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RADIOSYNTHESIS OF A PHOTOAFFINITY PROBE FOR THE COCAINE RECEPTOR OF THE DOPAMINE TRANSPORTER: 3β-(*p*-CHLOROPHENYL)TROPAN-2β-CARBOXYLIC ACID *m*-([^{125}I]-IODO)-*p*-AZIDOPHENETHYL ESTER ([^{125}I]-RTI-82)

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Summary

The tropane alkaloid RTI-82, an iodoarylazide, has been labeled with iodine-125 for use as a photoaffinity probe for the cocaine binding site of the dopamine transporter. The one-flask radiosynthesis involves no-carrier-added electrophilic radioiodination of the corresponding aniline followed by diazotization and treatment with sodium azide. Isolation by reverse phase HPLC and solid phase extraction gives [^{125}I]-RTI-82 in good yield (76 ± 7%, n = 6), high radiochemical purity (>99.8%), and high specific radioactivity (1490 - 1880 mCi/μmol).

Keywords: Radioiodination, iodine-125, dopamine transporter, photoaffinity labeling, cocaine

Introduction

Cocaine abuse, one of the greatest public health concerns in the United States, is linked to neuropsychological impairment (1), and is recognized as a leading cause of drug-related deaths (2,3). Cocaine use during pregnancy has reached epidemic proportions, and is a contributing factor to a number of neonatal problems including cardiovascular and central nervous system disorders (4,5). A number of pathways may mediate the diverse central and peripheral effects of cocaine, but inhibition of

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dopamine transport is a key neurochemical determinant of the potent reinforcing properties and abuse liability of the drug (6). Consequently, there is much interest in gaining a better understanding of this process at the molecular level.

A particularly intriguing question is whether or not different recognition sites are associated with the binding of cocaine and dopamine to the dopamine transporter (7). Several other structurally distinct compound classes, such as mazindol derivatives and the GBR series of functionalized piperazines, also bind to the dopamine transporter (7). There are differences in the binding behavior and pharmacological action of these agents with respect to cocaine, and multiple recognition sites or different affinity states have been postulated (7-11). The development of radiolabeled, irreversible ligands from the various structural classes of dopamine uptake inhibitors would help elucidate the factors responsible for these differences, and would facilitate biochemical studies of the glycoprotein nature of the dopamine transporter.

Radiolabeled photoaffinity ligands based upon the GBR framework (12-15), such as [^{125}I]-DEEP ([^{125}I]-1-[2-diphenylmethoxy)ethyl]-4-[2-(4-azido-3-iodophenyl)ethyl]piperazine; Figure 1) (14,15), have been used for characterization of the dopamine transporter. A complementary series of potentially irreversible ligands have been described which are tropane alkaloid congeners of cocaine (16). One of these, 3β-(*p*-chlorophenyl)tropan-2β-carboxylic acid *m*-iodo-*p*-azidophenethyl ester (RTI-82; Figure 1), potently inhibits binding of another cocaine analog, [^{3}H]-WIN 35,428, to the dopamine transporter in rat striatal membranes (IC_{50} 14.5 nM). Inhibition becomes wash-resistant under exposure to UV light, in accord with covalent bonding at or near the ligand recognition site. We prepared [^{125}I]-RTI-82, and found that this cocaine analog irreversibly labels the same 80 kDa glycoprotein in rat striatal membranes as the GBR analog [^{125}I]-DEEP (17). The pharmacology observed for both radioligands was consistent with selective photoaffinity labeling of the dopamine transporter. Here we provide the details of the radiosynthesis of [^{125}I]-RTI-82, a new tool for investigations of the cocaine receptor of the dopamine transporter.

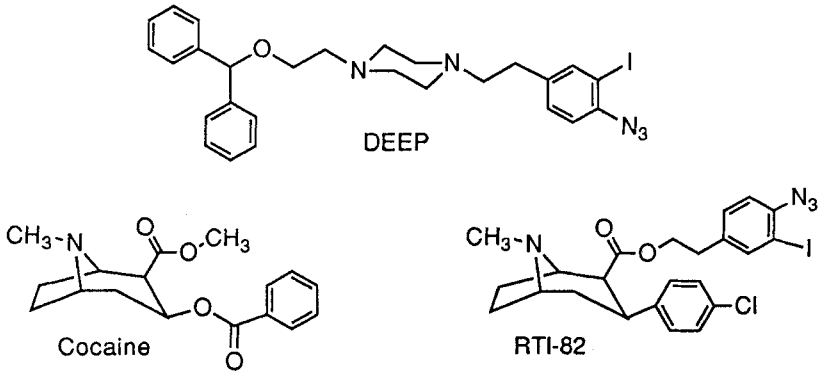
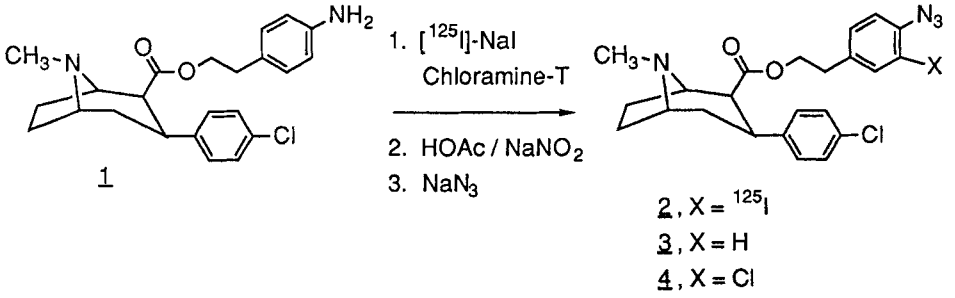


Figure 1. Structures of DEEP, cocaine and RTI-82.

Results and Discussion

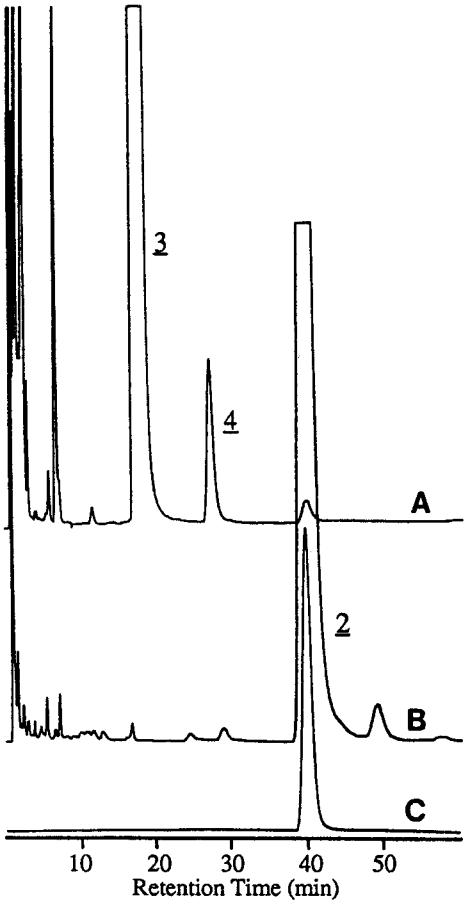
The synthetic route used for the preparation of [^{125}I]-RTI-82 (2) is shown in Scheme 1. The three-step sequence involves electrophilic radioiodination of aniline 1 under no-carrier-added conditions, diazotization, and substitution of the diazo moiety by azide. The one-flask procedure requires approximately 1 hour, and is conducted according to the general method reported by Wilson *et al.* (18). Superior overall yields of radioiodinated arylazides are obtained using this approach rather than the conventional two-flask method requiring isolation of the radioiodinated aniline intermediate (18).



Scheme 1. Radiosynthesis of [^{125}I]-RTI-82 (2).

In preliminary experiments, treatment of 1 with [^{125}I]-NaI at ambient temperature using chloramine-T as oxidant in aqueous acetate / acetic acid buffer (pH 4.9) for 30 minutes gave 85 - 95% incorporation of radioiodine. In one case, the radioiodinated product was purified by reverse phase HPLC, and isolated by solid phase extraction in 65% yield. This material co-eluted under several different HPLC conditions with a standard sample of non-radioactive 3β-(*p*-chlorophenyl)tropan-2β-carboxylic acid *m*-iodo-*p*-aminophenethyl ester. Having established the identity of the radiolabeled intermediate, we turned our attention to implementation of the entire sequence in a single vessel.

Acidification of the crude radioiodination reaction mixture with acetic acid (3.0 M), followed by addition of aqueous $NaNO_2$ at -10 °C, sufficed for generation of the aryl diazonium salt *in situ*. Treatment with aqueous NaN_3 and warming to ambient temperature completed the Sandmeyer reaction. Analysis by reverse phase HPLC using a ternary mobile phase showed nearly quantitative conversion to one major radioiodinated product, [^{125}I]-RTI-82 (2), having a chromatographic profile (t_R = 40.5 min, k'= 56.8) identical to that of non-radioactive RTI-82 (Figure 2B). Aniline 1 was converted in high yield to azide 3 (t_R = 17.8 min, k'= 24.4), the predominant non-radioactive material (Scheme 1, Figure 2A). Under these conditions, [^{125}I]-RTI-82 was also well separated from any residual radioiodinated aniline intermediate (t_R = 7.4 min, k'= 9.6) or non-radioactive precursor 1 (t_R = 2.3 min, k'= 2.3).



UV absorbance (λ 254 nm) chromatogram for purification of a typical [^{125}I]-RTI-82 reaction mixture.

Radioactivity chromatogram associated with chromatogram A.

Radioactivity chromatogram for the final formulation of purified [^{125}I]-RTI-82.

Figure 2. HPLC chromatograms for the radiosynthesis of [^{125}I]-RTI-82 (2).

In fact, these particular HPLC conditions were chosen to ensure good resolution of a minor non-radioactive component which displayed chromatographic lipophilicity (t_R = 28.0 min, k'= 39.0) between that of azide 3 and the iodoarylazide 2 (Figure 2A). This product has been tentatively assigned as chloroarylazide 4 (Scheme 1) on the basis of the HPLC characteristics, and the literature precedent for substrate chlorination as a common side reaction encountered during electrophilic radioiodination with *N*-chloroamine oxidants (18,19).

To test this hypothesis, model reactions were performed where the amount of chloramine-T was varied in the absence of radioiodide. Consistent with oxidant-promoted chlorination, the proportion of 4 grew with increasing chloramine-T. For example, when the molar ratio of aniline 1 to chloramine-T was 10 to 1, the ratio of arylazide 3 to chloroarylazide 4 was 97 to 3. With a 2 to 1 ratio of precursor to oxidant, the ratio of 3 to 4 reached 70 to 30. Interestingly, when no chloramine-T was added, a trace of 4 (*ca.* 0.5%) was still observed along with the nearly quantitative conversion of 1 to 3. Presumably, 4 is formed in this case by limited

nitrous acid oxidation of the chloride which is present in the reaction mixture as a consequence of the use of precursor 1 as the dihydrochloride salt.

Although 4 is readily separated by HPLC from [^{125}I]-RTI-82, the presence of any chloroarylazide pseudocarrier would lower the effective specific radioactivity of the final product. Therefore, preparative reaction conditions were chosen which should minimize chlorination, and also ensure efficient radioiodide oxidation (1 : chloramine-T : radioiodide approximately 150 : 14 : 1). From 6 runs conducted with [^{125}I]-NaI on a 2 mCi (*ca.* 1.0 nmol) scale, [^{125}I]-RTI-82 was obtained after HPLC purification (Figure 2) and solid phase extraction in a radiochemical yield of 76 ± 7% (mean ± SEM; n = 6) as a solution (*ca.* 0.5 μM) in buffered ethanol. Specific radioactivities for [^{125}I]-RTI-82 (1490 - 1880 mCi/μmol) were calculated for aliquots of known radioactivity using HPLC data to establish the mass of carrier. These values were in good agreement (± 5 - 15%) with the specific radioactivities quoted for the individual batches of radioiodide.

Analytical HPLC also indicated high radiochemical purity (>99.8%, Figure 2C) for the final radioligand formulation, with no chemical impurities observed by ultraviolet absorbance (λ 254 nm) detection. Stock solutions of [^{125}I]-RTI-82 were quite stable (91%) upon prolonged storage (18 weeks) at -20 °C in the dark. Approximately 6% of the radioactivity eluted as an impurity at the solvent front, consistent with decomposition to free radioiodide, while an additional 3% of the radioactivity was associated with an unidentified radiochemical impurity much less lipophilic than [^{125}I]-RTI-82. Thus, [^{125}I]-RTI-82 has a good shelf-life for routine use in biochemical studies.

Experimental

Materials and Methods. Fully characterized, non-radioactive samples of all tropane alkaloids except the putative chloroarylazide 4 were prepared as previously described (16). Other chemicals and solvents were reagent or HPLC grade, and were used as received from commercial sources. No-carrier-added [^{125}I]-NaI was obtained from Amersham Corp. (1 mCi / 10 μL dilute aqueous NaOH, pH 7-11). HPLC equipment consisted of a Rheodyne 7125 injector, Waters 510EF pumps, Waters 490 UV absorbance detector (λ 254 nm), a flow-through NaI(Tl) crystal scintillation detector comprised of EE&G Ortec components, and Shimadzu CR-3A integrating recorders. A Waters C-18 Nova-Pak column (radial compression module, 8 mm x 10 cm, 4μm), protected by an Alltech C-18 Adsorbosphere guard column, was used for preparative and analytical HPLC. Activated Waters SEP-PAK Light *t*-C-18 cartridges were employed for solid phase extraction. Radioactivity was measured with a radioisotope dose calibrator (Capintec CRC-7). Similar counting geometry and vessel type were used for each reading. Specific radioactivities were calculated for aliquots of known radioactivity using HPLC data for determination of the mass associated with the UV absorbance peak area of the carrier. The HPLC

response curve relating mass to peak area was established using non-radioactive RTI-82 standards chosen to bracket the region of interest.

3β-(*p*-Chlorophenyl)tropan-2β-carboxylic Acid *m*-([^{125}I]-Iodo)-*p*-azidophenethyl Ester ([^{125}I]-RTI-82, 2). A solution of 3β-(*p*-chlorophenyl)tropan-2β-carboxylic acid *p*-aminophenethyl ester dihydrochloride (1; 50 μL, 3.0 mM; 150 nmol) in aqueous buffer (pH 4.91; 0.1 M AcOH, 0.22 M NaOAc) was added to an amber glass vial sealed with a Teflon-faced septum. [^{125}I]-NaI (20 μL, 1.85 mCi; *ca.* 1.0 nmol) was added, followed by aqueous *N*-chloro-4-toluenesulfonamide (Chloramine-T) trihydrate (4 μL, 3.5 mM; 14 nmol). The mixture was incubated at ambient temperature for 30 min, and then chilled at -10 °C in an ice / MeOH bath. Cold aqueous AcOH (50 μL, 3.0 M; 150 μmol) was then added, followed by cold aqueous $NaNO_2$ (10 μL, 0.5 M; 5 μmol). After 15 min, aqueous NaN_3 (10 μL, 0.5 M; 5 μmol) was added, and the mixture allowed to warm to ambient temperature. After 10 min, the reaction was quenched with $Na_2S_2O_5$ (10 μL, 50 mM; 0.5 μmol) and diluted with EtOH (100 μL). The mixture was taken up in a syringe, and combined with a rinse of the vial with 200 μL of the ternary HPLC mobile phase consisting of MeOH (22.5%), CH_3CN (22.5%), and an aqueous solution (55%) of Et_3N (2.1% *v/v*) and HOAc (2.8% *v/v*). At a flow rate of 4 mL/min, [^{125}I]-RTI-82 (t_R = 40.5 min, k'= 56.8) was well separated from 1 (t_R = 2.3 min, k'= 2.3), the iodoaniline (t_R = 7.4 min, k'= 9.6), 3 (t_R = 17.8 min, k'= 24.4), and 4 (t_R = 28.0 min, k'= 39.0). The radioligand was collected in a 20 mL volume, diluted to 100 mL with distilled water, and passed through an activated SEP-PAK which was then flushed with water (5 mL) to remove salts, and then with air. Approximately 97% of the radioactivity in the original solution was retained by the cartridge. Elution with EtOH (2.0 mL) containing 1% *v/v* TRIS buffer (5 mM, pH 7.4) gave a 99% recovery of radioactivity, and provided [^{125}I]-RTI-82 (1.40 mCi) in 76% radiochemical yield. Using the HPLC conditions described above, aliquots of the [^{125}I]-RTI-82 solution were determined to be radiochemically pure with a specific radioactivity of 1740 mCi/μmol.

Acknowledgments

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