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Alternative Labelling of the Cocaine Analogue Isomers α-CIT and ß-CIT by Direct Iodination with No-Carrier-Added $Na^{125}I$.

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SUMMARY

An alternative labelling of the cocaine analogue isomers α-CIT and β-CIT with no-carrier-added ^{125}I by direct iodination of 2α- and 2ß-carbomethoxy-3β-phenyltropane with $Na^{125}I$ / sulfuric acid / nitric acid / acetic acid and peracetic acid under different reaction conditions, is described. The maximum radiolabelling yield obtained with the two isomers was 48% for [^{125}I]α-CIT and 28% for [^{125}I]ß-CIT.

Key words: Dopamine transporter, CIT, ^{123}I, ^{125}I, SPECT

INTRODUCTION

Cocaine binds to several receptors in the brain and abuse of cocaine is related to drug-induced feelings of well-being and euphoria. Experimental studies indicate that a specific binding site in the brain for cocaine is related to this pharmacological effect[1].

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This binding site is mainly localized presynaptically on dopaminergic neurons in the striatum[2].

In spite of cocaine's rather low affinity for the dopamine transporter, (IC_{50}=67.8 nM) tested in competition experiments with [^{3}H]cocaine[3], cocaine has been labelled with ^{11}C and used in studies with positron emission tomography (PET)[4,5]. This low affinity limits the potential of radiolabelled cocaine for quantitative examination of regional binding and stimulates the search for more potent cocaine analogues.

Two such analogues are 2α-carbomethoxy-3β-(4-iodophenyl) tropane and its isomer 2ß-carbomethoxy-3β-(4-iodophenyl)tropane (referred to as α-CIT and β-CIT respectively). In *in vitro* test with α-CIT and ß-CIT, binding to the dopamine transporter has been tested in competition experiments with [^{3}H]CFT and compared to cocaine[6]. The results demonstrated that both α-CIT (IC_{50}=87.6 nM) and ß-CIT (IC_{50}=1.6 nM) have a higher affinity for the dopamine transporter than cocaine (IC_{50}=221 nM). β-CIT has been labelled with the positron emitting isotope ^{11}C and characterized *in vivo* in PET-studies[7,8]. Neumeyer et al.[6] report on the synthesis of [^{123}I]β-CIT using nonradioactive β-CIT, as starting material. The corresponding trialkyltin derivative was synthesized from the iodo precursor and treated with no-carrier-added Na^{123}I. The product, [^{123}I]β-CIT, was used for single photon emission computed tomography (SPECT)[6,9]. [^{125}I]β-CIT has been used in *in vitro* studies[10].

A preliminary PET examination of [^{11}C]α-CIT in a Cynomolgus monkey demonstrated different binding kinetics compared to [^{11}C]ß-CIT (Farde, unpublished results). Due to this difference in kinetics, α-CIT may be used as an alternative to ß-CIT as a radioligand for PET and SPECT.

Our aim in this work was to employ precursors for labelling of [^{125}I]α-CIT and [^{125}I]β-CIT that were more easy to prepare than the trialkyltin precursor previously reported[6]. In addition, using the deiodo precursor would exclude the risk of reducing the specific radioactivity in the final product due to the presence of CIT in the trialkyltin precursor[6,11].

In the present communication, the synthesis of no-carrier-added [^{125}I]α-CIT and [^{125}I]β-CIT, by direct iodination of the corresponding deiodo precursors is described (Fig. 1). The labelling yields of the syntheses were determined in the radio HPLC as the amount of ^{125}I incorporated in the product at different time points.

EXPERIMENTAL

General:

No-carrier-added Na^{125}I was obtained from Amersham International (specific radioactivity about 2 Ci/μmol). ß-CIT was obtained from Research Biochemical Incorporated (RBI), Natick, USA. α-CIT was prepared as previously reported[12]. Dichloramine-T is available from TCI, Portland, Oregon. All other chemicals were obtained from commercial sources and were of analytical grade. Water was purified by reverse osmosis (Milli-Q, Millipore/Waters)

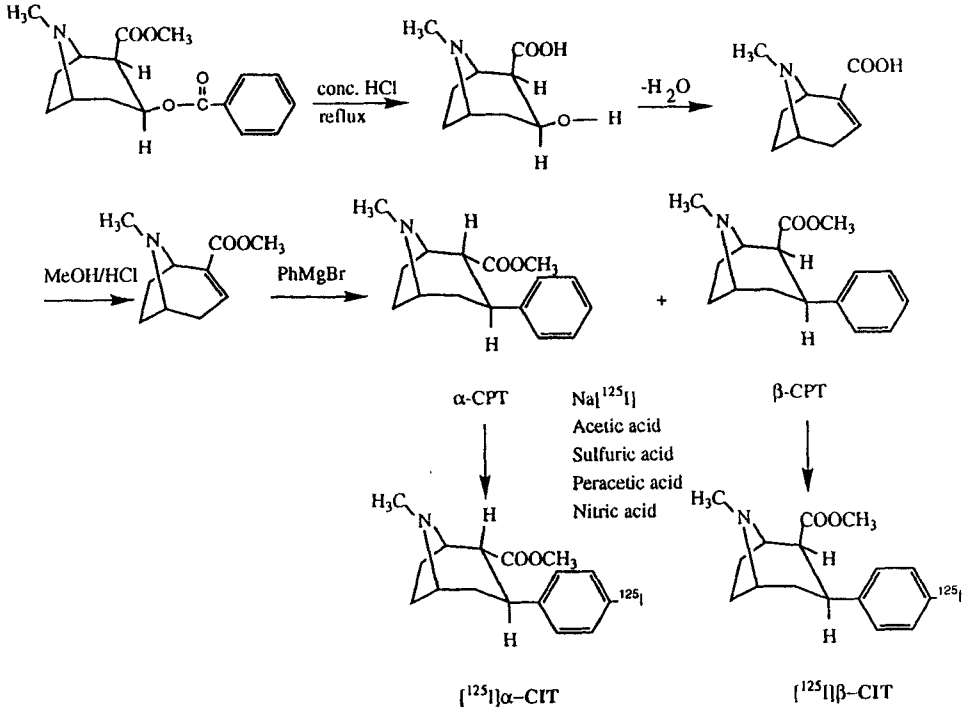

FIGURE 1

Synthesis of [^{125}I]α-CIT and [^{125}I]ß-CIT.

The analyses of the reaction mixture were performed on a reverse phase HPLC system at different time points with a Waters HPLC pump 600E with a Rheodyne Injector and a Waters UV-detector 490E (operated at 214 nm and 254 nm).

Separations were accomplished with a Novo C-18 column (250 x 3.9 mm) at 30°C by use of an eluent of, A: water 0.1% TFA / acetonitrile 90/10 and B: acetonitrile 0.1% TFA /water 90/10 in an isocratic system with 76% A and 24% B. The flow rate was 1.0 ml/minutes. The radioactivity in the column effluent was monitored with a Radiomatic/Canberra Flo-One Beta detector A-280, with a 250 µl Gamma-C flow cell. The data were collected by FLO-ONE/Data software on a PC-XT computer.

Total radioactivity was determined with a Packard 2000 CA tri-carb liquid scintillation analyzer, with 20 ml counting vials and Pico-aqua™ Packard liquid scintillator. Counting efficiency was determined with an internal standard made of a diluted I^{125} reference sample from Amersham International. [^{1}H]NMR-spectra were recorded in $CDCl_3$ in a 400 MHz Bruker spectrometer.

Preparation of 2α- and 2ß- carbomethoxy-3ß-phenyltropane.

The two 3ß-phenyltropane isomers were synthesized according to procedures analogous to those of Clarke et al.[13] and Carroll et al.[14] in our laboratories (Fig. 1). Briefly, anhydroecgonine methyl ester was prepared by dehydration of cocaine followed by esterification in methanol-hydrogen chloride[15]. Conjugate addition of phenylmagnesium bromide at -40°C and hydrolysis of the resulting magnesium compound with trifluoroacetic acid at -78°C gave a mixture of the 2α- and 2ß-carbomethoxy-3ß-phenyltropane derivatives which were separated by column chromatography (SiO_2, diethylether: triethylamine, 9:1). On TLC, the α-isomer had a Rf=0.54 and the ß-isomer had a Rf=0.76 (SiO_2, Kiselgel 60, diethylether:triethylamine, 9:1).

[^{1}H]NMR 2α-carbomethoxy-3ß-phenyltropane: δ = 7.25 (m, 5H, ar), 3.5 (s, 3H, O-CH_3), 3.4 (d, 1H), 3.25 (m, 1H), 3.1 (m, 2H), 2.4 (s, 3H, N-CH_3), 2.1 (m, 2H), 1.6 (m, 4H).

[^{1}H]NMR 2ß-carbomethoxy-3ß-phenyltropane: δ = 7.20 (m, 5H, ar), 3.5 (m, 1H), 3.4 (s, 3H, O-CH_3), 3.30 (d, 1H), 3.0 (m, 2H), 2.1 (s, 3H, N-CH_3), 2.0 (m, 2H), 1.5 (m, 4H).

[^{125}I]α-CIT and [^{125}I]β-CIT by direct iodination:

The incorporation of ^{125}I was investigated at 60°C and 90°C, respectively. In each experiment 5 mg 2α-carbomethoxy-3ß-phenyl tropane and 5 mg 2ß-carbomethoxy-3ß-phenyl tropane, respectively, was used. The precursor was dissolved in ethanol in a 1.5 ml micro tube. The ethanol was evaporated under nitrogen and to the residual yellow oil were added acetic acid (200 µl), sulfuric acid (25 µl), peracetic acid (10 µl, conc. 32%), nitric acid (5 µl) and Na^{125}I (100 µl, 1 mCi). The vial was sealed and placed in a heating block. Samples were taken and analysed by HPLC at different time points (Fig. 2 and 3). All samples were diluted in the mobile phase and placed on ice until analysis.

As alternative methods we tried iodination of 2ß-carbomethoxy-3ß-phenyltropane with chloramine-T and dichloramine-T, respectively, as oxidative reagents.

Chloramine-T method:

Two experiments were performed at 20°C. In each experiment 5 mg 2ß-carbomethoxy-3ß-phenyltropane dissolved in ethanol was added to a 1.5 ml micro tube. The ethanol was evaporated and NaH_2PO_4 (50 µl, 0.5M pH=7.4) and Na^{125}I (100 µl, 1 mCi) were added. Freshly prepared chloramine-T (10 µl, 1 mg/ml in ethanol) was added and the tube was stirred during the reaction. Samples were taken and analysed by HPLC at 1, 2, 5, 10 and 20 minutes.

Dichloramine-T method:

Three experiments were performed at 20°C, with 5 mg 2ß-carbomethoxy-3ß-phenyltropane in each. Acetonitrile (200 µl), H_3PO_4 (20 µl, 2M) and Na^{125}I (2 µl, 150

μCi) were added and the tube stirred. Freshly prepared dichloramine-T (40 μl, 1 mg/ml in ethanol) was added and the tube was stirred during the reaction. Samples were taken and analysed by HPLC at 1, 2, 5, 10 and 20 minutes.

RESULTS AND DISCUSSION

The incorporation of ^{125}I into [^{125}I]α-CIT was very slow at 60°C. The yield of [^{125}I]α-CIT was 35% after 300 minutes (Fig 2), and increased to 47% after 360 minutes. At 1300 minutes the yield had decreased to 9%. The temperature was increased to 90°C in a second experiment (Fig. 3). The maximal yield at 90°C was 48% after only 15 minutes. As seen from Figure 3 the yield at 90°C declined very fast with time after the maximum was reached.

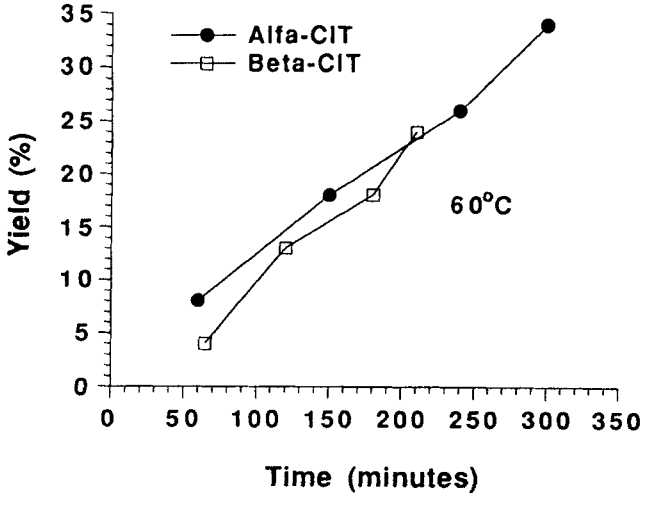

FIGURE 2

Radiochemical yield of [^{125}I]α-CIT and [^{125}I]ß-CIT by direct iodination at 60°C.

Incorporation of ^{125}I into [^{125}I]ß-CIT had a sligthly slower kinetics than that observed for [^{125}I]α-CIT. At 60°C the yield was 24% after 210 minutes, whereas it reached maximum

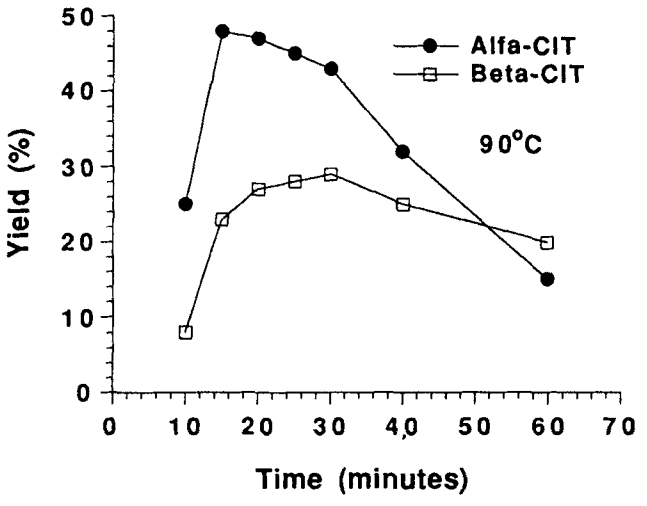

FIGURE 3.

Radiochemical yield of [^{125}I]α-CIT and [^{125}I]ß-CIT by direct iodination at 90°C.

after 30 minutes at 90°C (28%) (Figs. 2 and 3). As for the [^{125}I]α-CIT, we observed a decline in yield after the maximum had been reached for [^{125}I]ß-CIT at 90°C.

A ^{125}I-labelled degradation product was formed during the labelling of both α-CIT and ß-CIT. This byproduct was more hydrophilic than α- and ß-CIT and had a retention time of 11 minutes (maximum appearence 33% after 40 minutes for α-CIT and maximum 32% after 60 minutes for ß-CIT). The same byproduct was formed at 60°C, but not to the same extent (maximum appearence 22% after 360 minutes for α-CIT and maximum 26% after 210 minutes for ß-CIT). Since no-carrier-added Na^{125}I was used and since both [^{125}I]α-CIT and [^{125}I]ß-CIT eluted without contamination from precursor and "cold" impurities in the HPLC system, the specific radioactivity for the two iodinated products peaks should be around 2 Ci/μmol. The following retention times were obtained in the HPLC separation; Na^{125}I: 4.5 min, 2α-carbomethoxy-3ß-phenyl tropane: 7.8 min, 2ß-carbomethoxy-3ß-phenyltropane: 6.6 min, [^{125}I]ß-CIT: 23.0 min, [^{125}I]α-CIT: 25.0 min.

The direct iodination method has the potential of giving ortho, meta and para substituted products. The substituent on the phenyl group, the tropane, will slightly

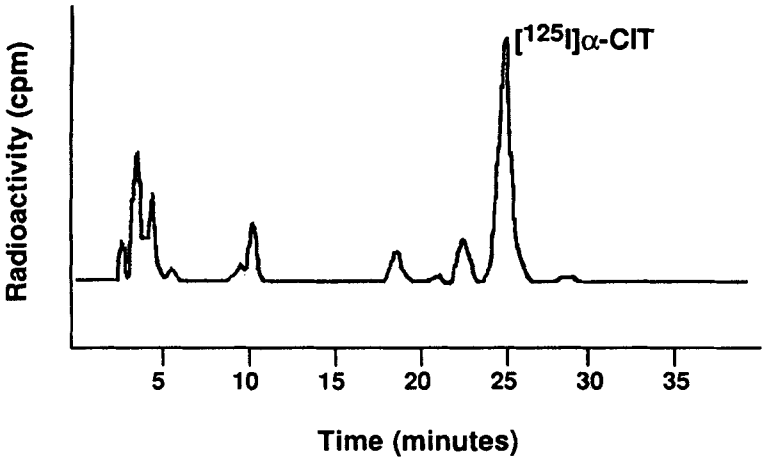

FIGURE 4

Typical radio-HPLC chromatogram from the synthesis of [^{125}I]α-CIT after 15 min at 90°C

activate the ortho and para positions. Since the ortho positions probably have steric hindrence, the major product is more likely to be the para iodinated product, [^{125}I]-CIT, which actually proved to be the case. As seen in the chromatogram (figure 4), small amounts of iodinated impurities, eluting closely to the [^{125}I]α-CIT were formed. Similar impurities were formed, when iodinating ß-CIT. The impurities could represent ortho and meta iodinated analogous of CIT, but since only the para substituted α- and ß-CIT were available as reference compounds, the impurites could not be identified.

The incorporation of ^{125}I into [^{125}I]ß-CIT with chloramine-T as oxidizing agent gave a maximum of 2% yield after 2 min, whereas we did not observe any product with dichloramin-T.

We have developed an alternative method for labelling of α- and ß-CIT, which consists of a direct radioiodination of 2α-carbomethoxy-3ß-phenyltropane and 2ß-carbomethoxy-3ß-phenyltropane. Neumeyer et al.[6] reported the synthesis of [^{123}I]ß-CIT for SPECT applications using the nonradioactive ß-CIT as starting material to synthesize the corresponding trialkyltin derivative which is reacted with Na^{125}I. The trimethyltin

precursor of ß-CIT is commercially available from Research Biochemical Incorporated (RBI). Contamination of ß-CIT in the trialkyltin precursor, has been reported as a problem[6,11]. This problem is due to an incomplete separation of ß-CIT from the trialkyltin precursor in the purification and can be eliminated by the use of the direct iodination method.

In conclusion, the two α- and ß-CIT isomers have been labelled with ^{125}I with maximum yields of 48% and 28%, respectively. The advantages of using the direct iodination are easier access to the precursor whereas the synthesis of the trialkyltin precursor requires extra steps and carefull purification to ensure the absence of carrier. Advantages of the iodostannylation method are rapid high yield under mild radioiodination conditions and regiospecific placement of the label whereas the direct iodination requires heating and results in lower yields. [^{125}I]α-CIT and [^{125}I]ß-CIT are well suited for *in vitro* studies in rodents and man and if the chemistry can be extended to ^{123}I, this method may be useful when labelling α-CIT and ß-CIT for SPECT-studies.

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REFERENCES

1. Ritz M.C., Lamb R.J., Goldberg S.R. and Kuhar M.J. - Science 237: 1219-1223 (1987).

2. Kuhar M., Sanchez-Roa P., Wong D., Dannals R., Grigoriadis D., Lew R. and Milberger M. - Eur. Neurol 30: 15-20 (1990).

3. Madras B.K., Fahey M.A., Bergman J., Canfield D.R. and Spelman R.D. - J. Pharmacol. Exp. Ther. 251: 131-141 (1989).

4. Fowler J.S., Volkow N.D.,Wolf A.P., Dewey S.L., Schlyer D.J., MacGregor R.R., Hitzemann R., Logan J., Bendriem B., Gatley S.J. and Christman D.- Synapse 4: 371-377 (1989).

5. Volkow N.D., Fowler J.S., Wolf A.P., Wang G-J., Logan J., MacGregor R., Dewey S.L., Schlyer D. and Hitzemann R. - J. Nucl. Med. 33: 521-525 (1992).

6. Neumeyer J.L.,Wang S., Milius R.A., Baldwin R.M., Zea-Ponce Y., Hoffer P., Sybirska E., Al-Tikriti M., Charney D.S., Malison R.T., Laruelle M. and Innis R.B. - J. Med. Chem. 34: 3144-3146 (1991).

7. Müller L., Halldin C., Farde L., Karlsson P., Hall H., Swahn C.-G., Neumeyer J., Gao Y., Milius R. - Nucl. Med. Biol. 20: 249-255 (1993).

8. Farde L., Halldin C., Müller L., Suhara T., Karlsson P. and Hall H. - Synapse 4: *93-103* (1994).

9. Innis R., Baldwin R., Sybirska E., Zea Y., Laruelle M., Al-Tikriti M., Charney D., Zoghbi S., Smith E., Wisniewski G., Hoffer P., Wang S., Milius R. and Neumeyer J. - Eur. J. Pharmacol. 200: 369-370 (1991).

10. Boja J.W., Patel A.P., Carroll F.I., Rahmann M.A., Phillip A., Lewin A.H., Kopatjic T.A. and Kuhar M.J. - Eur. J. Pharmacol. 194: 133-134 (1991).

11. Baldwin R.M., Zea-Ponce Y., Zoghbi S.S., Laurelle M., Al-Tikriti M.S., Sybirska E.H., Malison R.T., Neumeyer J.L., Milius R., Wang S., Stabin S., Smith E.O., Charney D.S., Hoffer P.B. and Innis R. - Nucl. Med. Biol. 20: 597-606 (1993)

12. Någren K., Müller L., Halldin C., Swahn C.-G. and Lehikoinen P. - Nucl. Med. Biol. (1994) *(in press)*.

13. Clarke R.L., Daum S.J., Gambino A.J., Aceto M.D., Pearl J., Levitt M., Cumiskey W.R. and Bogado E.F. - J. Med. Chem. 16: 1260 (1973)

14. Carroll F.I., Gao Y., Rahman M.A., Parham K., Lewin A.H., Boja J.W. and Kuhar M.J. - J. Med. Chem. 34: 2719-2725 (1991).

15. Zirkle C.L., Geissman T.A., Bloom M., Craig P.N., Gerns F.R., Indik Z.K. and Pavloff A.M. - J. Org. Chem. 27: 1269-1279 (1962).