Structure Biodistribution Relationship of Radioiodinated Tropeines: Search for a Molecular Probe for the Characterization of the Cocaine Receptor.

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SUMMARY

Three iodinated analogs of cocaine were synthesized and radiolabeled by isotope exchange in the presence of Cu^{++} using a "kit" procedure. 2'-Iodococaine, 2'-iodotropacocaine and 2'-iodobenzoyl tropine radiolabeled with ^{125}I were injected in mice and their biodistribution studied. Activity with all three radiolabeled compounds peaked in the brain and heart between 5 and 30 min. post injection. Activity from ^{125}I-2'-iodococaine (S.A.=468 mCi/mmol), peaked in the brain at 15 min (2.38% injected dose/g) and showed a plateau in the heart between 5 and 60 min post injection (1.67-2.16%). ^{125}I-2'-iodotropacocaine (S.A.=407 mCi/mmol) at 5 min had the highest uptake in the brain (8.16%) and the heart (4.44%) while activity from ^{125}I-2'-iodobenzoyl tropine (S.A.=407 mCi/mmol) peaked in the brain at 15 min (4.19%) and at 5 min in the heart (3.33%). The biodistribution of ^{125}I-2'-iodococaine paralleled literature values obtained with ^{3}H-/^{14}C-cocaine. We conclude that radioiodinated tropeines radiolabeled with ^{123}I may be developed into useful probes to examine and characterize the cocaine receptor in-vivo by SPECT imaging.

Keywords: Radioiodinated Tropeines, Cocaine, Tropacocaine, Brain uptake, Heart uptake.

INTRODUCTION

Cocaine radiolabelled with ^{3}H and ^{14}C has long been used by researchers to study the metabolic disposition and pharmacokinetics of cocaine in animals and humans (1-5). In the early 1980's ^{3}H-cocaine was introduced as a tool to further evaluate the molecular mechanism of neuronal dopaminergic transport in the brain (6-7). Schoemaker et al concluded in 1985 that ^{3}H-cocaine may be a useful ligand to examine the dopamine transporter in the rat striatum and the human putamen (8).

More recently, Galloway (9) reviewed new biochemical findings on the site of action of cocaine and its effects on dopamine systems, while Calligaro et al (10) suggested that the DA transporter in the striatum is the putative "cocaine receptor". Ritz et al (11) in their work,

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implied that cocaine receptors, specifically those on dopamine terminals, are related to cocaine self-administration.

Recently Fowler et al (12,13) synthesized [N-^{11}C-methyl]cocaine, and studied its regional distribution and kinetics in human and baboon brain using Positron Emission Tomography (PET). Fowler's work showed that regional binding of ^{11}C-cocaine in the striatum was reduced by carrier cocaine and nomifensine but not desipramine, suggesting that the cocaine binding occurs predominantly at the dopamine reuptake site.

Som et al (2), using ^{14}C-cocaine and autoradiography showed high uptake of radioactivity at short intervals, 2-4 min, in the brain, heart, adrenals and eyes of rats.

We have initiated a Structure Biodistribution Relationship (SBR) approach to study radioiodine labeled tropeines, esters of tropine, pseudotropine, ecgonine and derivatives, as molecular probes for the characterization of the cocaine receptor in-vitro, and to potentially reveal in-vivo cocaine receptor populations. Such a probe could also be used to unravel the mechanism of cocaine cardiotoxicity.

The radionuclides chosen were ^{125}I ($t_{1/2}$=60 days, 35 keV) for in-vitro studies and ^{123}I ($t_{1/2}$=13.3 hrs, 159 keV) for SPECT, both easily available to researchers and the Nuclear Medicine community.

This paper deals with the synthesis, radiolabeling and preliminary biodistribution studies of three analogs of cocaine radiolabeled with radioiodines (Fig. 1).

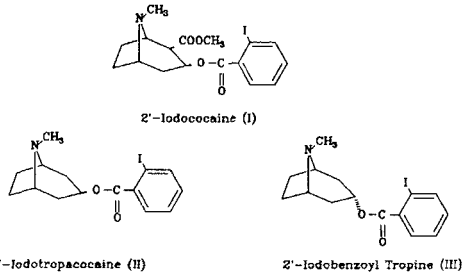

Figure 1. Structures of iodinated tropeines.

EXPERIMENTAL

MATERIALS AND METHODS

All reagents and solvents (Aldrich Chemical Co., Milwaukee, WI) were reagent grade and were used without further purification unless otherwise specified. Cocaine HCl was obtained from Sigma Chemical Corp., St. Louis, MO. Sodium iodide-^{125}I (no-carrier added, 10 mCi/0.1 ml aqueous 0.1N NaOH) was purchased from ICN Chemical & Isotope Division. Sodium iodide-^{131}I (radiopharmaceutical grade, 7.05 mCi/ml) was purchased from Squibb Diagnostics. Sodium

iodide-^{123}I (radiochemical grade, no-carrier added) was purchased from Nordion, Canada. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Elemental analyses were performed at Midwest Microlab Inc., Indianapolis, IN, and were within 0.4% of calculated values. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator (Eastman, cat #13181). The solvent systems were: I: Methanol:ethyl acetate: concentrated ammonium hydroxide (80:3:3 v/v); II: Chloroform: glacial acetic acid (9:1 v/v). Visualization of tropeines on TLC was by UV (254 nm) and exposure to iodine vapors. Radiochromatograms were scanned on a LB2723 Dunnschicht-Scanner II auto scanner (Berthold, Germany) and evaluated with a Shimadzu C-R1B integrator. Radioactivity in tissue samples was assayed with a Packard Multi-Prias-2 gamma counter (United Technologies). Tissues were weighed on a Mettler AC100 Digital Electronic balance.

Synthesis

Ecgonine methyl ester. Ecgonine HCl (2 g.) (prepared from cocaine HCl according to Bell & Archer (14)) was dissolved in 50 ml dry methanol (distilled after drying over molecular sieves) saturated with dry HCl gas (passed through 100 ml concentrated sulfuric acid). The mixture was heated at 75°C in a water bath for 3 hrs. Dry HCl gas was again bubbled through the solution for 5 min. and the flask was capped and left at room temp. for 24 hrs. The solvent was evaporated in vacuo and the white solid was dissolved in hot absolute ethanol. White crystals of ecgonine methyl ester HCl deposited upon cooling. 1.8 g., m.p. 212.5-213°C (lit. 215°C (15)). TLC in System I, showed a single spot at R_f 0.7.

2'-Iodococaine (I). Ecgonine methyl ester HCl (236 mg, 1 mmol) was dissolved in 5 ml water, the solution made basic with solid Na_2CO_3 and extracted with 3x10 ml $CHCl_3$. The $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. To the ecgonine methyl ester oil was added 5 ml of benzene, 400 mg (1.5 mmol) o-iodobenzoyl chloride in 5 ml benzene, and 1 g of anhydrous solid Na_2CO_3. The mixture was stirred and heated under reflux for 18 hrs, cooled to room temperature, and 20 ml water was added to dissolve the Na_2CO_3. The benzene layer was collected and the basic aqueous layer extracted with 2x10 ml benzene. The combined benzene layers were extracted with 4x10 ml 0.5N H_2SO_4. The acid aqueous layers were made basic with 10% $(NH_4)_2CO_3$ and extracted with 4x20 ml diethyl ether. The ether layers were dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to yield a colorless oil (0.27 g, 63%). The hydrochloride salt (precipitated from an ether solution of the base by bubbling dry HCl gas through the solution) was recrystallized from ethanol and dried under vacuum: m.p. 78-79°C (very hygroscopic). The tartrate salt (precipitated from an ether solution of the base by the addition of a 1% methanolic solution of tartaric acid) was recrystallized twice from absolute ethanol: m.p. 154°C. TLC in System I, showed a single spot at R_f 0.85. C,H,O analysis of tartrate salt confirmed structure. Partition coefficient (0.15M Phosphate Buffer pH 7.4/1-Octanol) = 8.4 ± 0.1.

Pseudotropine. Sodium metal pellets (11.5 g, 0.5 mol) were added to 55 ml (0.5 mol) of 1-pentanol in a 250 ml round bottom flask. The mixture was stirred vigorously and heated under reflux to dissolve the sodium. To this was added 14.1 g (0.1 mol) of tropine in 25 ml 1-pentanol. The mixture was heated under reflux for 18 hrs, allowed to cool to room temperature, and 25 ml of water was added dropwise with stirring. Concentrated HCl was added dropwise to neutralize and finally acidify the aqueous layer to pH 2.0. The two layers were shaken in a separatory funnel, and the aqueous layer was collected, made basic with solid KOH and extracted with 4x50 ml of $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to yield a colorless oil which solidified upon trituration with petroleum ether to yield a white solid (9.3 g, 66%): m.p. 110°C (lit.109-110°(14)).

2'-Iodotropacocaine (II). To pseudotropine (2.83 g, 20 mmol) in 25 ml benzene in a 100 ml round bottom flask, was added 6.38 g (24 mmol) of o-iodobenzoyl chloride in 25 ml benzene and 1 g of anhydrous solid Na_2CO_3. The mixture was stirred and heated under reflux for 3 hrs. The workup was the same as (I). The pale yellow oil (5.3 g, 71.4%) was converted to the hydrochloride (as described for I) to yield a white solid which was recrystallized from acetone: m.p. 223°C (with decomp.). TLC in System I, showed a single spot at R_f 0.72. C,H,O analysis of HCl salt confirmed structure. Partition coefficient = 8.7 ± 0.2.

2'-Iodobenzoyl Tropine (III). To tropine (2.83 g, 20 mmol) in 25 ml benzene in a 100 ml round bottom flask, was added 6.38 g (24 mmol) of o-iodobenzoyl chloride in 25 ml benzene and 1 g of anhydrous solid Na_2CO_3. The mixture was stirred and heated under reflux for 2 hrs. The workup was the same as (I). The pale yellow oil (3.7 g, 50%) was converted to the hydrochloride salt (as described for I) to yield a white solid which was recrystallized twice from acetone : m.p. 218-219°C. TLC in System I, showed a single spot at R_f 0.61. C,H,O analysis of HCl salt confirmed structure. Partition coefficent = 8.9 ± 0.1.

General Procedure for Radioiodination of I, II, & III.

A. Preparation of Kits:

To a 10 ml serum vial was added 0.1 ml of a 10 mg/ml of a solution of the hydrochloride salt of I, II or III (1 mg) in distilled water, 1 ml of 0.2M sodium acetate buffer pH 4.0 and 0.2 ml of a 5 mg/ml solution of $CuSO_4.5H_2O$ in distilled water (1 mg). The solution was frozen and lyophilized overnight. The vials were stoppered under vacuum and stored at 4°C.

B. Labeling Method:

Radiolabeling was performed by adding up to 0.5 ml of radioactive NaI (^{125}I, 0.5-2 mCi; ^{131}I, up to 10 mCi; ^{123}I, 5 mCi) to the freeze-dried contents of a vial. The contents of the vial dissolved yielding a pale blue solution. The vial was placed in a boiling water bath for 10 min, cooled to room temperature, and 1-2 ml of a 0.44M Na_2HPO_4 solution was added to precipitate the copper as copper phosphate and raise the pH to 7-8. The precipitate was filtered through

a 0.22 μm filter. Labeling yields were determined by TLC in systems I & II: the radiochromatograms were scanned and the peaks integrated.

Animal Biodistribution Studies

CF-1 male mice (27-32 g) were weighed, placed in a mouse restrainer and injected into a tail vein with 0.1 ml of a solution (as prepared above; diluted with saline to desired specific concentration) of I-125 labeled I (I'), II (II'), and III (III") (10-20 μCi, specific activity of I≈468 mCi/mmol; specific activity of II & III≈407 mCi/mmol). Three mice were sacrificed by cervical dislocation at 2,5,15,30 and 60 min following injection. Duplicate samples (30 mg to whole organ) of the following tissues: liver, lungs, kidneys, spleen, muscle, small intestine, large intestine, bone, thyroid, adrenals, heart, brain, fat and testes, were removed, rinsed with distilled water, blotted dry, weighed and placed in counting tubes. Blood samples taken by cardiac puncture and urine samples taken by bladder puncture were weighed and placed in counting tubes. The tail was also excised and counted. A standard dose was prepared for counting by diluting an equal volume of the dosage to 100 ml with water, and 0.1 ml was counted with the tissues. The injected dose was adjusted by subtracting the activity left in the tail. This was then used to calculate the % injected dose/g for the tissues/body fluids.

Extraction of Radioactivity from Tissues:

Brain and heart tissues were homogenized in 0.9% saline to give a 20% homogenate (w/v). Esterases in the blood were inhibited by the addition of stannous fluoride when blood was collected. 1 mg of I, II, or III was added to 2 ml of the homogenate or the whole blood followed by 2 g solid K_2CO_3. 15 ml of a mixture of $CHCl_3$:Isopropanol (2:1 v/v) was added followed by shaking for 20 min. The mixture was centrifuged at 1000xg for 10 min. The radioactivity in the aqueous layer as well as the organic phase was measured. The organic phase was collected, filtered through anhydrous Na_2SO_4, evaporated to dryness with N_2 and reconstituted with 0.5 ml methanol. The methanol layer was chromatographed on TLC in systems I & II. The aqueous layer was made acidic with conc. HCl and extracted with ether. The ether layer was assayed for radioactivity (check for o-iodobenzoic acid).

RESULTS AND DISCUSSION

The desired non-radioactive compounds were synthesized by esterifying their respective alcohols: ecgonine methyl ester in the case of 2'-iodococaine (I), pseudotropine, for 2'-Iodotropacocaine (II) and tropine for 2'-Iodobenzoyl tropine (III) with the acid chloride of o-iodobenzoic acid.

The kit radiolabeling of I, II and III yielded a single radioiodinated product with no more than 2% free iodide ion. No other radioactive or chemical impurity was detected by TLC.

During trial labeling experiments, the optimum volume of radioiodine solution (radiochemical grade, no-carrier added , NaI-125/NaI-123 or radiopharmaceutical grade I-131), was found to be 0.5 ml. The pH of the medium was thus kept below pH 5. Larger volumes gave lower yields with as high as 20% free iodide ion being left unreacted.

The biodistribution in mice of ^{125}I-2'-iodococaine (I') (Table 1) shows high transient uptake in the brain peaking at 15 min post injection, while other tissues continue to accumulate activity up to 30 min. At 60 min post injection, only the urine and large intestine contained more activity than at 30 min, showing urinary and hepatobiliary clearance. Other organs of interest i.e. heart, spleen, adrenals, testes also show continous accumulation of activity up to 30 min. Brain-to-blood ratios are >1 up to 15 min post injection.

Table 1. Biodistribution of [^{125}I]-2'-Iodococaine* (I') in Male Mice

	Time after Injection				
Tissue	2 Min	5 Min	15 Min	30 Min	60 Min
Blood	0.41 ± 0.12	1.05 ± 0.20	1.43 ± 0.38	2.22 ± 0.41	1.72 ± 0.10
Urine	0.03 ± 0.02	1.32 ± 0.97	1.26 ± 0.77	33.77 ± 3.88	41.60 ± 39.53
Liver	0.72 ± 0.39	3.67 ± 0.87	6.81 ± 2.42	8.95 ± 0.86	5.63 ± 0.88
Lungs	3.06 ± 1.10	4.99 ± 1.73	4.59 ± 1.00	2.69 ± 0.36	1.58 ± 0.45
Kidneys	2.16 ± 0.70	4.91 ± 1.73	6.22 ± 1.06	7.74 ± 1.12	6.60 ± 0.10
Spleen	0.58 ± 0.19	2.68 ± 1.07	3.05 ± 0.63	2.83 ± 0.06	1.66 ± 0.08
Muscle	0.53 ± 0.13	0.95 ± 0.41	1.14 ± 0.26	1.39 ± 0.19	1.20 ± 0.21
Small Intestine	0.73 ± 0.22	3.30 ± 1.21	3.95 ± 0.54	5.44 ± 1.89	2.60 ± 0.95
Large intestine	0.56 ± 0.18	1.84 ± 0.74	3.07 ± 0.53	2.25 ± 0.32	2.31 ± 0.50
Bone	0.29 ± 0.09	1.00 ± 0.35	1.18 ± 0.22	1.38 ± 0.06	1.11 ± 0.19
Adrenals	0.71 ± 0.21	2.48 ± 1.36	2.36 ± 1.18	2.68 ± 0.21	0.54 ± 0.40
Heart	1.14 ± 0.37	1.89 ± 0.63	1.87 ± 0.38	2.16 ± 0.29	1.67 ± 0.25
Brain	0.74 ± 0.38	2.06 ± 1.27	2.38 ± 0.59	1.53 ± 0.28	0.67 ± 0.07
Fat	0.12 ± 0.06	0.50 ± 0.29	0.98 ± 0.39	1.40 ± 0.44	0.68 ± 0.05
Testes	0.25 ± 0.13	0.80 ± 0.17	1.62 ± 0.36	2.48 ± 0.19	1.84 ± 0.14

*10-20μCi was injected into each mouse. Values represent the average % injected dose/g ± 1 standard deviation for three animals per time period.

The biodistribution of ^{125}I-2'-iodotropacocaine (II') and ^{125}I-2'-iodobenzoyl tropine (III') have several similarities but show dissimilarities to I' when certain key tissues are compared. Tables 2 & 3 summarize the biodistribution results of II' & III'. The peaking of the activity in the brain is at 5 min with II' while it is at 15 min with III' (8.16% vs. 4.19% injected dose/g) with both values being higher than the peak uptake with I' (15 min, 2.38%). Heart uptake peaks at 5 min with both (II' 4.44% & III' 3.33%) while with I' it peaks at 30 min (2.16%). One major

difference noted in the biodistribution of the three compounds is the immediate very high and subsequent washout of lung activity in II' & III' compared to I'; also II' & III' show much faster kidney uptake and urinary excretion than I'. III' shows the lowest blood levels over the 60 min study period.

The accumulation and washout of II' and III' in other organs is very much comparable to that of I' i.e. in the spleen, adrenals, testes etc. Figure 2 shows the differences in peaking and washout of activity among the three compounds in the heart and brain.

The compounds chosen for this study are structural analogs of cocaine. I & II have a β-configuration at the ester attachement to the tropane unit while III has a α-configuration.

Table 2. Biodistribution of [^{125}I]-2'-Iodotropacocaine* (II') in Male Mice

	Time after Injection				
Tissue	2 Min	5 Min	15 Min	30 Min	60 Min
Blood	1.92 ± 0.21	3.01 ± 0.09	2.20 ± 0.81	2.39 ± 0.24	0.90 ± 0.09
Urine	0.06 ± 0.02	3.59 ± 3.75	12.57 ± 4.10	23.48 ± 2.31	65.32 ± 11.45
Liver	2.20 ± 0.64	5.12 ± 0.96	6.32 ± 1.67	6.10 ± 1.00	3.13 ± 0.90
Lungs	32.30 ± 11.52	24.75 ± 4.96	12.19 ± 4.07	6.59 ± 1.40	1.98 ± 0.52
Kidneys	12.01 ± 3.63	12.45 ± 4.62	10.41 ± 3.29	9.12 ± 0.98	2.91 ± 0.63
Spleen	3.45 ± 1.23	5.49 ± 3.73	4.59 ± 1.48	4.67 ± 0.75	1.21 ± 0.34
Muscle	2.47 ± 0.57	2.54 ± 0.32	1.31 ± 0.38	1.15 ± 0.25	0.32 ± 0.03
Small Intestine	4.17 ± 1.59	5.61 ± 1.86	3.50 ± 0.76	4.05 ± 0.60	1.77 ± 0.66
Large intestine	2.09 ± 0.48	3.36 ± 0.70	2.25 ± 0.85	1.97 ± 1.09	1.34 ± 0.27
Bone	2.22 ± 0.17	3.30 ± 0.04	2.02 ± 0.04	1.18 ± 0.00	0.63 ± 0.16
Adrenals	2.38 ± 1.40	6.67 ± 4.74	6.68 ± 2.72	3.66 ± 1.00	1.29 ± 0.60
Thyroid	1.98 ± 0.72	2.25 ± 0.01	1.00 ± 0.00	1.49 ± 0.01	0.51 ± 0.02
Heart	4.40 ± 1.18	4.44 ± 0.45	2.22 ± 0.66	1.97 ± 1.09	0.46 ± 0.06
Brain	5.92 ± 1.01	8.16 ± 1.05	4.82 ± 1.13	3.94 ± 0.23	1.16 ± 0.25
Fat	0.82 ± 0.44	0.82 ± 0.14	1.09 ± 0.47	0.97 ± 0.30	0.37 ± 0.09
Testes	1.22 ± 0.44	1.90 ± 1.04	2.10 ± 0.52	2.35 ± 0.30	2.42 ± 0.57

*10-20μCi was injected into each mouse. Values represent the average % injected dose/g ± 1 standard deviation for three animals per time period.

II and III are isomers and lack the -$COOCH_3$ at C-2 of the tropane unit present in I and cocaine (Fig. 1).

The introduction of the iodine moiety in the molecule was decided from literature metabolic studies of cocaine, to be best on the benzoic acid portion of the molecule. The o-iodo position was chosen over the meta-or para- for the following reasons: 1) The stability to in-vitro and in-vivo deiodination of the o-iodobenzoyl moiety is known to be

Table 3. Biodistribution of [^{125}I]-2'-Iodobenzoyl Tropine* (III') in Male Mice

Tissue	Time after Injection				
	2 Min	5 Min	15 Min	30 Min	60 Min
Blood	0.94 ± 0.35	0.67 ± 0.11	0.76 ± 0.09	0.82 ± 0.15	0.55 ± 0.11
Urine	0.06 ± 0.03	0.46 ± 0.12	14.07 ± 2.33	30.66 ± 12.11	70.80 ± 25.42
Liver	1.46 ± 0.79	1.58 ± 0.38	5.88 ± 0.41	8.45 ± 0.94	6.02 ± 0.60
Lungs	19.42 ± 4.70	18.06 ± 2.60	14.39 ± 4.13	11.17 ± 1.17	4.93 ± 0.99
Kidneys	4.96 ± 0.63	6.58 ± 0.61	8.51 ± 0.50	6.97 ± 0.38	4.05 ± 1.93
Spleen	1.31 ± 0.37	2.20 ± 0.21	4.19 ± 0.97	3.94 ± 0.23	1.68 ± 0.44
Muscle	1.06 ± 0.35	1.21 ± 0.21	1.34 ± 0.10	1.01 ± 0.06	0.58 ± 0.19
Small Intestine	1.68 ± 0.52	2.68 ± 0.09	4.95 ± 0.71	10.75 ± 1.55	6.19 ± 2.06
Large intestine	1.13 ± 0.31	2.06 ± 0.57	3.44 ± 0.21	3.60 ± 0.59	5.64 ± 2.91
Bone	0.84 ± 0.27	0.96 ± 0.11	1.63 ± 0.28	1.29 ± 0.15	0.70 ± 0.01
Adrenals	3.32 ± 0.87	6.72 ± 2.25	4.20 ± 0.90	5.43 ± 1.27	2.87 ± 0.36
Heart	3.18 ± 0.75	3.33 ± 0.51	2.06 ± 0.16	1.54 ± 0.12	0.70 ± 0.01
Brain	1.50 ± 0.45	2.62 ± 0.02	4.19 ± 0.16	3.58 ± 0.08	2.04 ± 0.02
Fat	0.15 ± 0.06	0.36 ± 0.09	0.73 ± 0.13	0.69 ± 0.09	0.74 ± 0.09
Testes	0.35 ± 0.13	0.58 ± 0.04	1.68 ± 0.32	2.06 ± 0.29	2.64 ± 0.54

*10-20μCi was injected into each mouse. Values represent the average % injected dose/g ± 1 standard deviation for three animals per time period.

excellent. ^{131}I-/^{123}I-ortho-iodohippuran, clinically used radiopharmaceuticals, have such a stability; 2) It has been shown (16) that the meta- & para-positions on the benzoyl moiety in cocaine are known to be hydroxylated in humans to form polar metabolites, while no ortho- hydroxylation has been shown to occur; 3) Steric crowding of the iodine at the ortho- position on the benzoic acid could help prevent blood and tissue esterases from hydrolyzing the tropeine esters, a known metabolic degradation of cocaine (1,3,5); 4) Using the analogy of o-iodohippuran, the exchange reaction between the radioactive iodide ion and the o-iodo group is relatively fast at pH <7 in the presence of Cu^{++} ions at 100°C (17).

In the biodistribution studies it was observed that radioactivity from II' & III' clears the blood much faster than radioactivity from I' indicating that demethoxylation of iodococaine occurs in the mouse, releasing iodobenzoyl ecgonine, a hydrophilic metabolite that is retained in circulation for longer times. From the tissue extraction experiments, ^{125}I-2'-iodobenzoyl ecgonine was the only metabolite of I' that was detected in the blood (30% at 2 min to 85% at 60 min) and the heart (14% at 2 min to 37% at 60 min), but not in the brain (18). II' & III' do not have a -$COOCH_3$ group and thus no radioactive hydrophilic metabolite (except o-iodobenzoic acid). This metabolite was not detected in the blood, brain or heart of mice treated with II' or III'. In the case of II', the thyroid was sampled and it showed low activity. This confirmed the in-vivo stability of the compounds to deiodination, at least up to the duration of this study.

The specific activities of the I', II' and III' tropeines used in the present biodistribution studies were in the range of about 400 mCi/mmol much higher than the specific activities of ^{3}H-/^{14}C-cocaine (5.8-250 mCi/mmol) used in the metabolism and disposition studies mentioned in the introduction (2-5). This specific activity is about 88 times lower than the specific acitivity of ^{3}H-labeled cocaine (35200 mCi/mmol) used in cocaine receptor identification work (1,7,8,10) and 250 times lower than the ^{11}C-labeled cocaine (100,000 mCi/mmol) used by Fowler et al (13) in PET scanning.

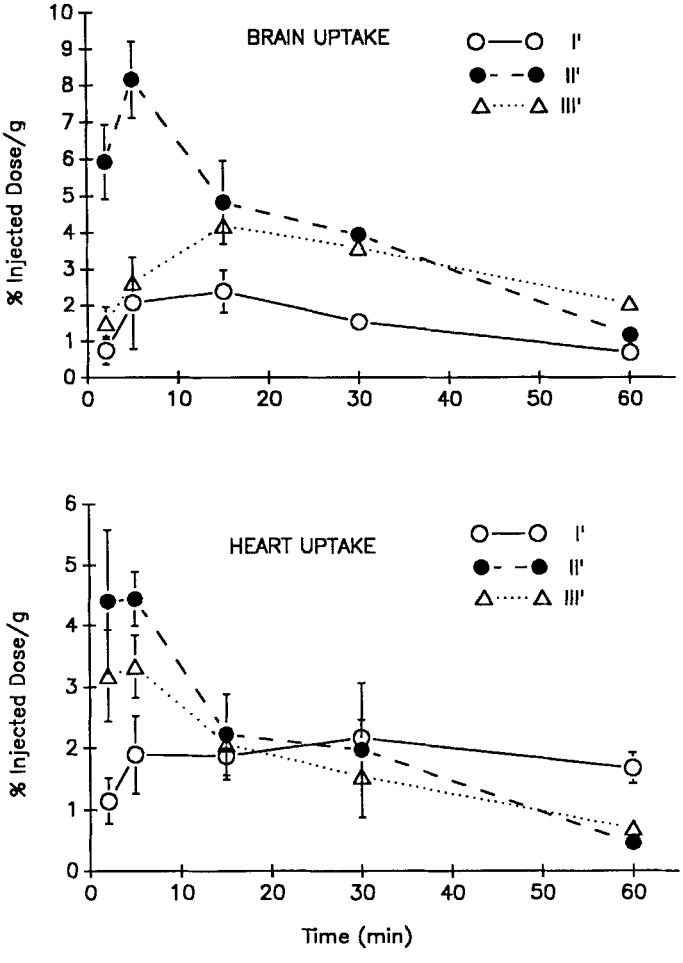

Figure 2. Uptake of the radioiodinated tropeines in the brain and heart of mice.

CONCLUSIONS

The biodistribution of all three cocaine analogs support the contention that radioiodination of tropeines in the 2'-position of the benzoyl group, yield compounds that behave

in-vivo like ^{3}H-/^{14}C-/^{11}C-cocaine but with slightly different peak and washout times in such target organs as the brain and the heart. We have also recently shown that a ten fold increase in the specific activity of ^{125}I-2'-iodococaine showed an 8.8 fold increase in uptake in the brain at 2 min post injection. In addition the heart of three different strains of mice showed significantly different uptakes of radioactivity when injected with ^{125}I-2'-iodococaine (S.A.=4 Ci/mmol) while the brain activities were identical (18). ^{123}I-2'-Iodococaine or structural analogs less prone to metabolism, prepared by a no-carrier added radiolabeling technique, which we are presently developing, to obtain much higher specific activities, could behave in humans similar to ^{11}C-cocaine (12). Such compound(s) could be used as a molecular probe for the characterization of the cocaine receptors in the brain and the heart by SPECT imaging.

REFERENCES

1. Nayak P.K., Misra A.L., Mulé S.J.- J Pharmacol Exp Ther 196: 556 (1976)
2. Som P., Oster Z.H., Volkow N.D., Sacker D.F., and Weber D.A. - J Nucl Med 30: 831 (1989)
3. Misra A.L., Patel M.N., Alluri V.R., Mulé S.J., and Nayak P.K. - Xenobiotica 6: 537 (1976)
4. Misra A.L., Nayak P.K., Patel M.N., Vadlamani N.L., and Mulé S.J. - Experientia 30: 1312 (1974)
5. Inaba T., Stewart D.J., Kalow W. - Clin Pharmacol Ther 23: 547 (1978)
6. Kennedy L.T., Hanbauer I. - J Neurochem 41: 172 (1983)
7. Pimoule C., Schoemaker H., Javoy-Agid F., Scatton B., Agid Y., and Langer S.Z. - Eur J Pharmacol 95: 145 (1983)
8. Schoemaker H., Pimoule C., Arbilla S., Scatton B., Javoy-Agid F., and Langer S.Z. - Naunyn-Schmiderberg's Arch Pharmacol 329:227 (1985)
9. Galloway M. - Trends Pharmacol Sci 9: 451 (1988)
10. Calligaro D.O., Eldefrawi M.E. - Membrane Biochem 7: 87 (1988)
11. Ritz M.C., Lamb R.J., Goldberg S.R., and Kuhar J.M. - Science 237: 1219 (1987)
12. Fowler J.S., Volkow N.D., Wolf A.P., Dewey S., Christman D., MacGregor R., Logan J., Schlyer D., and Bendriem B. - J Nucl Med 30: 761 (1989)
13. Logan J., Fowler J.S., Dewey S.L., Wolf A.P., Volkow N.D., Christman D.R., MacGregor R., Schlyer D.J., and Bendriem B. - J Nucl Med 30: 898 (1989)
14. Bell M.R., Archer S. - J Amer Chem Soc 82: 4642 (1960)
15. Findlay S.P. - J Amer Chem Soc 76: 2855 (1954)
16. Smith R.A. - J Anal Toxicology 8: 35 (1984)
17. Hinkle G.H., Basmadjian G.P., Kirschner A.S., and Ice, R.D. - J Pharm Sci 70: 312 (1981)
18. Basmadjian G.P., Kanvinde M., Mills S.L., Nasr F. - Manuscript in preparation.