SYNTHESIS OF 2β-CARBOMETHOXY-3β-(4-[^{76}Br]BROMOPHENYL)TROPANE ([^{76}Br]β-CBT), A PET TRACER FOR IN VIVO IMAGING OF THE DOPAMINE UPTAKE SITES.

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SUMMARY

2β-carbomethoxy-3β-(4-[^{76}Br]bromophenyl)tropane ([^{76}Br]β-CBT) was prepared either by electrophilic substitution from the tributyl-stannyl derivative and peracetic acid as oxidant or by nucleophilic substitution from the iodo analogue (β-CIT) and a Cu^{+} assisted bromodeiodination exchange. After purification by solid phase extraction and reverse phase HPLC, the chemical and radiochemical purities of [^{76}Br]β-CBT were >98% and the specific radioactivity was 20 GBq/μmol. Using the two labelling techniques, the radiochemical yields were 80% and 60%, respectively. From the deshalogeno compound and different oxidizing conditions, the radiolabelling yields were <5%. *In vitro* competition and saturation pharmacological studies showed that [^{76}Br]β-CBT mainly labelled the dopamine transporter and bound to a single population of sites in striatal membranes (B_{max}= 6.5 pmol/mg protein) with an apparent dissociation constant of 2.8 nM. Biodistribution and autoradiography studies of the title compound in rats showed that 3 h post injection, the highest concentration in the brain was found in the striata (2.5% ID/g). 24 h post injection, the striatum to cerebellum radioactive concentration ratio was still 17.

Keywords: Dopamine transporter, Cocaine, 2β-carbomethoxy-3β-(4-[^{76}Br]bromophenyl)tropane Positron emission tomography.

INTRODUCTION

Alteration in monoamine transporters have been described in neurodegenerative and neuropsychiatric conditions. In patients suffering of Parkinson's disease or Alzheimer's disease, decreases of dopamine transporter density in the striatum and decreases of serotonine

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transporter density in cortex have been described in post-mortem studies (1-5). In the brain, cocaine binds to specific sites which are mainly associated to dopamine, norepinephrine and serotonine transporters. To study these binding sites *in vivo* in the brain, by positron emission tomography (PET) cocaine was labelled with [^{11}C] (6,7). Nevertheless, its rapid metabolism and its low affinity limited its use in *in vivo* studies. To examine the dopamine transporter by PET, several cocaine congeners such as: [^{11}C]-2β-carbomethoxy-3β-(4-fluorophenyl)tropane: [^{11}C]β-CFT (8) and [^{11}C]-2β-carbomethoxy-3β-(4-iodophenyl)tropane: [^{11}C]β-CIT (9) were developed. To study by single photon emission tomography (SPET) the monoamine reuptake sites, β-CIT was also labelled with [^{123}I] (10). The comparison of the pharmacological properties of β-CFT and β-CIT showed that β-CFT is more selective for the dopamine transporter while β-CIT also labelled the serotonine transporter. To study the role of the halogen in the selectivity of the tracers for the dopamine reuptake sites and to follow the kinetics by PET for a longer time period, we have labelled the bromo analogue methyl 2β-carbomethoxy-3β-(4-bromophenyl)tropane, (β-CBT) with [^{76}Br] ($T_{1/2}$=16 h) using electrophilic or nucleophilic substitution reactions.

MATERIALS AND METHODS

General

2β-carbomethoxy-3β-(4-iodophenyl)tropane, 2β-carbomethoxy-3β-(4-tributylstannylphenyl)-tropane, GBR12909, desipramine and mazindol were purchased (Research Biochemicals Inc). 2β-carbomethoxy-3β-phenyltropane was synthesised as reported in detail elsewhere (11,12). 2β-carbomethoxy-3β-(4-bromophenyl)tropane was a generous gift from Dr Coenen (Essen, Germany). Citalopram was kindly provided by Lundbeck (Denmark). All the others chemicals were reagent grade obtained from conventional sources.

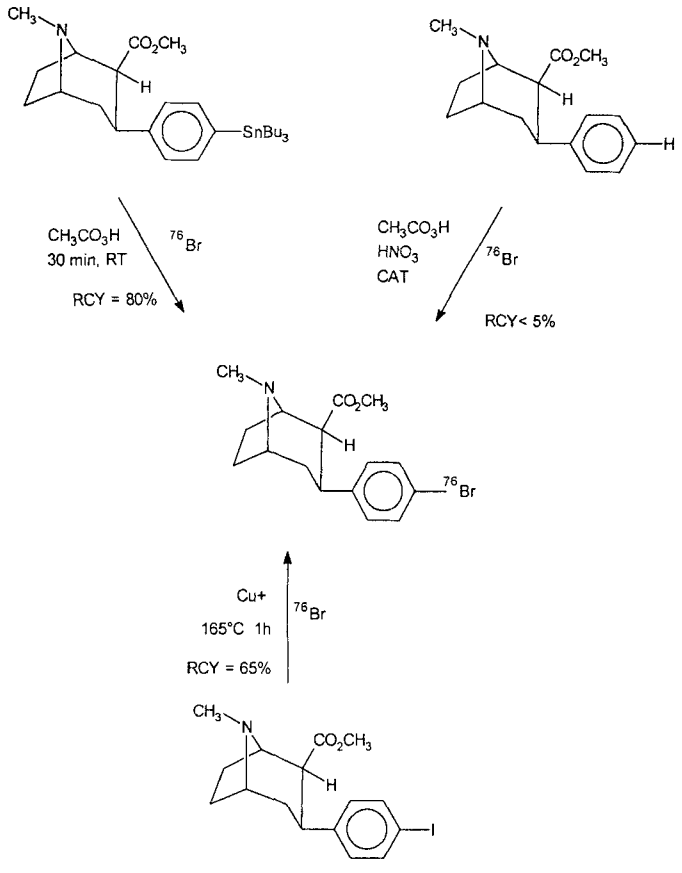
[^{76}Br] was produced by irradiation of natural arsenic (1.7 g) with a beam of 30 Mev [^{3}He] ions. After decay of [^{75}Br] ($T_{1/2}$= 1.6 h), the target was dissolved in 40 mL concentrated sulphuric acid at 180°C. After cooling to 50°C, 4 g chromic acid in 12 mL water were added. The radioactive bromine was carried out with a nitrogen stream and trapped as bromide in 2 mL 1M ammonia that was later taken to dryness.

For purification, the crude labelling mixtures were poured onto a C18 cartridge (Sep-Pak, Waters). Polar by-products and unreacted [^{76}Br]NH_4 were washed from the cartridge with 5 mL water and 3 mL of water-methanol (90-10, v/v). [^{76}Br]β-CBT was eluted by 3 mL of methanol. After evaporation, the product was purified by reverse-phase HPLC using a Waters 450 pump, an automatic sample injector with a 2 mL loop (Vici) and a Waters μ-Bondapak C18 column (300x3.9 mm, 10 μm). The effluent (water-acetonitrile-triethylamine, 75-25-0.1,v/v/v) eluted at a 1.5 mL/min flow rate, was monitored with an UV detector at 254 nm (M440, Waters) and a Geiger-Muller radioactivity detector. The radioactive peak eluted at the retention time of

authentic β-CBT was collected in a flask and the solvent evaporated. The residue was dissolved in sterile saline and filtered through a 0.22 μm sterile membrane.

The time course of the radiolabelling yield during the synthesis and the radiochemical purity of the radiopharmaceutical preparation of [^{76}Br]β-CBT was determined by TLC using a silica gel plate with a chloroform-methanol-triethylamine mixture (90-10-0.1,v/v/v). The chemical purity of [^{76}Br]β-CBT was checked by RP-HPLC in the same conditions described above.

The specific radioactivity was calculated from the HPLC chromatogram obtained in the purification step. The area of the UV absorbance peak of [^{76}Br]β-CBT corresponding to the carrier product was measured and compared to a standard curve relating mass to UV absorbance. The radioactivity of the [^{76}Br]β-CBT fraction was measured in a radioactive dose calibrator (Capintec).



Scheme 1

Radiolabelling

Electrophilic substitution

The radiolabelling of [^{76}Br]β- CBT was performed using peracetic acid as previously described for [^{123}I]β-CIT preparation (10). To a vial containing [^{76}Br]NH_4 in 200 μL water was added 50 μg (94 nmol) 2β-carbomethoxy-3β-(4-tributylstannylphenyl)tropane in 50 μL ethanol, 20 μL 5M H_3PO_4 and 20 μL (4.2 μmol) of 1.6% peracetic acid in concentrated acetic acid. The vial was sealed and the mixture was placed in room temperature for 30 min. The labelling process was stopped by addition of 1 mg of $Na_2S_2O_5$ then diluted with 1 mL water for later purification.

Nucleophilic substitution

[^{76}Br]β-CBT was prepared from the iodinated analog 2β-carbomethoxy-3β-(4-iodophenyl)tropane using a Cu^+ assisted nucleophilic substitution reaction (13,14). β-CIT (0.2 mg, 0.52 μmol), gentisic acid (0.66 mg, 4.2 μmol), ascorbic acid (0.66 mg, 3.7 μmol) and citric acid (1 mg, 5.2 μmol) were dissolved in 50 μL 1M acetic acid. $CuSO_4$, $5H_2O$ (0.14 μmol) dissolved in 10 μL 1M acetic acid and 370 MBq [^{76}Br]NH_4 were added and the reaction vial was hermetically sealed. The exchange between bromine and iodine atoms was performed at 165°C for 60 min in a dry oven (Reacti-Therm, Pierce). The vial was cooled to room temperature and the reaction mixture was diluted with 1 mL water.

In vitro studies

The binding parameters of [^{76}Br]β-CBT were evaluated *in vitro* on homogenates of rat cerebral striatal membranes. Dopaminergic reuptake sites were labelled by incubating membranes with increasing concentrations of [^{76}Br]β-CBT from 0.1 to 20 nM in ACFS buffer (15). Non-specific binding was measured in presence of 30 μM cocaine. Selectivity of [^{76}Br]β-CBT was assessed in competition experiments with selective monoaminergic transport inhibitors: GBR 12909 (a dopamine transport inhibitor), citalopram (a serotonin transport inhibitor), desipramine (a norepinephrine transport inhibitor) and mazindol (a monoamine reuptake inhibitor) which were added to the incubating mixture.

Ex vivo biodistribution studies

The regional brain uptake of the radiobromocompound was followed for 24 h in Wistar male rats which were injected in the tail vein with 0.2 MBq of [^{76}Br]β-CBT. Rats were sacrificed by decapitation. The brain structures were dissected and the radioactivity of aliquots were measured. The radioactivity concentrations expressed as percent of injected dose per gram of tissue (% ID/g) were plotted versus time. The *in vivo* specific striatal localisation of [^{76}Br]β-CBT was studied by autoradiography 2 h after injection of 8 MBq of the labelled compound in rats. The brains were recovered, frozen and cut in 20 μm thick horizontal sections with a

cryomicrotome (Leitz 1720). The slices were put into X-ray cassettes together with β-radiation sensitive film (Hyperfilm β-max, Amersham) for a 2 day exposure. The films were analysed and colour coded using a computerised densitometric system.

RESULTS AND DISCUSSION

Radiolabelling

The preparation of [^{76}Br]β-CBT from the deshalogeno precursor was unsuccessful. Using various oxidizing agents (chloramine-T, dichloramine-T, peracetic and nitric acids) and different reaction conditions (temperature, solvents and reagent concentrations), the labelling yields were < 5%. These low radiolabelling yields are explained by the absence of organic substituent on the phenyl ring enhancing the electrophilic substitution. In the contrary, by using the tributylstannyl precursor and peracetic acid as oxidant, a high (80%) and regioselective labelling was obtained during 30 min reaction.

[^{76}Br]β-CBT was also prepared from β-CIT by a Cu^+ nucleophilic aromatic bromodeiodination reaction: iodine is able to coordinate with copper that initiates halogen exchange (16). The copper-to-β-CIT molar ratio was 4, in good agreement with the theoretical optimal value for Cu^+ assisted radiohalogenation (17). After optimisation of the reaction conditions (reaction volume: 260 μL) and 1h at 165°C, the radiolabelling yield, measured by TLC, was 60-65%.

For the purification of the radiotracer, whatever the radiolabelling conditions, the unreacted [^{76}Br]-bromide, the radiolabelled polar by-products and the excess of unreacted organic acids were eliminated using solid phase extraction. More than 98% of [^{76}Br]β-CBT was recovered by methanol.

By using an analytical reverse phase column for HPLC purification, [^{76}Br]β-CBT (t_R=12 min) eluted before the unlabelled precursors: β-CIT (t_R=15 min) or tributystannyl analogue, avoiding any risk of contamination of the radiopharmaceutical by the iodo compound.

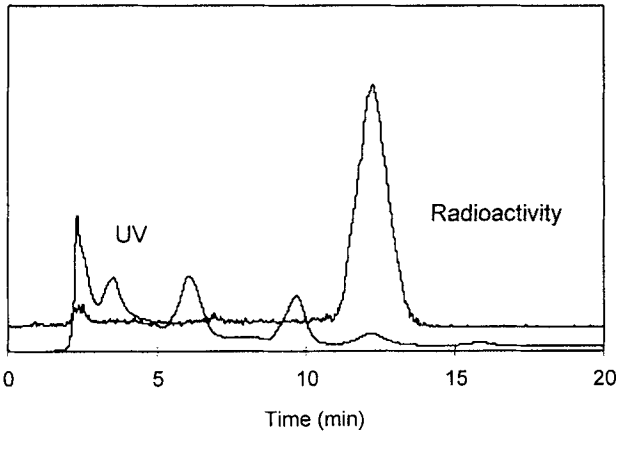


Figure 1. HPLC radiochromatogram of [^{76}Br]β-CBT purification

The total synthesis time including [^{76}Br]bromide preparation was 2.5 to 3.5 h, depending on the radiolabelling process. The total radiochemical yield was 60 to 80% allowing the preparation of more than 5 mCi (~200 MBq) of [^{76}Br]β-CBT in a sterile solution. The radiochemical purity checked by the TLC system was >98%. The specific radioactivity determined by HPLC was 0.5 Ci/μmol (18.5 GBq/μmol) which is satisfactory for studying the dopamine transporter. Preliminary quality control studies have shown that this radiopharmaceutical was sterile and pyrogen-free.

Pharmacological evaluation

The *in vitro* and *in vivo* pharmacological properties of β-CBT have been determined and compared to that of β-CIT. The binding of [^{76}Br]β-CBT to the striatal membranes was saturable and the specific binding i.e. the difference between the total binding minus the non specific binding showed a typical hyperbolic saturation curve (Figure 2). Analysis of saturation data using a non linear least square regression method revealed a single population of binding sites (nH = 1.03 and B_{max} = 6.5 pmol/mg protein) with an apparent dissociation constant of 2.8 nM.

The specificity of [^{76}Br]β-CBT to the dopamine transporter was checked by measuring the ability of selective monoaminergic agents to block the radiotracer binding to striatal membranes. The concentration of these agents that inhibited 50% of the radiotracer are: GBR 12909: 10 nM, mazindol: 50 nM, desipramine: 6 μM and citalopram: 8 μM. These values obviously indicated that β-CBT has pharmacological characteristics mainly associated with a binding to the dopamine transporter.

In the biodistribution studies and autoradiographic studies, the regional distribution of the radioligand paralleled the morphological localisation of dopamine nerve terminals which are

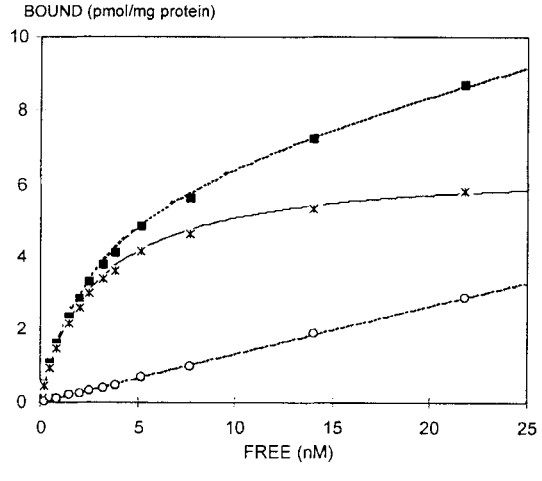


Figure 2. In vitro saturation curves of [^{76}Br]β-CBT to rat striatal membranes.
Total binding (■), specific binding (*), non specific binding (o).

known to be dense in striatum and less abundant in other brain regions especially in the cerebellum. Three hours post injection, the highest concentration in the brain was found in the striata (~2.5 % ID/g) while the cerebellar radioactive concentration (0.11 % ID/g) remained very similar to that of plasma. The striatum to cerebellum concentration ratio which reached a value of 22, 5 h post injection, was still 17, 24 h post injection.

CONCLUSION

In conclusion, [^{76}Br]β-CBT was prepared by bromination via electrophilic destannylation of the tributylstannyl analogue or via copper assisted bromodeiodination of β-CIT with no carrier added [^{76}Br]NH_4. The radiolabelling and the purification used resulted in radiochemical and chemical pure products. The pharmacological data obtained *in vitro* and *in vivo* indicated that [^{76}Br]β-CBT has the potential of being developed as a useful PET radiotracer for imaging dopamine uptake sites in pathological conditions.

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