Carbon-11-*d-threo*-Methylphenidate Binding to Dopamine Transporter in Baboon Brain

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The more active *d*-enantiomer of methylphenidate (*dl-threo*-methyl-2-phenyl-2-(2-piperidyl)acetate, Ritalin) was labeled with ^{11}C ($t_{1/2}$: 20.4 min) to characterize its binding, examine its specificity for the dopamine transporter and evaluate it as a radiotracer for the presynaptic dopaminergic neuron. **Methods:** PET studies were carried out in the baboon. The pharmacokinetics of [^{11}C]*d-threo*-methylphenidate ([^{11}C]*d-threo*-MP) were measured and compared with [^{11}C]*l-threo*-MP and with its racemate ([^{11}C]*dl-threo*-methylphenidate, [^{11}C]MP). Nonradioactive methylphenidate was used to assess the reversibility and saturability of the binding. GBR 12909, 3β-(4-iodophenyl)tropane-2-carboxylic acid methyl ester (β-CIT), tomoxetine and citalopram were used to assess the binding specificity. **Results:** The ratio between radioactivity in the striatum and that in the cerebellum (ST/CB) after injection of [^{11}C]*d-threo*-MP was higher than that for [^{11}C]MP and [^{11}C]*l-threo*-MP (3.3 for *d*-, 2.2 for racemic and 1.1 for *l*- in the same baboon). Most of the striatal binding of [^{11}C]*d-threo*-MP was displaceable by injection of nonradioactive MP. Pretreatment with nonradioactive MP (0.5 mg/kg), GBR12909 (1.5 mg/kg) and RTI-55 (0.3 mg/kg) markedly reduced striatal but not cerebellar uptake of [^{11}C]*d-threo*-MP. In all cases, the ST/CB after pretreatment was reduced by about 60% compared to 43% for [^{11}C]MP. The ratios of distribution volumes at steady-state for the ST/CB for the three separate studies in the same baboon were reduced by about 50%, as compared with 37% for [^{11}C]MP. In contrast, pretreatment with tomoxetine (3.0 mg/kg) or citalopram (2.0 mg/kg) did not change [^{11}C]*d-threo*-MP kinetics; the ST/CB after pretreatment was similar to that for the control. **Conclusion:** These results demonstrate the saturable, reversible and specific binding of [^{11}C]*d-threo*-MP to the dopamine transporter in the baboon brain, suggesting that [^{11}C]*d-threo*-MP will be a useful PET tracer for the presynaptic dopaminergic neuron in living human brain.

Key Words: carbon-11-*d-threo* methylphenidate; dopamine transporter; stereoselectivity; dopaminergic neuron

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Methylphenidate (MP, Ritalin) is the drug of choice for the treatment of attention deficit hyperactivity disorder (ADHD), which is estimated to affect 2%–5% of school age children (*1*). MP is also used to treat narcolepsy (*2*). The psychostimulant properties of MP have been linked to its binding to a site on the dopamine transporter, resulting in inhibition of dopamine reuptake and enhanced levels of synaptic dopamine.

We have developed a rapid synthesis of [^{11}C]*dl-threo*-methylphenidate ([^{11}C]MP) to examine its pharmacokinetics and pharmacological profile in vivo and to evaluate its suitability as a radiotracer for the presynaptic dopaminergic neuron (*3,4*). These first PET studies of MP in the baboon and human brain demonstrated the saturable [^{11}C]MP binding to the dopamine transporter in the baboon brain and its sensitivity to dopamine neuron degeneration in Parkinson's disease. Although these results are promising in terms of labeled MP as a ligand for the presynaptic dopaminergic neuron, the use of *dl-threo*-methylphenidate, the form which is marketed, is not ideal because it is a mixture of enantiomers. It has been shown that *d-threo*-MP is more potent in the induction of locomotor activity and has a higher affinity for the dopamine transporter than *l-threo*-MP (*5*). The potency (IC_{50}) of *dl-threo*-MP in displacing [^{3}H]*dl-threo*-MP from striatal synaptosomal membrane binding sites is 0.21 μM compared to 0.088 μM for the *d*-enantiomer and 1.2 μM for the *l*-enantiomer (*6*). The relative affinities of racemic MP and its individual enantiomers to monoamine transporters have been reported (*5,7,8*) (Table 1). Thus, while [^{11}C]*d-threo*-MP would be predicted to be a promising PET ligand, [^{11}C]*l-threo*-MP would be expected to contribute to nonspecific binding and to the absorbed radiation burden. Additionally, while it has been demonstrated that the two enantiomers have a similar bioavailability after intravenous (but not oral) administration (*9,10*), the use of [^{11}C]*d-threo*-MP, rather than the racemic mixture, is preferred because it would allow measurement of an arterial input function from a single labeled compound, thus facilitating quantitation.

We report here the characterization of [^{11}C]*d-threo*-MP binding in baboon brain which include the following: measurement of regional brain uptake and clearance; an assessment of the reproducibility of repeated measures; the effect of pharmacological interventions with various drugs to assess if the binding is saturable and specific. The saturability of the binding was assessed with unlabeled methylphenidate. An assessment of the specificity of [^{11}C]*d-threo*-MP for the presynaptic dopaminergic neuron was determined

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TABLE 1
Binding of Methylphenidate to Monoamine Transporters

	Dopamine	Norepinephrine	Serotonin
Binding (Ki, n*M*)			
d-threo-MP*	27	65	20,000
l-threo-MP*	360	2800	23,000
dl-threo-MP†	390	1900	15,000
Transport (IC_{50}, n*M*)			
d-threo-MP‡	1300	190	—
l-threo-MP‡	11,000	1200	—

*Because of a limited amount of material, these are the results of a single experiment (10 concentrations of compound, in triplicate). For the dopamine, norepinephrine and serotonin transporters, [^{3}H]WIN 35,428, [^{3}H]nisoxetine and [^{3}H]paroxetine, respectively, were used with membranes prepared from the striatum, frontal cortex and brainstem of rat brain, respectively, as described by Pan et al. (*7*).

†Adapted from Ritz et al. (*8*).

‡Adapted from Patrick et al. (*5*).

by pretreatment with GBR 12909 (a selective dopamine uptake blocker) (*11*), 3β-(4-iodophenyl)tropane-2-carboxylic acid methyl ester (β-CIT) (an inhibitor of both the dopamine and serotonin transporters) (*12*), tomoxetine (a selective norepinephrine uptake inhibitor) (*13*) or citalopram (a selective serotonin uptake inhibitor) (*14*). A chase experiment with unlabeled MP was also performed to further verify binding reversibility and specificity. The time course of unchanged tracer in arterial plasma was also measured and used to calculate the steady-state distribution volume in brain (*15*).

MATERIALS AND METHODS

Unlabeled *dl-threo*-MP · HCl and ritalinic acid were provided by Ciba-Geigy Corp., Summit, NJ. The individual enantiomers (*d-threo*-MP and *l-threo*-MP) were prepared in our laboratory according to a previously published method for preparative separation of *dl-threo*-MP using (R)-(−) and (S)-(+) 1,1′-binaphthyl-2-2′-diyl hydrogen phosphate as the resolving reagents (*5*). Citalopram and tomoxetine were provided by Lundbeck (Sweden) and Eli Lilly (Indianapolis, IN), respectively, and GBR12909 was purchased from Research Biochemicals Inc., Natick, MA.

Synthesis of Carbon-11-*d-threo*-Methylphenidate

Carbon-11-*d-threo*-MP was prepared in two steps: O-methylation of the N-protected *d-threo*-ritaliniic acid derivative with [^{11}C]H_3I followed by hydrolysis. The total synthesis time was 40 min, with an average specific activity of 1.5 Ci/μmole (EOB) and radiochemical (>98%) and enantiomeric purity (99%) (*3*).

PET Studies of Carbon-11-*d-threo*-Methylphenidate

Baboon Studies. Two adult female baboons (*Papio anubis*) were used in six paired PET studies over an 8-mo period with at least 4 wk between studies. The baboon was anesthetized and prepared for PET studies as described previously (*16*). Animals were initially anesthetized with an intramuscular injection of ketamine hydrochloride (10 mg/kg), intubated and transported to the PET facility. They were maintained on oxygen, nitrous oxide and isoflurane throughout the study. Catheters were placed in an antecubital vein for radiotracer injection and femoral artery for blood sampling. For each paired study, two tracer doses of [^{11}C]*d-threo*-MP (5–9 mCi in 3 ml saline, 0.017–0.03 μmole (4–7 μg) per injection; i.v.) was administered with a 2–3-hr time period between doses to test the reproducibility of measurements or to examine the effects of drug pretreatment on the binding of [^{11}C]*d-threo*-MP. The same scanning protocol was performed as described for [^{11}C]*dl-threo*-MP (*4*). Arterial blood sampling and plasma assay for the presence of unchanged labeled MP were carried out following the same procedure as reported for [^{11}C]*dl-threo*-MP. Vital signs, including heart and respiratory rate, were monitored and recorded throughout the study.

Drug Pretreatment

The pharmacological profile of [^{11}C]*d-threo*-MP binding in baboon was determined by carrying out a baseline PET study and then pretreating with an intravenous injection of the following drugs at the pharmacological doses prior to the second injection of [^{11}C]*d-threo*-MP: *dl-threo*-MP (0.5 mg/kg, 24 min prior); GBR 12909 (1.5 mg/kg, 23 and 45 min prior); β-CIT (0.32 mg/kg, 90 min prior); tomoxetine (3.0 mg/kg, 20 min prior); and citalopram (2.0 mg/kg, 30 and 120 min prior). The timing for drug administration was chosen for maximum drug uptake at the time of tracer administration. This information was available from PET studies for GBR 12909 (*17*) and *dl-threo* MP (*4*). Citalopram has been labeled with ^{11}C and tissue distribution performed in mice (*18*). Pretreatment times of 30 min and 2 hr were chosen. The timing for β-CIT was obtained from a SPECT study with [^{123}I]β-CIT (*12*). The timing for tomoxetine was based on the report of Kleven et al. (*13*).

Chase Experiment

To investigate the reversibility and specificity of [^{11}C]*d-threo*-MP binding, unlabeled MP (0.5 mg/kg) was injected 15 min after the second tracer dose of [^{11}C]*d-threo*-MP.

Assay of Carbon-11-*d-threo*-Methylphenidate in Plasma

Unchanged [^{11}C]*d-threo*-MP in plasma was determined by a solid-phase extraction method as described for [^{11}C]MP (*4*).

Image and Data Analysis

Regions of interest (ROIs) on baboon brain were drawn directly on the PET scans as described previously (*16*). The striatal ROIs were drawn in two sequential planes at the level of the genu of the corpus callosum. The thalamic ROI was drawn in the lower plane from which the striatal regions were obtained. The brainstem ROIs were drawn on the slice immediately inferior to the level of the thalamic region. The thalamic ROI was drawn across the midline to include both the right and left sides. Cerebellar ROIs were drawn in the plane that intersected the middle of the cerebellum and ROIs were obtained in the left and right hemispheres.

Time-activity data (%ID/cc) at 60 min for tissue ^{11}C concentration were used to calculate the striatum-to-cerebellum ratio. Time-activity curves for ^{11}C and the time course of unchanged tracer in plasma were used to calculate the distribution volume in the in striatal, thalamic, cerebellar and midbrain regions using a graphical analysis method for reversible systems (Logan plots) as previously described (*15*). The ratio of the distribution volume in striatum to that in the cerebellum was used as the model parameter to calculate dopamine transporter availability at baseline and after drug interventions.

The distribution volume provides a measure of binding that is a linear function of transporter availability given by:

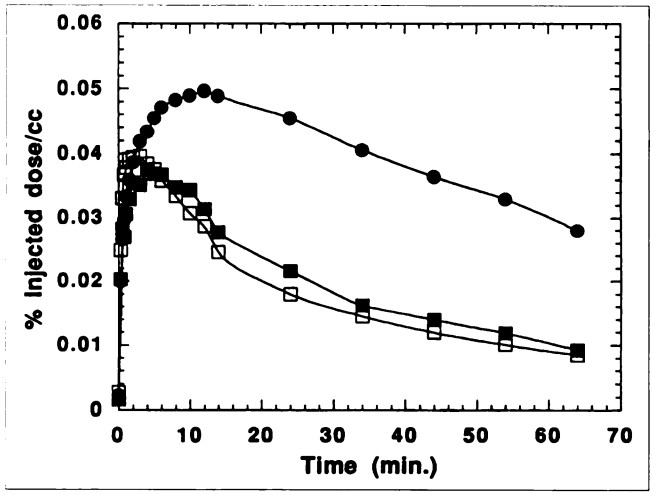

FIGURE 1. Time-activity curves for ^{11}C in the striatum (solid circles), thalamus (solid squares) and cerebellum (open squares) of baboon brain after injection of [^{11}C]*d-threo*-MP. Curves represent an average of six different runs in the same baboon (Brie).

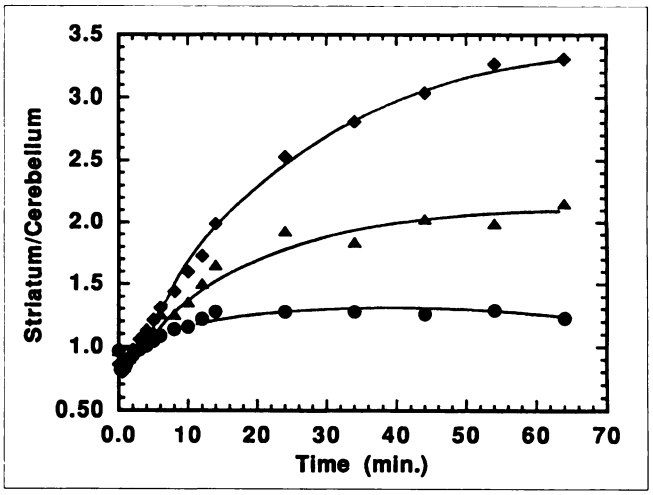

FIGURE 2. Ratios of striatum-to-cerebellum for [^{11}C]*d-threo*-MP (diamonds), [^{11}C]MP (triangles) and [^{11}C]*l-threo*-MP (circles) in the same baboon (Brie).

$$DV = K_1/k_2(1 + B'_{max}/K'_d) \quad \text{Eq. 1}$$

for regions containing transporter sites characterized by an equilibrium dissociation constant K'_d ($K'_d = K_d/f_{NS}$; f_{NS} means free fraction of tracer in tissue) and transporter concentration B'_{max}. K_1 and k_2 are the plasma-to-tissue and the tissue-to-plasma transport constant, respectively. For regions with no transporter, the distribution volume is given by:

$$DV = K_1/k_2. \quad \text{Eq. 2}$$

A parameter proportional to free transporter concentration can be obtained from Equations 1 and 2 giving:

$$B'_{max}/K'_d = [DV_{ROI}/DV_{CB}] - 1, \quad \text{Eq. 3}$$

where K'_d and k_2 include the free fraction of tracer in tissue. Equations 1 and 2 are based on classical compartmental analysis in which the effects of CBF and capillary permeability are implicitly included in the parameters K_1 and k_2.

Changes in the distribution volume ratio for a ROI to that in the cerebellum after drug treatment were considered significant if they were greater than 3 s.d.s from the average distribution volume ratio at baseline.

RESULTS

Kinetics of Brain Regional Activities

In each baseline study, brain activity distributed heterogeneously, with the highest uptake occurring in striatum. Peak uptake (average 0.05% ID/cc) occurred 5–15 min postinjection for the striatum, 5–10 min for the thalamus and 3–5 min for the cerebellum (Fig. 1). The half-times for clearance from peak uptake for [^{11}C]*d-threo*-MP (n = 12) were 70 ± 10, 35 ± 5 and 25 ± 5 min for the striatum, thalamus and cerebellum, respectively, compared to 60 ± 5, 35 ± 5, 25 ± 5 min for [^{11}C]MP (n = 8) (*4*). The striatum-to-cerebellum ratio was about 2.8 for baboon Angel and 3.3 for baboon Brie at 60 min. Comparative studies of three ^{11}C-labeled MP tracers (individual enantiomers and racemate) on the same baboon (Brie) demonstrated a difference in the striatum-to-cerebellum ratio: 3.3 for [^{11}C]*d-threo*-MP, 2.2 for [^{11}C]MP and 1.1 for [^{11}C]*l-threo*-MP (Fig. 2).

The average distribution volumes of the striatum and cerebellum for baseline studies of [^{11}C]*d-threo*-MP, [^{11}C]MP and [^{11}C]*l-threo*-MP for baboons Brie and Angel are presented in Table 2. The distribution volume for the striatum decreased in the order $d > dl > l$. The ratio of the distribution volumes (striatum-to-cerebellum) averaged 2.4 for baboon Brie and 2.2 for baboon Angel. Although we observed high intersubject variability in the absolute values for the striatal and the cerebellar distribution volumes (compare Brie and Angel), the distribution volume ratios for the *d* and *l* enantiomers for the two baboons were similar. The cerebellar values for the *d* and *l* isomers differed from one another ($p < 0.02$). It is noted that the distribution volume for both the striatum and cerebellum was smaller for the *l* isomer than the *d* isomer, which may be due to pharmacokinetic factors such as plasma protein binding.

The average distribution volumes for three different ba-

TABLE 2
Stereoselectivity of Carbon-11-Methylphenidate Binding in Baboon Brain

Baboon	Tracer	DV_{ST}	DV_{CB}	DV_{ST}/DV_{CB}
Brie	[^{11}C]*d-threo*	33.60 ± 3.44	13.78 ± 1.77	2.45 ± 0.20 (n = 8)
	[^{11}C]*dl-threo*	22.19 ± 1.71	12.28 ± 1.50	1.82 ± 0.13 (n = 5)
	[^{11}C]*l-threo*	12.33 ± 0.78	10.80 ± 0.67	1.14 ± 0.01 (n = 3)
Angel	[^{11}C]*d-threo*	25.54 ± 0.75	11.7 ± 0.20	2.18 ± 0.03 (n = 3)
	[^{11}C]*dl-threo*	8.5	7.23	1.17 (n = 1)

DV = distribution volume; ST = striatum; CB = cerebellum.

TABLE 3
Average Distribution Volumes ± s.d. for Different Brain Regions

Baboon	Striatum	Thalamus	Midbrain	Cerebellum
Brie (n = 8)	33.61 ± 3.44	15.78 ± 1.78	15.33 ± 1.86	13.78 ± 1.78
Angel (n = 2)	25.54 ± 1.06	14.25 ± 1.35	13.26 ± 0.66	11.67 ± 0.46
Carm (n = 1)	27.41	14.76	15.22	13.73

boons for the striatum, thalamus, midbrain and cerebellum are given in Table 3. The average distribution volume was greatest in the striatum with lower values in the thalamus, midbrain and cerebellum.

Time-activity curves for the striatum and cerebellum at baseline and after treatment with β-CIT are shown in Figure 3A and the corresponding plots for the striatum-to-cerebellum ratio are shown in Figure 3B. β-CIT markedly reduced the striatal but not cerebellar binding of [^{11}C]*d-threo*-MP, demonstrating the saturable and specific binding of [^{11}C]*d-threo*-MP to the dopamine transporter in the brain. For MP pretreatment, the striatum-to-cerebellum ratio also approached one. For GBR 12909 pretreatment, the ratio was reduced to 1.4–1.5.

The average and individual ratios for baseline studies and individual values for drug treatment studies are presented in Table 4. The standard deviations for the average distribution volume ratios were used to assess the significance of drug-induced changes. We considered changes greater than 3 s.d.s from the baseline average to be significant. The ratios of distribution volume for the striatum to cerebellum were reduced by 54%, 50% and 55% for unlabeled MP, GBR 12909 and β-CIT, respectively. A parameter proportional to free transporter concentration (B'_{max}/K'_d) also showed a large change after pretreatment, with 91%, 79% and 100% reduction for unlabeled MP, GBR 12909 and β-CIT, respectively (data not shown). In contrast, pretreatment with citalopram and tomoxetine did not produce a significant change in the distribution volume ratios in any brain region.

In a chase experiment, [^{11}C]*d-threo*-MP binding proved to be reversible as the injection of nonradioactive MP at 15 min induced a displacement of striatal activity to a level similar to the cerebellar activity. As a result, the striatum-to-cerebellum ratio dropped significantly and approached one. In contrast, it steadily increased during that period in the control experiment (Fig. 4), indicating that, at 15 min, most of the striatal activity was associated with saturable sites.

The results of the assay for unchanged tracer in baboon plasma after intravenous injection of [^{11}C]*d-threo*-MP were similar to that for [^{11}C]MP (Fig. 5), which are consistent with other existing MP pharmacokinetic data that MP is rapidly metabolized and the two enantiomers have a similar bioavailability after intravenous (but not oral) administration (*9,10,19–21*).

DISCUSSION

A number of trends characterize today's chiral drug industry. Perhaps the most important one is that a steady, rising flow of single-isomer forms of chiral drugs in the U.S. and world markets is creating an increasing demand for enantiomeric intermediates and bulk active compounds, as well as enantioselective technology and services (*22*). The driving force is that single enantiomers can be more selec-

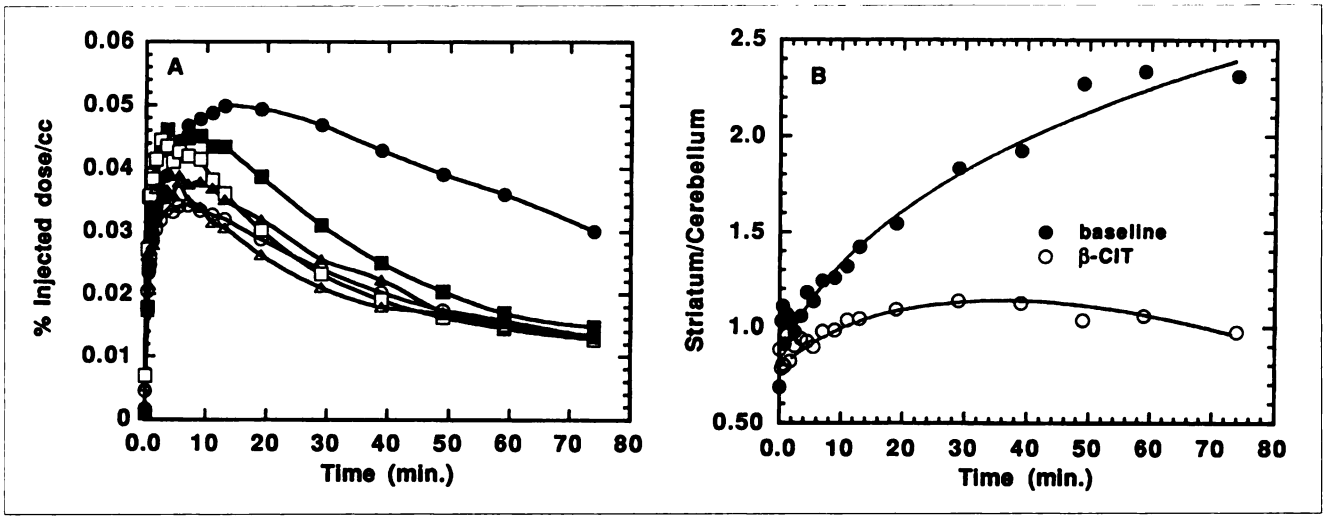

FIGURE 3. (A) Time-activity curves for [^{11}C]*d-threo*-MP in striatum (circles), thalamus (squares) and cerebellum (triangles) of baboon brain before (solid) and after (open) β-CIT pretreatment striatum-to-cerebellum ratios are presented for the same data in Figure 3B (angle). There is marked change in striatal uptake (A) and in the striatum-to-cerebellum ratio (B) after pretreatment with β-CIT.

TABLE 4
Distribution Volume Ratios (ROI/Cerebellum) for Carbon-11-*d-threo*-MP for Baseline (Run 1) and Drug Treatment (Run 2)

Study average	Drug treatment	DV_{ST}/DV_{CB}	% Change*	DV_{TH}/DV_{CB}	% Change*	DV_{MB}/DV_{CB}	% Change*
Brie (n = 6)	none	2.43 ± 0.24		1.17 ± 0.11		1.09 ± 0.05	
Angel (n = 2)	none	2.19 ± 0.04		1.22 ± 0.09		1.14 ± 0.04	
Carm (n = 1)	none	2.0		1.08		1.11	
Saturability							
Brie	Baseline	2.46		1.25		1.14	
	MP	1.13	−54†	1.06	−15	1.03	−10
DA transporter							
Brie	Baseline	2.75		1.31			
	GBR; 45 min	1.38	−50†	1.13	−14		
Brie	Baseline	2.66		1.26		1.14	
	GBR; 23 min	1.34	−50†	1.20	−5	1.09	−4
Angel	Baseline	2.16		1.16		1.11	
	β-CIT	1.04	−52†	1.03	−11	1.00	−10
Brie	Baseline	2.22		1.07		1.05	
	β-CIT	1.00	−55†	0.96	−10	0.94	−10
NE transporter							
Brie	Baseline	2.24		1.05		1.08	
	Tomoxetine	2.10	−11	1.05	0	1.12	4
Carm	Baseline	2.00		1.08		1.11	
	Tomoxetine	1.90	−10	1.13	5	1.11	0
5HT Transporter							
Angel	Baseline	2.22		1.28		1.16	
	Citalopram; 2 hr	2.18	−2	1.27	−1	1.13	−2.2
Brie	Baseline	2.22		1.10		1.04	
	Citalopram; 30 min	2.30	3.6	1.13	2	1.11	6

*The % change is [(run 2 − run 1)/run 1] × 100.
†Drug treatment changed the value of the DV ratio by more than 3 s.d. from the average of the baseline values for that animal.
DV = distribution volume; ST = striatum; CB = cerebellum; TH = thalamus; MB = midbrain; DA = dopamine; NE = norepinephrine; 5HT = serotonin.

tively effective. Methylphenidate is a central nervous system stimulant widely used to treat children with attention-deficit hyperactivity disorder and is marketed as *dl-threo* racemic form. It has been shown, however, that the pharmacological activity resides almost entirely in the *d-threo*-enantiomer (*5*). The rapid synthesis we have developed to obtain enantiomerically pure ^{11}C-labeled *d-threo*-MP allows

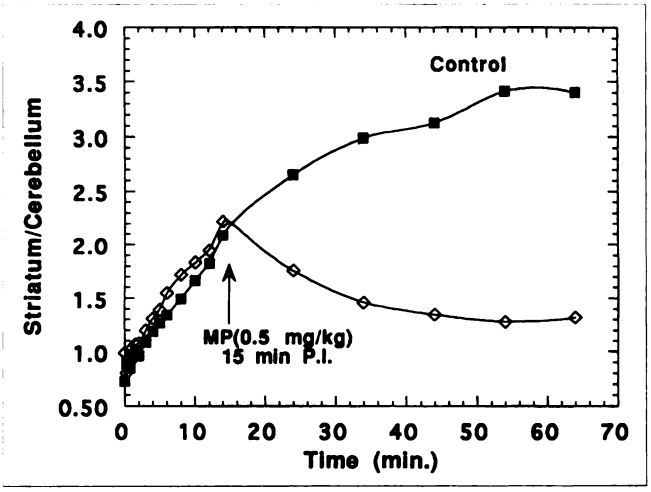

FIGURE 4. Effect of MP on the striatum-to-cerebellum ratio for [^{11}C]*d-threo*-MP (Brie). Methylphenidate (0.5 mg/kg, i.v.) was injected 15 min postinjection of [^{11}C]*d-threo*-MP and induced a displacement of [^{11}C]*d-threo*-MP uptake in the striatum, resulting in the reduction of the striatum-to-cerebellum ratio.

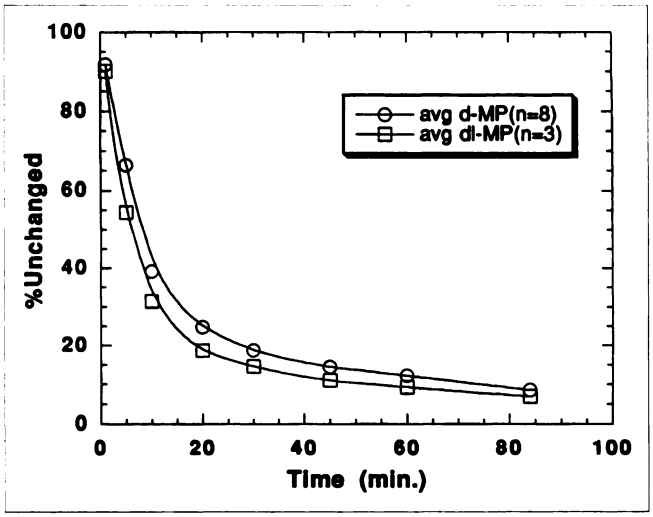

FIGURE 5. Percentage of unchanged tracer in baboon plasma after intravenous injection of [^{11}C]*d-threo*-MP (circles) and [^{11}C]MP (squares).

us to characterize its binding, to compare it with the *l*-enantiomer and racemic mixture, to examine its specificity to dopaminergic neurons and to evaluate it as a radiotracer for the presynaptic dopaminergic neuron.

Binding Characteristics

In our previous study using [^{11}C]MP (*dl-threo* racemic form), we demonstrated the specific binding to dopamine transporters as well as the sensitivity to degeneration of the dopaminergic neurons in early Parkinson's disease (*4*). Comparative studies of [^{11}C]*d-threo*-MP and [^{11}C]MP in the same baboon (Brie) demonstrated a large difference in the ratio of specific-to-nonspecific binding, as indicated by striatal versus cerebellar uptake: 3.3 for [^{11}C]*d-threo*-MP and only 2.2 for [^{11}C]MP (Fig. 2). This is consistent with the potency in displacing [^{3}H]*dl-threo*-MP from striatal synaptosomal membrane binding sites—IC_{50} corresponding to 0.088 μM for *d-threo*-MP and 0.21 μM for *dl-threo*-MP (*6*).

The ability of [^{11}C]*d-threo*-MP to label dopamine transporter was primarily demonstrated by blocking studies with various monoamine uptake inhibitors. GBR 12909 is a selective inhibitor of dopamine uptake. The IC_{50} of GBR 12909 to inhibit uptake of radiolabeled monoamines into rat synaptosomes is 1 nM for dopamine, 170 nM for 5-HT and 440 nM for NE (*11*). GBR 12909 inhibited [^{11}C]*d-threo*-MP uptake in the striatum but not in the thalamus or cerebellum, which is consistent with [^{11}C]*d-threo*-MP binding to dopamine transporters in the striatum. A decrease of 50% in the distribution volume ratio in the striatum to that in the cerebellum (DV_{ST}/DV_{CB}, Table 4), which corresponds to $(B'_{max}/K'_d) + 1$, was observed after pretreatment with GBR 12909. If one quantifies the percent change in B'_{max}/K'_d in the striatum with GBR 12909, this corresponds to 78%. The failure to totally block MP binding by GBR 12909 was probably related to a single dose being used (insufficient dose) as well as to differences in the binding patterns within the dopamine transporter between these two drugs (*23,24*). In fact, when we pretreat with the cocaine analog β-CIT (0.3 mg/kg i.v.), the distribution volume in the striatum for [^{11}C]*d-threo*-MP is reduced almost to the distribution volume value of the cerebellum (Table 4). It has been reported that β-CIT binds to both dopamine and 5-HT transporters with in vitro IC_{50}, 1.6 nM for displacing [^{3}H]CFT from dopamine transporter sites in monkey striatum and 3.8 nM for displacing [^{3}H]paroxetine from 5-HT transporter sites in rat cortical membranes (*25*). The percent change in B'_{max}/K'_d in striatum with β-CIT treatment corresponds to 97%–100%, which is consistent with the specific binding of the tracer to dopamine transporter in the striatum. We observed a 5%–15% change in the distribution volume ratio (DV_{TH}/DV_{CB}) for the thalamus with β-CIT, GBR 12909 and MP. The variability in the response, however, as well as the fact that it was small (less than 3 s.d. from the average of the baseline measures) is only suggestive of a trend. A similar small reduction was observed with the distribution volume ratio for the midbrain after β-CIT and MP treatment.

The binding specificity of [^{11}C]*d-threo*-MP to dopamine transporters in the striatum was further supported by the lack of blocking effect after pretreatment with tomoxetine or citalopram (inhibitors of the norepineprine and serotonin transporter, respectively) (Table 4). This result is consistent with a report that the distribution of *d-threo* MP is inhibited by mazindol (which inhibits both dopamine and norepinephrine transport) but not by desipramine (norepinephrine reuptake inhibitor) (*26*). It also supports the prevailing view that the stimulant effects of MP and related drugs are related more consistently to their inhibitory actions on the dopamine transporter than on the norepinephrine or the serotonin transporter.

A number of PET and SPECT radioligands that bind to the neuronal dopamine transporter in vivo and have been shown to be sensitive to the loss of dopamine transporters (either in vivo or in vitro with tritium- or ^{125}I-labeled tracers) have been reported, including [^{11}C]nomifensine (*27–29*), [^{11}C]cocaine and [^{3}H]cocaine (*30–32*), [^{18}F]GBR 13119 and [^{3}H]GBR 12935 (*33,34*) and ^{11}C- and [^{3}H] WIN 35428 (*35–39*) and ^{123}I- and ^{125}I-labeled cocaine analogs (*12,40–44*). In comparison with these ligands, [^{11}C]MP has several attractive features for PET, including its high uptake and reversibility in the brain, which facilitates kinetic modeling (*15*). The fact that MP is an approved drug expedites approval for human studies. It also makes it possible to use unlabeled MP to assess saturability of binding in humans. The present study demonstrates a higher ratio for specific versus nonspecific binding for [^{11}C]*d-threo*-MP, the ratio of striatum-to-cerebellum after pretreatment with unlabeled MP and GBR 12909 was reduced by about 60% for [^{11}C]*d-threo*-MP (data not shown) compared to 43% for [^{11}C]MP. The ratio of distribution volumes for the striatum-to-cerebellum for these two separate studies was reduced by 54% and 50%, respectively, for [^{11}C]*d-threo*-MP (Table 4) compared to 37% and 38% for [^{11}C]MP (*4*) Thus, [^{11}C]*d-threo*-MP is characterized by a higher striatal uptake with higher striatal-to-cerebellar ratios, higher degree of the specificity to dopamine transporter and the reversibility which facilitates kinetic modeling.

Evaluating Dopaminergic Neuron Loss

In anticipation of the use of [^{11}C]*d-threo*-MP to evaluate to loss of dopaminergic neurons in Parkinson's disease, we examined the potential effect of elevated striatal dopamine produced by L-DOPA therapy on radiotracer binding. We found that treatment with L-DOPA (50 mg/kg) plus benserazide, an aromatic amino acid decarboxylase inhibitor (5 mg/kg) did not change the binding of [^{11}C]*d-threo*-MP in the baboon brain (*45*). This result suggests that the sensitivity of [^{11}C]*d-threo*-MP to changes in dopamine concentration is low and the uptake would not be affected by administration of L-DOPA in Parkinson patients. These PET studies as well as a previous PET study with [^{11}C]MP (*4*) suggest that [^{11}C]*d-threo*-MP may be sensitive to the loss in dopamine neurons associated with normal aging and

may also be a marker of dopamine transporters in neurodegenerative diseases such as Parkinson's. A particularly important characteristic of such a radiotracer is high sensitivity to early or presymptomatic changes that may identify subjects at risk who may optimally benefit from therapy. We view reversibility of [^{11}C]*d-threo*-MP binding as a particularly important characteristic that facilitates quantification relative to irreversibly trapped tracers which may exhibit flow-limited binding.

CONCLUSION

We have characterized [^{11}C]*d-threo*-MP binding in the baboon brain. The availability of this labeled drug makes it possible to compare the pharmacokinetics with its racemic form ([^{11}C]MP) in the human brain. The observation of higher specific-to-nonspecific binding and selectivity to dopamine transporters and reversibility suggests that this may be a potentially useful tracer to evaluate the degeneration of dopaminergic neurons and the associated loss of nerve terminals in the striatum. This study supports further evaluation of [^{11}C]*d-threo*-MP as a PET radiotracer for the presynaptic dopaminergic neuron in human brain. Studies are now underway to determine the sensitivity of *d-threo*-MP to neuronal loss occurring in normal aging and in neurodegenerative disease and to monitor the effect of drugs on the rate of dopaminergic neuron loss.

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