt: mp 72-74 °C; $[\alpha]^{25}_D$ -87.7° (c = 1.5, CH_3OH). Anal. C, H, N, I (Scheme I). Iodination of **7** by the same procedure gave **3b** (α-CIT) as an oil: 39%; $[\alpha]^{25}_D$ +44° (c = 2.5, $CHCl_3$). 1,5-Naphthalenedisulfonate salt: mp 139-140 °C. Anal. C, H, N, I. N-Demethylation of **6** was accomplished by conversion to its 2,2,2-trichloroethyl carbamate followed by reduction (Zn/acetic acid) to yield **8** by the procedure previously described by Milius et al.,[5] followed by iodination to yield *nor*-CIT (**4**), which was isolated as a yellow crystalline solid (free base 48% from **6**): mp 149-151 °C; $[\alpha]^{25}_D$ -67.4° (c = 1, $CHCl_3$). Anal. C, H, N.

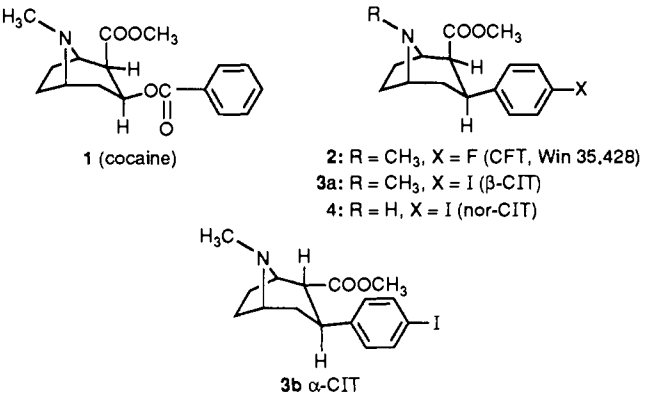
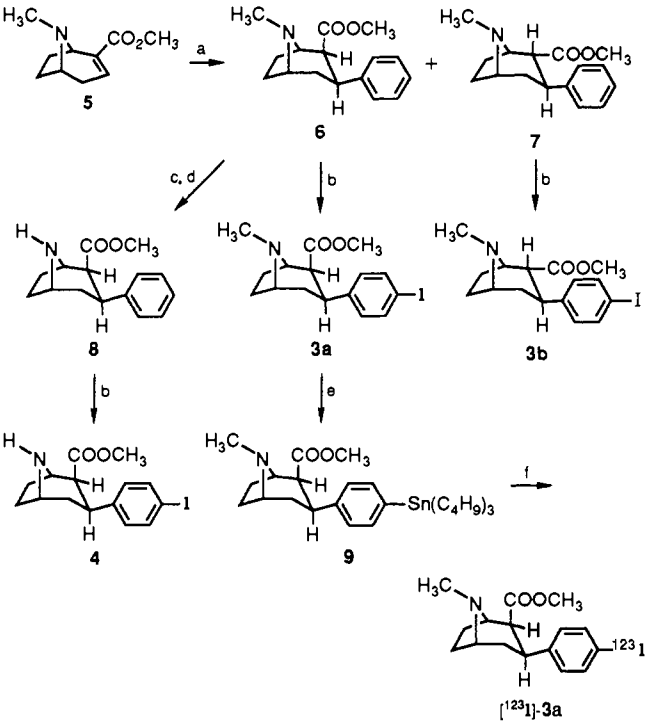


Figure 1. Cocaine and 3β-(4-halophenyl) anologues.

Scheme I. Synthesis of 2-Carbomethoxy-3β-(4-iodophenyl)tropanes[a]



[a] (a) C_6H_5MgBr. (b) I_2, HNO_3-H_2SO_4. (c) Cl_3CCH_2OCOCl. (d) Zn, CH_3CO_2H. (e) $(C_4H_9Sn)_2$, $[(C_6H_5)_3P]_4Pd/(CH_3CO_2)_2Pd$. (f) $Na^{123}I$, CH_3CO_3H.

[^{123}I]CIT [^{123}I-**3a**] was synthesized from nonradioactive β-CIT (**3a**) by conversion to the corresponding tributyltin derivative **9** (Scheme I). Treatment of **3a** with bis(tributyltin), tetrakis(triphenylphosphate)palladium(0), and palladium(II) acetate in refluxing tetrahydrofuran gave **9** as a colorless waxy solid after flash chromatography (silica, stepwise gradient, hexane to hexane/ether, 75:25) in 26% yield from **3a**. The 300-MH_3 NMR ($CDCl_3$) of **9** was consistent with the assigned structure. Reaction of **9** with no-carrier-added $Na^{123}I$ in the presence of peracetic acid gave [^{123}I]-**3a**. The radioiodinated product was purified by preparative HPLC (Novapak C_{18}, $MeOH/H_2O/Et_3N$, 75:25:0.2, 1.0 mL/min; t_R 6.7 min) and formulated in normal saline containing 5% ethanol and 1% ascorbic acid. [^{123}I]-**3a** was obtained in average overall yield of 60.0 ± 13.4% and with radiochemical purity of 97.6 ± 1.6%. The tributyltin precursor used in radiolabeling contained about 7 mol% CIT carrier, resulting in an ^{123}I product having

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Table I. In Vitro Radioligand Binding Data for Cocaine and 3-(4-Halophenyl) Analogues[a]

analogue	displacement of [^{3}H]CFT		displacement of [^{3}H]paroxetine	
	IC_{50} (nM)	Hill slope (nH)	IC_{50} (nM)	Hill slope (nH)
1 (cocaine)	221 ± 14	0.69 ± 0.06 (3)	207 ± 66	0.73 ± 0.12 (5)
2 (β-CFT)	15.3 ± 1.2	0.75 ± 0.01 (3)	479 ± 59	1.34 ± 0.22 (3)
3b (α-CIT)	87.6 ± 2.9	0.70 ± 0.07 (2)	210 ± 86	0.73 ± 0.04 (2)
3a (β-CIT)	1.6 ± 0.15	0.79 ± 0.04 (3)	3.78 ± 0.53	0.82 ± 0.08 (6)

[a] Radioligand binding of [^{3}H]CFT (0.5 nM) to dopamine reuptake sites in tissue homogenates prepared from primate striatum was performed as described by Madras et al.[9] Binding of [^{3}H]paroxetine to serotonin reuptake sites in homogenates prepared from rat cortical membranes was performed according to Laruelle et al.[10] The IC_{50} value is the concentration of displacing analogue required to decrease specific radioligand binding by 50%. Values represent mean ±SEM (of n experiments).

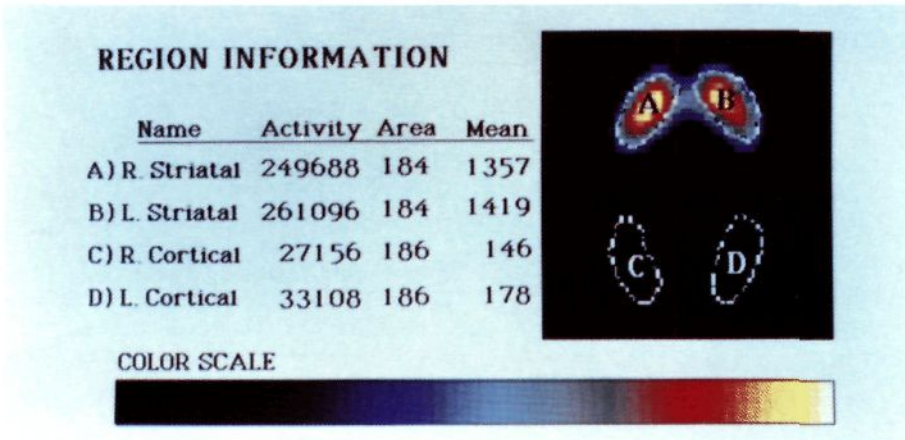

Figure 2. Transaxial slice through the baboon brain at the level of the occipital cortex and striatum 360 min postinjection 3.3 mCi [^{123}I]CIT. This image was reconstructed from data acquired by the Strichman 810X over 2 min from a plane parallel and approximately 15 mm superior to the canthomeatal line. Typical regions of interest drawn on the computer screen are used for radioactivity measurements. Figure shows right (R) and left (L) regions from striatum and occipito-parietal cortex (cortical). The area of the region is expressed in pixels, with each pixel corresponding to approximately 1.6 × 1.6 mm. The "mean" regional activities are equal to total "activity" divided by "area" and are the values represented in Figure 3.

a specific activity of about 2000 Ci/mmol.

The affinities of cocaine (1), α-CIT (**3b**), β-CIT (**3a**), and β-CFT (**2**) for the dopamine and serotonin reuptake sites were determined from radioligand displacement studies using tissue homogenates prepared from baboon and rat brain (Table I).

Five SPECT (single photon emission computed tomography) experiments were performed with four female baboons (*Papio anubis*, 10–12 kg) under isoflurane anesthesia. Animals were injected iv with 8.1 ± 1.4 mCi [^{123}I]-β-CIT (with these and subsequent data expressed as mean ± SEM) and scanned for 300 ± 41 min in the 810X Brain Imager (Strichman Medical Equipment, Medfield, MA). Serial 1–2 min images were reconstructed assuming uniform attenuation equal to that of water in an ellipse drawn around the brain. Data were decay corrected to time of injection.

Highest brain uptake overlay the striatal region and peaked at 154 ± 19 min postinjection (pi) radioligand and showed striatal to cerebellar ratios at that time of 9.8 ± 1.6 (n = 5; Figure 2). Washout of striatal activity was followed for an additional 200 and 260 min in two of the three control animals and showed 0% and 12% decreases, respectively, from time of striatal peak to end of the experiment.

The brain area with second highest activities approximately overlay the hypothalamic and midbrain substantia nigra regions and showed peak levels at 43 ± 5 min pi (n = 5) and had a faster washout than striatal activity (Figure 3A).

The pharmacological specificity of the in vivo labeling of [^{123}I]-β-CIT was examined with displacement of brain activity by indatraline (also designated Lu 19-005; source, Research Biochemicals Inc.), a potent agent for the dopamine and serotonin reuptake sites,[11] and citalopram, an agent selective for the serotonin reuptake site,[12] following an in vivo displacement paradigm described by Innis et al.[13] Indatraline (3 μmol/kg iv) injected at 200 min pi radioligand caused significant decrease of both striatal and hypothalamic activity (Figure 3A). During the 100-min period after injection of Lu 19-005, striatal activity decreased by 65% compared to a mean decrease of 2% during the same period in the two control animals followed for that length of time. In contrast, citalopram (7.4 μmol/kg iv) injected 60 min pi radioligand showed a selective decrease of hypothalamic activity (Figure 3B). Citalopram caused a 48% decrease of hypothalamic activity during the 60-min period after injection, in comparison to 16 ± 3% decrease (n = 3) of hypothalamic activity in control animals followed during this same period.

These results showed that [^{123}I]-β-CIT was a useful SPECT probe of monoamine reuptake sites in primate

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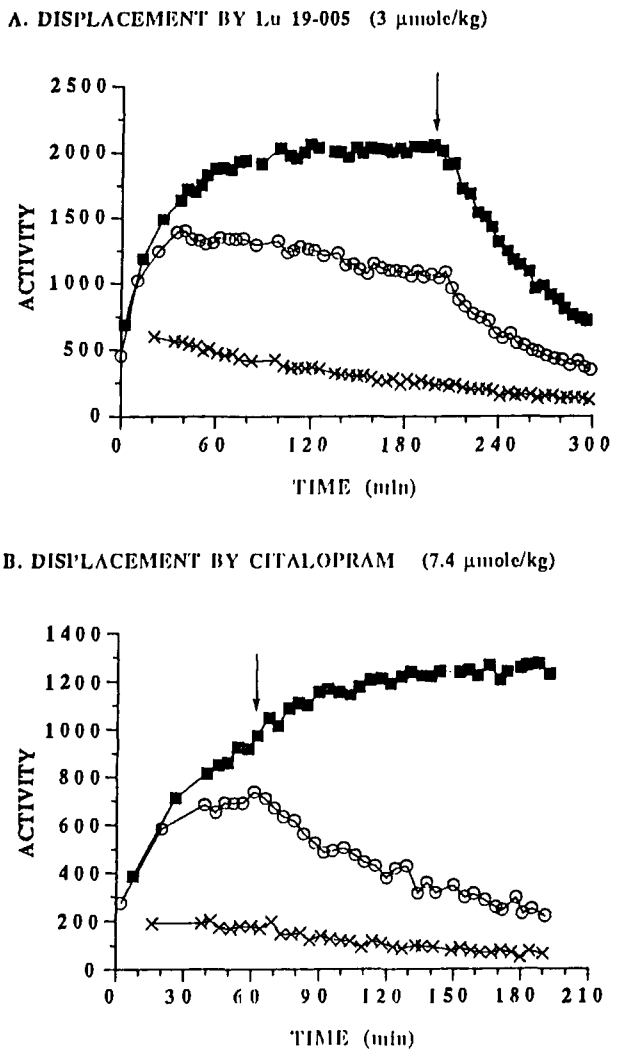

Figure 3. Regional activity in baboon brain following iv injection of 12.1 mCi (A) and 4.2 mCi (B) [^{123}I]CIT. Activity is expressed in arbitrary units known from phantom studies to be linear with radioactive concentrations. Displacing agents were injected iv at the times marked with arrows. Activities in three brain regions are graphed: ■, striatum; ○, hypothalamus; and x, cerebellum.

brain and suggest that [^{11}C]-β-CIT may be a similarly useful PET tracer. The in vivo displacement SPECT experiments support the notion that the majority of striatal activity following injection of [^{123}I]-β-CIT was associated with dopamine reuptake sites and that the majority of hypothalamic activity was associated with serotonin reuptake sites, which is consistent with the densities of these monoamine transporters measured in postmortem primate brain.[14] Brain washout of activity was relatively slow, in part because of the high affinities of **4a** for the monoamine transporters. In addition, the iodine appears to be in a relatively metabolically resistant position, since whole body scanning showed low thyroid uptake, indicative of a slow in vivo rate of deiodination (unpublished results). [^{123}I]-β-CIT and [^{11}C]-β-CIT may be useful clinical markers of dopaminergic and serotonergic innervation in human disorders such as Parkinson's disease and depression, which are thought to have abnormalities in these neurotransmiter systems.

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