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ORIGINAL INVESTIGATION

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Chiral drugs: comparison of the pharmacokinetics of [^{11}C]*d-threo* and *l-threo*-methylphenidate in the human and baboon brain

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Abstract Methylphenidate (Ritalin) is the most commonly prescribed psychoactive medication for children in the US where it is used for the treatment of attention deficit hyperactivity disorder. Methylphenidate is marketed as a racemic mixture of the *d-threo* and *l-threo* enantiomers. It is believed that the *d* enantiomer is responsible for the therapeutic effect of methylphenidate. In this study we labeled the individual enantiomers with carbon-11 and compared their binding and pharmacokinetics in the human and baboon brain. Microdialysis studies in the rat were performed to compare their potency in elevating striatal dopamine concentration. Positron emission tomographic (PET) studies with [^{11}C]*d-threo*-methylphenidate ([^{11}C]*d-threo*-MP) demonstrated highest regional uptake in basal ganglia. In contrast, [^{11}C]*l-threo*-methylphenidate ([^{11}C]*l-threo*-MP) displayed similar uptakes in all brain regions. The ratios of distribution volumes at the steady-state for the basal ganglia to cerebellum (DV_{BG}/DV_{CB}) ranged from 2.2 to 3.3 for [^{11}C]*d-threo*-MP in baboon and human, and only 1.1 for [^{11}C]*l-threo*-MP. Pretreatment with unlabeled methylphenidate (0.5 mg/kg) or GBR12909 (1.5 mg/kg) markedly reduced the striatal but not the cerebellar uptake of [^{11}C]*d-threo*-MP, whereas there was no effect on DV_{BG}/DV_{CB} for [^{11}C]*l-threo*-MP. In the rat, *d-threo*-MP increased extracellular dopamine concentration by 650% whereas *l-threo*-MP did not affect dopamine levels. These results indicate that pharmacological specificity of MP resides entirely in the *d-threo* isomer and directly show that binding of the *l*-isomer in human brain is mostly nonspecific.

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Introduction

Attention deficit hyperactivity disorder (ADHD), which is estimated to affect more than 2 million children (or 3–5%), has become America's no. 1 childhood psychiatric disorder (Barkley 1977). Methylphenidate [MP, *dl-threo*-methyl-2-phenyl-2-(2-piperidyl)acetate (Ritalin)] is the drug of choice for the treatment of ADHD. It is a chiral drug and is marketed as the racemic mixture of the *d-threo* and *l-threo* forms. 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. It is this stimulation which is believed to regulate attention and impulsivity of ADHD children. However, 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 (Barkely 1977; Schweri et al. 1985; Patrick et al. 1987; Ritz et al. 1987; Pan et al. 1994). An important concern is, "should we use racemic drug or single enantiomer?" For most chiral drugs, the advantages of using a single enantiomer are evident: smaller doses, products twice as active, fewer side effects, and superior pharmacological profiles of the active compound (Thall 1996). In order to establish the comparative in vivo studies, we have developed the synthesis of enantiomerically pure C-11 labeled *d*- as well as *l*-MP ([^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP) and carried out initial studies in baboon as part of the development of [^{11}C]*d-threo*-MP as a radiotracer for studies of the dopamine transporter in human using positron emission tomography (PET).

Our first PET studies of C-11 labeled racemic *dl-threo*-methylphenidate ([^{11}C]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 (Ding et al. 1994a, b). The binding of [^{11}C]*d-threo*-methylphenidate in the baboon and human brain has also been characterized (Ding et al. 1995; Volkow et al. 1995). The observation of higher specific-to-nonspecific binding as compared to the racemic [^{11}C]MP and the selectivity to dopamine transporters and reversibility demonstrates that [^{11}C]*d-threo*-MP is a suitable PET radiotracer to probe the neuronal loss occurring in normal aging and in neurodegenerative disease (Wang et al. 1995; Volkow et al. 1996a, b, c).

We now report the comparative PET studies of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP in both baboon and human brain. We extend previous baboon studies to include assessment of the saturability and specificity of [^{11}C]*l-threo*-MP. The saturability of the binding was assessed with unlabeled methylphenidate. An assessment of the specificity for the presynaptic dopaminergic neuron was determined by pretreatment with GBR 12909 (a selective dopamine uptake blocker) (Anderson 1989). Furthermore, regional brain uptakes and clearances of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP were directly compared in the same human volunteers. The time courses of unchanged individual radiotracers in arterial plasma were also measured and used to calculate the steady-state distribution volume in brain (Logan et al. 1990). We also carried out microdialysis studies in free-moving rats in order to probe the changes in extracellular dopamine concentrations produced by individual unlabeled *d-threo*-MP and *l-threo*-MP.

Materials and methods

Unlabeled *dl-threo*-MP · HCl was purchased from Research Biochemicals Incorporated (Natick, Mass., USA). The individual enantiomers (*d-threo*-MP and *l-threo*-MP) were prepared in our laboratory according to a published method (Patrick et al. 1987). GBR 12909 was purchased from Research Biochemicals Incorporated.

Synthesis 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 in two steps: *O*-methylation with [^{11}C]CH_3I followed by hydrolysis. 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 [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP

Baboon studies

Two adult female baboons (*Papio anubis*) were used in the PET studies over an 8-month period with at least 4 weeks between studies. The baboons were anesthetized and prepared for PET studies as described previously (Ding et al. 1995). For each paired baseline study, tracer doses of [^{11}C]*d-threo*-MP followed by [^{11}C]*l-threo*-MP (5–9 mCi in 3 ml of saline, 0.017–0.03 μmol (4–7 μg) per injection; IV) were administered (or the order of the injection was reversed) with a 2 to 3-h time period between doses to compare the kinetics of each enantiomer in the same baboon. An examination of the effects of drug pretreatment on the binding of individual radiotracers was also carried out in paired studies, with and without pharmacological intervention. The same scanning protocol was performed as described for [^{11}C]*dl-threo*-MP (Ding et al. 1994a). Arterial blood sampling and plasma assay for the presence of unchanged labeled methylphenidate were carried out following the same procedure as reported for [^{11}C]*dl-threo*-methylphenidate. Vital signs, including heart and respiratory rate, were monitored and recorded throughout the study.

Human studies

Two healthy human volunteers were studied (males, subjects 1 and 2 were 67 and 63 years old, respectively). Both subjects were free from medication. An individually molded headholder was made for each subject. The head of the subject was then positioned in the gantry with the aid of two orthogonal laser lines one of which was placed parallel to the canthomeatal line and the other parallel to the sagittal plane. A chin strap device was used to minimize movement of the head during the scan and to assure accurate repositioning. Prior to radiotracer injection, transmission scans were obtained to correct for attenuation. In preparation for the study, subjects had two catheters implanted, one in an antecubital vein for tracer injection and the other in the radial artery for blood sampling. Both normal volunteers had two PET scans (tracer doses (6–7 mCi) of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP) with a 2-h interval between injections to assess the differences in kinetics of individual enantiomers in the same subject. Arterial plasma sampling and analysis were carried out as described for the baboon studies. After injection of [^{11}C]*d-threo*-MP or [^{11}C]*l-threo*-MP, a series of 20 emission scans was obtained from time of injection up to 84 min (four 15-s, two 30-s, four 1-min, four 2-min, five 10-min and one 20-min scans).

Drug administration

The pharmacological profiles of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP binding in baboon were determined by carrying out a baseline PET study and then pretreating with an IV injection of the following drugs prior to the second injection of [^{11}C]*d-threo*-MP or [^{11}C]*l-threo*-MP: *dl-threo*-methylphenidate (0.5 mg/kg, 20 min prior) and GBR 12909 (1.5 mg/kg, 23–45 min prior).

Assay of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP in plasma

Unchanged [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP in plasma was determined by a solid phase extraction method as described for [^{11}C]MP (Ding et al. 1994a).

Image and data analysis

Regions of interest (ROI) on baboon brain were drawn directly on the PET scans as described previously (Dewey et al. 1995). The striatal ROI were drawn in two sequential planes at the level of the genu of the corpus callosum. Cerebellar ROIs were drawn in the plane that intersected the middle of the cerebellum and regions were obtained in the left and right hemispheres. For the purpose of drawing regions of interest on the human brain, an averaged emission scan representing the activity from 10 to 90 min was obtained after injection of the tracer. Regions of interest for basal ganglia and cerebellum were drawn on these averaged images and then projected to the dynamic emission scans using a template previously described.

For the baboon and human studies, time-activity curves for tissue carbon-11 concentration were used to calculate the ratio of basal ganglia to cerebellum. Time-activity curves for carbon-11 and the time course of unchanged tracer in plasma were used to calculate distribution volume (DV) in basal ganglia (BG) and cerebellum (CB) regions using a graphical analyses method for revers-

ible systems (Logan plots) as previously described (Logan et al. 1990). The ratio of the DV in BG to that in CB was used as the model parameter to calculate dopamine transporter availability at baseline and after drug interventions.

DV provides a measure of binding that is a linear function of transporter availability given by

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

for regions containing transporter sites characterized by an equilibrium dissociation constant K'_d ($K'_d = K_d / f_{NS}$; f_{NS} = 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 DV is given by

$$DV = K_1 / k_2. \quad (2)$$

A parameter proportional to free transporter concentration can be obtained from equations (1) and (2) giving

$$B'_{max} / K'_d = [DV_{ROI} / DV_{CB}] - 1 \quad (3)$$

where K'_d and k_2 include the free fraction of tracer in tissue. Eq. (1) and Eq. (2) are based on classical compartmental analysis in which the effects of cerebral blood flow and capillary permeability are implicitly included in the parameters K_1 and k_2.

Microdialysis studies

Adult male Sprague-Dawley rats (200–300 g, Taconic Farms) were anesthetized and siliconized guide cannulae were stereotaxically implanted into the right antero-lateral basal ganglia (0.5 mm anterior and 2.5 mm lateral to bregma, and 2.5 mm ventral to the cortical surface) under chloral hydrate anesthesia (400 mg/kg IP) at least 3 days prior to study (Dewey et al. 1995). On the day of study, rats were placed in a bowl for at least 30 min prior to fraction collection. Microdialysis probes (4.0 mm, Bioanalytical Systems, BAS) were positioned within the guide cannulae and artificial cerebral spinal fluid (ACSF, 155.0 mM Na^+, 1.1 mM Ca^{2+}, 2.9 mM K^+, 132.76 mM Cl^-, and 0.83 mM Mg^{2+}, pH 7.4) was administered through the probe using a CMA/100 microinfusion pump (BAS) at a flow rate of 2.0 µl/min. Twenty minute samples were collected within the injection loop (50 µl), injected on line (CMA/160, BAS) and analyzed until three sequential injections differed by less than 10%. The average DA concentration of these three stable samples was defined as control (100%) and all subsequent treatment values were transformed to a percentage of that control. Upon establishing this baseline measurement, *dl-threo*-MP, *d-threo*-MP or *l-threo*-MP (20 mg/kg, 1.0 cc, $n = 6$), dissolved in sterile physiological saline, was injected IP. Control animals received saline injections using a similar injection protocol ($n = 10$). The HPLC system consisted of a BAS reverse phase column (3.0 µC-18), a BAS LC-4C electrochemical transducer with a dual glassy carbon electrode set at 650 mV, and a dual pen chart recorder. The mobile phase (flow rate = 1.0 ml/min) consisted of 9.5% methanol, 50 mM sodium phosphate monobasic, 1.0 mM sodium octyl sulfate, and 0.1 mM EDTA, pH 4.0. Dopamine eluted at 7.5 min. Upon completion of the study, rats were killed and brains were sectioned to verify probe placement.

Results

Baboon studies

In each baseline study, the distribution of [^{11}C]*d-threo*-MP in the brain was heterogeneous with the highest uptake (average 0.05% of the injected dose/cc) occurring in the basal ganglia. The half-times for clearance from peak uptake were 70, 30 and 21 min for basal ganglia, thalamus and cerebellum, respectively. In contrast, [^{11}C]*l-threo*-MP displayed similar uptakes in all brain regions (average 0.03% of the injected dose/cc) with a half-time for clearance of approximately 25 min for all the regions [see Fig. 1; solid symbols represent baseline of [^{11}C]*d-threo*-MP (a) and [^{11}C]*l-threo*-MP (b)]. As a result, the basal ganglia to cerebellum ratio was 3.3 for [^{11}C]*d-threo*-MP and only 1.1 for [^{11}C]*l-threo*-MP. The average distribution volumes of basal ganglia and cerebellum for the 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 1. The distribution volumes for the basal ganglia decreased in the order *d* > *dl*> *l*.The ratio of the distribution volumes (basal ganglia to cerebellum) for [^{11}C]*d-threo*-MP averaged 2.4 for baboon Brie and 2.2 for baboon Angel; and that for [^{11}C]*l-threo*-MP was 1.1 for both baboons. Though we observed a high intersubject variability in the absolute values for the striatal and the cerebellar DVs (compare Brie and Angel), the DV 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 DV for both the basal ganglia and the cerebellum was smaller for the *l* isomer, than for the *d* isomer, which may be due to pharmacokinetic factors such as plasma protein binding.

Pretreatment with unlabeled methylphenidate (0.5 mg/kg) or GBR 12909 (1.5 mg/kg) 23–45 min prior to [^{11}C]*d-threo*-MP injection markedly reduced the striatal but not the cerebellar uptake 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, whereas there was no effect on the binding of [^{11}C]*l-threo*-MP. The basal ganglia to cerebellum ratios

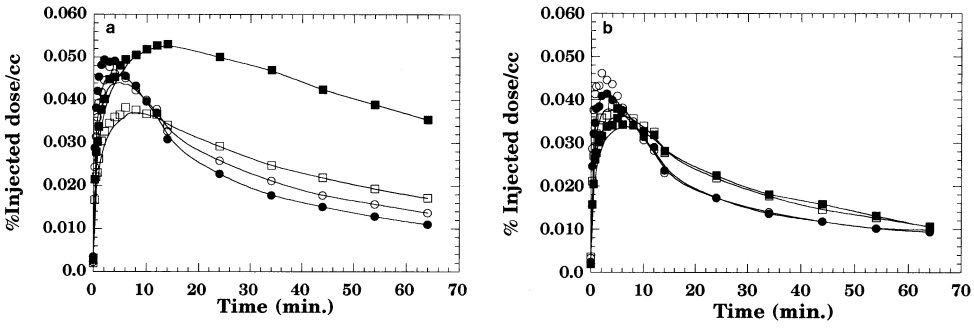

Fig. 1a, b Time-activity curves for (**a**) [^{11}C]*d-threo*-MP and (**b**) [^{11}C]*l-threo*-MP in basal ganglia (*squares*) and cerebellum (*circles*) of baboon brain before (*solid symbols*) and after (*open symbols*) unlabeled MP pretreatment. Note the marked change for [^{11}C]*d-threo*-MP in basal ganglia as compared to no significant change for [^{11}C]*l-threo*-MP after treatment with MP

Table 1 Comparison of C-11 labeled methylphenidate binding in baboon brain[a]. *d-MP* [^{11}C]*d-threo*-MP; *dl-MP* [^{11}C]*dl-threo*-MP; *l-MP* [^{11}C]*l-threo*-MP. Values are given for the distribution volume (*DV*) in the basal ganglia (*BG*) and cerebellum (*CB*) and for the ratio of the DV of BG to that of CB (DV_{BG}/DV_{CB})

Study	Tracer	Drug treatment	DV_{BG}	DV_{CB}	DV_{BG}/DV_{CB}	% Change
Reproducibility						
Angel	*d*-MP	None	25.54 ±0.75	11.7 ± 0.20	2.18 ± 0.03 (*n* = 3)	
	l-MP	None	8.5	7.23	1.17	
Brie	*d*-MP	None	33.60±3.44	13.78±1.77	2.45±0.20 (*n* = 8)	
	l-MP	None	12.33±0.78	10.80±0.67	1.14±0.01 (*n* = 3)	
Saturability						
Brie	*d*-MP	Baseline	27.96	11.37	2.46	–54
		MP	13.22	11.68	1.13	
Angel	*l*-MP	Baseline	8.49	7.22	1.17	–2
		MP	7.56	6.60	1.14	
DA Transporter						
Brie	*d*-MP	Baseline	32.59	11.83	2.75	–50
		GBR(45 min)	16.49	11.98	1.38	
Brie	*d*-MP	Baseline	36.84	13.86	2.66	–50
		GBR(23 min)	17.08	12.75	1.34	
Angel	*d*-MP	Baseline	41.24	15.99	2.58	–41
		GBR(30 min)	14.94	9.75	1.53	
Brie	*l*-MP	Baseline	12.174	10.86	1.12	–3
		GBR(30 min)	11.65	10.80	1.08	

[a] In order to facilitate comparison, some previously published data on *d-MP* were reproduced from Ding et al.(1995)

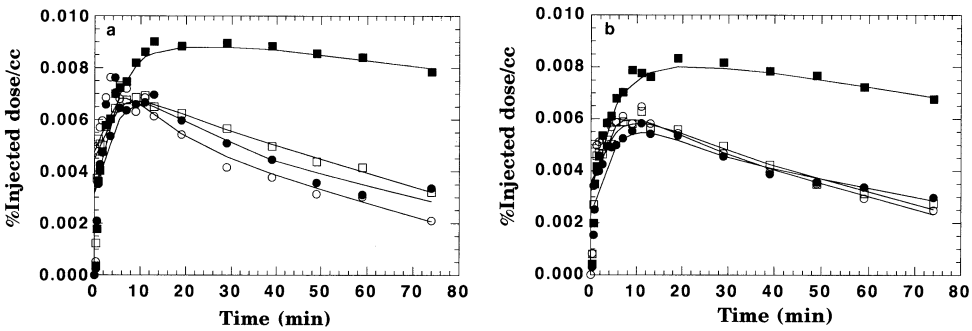

Fig. 2a, b Individual time-activity curves in basal ganglia (*squares*) and cerebellum (*circles*) for human (**a**) subject 1 and (**b**) subject 2 after injection of [^{11}C]*d-threo*-MP (*solid symbols*) and [^{11}C]*l-threo*-MP (*open symbols*). Note the much higher uptake of [^{11}C]*d-threo*-MP in the basal ganglia as compared to that of [^{11}C] *l-threo*-MP

before and after methylphenidate pretreatment are shown in Fig. 1. Additionally, for these two drug intervention studies, the ratios of distribution volumes for the basal ganglia to cerebellum for [^{11}C]*d-threo*-MP were reduced by 54 and 50% for methylphenidate and GBR 12909, respectively, and no significant change for [^{11}C]*l-threo*-MP was observed (see Table 1).

The results of the assays for unchanged tracer in baboon plasma after IV injection of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP were similar, with a slightly slower metabolism for [^{11}C]*d-threo*-MP. The fraction of [^{11}C]*d-threo*-MP in plasma was 95, 79, 50, 30, 25, 20, 15, and 13% at 1, 5, 10, 20, 30, 45, 60, and 84 min, respectively. This is consistent with other existing MP pharmacokinetic data reporting that MP is rapidly metabolized and the two enantiomers have a similar bioavailability with slightly higher ratio of *d* versus *l* isomer in the plasma after IV (but not oral) MP administration (Srinivas et al. 1992, 1993; Chan et al. 1980; Hungund et al. 1979; Wargin et al. 1983).

Human studies

Comparison of time-activity curves for the basal ganglia and cerebellum for two normal volunteers after IV injections of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP (Fig. 2) showed slow clearance of [^{11}C]*d-threo*-MP in the basal ganglia (only 10–15% clearance from peak activity had occurred at 74 min post-tracer injection), whereas the clearance rate of [^{11}C]*d-threo*-MP in cerebellum was similar to those of [^{11}C]*l-threo*-MP in most brain regions, including basal ganglia and cerebellum (more than 50% clearance occurred at 74 min). Figure 3 shows the brain images obtained from one of the subjects after intravenous injections of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP. Quantitative estimates for the plasma to tissue transfer constant (K_1) as well as the distribution volume measures are shown in Table 2. High distribution volume ratios for the basal ganglia to cerebellum for [^{11}C]*d-threo*-MP (2.9 for subject 1 and 2.3 for subject 2) as compared to 1.1–1.2 for [^{11}C]*l-threo*-MP

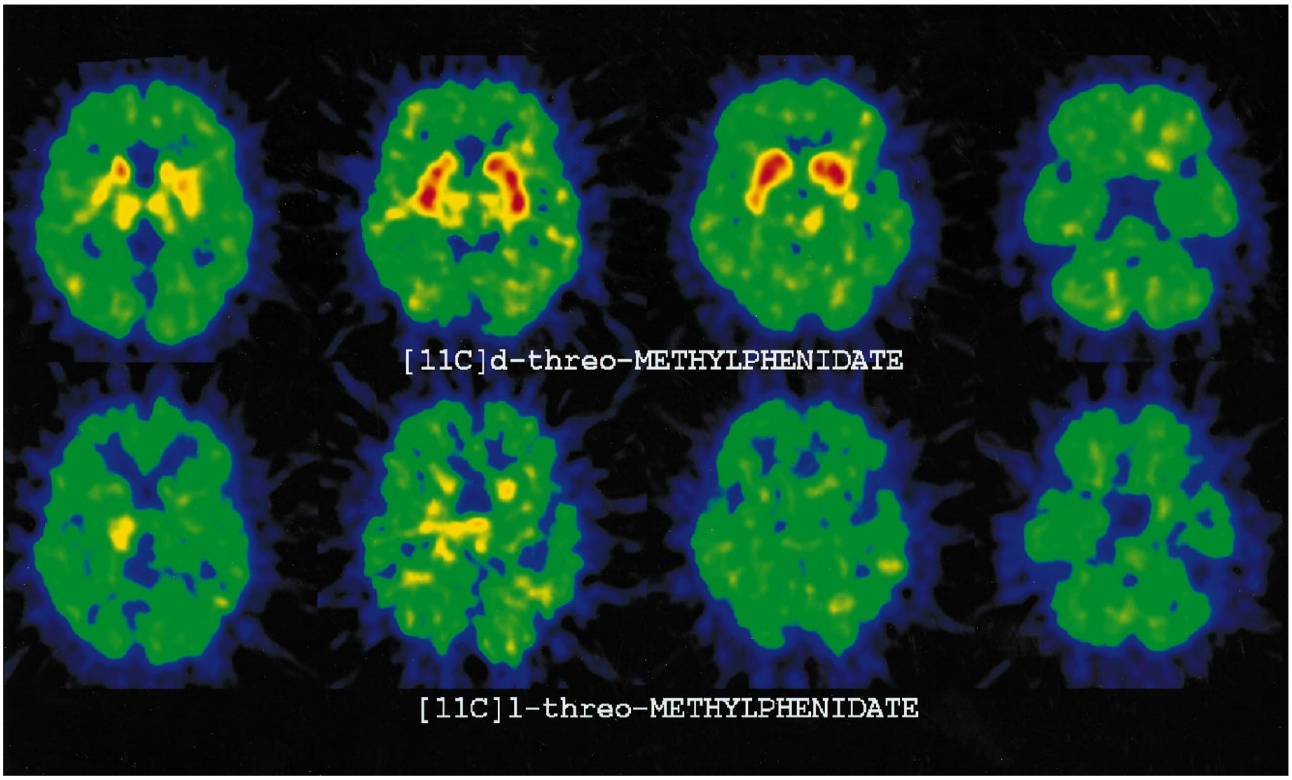

Fig. 3 Transaxial PET images (planes 8, 9, 10 and 12 on an averaged emission scan representing the activity from 10 to 90 min) of the human brain after injection of [^{11}C]*d-threo*-MP (*top panel*) and [^{11}C]*l-threo*-MP (*bottom panel*). Images are from the top of the brain to the base of the skull (*left to right*). Note the high accumulation of radioactivity in the basal ganglia for [^{11}C]*d-threo*-MP as compared to that for [^{11}C]*l-threo*-MP

for both subjects are consistent with results from baboon studies.

The results of the assays for unchanged tracer in human plasma after IV injection of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP indicated a higher percentage of unchanged [^{11}C]*d-threo*-MP as compared to that of [^{11}C]*l-threo*-MP for both subjects. The fraction of [^{11}C]*d-threo*-MP in plasma was 98, 95, 80, 52, 40, 25, and 21%, as compared to 96, 68, 44, 29, 21, 14 and 12% for [^{11}C]*l-threo*-MP, at 1, 5, 10, 20, 30, 60 and 84 min, respectively. However, plasma integrals of radioactivity due to parent tracer after [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP injections in the same subject are identical, suggesting a similar bioavailability for both tracers. Thus, the ratios of radioactivity in basal ganglia to that in cerebellum from the time-activity curves for both tracers are identical to the corresponding distribution volume measures.

Microdialysis studies

The position of the guide cannulae within the antero-lateral aspect of the right corpus basal ganglia was verified in all animals. Unlabeled *d-threo*-MP produced an average maximum increase in extracelluar striatal dopamine concentrations of 650% at 80 min following administration, as compared to 450% for *dl-threo* racemic MP, and only slight increase for *l-threo*-MP. The dose of each drug used in the studies was 20 mg/kg (Fig. 4).

Table 2 Distribution volumes (ml g^{-1}) for [^{11}C]*d-threo*-MP and [^{11}C]*l-threo* MP in different brain regions of normal subject. d-*MP* [^{11}C]*d-threo*-MP; *l-MP* [^{11}C]*l-threo*-MP. Values are given for the distribution volume (*DV*) and the tissue-to-plasma transport constant (K_1) in the basal ganglia (*BG*) and cerebellum (*CB*) and for the ratio of the *DV* of *BG* to that of *CB* (DV_{BG}/DV_{CB})

Subject	Tracer	ROI	K_1	DV	DV_{BG}/DV_{CB}
Subject 1	*d*-MP	Basal ganglia	0.450	31.263	2.865
		Cerebellum	0.434	10.911	1.000
	l-MP	Basal ganglia	0.343	13.578	1.346
		Cerebellum	0.324	10.090	1.000
Subject 2	*d*-MP	Basal ganglia	0.485	26.569	2.307
		Cerebellum	0.359	11.517	1.000
	l-MP	Basal ganglia	0.409	11.073	1.030
		Cerebellum	0.407	10.754	1.000

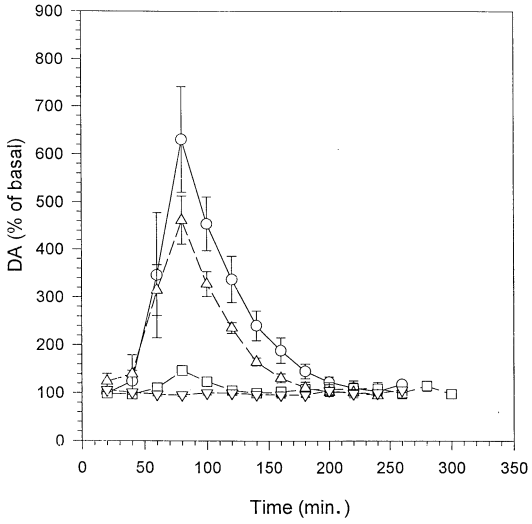

Fig. 4 Effects of methylphenidate on extracellular striatal dopamine concentrations in free-moving rats. *Reverse triangles* are from control studies. *Open circles* are from studies performed following *d-threo*-MP administration; *triangles* are from *dl-threo*-MP and *open squares* are from *l-threo*-MP. The dose of each drug used in the studies was 20 mg/kg

Discussion

MP (Ritalin) is a routinely prescribed oral drug that allows children and a growing number of adults to focus their minds. It is a chiral drug and is marketed as the *dl-threo* racemic form. As we know, differences in the biological activities of the individual enantiomers for dozens of racemic drugs has been reported in specialized journals and texts (Wainer and Drayer 1988; Krogsgaard-Larson and Bundgard 1991; Sheldon 1993). While therapeutic activity often resides in one enantiomer, the other can lead to undesirable side effects. The thalidomide tragedy (only the L enantiomer of the racemic thalidomide, a sedative, displayed therapeutic effects, whereas the D enantiomer caused pregnant women to give birth to deformed babies) offers a noteworthy example (Brown and Davies 1989). With the current trend in the pharmaceutical industry to develop optically pure products, the stereoselectivity of drugs has become a timely subject. Our comparative PET studies demonstrate the neurochemical specificity of *d-threo*-MP in vivo, supporting 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 other neurotransmitter transporters; however, *l-threo*-MP only contributes to non-specific binding. These results are consistent with a number of behavior, in vitro and ex-vivo studies (Ferris et al. 1972; Schweri et al. 1985; Patrick et al. 1987; Eckerman et al. 1991; Aoyama et al. 1994). Our microdialysis studies in free moving rats, which allow us directly to measure the effect of a drug on extracellular striatal dopamine in the brain, further demonstrate that pharmacological specificity of MP resides entirely in the *d-threo* isomer and the binding of *l*-isomer is mostly non-specific. This leads to the very important issue of whether or not we should use a racemic drug or a single enantiomer. It is estimated that approximately 1.5 tons of MP were used in the US in 1990, sales of the drug last year alone exceeded $350 million. Studies also indicate that those with untreated ADHD are more likely to become alcoholics, smokers or drug abusers than the general population (Gittelman et al. 1985). If the beneficial effects of the drug reside only in the *d-threo* form, then 50% of the weight of the administered drug may not contribute to its therapeutic effects, or may interact to influence the behavior of the active form by multiple mechanisms or it may contribute to its side effects.

The mechanism(s) which make methylphenidate work for ADHD patients is still unclear, though it has been suggested that the stimulant appears to increase the level of extracellular dopamine in the frontal lobe of the brain (Zetterstrom et al. 1988; Butcher et al. 1991; Volkow et al. 1994), where it regulates attention and impulsivity. The difference in terms of therapeutic effects and side effects that are involved by using the *d*-form alone as compared to the use of the racemic drug would in principle help us better to understand the mechanism(s). Of course, this requires large size and long-term human studies. Additionally, the results of the assays for unchanged tracer in baboon and human plasma after IV injection of [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP demonstrated a similar bioavailability for both tracers which are consistent with previous studies that the two enantiomers have a similar bioavailability after IV administration (Srinivas et al. 1992, 1993). Though the rates of metabolism are different with a higher percentage of unchanged [^{11}C]*d-threo*-MP as compared to that of [^{11}C]*l-threo*-MP for both baboon and human, this was also consistent with previous reports of a slightly higher ratio of *d* versus *l* isomer in the plasma after IV administration of MP. However, an extensive stereoselective presystemic metabolism after oral administration has been observed; that is, the drug enters the systemic circulation with a significant distortion in the enantiomeric ratio that favors the more active *d*-enantiomer (*d-threo*-MP found in both urine and plasma was 8 to 10 fold greater than *l-threo*-MP) (Hungund et al. 1979; Chan et al. 1980; Srinivas et al. 1990, 1992, 1993).

To date, efforts in clinical work have shown that it is most beneficial to use single-enantiomer drugs when one of the isomers proves too toxic or causes undesirable side effects; for example, many of the serious side effects encountered with racemic dopa, such as granulocytopenia, were not seen with L-dopa (Thall 1996). In another example, *R*(–)methadone, the more active component of racemic methadone which is used for the treatment of heroin addiction, is prescribed to patients suffering from severe liver damage in order to avoid the extra burden of metabolizing the inactive enantiomer (Kreek et al. 1979).

In conclusion, these comparative studies of enantiomerically pure [^{11}C]*d-threo*-MP and [^{11}C]*l-threo*-MP

in both baboon and human brain demonstrate high specific to non-specific binding, selectivity to DA transporters and reversibility of [^{11}C]*d-threo*-MP as compared to mostly non-specific binding of [^{11}C]*l-threo*-MP. These PET studies, along with microdialysis studies, strongly indicate that pharmacological specificity of MP resides entirely in the *d-threo* isomer, supporting the further evaluation of using a single enantiomer (*d-threo*-MP) instead of racemic MP as the commercial drug form.

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