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Carbon-11-NNC 112: A Radioligand for PET Examination of Striatal and Neocortical D_1-Dopamine Receptors

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The aim of this work was to explore the potential of a selective D_1-dopamine receptor antagonist as a new radioligand for PET examination of striatal and neocortical D_1-dopamine receptors. **Methods:** The active (+)- and inactive (−)-enantiomers of [^{11}C]NNC 112 were radiolabeled using the N-methylation approach and were examined by PET in cynomolgus monkeys and healthy men. Metabolite levels in plasma were measured by gradient high-performance liquid chromatography (HPLC). **Results:** N-methylation of the corresponding desmethyl precursors with [^{11}C]methyl triflate gave high total radiochemical yield (50%–60%) and specific radioactivity (110 GBq/μmol). (+)-[^{11}C]NNC 112 binding in cynomolgus monkeys was 5.77 ± 0.31 and 2.36 ± 0.14 times higher in the striatum and neocortex, respectively, than in the cerebellum at a transient equilibrium that appeared 40–50 min after injection. The binding of (+)-[^{11}C]NNC 112 is stereoselective, because the brain distribution of the inactive (−)-enantiomer was on an equally low level for all brain regions. Displacement and pretreatment experiments using unlabeled SCH 23390 and ketanserin confirms that (+)-[^{11}C]NNC 112 binds specifically and reversibly to D_1-dopamine receptors. The radioactivity ratios of the striatum, frontal cortex and nucleus accumbens to the cerebellum were 3.8–4.0, 1.7–2.0 and 2.8–3.1, respectively, at a transient equilibrium that appeared 40–50 min after injection in four healthy human subjects. Linear graphical analysis gave distribution volume ratios of 3.9 and 1.5 in the putamen and frontal cortex, respectively. The fraction of the total radioactivity in human plasma representing unchanged (+)-[^{11}C]NNC 112 was 85% at 5 min and 25% at 75 min after injection. **Conclusion:** (+)-[^{11}C]NNC 112 should be a useful PET radioligand for quantitative examination of not only striatal but neocortical D_1-dopamine receptors in man.

Key Words: brain; D_1-dopamine receptors; striatal; neocortical; extrastriatal; NNC 112; carbon-11; PET

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PET and the radioligand [^{11}C]SCH 23390 have been used to examine D_1-like dopamine receptors (D_1R) in the monkey and human brain (*1–5*). There is a high density of D_1R in the basal ganglia and also in the neocortex. The neocortical density is about 30% of that in the striatum as determined in vitro and in vivo (*6,7*).

Studies on brain morphology, biochemistry and physiology indicate that the function of extrastriatal brain regions may be disturbed in patients with schizophrenia (*8*). In a recent PET study, Okubo et al. (*9*) found a reduction in D_1R binding in the prefrontal cortex of patients with schizophrenia. The reduction was significantly correlated to negative symptoms.

A problem in using [^{11}C]SCH 23390 is that the neocortex-to-cerebellum ratio is lower than 1.5, which is not ideal for detailed quantitative examination of D_1R in the neocortex (*1*). It is important to develop a selective PET radioligand that provides a high signal-to-noise ratio in the neocortical brain region.

(+)-NNC 112 is a new benzazepine ((+)−8-chloro-5-(7-benzofuranyl)−7-hydroxy-3-methyl-2,3,4,5-tetra-hydro-1H-3-benzazepine, Fig. 1) that has high affinity for D_1R, 100-fold lower affinity for 5-HT_{2A}, and virtually no affinity for other putative central receptors (Tables 1 and 2) (*10–12*). In vivo binding studies in rodents have demonstrated that the behavioral and biochemical effects of NNC 112 are closely correlated with the D_1R occupancy (*10*).

The aim of this study was to develop the selective D_1R antagonist (+)-[^{11}C]NNC 112 as a new radioligand for PET. Stereoselectivity was examined with the two enantiomers of [^{11}C]NNC 112. The specificity of binding was examined by displacement and pretreatment experiments. Radioligand me-

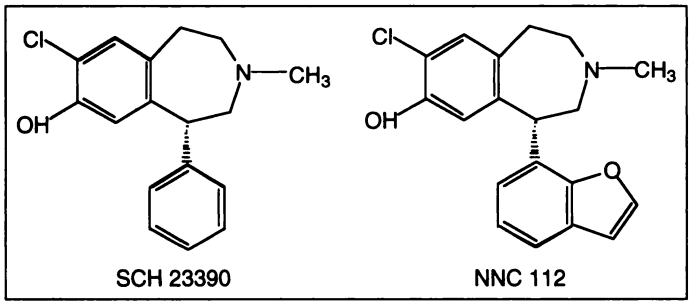

FIGURE 1. Structural formula for SCH 23390 and NNC 112.

tabolism was measured in plasma by gradient high-performance liquid chromatography (HPLC). Transient equilibrium (*13*) and linear graphical analysis (*14*) were used to calculate radioactivity ratios and volume-distribution ratios, respectively.

MATERIALS AND METHODS

General Chemistry

(+)−8-chloro-5-(7-benzofuranyl)−7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (+)-NNC 112 and (−)−8-chloro-5-(7-benzofuranyl)−7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (−)-NNC 112 were prepared according to the literature (U.S. Patent 4,751,222). Other chemicals were obtained from commercial sources and were of analytical grade whenever possible. [^{11}C]Methyl iodide was synthesized from [^{11}C]carbon dioxide using a one-pot reaction setup similar to that reported previously (*15*). The [^{11}C]methyl triflate was prepared on-line from [^{11}C] methyl iodide transferred through a soda glass column (oven temperature, 170°C) containing silver triflate-impregnated graphitized carbon. [^{11}C]methyl triflate was trapped at room temperature in a reaction vessel containing the precursor, solvent and base (*16–18*).

Semipreparative normal-phase HPLC was performed using a Kontron 420 pump (Kontron Instruments, Milano, Italy), an automatic sample injector (Type VICI with a 1-ml loop; VICIAG, Schenkon, Switzerland), a Waters μ-Porasil column (300 × 7.8 mm, 10 μm; Waters, Milford, MA) and a Kontron 432 ultraviolet (UV) detector (wavelength = 280 nm) in series with a Geiger-Mueller tube for radiation detection. (+)-NNC 112 and (−)-NNC 112 were purified using methylene chloride/methanol/triethylamine = 96/4/0.04 (methanol/triethylamine preadjusted to pH = 8 with concentrated acetic acid) as the mobile phase with a flow rate of 2.0 ml/min. The radiochemical purity of (+)- and (−)-[^{11}C]NNC 112 was analyzed by reversed-phase HPLC using a

TABLE 1
Affinities of NNC 112 for Various Brain Receptors As Studied in In Vitro Receptor Binding Assays

Receptor	Radioligand	IC_{50} (n*M*)
D_1	[H-3]SCH 23390	0.4
D_2	[H-3]Spiperone	1,857
α_1	[H-3]Prazosin	2,300
α_2	[H-3]RX781094	369
β	[H-3]DHA	>10,000
Muscarine	[H-3]QNB	>1,000
$5HT_{1A}$	[H-3]8-OHDPAT	1,500
$5HT_2$	[H-3]Ketanserin	37
GABA	[H-3]Muscimol	>10,000
BZ	[H-3]Diazepam	>10,000
H_1	[H-3]Pyrilamine	1,800

Data from References 10–12.

TABLE 2
Inhibition Constants (K_i, n*M*) of SCH 23390, NNC 756, NNC 112 and (−)-NNC 112 in Rat Brain Tissue In Vitro

Name	D_1	D_2	$5HT_2$
SCH 23390	0.14	895	37
NNC 756	0.17	942	4.5
NNC 112	0.18	898	18
NNC 112 (−)	170	988	—

Data from References 10–12.

Kontron 420 pump, a Rheodyne injector (7125 with a 100-μl loop) equipped with a Waters μ-Bondapak-C18 column (300 × 4.6 mm, 10 μm) and an LDC-Milton Roy 300 UV-spectrophotometer (280 nm; Milton Roy, Riviera Beach, FL) in series with a Beckman 170 radioactivity detector (Beckman, Fullerton, CA). Acetonitrile and 0.01 *M* phosphoric acid (30/70) were used as the mobile phase with a flow rate of 2.0 ml/min.

Nuclear magnetic resonance (NMR) spectra were recorded with a 200 MHz NMR spectrometer at 20°C with deutero-dimethyl sulfotide (DMSO) as solvent. Chemical shifts (δ) are given in parts per million downfield from tetramethylsilane.

Preparation of Precursors

(+)- and (−)−8-chloro-5-(7-benzofuranyl)−7-hydroxy-2,3,4,5-tetrahydro-1H-3-benzazepine ((+)- and (−)-desmethyl-NNC 112). The N-demethylations were performed after a modification of a published procedure (*19*). (+)- or (−)-NNC 112 (0.5 g) was dissolved in hot 1-chloroethyl chloroformate (ACE-Cl, 15 ml). The mixture was refluxed for 4 hr and then was concentrated in a vacuum. The residue was dissolved in methanol (20 ml) and refluxed for 16 hr. After evaporation in a vacuum, methanol (2 ml) and water (4 ml) were added. NaOH (0.2 *M*) was added until pH 7. A slightly brown solid precipitated. The solid was washed with water and dried. The yields of (+)-desmethyl-NNC 112 and (−)-desmethyl-NNC 112 were 220 and 200 mg, respectively.

^{1}H NMR: 2.85 (m) 2H, 3.20 (m) 2H, 3.50 (m) 2H, 4.85 (d) 1H, 6.15 (s) 1H, 7.00 (d) 1H, 7.08 (d) 1H, 7.20 (s) 1H, 7.30 (t) 1H, 7.65 (d) 1H, 8.00 (d) 1H. There was no peak for N-CH_3 (s) at 2.25.

Preparation of (+)-[^{11}C]NNC 112 and (−)-[^{11}C]NNC 112

(+)-[^{11}C]NNC 112 (Fig. 2). Carbon-11-methyl iodide or [^{11}C]methyl triflate, prepared as described in detail elsewhere (*15,17,18*), was trapped at room temperature in a reaction vessel (1.0 ml minivial, Alltech, Deerfield, IL), containing (+)-desmethyl-NNC 112 (1.0 or 0.5 mg for [^{11}C]methyl iodide or [^{11}C]methyl triflate, respectively) and acetone (300 μl). For [^{11}C]methyl iodide, the vessel was sealed and was heated at 110°C for 4 min. No further reaction after trapping was needed for [^{11}C]methyl triflate. Mobile phase (700 ml) was added before injection onto the semipreparative HPLC column. (+)-[^{11}C]NNC 112 eluted after 8–9 min with a retention time identical to a standard reference sample (Fig. 3). After evaporation of the mobile phase, the residue was dissolved in 8 ml sterile physiological phosphate buffered saline (pH = 7.4) and was filtered through a Millipore filter (0.22 mm), yielding a solution that was sterile and free from pyrogens.

(−)-[^{11}C]NNC 112 (Fig. 2). (−)-[^{11}C]NNC 112 was prepared from the corresponding (−)-desmethyl-NNC 112 precursor using similar reactions and purification conditions as described previously for (+)-[^{11}C]NNC 112. (−)-[^{11}C]NNC 112 eluted after 8–9 min with a retention time identical to a standard reference sample.

PET

Two PET systems at the Karolinska Hospital were used. The first was Scanditronix PC2048-15B, which measures radioactivity

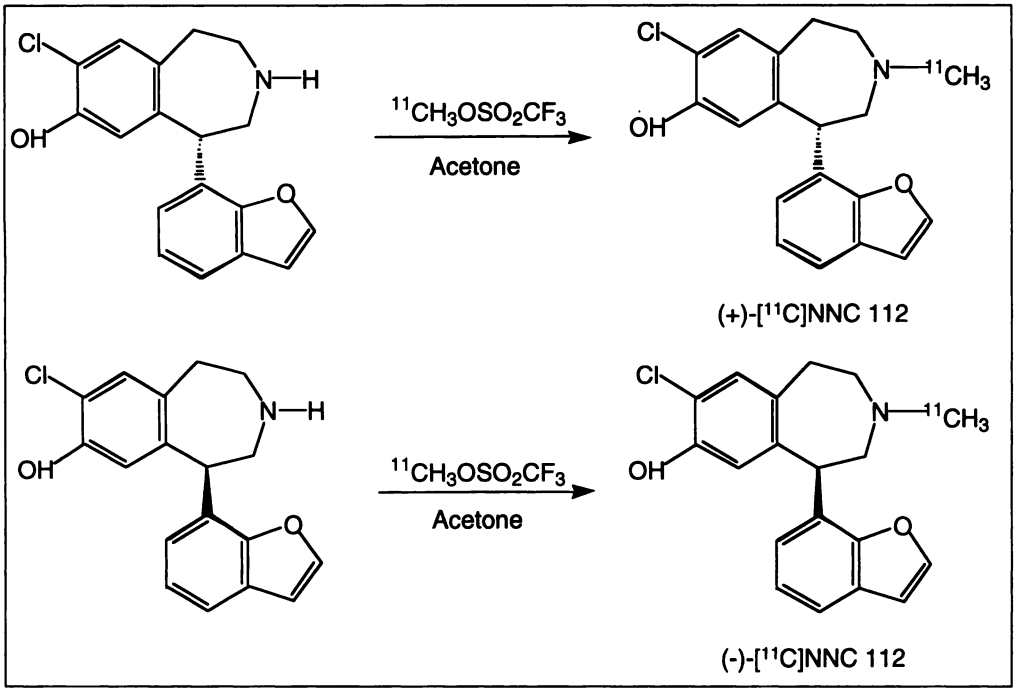

FIGURE 2. Incorporation of [^{11}C]methyl triflate to (+)-[^{11}C]NNC 112 and (−)-[^{11}C]NNC 112.

in 15 horizontal slices with a separation of 6.5 mm and a spatial resolution of about 4.5 mm full width at half maximum (FWHM) (*20*). The second was the recently installed Siemens ECAT EXACT HR (Siemens Medical Systems, Knoxville, TN), which measures radioactivity in 47 slices with a separation of 3.3 mm and a spatial resolution of about 3.8 mm FWHM (*21*).

PET Measurements in Monkeys

Four male cynomolgus monkeys weighing approximately 4 kg were supplied by the National Laboratory for Bacteriology, Stockholm. Anesthesia was induced by repeated intramuscular injection of ketamine (Ketalar® 15–25 mg kg^{-1} h^{-1}; Parke-Davis Scandinavia AB, Solna, Sweden). Two monkeys were examined with the Scanditronix PC2048-15 PET system and two were examined with the Siemens ECAT EXACT HR for three-dimensional imaging. A head-fixation system was used to secure a fixed position of the monkey head during the PET measurements (*22*). The monkey head was positioned so that the imaging planes were parallel to the cantomeatal line. Body temperature was controlled by a heating pad with a thermostat. In each PET study, 37–40 MBq [^{11}C]NNC 112 were injected intravenously in the left sural vein. Radioactivity in the brain was measured according to a preprogrammed sequence of frames up to 108 min after injection.

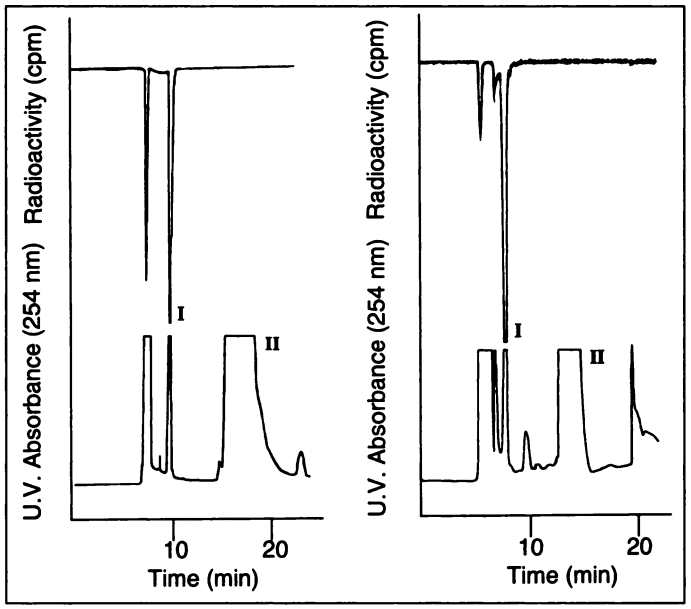

FIGURE 3. Semipreparative HPLC chromatograms (UV and radioactivity versus time) using a normal phase Waters μ-Porasil column. The left panel starts from [^{11}C]methyl iodide and the right panel starts from [^{11}C]methyl triflate. I, [^{11}C]NNC 112; II, desmetyl-NNC 112.

In the first monkey, two PET measurements were obtained. The first measurement was taken after intravenous injection of the active enantiomer (+)-[^{11}C]NNC 112 and the second was taken after injection of the inactive enantiomer (−)-[^{11}C]NNC 112.

Three measurements were obtained in the second monkey. The first was a baseline experiment with (+)-[^{11}C]NNC 112. The second was a displacement experiment, in which SCH 23390 (2.5 mg/kg) was injected intravenously 23 min after the injection of (+)-[^{11}C]NNC 112. The third was a displacement experiment, in which the 5-HT_{2A} antagonist ketanserin (2 mg/kg) was injected intravenously 21 min after the injection of (+)-[^{11}C]NNC 112.

Four measurements were obtained in the third monkey on two separate days. On the first day, a baseline experiment with (+)-[^{11}C]NNC 112 was followed by a displacement experiment in which ketanserin (2 mg/kg) was injected intravenously 21–23 min after the injection of (+)-[^{11}C]NNC 112. On the second day, a baseline experiment with (+)-[^{11}C]NNC 112 was followed by a displacement experiment in which SCH 23390 (2.5 mg/kg) was injected intravenously 21–23 min after the injection of (+)-[^{11}C]NNC 112.

Two measurements were obtained in the fourth monkey. The first was a baseline experiment with (+)-[^{11}C]NNC 112. The second was a pretreatment experiment in which ketanserin (2 mg/kg) was injected intravenously 10 min before the injection of (+)-[^{11}C]NNC 112.

To validate the assumption that the radioactivity in the cerebellum may be used as an estimate of the time curve for a free and nonspecifically bound radioligand concentration in the brain, we calculated the time curve for ratio of radioactivity in the cerebellum to blood for (+)-[^{11}C]NNC 112 in the baseline and displacement experiments on the monkeys.

PET Measurements in Healthy Human Subjects

The study was performed in compliance with the Declaration of Helsinki and was approved by the Ethics and Radiation Safety Committees of the Karolinska Hospital, Stockholm. Four men (aged 20, 22, 26 and 24 yr and weighing 61, 74, 85 and 94 kg, respectively) participated after giving informed consent. The subjects were healthy according to history, physical examination, blood and urine chemistry and MRI examination of the brain. Exclusion criteria were psychiatric disorder according to *DSM IV*, significant somatic disorder, previous intake of any psychotropic drug, history of alcoholism or drug addiction.

An individualized plaster helmet was made for each subject and was used with a head-fixation system during both MRI and PET (*23*). An arterial cannula was inserted into the brachial artery of the left arm and a venous cannula was inserted into the antecubital vein of the right arm. The radioligand was injected intravenously as a bolus during 2 sec into the right antecubital vein. Each PET measurement comprised a series of sequential frames during 68 or 93 min.

Subject 1 was examined with the Scanditronix PC2048-15 PET system, Subject 2 was examined with the Siemens ECAT EXACT HR in the two-dimensional mode and Subjects 3 and 4 were examined with the Siemens ECAT EXACT HR in the three-dimensional mode. Subject 1 participated in one PET measurement in which 310 MBq (+)-[^{11}C]NNC 112 were injected. Subject 2 participated in two measurements performed on the same day. In the first measurement, (+)-[^{11}C]NNC 112 was injected; in the second measurement 3.5 hr later, (−)-[^{11}C]NNC 112 was injected. Subjects 3 and 4 participated in one PET measurement each in which 406 and 257 MBq (+)-[^{11}C]NNC 112 were injected, respectively.

Blood was drawn from the arterial cannula, and radioactivity in the blood was measured using a well-counter (*24*). Blood samples (2 ml) were drawn manually at each time frame until the end of the experiment. One milliliter of the blood sample was immediately pipetted and radioactivity was measured in a well-counter for 10 sec. After centrifugation of the remaining blood sample, 0.2 ml plasma was pipetted and plasma radioactivity was measured in the well-counter. Radioactivity in whole blood and plasma was corrected for decay and was plotted versus time.

Regions of Interest

In monkeys, the regions of interest (ROIs) were drawn on the PET summation images that represent radioactivity measured from 9 min after intravenous injection to the end of the measurement. The striatum, neocortex, cerebellum and the whole brain contour were defined according to an atlas of a cryosected cynomolgus monkey head in situ (*22*). Radioactivity was calculated for the sequence of time frames, was corrected for the radioactivity decay and was plotted versus time. To calculate the percentage of [^{11}C]NNC 112 injected that was present in the brain at the time of maximal radioactivity, the radioactivity concentration in the ROI for the whole brain was multiplied with the 65-ml brain volume of a cynomolgus monkey weighing 4 kg. The calculated value for total radioactivity in the brain was then divided with the radioactivity injected and was multiplied by 100.

In the human subjects, ROIs were delineated manually on MR images for the putamen, the caudate nucleus, the nucleus accumbens, the frontal cortex and the cerebellum. The ROIs were transferred to the corresponding PET images. Regional radioactivity was measured for each sequential scan, was corrected for ^{11}C decay and was plotted versus time. The percentage of radioligand in brain was calculated as described above for the monkey brain. The estimated average brain size of a healthy man was 1250 ml.

Quantitative Analyses

The cerebellum is a region with negligible density of D_1R (*6*). The radioactivity in the cerebellar cortex was therefore used as an approximation of free and nonspecifically bound radioligand concentration in the brain.

The time curve for specific (+)-[^{11}C]NNC 112 binding to D_1R in the putamen and the cortex was defined as the difference between the total radioactivity concentration in an ROI and in the cerebellum. Time for transient equilibrium was defined as the moment when the curve for specific binding reached a peak (*13*).

The ratio of binding in an ROI to the cerebellum was calculated using a time interval during 40–50 min in which a transient equilibrium occurs. This ratio can be viewed as an index for the density of available receptors. All calculations were based on the assumption that radioactivity in the brain represents unchanged radioligand.

(+)-[^{11}C]NNC 112 binding was also analyzed using a linear graphical analysis for reversible radioligand binding to receptors (*14*). The radioactivity of unchanged [^{11}C]NNC 112 in arterial plasma was used as an input function. The regional distribution volume (DV^{Logan}) was determined from the slope of the linear plots obtained.

Plasma Metabolite Studies and Protein Binding

The fractions of the radioactivity in monkey and human plasma that correspond to unchanged (+)- or (−)-[^{11}C]NNC 112 and labeled metabolites were determined using an HPLC method previously developed for other PET ligands (*25,26*). Blood samples (2 ml) were obtained at approximately 4, 10, 20, 30, 40, 50, 60 and 75 min after injection of (+)- or (−)-[^{11}C]NNC 112. The supernatant (0.5 ml) obtained after centrifugation at 2000 *g* for 1 min was mixed with acetonitrile (0.7 ml) containing a standard of NNC 112. The radioactivity in the supernatant (1.1 ml) obtained after centrifugation at 2000 *g* 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, the UV-absorption and radioactivity peaks were integrated and the data were stored in a personal computer (PC).

The reversed-phase HPLC Kontron system consists of: two Kontron 420 pumps, a Rheodyne injector (7125 with a 1.0-ml loop) equipped with a Waters μ-Bondapak-C18 column (300 × 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; Packard Instruments, Meriden, CT). Phosphoric acid (0.01 *M*) (A) and acetonitrile (B) were used as the mobile phases with a flow rate of 6.0 ml/min. Gradient elution was used on all metabolite analyses. The gradient profile was the following: HPLC time 0–5.5 min, (A/B) 80/20–40/60; 5.5–6.0 min, (A/B) 40/60–80/20; 6.0–7.5 min (A/B) 80/20 isocratic; 7.5 min end. The Kontron 450 Multitasking system was used as an efficient controller and PC-integration system. Fractions that correlated with standards of NNC 112 and the corresponding radioactive peaks were also taken and counted in a well-counter. The radioactivity in a certain fraction was divided by the total radioactivity and was expressed as a percentage of the total.

Protein binding was determined by ultrafiltration. To monkey plasma (0.5 ml) was added 20 μg of carrier and ~30,000 Hz of (+)-[^{11}C]NNC 112. The mixture was transferred to the Centrisart I (Sartorius AG, Göttingen) ultrafiltration devices with a cutoff of 10,000 and counted in a well-detector. The samples were preincubated for 5 min and after that time were centrifuged for 10 min. The volume of the ultrafiltrate was recorded and the sample was counted in a well-detector. The counts obtained were compared to what should have been obtained after decay correction for the nonfiltrated solution of an equal volume.

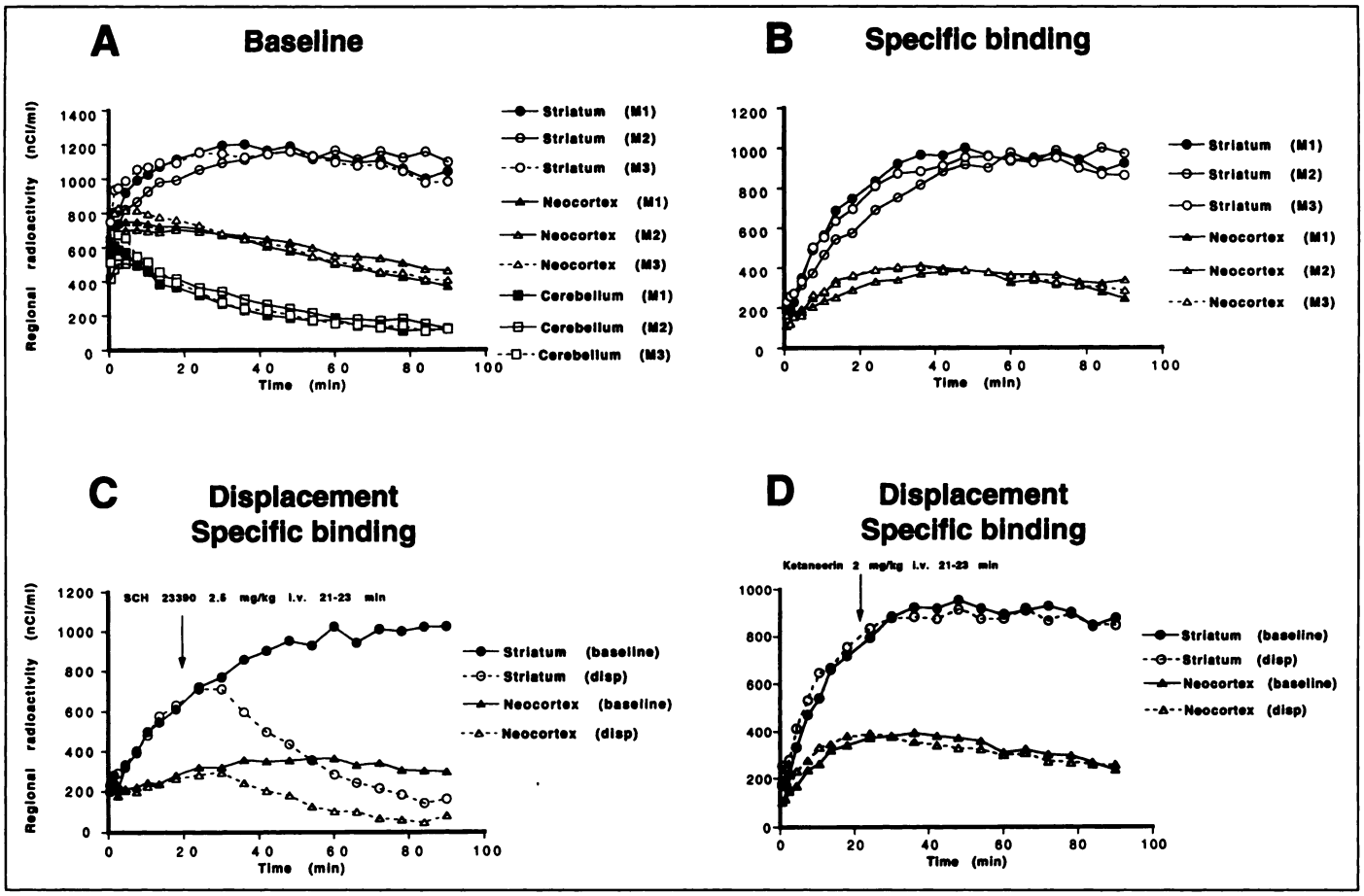

FIGURE 4. Time course for brain radioactivity (nCi/ml) in cynomolgus monkeys. (A) Regional brain radioactivity after intravenous administration of (+)-[^{11}C]NNC 112 (n = 3). (B) Specific binding in striatum and neocortex (n = 3). (C) Specific binding of a baseline and a displacement experiment with SCH 23390 (2.5 mg/kg) at 21–23 min after intravenous injection of (+)-[^{11}C]NNC 112. (D) Specific binding of a baseline and a displacement experiment with ketanserin (2.0 mg/kg) at 21–23 min after intravenous injection of (+)-[^{11}C]NNC 112. PET-camera system was a Siemens ECAT EXACT HR.

RESULTS

Chemistry

The N-demethylation of (+)−8-chloro-5-(7-benzofuranyl)−7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (+)-NNC 112 and (−)−8-chloro-5-(7-benzofuranyl)−7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (−)-NNC 112 was performed after a procedure described by Olofson (*19*) giving the pure desmethyl precursors of NNC 112 in 40% chemical yield.

The incorporation of [^{11}C]methyl iodide to (+)- or (−)-[^{11}C]NNC 112 was 50% (using 1.0 mg of the desmethyl precursors). The total radiochemical yield of (+)- or (−)-[^{11}C]NNC 112, calculated from end of bombardment (EOB) and decay corrected, was 35%–40% with a total synthesis time of 30–35 min. The incorporation of [^{11}C]methyl triflate to (+)- or (−)-[^{11}C]NNC 112 was 70%–90% (using 0.5 mg of the desmethyl precursors). No further reaction after trapping was needed for [^{11}C]methyl triflate, which simplifies the experimental setup. The total radiochemical yield of (+)- or (−)-[^{11}C]NNC 112, calculated from EOB and decay corrected, was 50%–60% with a total synthesis time of 25–30 min.

Purification was performed by semipreparative normal-phase HPLC (Fig. 3) yielding (+)- or (−)-[^{11}C]NNC 112 with a radiochemical purity better than 99%. The retention times of [^{11}C]NNC 112 and desmethyl-NNC 112 were 8–9 and 14–18 min, respectively (Fig. 3). The retention times of [^{11}C]NNC 112 on the analytical reversed-phase HPLC system was 4.1 min. The specific radioactivity obtained at time of injection of [^{11}C]NNC 112 was about 1500–3000 Ci/mmol (56–110 GBq/μmol), corresponding to a total injected dose of about 0.2–0.3 μg in the monkey and 1.0–1.5 μg in the human experiments.

PET Measurements in Monkeys

At 4 min after intravenous injection of (+)-[^{11}C]NNC 112 in two cynomolgus monkeys, 4.2% of the total radioactivity injected was present in the brain. The uptake in the striatum was much higher than in the cerebellum (Fig. 4A). The radioactivity ratios of the striatum and neocortex to the cerebellum increased continuously with time and were 5.77 ± 0.31 and 2.36 ± 0.14 (n = 6) at a transient equilibrium that was reached at about 40–50 min after injection (Fig. 4B).

After intravenous injection of the inactive (−)-enantiomer of [^{11}C]NNC 112, there was an even distribution of radioactivity in the brain. There was no conspicious accumulation of radioactivity in brain regions known to contain D_1R.

The specificity of (+)-[^{11}C]NNC 112 binding was examined in displacement and pretreatment experiments. After injection of SCH 23390 (2.5 mg/kg), the radioactivity decreased rapidly and approached the curve for the cerebellum. The reduction of (+)-[^{11}C]NNC 112 binding in the striatum and the neocortex was about 80% at 72 min after injection (n = 2) (Fig. 4C).

After injection of ketanserin (2 mg/kg) in two displacement and one pretreatment experiment (Fig. 4D), the striatal and neocortical curves were similar to those in the baseline experiments. There was no reduction of the ratio of (+)-[^{11}C]NNC 112 binding in the striatum to the cerebellum (data not shown).

There was no effect of SCH 23390 on the curve for the ratio

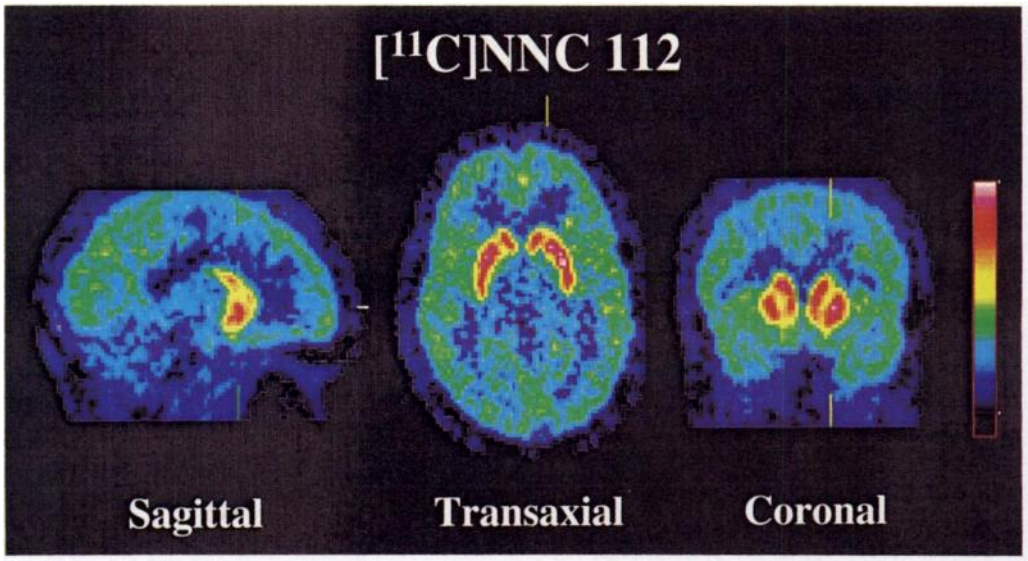

FIGURE 5. Color-coded PET image showing distribution of radioactivity in human brain (Subject 3) after intravenous administration of ~409 MBq (+)-[^{11}C]NNC 112 (measured between 9 and 76 min). The PET system was a Siemens ECAT EXACT HR.

of radioactivity in the cerebellum to blood that was calculated for (+)-[^{11}C]NNC 112 in the displacement experiments.

PET Measurements in Humans

After injection of (+)-[^{11}C]NNC 112 in Subject 1 who was examined with the Scanditronix PC2048-15 PET system, the radioactivity ratios of the striatum and the neocortex to the cerebellum were 3.8 and 1.8, respectively. Transient equilibrium was reached at about 40–50 min after injection.

(+)- and (−)-[^{11}C]NNC 112 were injected in separate experiments in Subject 2, who was examined with the Siemens ECAT EXACT HR PET system in the two-dimensional mode. Initially, there was a high transient peak of radioactivity in whole blood and plasma. Four minutes after the injection, 4.5% of injected (+)-[^{11}C]NNC 112 or (−)-[^{11}C]NNC 112 was present in the brain. After intravenous injection of (+)-[^{11}C]NNC 112, the radioactivity in the striatum increased rapidly and reached a maximum level within 10 min. The radioactivity ratio of the putamen to the cerebellum was 4.0. The corresponding ratios of the frontal cortex to cerebellum and the nucleus accumbens to the cerebellum were 2.0 and 2.8, respectively. The brain uptake of the inactive (−)-[^{11}C]NNC 112 enantiomer did not accumulate conspicuously in brain regions known to have D_1R.

Subjects 3 and 4 were examined with the Siemens ECAT EXACT HR PET system in the three-dimensional mode (Fig. 5). Intravenous injection of (+)-[^{11}C]NNC 112 gave radioactivity ratios of the striatum, frontal cortex and nucleus accumbens to the cerebellum of 3.8–3.9, 1.7 and 2.8–3.1, respectively (Fig. 6A) at transient equilibrium that was reached at 40–50 min after injection (Fig. 6B). In the linear graphical analysis (*14*), a linear phase was observed for all regions including the cerebellum. The slopes were determined by linear fitting to the last five points for each region. Theoretically, these slopes correspond to the total volume of distribution (Vt), which were 15.2, 5.8 and 3.9 for putamen, frontal cortex and cerebellum, respectively. The calculated distribution volume ratios were 3.9 and 1.5 in the putamen and frontal cortex, respectively (Fig. 7).

Plasma Metabolite Studies

Blood samples were processed and plasma was isolated and extracted. The recovery of radioactivity was higher than 95%. The peaks of UV and radioactivity were integrated simultaneously by the PC and the chemical identity was determined by the simultaneous addition of a standard of NNC 112. The integrated values obtained were in good agreement with the collected fractions measured in the well-counter. The resolution between radioligand and labeled metabolites is sufficient. Experiments showed that >98% of the injected radioactivity was recovered from the column. Compared to the experimentally simple but time-consuming thin-layer chromatography procedure that allows only a few samples to be processed (*27*), the HPLC method is rapid, efficient and reliable.

The percentage of total radioactivity in plasma representing unchanged compound after injection of (+)-[^{11}C]NNC 112 or (−)-[^{11}C]NNC 112 in a human subject is shown in Figure 8. The fraction of the total radioactivity representing (+)-[^{11}C]NNC 112 in human plasma was 80% at 5 min and 25% at 75 min after injection. The fraction of the total radioactivity representing (−)-[^{11}C]NNC 112 in plasma was 80% at 5 min

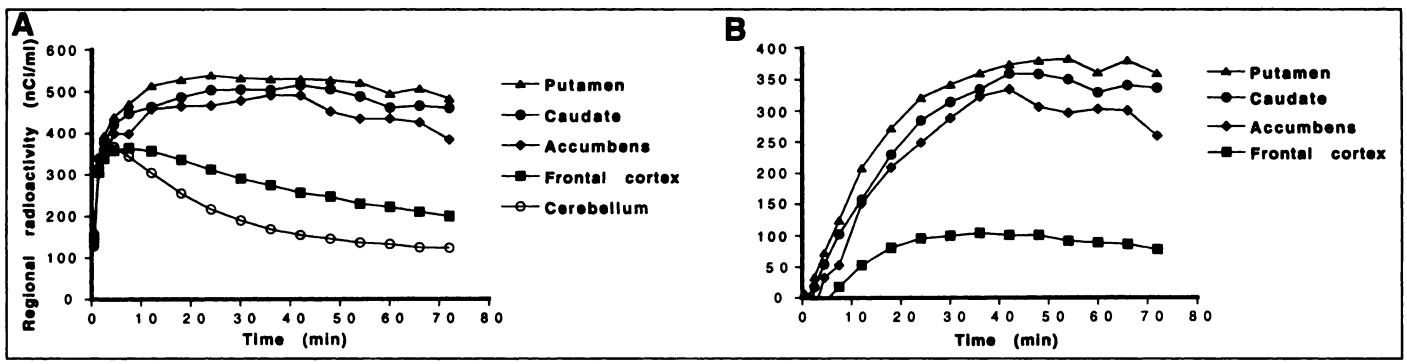

FIGURE 6. Time course for brain radioactivity (nCi/ml) in healthy human subject (Subject 3). (A) Regional brain radioactivity after intravenous administration of (+)-[^{11}C]NNC 112. (B) Specific binding in putamen, caudate, accumbens and frontal cortex. PET-camera system was a Siemens ECAT EXACT HR.

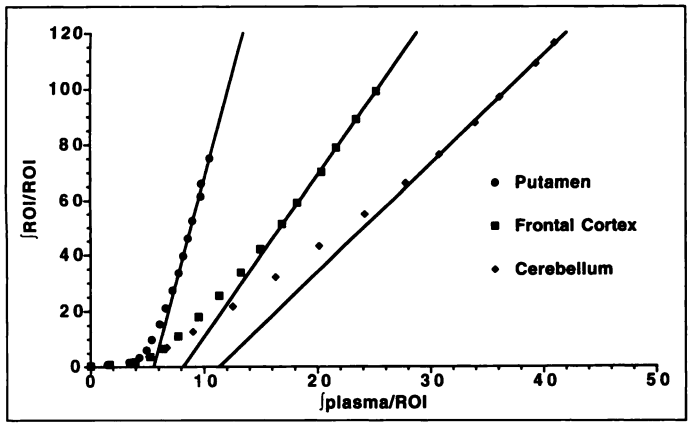

FIGURE 7. Graphical analysis according to Logan et al. (*14*) in three brain regions. Slopes were determined by linear fitting to the last five points for each ROI (Subject 3).

and 6% at 75 min after injection. A preliminary observation in monkey plasma indicates that (+)-[^{11}C]NNC 112 has a slower metabolism than (−)-[^{11}C]NNC 112. This observation was confirmed in human plasma (Fig. 8). The analysis of the labeled metabolites indicates that they are more polar than the parent compound and that NNC 112 is conjugated. Therefore, it is unlikely that significant amounts of the major labeled metabolites might pass the blood–brain barrier. The results obtained in monkey plasma were in agreement with the human results. The free (not protein-bound) fraction measured by ultrafiltration was 1%–2% (n = 7).

DISCUSSION

Chemistry

Several D_1R antagonists such as SCH 23390, SCH 39166, NNC 687, NNC 756 and NNC 22-0010 have previously been labeled with ^{11}C by N-methylation of the corresponding desmethyl compound with [^{11}C]methyl iodide (*2,4,28–30*). The yield obtained after incorporation of [^{11}C] methyl iodide is usually sufficient for routine PET examinations. [^{11}C]Methyl triflate has recently been introduced as a more powerful methylating agent than [^{11}C]methyl iodide (*16–18*). The use of [^{11}C]methyl triflate results in higher yields, shorter reaction times and lower reaction temperatures, which may be of importance for reliable routine production. In addition, a smaller amount of precursor can be used that is important when considering the final purification of the radioligands and the cost and availability of the precursor. Several examples of ^{11}C methylations of amines, phenols and carboxylic acid with [^{11}C]methyl triflate have been reported recently (*17,18*). When comparing the radiochemical yields obtained in this study from the labeling of either (+)-or (−)-[^{11}C]NNC 112 from [^{11}C]methyl iodide or [^{11}C]methyl triflate, it can be concluded that [^{11}C]methyl triflate is superior to [^{11}C]methyl iodide.

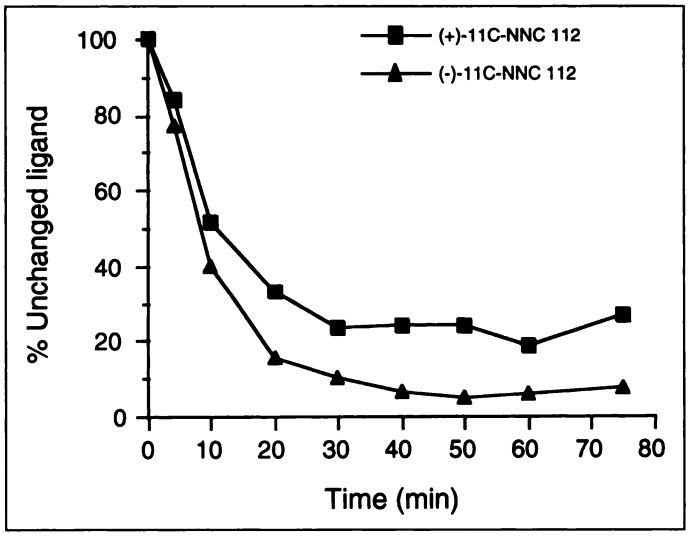

FIGURE 8. Determination of unchanged radioligand in human plasma (percentage of total radioactivity versus time) of (+)-[^{11}C]NNC 112 and (−)-[^{11}C]NNC 112.

PET Measurements

Four minutes after injection of (+)-[^{11}C]NNC 112, 4.2%–4.5% of the total radioactivity injected was present in the monkey or human brain. This uptake is higher than the total uptake of 1%–2% previously demonstrated for useful PET ligands such as [^{11}C]SCH 23390 or [^{11}C]raclopride (*1,31,32*) and should be advantageous for regional analysis of D_1R binding.

The regional distribution of radioactivity was in accordance with the known distribution of D_1R, with highest density in the basal ganglia, with lower density in the neocortex and with low density in the cerebellum (Figs. 4A, 5 and 6A). The striatum- and neocortex-to-cerebellum ratios (5.77 ± 0.31 and 2.36 ± 0.14, respectively) obtained in the monkey brain are higher than those previously reported for SCH 23390 and other benzazepines (*2*).

The cerebellum is a region with a negligible density of D_1R (*6*). The radioactivity in the cerebellum was therefore used as an estimate of the time curve for free and nonspecifically bound radioligand concentration in brain. There was no effect of SCH 23390 or ketanserin on the ratio of radioactivity in the cerebellum to blood, indicating that the cerebellum may be used as a valid reference region.

The specificity of (+)-[^{11}C]NNC 112 binding to D_1R was demonstrated by the displacement and pretreatment experiments. The radioactivity in the striatum and the neocortex was markedly reduced after intravenous injection of unlabeled SCH 23390 (2.5 mg/kg) (Fig. 4C). There was no obvious effect of SCH 23390 on the cerebellar curve. The 5-HT_{2A} antagonist ketanserin (2 mg/kg) had no effect on the regional time curves using either a displacement (Fig. 4D) or pretreatment protocol. These observations are consistent with a more extensive pharmacological characterization that was performed in rodents in vivo demonstrating selectivity for D_1R (*10,11*) (Tables 1 and 2). The results of the present PET study and in vivo studies in rodents indicate that (+)-NNC 112 binds selectively to D_1R in vivo.

In the human brain, high ratios were obtained for the striatum, nucleus accumbens and the frontal cortex (3.9–4.0, 2.9–3.1 and 1.7–2.0, respectively). These ratios are higher than the ratios previously obtained for [^{11}C]SCH 23390 (*1*). The transient equilibrium and the Logan analysis are dependent on the cerebellum as a reference region and gave very good agreement. Transient equilibrium was reached during the time of a PET measurement (40–50 min). This time is earlier than that previously observed for [^{11}C]NNC 756, a PET radioligand that like (+)-[^{11}C]NNC 112 provides a high binding ratio but has a significant affinity for 5-HT_2 receptors (*22*). The cortical uptake of [^{11}C]NNC 756 was reduced in displacement and pretreatment experiments with ketanserin by 24%–28% (1.5 mg/kg) (*22*). The earlier peak and the binding selectivity indicate that (+)-[^{11}C]NNC 112 should be a suitable radioligand for quantitation of neocortical D_1R.

Plasma Metabolite Studies

Both (+)- and (−)-[^{11}C]NNC 112 were rather rapidly metabolized in monkey and human plasma (Fig. 8). Rapid metabolism is seen for many radioligands and does not preclude that (+)-[^{11}C]NNC 112 may be useful in PET studies (*25*). A problem may arise if the metabolites pass the blood–brain barrier and bind to dopamine receptors. It is known, however, that the benzazepine derivative [^{11}C]SCH 23390 is metabolized mainly to polar conjugates (*26*), and it is likely that (+)-[^{11}C]NNC 112 is metabolized in an analogous way. This would lead to polar glucuronide and sulfate conjugates that should poorly penetrate the blood–brain barrier and are also devoid of affinity for the dopamine receptor. The protein binding (98%–99%) is in accordance with data obtained with the benzazepine analog, NNC 22-0010, which has shown high protein binding to rat, dog and human plasma proteins (98%–99%; Novo Nordisk, unpublished results).

CONCLUSION

(+)-[^{11}C]NNC 112 bound with high affinity and selectivity to central D_1R, as demonstrated by displacement experiments in the monkey brain in vivo. In human subjects, (+)-[^{11}C]NNC 112 reached transient equilibrium and gave a high signal-to-noise ratio not only in the striatum but also in the neocortical brain regions. (+)-[^{11}C]NNC 112 should be suitable for quantitation of both striatal and neocortical D_1R in man. This is particularly useful for research on schizophrenia and drug challenges.

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