

Brain Kinetics of Methylphenidate (Ritalin) Enantiomers After Oral Administration

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KEY WORDS methylphenidate; Ritalin; ADHD; dopamine transporter; positron emission tomography; chiral drugs

ABSTRACT Methylphenidate (MP) (Ritalin) is widely used for the treatment of attention deficit hyperactivity disorder (ADHD). It is a chiral drug, marketed as the racemic mixture of *d*- and *l*-threo enantiomers. Our previous studies (PET and microdialysis) in humans, baboons, and rats confirm the notion that pharmacological specificity of MP resides predominantly in the *d*-isomer. A recent report that intraperitoneally (i.p.) administered *l*-threo-MP displayed potent, dose-dependent inhibition of cocaine- or apomorphine-induced locomotion in rats, raises the question of whether *l*-threo-MP has a similar effect when given orally. It has been speculated that *l*-threo-MP is poorly absorbed in humans when it is given orally because of rapid presystemic metabolism. To investigate whether *l*-threo-MP or its metabolites can be delivered to the brain when it is given orally, and whether *l*-threo-MP is pharmacologically active. PET and MicroPET studies were carried out in baboons and rats using orally delivered C-11-labeled *d*- and *l*-threo-MP ([methyl-¹¹C]*d*-threo-MP and [methyl-¹¹C]*l*-threo-MP). In addition, we assessed the effects of i.p. *l*-threo-MP on spontaneous and cocaine-stimulated locomotor activity in mice. There was a higher global uptake of carbon-11 in both baboon and rat brain for oral [¹¹C]*l*-threo-MP than for oral [¹¹C]*d*-threo-MP. Analysis of the chemical form of radioactivity in rat brain after [¹¹C]*d*-threo-MP indicated mainly unchanged tracer, whereas with [¹¹C]*l*-threo-MP, it was mainly a labeled metabolite. The possibility that this labeled metabolite might be [¹¹C]methanol or [¹¹C]CO₂, derived from demethylation, was excluded by ex vivo studies in rats. When *l*-threo-MP was given i.p. to mice at a dose of 3 mg/kg, it neither stimulated locomotor activity nor inhibited the increased locomotor activity due to cocaine administration. These results suggest that, in animal models, *l*-threo-MP or its metabolite(s) is (are) absorbed from the gastrointestinal tract and enters the brain after oral administration, but that *l*-threo-MP may not be pharmacologically active. These results are pertinent to the question of whether *l*-threo-MP contributes to the behavioral and side effect profile of MP during treatment of ADHD. **Synapse 53: 168–175, 2004.** © 2004 Wiley-Liss, Inc.

INTRODUCTION

Racemic methylphenidate (MP, *dl*-threo-methyl-2-phenyl-2-(2-piperidyl)acetate, Ritalin) is a CNS stimulant that is the most commonly prescribed drug for the treatment of attention deficit hyperactivity disorder (ADHD). ADHD has become America's No. 1 childhood psychiatric disorder (Barkley, 1977) with an estimated prevalence of 5–10% of the general population (Swanson et al., 1998). The therapeutic effect of MP has been linked to its blockade of the dopamine transporter, resulting in enhanced levels of synaptic dopamine (Volkow et al., 2001). We have labeled both *d*- and *l*-threo-MP with carbon-11 ([¹¹C]*d*-threo-MP and [¹¹C]*l*-

threo-MP) and used positron emission tomography (PET) (1994a,b, 1995, 1997) to show high specific bind-

Contract grant sponsor: U.S. Department of Energy and Office of Biological Environmental Research; Contract grant number: DE-AC02-98CH10886; Contract grant sponsor: National Institutes of Health, National Institute for Biomedical Imaging and Bioengineering; Contract grant number: EB002630; Contract grant sponsor: National Institute on Drug Abuse; Contract grant number: DA-06278; Contract grant sponsor: Office of National Drug Control Policy.

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Received 14 October 2003; Accepted 26 April 2004

DOI 10.1002/syn.20046

Published online in Wiley InterScience (www.interscience.wiley.com).

ing of [^{11}C]*d-threo*-MP as compared to mostly nonspecific binding of [^{11}C]*l-threo*-MP after i.v. injection into humans and baboons (Ding et al., 1997). Studies that employed computer testing of children with ADHD suggested a drug-induced improvement in sustained attention that was entirely attributable to the *d*-enantiomer (Srinivas et al., 1992b). Microdialysis studies in free-moving rats, used for measuring the changes in extracellular dopamine concentrations produced by i.p. injection of individual unlabeled *d-threo*-MP and *l-threo*-MP, further demonstrate that pharmacological specificity of MP with respect to MP-induced DA increases resides predominantly in the *d-threo* isomer and the binding of *l*-isomer is mostly nonspecific (Aoyama et al., 1994, 1996).

Methylphenidate undergoes extensive and stereospecific presystemic metabolism (Aoyama et al., 1990; Hubbard et al., 1989; Srinivas et al., 1987) in both human and animals to form predominantly the hydrolysis product, ritalinic acid (RA), resulting in a low absolute oral bioavailability, and an oral bioavailability of *d*- and *l*-MP of 22% and 5%, respectively (Srinivas et al., 1993). Animal studies have suggested that the primary site of presystemic metabolism is in the gut and/or intestinal wall and not the liver or lungs (Aoyama et al., 1990). RA concentrations in the blood may be 30–60-fold higher than MP concentrations. Although RA is a polar compound and circulates in higher concentration than MP, it does not appear to have CNS activity even when administered intracerebroventricularly (Patrick et al., 1984; Sheppard et al., 1960).

Studies from our laboratory and other research groups have shown that injections of racemic MP and *d-threo*-MP increase locomotor activity in rodents (Ding et al., 1997; Gerasimov et al., 2000). A recent report that i.p. administered *l-threo*-MP displayed a dose-dependent inhibitory effect on the locomotor activity induced by *d-threo*-MP, cocaine, or apomorphine in intact adult rats (Baldessarini, 2001) raised the question of whether *l-threo*-MP has a similar effect when given orally. This is relevant since ADHD subjects are treated with oral MP and it is therefore crucial to assess whether *l-threo*-MP plays a role or is inactive in the brain when used therapeutically.

We report here our studies using PET and C-11-labeled *d*- and *l-threo*-MP ([^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP) to compare the uptake of these tracers in the brain after oral administration to individual baboons and rats. Rats were sacrificed after tracer administration to investigate the chemical form of C-11 in brain. To evaluate whether *l-threo*-MP interfered with cocaine-induced locomotion, we conducted experiments with these two drugs in mice.

MATERIALS AND METHODS

Drugs

Unlabeled *dl-threo*-MP.HCl was purchased from Research Biochemicals (Natick, MA). The individual unlabeled enantiomers (*d-threo*-MP and *l-threo*-MP) were separated in our laboratory according to a literature method (Ding et al., 1994b; Patrick et al., 1987). Cocaine was obtained from the NIDA.

Animals

All studies were conducted in accordance with the guidelines established by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Brookhaven National Laboratory. Adult female baboons (*Papio anubis*) housed at BNL for several years were used for PET studies. Adult male Sprague Dawley rats and Swiss-Webster mice were purchased from Taconic Farms (Germantown, NY). Rats had a mean weight of 375 ± 12 g when utilized, and mice had a mean weight of 30 ± 5 g. Animals were individually housed in a room controlled for temperature and humidity as well as a 12-h light/dark cycle. Water (except where indicated) and food were available ad libitum.

Radiosynthesis of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP

[^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP were prepared from an N-protected *d-threo*- or *l-threo*-ritalinic acid derivative in two steps: O-methylation with [^{11}C]CH₃I followed by hydrolysis of the protective group. The total synthesis time was 40 min, with an average specific activity of 1.5 Ci/ μmol (EOB), radiochemical purity > 98%, and enantiomeric purity of 99% (Ding et al., 1994b).

PET studies of oral [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP in baboons

Three baboons were used in the PET studies over an 8-month period with at least 3 weeks between studies to allow recovery from anesthesia and blood sampling. The baboons were anesthetized and prepared for PET studies as described previously (Ding et al., 1995). Briefly, animals were anesthetized with an intramuscular dose of ketamine (10 mg/kg) and then intubated and ventilated with a mixture of isoflurane (Forane, 1–4%), nitrous oxide (1,500 ml/min), and oxygen (800 ml/min). Catheters were inserted in a popliteal artery and a radial arm vein for arterial sampling and radiotracer injection, respectively. An oral gastric tube was inserted. The baboons were positioned on an individualized padded restraining table to minimize motion and repositioning errors. An attenuation scan was performed prior to radiotracer administration. During the study, heart rate, respiration rate, PO₂, and temperature were measured using a pediatric monitor

(SpaceLabs Pediatric Patient Care Monitoring System). For each paired study, tracer doses (4–7 μg per injection) of [^{11}C]d-threo-MP and [^{11}C]l-threo-MP were administered via a gastric tube with a 2–3-h time period between the two doses. Each injection contained 3–10 mCi of radioactivity in 10 mL of H_2O , and the gastric tube was then flushed with 30–50 mL water. This procedure allowed us to compare the kinetics of each enantiomer in the brain of the same baboon. The order of administration of the enantiomers was varied to control possible effects of prolonged anesthesia. Scanning was performed for 90 min on an HR+ high-resolution PET scanner (63 slices; $4.5 \times 4.5 \times 4.5$ mm) operating in 3D mode. The following scanning sequence was used: 10 frames for 1 min each; four frames for 5 min each, eight frames for 7.5 min each. Gamma shielding was placed across the baboon body to reduce effects of scattered radiation on brain images after the administration via a gastric tube. Arterial blood sampling and determination of unchanged labeled methylphenidate in blood plasma were carried out as reported previously (Ding et al., 1995). The gastric tube was taken out at the end of the second study. The residual radioactivity in the gastric tube was measured and decay-corrected to the time of the injection to determine the amount of radioactivity that was delivered to the stomach. To minimize the movement and disturbance of the baboon, the gastric tube was not removed after the first study.

Image and data analysis

In our previous studies, after the i.v. administration of [^{11}C]d-threo-MP or [^{11}C]l-threo-MP, regions of interest (ROIs) of the baboon brain were clearly defined and were drawn directly on the PET scans (Ding et al., 1997). However, there was much less radioactivity in the brain after oral administration of [^{11}C]d-threo-MP or [^{11}C]l-threo-MP, and thus only the uptakes of the C-11-labeled individual enantiomers in the whole brain were determined and compared. In one of the studies that showed the highest uptakes in the brain, radioactivity in the striatum and cerebellum was also compared. For studies performed on the HR+ scanners, time frames were summed to obtain an image on which a global region comprised 5–7 central slices. The summed image was then projected onto the dynamic sequence to obtain time–activity data. Time–activity curves were compared for [^{11}C]d-threo-MP vs. [^{11}C]l-threo-MP in the whole brain in the same animal studied on the same day.

MicroPET studies in rats

Rats were anesthetized i.p. with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic head holder in a prone position on the bed of a microPET R4 scanner (Concorde Microsys-

tems, Knoxville, TN). The methodology of Thanos et al. (2002) was used. The total acquisition time was 75 min [(26 frames: 6 (10 sec); 3 (20 sec); 8 (60 sec); 5 (300 sec); 4 (600 sec)] and data was acquired in fully 3D mode with maximum axial acceptance angle ($\pm 28^\circ$). Images were reconstructed using FORE rebinning followed by 2D filtered backprojection with a ramp filter cutoff at Nyquist. Using the rat stereotaxic atlas (Paxinos and Watson, 1993) and the harderian glands as a reference point, the coronal planes of striatum and cerebellum were identified in the same manner. Specifically, for each animal the striatum and cerebellum was identified as 3 and 7 slices, respectively, caudal to the harderian glands (slice thickness was 1.2 mm). It has been previously shown that the harderian glands (located just rostral to the brain) because of their uptake of radioactivity are convenient anatomical markers in rodent PET studies (Thanos et al., 2002).

Animals were injected via the tail vein with [^{11}C]d-threo-MP (244 $\mu\text{Ci/cc}$) or [^{11}C]l-threo-MP (291 $\mu\text{Ci/cc}$) in a volume of 0.2 ml of sterile saline. The same two rats were used for oral administration studies 3 weeks after the i.v. administration experiments. Between the i.v. and oral studies they were trained to drink water on demand by depriving them of access to water for 24-h periods. This strategy avoided the need for surgery to implant gastric tubes and thus reduced the animal's levels of stress. The exact amount of radioactivity consumed by each animal was determined by measuring the activity in the drinking tube before and after drinking.

Analysis of the chemical form of carbon-11 in tissues

Rats were sacrificed at about 100 min after administration of tracers, when microPET imaging had been completed. Striatum (ST), cerebellum (CB), and the rest of the brain (ROB) were dissected out, weighed, and homogenized in a 2:1 acetonitrile/methanol mixture (volumes ranged from 0.5–1.0 mL depending on the weight of tissue) using a Tissue Tearor (Biospec Products, Bartlesville, OK) at full speed for 1 min. The homogenates were transferred to 2-mL Eppendorf tubes and centrifuged to produce clear supernatants. Supernatants and pellets were counted to determine extraction efficiency. All counting was done on a Minaxi 5000 auto gamma counter (Packard Instruments, Meriden, CT). Supernatants were spiked with unlabeled methylphenidate standard and analyzed by HPLC to determine percent unchanged methylphenidate in the tissues (HPLC conditions: Phenomenex Spherisorb 10 ODS1 250×4.6 , 60:40 acetonitrile/0.15M ammonium phosphate, flow 1.3 mL/min). The fraction of the labeled MP in each sample was taken as the amount of radioactivity co-eluting with the unlabeled MP (identified by monitoring UV activity at 254 nm) relative to the total amount injected into the

HPLC. Radioactivity in the entire gastrointestinal tract was also measured. Tissue radioactivity concentrations were expressed as percent injected radioactivity per gram (%IA/g).

In another study, rats ($n = 2$ for each enantiomer) were sacrificed 30 min after drinking aqueous solutions of [^{11}C]d-threo-MP or [^{11}C]l-threo-MP and tissue radioactivity concentrations determined as described above. In addition, aliquots of the supernatants were also analyzed to determine the extent to which radioactivity in the brain could be [^{11}C]methanol or [^{11}C]CO₂, derived from demethylation of the labeled MP. For each brain sample, a paired study was carried out (Fox et al., 1985). Aliquots of the supernatants from various brain regions were placed into two tubes containing 3 mL of isopropyl alcohol and 1.0 mL of 0.9 M sodium bicarbonate. One mL of 6 N HCl was added to one member of each pair and 1 mL of 0.1 N NaOH was added to the other. Radioactivity in both samples was assayed with a gamma-well counter before and after sonication in a heated ultrasonic bath for 10 min.

Cocaine induced locomotor activity

Outbred male Swiss-Webster mice (25–30 g) were purchased from Taconic Farms and maintained in our animal facility for at least 5 days before use on a 12-h light/dark cycle. Locomotor experiments were performed 1 h into the dark cycle, to maintain relatively high levels of spontaneous activity. Activity monitors were obtained from San Diego Instruments (San Diego, CA). They consisted of 16-inch Plexiglas cubical chambers in which horizontal movements of the mice interrupted infrared beams. Two mice were placed in each of the four chambers available, in order to increase the number of beam crossings recorded. The mice were allowed to explore the chambers for 1 h before administration of drugs. In a typical experimental session, one pair of mice was then injected with vehicle, one pair with l-threo-MP (3 mg/kg) alone, one pair with cocaine (10 mg/kg) alone, and one pair with l-threo-MP (3 mg/kg) plus cocaine (10 mg/kg). A total of six sessions were conducted, with chambers matched with drug group in a randomized manner on each day of testing. Average beam crossings for the six pairs of mice in each of the four conditions were graphed. Additionally, for each pair of animals, the mean activity (number of beam breaks) between 65 and 130 min was divided by the mean activity between 45 and 60 min. These activity ratios were used to calculate normalized average values for the four groups—mean \pm SE ($n = 6$). We routinely placed mice in pairs in the locomotor activity boxes to increase the number of beam breaks. In our hands this gives more reproducible results, probably because animals placed individually in boxes occasionally remain in one place for long periods. This strategy could be criticized on the grounds that group differences could reflect drug effects on interactions

TABLE I. Baboon studies with oral administration of [^{11}C]d-threo-MP or [^{11}C]l-threo-MP

Tracer	Baboon	Dose (mCi)	Global brain uptake ^a (%injected dose/cc)
[^{11}C]l-threo	A	5.22	0.005%
[^{11}C]d-threo		3.37	0.001%
[^{11}C]l-threo	A	5.58	0.0037%
[^{11}C]d-threo		9.38	0.0027%
[^{11}C]d-threo	A	6.44	<0.001%
[^{11}C]l-threo		— ^b	—
[^{11}C]d-threo	B	10.88	0.0013%
[^{11}C]l-threo		6.01	0.0020%
[^{11}C]l-threo	B	4.24	0.0011%
[^{11}C]d-threo		6.26	0.0008%

^aMaximum global brain uptake value based on the time-activity curve.

^bThis second study was cancelled due to a technical problem.

between animals, rather than drug effects on locomotor activity. However, we consider this unlikely in the present instance.

RESULTS

PET studies of oral [^{11}C]d-threo-MP and [^{11}C]l-threo-MP in baboons

In our previous studies, after i.v. injections of [^{11}C]d-threo-MP or [^{11}C]l-threo-MP the radioactivity reached a peak uptake ($\sim 0.05\%$ injected dose/cc) in baboon brain in less than 8 min (Ding et al., 1997). As expected, much slower brain uptake of carbon-11 was observed when these two tracers were given orally via a gastric tube. In most cases the uptake in the brain did not reach peak concentrations until at least 30 min after injection of [^{11}C]l-threo-MP and 40 min after injection of [^{11}C]d-threo-MP. This slow kinetics was consistent with our previous baboon PET study with orally administered racemic [^{11}C]MP (Volkow et al., 1998). Peak uptake values for five studies are summarized in Table I. Time-activity curves for a representative paired study are shown in Figure 1. Uptake in the whole brain for [^{11}C]l-threo-MP was always higher than for [^{11}C]d-threo-MP in all of the studies, regardless of the injected dose or the order of the injections. However, the ratio of the radioactivity in striatum to that in cerebellum was higher for oral injection of [^{11}C]d-threo-MP than that for [^{11}C]l-threo-MP (data not shown).

Measurement of total carbon-11 in baboon plasma after oral injection of radiotracers indicated a higher plasma integral for [^{11}C]l-threo-MP than for [^{11}C]d-threo-MP. However, plasma integrals for parent radiotracer (i.e., with the metabolite correction) were similar after [^{11}C]d-threo-MP and [^{11}C]l-threo-MP injections in the same baboon, and represented a small fraction of total radioactivity (Fig. 2).

The decay-corrected radioactivity for the two enantiomers remaining in the gastric tube was negligible. Thus, the measured injected dose was not confounded by the possibility that part of the activity adhered to the tube and was not available for biodistribution.

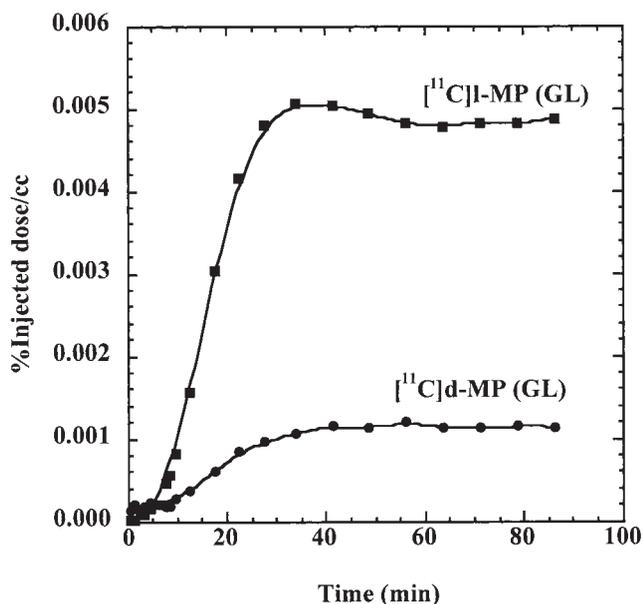


Fig. 1. Global uptake in baboon brain after oral administration of $[^{11}\text{C}]l\text{-threo-MP}$ (solid squares) and $[^{11}\text{C}]d\text{-threo-MP}$ (solid circles) in the same baboon.

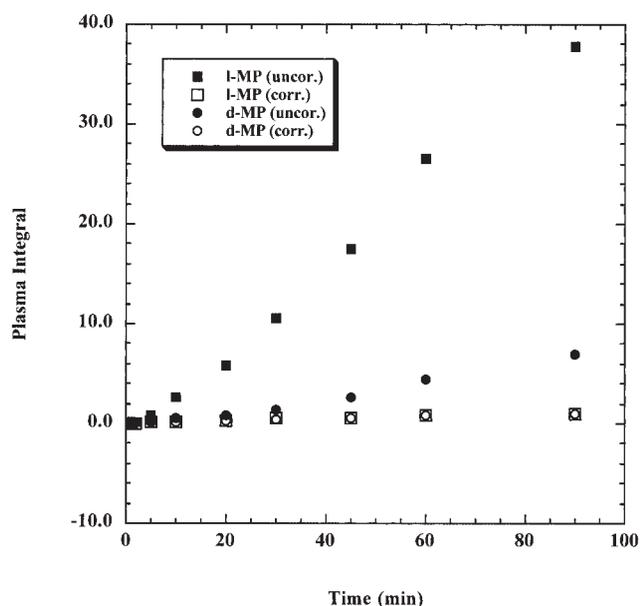


Fig. 2. Plasma integral of the radioactivity in baboon after oral injection of $[^{11}\text{C}]d\text{-threo-MP}$ (circles) and $[^{11}\text{C}]l\text{-threo-MP}$ (squares). Solid symbols represent data that have not been corrected for metabolite, open symbols represent data that have been corrected for metabolite. Note that the plasma integrals for both $[^{11}\text{C}]d\text{-threo-MP}$ and $[^{11}\text{C}]l\text{-threo-MP}$ were similar after they were corrected for the presence of labeled metabolite(s).

MicroPET studies of i.v. and oral $[^{11}\text{C}]d\text{-threo-MP}$ and $[^{11}\text{C}]l\text{-threo-MP}$ in rats

A clear and significant difference was observed between the two isomers with $[^{11}\text{C}]d\text{-threo-MP}$, but not $[^{11}\text{C}]l\text{-threo-MP}$, showing high striatal uptake follow-

TABLE II. Oral $[^{11}\text{C}]d\text{-threo-MP}$ and $[^{11}\text{C}]l\text{-threo-MP}$ in rats

Oral $[^{11}\text{C}]d\text{-threo-MP}$ at 30 min		
ROI	%IA/g	
ST	Rat1	Rat2
CB	0.042%	0.040%
ROB	0.055%	0.034%
G.I. tract	0.060%	0.039%
6.42%		
>50% of the C-11 radioactivity in the brain was MP		
Oral $[^{11}\text{C}]l\text{-threo-MP}$ at 30 min		
ST	Rat1	Rat2
CB	0.072%	0.088%
ROB	0.074%	0.096%
G.I. tract	0.198%	0.098%
1.97%		
<5% of the C-11 radioactivity in the brain was MP		
Oral $[^{11}\text{C}]d\text{-threo-MP}$ at 100 min		
ROI	%IA/g	
ST	0.030%	
CB	0.018%	
ROB	0.016%	
G.I. tract	0.02%	
~30% of the C-11 radioactivity in the brain was MP		
Oral $[^{11}\text{C}]l\text{-threo-MP}$ at 100 min		
ST	0.030%	
CB	0.020%	
ROB	0.048%	
G.I. tract	0.064%	
The C-11 radioactivity in the brain was not MP, $^{11}\text{CO}_2$, or $^{11}\text{CH}_3\text{OH}$		

Values are given for the percent injected activity per gram (%IA/g) in the striatum (ST), cerebellum (CB), the rest of brain (ROB), and the gastrointestinal tract. Percent unchanged tracer was determined by HPLC.

ing i.v. injection in rats. These results are consistent with our previous studies in baboon and human (Ding et al., 1997). However, after oral administration of the two enantiomers microPET did not give good images of the brain, possibly due to more rapid metabolism in rodents than primates, and the insensitivity of microPET to very low radioactivity concentrations.

Radioactivity assay in various tissues after oral $[^{11}\text{C}]d\text{-threo-MP}$ and $[^{11}\text{C}]l\text{-threo-MP}$ in rats

At both 30 and 100 min after oral administration, the total fraction of radioactivity in the whole brain after $[^{11}\text{C}]l\text{-threo-MP}$ was higher than that after $[^{11}\text{C}]d\text{-threo-MP}$ (Table II). The radioactivity in the gastrointestinal tract was highest for oral $d\text{-threo-MP}$ at 30 min, but highest for $l\text{-threo-MP}$ at 100 min postoral injection. HPLC analysis indicated that radioactivity in the brain regions after oral $l\text{-threo-MP}$ administration did not represent the parent labeled compound ($[^{11}\text{C}]l\text{-threo-MP}$); in contrast, most of the radioactivity in the brain after oral $[^{11}\text{C}]d\text{-threo-MP}$ was unmetabolized.

Determination of the contributions to total radioactivity of $[^{11}\text{C}]$ methanol or $[^{11}\text{C}]\text{CO}_2$

The evaporation experiments performed under either acidic or basic conditions did not produce any significant loss of radioactivity before and after sonication in a heated ultrasonic bath (data not shown).

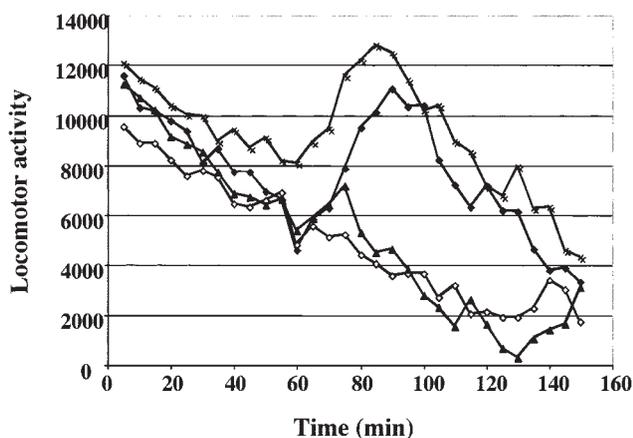


Fig. 3. The effect of *l*-threo-MP (3 mg/kg, i.p.) on cocaine induced locomotor activity. Y axis indicates movements (i.e., locomotion). Saline (open diamonds); *l*-threo-MP (triangles); cocaine (10 mg/kg, black diamonds); and combination of *l*-threo-MP and cocaine (double crosses). Drugs were injected i.p. in mice. Each of the four curves was generated based on the data from 12 mice.

These results suggest that the observed radioactivity in the brain was not due to either [^{11}C]methanol or [^{11}C]CO₂.

Effect of *l*-threo-MP on cocaine-induced locomotor activity

Averaged time-activity data for mice treated with saline, *l*-threo-MP, cocaine, or cocaine plus *l*-threo-MP are shown in Figure 3. The combination of *l*-threo-MP and cocaine appeared to slightly enhance the activity over that seen with cocaine alone. However, when data for each pair of animals during the 70-min period after drug treatment was normalized to activity in that pair in the 15-min period before drug, we obtained the following ratios. Saline, 0.56 ± 0.04 ; *l*-threo-MP, 0.60 ± 0.07 ; cocaine, 1.33 ± 0.17 ; cocaine plus *l*-threo-MP, 1.22 ± 0.06 . There was no significant difference between the two groups without cocaine, or between the two groups with cocaine. However, either group with cocaine had a significantly higher ratio than either group without cocaine (all four group differences were $P < 0.01$). Thus, cocaine, as expected, was stimulatory. However, *l*-threo-MP neither stimulated locomotion nor, in contrast to the report of Baldessarini et al. (2002), did this compound reduce cocaine-stimulated locomotion. A similar experiment using four pairs of mice per condition and drug doses of 6 mg/kg *l*-threo-MP and 10 mg/kg cocaine also did not find a reduction of cocaine-stimulated locomotor activity (data not shown).

DISCUSSION

Methylphenidate (MP, Ritalin) treatment for ADHD has been considered one of the great successes in psychiatry. It is a commonly prescribed oral drug for children and a growing number of adults with ADHD. It is

a chiral drug and is marketed as the *dl*-threo racemic form. There is no evidence of interconversion between the two enantiomers in vivo. Racemic MP is absorbed quickly and completely after oral administration in humans (Faraj et al., 1974), but *d*-threo-MP is found in the urine at 10-fold higher levels than *l*-threo-MP. Conversely, urine contains 2–3-fold higher levels of *l*-threo-ritalinic acid than *d*-threo-ritalinic acid after oral administration of MP. In contrast, no significant enantiomeric difference was found for MP or ritalinic acid in urine after i.v. administration. These findings have been attributed to enantioselective presystemic conversion of MP to ritalinic acid rather than enantioselective excretion (Srinivas et al., 1992a) and have led to the speculation that *l*-threo-MP given orally would not enter the brain (Srinivas et al., 1992b). It has also been suggested by some investigators (Srinivas et al., 1992b), but not others (Jonkman et al., 1998), that *l*-threo-MP is not absorbed by the gastrointestinal tract as evidenced by the failure to detect *l*-threo-MP in plasma after oral MP administration.

To our surprise, a higher global uptake of radioactivity in the brain was observed for [^{11}C]*l*-threo-MP than for [^{11}C]*d*-threo-MP when they were given orally in both baboons and rats. One of the advantages of our using MP labeled with C-11 on the methyl ester moiety is that the demethylated metabolite RA is no longer radioactive; that is, it does not contribute to the PET images and does not complicate interpretation of results. Therefore, brain radioactivity after *l*-threo-MP is not in the form of ritalinic acid. Our results in rats also exclude unchanged [^{11}C]*l*-threo-MP as well as the demethylation products [^{11}C]methanol and [^{11}C]CO₂. Although our data are consistent with previous observations that *l*-threo-MP cannot be detected in plasma samples after administration of pharmacological doses of *l*-threo-MP (Srinivas et al., 1992a), they indicate formation of a metabolite bearing the methyl ester group that is able to cross the blood-brain barrier. At present, we have not definitively identified the metabolite, although it has the same HPLC retention time as *p*-hydroxy-MP (Ding et al., unpubl.), of which the hydrolyzed form has been previously recognized as a metabolite of racemic MP in human urine (Faraj and Jenkins, 1973; Srinivas et al., 1991). Other polar metabolites that have been identified in dog and rat urine after oral administration of ^{14}C -labeled MP are methyl 6-oxo- α -phenylpiperidineacetate, the glucuronide of *p*-hydroxy-MP and their hydrolyzed forms (oxo-RA and *p*-hydroxy-RA) (Egger et al., 1981). An argument against the assignment of the labeled metabolite of *l*-threo-MP as *p*-hydroxy-*l*-threo-MP is the reported low brain penetrability of racemic *p*-hydroxy-MP (Patrick et al., 1984). Further studies to elucidate these issues are necessary. However, our results with oral administration to monkeys and rats of the enantiomers of [^{11}C]MP do not contradict the common assumption that

the pharmacological specificity of MP resides entirely in the *d-threo* isomer. The key observations are that, after oral administration of [¹¹C]*d-threo*-MP, most of the radioactivity in the brain represented the parent compound and a higher uptake was observed in striatum than that in cerebellum. These findings were consistent with our previous studies when the tracers were given intravenously (Ding et al., 1997).

In our locomotor activity studies in mice, *l-threo*-MP given alone did not affect locomotor activity, which is consistent with our previous finding in rat brain microdialysis studies that *l-threo*-MP did not alter extracellular dopamine levels (Ding et al., 1997). These results are also in agreement with the recent report by Davids et al. (2002). However, our data did not support the finding of an inhibitory effect of *l-threo*-MP on cocaine-stimulated locomotor activity that was recently reported by Baldessarini et al. (2001). This would be an important finding, if verified by other workers, because of its possible implications for the treatment of cocaine dependency.

Taken together, our studies indicate for the first time that there is a compound that is absorbed by the gastrointestinal tract and then enters the brain after the oral administration of *l-threo*-MP. Although our baboon and microPET studies were done at tracer doses of the C-11-labeled MPs and they do not perfectly mimic the in vivo situation when a person takes a pill, and there may be differences in the metabolism and kinetics of MP among different species, these results raise the question of whether *l-threo*-MP contributes to the behavioral and side-effect profile of MP during treatment of ADHD. Numerous examples from a range of therapeutic areas confirm that single enantiomers can enhance clinical efficacy, reduce adverse effects, cause fewer interactions with other drugs, and minimize response variations among patients by offering more predictable pharmacokinetics and greater selectivity (Rosenbaum, 2002). In some cases, these advantages are simply due to the removal of an inactive enantiomer; but in other cases, a given dose of a single isomer offers greater benefits when administered alone than when administered as the racemic mixture, suggesting that the opposite enantiomer actually has detracting effects. This assumes the inactive enantiomer is always detrimental. On the other hand, one can speculate that perhaps in some cases the presence of the inactive enantiomer may contribute to therapeutic efficacy of a drug by enhancing bioavailability and other factors. The racemic form of MP has been widely used as the treatment of ADHD; however, neither the therapeutic mechanism(s) nor side effects have been well characterized. Our findings strongly suggest the importance and urgent need to better understand this drug.

In conclusion, these comparative studies of enantiomerically pure [¹¹C]*d-threo*-MP and [¹¹C]*l-threo*-MP in both baboon and rat brain demonstrate a higher global

uptake of radioactivity for [¹¹C]*l-threo*-MP than for [¹¹C]*d-threo*-MP when they were given orally. However, although we confirm that [¹¹C]*d-threo*-MP enters the brain after oral administration, the chemical form of radioactivity in brain after administration of [¹¹C]*l-threo*-MP is not the administered labeled compound but a metabolite tentatively identified as *p*-hydroxy-MP. These PET and microPET studies, combined with our previous studies with i.v. injection of [¹¹C]*d-threo*-MP and [¹¹C]*l-threo*-MP, strongly indicate that pharmacological specificity of MP resides predominantly in the *d-threo* isomer. This conclusion is supported by studies in mice indicating that *l-threo*-MP may be behaviorally inactive. Most important, these results support the further examination in humans of the comparative absorption, metabolism, and pharmacological activity of the individual enantiomers of MP and the nature of their interaction when the drug is given as a racemic mixture.

ACKNOWLEDGMENTS

This research was carried out at Brookhaven National Laboratory. The authors thank D. Alexoff, R. Ferrieri, and D. Schlyer for their assistance in various aspects of this research.

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