Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 1033-1046 (2000)

SYNTHESIS OF [^{11}C]2ß-CARBOMETHOXY-3ß-(3´-IODO-4´-METHYL, -ETHYL AND ISOPROPYL PHENYL)NORTROPANE AS POTENTIAL RADIOTRACERS FOR EXAMINATION OF THE SEROTONIN TRANSPORTER WITH POSITRON EMISSION TOMOGRAPHY

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Summary

Research on depression and anxiety disorders would benefit from the development of suitable radioligands for PET-imaging of the serotonin transporter. Three cocaine analogues, 2ß-carbomethoxy-3ß-(3´-iodo-4´-methyl, -ethyl and isopropyl phenyl)nortropane (LBT-14, EINT and LBT-44), were prepared in a three-step synthesis by 1-4 addition of the appropriate Grignard reagent to anhydroecgonine methyl ester as first step. Iodination of the phenyl ring was accomplished with a mixture of yellow mercuric oxide, perchloric acid and acetic acid followed by a solution of iodine in dichloromethane. *N*-desmethylation was performed by using 2,2,2-trichloroethylchloroformiate followed by treatment of zinc in acetic acid. Acidic hydrolysis of the ester functions gave the carboxylic acid analogues of LBT-14, EINT and LBT-44. The precursors were labelled with ^{11}C using [^{11}C]methyl iodide or [^{11}C]methyl triflate in dimethyl formamide (DMF) and tetrabutyl ammonmium hydroxide (TBAH) as base. [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 were all examined in Cynomolgus monkey with PET. All three compounds entered the monkey brain to a high degree (~5-10% of injected dose). There was a marked uptake of radioactivity in the thalamus and the brainstem, regions known to contain serotonin transporters. Pre-treatment with the selective serotonin transporter inhibitor citalopram had minor effect on the binding ratios, suggesting that none of the three examined radioligands are preferable to the previously examined non-iodinated 4´-isopropenyl analogue [^{11}C]RTI-357 for the study of the serotonin reuptake system with PET.

Key Words : Positron Emission Tomography, EINT, LBT-14, LBT-44, Serotonin transporter, carbon-11.

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Received 12 May 2000
Accepted 24 May 2000

Introduction

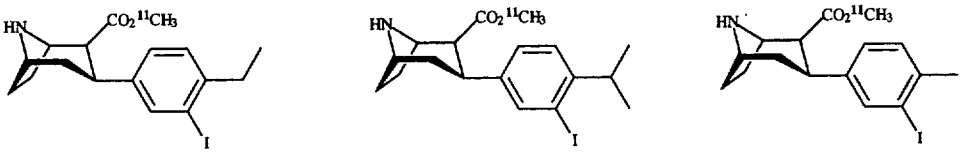
The serotonin transporter (5-HTT) is a membrane bound protein of clinical interest in the pathophysiology and treatment of depression and anxiety disorders. [^{11}C](+)McN5652 is so far the most promising PET radioligand proposed for examination of the 5-HTT in the human brain (1,2). However, [^{11}C](+)McN5652 do not fulfil all binding criteria for a suitable ^{11}C-radiolabeled ligand (3,4). The phenyltropanes ß-CIT and nor-ß-CIT are nonselective monoamine transporter ligands with high affinity for the dopamine transporter (DAT) as well as the 5-HTT. Both ligands have been used for imaging of the 5-HTT with PET and SPECT, but poor selectivity limits their use for quantitative studies (5-8).

Structure-activity relationship studies have shown that several phenyl nortropane derivatives bearing an alkyl or alkenyl group at the 4'-position of the aromatic ring have higher selectivity for the 5-HTT over the DAT when compared with nor-ß-CIT (9). From this series of compounds the [*O*-methyl-^{11}C]2ß-carbomethoxy-3ß-(4'-isopropenylphenyl)nortropane ([^{11}C]RTI-357) has earlier been evaluated as a potential PET radioligand for the 5-HTT (10). [^{11}C]RTI-357 has shown high uptake in brain structures such as the thalamus and the brainstem, regions known to contain high densities of 5-HTT. The binding was shown to be specific since [^{11}C]RTI-357 accumulation could partially be blocked with the selective serotonin transporter inhibitor citalopram.

The relative selectivity for 5-HTT over DAT can be further improved by the incorporation of iodine at the 3′-position of the aromatic ring (11). Blough *et al.* (11) have reported the synthesis of 2ß-carbomethoxy-3ß-(4'-ethyl-3'-iodophenyl)nortropane (EINT) a compound with sufficiently high affinity and specificity for the 5-HTT *in vitro* (IC_{50} = 0.6 nM) for successful visualisation of the 5-HTT with PET. Consequently, introduction of a γ-emitting isotope such as iodine-123 may provide a much-needed SPECT radioligand. Furthermore, the 3′-iodinated analogue 2ß-carbomethoxy-3ß-(4'-isopropyl-3'-iodophenyl)nortropane (LBT-44) has recently been synthesised and labelled with iodine-125 (12). *Ex vivo* experiments in rodents confirmed that [^{125}I]LBT-44 accumulated in brain regions rich on serotonin transporters.

To develop tracers suitable for both PET and SPECT, we report here the synthesis and carbon-11 labelling of three iodinated nortropane analogues: 2ß-carbomethoxy-3ß-(3'-iodo-4'-methyl, ethyl and isopropyl-phenyl)nortropane (LBT-14, EINT and LBT-44). The 4′-methyl analogue [^{11}C]LBT-14 (2ß-carbomethoxy-3ß-(4'-methyl-3′-iodophenyl)nortropane) was chosen due to being less lipophilic than EINT and LBT-44. The regional brain distribution and specific binding to

the 5-HTT *in vivo* was examined with PET in Cynomolgus monkeys. Unchanged radioligand in monkey plasma was determined by HPLC.



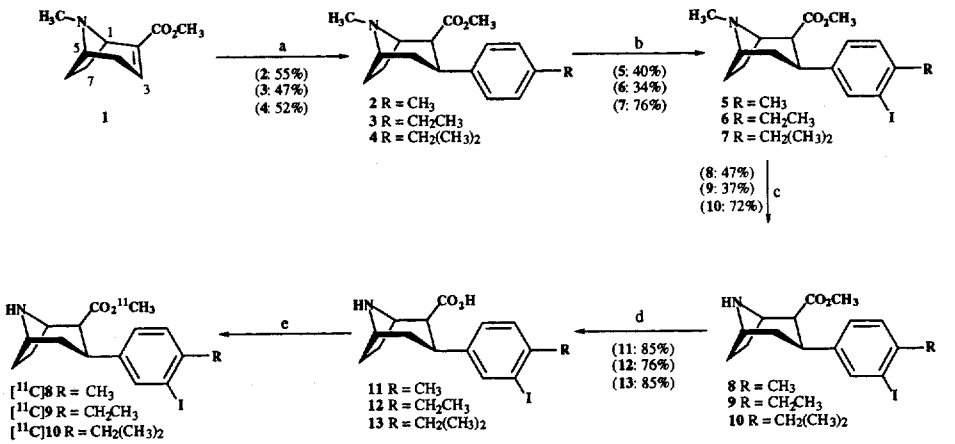
[^{11}C]EINT [^{11}C]LBT-44 [^{11}C]LBT-14

FIG. 1. Chemical structures of [^{11}C]EINT, [^{11}C]LBT-44 and [^{11}C]LBT-14.

Results and Discussion

Chemistry

The precursor syntheses (Scheme 1) for carbon-11 radiolabelling followed synthetic pathways already described (11,13,14). The corresponding Grignard reagent was added at –40°C to ecgonidine methyl ester followed by a hydrolysis at –78°C to afford compounds **2-3-4** after flash



Scheme 1 : Synthesis and radiolabelling of LBT-14, EINT and LBT-44. Reagents: (a) 4-alkylphenylmagnesium bromide/Et_2O; (b) (1) HgO/$HClO_4$/AcOH, (2) I_2/CH_2Cl_2; (c) (1) Cl_3CCH_2OCOCl, (2) Zn/CH_3COOH; (d) HCl/H_2O; (e) [^{11}C]MeI/TBAH/DMF ([^{11}C]***8***,[^{11}C]***9***) or [^{11}C]$MeSO_2CF_3$/TBAH/DMF ([^{11}C]***10***).

chromatography separation on silica gel. Direct iodination of the phenyl ring was conducted using a mixture of yellow mercuric oxide, perchloric acid and acetic acid followed by a solution of iodine in dichloromethane. The introduction of iodine at the 3' position of the phenyl ring was confirmed by NMR hydrogen carbon three bonds correlation, which exhibited interaction between carbon 3 of the tropane structure and two aromatic hydrogens. *N*-Desmethylation using 2,2,2-trichloroethylchloroformiate followed by a zinc-acetic acid treatment prepared nortropane analogues **8-9-10**. Finally hydrolysis of compounds **8-9-10** in aqueous hydrochloric acid gave the corresponding acid precursors **11-12-13** which were used in the labelling with [^{11}C]methyl iodide or [^{11}C]methyl triflate.

Radiochemistry

The preparation of [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 is based on an *O*-alkylation reaction of the free acid precursor using [^{11}C]methyl iodide or [^{11}C]methyl triflate in DMF with TBAH as base (Scheme 1). The incorporation of [^{11}C]methyl iodide to [^{11}C]LBT-14 and [^{11}C]EINT and [^{11}C]methyl triflate to [^{11}C]LBT-44 was in the range of 15-30% using 0.5 mg of the free acid precursor (Fig. 1). The total synthesis time was 30-35 minutes. The relatively low radiochemical yield is probably due to the alternative side-reaction which is an incorporation of [^{11}C]methyl iodide/triflate to the secondary amine function (Fig. 1). The use of either [^{11}C]methyl iodide or triflate did not affect the radiochemical yield. However, the yield was sufficient for initial studies in Cynomolgus monkey. The specific radioactivity for [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44, was about 200 Ci/mmol at time of injection and the radiochemical purity was in general better than 99%.

Positron Emission Tomography

[^{11}C]LBT-14 After intravenous injection of 52 MBq of [^{11}C]LBT-14 there was a high accumulation of radioactivity in the brain. Fifty minutes after injection 8% of the total radioactivity was present in the monkey brain (Fig. 3**A**). The highest uptake of radioactivity was observed in occipital and insular cortex whereas radioactivity was lower in the 5-HTT rich areas thalamus and brainstem. Hence, it could be concluded that the distribution of radioactivity was not in accordance with the known distribution of serotonin transporters. The uptake in thalamus and brainstem was still higher than in the cerebellum, a reference region with low amount of 5-HTT (Fig. 4**A**). When

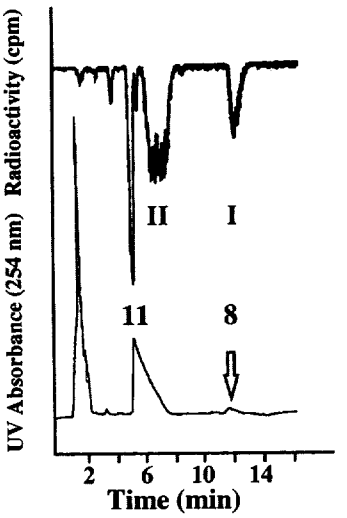


FIG. 2. Semipreparative HPLC chromatogram (U.V. and radioactivity versus time) using a Waters μ-Bondapak-C18 column for the preparation of [^{11}C]LBT-14. **I**, [^{11}C]LBT-14; **8**, LBT-14; **11**, LBT-14 acid precursor; **II**, [*N*-CH_3-^{11}C]LBT-14 acid.

citalopram was injected 10 minutes before injection of the radioligand there was no reduction of the regions of interest to cerebellum ratios. However, the pre-treatment with citalopram led to an increase in the total uptake of radioactivity in brain.

[^{11}C]EINT The accumulation of radioactivity in the monkey brain after injection of [^{11}C]EINT reached its maximum at 30 minutes when 4% of the total radioactivity injected was present in the brain (Fig. 3**B**). Highest uptake of radioactivity was observed in the thalamus and brainstem (Fig. 4**B**). At peak equilibrium (5), which was reached at 20 minutes after injection, the thalamus to cerebellum and brainstem to cerebellum ratios were 2.1 and 1.7, respectively. Following injection of citalopram in the pre-treatment experiment there was only a 10-20% reduction in the radioactivity ratios between [^{11}C]EINT binding in the thalamus or the brainstem to the cerebellum.

[^{11}C]LBT-44 Following injection of [^{11}C]LBT-44 radioactivity accumulated in the monkey brain over 25 minutes at which time 5% of the total radioactivity injected was present (Fig. 3**C**). Highest accumulation of radioactivity was observed in the thalamus and brainstem (Figs. 4**C**). The thalamus to cerebellum and brainstem to cerebellum ratios were 1.8 and 1.5, respectively, at peak

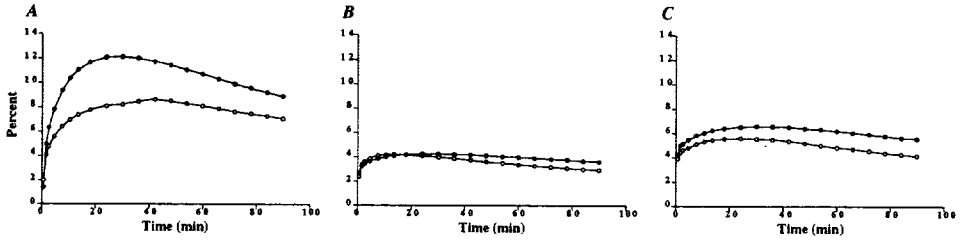


FIG. 3. Brain radioactivity in percent of injected dose of [^{11}C]LBT-14 (*A*), [^{11}C]EINT (*B*) and [^{11}C]LBT-44 (*C*) in Cynomolgus monkey brain, at baseline conditions (○) and after pre-treatment with citalopram (5 mg/kg) (●).

equilibrium, which was reached at 15 minutes after injection. The pre-treatment experiment with citalopram (5 mg/kg) did not reduce the radioactivity ratios of the thalamus to the cerebellum and the brainstem to the cerebellum to any significant degree. The total uptake of [^{11}C]LBT-44, however, was slightly higher in the pre-treatment experiment compared to the baseline study reaching a maximum of 6% at 10 minutes (Fig. 3C).

Only [^{11}C]EINT and [^{11}C]LBT-44 displayed uptake in accordance with the known distribution of 5-HTT. The signals could however, not be successfully inhibited by the selective 5-HTT-inhibitor citalopram. The introduction of iodine in the phenyl ring makes the compounds more lipophilic than their non-iodinated counterparts, and this increase in lipophilicity could contribute to a higher degree of non-specific binding. It is thus likely that a considerable part of the recorded signal has its origin in non-displaceable non-specific binding.

The increased brain uptake of [^{11}C]LBT-14 and [^{11}C]LBT-44 in pre-treatment experiments compared to controls could mainly be explained by displacement of peripheral binding to 5-HTT sites (i.e. in lung and intestinal tract) (Figs. 3A and 3C). Similar observations have been reported previously for other 5-HTT radioligands (10,15).

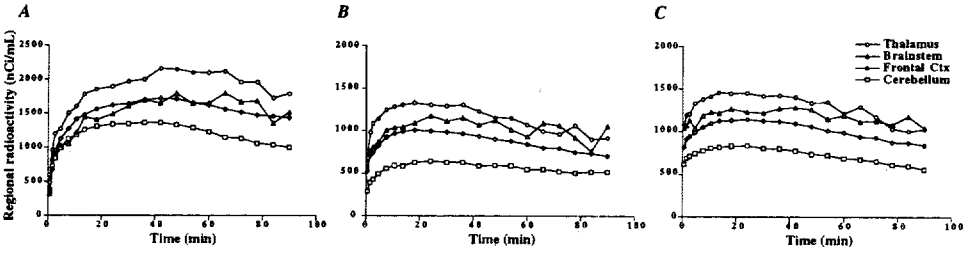


FIG. 4. Time courses for regional radioactivity (nCi/mL) in the brain of a Cynomolgus monkey after intravenous injection of [^{11}C]LBT-14 (*A*), [^{11}C]EINT (*B*) and [^{11}C]LBT-44 (*C*).

Plasma Metabolite Studies

The percentages of total radioactivity in plasma representing unchanged radioligand are shown in Figure 5A. [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 were all metabolised at a similar rate. The percentage of unchanged [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 in monkey plasma was about 35% at 45 minutes after injection. The injected radioactivity eluted from the HPLC column with a good resolution of unchanged radioligand from the labelled metabolites. It was observed that the radioligands were metabolised mainly to polar labelled metabolites, which should penetrate the blood-brain barrier poorly (Fig. 5B).

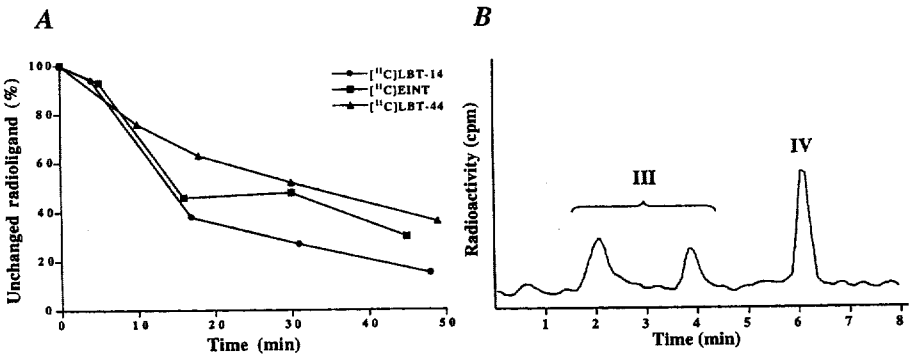


FIG. 5. Determination of unchanged radioligand in monkey plasma (% of total radioactivity versus time) of [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 (A). Gradient HPLC chromatogram of a plasma sample from a Cynomolgus monkey after i.v. injection of [^{11}C]EINT, obtained 16 min after administration of the radioligand. **III**, polar labelled metabolites; **IV**, [^{11}C]EINT (B).

Conclusion

In conclusion, it was demonstrated that the distribution of radioactivity after i.v. injection of [^{11}C]EINT and [^{11}C]LBT-44 was in accordance with the known distribution of serotonin transporters with high uptake in the thalamus and the brainstem. Pre-treatment experiments indicated that only [^{11}C]EINT may have potential as a radioligand for *in vivo* PET studies of the 5-HTT. However, none of the radioligands examined seem to be preferable to the previously examined non-iodinated 4′-isopropenyl analogue [^{11}C]RTI-357 (10).

Experimental

General

All chemicals were obtained from commercial sources and were of analytical grade. $^{11}CO_2$ was produced batchwise using the Scanditronix MC 16 cyclotron at the Karolinska Hospital/Institute by bombardment of a nitrogen gas target with 16 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction. Carbon-11-methyl iodide was synthesised from $^{11}CO_2$ utilising a one-pot reaction set-up similar to that reported previously (16).

Semipreparative reversed-phase HPLC was performed using a Waters μ-Bondapak C-18 column (300 x 7.8 mm, 10 μm) and a UV-detector (wavelength = 254 nm) in series with a GM-tube for radiation detection. [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 were purified with acetonitrile and 0.01 M H_3PO_4 (30/70, 35/65, 34/66) as the mobile phase with a flow rate of 6 mL/min. The radiochemical purity was analysed by reversed phase HPLC with a Waters μ-Bondapak C-18 column (300 x 3.9 mm, 10 μm) and a UV-detector (wavelength = 234 nm) in series with a Beckman ß-flow radiodetector for radiation detection. Acetonitrile and 0.01 M H_3PO_4 (30/70, 35/65, 34/66) were used as the mobile phase with a flow rate of 3 mL/min. The chemical identity of [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 were determined by coinjection of unlabeled LBT-14, EINT and LBT-44, respectively.

Chemistry

GENERAL PROCEDURE OF IODINATION OF 2β-CARBOMETHOXY-3β-(4′-ALKYL PHENYL)TROPANE (5-6-7)

Compounds 2,3 or 4 were dissolved in a mixture containing acetic acid (3.2 mL/mmol), $HClO_4$ (1.1 mL/mmol) and yellow mercuric oxide (212 mg/mmol). Iodine (685 mg/mmol) in CH_2Cl_2 (9 ml/mmol) and acetic acid (4.5 mL/mmol) was added dropwise. The mixture was allowed to stir at room temperature for one night, filtrated, basified with NH_4OH and extracted with CH_2Cl_2. The organic layers were dried and the solvents evaporated. After flash chromatography (**5**: hexane/ethylacetate/triethylamine 70/20/10 **6**: hexane/ ethylacetate/triethylamine 50/50/2 **7**: diethylether/ triethylamine 95/5), pure **5**, **6** and **7** was obtained (yields: derivative **5** 40%, derivative **6** 34%, derivative **7** 76%).

5: ^{1}H NMR ($CDCl_3$), δ: 1.52-1.62 (m, 3H, H-4α, H-6α, H-7α); 1.96-2.10 (m, 2H, H-6ß, H-7ß); 2.14 (s, 3H, N-CH_3); 2.28 (s, 3H, Ph-CH_3); 2.43 (td, 1H, $^3J_{3\text{-}4\beta} = {}^2J_{4\alpha\text{-}4\beta}$ = 12.6 Hz, $^3J_{4\beta\text{-}5}$ = 3.8 Hz, H-4ß); 2.77-2.88 (m, 2H, H-2, H-3); 3.26 (m, 1H, H-5); 3.45 (s, 3H, O-CH_3); 3.49 (m, 1H, H-1); 7.04 (d, 1H, $^3J_{1H}$ = 7.9 Hz, H-5´); 7.13 (dd, 1H, $^3J_{1H}$ = 7.9 Hz, $^4J_{1H}$ = 1.6 Hz, H-6´); 7.6 (s, 1H, $^4J_{1H}$ = 1.6 Hz, H-2´).

^{13}C NMR ($CDCl_3$), δ: 25.1, 25.7 (C6, C7); 27.4 (Ph-$\underline{C}H_3$); 31.8 (C-3); 33.9 (C4); 52.5 (C2); 62.0 (C5); 65.1 (C1); 100.8 (C-I); 129.1, 137.7 (3 CH_{ar}); 138.5 (C_{ar}); 142.5 (C_{ar}); 171.9 (C=O).

MS: m/z = 399 (M^+;9); 97 (34); 96 (37); 83 (100); 82 (79); 42 (30).

GENERAL PROCEDURE OF N-DESMETHYLATION OF 2β-CARBOMETHOXY-3β-(3´-IODO-4´-ALKYLPHENYL)TROPANE (8-9-10)

The tropane derivative was treated with 2,2,2-trichloroethylchloroformate (2.4 eq) and the mixture was heated at 120°C for 75 min. The excess of reagent was distilled off by *vacuum* (60-62°C/15 torr) yielding the corresponding crude carbamate which was dissolved in 95% acetic acid (7.6 mL/mmol) containing freshly activated zinc dust (12.3 eq). The reaction mixture was stirred at room temperature for 24 hours after which the solution was filtered through celite, treated with 15% ammonia (30 mL) and extracted twice with $CHCl_3$. The crude product was purified by means of flash chromatography (derivatives **5**-**6**: diethyl ether/triethylamine 75/25 and derivative **7** : ethyl acetate/hexane/triethylamine 50/40/10). The pure compound was isolated as a waxy substance (yields: derivative **8** 47%, derivative **9** 37%, derivative **10** 72%).

8: ^{1}H NMR ($CDCl_3$), δ: 1.42-1.68 (m, 3H, H-4α, H-6α, H-7α); 1.83-2.00 (m, 2H, H-6ß, H-7ß); 2.15-2.29 (td, 1H, , $^3J_{3\text{-}4\beta} = {}^2J_{4\alpha\text{-}4\beta}$ = 13.0 Hz, $^3J_{4\beta\text{-}5}$ = 2.9 Hz, H-4ß); 2.26 (s, 3H, Ph-C$\underline{H}_3$); 2.59 (dd, 1H, $^3J_{2\text{-}3}$ = 5.7 Hz, $^3J_{2\text{-}1}$ = 1.9 Hz, H-2); 3.04 (dt, 1H, $^3J_{3\text{-}4\beta}$ = 12.8 Hz, $^3J_{2\text{-}3} = {}^3J_{3\text{-}4\alpha}$ = 5.7 Hz, H-3); 3.32 (s, 3H, O-CH_3); 3.59 (m, 2H, H-1, H-5); 6.96 (dd, 1H, $^3J_{1H}$ = 7.8 Hz, $^4J_{1H}$ = 1.9 Hz H-6´); 7.02 (d, 1H, $^3J_{1H}$ = 7.8, H-5´); 7.51 (d, 1H_{ar}, $^4J_{1H}$ = 1.9 Hz, H-2´).

^{13}C NMR ($CDCl_3$), δ: 27.4 (Ph-$\underline{C}H_3$); 27.6, 29.0 (C6, C7) (C-3); 33.7 (C4); 34.8 (C3) 50.9 (C2); 51.1 (O-CH_3); 53.5, 56.2 (C1, C5); 101.0 (C-I); 127.0 (C6´); 129.3 (C-5´); 137.8 (C2´); 139.3, 141.8 (C1´, C4´); 173.6 (C=O).

MS: m/z = 385 (M^+;13); 141 (10); 83 (93); 82 (55); 59 (100); 68 (74).

GENERAL PROCEDURE OF HYDROLYSIS OF 2β-CARBOMETHOXY-3β-(3´-IODO-4´-ALKYLPHENYL)NORTROPANE (11-12-13)

The methyl ester derivative (**5**) (50 mg, 0.2 mmol) was refluxed in 11M aqueous hydrochloric acid solution (600 µL) overnight. Water was removed under *vacuum*. The compounds were isolated as hydrochloride salts. (yields: derivative **11** 85%, derivative **12** 76%, derivative **13** 85%).

11: ^{1}H NMR (D_2O), δ: 1.61-1.78 (m, 1H, H-4α); 2.00-2.24 (m, 4H, H-6α, H-7α, H-6ß, H-7ß); 2.15-2.29 (td, 1H, $^3J_{3\text{-}4ß} = {}^2J_{4α\text{-}4ß} = 13.0$ Hz, $^3J_{4ß\text{-}5} = 2.9$ Hz, H-4ß); 2.24 (s, 3H, Ph-CH_3); 2.88 (d, 1H, $^3J_{1H} = 6.6$ Hz, H-2); 3.35 (t, 1H, $^3J_{1H} = 7.0$ Hz, H-3); 4.17 (m, 2H, H-1, H-5); 7.09 (d, 1H, $^3J_{1H} = 7.9$ Hz, H-6´, H-5´); 7.16 (d, 1H$_{ar}$, $^3J_{1H} = 7.9$ Hz, H-6´, H-5´) 7.51 (s, H-2´).

^{13}C NMR (D_2O), δ: 25.2 (Ph-$\underline{C}H_3$); 26.1, 28.5 (C6, C7); 30.5 (C4); 33.4 (C3); 48.8 (C2); 55.5, 56.5 (C1, C5); 101.3 (C-I); 128.1, 130.4 (C-5´, C-6´); 138.3 (C-2´); 139.2, 141.6 (C-1´, C-4´); 176.4 (C=O).

12: ^{1}H NMR (D_2O), δ : 0.99 (t, 3H, $^3J_{2H} = 7.4$ Hz, $C\underline{H}_3$-CH_2); 1.36-2.15 (m, 5H, H-4α, H-6α, H-7α, H-6ß, H-7ß); 2.47-2.51 (m, 3H, CH_3-$C\underline{H}_2$, H-4ß); 2.85 (m, 1H, H-2); 3.17 (m, 1H, H-3); 4.10-4.18 (m, 2H, H-1, H-5); 7.09 (m, H-5´, H-6´); 7.58 (s, 1H, H-2´).

^{13}C NMR (D_2O), δ: 14.5 (C9); 24.8, 25.6 (C6, C7); 30.2 (C3); 33.0 (C-8); 33.5 (C4); 48.3 (C-2); 54.6, 55.9 (C1, C5); 100.6 (C-I); 127.7 (C-6´); 128.7 (C-5´); 138.1 (CH_{ar}); 139.1 (CH_{ar}), 145.2 (CHar); 175.4 (C=O).

13: ^{1}H NMR (MeOH D_4), δ: 1.10 (d, 6H, $^3J_{1H} = 6.8$ Hz); 1.79 (d, 1H, $^3J_{3,4ß} = 13.6$ Hz, H-4α); 2.02-2.30 (m, 4H, H-6α, H-7α, H-6ß, H-7ß); 2.27 (t, 1H, $^2J_{4α,4ß} = {}^3J_{3,4ß} = 13.6$ Hz, H-4ß); 2.90 (bd, 1H, H-2); 3.03-3.17 (m, 3H, H-3, N-H, $C\underline{H}(CH_3)_2$); 3.31-3.47 (m, 1H, H-3); 4.13 (m, 2H, H-1, H-5); 7.16 (m, 2H$_{ar}$); 7.63 (m, 1H$_{ar}$).

^{13}C NMR (MeOH D_4), δ: 22.6 ($CH(\underline{C}H_3)_2$); 25.3, 26.1 (C6, C7); 30.2 (C4); 33.0 (C3); 33.5 ($\underline{C}H(CH_3)_2$); 48.3 (C2); 55.7, 56.9 (C1, C5); 100.6 (C-I); 126.1 (CH_{ar}); 128.5 (CH_a); 140.0 (CH_{ar}); 139.8 (C_{ar}), 149.9 (Car); 175.6 (C=O).

Radiochemistry

[^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 were prepared in a similar procedure; [^{11}C]Methyl iodide ([^{11}C]LBT-14, [^{11}C]EINT) or [^{11}C]methyl triflate ([^{11}C]LBT-44) was trapped at room temperature in a reaction vessel (1.0 mL) containing the precursor (0.5 mg of the free acid precursor), DMF (300 µL) and TBAH (0.4 M, 6 µL). The vessel was sealed and heated at 80°C for 2 min. Mobile phase (600 µL) was added prior to injection into the semipreparative HPLC

column. The radioactive fraction containing the methylated radioligand was collected and after evaporation of the mobile phase the residue was dissolved in 6 mL sterile physiological phosphate buffer (pH = 7.4) solution and filtered through a Millipore filter (0.22 μm) yielding a solution which was sterile and free from pyrogens. The retention times of [^{11}C]LBT-14, [^{11}C]EINT and [^{11}C]LBT-44 on the preparative HPLC system were 11, 12 and 13 minutes, respectively.

Positron Emission Tomography

The PET-system used was Siemens ECAT EXACT HR which measures radioactivity in 47 slices with a separation of 3.3 mm and a spatial resolution of about 3.8 mm FWHM (Full Width Half Maximum) (17). Acquisition of PET data was performed in 3D mode.

Three Cynomolgus monkeys weighing 3.9, 5.7 and 4.1 kg were supplied by the Swedish Institute of Infectious Disease Control, Solna, Sweden. Anaesthesia was induced by repeated i.m. injection of ketamine (Ketalar® 10-15 mg kg^{-1} h^{-1}). The head was positioned so that the imaging planes were parallel to the canto-meatal line. A head fixation system was used to secure a fixed position of the monkey head during PET measurements (18). Body temperature was controlled by a heating pad with thermostat.

A total of six PET measurements were performed. Monkey number one was examined in a baseline study after injection of 52 MBq of [^{11}C]LBT-14 and a pre-treatment study in which the selective serotonin reuptake inhibitor Citalopram (5 mg/kg) was injected i.v. 10 minutes before injection of 52 MBq of [^{11}C]LBT-14. A similar protocol was used in monkey number two and three for the PET examination of [^{11}C]EINT (51 and 49 MBq injected) and [^{11}C]LBT-44 (49 and 50 MBq injected), respectively. The radioligands were injected i.v. in the left sural vein. Radioactivity in brain was measured according to a pre-programmed sequence of frames up to 108 minutes after radioligand injection.

Regions of interest were drawn on the PET summation images, which represent radioactivity measured from 9 min after i.v. injection to the end of the measurement. The thalamus, brainstem, cerebellum and the whole brain contour were defined according to an atlas of a cryosected Cynomolgus monkey head *in situ* (18). Radioactivity was calculated for the sequence of time frames, corrected for the radioactivity decay, and plotted versus time. The percent of radioligand injected that was present in brain at the time of maximal radioactivity was used as an index of radioligand uptake in brain. To calculate the percentage of injected radioligand the radioactivity

concentration in the ROI for the whole brain was multiplied with the brain volume (about 65 mL). The calculated value for radioactivity in the brain was then divided by the radioactivity injected and multiplied by 100.

Plasma Metabolite Studies

The percentage of unchanged radioligand in plasma was determined by an HPLC method that has been shown to be useful for a series of other PET radioligands (19). Blood samples (2 mL) were obtained at about 5, 15, 35 and 45 minutes after injection of radioligand. The supernatant (0.5 mL) obtained after centrifugation at 2000g for 1 min was mixed with acetonitrile (0.7 mL) containing a standard. The radioactivity in the supernatant (1.1 mL) obtained after centrifugation at 2000g for 1 min was measured in the well counter and 1 mL was subsequently injected into the HPLC column. The mixture was chromatographed through the column and the UV-absorption and radioactivity peaks were integrated and the data were stored in a PC.

The reversed-phase HPLC Kontron system consists of: 2 Kontron 420 pumps, a Rheodyne injector (7125 with a 1.0 mL loop) equipped with a Waters μ-Bondapak-C18 column (300 x 7.8 mm, 10 μm) and a Kontron 432 UV-spectrophotometer (254 nm) in series with a Packard Radiochromatography detector Series A-100 (1 mL cell). A mixture of phosphoric acid (0.01 M) (A) and acetonitrile (B) was used as the mobile phase with a flow rate of 6.0 mL/min. Gradient elution was employed on all metabolite analyses. The gradient profile was the following: HPLC time 0-5.5 minutes, (A/B) 75/25-40/60; 5.5-6.5 minutes, (A/B) 40/60-75/25; 6.5-8.0 minutes (A/B) 75/25 isocratic; 8.0 minutes end. The Kontron 450 Multitasking system was used as an efficient controller and PC-integration system.

Acknowledgements

The authors would like to thank Mr Göran Printz and Mr Dennis Eriksson for assistance with the radionuclide production and Ms Katarina Holmborn for technical assistance. The assistance of the members of the Stockholm PET group involved in the PET experiments is also greatly acknowledged. This work was supported by grants from the Swedish Medical Research Council (12983-01A), Karolinska Institutet, the Region Centre (France) and the French Ministry of Research and Technology.

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