Synthesis of Carbon-11 Labeled Iodinated Cocaine Derivatives and Their Distribution in Baboon Brain Measured Using Positron Emission Tomography

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Three iodine-substituted derivatives of cocaine, methyl esters of 3-[(2′-, 3′-, and 4′-iodobenzoyl)oxy]-8-methyl-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]octane-2-carboxylic acid (**2a–c**), were synthesized and subjected to N-demethylation to give the corresponding noriodococaines 3-[(2′-, 3′-, and 4′-iodobenzoyl)oxy]-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]-octane-2-carboxylic acid (**3a–c**). These were remethylated with [^{11}C]CH_3I to give the [*N*-^{11}C-*methyl*]iodococaines **4a–c** which were examined in baboon brain in vivo using positron emission tomography (PET). Compared to [*N*-^{11}C]cocaine itself the regional distributions were changed from a highly specific localization in the corpus striatum to more diffuse patterns which included the cerebellum and cortex. Peak brain uptakes and clearance kinetics were also changed. [*N*-^{11}C]-*o*-Iodococaine (**4a**) had a peak uptake in the striatum at 4–5 min after injection of only 17% that of cocaine in the same animal. The peak uptake of [*N*-^{11}C]-*p*-iodococaine (**4c**) was 60% of that of [*N*-^{11}C]cocaine and a clearance half-time of approximately 55 min, twice that of [*N*-^{11}C]cocaine. [*N*-^{11}C]-*m*-Iodococaine (**4b**) displayed half the uptake of [*N*-^{11}C]cocaine, buts its clearance was similar to that of the parent molecule. The fractions of unmetabolized tracer in blood plasma at 1–30 min were higher for **4a–c** than for [*N*-^{11}C]cocaine. Plasma protein binding experiments showed 10%, 0.3%, 1.6%, and 6% as the free fraction for cocaine and *o*-, *m*-, and *p*-iodococaines respectively, consistent with the low brain uptake observed for the ortho isomer, and implicated α_1-acid glycoprotein as responsible for the low free fraction of *o*-iodococaine. The potencies of **2a–c** to displace tritiated cocaine from striatal membranes were *p*-iodo ≈ cocaine > *m*-iodo ≈ *o*-iodo.

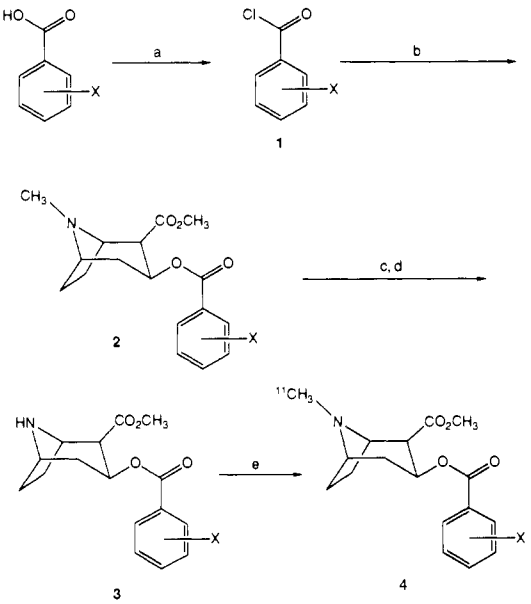
Introduction

Although cocaine has a variety of effects on processes involved in nervous transmission,[1-5] the powerful euphorigenic properties of cocaine which lead to its abuse probably stem from its inhibition of presynaptic dopamine reuptake.[6-9] Recent studies from our group showed that the regional binding and kinetics of cocaine in the brain could be directly measured by using positron emission tomography (PET) and a tracer dose of [*N*-^{11}C-*methyl*]-cocaine in normal human volunteers and nonhuman primates.[10] The highest regional concentration occurred in the corpus striatum and this was significantly reduced by pretreatment with the dopamine reuptake inhibitor nomifensine but not by the norepinephrine uptake blocker desipramine. Furthermore, the tissue concentration of [*N*-^{11}C-*methyl*]cocaine in the human striatum following intravenous administration exhibited a similar time-course to that of the euphoria reported by abusers of the drug. These studies demonstrated the feasibility of using labeled cocaine for the examination of pharmacologically relevant cocaine binding sites (presumably dopamine transporters) in the striatum.

Recently, Ritz et al. reported a study of structure–activity relationships for binding of several compounds structurally related to cocaine to the dopamine, norepinephrine, and serotonin transporters in vitro.[11] Basmadjian et al. have reported that radioiodination of tropeines in the ortho position of the benzoyl group yields compounds with mouse biodistributions like [^{3}H]-/[^{14}C]-/[^{11}C]-cocaine but with slightly different peak and washout times in the brain and the heart.[12] Although the mouse studies were done with labeled compounds of too low specific radioactivity to evaluate binding to reuptake sites, no-carrier-added *o*-[^{123}I]iodococaine was subsequently prepared[13] and suggested as a probe for the characterization of the cocaine receptors in the human brain and heart by single photon emission computed tomography (SPECT) imaging.

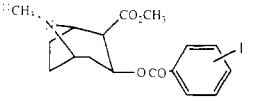
In order to examine both the sensitivity of cocaine to structural modification and the possible usefulness of labeling cocaine derivatives with the longer lived nuclide ^{123}I for SPECT studies, we synthesized three [*N*-^{11}C-*methyl*]iodobenzoyl substituted (ortho, meta, para) analogues of cocaine (**4a–c**)[14] and compared their regional

Scheme I.[a] Synthesis of Iodinated [*N*-^{11}C]Cocaine



X = *o*-, *m*-, *p*-I; EME = ecgonine methyl ester

[a] (a) $SOCl_2$; (b) NaH (or Na_2CO_3), EME/THF/Δ; (c) $CH_3CHClOCOCl$/Proton Sponge; (d) MeOH/Δ; (e) $^{11}CH_3I$.



(-)-(*o*-, *m*-, *p*-)-[N-^{11}CH_3]cocaine (**4a-c**)

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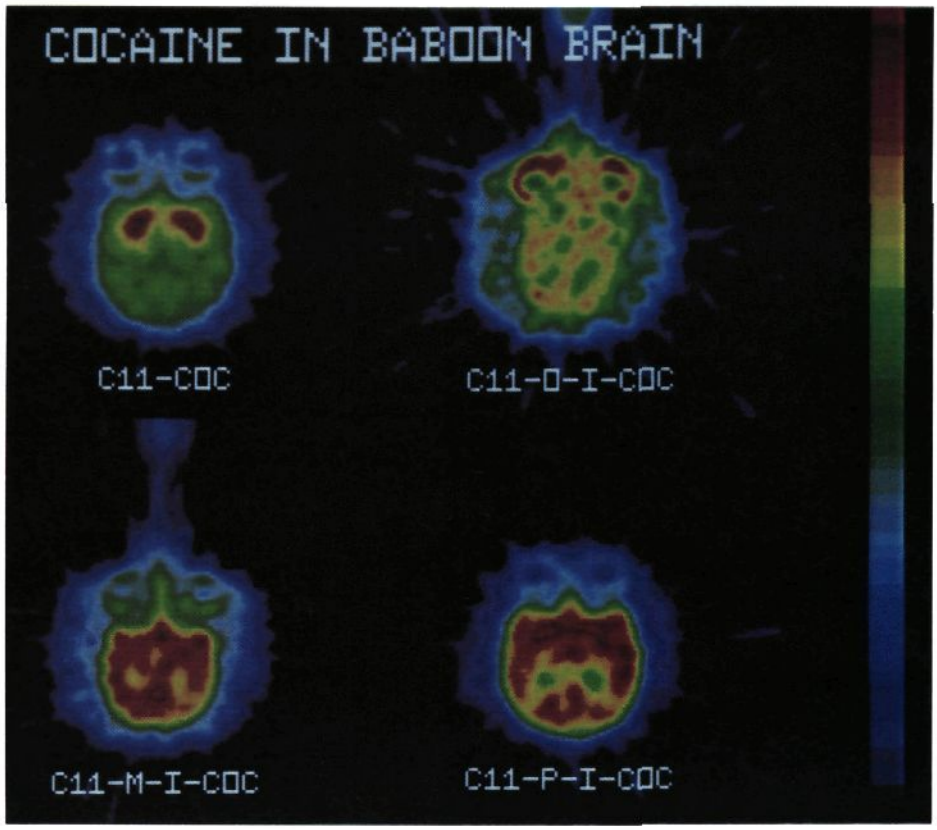

Figure 1. Uptake of carbon-11 in baboon brain. Images show the distribution of radioactivity 15 min after injection of [*N*-^{11}C-*methyl*]cocaine or [*N*-^{11}C-*methyl*]iodococaine. Each image is normalized to its maximum pixel value, so that the four compounds can be compared with respect to regional distribution but not to absolute uptake. The color scale at the right shows that red indicated the highest radioactivity concentration.

distribution and kinetics to those of the parent molecule in the baboon brain using PET, along with the spectrum of labeled metabolites in plasma. To help interpret the PET data, we measured the plasma free fractions of the [*N*-^{11}C-*methyl*]iodococaines by ultrafiltration, and the abilities of the unlabeled iodococaines to compete with [^{3}H]cocaine for binding to rat striatal membranes.

Results

Chemistry and Radiochemistry. Syntheses of carbon-11-labeled iodobenzoyl-substituted derivatives of cocaine (**4a–c**) are illustrated in Scheme I. The iodobenzoyl chlorides **1a–c** were either obtained from commercial sources or prepared from the appropriate iodobenzoic acid using thionyl chloride. Coupling of iodobenzoyl chlorides **1a–c** and ecgonine methyl ester (EME) in the presence of base (NaH or Na_2CO_3) gave moderate yields of corresponding iodococaines **2a–c**, which were purified by column chromatography. N-Demethylation of iodococaines **2a–c** with α-chloroethyl chloroformate[15] gave noriodococaines **3a–c**. (−)-[*N*-^{11}C-*methyl*]Iodococaines **4a–c** were prepared by the reaction of [^{11}C]CH_3I[16] with noriodococaines **3a–c**. The radioactive syntheses took less than 1 h and achieved specific activities of ca. 0.25 Ci/μmol (end

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Table I. Properties of Cocaine and Iodococaines in Vivo and in Vitro

compound	peak striatal uptake[a]	% free fraction	IC_{50} (nM)	% unmetabolized at 30 min[b]	plasma integral at 30 min (μCi min/mL)[b]
o-iodococaine	0.01	0.3	350	33	6.1
m-iodococaine	0.03	1.6	325	36	2.8
p-iodococaine	0.04	6.0	100	22	1.8
cocaine	0.06	10.0	70	17	2.1

[a] Percent of injected dose per mL of tissue. [b] Values are the mean of two experiments.

Table II. Metabolite Profiles for Cocaine and Derivatives[a]

compound	metabolite	% tracer or metabolite at time (min) 1	5	10	30
cocaine	unchanged	70	44	31	17
	EME	12	13	11	12
	acidic metabolites	0.4	22	38	60
	CO_2	6	30	37	44
o-iodococaine	unchanged	97	79	48	33
	EME	0.3	0.2	0.2	0.2
	acidic metabolites	0.02	15	52	69
	CO_2	−7	15	21	29
m-iodococaine	unchanged	95	77	60	36
	EME	0.7	3	1	4
	acidic metabolites	0.4	18	35	51
	CO_2	3	17	33	43
p-iodococaine	unchanged	90	60	48	22
	EME	1.5	2.5	2	2.6
	acidic metabolites	0.5	17	45	71
	CO_2	1	7	21	32

[a] Values are the mean from two experiments. For each administered ^{11}C compound HPLC assay was used to separate unchanged tracer and ecgonine methyl ester from acidic metabolites (ecgonine, arylecgonine and bicarbonate) which eluted at or very near the void volume. Labeled bicarbonate was independently determined by a volatilization assay.

of synthesis). The labeled [*N*-^{11}C-*methyl*]iodococaines **4a–c** were identified by carrier-added TLC and HPLC analysis and purified by HPLC with radiochemical purity >98%.

Brain Uptake and Kinetics. The iodococaine isomers behaved very differently from each other, and from cocaine itself (Figure 1). After injection of *o*-, *m*-, and *p*-[*N*-^{11}C-*methyl*]iodococaine, radioactivity was homogeneously distributed throughout all cortical structures including parietal, frontal, temporal, and occipital cortices as well as subcortical structures including the thalamus and brain stem. In contrast, [*N*-^{11}C-*methyl*]cocaine concentrated predominantly in the striatum.

Time courses of radioactivity concentration for the baboon striatum, cerebellum, and frontal cortex for representative PET studies with each iodococaine are shown in Figure 2. [*N*-^{11}C-*methyl*]Cocaine reached peak concentrations within 4 min and cleared to 50% of the peak value in the corpus striatum by 20 min after injection. While the parenchymal level of radioactivity from [*N*-^{11}C-*methyl*]-*o*-iodococaine exceeded that of the ventricular spaces, the peak value of the striatal uptake of *o*-iodococaine at 4.5 min postinjection was only about 17% of that of cocaine. Although [*N*-^{11}C-*methyl*]-*m*-iodococaine displayed a similar uptake and clearance pattern in the striatum to cocaine itself, its peak striatal uptake was only half that of cocaine, and uptake in both cerebellum and frontal cortex was similar to that in striatum. For [*N*-^{11}C-*methyl*]-*p*-iodococaine, the accumulation of radioactivity was slower than for the ortho and meta isomers in corpus striatum, cerebellum, and frontal cortex. Striatal clearance was also slower, with 50% of peak radioactivity remaining at 45 min.

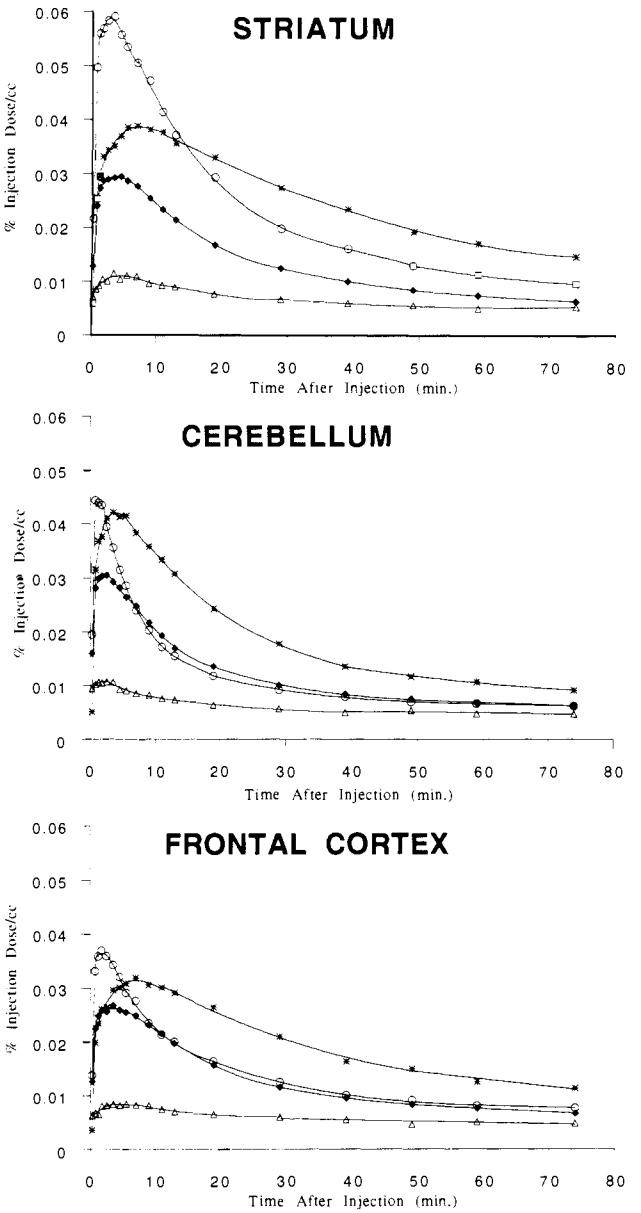

Figure 2. Kinetics of carbon-11 in brain regions following administration of labeled cocaine (circles), *o*-iodococaine (triangles), *m*-iodococaine (diamonds), or *p*-iodococaine (stars).

Blood Kinetics and Metabolism. Both *m*- and *o*-iodococaines had slower clearance from blood plasma than cocaine, with metabolite-corrected plasma radioactivity–time integrals at 30 min after injection about 150% and 300% that of cocaine, respectively. The integrals for *p*-iodococaine and cocaine were similar (Table I).

Analyses of radioactivity in plasma after injection of *o*-, *m*-, and *p*-iodococaines are shown in Table II. Disappearance of labeled compound was slower for *o*- and *m*-iodococaine than for cocaine itself. However, *m*-iodococaine was most similar to cocaine in terms of metabolism to labeled CO_2. Very little labeled EME was observed for

the iodococaines (<4% compared to 10–15% for cocaine).

Binding to Rat Striatal Membranes and Plasma Proteins. *p*-Iodococaine was about equipotent at displacing [^{3}H]cocaine from membrane binding sites as cocaine itself, while the ortho and meta isomers were weaker than cocaine (Table I).

In baboon plasma protein binding experiments the rank order of free fractions followed that of peak striatal uptake, with the free fraction for *o*-iodococaine being only 0.3%, versus 10% for cocaine (Table I). *o*-Iodococaine gave a free fraction of 5.1% in an ultrafiltration experiment with 0.5 mg/mL purified human α_1-acid glycoprotein, and free fractions of 86% and 91% with purified albumin (40 mg/mL) and thyroid hormone binding globulin (0.1 mg/mL), respectively (data not shown). Similarly, its free fraction in whole human plasma was elevated from 0.6% to 3.5% by 0.1 mM propranolol, a ligand for α_1-acid glycoprotein,[17] but was little affected by 0.1 mM thyroxine (0.6%) or benzoic acid (<0.8%) (data not shown), which were used as competitive ligands for thyroid hormone binding globulin and albumin, respectively.

Discussion

Our previous studies[10] demonstrated that [^{11}C]cocaine is a suitable radiotracer for PET studies of cocaine binding and kinetics. In order to explore the possibility of labeling this molecule with ^{123}I for SPECT studies, we examined the effect of iodine substitution on the biological properties of [^{11}C]cocaine.

In spite of the abilities of the iodococaines to compete with cocaine for binding to rat striatal membranes, the regional distribution and brain kinetics of ^{11}C-labeled iodinated cocaine derivatives up to 1 h after administration bore little resemblance to those of the parent molecule in primate brain (Figures 1 and 2), and therefore are not suitable for mapping cocaine binding sites in vivo.

Neither peak striatal uptakes nor striatal clearance times were well correlated with results of [^{3}H]cocaine competition measurements (Table I). However, the longer striatal clearance time for *p*-iodococaine (45 versus about 15 min for the other compounds), together with the smaller IC_{50} for *p*-iodococaine, suggest binding to cocaine receptors in vivo. Slower clearance of *p*-iodococaine from striatum than from cerebellum also supports the idea that striatal uptake includes binding to dopamine reuptake sites, which are present in far lower concentration in the cerebellum than in striatum.[18] Nevertheless it appears that all three iodococaines bind to one or more sites in the cerebellum and in cortical areas which have little affinity for cocaine itself. Whatever the nature of the cerebellar binding, it prevents the customary use of the cerebellum as a "reference area" with PET and SPECT radiotracers of the dopamine system.

There was good correlation between peak striatal uptakes and plasma-free fractions of cocaine and its derivatives (Table I). It is particularly interesting that substitution of iodine in the ortho position drastically increased the bound fraction, which no doubt accounts for its poor uptake in brain. Experiments with competitive ligands and purified plasma proteins implicate tight interaction with α_1-acid glycoprotein as the cause of the low free fraction for *o*-iodococaine. Neither thyroid hormone binding globulin (whose substrates are also *o*-iodo compounds) nor albumin appear to bind *o*-iodococaine strongly. Since cocaine and the iodococaines should be sufficiently lipophilic to readily cross the blood–brain barrier, the binding of the compounds in blood is clearly a major determinant of the magnitude of their brain uptake.[19] However, cocaine's brain uptake of about 0.05%/cm^3 within 2 min implies a single pass extraction fraction considerably greater than the plasma-free fraction of 10%. It would be 50% if 5% of the cardiac output goes to a 200-cm^3 brain. Therefore a substantial portion of the protein-bound cocaine must dissociate during transit through brain capillaries. Nevertheless, since the same rank order was found for the peak extraction and free fraction for the present series of compounds, the fractions available for extraction clearly parallel the free fractions. The greater plasma protein binding of *o*- and *m*-iodococaines than cocaine is also reflected in the higher plasma integrals for these derivatives (Table I). The integral for *p*-iodococaine was not significantly different from that of cocaine, in spite of the lower free fraction of *p*-iodococaine. More efficient removal from blood of the *p*-iodo compound than its isomers may reflect its higher affinity for tissue cocaine binding sites (Table I).

The fact that iodine substitution in the para position of the benzoyl ring in cocaine results in a molecule with similar potency for displacing [^{3}H]cocaine from striatal membranes resembles observations in another series of novel cocaine analogues, the WIN series, where the ester linkage of the benzoyl function has been eliminated. Here the substitution of iodine in the para position of the benzoyl ring results in enhanced binding.[20–22]

Conclusions

Introducing an iodine atom onto any position of the benzoyl ring of cocaine changes its regional biodistribution and pharmacokinetics. The relative uptake of all three isomers in cerebellum and cortex was greater (relative to striatum) than for cocaine itself. *o*-Iodococaine showed very low brain uptake (17% of cocaine) due to high plasma protein binding. Although the striatal clearance of *m*-iodococaine was most similar to that of cocaine, the poor in vitro binding to striatal membranes suggests that the uptake of *m*-iodococaine in baboon striatum is not mediated by cocaine binding sites. Of the three iodinated cocaines, *p*-iodococaine exhibited both the most prolonged striatal clearance and the greatest ability to displace [^{3}H]cocaine from binding sites in striatal membranes. Therefore, in spite of its concentration in nondopaminergic regions, *p*-iodococaine labeled with ^{123}I may be a useful

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SPECT radiopharmaceutical for imaging at times >1 h if further studies confirm that binding in the striatum is predominantly to dopamine reuptake sites. A complete pharmacological characterization of the binding of *p*-iodococaine in vivo as well as in vitro would be necessary to fully assess its potential for imaging studies. In contrast to iodination, we have shown in parallel studies that substitution at the para position with fluorine does not significantly alter the regional or temporal characteristics of [*N*-^{11}C]cocaine in the baboon brain.[14]

Experimental Section

Materials and Methods. Commercial reagents were utilized without further purification unless otherwise noted. *o*-Iodobenzoyl chloride and *p*-iodobenzoyl chloride were purchased from Aldrich, and plasma proteins from Sigma. Ecgonine methyl ester was obtained from the National Institutes on Drugs Abuse. [*benzoyl*-3,4-^{3}H(N)]Cocaine (specific activity 25 Ci/mmol) was purchased from Dupont. Anhydrous solvents were distilled before use: tetrahydrofuran was distilled from benzophenone ketyl. Reactions carried out "under nitrogen" indicate the utilization of a positive pressure of N_2 successively bubbled through concentrated sulfuric acid and dried through sodium hydroxide and Drierite. ^{1}H-NMR spectra were obtained on a Brucker 300-MHz instrument in $CDCl_3$ using TMS as internal reference. Mass spectra were obtained on a Finnegan-Mat GC-MS 5100 mass spectrometer (LRMS) or a Kratos MS50TC spectrometer (LRMS and HRMS). HPLC analysis was performed on a Phenomenex column (Ultremex, 5 Si, 250 × 4.6 mm), eluting with a mixture of CH_3CN/0.004 M $(NH_4)_2HPO_4$ (4:1, v/v) at flow rate of 2 mL/min. TLC analysis was conducted on Bakerflex IB2-F plates utilizing UV and iodoplatinate reagent visualization.[23] Column chromatography was performed on Merck silica gel 60 (240–400 mesh).

***m*-Iodobenzoyl Chloride.** A suspension of *m*-iodobenzoic acid (1.25 g, 5.0 mmol) in 15 mL of thionyl chloride was heated at reflux with exclusion of moisture for 25 h. Excess thionyl chloride was removed by distillation. Benzene (10 mL) was added to the residue and this was distilled to remove the last traces of thionyl chloride. The residue was dried under vacuum for 16 h to afford the crude *m*-iodobenzoyl chloride, which was used immediately for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 8.44 (t, 1 H), 8.09 (dt, 1 H), 8.01 (dt, 1 H), 8.27 (t, 1 H).

General Procedure for Iodococaines 2a–c. The HCl salt of ecgonine methyl ester (EME) (0.118 g, 0.5 mmol) was dissolved in 1 mL of ammonium hydroxide (58%) saturated with NaCl. This aqueous solution was extracted with ether (5 mL × 3). The combined ether layers were then dried (anhydrous potassium carbonate) and evaporated under reduced pressure to yield EME as a gummy oil. The EME was then dissolved in dry tetrahydrofuran (THF) (2 mL) and added to a suspension of NaH (80% dispersion in mineral oil, 18 mg, 0.6 mmol) in dry THF (2 mL). The reaction mixture was stirred under nitrogen for 10 min. Iodobenzoyl chloride (0.134 g, 0.51 mmol) in 2 mL of dry THF was added via a syringe. The reaction mixture was heated at reflux for 10–40 h. The course of the reaction was monitored by TLC in $CH_3CN/H_2O/NH_4OH$ 90:10:1 (iococaine $R_f \approx 0.8$). The solvent was removed under reduced pressure, and the reaction mixture was quenched with water (2 mL) and extracted with ether (3 mL × 3). The combined organic layers were dried (anhydrous potassium carbonate) and evaporated. The residue was chromatographed on silica gel with ethyl acetate/2-propanol (9:1, v/v). The iodococaine was collected in the second fraction. Removal of the solvent under reduced pressure gave the product as a gummy oil.

3-[(2-Iodobenzoyl)oxy]-8-methyl-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]octane-2-carboxylic Acid, Methyl Ester (2a, *o*-Iodococaine). Yield: 101 mg (47%). ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 1 H), 7.88 (d, 1 H), 7.40 (t, 1 H), 7.14 (t, 1 H), 5.23 (m, 1 H), 3.72 (s, 3 H), 3.58 (br, 1 H), 3.30 (br, 1 H), 3.09 (m, 1 H), 2.44 (m, 1 H), 2.23 (s, 3 H), 2.23–2.06 (m, 2 H), 1.94–1.87 (m, 1 H), 1.78–1.68 (m, 2 H). Mass spectrum, *m/z* (relative intensity): 430 (M^+, 0.79), 429 (3.40), 182 (12.82), 96 (17.01), 94 (22.20), 82 (100.00). HRMS calcd for $C_{17}H_{20}INO_4$ 429.0439, found 429.0429.

3-[(3-Iodobenzoyl)oxy]-8-methyl-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]octane-2-carboxylic Acid, Methyl Ester (2b, *m*-Iodococaine). Yield: 123 mg (58%). ^{1}H NMR ($CDCl_3$): δ 8.33 (s, 1 H), 7.98 (d, 1 H), 7.86 (d, 1 H), 7.16 (t, 1 H), 5.20 (m, 1 H), 3.73 (s, 3 H), 3.57 (m, 1 H), 3.29 (m, 1 H), 3.01 (m, 1 H), 2.42 (dt, 1 H), 2.23 (s, 3 H), 2.20–2.00 (m, 2 H), 1.99–1.83 (m, 1 H), 1.75–1.70 (m, 2 H). Mass spectrum, *m/z* (relative intensity): 431 (M + 1, 0.97), 430 (M^+, 2.31), 429 (13.04), 182 (6.32), 96 (15.57), 94 (22.95), 82 (100.00). HRMS calcd for $C_{17}H_{20}INO_4$ 429.0439, found 429.0442.

3-[(4-Iodobenzoyl)oxy]-8-methyl-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]octane-2-carboxylic Acid, Methyl Ester (2c, *p*-Iodococaine). Yield: 90 mg (43%). ^{1}H NMR ($CDCl_3$): δ 7.80–7.71 (q, 4 H), 5.26–5.18 (m, 1 H), 3.71 (s, 3 H), 3.57 (m, 1 H), 3.30 (m, 1 H), 3.01 (m, 1 H), 2.42 (dt, 1 H), 2.26 (s, 3 H), 2.16 (m, 2 H), 1.89–1.84 (m, 1 H), 1.71 (m, 2 H). Mass spectrum, *m/z* (relative intensity): 430 (M^+, 2.47), 429 (7.59), 231 (22.20), 198 (16.32), 182 (100.00), 96 (19.54), 94 (24.57), 82 (80.65). HRMS calcd for $C_{17}H_{20}INO_4$ 429.0439, found 429.0437.

General Procedure of Iodonorcocaines 3a–c. To a solution of the (*o*-, *m*-, *p*-)iodococaine (50 mg, 0.11 mmol) and 1,8-bis(dimethylamino)naphthalene (Proton Sponge, 11.5 mg, 0.06 mmol, 0.55 equiv) in ethylene dichloride (2.5 mL) was added α-chloroethyl chloroformate[15] (92 mL, 122 mg, 0.85 mmol, 7.8 equiv) by syringe. This mixture was warmed to reflux for 1.5 h. After cooling to room temperature, 95 mL of 1 M anhydrous hydrogen chloride in diethyl ether solution was added, and the mixture was passed through a silica gel plug and chased with methylene chloride (2 mL × 3). The combined eluents were evaporated under a stream of nitrogen to afford the (*o*-, *m*-, *p*-)iodococaine *N*-(α-chloroethylformamide), which was analyzed by TLC (R_f = 0.4, silica gel, ether/hexanes 2:1). Methanol (2.5 mL) was added to the carbamate intermediate and the reaction solution was heated at reflux for 1 h. After evaporation of the solvent under reduced pressure, a mixture of 2.5 mL of H_2O and 2.5 mL of 1 M KOH aqueous solution was added to the residue. The mixture was extracted with ether (5 mL × 3). The combined ether layers were dried (anhydrous K_2CO_3) and evaporated under reduced pressure.

3-[(2-Iodobenzoyl)oxy]-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]octane-2-carboxylic Acid, Methyl Ester (3a, *o*-Iodonorcocaine). Yield: 35 mg (76.5%). ^{1}H NMR ($CDCl_3$): δ 8.00 (dd, 1 H), 7.72 (dd, 1 H), 7.39 (dt, 1 H), 7.15 (dt, 1 H), 5.44 (m, 1 H), 3.72 (m, 2 H), 3.67 (s, 3 H), 3.11 (dd, 1 H), 2.71 (br, 1 H), 2.19–1.94 (m, 4 H), 173 (m, 2 H). Mass spectrum, *m/z* (relative intensity): 430 (M^+, 0.79), 429 (3.40), 182 (12.82), 96 (17.01), 94 (22.20), 82 (100.00).

3-[(3-Iodobenzoyl)oxy]-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]octane-2-carboxylic Acid, Methyl Ester (3b, *m*-Iodonorcocaine). Yield: 28 mg (61.2%). ^{1}H NMR ($CDCl_3$): δ 8.26 (t, 1 H), 7.94–7.86 (m, 2 H), 7.18 (t, 1 H), 5.42 (m, 1 H), 3.74–3.70 (m, 2 H), 3.68 (s, 3 H), 3.03 (dd, 1 H), 2.09–1.92 (m, 4 H), 1.74–1.65 (m, 3 H). Mass spectrum, *m/z* (relative intensity): 431 (M + 1, 0.97), 430 (M^+, 2.31), 429 (13.04), 182 (6.32), 96 (15.57), 94 (22.95), 82 (100.00).

3-[(4-Iodobenzoyl)oxy]-[1*R*-(*exo*,*exo*)]-8-azabicyclo[3.2.1]octane-2-carboxylic Acid, Methyl Ester (3c, *p*-Iodonorcocaine). Yield: 32 mg (69%). ^{1}H NMR ($CDCl_3$): δ 7.81–7.63 (q, 4 H), 5.42 (m, 1 H), 3.72 (t, 2 H), 3.63 (s, 3 H), 3.05 (dd, H), 2.15–1.90 (m, 5 H), 1.75–1.68 (m, 2 H). Mass spectrum, *m/z* (relative intensity): 430 (M^+, 2.47), 429 (7.59), 231 (22.20), 198 (16.32), 182 (100.00), 96 (19.54), 94 (24.57), 82 (80.65).

Synthesis of [*N*-^{11}C-*methyl*]-(−)-Iodococaines 4a–c. [^{11}C]CH_3I[16] was reacted with (o-, m-, p-)noriodococaine (3a–c) (2 mg) dissolved in 300 mL of acetonitrile and 200 mL of dimethyl sulfoxide dimethylformamide (1:4) at 135 °C. The labeled iodococaine was purified by semipreparative HPLC (silica gel, Ultrasphere-Si, 10 × 250 mm) with acetonitrile/0.004 M $(NH_4)_2HPO_4$ (70:30; v/v) at 6 mL/min. The retention time of noriodococaine in this system was 10–11 min and the retention time of iodococaine was 15–16 min. The solvent was evaporated from the fraction containing the labeled iodococaine and the residue was dissolved in 3 mL of isotonic silane (USP) for injection. The total synthesis time was 35 min. The radiochemical purity was determined to be >98% by TLC (acetonitrile/water/am-

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monium hydroxide (90:10:1); $R_f = 0.8$) and HPLC, and the specific activity determined by HPLC (by comparison with a standard curve) was typically 250 mCi/μmol at time of injection into the baboon.

Positron Emission Tomography Scanning Procedures. Adult female baboons (n = 7, 14.2–18.5 kg) were initially anesthetized with an intramuscular injection of ketamine hydrochloride (10 mg/kg), intubated, and transported to the PET facility. Animals were maintained on gas anesthesia (oxygen, nitrous oxide, and isofluorane) throughout the study. Vital signs including heart rate, blood pressure, and respiratory rate were monitored and recorded for all studies. Catheters were placed in an antecubital vein for isotope injection and a femoral artery for blood sampling. Baboons were positioned in the tomograph using a horizontal and vertical laser alignment system. Each animal had its own individual head holder.[24] In addition, an infrared motion-monitoring device (Tri-Tronics, Smarteye Model SD) attached directly to the head holder and aimed at the tip of the baboons' nose was used in order to alert the investigators of any head movement (>1.0 mm). Scanning was performed for 58 min in a Computer Technology Imaging (CTI) positron tomograph (Model 931-08/12; 15 slice, 6.5-mm slice thickness, full width at half-maximum (FWHM)) with an in plane resolution of 6.0 × 6.0 mm FWHM.

Each iodococaine isomer was scanned twice in different baboons, and in at least one of the scanning sessions with each isomer, a study was also performed with [N-^{11}C]cocaine. In the remaining sessions two iodococaine isomers were studied. From 4.7 to 8.4 mCi of ^{11}C compound was injected per scan. Continual arterial blood sampling was performed by an automated device (Ole Dich) for the first 2 min in order to accurately sample the plasma radioactivity peak, with manual sampling being used for the remainder of the study as described previously.[10,25] Plasma was counted for total radioactivity, and 1-, 5-, 10-, and 30-min samples were analyzed for the proportions of unchanged tracer and labeled metabolites. Metabolite-corrected time–radioactivity integrals were calculated using two-component exponential fits to the metabolite data and a cubic spline integration method.

Assay of Labeled Iodococaine in Plasma. Plasma (0.2 mL) was added to 1 mL of acetonitrile and the mixture was sonicated for 20 s and centrifuged. The supernatant which contained >90% of the radioactivity was analyzed by HPLC after addition of unlabeled iodococaine. A 4.6 × 250 mm Ultrasphere-Si column was used with acetonitrile/0.004 M $(NH_4)_2HPO_4$ (70/30, v/v) at 2 mL/min using UV and radioactivity assay of fractions. The fraction of labeled iodococaine in each sample was taken as the amount of radioactivity in the iodococaine UV peak relative to the total. Similar fractions were calculated for EME, which eluted at about twice the retention time of the iodococaine, and for ^{11}C eluting near the void volume (including iodobenzoyl ecgonine, ecgonine, and [^{11}C]CO_2). Plasma samples were also assayed separately for [^{11}C]CO_2 as described previously.[26]

Plasma Protein Binding.[27] ^{11}C-labeled compounds were added to 0.18 mL of baboon plasma plus 0.02 mL of 100 mM sodium phosphate buffer, pH 7.4, containing 1 μg of physostigmine to inhibit esterases. Each mixture was incubated for 15 min at room temperature with vortex mixing every 5 min and transferred to the upper chamber of a Centrifree filter (Amicon Div., W.R. Grace & Co.) which was then centrifuged for 5 min to collect 50–100 μL of ultrafiltrate. Free fractions were calculated as the ratio of ^{11}C concentration in the ultrafiltrate to that in incubation mixture.

Binding to Rat Striatal Membranes.[28] Striata were rapidly dissected and homogenized (Polytron setting 6; 2 × 10 s) in 20 volumes of ice-cold 320 mM sucrose containing 10 mM NaH_2PO_4, pH 7.45. The homogenate was centrifuged for 3 min, 1000g, and the supernatant recentrifuged for 30 min at 30000g. The pellet was resuspended in isolation buffer (1.5 mL per rat). Incubations were done at room temperature with 2 nM [^{3}H]cocaine, ca. 0.25 mg of protein, and a total volume of 0.25 mL. Inhibitors were added over the range 6 nM to 20 μM. After 20 min the incubation mixtures were rapidly passed through glass-fiber filters (Sigma F-4144 presoaked for 20–60 min in 0.05% polyethylenimine) held in a porcelain filter cup. Filters were quickly washed with 5 mL of ice-cold buffer, transferred to a scintillation vial, and counted in a Packard liquid scintillation counter after at least 8 h at about 50% efficiency. Nonspecific binding was defined by 20 μM cocaine. Results were expressed as percent control binding and IC_{50} values estimated graphically.

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Registry No. **1a**, 609-67-6; **1b**, 1711-10-0; **1b** acid, 618-51-9; **1c**, 1711-02-0; **2a**, 130022-30-9; **2b**, 141120-38-9; **2c**, 141120-39-0; **3a**, 141120-40-3; **3b**, 141170-82-3; **3c**, 141120-41-4; **4a**, 141120-42-5; **4b**, 141120-43-6; **4c**, 141120-44-7; EME·HCl, 38969-40-3.

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