SYNTHESIS AND BINDING TO STRIATAL MEMBRANES OF NO CARRIER ADDED I-123 LABELED 4'-IODOCOCAINE.

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SUMMARY

An ^{123}I labeled cocaine analog, 4'-[^{123}I]iodococaine, has been prepared by oxidative destannylation of the tributyltin analog and shown to interact with cocaine binding sites in rat brain striatal membranes. It may thus be a suitable SPECT radiotracer for studies of the dopamine reuptake site in neurodegenerative diseases.

Key Words: Cocaine, dopamine, radioiodination, iododestannylation, 4'-[^{123}I]iodococaine

INTRODUCTION

There is evidence that the powerful reinforcing properties of cocaine administration stem from its inhibition of pre-synaptic neurotransmitter re-uptake at dopaminergic nerve terminals [1-4]. Cocaine also binds to and inhibits noradrenergic and serotonergic re-uptake sites [5], and has a variety of effects on other processes involved in nervous transmission [6-10]. We have previously demonstrated in PET experiments that the tissue concentration of [N-$^{11}CH_3$]cocaine in the human striatum following intravenous administration exhibits a similar time-course to that of the euphoria reported by abusers of the drug [11]. Furthermore, peak radioactivity from [^{11}C]cocaine in baboon striatum was reduced by the dopamine re-uptake blocker nomifensine, whereas the norepinephrine re-uptake blocker desipramine was without effect in human striatum [11]. In subsequent work we prepared the three isomeric N-$^{11}CH_3$ labeled ring iodinated cocaine derivatives [12, 13]. Their behaviors in baboon brain differed substantially from each other, and

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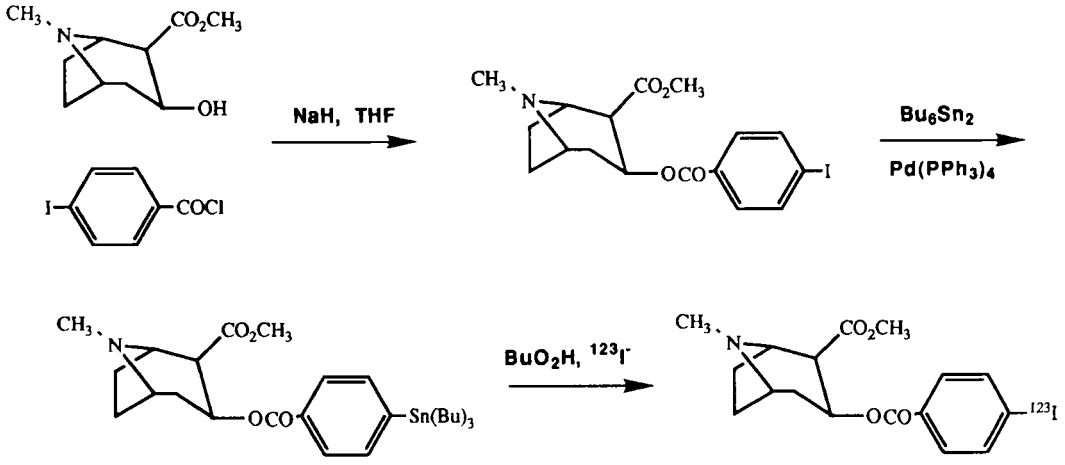
unlike [^{11}C]cocaine itself none of the iodinated compounds preferentially localized in striatum at early times. Brain uptake of 2'-[^{11}C]iodococaine was slight, probably because tight binding to plasma proteins limited extraction from the blood [14]. Widespread uptake of 3'-[^{11}C]iodococaine was seen in the brain, and time-courses of radioactivity in striatum and cerebellum were quite similar to those of [^{11}C]cocaine. However, striatal ^{11}C was less for 3'-iodococaine than for cocaine at all times up to 75 minutes. In contrast, although the initial striatal uptake of 4'-[^{11}C]iodococaine was less than that of cocaine, clearance was slower, and by 75 minutes the striatal radioactivity was higher than that in cerebellum or frontal cortex [13]. 4'-Iodococaine was almost as effective as cocaine in displacing ^{3}H-cocaine from rat striatal membranes [13], indicating a similar affinity of 4'-iodococaine for the dopamine re-uptake site. The 2'-iodo- and 3'-iodo-isomers were about 4 times less effective than cocaine [13]. These studies suggested that 4'-iodococaine labeled with ^{123}I might provide SPECT images of dopaminergic innervation at later times after administration, and thus have potential in the clinical setting for monitoring its deterioration in early stages of diseases such as Parkinsonism. A series of carrier added radioiodinated cocaine analogs was prepared by Basmadjian et al [15], but specific radioactivities were too low for in vivo imaging of binding sites. A subsequent abstract [16] describes the synthesis of high specific activity 2'-[^{123}I]iodococaine, but the limited brain uptake of 2'-[^{11}C]iodococaine [13, 14] suggests that this ^{123}I compound will not be useful. However, the structurally related compound 2β-carbomethoxy-3β-(4-iodophenyl)tropane (RTI-55 [17] or β-CIT [18]) has been labeled with ^{123}I and found to bind strongly to dopamine reuptake sites. We prepared no carrier added 4'-[^{123}I]iodococaine to directly examine its ability to interact with cocaine binding sites in brain striatal membranes.

RESULTS

Non-radioactive 4'-iodococaine was prepared by reaction of 4-iodobenzoyl chloride and ecgonine methyl ester (Scheme I) [13]. Hexabutylditin with Pd(0) catalysis [19, 20] subsequently provided 4'-tributylstannylcocaine, which was used as the starting material for 4'-[^{123}I]iodococaine via oxidative iododestannylation [21]. Initial experiments gave high yields (>50%) but disappointing specific radioactivities (<0.1 Ci/μmol) due to traces of stable iodide in the tributyltin compound. When these were removed by washing with $AgNO_3$ solution the radiochemical yield was lower (28%) but specific radioactivity was improved (1.95 Ci/μmol).

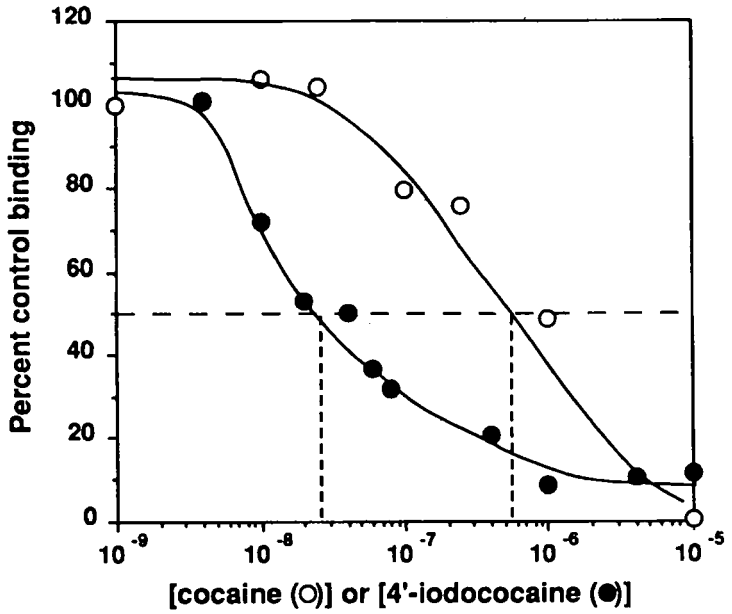
Preparation of 4'-[123I]iodococaine.

Scheme I. Preparation of ^{123}I labeled 4'-iodococaine.



Binding of 4'-[^{123}I]iodococaine to rat brain striatal membranes was seen. A 10-100 fold higher concentration of cocaine than 4'-iodococaine was required to provide equivalent competition with the ^{123}I compound (Figure 1). Binding of 4'-[^{123}I]iodococaine was also reduced by 10μM nomifensine to the same extent as by 10μM cocaine (not shown).

Figure 1 Competition of cocaine and 4'iodococaine with I-123 iodococaine.



DISCUSSION

We have prepared 4'-[^{123}I]iodococaine in purity and specific radioactivity suitable for radiopharmaceutical use. Although the radiochemical yield was moderate, experience with other iododestannylation reactions suggests that further optimization would be possible.

Our preliminary brain membrane experiments (Figure 1) indicate that 4'-[^{123}I]iodococaine binds to striatal sites in a cocaine and nomifensine-sensitive manner, and more strongly than cocaine. Similar binding studies with [^{3}H]cocaine and non-radioactive 4'-iodococaine, however, indicated slightly lower affinity for 4'-iodococaine than cocaine. The discrepancy suggests differences in binding sites or in binding kinetics. The previous PET 4'-[^{11}C]iodococaine study indicates that 4'-iodococaine also binds to non-dopaminergic sites [13]. Nevertheless it can be concluded that 4'-iodococaine interacts with the dopamine reuptake site, and may be viable as a SPECT radiotracer. 4'-Iodococaine differs from RTI-55 (β-CIT) [17, 18] in that the aromatic ring is linked to the tropane moiety via an ester linkage, rather than a direct C—C bond. It is thus expected to be a substrate for hydrolytic enzymes such as butyrylcholinesterase [7]. Indeed at 30 minutes after injection only 25% of the ^{11}C in blood plasma of baboons given 4'-[^{11}C]iodococaine was unmetabolized [13]. While the susceptibility of a radiotracer to metabolism may be expected to decrease target tissue uptake, it may also increase clearance of radioactivity in blood and non-target tissues and therefore allow imaging at earlier times and/or favorably modify radiation dosimetry. It may thus be of value to examine both 4'-[^{123}I]iodococaine and and its analog RTI-55 as candidate SPECT radiopharmaceuticals targeted to the dopamine reuptake site.

EXPERIMENTAL

General. Ecgonine methyl ester HCl, and 4-iodobenzoyl chloride were obtained from the National Institute on Drug Abuse and the Aldrich Chemical Co., respectively. ^{123}I was obtained from Nordion. Other reagents and solvents were purchased commercially. A Brucker 300 MHz instrument was used for ^{1}H-NMR spectra, and a Finnegan-Mat 5100 for GC-MS. HPLC was performed using 4.6 x 250 mm Phenomenex columns containing 5μm Si or C-18 particles. Mobile phases were CH_3CN/0.004 M $(NH_4)_2HPO_4$ at 70:30 and 90/10, v/v, respectively. TLC was conducted with Merck PE-backed silica gel plates pre-washed with 0.1M KOH in methanol, developed in $CH_3CN/H_2O/NH_4OH$ (90:10:1, v/v) and visualized with UV. Radioactive spots were located using a Berthold scanner. Column chromatography was performed with Merck silica gel 60 (200-400 mesh). The mass of no carrier added 4'iodococaine was measured by comparing

the area under the HPLC UV peak with that of standards. Specific radioactivity was then calculated by dividing the collected radioactivity by the mass.

4'-Iodococaine. A solution of 0.23 g (1 mmol) of ecgonine methyl ester HCl in 2 mL NaCl-sat. NH_4OH was rapidly extracted with 3 x 10 mL ether. After drying with anh. K_2CO_3 and removal of solvent the gummy residue was dissolved in 8 mL dry THF together with a mineral oil suspension of 36 mg (1.2 mmol) of NaH. 4-Iodobenzoyl chloride (0.27 mg, 1 mmol) was added in 4 mL dry THF. and the mixture heated at reflux for 10 hr. Solvents were removed under reduced pressure and 4 mL H_2O added. The mixture was extracted with 3 x 5 mL ether which was then dried (anh. K_2CO_3) and evaporated. Column chromatography with ethyl acetate/isopropanol (9:1, v/v) yielded 180 mg (45%) of a colorless oil which was used without further purification.

NMR: δ 7.75 (q, 4H), 5.2 (m, 1H), 3.71 (s, 3H), 2.26 (s, 3H), 2.15 (m, 2H) 1.7 (m, 2H).

MS: m/z (rel. intensity) 430 (2), 429 (9.7), 398 (2.8), 231 (2.3), 182 (10), 94 (3.8), 82 (7.6).

4'Tributylstannylcocaine. Iodococaine (429mg, 1mmol), hexabutylditin (1734 mg, 3mmol) and $Pd(PPh_3)_4$ (15 mg, 0.013 mmol) were dissolved in 20 mL toluene and stirred at 60° for 72 hr under N_2. The precipitated black Pd metal was filtered and the filtrate was evaporated under reduced pressure. Excess hexabutylditin was removed by column chromatography with n-hexane. p-Tributylstannylcocaine was eluted with ethyl acetate/isopropanol (0:1, v/v) to yield 350 mg (60%) as an oil which was stored at -15°.

NMR: δ 7.86 (d, 2H), 7.46 (d, 2H), 5.18 (t, 1H), 3.65 (s, 3H), 3.52 (m, 1H), 3.27 (m, 1H), 2.95 (m, 1H), 2.19 (s, 3H), 2.05 (m, 2H), 1.66 (d, 2H), 1.40 (m, 6H), 1.25 (m, 7H), 1.0 (m, 6H), 0.75 (t, 9H).

MS: m/z (rel. intensity) 536 (8.9), 355 (4.3), 331 (3.6), 315 (3.2), 281 (8.2), 241 (2.7), 207 (10), 182 (9.3), 82 (7.6).

Removal of trace iodide from tributyltin compound. 4'-Tributylstannylcocaine (3-mg) was dissolved in 5 mL of ether and shaken (vortex mixer) with 1 mL 0.1M $AgNO_3$. The ether layer was carefully removed from the turbid aqueous phase, washed with H_2O and dried over anh. Na_2SO_4. The ether was evaporated in a N_2 stream and the residue dissolved in 50 µL $CHCl_3$.

Radiolabeling. Stock solutions in $CHCl_3$ were prepared of CH_3CO_2H (1M), $tBuO_2H$ (0.88M) and 4'-tributylstannylcocaine (0.1M). To the shipping vial containing 1 mCi $^{123}I^-$ were added in turn 8 μL, 8 μL and 20 μL of these solutions, respectively. The vial was then heated at 60° (sand-bath) for 30 min. After cooling, CH_3CN (250 μL) was added and the solution injected directly onto a silica HPLC column. The radioactive peak at 35 min. was collected and the solvents evaporated at 60° in a N_2 stream. The residue was dissolved in 0.9% NaCl and stored at 0-4°. Radiochemical yield 28%. Specific activity 1.95 Ci/μmol. TLC showed a single radioactive spot at Rf = 0.8, coincident with UV absorption of a 4'iodococaine standard. Reverse-phase HPLC showed a single radioactive peak at 36 minutes.

Rat brain striatal membrane binding [22]. Striata were homogenized at 0° in 2.5 mL of 320 mM sucrose/10 mM Na_2HPO_4, pH 7.45 with 2 x 10 sec. bursts using a Polytron at setting 6. Cell debris was removed by centrifuging at 1,000 x g for 10 minutes, and membranes were harvested at 30,000 x g for 30 minutes. The pellet was resuspended in homogenization buffer at 1 mg tissue weight/mL.

Incubations were done in triplicate with 2 nM 4'-[^{123}I]iodococaine and varying concentrations of cocaine or 4'-iodococaine. Approximately 0.25 mg tissue wet weight was incubated per tube in a total volume of 0.25 mL for 30 min at room temperature, before rapidly filtering through Whatman glass fiber filters pre-soaked in 0.05% polyethyleneimine. The filters were rapidly washed with 2x5 mL ice-cold buffer, transferred to a scintillation mini-vial and counted after more than 5 hours with Ultima Gold scintillation fluid in a Packard Tri-carb 1600 TR counter at about 90% efficiency. Nonspecific binding was that observed with 30 μM 4'-[^{123}I]iodococaine, and this amount was subtracted from other values before graphical display.

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