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# Pharmacological Characterization of (*E*)-*N*-(4-Fluorobut-2-enyl)-2β-carbomethoxy-3β-(4′-tolyl)nortropane (LBT-999) as a Highly Promising Fluorinated Ligand for the Dopamine Transporter

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## ABSTRACT

In the aim to develop an efficient fluorinated probe for positron emission tomography (PET) exploration of the dopamine transporter (DAT), we studied several in vitro and in vivo characteristics of the phenyltropane derivative (*E*)-*N*-(4-fluorobut-2-enyl)-2β-carbomethoxy-3β-(4′-tolyl)nortropane (LBT-999). In vitro on rat striatal membrane, [$^{3}$H]LBT-999 bound to a single site with a $K_d$ of 9 nM, $B_{max}$ of 17 pmol/mg protein, and a very high selectivity for the DAT [$IC_{50}$ for 1-{2-[bis-(4-fluorophenyl)-methoxy]ethyl}-4-(3-phenylpropyl)piperazine (GBR 12909) and (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-methylphenyl)nortropane (PE2I): 2.4 and 18 nM, respectively; $IC_{50}$ for paroxetine, citalopram, *N,N*-dimethyl-2-(2-amino-4-methylphenyl thio)benzylamine, nisoxetine, and desipramine >1 μM]. In vitro on post-mortem human brain sections, LBT-999 bound with high intensity to the caudate-putamen, weakly to the thalamus, and not in the neocortex and cerebellum. This binding was totally abolished in the presence of PE2I. Ex vivo cerebral biodistribution of [$^{11}$C]LBT-999 in rats showed striatum/cerebellum radioactivity ratios of 18 and 25 at 30 and 60 min postinjection, respectively. This accumulation was strongly prevented by preinjection of GBR 12909, whereas paroxetine and nisoxetine had no effect. An in vivo kinetic PET study in three baboons showed a fast and very high uptake in the striatum, with a plateau at 30 min postinjection and a maximal putamen/cerebellum ratio of 30. Taken together, these findings demonstrate that LBT-999 is a highly promising agent for in vivo exploration of the DAT. This probe is currently labeled with $^{18}$F for further characterizations.

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As the membrane dopamine transporter (DAT) is very involved in a number of brain functions, in vivo exploration of this molecular target could become a relevant tool for the knowledge of pathophysiological mechanisms and diagnosis and follow-up of several neurological and psychiatric conditions. In this field, scintigraphic exploration of the DAT could be very useful for the early diagnosis and follow-up of Parkinson's disease. Most of these studies have been performed using single photon emission computerized tomography with radioligands chemically derived from the cocaine structure and labeled with $^{123}$I such as 2β-carbomethoxy-3β-(4-iodophenyl)tropane (Innis et al., 1993; Seibyl et al., 1998;

**ABBREVIATIONS:** DAT, dopamine transporter; FPCIT, *N*-3-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane; PET, positron emission tomography; FPCBT, *N*-(3-fluoropropyl)-2β-carbomethoxy-3β-(4-bromophenyl)nortropane; CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; FPCT, 2β-carbomethoxy-3β-(4-chlorophenyl)-8-(3-fluoropropyl)nortropane; FECNT, *N*-2-fluoroethyl-2β-carbomethoxy-3β-(4-chlorophenyl)-nortropane; PE2I, (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-methylphenyl)nortropane; LBT-999, (*E*)-*N*-(4-fluorobut-2-enyl)-2β-carbomethoxy-3β-(4′-tolyl)nortropane; HPLC, high-performance liquid chromatography; MADAM, *N,N*-dimethyl-2-(2-amino-4-methylphenyl thio)benzylamine; GBR12935, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine; ID, injected dose; ROI, region of interest; GBR 12909, 1-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine.

Schwarz et al., 2000), *N*-3-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (FPCIT) (Booij et al., 1999), altropane (Fischman et al., 1998), *N*-(3′-iodopropen-2′-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (Schwarz et al., 2000) or with $^{99m}$Tc such as TRODAT (Mozley et al., 2000). A major challenge of these investigations is to approach the most accurate in vivo quantification of the DAT density. For this specific aim, it seems that positron emission tomography (PET) imaging performs better than single photon emission computerized tomography because of its higher resolution. For PET imaging, several DAT radioligands labeled with $^{11}$C have now been proposed (Leenders et al., 1990; Guttman et al., 1997; Ilgin et al., 1999). However, as $^{18}$F can be more largely used for clinical purposes than $^{11}$C because of its longer physical period (110 versus 20 min), there is an increasing need to make available high-performance radioligands labeled with $^{18}$F. To date several cocaine derivatives labeled with $^{18}$F have been developed such as FPCIT (Chaly et al., 1996; Kazumata et al., 1998; Ma et al., 2002), *N*-(3-fluoropropyl)-2β-carbomethoxy-3β-(4-bromophenyl)nortropane (FPCBT) (Chaly et al., 2004), 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT) (Haaparanta et al., 1996; Laakso et al., 1998; Nurmi et al., 2000), 2β-carbomethoxy-3β-(4-chlorophenyl)-8-(3-fluoropropyl)nortropane (FPCT) (Goodman et al., 1997), and *N*-2-fluoroethyl-2β-carbomethoxy-3β-(4-chlorophenyl)-nortropane (FECNT) (Goodman et al., 2000; Deterding et al., 2001; Davis et al., 2003). Although they are interesting potential tools for PET exploration of the DAT, most of them require a prolonged period (>100 min or more) to reach specific binding equilibrium with the DAT and/or have a moderate selectivity for the DAT over serotonin transporter. The most adapted available ligand seems to be FECNT, with the most attractive kinetic characteristics by achieving peak striatal uptake at 60- to 75-min postinjection. As we had previously developed the tropane derivative (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-methylphenyl)nortropane (PE2I) characterized by the presence of a methyl group on the phenyl ring (Emond et al., 1997), which is highly efficient in terms of DAT selectivity and in vivo kinetics (Chalon et al., 1999; Pinborg et al., 2002; Halldin et al., 2003), we recently developed a fluorinated compound derived from the PE2I structure (Dollé et al., 2005). We present here the pharmacological properties of this new compound, (*E*)-*N*-(4-fluorobut-2-enyl)-2β-carbomethoxy-3β-(4′-tolyl)nortropane (LBT-999). For this characterization, we labeled LBT-999 with $^{3}$H for in vitro studies on brain tissue and with $^{11}$C for in vivo studies in the rat and monkey brain. As the present results show that it could be a very efficient tool for in vivo DAT quantification, LBT-999 is currently labeled with $^{18}$F for a detailed study of its in vivo properties in the primate.

## Materials and Methods

### Preparation of Nonlabeled LBT-999 and [$^{3}$H/$^{11}$C]LBT-999

LBT-999 and its *N*-desmethylated precursor for labeling (nor-LBT-999) were synthesized as described elsewhere (Dollé et al., 2005). The labeling of LBT-999 with tritium at its methyl ester function was performed as follows: 0.7 mg of nor-LBT-999 diluted in 300 μl of *N,N*-dimethyl formamide was mixed with 150 μl of [$^{3}$H]-methyliodide (370 MBq/ml, specific activity 3.1 TBq/mmol; Amersham, Uppsala, Sweden) and heated for 15 min at 90°C. After cooling to room temperature, 300 μl of acetonitrile was added, and the [$^{3}$H]LBT-999 was purified by HPLC using a $C_{18}$ reverse phase column (μBondapak; Waters, Milford, MA) and $CH_3CN$-$H_2O$-trifluoroacetic acid (35:65:0.1) as mobile phase. [$^{3}$H]LBT-999 was easily separated from the precursor and nonreacted [$^{3}$H]methyliodide and obtained with a specific activity of 3.1 TBq/mmol.

The labeling of LBT-999 with $^{11}$C ($t_{1/2}$: 20.4 min) at its methyl ester function was performed using standard conditions that have so far been used for the routine radiosynthesis of several radiotracers (Langer et al., 1999; Dollé et al., 2000, 2002; Sandell et al., 2000) and the efficient methylation reagent [$^{11}$C]methyl triflate. The conditions used were the following: 1) trapping at room temperature of [$^{11}$C]-methyl triflate in 300 μl of acetone containing 0.7 mg of nor-LBT-999 and 3 μl of a 3 M solution of NaOH in water; 2) concentration to dryness of the reaction mixture (at 110°C, using a helium stream for 1 min); 3) taking up the residue with 0.5 ml of the HPLC mobile phase; and 4) HPLC purification using a semipreparative $C_{18}$ reverse phase column (Symmetry C-18; Waters) and $CH_3CN$-$H_2O$-trifluoroacetic acid (35:65:0.1) as mobile phase. Typically, 5.55 to 9.25 GBq of [$^{11}$C]LBT-999 were routinely obtained within 30 min of radiosynthesis (including HPLC purification) with specific radioactivity ranging from 29.6 to 44.4 GBq/μmol.

### Animals and Drugs

All animal use procedures were in accordance with the recommendations of the European Community (86/609/CEE) and the French National Committee (décret 87/848) for the care and use of laboratory animals. Experiments in rats were conducted on male Wistar animals weighing 250 to 300 g (Centre d'Elevage R. Janvier, Le Genest St. Isle, France). The PET experiments were conducted in three male *Papio anubis* baboon weighing 10 to 15 kg.

Desipramine and nisoxetine were obtained from RBI Bioblock (Illkirch, France), paroxetine was a gift from Smith-Kline-Beecham (Nanterre, France), and citalopram was a gift from Lundbeck (Copenhagen, Denmark). PE2I and *N,N*-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine (MADAM) were synthesized according to previously published data (Emond et al., 1997, 2002).

### In Vitro Binding Assays on Rat Striatal Membranes

**Tissue Preparation.** Male rats were killed by decapitation on the day of the assay, and both striata of each animal were removed on ice and weighed (two rats were used for each experiment). The tissue was homogenized in 10 volumes of 0.32 M sucrose using an Ultraturrax T25. After 1000*g* centrifugation at 4°C for 10 min, the supernatant was kept, and the pellet was treated as described above. Both supernatants were then pooled and centrifuged at 17,500*g* for 30 min at 4°C. Twenty volumes of the incubation buffer was added to the pellet, and the mixture was homogenized and centrifuged at 50,000*g* for 10 min at 4°C. The final pellet was suspended in a minimum volume of the assay buffer, and the protein concentration was measured according to Bradford (1976) using bovine serum albumin as the standard.

**Saturation Studies.** [$^{3}$H]LBT-999 (3 KBq, 10 pmol) was incubated with unlabeled LBT-999 at concentrations varying between 0.2 and 50 nM with 30 μg of protein in a total volume of 1 ml in a pH 7.4 Tris-HCl buffer (50 mM Tris-HCl, 120 mM NaCl, and 5 mM KCl) for 90 min at 22°C. Nonspecific binding was determined in the presence of 1 μM PE2I. Samples were then rapidly filtered through Whatman GF/C fiber filters soaked with 0.05% polyethylenimine (Sigma Chemical, St. Quentin-Fallavier, France). The filters were washed twice with 4 ml of cold buffer, and the residual radioactivity was measured in a beta counter (LKB 1215 Rackbeta) in the presence of 6 ml of scintillator (LKB Optiphase). Results were analyzed with the EBDA RADLIG program (Biosoft, Cambridge, UK).

**Competition Studies.** For these studies, [$^{3}$H]LBT-999 (3 KBq, 10 pmol) together with 10 nM unlabeled LBT-999 were incubated with 30 μg of protein in a total volume of 1 ml in a pH 7.4 Tris-HCl buffer

(50 mM Tris-HCl, 120 mM NaCl, and 5 mM KCl) for 90 min at 22°C, in the presence of different drugs: PE2I or GBR12935 at concentrations of $10^{-8}$ to $10^{-11}$ M or paroxetine, citalopram, MADAM, desipramine, or nisoxetine at concentrations of $10^{-5}$ to $10^{-10}$ M. Samples were then treated as described above. Total binding was determined in the absence of any drug, and nonspecific binding was measured in the presence of 1 $\mu$M PE2I. The $IC_{50}$ values were determined graphically for each compound.

### In Vitro Autoradiographic Studies on Human Post-Mortem Brain

The human brain used was obtained from clinical autopsy at the National Institute of Forensic Medicine (Karolinska Institutet, Stockholm, Sweden) and handled as previously described (Hall et al., 1998, 2001). The study was approved by the Ethics Committee at Karolinska Institutet and the Swedish Board of Social Welfare. Cryosectioning on whole hemisphere sections was performed as previously described (Hall et al., 1998, 2001). Experiments were performed on 100-$\mu$m horizontal sections.

For the autoradiography studies, sections were incubated for 60 min at 22°C with 2 nM [$^3$H]LBT-999 in 10 ml of a phosphate buffer (10.14 mM $NaH_2PO_4$, 137 mM NaCl, 2.7 mM KCl, and 1.76 mM $KH_2PO_4$), pH 7.4. Nonspecific binding was defined on adjacent sections incubated in the presence of an excess (10 $\mu$M) of PE2I. Sections were then washed twice for 10 min in cold phosphate buffer and rinsed for 1 s in distilled water. After drying, the sections were exposed to film (Kodak Biomax MR; Amersham) for 12 weeks before development (developer: Kodak D19; fixation: Kodak Fixer 3000). The autoradiograms were digitized using a Scan Maker E6 high-resolution scanner (Microtek) and Adobe PhotoShop 6.5.

### Ex Vivo Cerebral Biodistribution in Rats

**Kinetic Study.** Twelve rats received an i.v. injection of [$^{11}$C]LBT-999 (2.22 MBq) and were sacrificed 10, 30, or 60 min postinjection (four animals per group). Samples of blood and cerebral regions (cerebellum, frontal cortex, and striatum) were removed, rapidly wiped (brain samples), and weighed, and radioactivity was measured in a gamma counter (Cobra Quantun; Packard). Uptake was expressed as the percentage of injected dose (ID) per gram of tissue.

**Competition Study.** Six rats per group received an i.v. injection of [$^{11}$C]LBT-999 (0.74 MBq) without (control) or with a preinjection (3 min) of drug (GBR 12909, paroxetine, or nisoxetine) at a dose of 5 mg/kg i.v. They were sacrificed 30 min after injection of [$^{11}$C]LBT-999. Samples of blood and cerebral regions (cerebellum, frontal cortex, and striatum) were removed, rapidly wiped (brain samples) and weighed, and radioactivity was measured in a gamma counter (Cobra Quantun; Packard). Uptake was expressed as the percentage of injected dose per gram of tissue.

### In Vivo Positron Emission Tomography Studies in Baboons

The PET study was performed with a CTI HR + Exact positron tomograph (CTI PET Systems, Knoxville, TN). This scanner allowed simultaneous acquisition of 63 slices every 2.2 mm with spatial and axial resolutions of 4.5 mm. Each animal was anesthetized using 1% isoflurane and 33% $O_2$/66% $N_2O$, controlled by an Ohmeda ventilator (OAV 7710; Ohmeda, Madison, WI). The tidal volume was adjusted to achieve stable end-tidal carbon dioxide tension between 38 and 40 mm Hg. The baboon's head was fixed in a head-holder and positioned in the scanner gantry for axial plane acquisition. A venous catheter was inserted for [$^{11}$C]LBT-999 administration and a femoral arterial catheter was used for blood sampling.

A transmission scan ($^{68}$Ge rods) was acquired for 15 min and used for subsequent correction attenuation of emission scans. The image acquisition started (T0 min) at the intravenous injection of [$^{11}$C]LBT-999 (419 MBq; specific radioactivity, 14 GBq/$\mu$mol) and lasted 80 min (T80 min). Twenty-four images were acquired with scan duration starting from 30 s and increasing up to 10 min during each experiment.

**PET Data Analysis.** The regions of interest (ROIs) were identified and drawn on 1.5-T magnetic resonance imaging scans images (SIGNA system; General Electric, Milwaukee, WI), which were matched with PET images (anatomist) and resliced according to the PET images. The ROIs were then copied onto the PET images. The averages of the radioactivity concentration in the different ROIs were then calculated and expressed as Becquerels per milliliter. The means of the corresponding left and right hemisphere ROI values were averaged. Tissue-time activity curves were constructed by plotting the percentage of injected dose per 100 ml of tissue. The cerebellum was used as reference in the analysis. The ROIs to cerebellum ratios were determined at 110 min postinjection.

### Statistical Analysis

For ex vivo cerebral biodistribution studies in rats, comparisons between groups (competition study) were performed using Student's $t$ test.

## Results

### In Vitro Binding on Rat Striatal Membranes

**Saturation Studies.** The affinity and density of specific [$^3$H]LBT-999 binding sites were measured by saturation experiments on rat striatal membranes. In our experimental conditions, the nonspecific binding, determined in the presence of 1 $\mu$M PE2I was ~1.5%. The Scatchard transformation of the resulting data (Fig. 1) revealed a linear curve suggesting a one-site model (Hill coefficient = 1) with a $K_d$ value of 9.15 ± 2.8 nM (mean ± S.D. of three independent determinations, each performed in triplicate) and a $B_{max}$ value of 17.47 ± 2.73 pmol/mg protein (mean ± S.D.).

**Competition Studies.** The pharmacological profile of specific [$^3$H]LBT-999 binding in the striatum was consistent

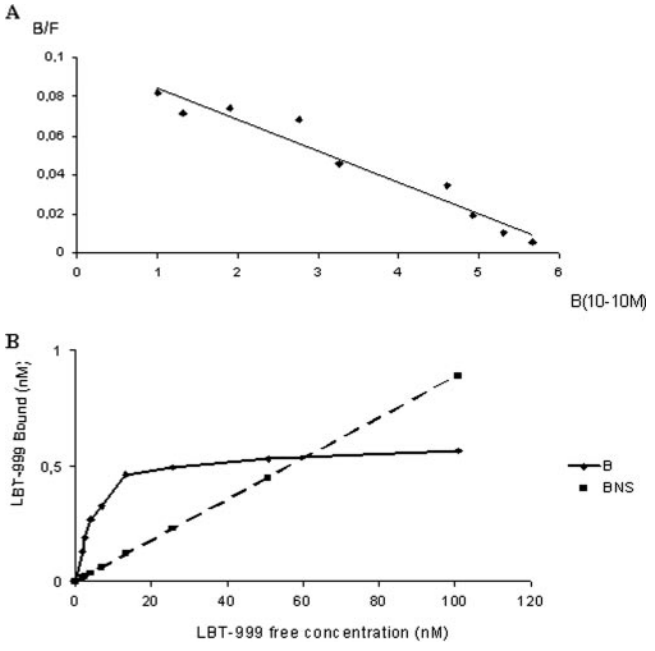


**Fig. 1.** Saturation experiments of [$^3$H]LBT-999 binding in the rat striatum. Striatal membranes (30 $\mu$g of protein/assay) were incubated with a constant concentration of [$^3$H]LBT-999 (10 pmol) and increasing concentrations of unlabeled LBT-999 (0.2–50 nM) in pH 7.4 Tris-HCl buffer for 90 min at 22°C. Nonspecific binding was determined in the presence of 1 $\mu$M PE2I. A, one representative saturation curve. B, Scatchard transformation of the data. B/F, bound/free.

with binding to the DAT (Fig. 2), as the rank order of potency of membrane transporter-drugs was GBR 12909 = PE2I > nisoxetine > desipramine = citalopram = paroxetine = MADAM (Table 1).

### Autoradiographic Studies in Human Post-Mortem Brain Sections

As shown in Fig. 3, [$^3$H]LBT-999 bound with high intensity to the basal ganglia (putamen and nucleus caudatus) of the human brain section. Weak binding could be seen in the thalamus, whereas there was virtually no or very low [$^3$H]LBT-999 binding in the cortical regions. The labeling of DAT with [$^3$H]LBT-999 was totally abolished in the presence of 10 $\mu$M PE2I.

### Ex Vivo Cerebral Biodistribution in Rats

**Kinetic Study.** Whole-blood radioactivity showed stable values 10 to 60 min postinjection (0.25 ± 0.02, 0.22 ± 0.02, and 0.15 ± 0.01% ID/g, respectively). Whatever the time postinjection, the highest accumulation of [$^{11}$C]LBT-999 was observed in the striatum compared with the cortex and cerebellum (Fig. 4). Radioactivity in the striatum was stable from 10 to 30 min postinjection (4.90 ± 0.36 and 4.95 ± 0.25% ID/g, respectively) and was slightly decreased at 60 min (3.37 ± 0.61% ID/g). The cortex and cerebellum displayed a weak degree of radioactivity from 10 to 60 min postinjection. The striatum/cerebellum radioactivity ratio reached 7, 18 and 25 at 10, 30 and 60 min postinjection, respectively.

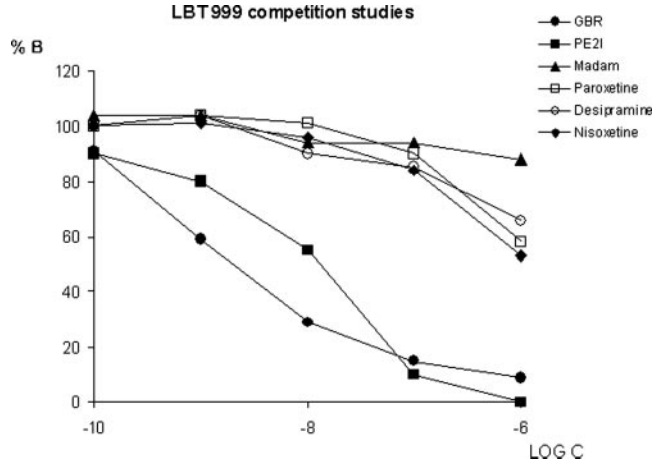


**Fig. 2.** Displacement of [$^3$H]LBT-999 binding in rat striatum by various drugs. Striatal membranes were incubated with 10 pmol of [$^3$H]LBT-999 together with 10 nM LBT-999 and increasing quantities of drugs ($10^{-11}$–$10^{-5}$ M). Each curve is representative of three independent experiments, each performed in triplicate. The inhibitory constants of the drugs are given in Table 1.

TABLE 1

Inhibition of specific [$^3$H]LBT-999 binding in rat striatal membranes

A mixture of 10 pmol of [$^3$H]LBT-999 and 10 nM unlabeled LBT-999 was incubated with 30 $\mu$g of protein in Tris-HCl buffer, pH 7.4, for 90 min at 22°C in the presence of different drugs at concentrations of $10^{-11}$ to $10^{-5}$ M. Total binding was determined in the absence of drugs and nonspecific binding in the presence of 1 $\mu$M PE2I. $IC_{50}$ values were determined graphically. Values are means ± S.D. of three independent experiments each performed in triplicate.

| Drug | $IC_{50}$ |
|---|---|
| | *nM* |
| GBR12935 | 2.4 ± 0.5 |
| PE2I | 18.0 ± 3.0 |
| Paroxetine | >1000 |
| Citalopram | >1000 |
| MADAM | >1000 |
| Nisoxetine | >1000 |
| Desipramine | >1000 |

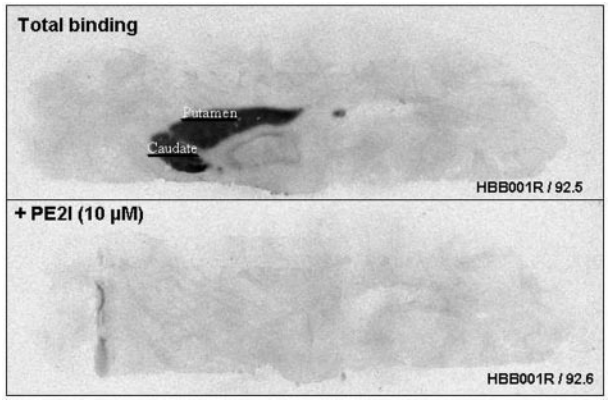


**Fig. 3.** Distribution of dopamine transporters in the human brain (horizontal sections) as visualized using [$^3$H]LBT-999 (2 nM). Nonspecific binding was determined in the presence of 10 $\mu$M PE2I. Numbers in the bottom right corner represent brain number and distance from vertex in millimeters.

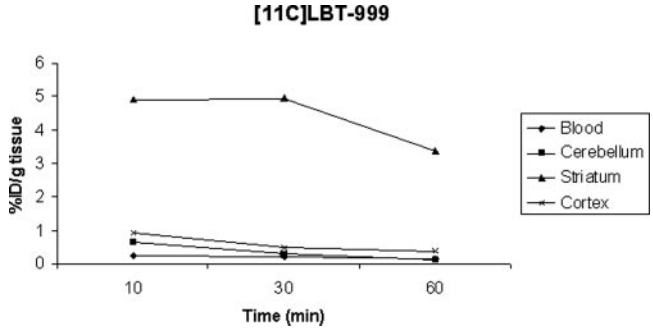


**Fig. 4.** Time course of [$^{11}$C]LBT-999 concentration in blood and cerebral areas (cerebellum, striatum, and cortex) at 10, 30, and 60 min after i.v. injection in the rat. $n$ = 4 per time.

**Competition Study.** As shown in Fig. 5, in vivo cerebral uptake of [$^{11}$C]LBT-999 in control rats reached 5.03 ± 0.7% ID/g in the striatum, with a striatum/cerebellum ratio of 15 at 30 min postinjection. Preinjection of GBR 12909 (5 mg/kg i.v.) strongly decreased the uptake of [$^{11}$C]LBT-999 (~70%) in the striatum (1.16 ± 0.3% ID/g, $p < 0.01$), whereas no effect was observed consecutively to a paroxetine preinjection (3.99 ± 0.3% ID/g), and a slight effect was observed with a nisoxetine preinjection (3.44 ± 0.5% ID/g, $p < 0.05$).

In the cerebellum, the radioactivity level remained low and similar in the four groups of animals (0.29 ± 0.07, 0.27 ± 0.06, 0.23 ± 0.06, and 0.26 ± 0.07% ID/g for the control, GBR 12909, paroxetine, and nisoxetine groups, respectively). In the cortex, a 30% decrease in the accumulation of [$^{11}$C]LBT-999 was observed in the GBR 12909 compared with the control group (0.36 ± 0.04 versus 0.58 ± 0.18% ID/g, $p < 0.05$), whereas no significant difference was observed for the paroxetine (0.51 ± 0.10% ID/g) and nisoxetine (0.64 ± 0.07% ID/g) groups.

### In Vivo PET Study in Baboon

Time activity curves (Fig. 6) of the concentration of [$^{11}$C]LBT-999 in different regions of the brain show that the highest accumulation was observed in the striatum with a peak uptake at 40 min (16.3 and 13.3% ID/100 ml in putamen

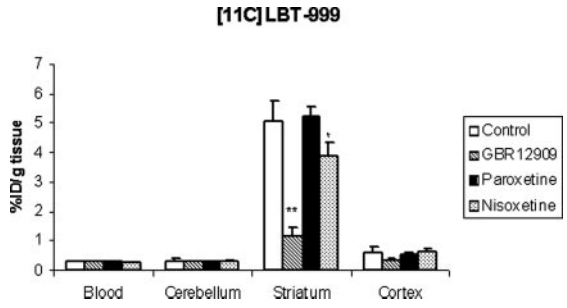


**Fig. 5.** Cerebral biodistribution of [$^{11}$C]LBT-999 in rats: competition study. Animals received a preinjection of GBR 12909, paroxetine, or nisoxetine at the dose of 5 mg/kg 3 min before [$^{11}$C]LBT-999 i.v. injection and were sacrificed 30 min later. $n = 6$ per group. **, $p < 0.01$; *, $p < 0.05$.

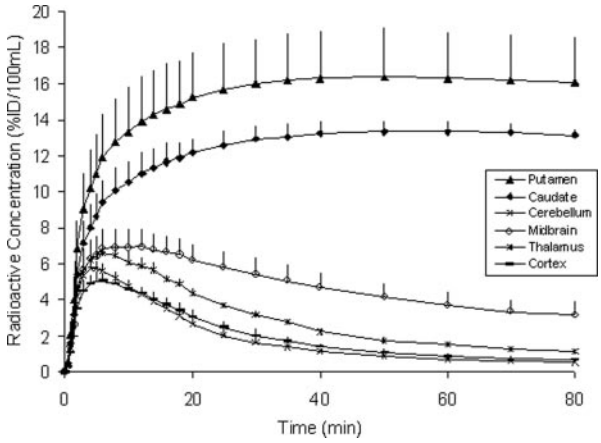


**Fig. 6.** Time-activity curves in various brain structures in baboon after intravenous injection of [$^{11}$C]LBT-999.

and caudate, respectively). The washout was then very slow from 30 to 80 min. Radioactivity uptake in the midbrain peaked at 12 min (6.9% ID/100 ml) and decreased with time. The lowest uptake was observed in the cortex and cerebellum (1.37 and 1.09% ID/100 ml, respectively).

The ROIs to cerebellum radioactivity ratio were calculated at 80 min postinjection. This ratio was very high in the striatum (30 and 24.6 in the putamen and caudate, respectively), intermediate in the midbrain (5.9), and low in the thalamus (2.1) and cortical structure (1.2).

## Discussion

With the aim of developing at term an efficient DAT probe for PET exploration with $^{18}$F, we studied several in vitro and in vivo characteristics of the phenyltropane derivative LBT-999, labeled in the present study with $^{3}$H or $^{11}$C, and demonstrated that this ligand is a highly promising imaging agent. Its in vitro pharmacological properties on rat striatal membranes are very close to those of PE2I (Chalon et al., 1999). It binds to a single high-affinity site with a moderate affinity (9 nM) closely related to that of other fluorinated tropane derivatives such as FPCIT (Neumeyer et al., 1996), FPCBT (Chaly et al., 2004), CFT (Meltzer et al., 1993), FPCT (Goodman et al., 1997), FECNT (Goodman et al., 2000), and PE2I (Chalon et al., 1999) and has a very high selectivity for the DAT. The $IC_{50}$ values of GBR12935 and PE2I versus LBT-999 were 2.4 and 18 nM, respectively, and we did not find competition ($IC_{50} > 1$ $\mu$M) between LBT-999 and several serotonin transporter ligands [citalopram, paroxetine, and *N,N*-dimethyl-2-(2-amino-4-methylphenyl thio)benzylamine] as well as norepinephrine transporter ligands (nisoxetine and desipramine). This selectivity is higher than that observed in vitro for other DAT ligands from the chemical tropane family such as FPCIT (Neumeyer et al., 1996), FPCBT (Chaly et al., 2004), CFT (Meltzer et al., 1993), FPCT (Goodman et al., 1997), and FECNT (Goodman et al., 2000). The selectivity of LBT-999 as indicated from the in vitro binding data on rat brain was clearly confirmed in the postmortem human autoradiographic study in which it bound highly to the caudate-putamen and only weakly to the thalamus and neocortical areas, as already observed for PE2I (Hall et al., 1999). This binding was achieved at a concentration (2 nM) that was near to the $K_d$ and was totally abolished in the presence of a saturating concentration of PE2I.

These homogenate and autoradiographic assessments were thus very useful as an initial view of the affinity/selectivity properties of LBT-999 as a DAT probe but did not totally predict its in vivo characteristics. In a first step, ex vivo cerebral biodistribution in rats demonstrated that the ligand had a rapid and high entry into the brain where its distribution was in agreement with specific binding to the DAT. The brain entrance of LBT-999 in rats (mean value of 4.95% ID/g in the striatum at 30 min postinjection) was higher than that for PE2I (1.06% in same experimental design) (Guilloteau et al., 1998), as well as the striatum/cerebellum ratio (18 for LBT-999 versus 10 for PE2I). The striatal accumulation of LBT-999 was around 70% decreased in the presence of a saturating dose of GBR 12909, as already observed for PE2I. In contrast, no significant effect was observed with a preinjection of paroxetine and a low significant 18% decrease was observed with nisoxetine. It can be hypothesized that this latter corresponds to a nonspecific binding rather than a binding of LBT-999 to the norepinephrine transporter, which is practically absent from the striatum (Sanders et al., 2005).

An in vivo kinetic study in the baboon confirmed that LBT-999 brain uptake was fast, high, and mainly localized in the putamen and caudate. The peak uptakes in these regions reached 17.1 and 12.8% ID/100 ml, respectively, at 30 min postinjection. This peak appeared earlier than that observed for [$^{11}$C]PE2I in human brain (55–65 min postinjection) (Halldin et al., 2003) and for $^{18}$F-labeled DAT tracers such as CFT (225 min postinjection) (Laakso et al., 1998), FPCT (60 min postinjection) (Goodman et al., 1997), FECNT (90 min postinjection) (Davis et al., 2003), and FPCIT (40 min postinjection) (Kazumata et al., 1998). An undesired property accompanying DAT binding by the fluorinated tracers available to date is the need for a prolonged time required to reach binding equilibrium, and it can be hypothesized from these preliminary data that LBT-999 would not have this drawback. The regional distribution of LBT-999 in baboon brain was in agreement with the known DAT localization with the highest density in the putamen and caudate, lower in the midbrain, much lower in the thalamus, and very low in the cortex and cerebellum. The maximum striatum/cerebellum ratio reached the very high values of 30 and 24.6 in the putamen and caudate, respectively. This ratio was higher than the ratios previously obtained for all previously cited tracers, thus showing that LBT-999 is the in vivo DAT probe that possesses the highest signal/noise ratio. The specificity

of LBT-999 in vivo binding on the DAT in the baboon brain is currently studied using competition experiments. From the present data obtained in vitro on rat striatal membranes and post-mortem human brain as well as ex vivo in the rat, it can be strongly hypothesized that LBT-999 will selectively bind to the DAT in primate brain. Taken together, these findings suggest that LBT-999 could be a very efficient tool for in vivo detection of DAT variations, thus allowing, e.g., early diagnosis of Parkinson's disease, monitoring its progression, or assessing the efficacy of therapies as well as improving the etiology and pathophysiology of lesser known central nervous system conditions such as attention-deficit/hyperactivity disorders (Jucaite et al., 2005).

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