ABSTRACT:

A comparative study of the plasma pharmacokinetics and tissue distribution of the threo enantiomers of methylphenidate (MPH), para-bromomethylphenidate (p-Br MPH), and para-methoxymethylphenidate (p-OCH₃ MPH) was conducted in rats after i.p. administration of a 37 μmol/kg dose. The plasma kinetic data was fit to a two-compartment model with absorption and lag time as well as evaluated by noncompartmental methods. All three compounds attained maximal concentration within 10 min of injection. Calculated mean residence time and elimination half-life values for p-Br MPH were significantly longer than those for d-MPH and d-p-OCH₃ MPH, and clearance of the bromo derivative was substantially lower than the latter two compounds. Tissue distribution studies of the three threo enantiomers revealed that para-substitution of d-MPH had a profound effect on the distribution pattern of these drugs. The highest concentration of drug was found in the kidney and lung for d-MPH, lung and liver for d-p-Br MPH, and lung and brain for d-p-OCH₃ MPH. The bromo derivative was found in the highest concentration in the central nervous system at 30, 120, and 180 min whereas levels of d-MPH were twice as high as d-p-OCH₃ MPH at 30 min but slightly lower than the latter at 120 min. Related studies on the lipophilicity, plasma protein binding, and resistance to plasma degradation of these compounds were also conducted. The combined data from these experiments along with the pharmacokinetics and central nervous system distribution of these drugs provide explanations for discrepancies between the in vivo and in vitro activity of these compounds described in previous work.

Chirality is a key factor for the pharmacokinetics of methylphenidate (MPH; Ritalin, methyl threo-dl-2-phenyl-2,2'-piperidyl)acetate hydrochloride). (1) Racemic MPH is absorbed from the gut and metabolized with substantial first pass effects. Rats selectively oxidize the l-threo enantiomer of MPH quite extensively to its p-hydroxy derivative, which is ultimately excreted in the feces after glucuronidation (Faraj et al., 1974; Patrick et al., 1986a). In humans, the drug undergoes significant enantioselective presystemic clearance favoring removal of the l-threo isomer by deesterification to ritalinic acid (Srivinas et al., 1987). Despite the availability of kinetic data on racemic and optically pure MPH, there are still no reports in the literature on the pharmacokinetics, metabolism, and tissue distribution of enantiomeric MPH analogs.

MPH appears to exert its pharmacological effects through potent inhibition of dopamine and norepinephrine reuptake into the presynaptic nerve terminus (Patrick et al., 1986b). Many of the investigations of MPH and its derivatives have examined in vitro neurochemical activity or in vivo pharmacodynamics in rats using racemic or diastereomeric mixtures. A recent study in our group developed new chemical syntheses and compared the neurochemical and locomotor activity in rats of optically enriched threo MPH enantiomers and their p-bromo and p-methoxy derivatives (Thae et al., 1998). Similar to the case with the parent compound, the pharmacological profile of the para-substituted MPH derivatives was such that the d-isomers were more active than the l-isomers. When comparing the d-threo enantiomers of MPH, p-bromo MPH, and p-methoxy MPH, we also noted a disparity between the in vitro and in vivo activities of these drugs. In particular, the neurochemical potency of d-methoxy MPH at dopaminergic and noradrenergic uptake sites was similar to d-MPH, yet its locomotor-inducing effects were much less pronounced than the latter in terms of both amplitude and time duration. The d-p-bromo analog was also shown to sustain locomotor activity for at least 180 min, which was more than twice the duration of any other compound tested. To define the role of metabolism in the overall in vitro/in vivo profile of these drugs, we investigated the pharmacokinetics and distribution of the threo enantiomers of MPH and its para-substituted derivatives in rats after i.p. administration, the same route of drug delivery used in the locomotor studies reported previously. The data from these studies would provide valuable preliminary information on the potential of these derivatives as alternative therapies for treatment of attention deficit hyperactivity disorder as well as imaging probes for central nervous system (CNS) dopaminergic activities.
Materials and Methods
Male Sprague-Dawley rats (225–250 g) were obtained from Hilltop Lab Animals, Inc. (Scottsdale, PA). Drug-free rat plasma was obtained from Zivic-Miller Laboratories, Inc. (Zelienople, PA). The d-threo enantiomers of MPH, p-bromo MPH, and p-methoxy MPH were prepared by asymmetric synthesis and recrystallized as white hydrochloride salts. The enantiomeric purities of these optically active compounds were determined by a previously described gas chromatographic derivatization technique and found to be >98% (Thai et al., 1998). Racemic threo ethylphenidate (EPH) was obtained by conventional esterification methods from ritalinic acid. Derivatization grade pentafluoropro- pionic anhydride (PFPA) was obtained from Acros Organics, Fisher Scientific, Pittsburgh, PA. The medical-grade polysiloxane tubing used to construct the cannula was obtained from Baxter Scientific (McGraw Park, IL.).

Surgical Cannulation. Male Sprague-Dawley rats (225–250 g) were anesthetized by i.p. administration of a 200-mg/kg dose of chloral hydrate diluted to a concentration of 75 mg/ml in water. Supplemental methoxyflurane (Mefo- fane) was administered as needed. The right jugular vein was exposed and cannulated with a polysiloxane cannula, which was then passed under the skin and fixed near the base of the neck. Daily flushing of the cannula with 0.028% heparinized saline was necessary to maintain patency. Rats were allowed to recover for at least 60 h from time of surgery before pharmacokinetic studies. All procedures involving handling of animals were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Drug Treatment. Male Sprague-Dawley rats (225–250 g) were given food and water ad libitum. They were housed two to four per cage and maintained in a temperature-controlled colony room with a 12-h light/dark schedule. The animals were injected i.p. with a 37 μmol/kg dose of either d-threo MPH · HCl (10 mg/kg), d-threo para-bromomethylphenidate (p-Br MPH) · HCl (12.9 mg/kg), or d-threo para-methoxymethylphenidate (p-OCH 3 MPH) · HCl (11.1 mg/kg) dissolved in 1.0 ml/kg saline using a 26-gauge 1/2-inch needle. For plasma pharmacokinetic studies, blood samples (250 μl) were collected through the cannula at 2, 5, 10, 20, 30, 45, 60, 90, 120, and 180 min after injection. Heparinized (0.028%) saline (250 μl) was flushed through the cannula into the rat to purge the line and to replace lost fluid volume. The blood samples were transferred to a 500 μl polypropylene tube containing 7.5 μg of powder Na 2 EDTA, and the plasma was separated from whole cells by centrifugation at 3000g for 3 min and then stored at −80°C until analysis.

For the tissue distribution studies, rats were decapitated at 30, 60, and 180 min and trunk blood was collected into a Becton Dickinson & Co. (Lincoln Park, NJ) purple top vacutainer tube containing powder Na 2 EDTA. The whole blood was centrifuged at 3000g for 15 min and the plasma was transferred to a polypropylene tube for storage at −80°C until analysis.

After blood collection, whole brain, heart, left lung, liver, and left kidney were quickly dissected free and stored in polypropylene tubes until analysis. Analytical Methods. Plasma samples were analyzed for MPH, p-OCH 3 MPH, or p-Br MPH by a modification of a gas chromatography-mass spec- trometry (GC-MS) method described by Patrick et al. (1984). A 100 μl volume of plasma was added to an 8-ml glass screw top tube containing 1.0 ml of 0.1 M HCl and 100 ng of EPH as internal standard. A 4:1 v/v solution of heptane/ethyl acetate (3.0 ml) was added, and the tube was agitated at high speed on a mechanical shaker for 2.5 min and centrifuged for 10 min. The top organic layer was aspirated and discarded. The acidic aqueous layer was alkalized with 1.0 ml of 0.75 M aqueous Na 2 CO 3 and then extracted with 2.0 ml of heptane/ethyl acetate (4:1 v/v). After shaking for 5 min and centrifuging for 10 min, the top organic layer was transferred to a clear glass screw top tube and evaporated in a Savant Speed Vac concentrator (Savant, Inc., Holbrook, NY). The resulting residue was treated with 50 μl of PFPA in 25 μl of ethyl acetate at 60°C for 45 min. Samples were dried in a Savant Speed Vac concentrator for 30 min, reconstituted in 50 μl of amyl acetate, transferred to crimp-top vials with 100 μl volume V-type inserts, and injected into a GC-MS by auto-injection.

The GC-MS system includes a Hewlett-Packard 5890 GC, a 5970 mass selective detector (MSD) with a capillary direct interface and a 5940 HP-UX Chemstation. The MSD includes a Tharos HED (high energy dynode). The column was an HP Ultra-2 (cross-linked 5% phenyl methyl silicone) fused silica capillary column, 12 m in length, 0.20 mm i.d., film thickness 0.33 nm. Analytical conditions included an initial column oven temperature of 130°C held for 1.5 min and then increased at a rate of 7°C/min to a final temperature of 240°C, which was then maintained for 0.5 min. The injector temperature was 290°C, the detector temperature 300°C, the helium (carrier gas) column flow (linear velocity) 38 cm/s, septum purge flow 1.8 ml/min, and purge vent flow 61 ml/min. MSD was set for selective ion monitoring of peaks at m/z 84 and 230. Under these conditions, retention times were 10.30, 13.82, and 14.36 min for the PFPA derivatives of MPH, p-OCH 3 MPH, and p-Br MPH, respectively.

Standard calibration curves were constructed by analyzing a series of 100-μl aliquots of drug-free rat plasma containing 1 μg/ml of EPH as internal standard and varying concentrations between 10 and 10,000 ng/ml of the appropriate enantiomeric drug. The concentration of drug in each unknown sample was determined by solving the linear calibration curve equation for each corresponding drug/internal standard ratio.

For analysis of drug concentration in tissues, the brain, liver, and heart were suspended in a 5-volume dilution of 0.1 N HClO 4 whereas kidney and lung were suspended in a 10-volume dilution. After homogenization at setting 6 of a Beckman homogenizer, the slurry was centrifuged at 15,000 rpm for 30 min. The supernatant was stored at −80°C until analysis. The extraction procedure was carried out on 500 μl of supernatant in identical fashion to the 100 μl aliquot of plasma.

Standard curves were constructed from spiked blank tissue prepared under the conditions described above. Drug concentration in tissue is expressed in terms of micrograms of drug per grams of tissue. Range of concentrations for the standard curve was between 10 and 25,000 ng/g of tissue. For both rat plasma and tissue assays, the regression coefficient (r) > 0.99. Inter- and intra-assay variability were acceptable with no more than 14% coefficient of variation. Recovery of all compounds from tissue and plasma was >60%.

Partition Coefficients. The partition coefficients of d-MPH, d-OCH 3 MPH, and d-Br MPH were determined between peanut oil and 0.067 M NaPO 4 buffer (pH 7.4) at room temperature. Both phases were saturated with one another before the assay. Each compound was dissolved in buffer (5.0 ml) to a concentration of 1.0 μg/ml and shaken with peanut oil (5.0 ml) for 1 h. Layers were centrifuged and then separated. A 1.0-ml aliquot of the phosphate buffer phase was then withdrawn for analysis.

EPH (100 ng) was added to each 1.0-ml aliquot as internal standard. For analysis of the buffer layer, 1.0 ml of 0.75 M aqueous Na 2 CO 3 was then added and extracted with 2.0 ml of heptane/ethyl acetate (4:1 v/v). The organic layer was dried and derivatized with PFPA according to methods described above for plasma samples. Standard curves were prepared in 0.067 M phosphate buffer. After determining the drug concentration in buffer, peanut oil concen- tration was calculated assuming mass balance. Partition coefficients were obtained by dividing concentration of compound in peanut oil by the concentration of compound in phosphate buffer and were performed in triplicate.

Plasma Protein Binding. Binding experiments were performed using a Spectrum equilibrium dialyzer (Spectrum Medical Industries, Inc., Los Ange- les, CA). Teflon cells with two chambers of 1.3 ml volume each were separated by Spectra-Por 1 regenerated cellulose membranes (Spectrum Medical Indus- tries, Inc., Los Angeles, CA) with a molecular weight cut-off of 6000 to 8000 Da. The membranes were washed with water before use. After assembling the cell, one of the chambers was filled with 1.0 ml of NaCl/NaPO 4 buffer (66 mM NaPO 4 in 50 mM NaCl adjusted to pH 7.4). Rat plasma (1.0 ml) containing 0.15% Na 2 EDTA to inhibit plasma esterases along with 1 μg/ml of d-MPH, d-OCH 3 MPH, or d-Br MPH was added carefully to the other chamber.

Equilibrium dialysis was conducted in a 37°C water bath with a rotational speed of 10 rpm for 2 h. The contents of each chamber were transferred to a polypolypropylene vial for storage at −80°C before analysis. Aliquots of 400 μl volume of plasma and buffer were extracted, derivatized with PFPA, and analyzed by GC-MS. Standard calibration curves were generated by spiking drug-free Na 2 EDTA-treated plasma and sodium phosphate buffer. All experi- ments were performed in quadruplicate.

Rat Plasma Esterase Activity. For esterase activity, fresh plasma obtained by collecting trunk blood from decapitated rats in heparinized tubes was used. Each of the d-threo enantiomers of MPH, p-Br MPH, and p-OCH 3 MPH were incubated in plasma (100 μl) as well as 0.067 M phosphate buffer (100 μl) at 37°C in a final concentration of 1.0 μg/ml. At 1, 2, 4, 7, and 9 h, samples were removed and EPH (100 ng) was added as internal standard. After alkalizing
the solution with 1.0 ml of 0.75 M aqueous Na₂CO₃, 2.0 ml of heptane/ethyl acetate (4:1 v/v) was added and the compound was extracted into the organic layer by vortexing and centrifugation. The top organic layer was then transferred to a clean glass tube and stored at −80°C until time of analysis. Samples were dried down and derivatized according to methods described above for plasma samples to determine levels of the esterified compounds. The study was conducted in triplicate for each time point and initial pharmacokinetic parameters were determined from plots of log concentration versus time using a noncompartmental analysis function in the computer program WinNonlin (Scientific Consulting, Inc. Apex, NC).

**Pharmacokinetic Analysis.** Plasma concentration versus time data from each individual experiment was evaluated by noncompartmental analysis as well as a two-compartment model with an absorption phase and lag time using the computer programs PKAnalyst (Micromath, Salt Lake City, UT) and WinNonlin (Scientific Consulting, Inc.). Initial estimates of exponential parameters for compartmental analysis were obtained with the computer program V-Fit (R. Brueckner, M.D., Walter Reed Army Institute of Research). These estimates were then used to fit the data by nonlinear least-squares methods. The goodness of fits were evaluated with informational criteria.

The pharmacokinetic parameters, $T_{\text{max,calc}}$ (time at maximum drug concentration), $C_{\text{max,calc}}$ (maximum drug concentration), and $k_{\beta}$ (distributional rate constant) were obtained from compartmental analysis only and are expressed as the means ± S.E. of four experiments. The remaining parameters are the means ± S.E. of values calculated by both noncompartmental and compartmental methods. The distribution and elimination half-lives were calculated by dividing 0.693 by the rate constants, $k_{\beta}$ and $k_{\beta,c}$ respectively. The $T_{\text{max,obs}}$ (time at maximum drug concentration) and $C_{\text{max,obs}}$ (maximum drug concentration) are the means ± S.E. of actual observed values for the four experiments.

The mean residence time (MRT) to the last sampling time was calculated by the equation:

$$\text{MRT} = \frac{\text{AUMC}_{(0\rightarrow t)}}{\text{AUC}_{(0\rightarrow t)}}$$

where AUMC$_{(0\rightarrow t)}$ is the area under the first moment curve to the last time and AUC$_{(0\rightarrow t)}$ is the area under the curve to the last time.

Clearance (CL) was calculated as:

$$\text{CL} = \frac{\text{Dose}}{\text{AUC}_{(0\rightarrow \infty)}}$$

The apparent volume of distribution ($V_{\text{d,app}}$) was obtained using the equation:

$$V_{\text{d,app}} = \frac{\text{Dose}}{k_{\beta} \times \text{AUC}_{(0\rightarrow \infty)}}$$

where $k_{\beta}$ is the elimination rate constant.

**Results**

**Pharmacokinetics of d-MPH, d-Br MPH, and d-0CH₃ MPH.** The plasma pharmacokinetics of the d-threo isomers of MPH and its para-substituted analogs were investigated 3 days after surgery in rats surgically implanted with cannula. These animals were found not to be significantly affected by the surgical procedure or anesthesia after 24 h. They were active and possessed normal appetite on the day of drug administration.

Concentrations of the d-threo enantiomers of each compound were measured in plasma at various time points after i.p. administration of molar equivalents (37 μmol/kg) of drug. Semilogarithmic plots of mean plasma concentration-time curves of d-MPH, d-Br MPH, and d-0CH₃ MPH appear in Fig. 2. Rapid absorption of all three compounds is apparent by the absence of points in the absorption phase. Average peak levels of all three drugs occurred within 5 min of injection. In addition, the average maximum concentration of d-MPH was lower than that of both para-substituted derivatives, which were found to be similar. Biexponential decline of drug levels in plasma was slower for d-Br MPH than for both d-MPH and d-0CH₃ MPH, which followed one another closely during the β-elimination phase. At all time points after 20 min, concentrations of d-MPH and d-0CH₃ MPH were not significantly different from one another, but both were significantly lower than those of d-Br MPH.

Pharmacokinetic parameters calculated from the plasma data by both noncompartmental and compartmental methods are shown in Table 1. We report both observed values (obtained by averaging actual values) and calculated values (obtained by averaging values obtained from the pharmacokinetic modeling) for $T_{\text{max}}$ and $C_{\text{max}}$. The $T_{\text{max,calc}}$ value was earliest for d-0CH₃ MPH (2.00 ± 1.20 min) followed by d-MPH (4.88 ± 1.48 min) and d-Br MPH (9.53 ± 3.70 min). Observed $T_{\text{max}}$ followed the same order for the three compounds. Maximum concentrations of the para-substituted analogs were similar and found to be 2.4-fold higher than the parent compound. The β half-life of d-Br MPH (130 ± 25.8 min) was greater than twice the half-lives of the other two compounds (51.6 ± 3.56 min for d-MPH; 62.5 ± 15.3 min for d-0CH₃ MPH). The MRT was also greater for bromine-substituted MPH (62.6 ± 4.58 min) relative to
As compared with AUC \text{inf}, area under the concentration versus time curve to infinity; AUC \text{1t}, area under the concentration versus time curve to last sampling time; MRT, mean residence time; CL/F, clearance.

The rat brain contained much higher amounts of -Br MPH with respect to -OCH\textsubscript{3} MPH. Although AUC to infinity of -MPH, the order of tissue concentration at 30 min was kidney > heart > brain > liver. The -Br derivative was distributed with the order lung > liver > kidney > brain > heart. Levels of -OCH\textsubscript{3} MPH were ranked as lung > brain > kidney > heart > liver.

At all time points and in all tissues assayed, the -Br analog was found to be in higher concentrations than the other two compounds. The rat brain contained much higher amounts of -Br MPH at all time points and its decay from this site was relatively slow in comparison with the other two compounds. At 30 min, the mean -Br concentration (3.13 ± 0.589 \mu g/g) was twice as high as -OCH\textsubscript{3} MPH (1.54 ± 0.471 \mu g/g). A more rapid decay of these drugs from the CNS as compared with -Br MPH was apparent from the large drop in mean concentrations between 30 and 120 min.

A CNS/plasma ratio was calculated from the tissue distribution study as an indicator of CNS penetrability and retention of these compounds (Table 3). At 30 min, values for -MPH (7.27 ± 0.63) and -Br MPH (7.29 ± 0.59) were very similar and were significantly greater than that of -OCH\textsubscript{3} MPH (4.33 ± 0.70). These ratios remained fairly high for -Br MPH at all time points but declined for the other two compounds, which were similar at 120 min and less than one for -MPH at 180 min. Levels of -OCH\textsubscript{3} MPH at 180 min were below the limit of quantitation.

### Rat Plasma Esterase Activity

The in vitro degradation of the -theo isoforms and their aromatic-substituted derivatives was investigated in rat plasma as well as in 0.067 M phosphate buffer (pH 7.4) at 37°C. The -bromo derivative was most resistant to plasma esterases followed in order by -OCH\textsubscript{3} MPH and -MPH. The calculated plasma half-lives shown in Table 4 were obtained from plots of time versus log concentration of each drug over a 9-h time span. The half-lives of -OCH\textsubscript{3} and -Br MPH in rat plasma were 3- and 8-fold longer than that of -MPH.

Comparative deesterifications in phosphate buffer did not follow the same trend. The half-lives of the three compounds were in the order -MPH > -OCH\textsubscript{3} MPH > -Br MPH. Differences between the three were not as great in buffer as in plasma. Comparing each compound in the two media, both -MPH and its methoxy analog were more susceptible to deesterification in rat plasma. On the other hand, the bromine-substituted analog was more resistant to deesterification in plasma as compared with buffer.

### Partition Coefficients and Plasma Protein Binding

The partitioning of these drugs between peanut oil and Sorenson's phosphate buffer (pH 7.4) was evaluated as a model of their body lipid solubility. The partition coefficients reported in Table 5 were obtained by dividing the drug concentration in peanut oil by its buffer concentration. Thus, -Br MPH with a value of 14.8 was much more lipid-soluble than the other two compounds. The partition coefficient of -OCH\textsubscript{3} MPH was approximately 1.6 times greater than -MPH. Plasma protein binding of these compounds was also determined by equilibrium dialysis and is reported in Table 5. Aromatic substitution increased percentage binding by 2.2- and 3.5-fold for methoxy and bromine derivatives, respectively.
DA and [3 H]NE uptake inhibition relative to racemic MPH, yet it did not act as strongly as racemic MPH in locomotion assays. The in vitro activity in the same assays as compared with MPH, which possessed activity even at 180 min.

In particular, the 51.6-min value obtained by us for MPH as compared with Aoyama (1994; 1996) were 46.2 min and 42.7 min. These values are consistent with the findings for d-Br MPH but did not further elucidate potential mechanisms for the differences between d-OCH3 MPH and d-MPH. We looked to tissue distribution with particular emphasis on CNS penetrability for additional insight.

Much like the case with the plasma pharmacokinetic assessments, the tissue distribution data was difficult to place within the context of currently available literature because of the differences in route of delivery, dose, and form (enantiomeric versus racemic) of drug. Nonetheless, qualitative comparisons could be made between our results and the available literature in terms of rank of tissue concentrations. After a 1 mg/kg i.v. dose (Kotaki et al., 1988) as well as a 20 mg/kg i.p. dose (Patrick et al., 1984), the distribution of racemic MPH was in the order kidney > lung > brain > heart > liver. Our treatment with 10 mg/kg of d-MPH provided the same outcome. The actual tissue levels reported by Patrick et al. (1984) after the 20 mg/kg i.p. dose were found to be 4 to 7.5 times higher than those in Table 2 obtained 30 min after a 10 mg/kg i.p. dose. Drug levels at the 20 mg/kg dose may represent saturation conditions such that the liver is unable to absorb and metabolize MPH as efficiently from portal circulation.

Organ distribution patterns are complicated by several factors. The most important determinants of organ distribution appear to be plasma protein binding, lipid solubility, perfusion and extraction efficiency of each tissue, and inherent tissue affinity for the drug. Metabolic transformation will also alter the level of drug. MPH is known to bind very weakly to 4% albumin with a total free fraction of 85% in human plasma (Fara et al., 1974). In accordance with these results, a free fraction of 77.4% for MPH was obtained in our study using rat plasma. The d-OCH3 and d-Br derivatives were bound to plasma proteins to a greater degree. In particular, the free fraction of 77.4% for MPH was similar to hepatic blood flow in rats (~70 ml/min/kg). On the other hand, values for d-MPH and d-OCH3 MPH exceeded hepatic blood flow by approximately 4-fold. This is consistent with the notion that both d-MPH and d-OCH3 MPH are cleared by extra-hepatic mechanisms such as plasma hydrolysis by esterases. The half-lives of these compounds (in rat plasma as seen in Table 4) were much less than d-Br MPH.

Upon examination of Fig. 2, it is immediately apparent that during β-phase elimination, decay of p-Br MPH is much slower than the other two d-threo enantiomers as evidenced by its more gradual slope on the concentration-time curve. This observation is reinforced by the substantially longer elimination half-life of the bromo derivative obtained by both noncompartmental and compartmental analyses in relation to the parent compound and methoxy derivative (Table 1). In addition, the MRT of d-Br MPH was found to be longer and the clearance smaller for this drug. In light of these results, we felt that perhaps the locomotor profiles of the three drugs could be explained according to clearance arguments. That is, slower clearance may lead to a longer duration of activity. Although this was consistent with the findings for d-Br MPH, the explanation did not hold for d-OCH3 MPH, which had similar clearance patterns to d-MPH despite weaker locomotor stimulation. An interesting point concerning the absolute values of clearance for these compounds is that clearance of d-Br MPH was similar to hepatic blood flow in rats (~70 ml/min/kg).

The in vivo neurochemical potency of the enantiomers of MPH, p-Br MPH, and p-OCH3 MPH given i.p. at a dose of 37 μmol/kg. These drugs absorbed quickly and reached peak levels within the first 10 min of i.p. dosing in similar fashion to an oral route (Patrick et al., 1984). In addition, the elimination half-life of d-MPH obtained in two separate studies by Aoyama (1994; 1996) were 46.2 min and 42.7 min. These values are in agreement with the 51.6-min value obtained by us for d-MPH as well as the 50-min half-life reported for racemic MPH by Patrick et al. (1984). Further comparisons of other parameters were cautiously avoided because of the differences in the route, amount, and form of dose in each study.

We previously reported a nonlinear relationship between the in vitro neurochemical potency of the enantiomers of MPH, p-Br MPH, and p-OCH3 MPH at dopaminergic and noradrenergic sites and the in vivo locomotor-inducing activity of these isomers (Thai et al., 1998). In particular, d-OCH3 MPH possessed a potency of 2.1 and 2.3 for [3H]DA and [3H]NE uptake inhibition relative to racemic MPH, yet it did not act as strongly as racemic MPH in locomotion assays. The d-Br analog was also shown to possess approximately 8- to 14-fold greater in vitro activity in the same assays as compared with d-MPH. The locomotor activity of d-MPH was significantly shorter than d-Br MPH, which possessed activity even at 180 min.

### Table 3

<table>
<thead>
<tr>
<th>Time</th>
<th>30</th>
<th>120</th>
<th>180</th>
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</thead>
<tbody>
<tr>
<td>MPH</td>
<td>7.27 ± 0.63</td>
<td>2.96 ± 0.65</td>
<td>0.76 ± 0.36</td>
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<tr>
<td>p-OCH3 MPH</td>
<td>4.35 ± 0.70</td>
<td>2.93 ± 0.56</td>
<td>ND</td>
</tr>
<tr>
<td>p-Br MPH</td>
<td>7.29 ± 0.55</td>
<td>6.62 ± 0.17</td>
<td>4.37 ± 0.65</td>
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</table>

* a,b: ratios were calculated by dividing brain levels/plasma levels of drug obtained after single i.p. injection of 37 μmol/kg of drug.
* a: values represent the mean ± S.E. of three to five experiments.
* ANOVA: p < 0.05 for p-OCH3 MPH at 30 min; p < 0.05 for p-Br MPH at 120 and 180 min.

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>MPH</th>
<th>p-OCH3 MPH</th>
<th>p-Br MPH</th>
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<tbody>
<tr>
<td>Half-life h</td>
<td>8.71</td>
<td>7.37</td>
<td>5.91</td>
</tr>
<tr>
<td>Rat plasma</td>
<td>1.98</td>
<td>6.05</td>
<td>16.6</td>
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* a,b: A half-life was computed using WinNonlin.

### Table 5

<table>
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<tr>
<th>Compound</th>
<th>Partition coefficient</th>
<th>Plasma protein binding</th>
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<tr>
<td>MPH</td>
<td>0.550 ± 0.068</td>
<td>22.6 ± 1.45</td>
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<tr>
<td>p-OCH3 MPH</td>
<td>0.869 ± 0.060</td>
<td>49.0 ± 9.38</td>
</tr>
<tr>
<td>p-Br MPH</td>
<td>14.8 ± 0.622</td>
<td>78.5 ± 5.86</td>
</tr>
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</table>

* a: Partition between peanut oil and Sorenson’s Buffer (pH 7.4) and is calculated by the formula C_{oil}/C_{solute}.
* b: Plasma protein binding expressed as a percentage of drug bound.

### Discussion

This work evaluated the plasma pharmacokinetics and tissue distribution of the d-threo enantiomers of MPH, p-Br MPH, and p-OCH3 MPH given i.p. at a dose of 37 μmol/kg. These drugs absorbed quickly and reached peak levels within the first 10 min of i.p. dosing in similar fashion to an oral route (Patrick et al., 1984). In addition, the elimination half-life of d-MPH obtained in two separate studies by Aoyama (1994; 1996) were 46.2 min and 42.7 min. These values are in agreement with the 51.6-min value obtained by us for d-MPH as well as the 50-min half-life reported for racemic MPH by Patrick et al. (1984). Further comparisons of other parameters were cautiously avoided because of the differences in the route, amount, and form of dose in each study.

Upon examination of Fig. 2, it is immediately apparent that during β-phase elimination, decay of p-Br MPH is much slower than the other two d-threo enantiomers as evidenced by its more gradual slope on the concentration-time curve. This observation is reinforced by the substantially longer elimination half-life of the bromo derivative obtained by both noncompartmental and compartmental analyses in relation to the parent compound and methoxy derivative (Table 1). In addition, the MRT of d-Br MPH was found to be longer and the clearance smaller for this drug. In light of these results, we felt that perhaps the locomotor profiles of the three drugs could be explained according to clearance arguments. That is, slower clearance may lead to a longer duration of activity. Although this was consistent with the findings for d-Br MPH, the explanation did not hold for d-OCH3 MPH, which had similar clearance patterns to d-MPH despite weaker locomotor stimulation. An interesting point concerning the absolute values of clearance for these compounds is that clearance of d-Br MPH was similar to hepatic blood flow in rats (~70 ml/min/kg). On the other hand, values for d-MPH and d-OCH3 MPH exceeded hepatic blood flow by approximately 4-fold. This is consistent with the notion that both d-MPH and d-OCH3 MPH are cleared by extra-hepatic mechanisms such as plasma hydrolysis by esterases. The half-lives of these compounds (in rat plasma as seen in Table 4) were much less than d-Br MPH. In light of the above information, pharmacokinetic parameters, such as clearance, MRT, and elimination half-life, were consistent with the findings for d-Br MPH but did not further elucidate potential mechanisms for the differences between d-OCH3 MPH and d-MPH. We looked to tissue distribution with particular emphasis on CNS penetrability for additional insight.

Much like the case with the plasma pharmacokinetic assessments, the tissue distribution data was difficult to place within the context of currently available literature because of the differences in route of delivery, dose, and form (enantiomeric versus racemic) of drug. Nonetheless, qualitative comparisons could be made between our results and the available literature in terms of rank of tissue concentrations. After a 1 mg/kg i.v. dose (Kotaki et al., 1988) as well as a 20 mg/kg i.p. dose (Patrick et al., 1984), the distribution of racemic MPH was in the order kidney > lung > brain > heart > liver. Our treatment with 10 mg/kg of d-MPH provided the same outcome. The actual tissue levels reported by Patrick et al. (1984) after the 20 mg/kg i.p. dose were found to be 4 to 7.5 times higher than those in Table 2 obtained 30 min after a 10 mg/kg i.p. dose. Drug levels at the 20 mg/kg dose may represent saturation conditions such that the liver is unable to absorb and metabolize MPH as efficiently from portal circulation.

Organ distribution patterns are complicated by several factors. The most important determinants of organ distribution appear to be plasma protein binding, lipid solubility, perfusion and extraction efficiency of each tissue, and inherent tissue affinity for the drug. Metabolic transformation will also alter the level of drug. MPH is known to bind very weakly to 4% albumin with a total free fraction of 85% in human plasma (Fara et al., 1974). In accordance with these results, a free fraction of 77.4% for MPH was obtained in our study using rat plasma. The d-OCH3 and d-Br derivatives were bound to plasma proteins to a greater degree. In particular, the free fraction of d-Br MPH was 21.5%. This represents an approximately 3.6-fold decrease in the amount of drug available for distribution through lipid membrane layers in various tissues relative to d-MPH when total plasma drug concentrations are equivalent. Despite the increase in the plasma protein binding of d-Br MPH, there were still sufficient levels of free drug to produce significantly greater levels in all organs, particularly the brain.
One reason for these observations may be found in the relative lipid solubilities of these drugs. We chose peanut oil/pH 7.4 buffer as the organic/aqueous partitioning between lipid and serum in the body (Dayton and Perel, 1971). We found that the partition coefficient of d-Br MPH between peanut oil and phosphate buffer at pH 7.4 was several-fold greater than d-MPH and d-OCH3 MPH indicating greater lipid solubility of d-Br MPH. In accordance with the partition coefficient data, d-Br MPH’s CNS levels at 30, 120, and 180 min were at least 2-fold higher than d-MPH and d-OCH3 MPH. Trends in lipid solubility were paralleled by a high brain/plasma ratio for d-Br MPH at all time points shown in Table 3. The lipid solubility data for d-MPH and d-OCH3 MPH, on the other hand, were not in agreement with their relative brain/plasma ratios. The ratio for the methoxy derivative was nearly twice as low despite a slightly greater partition coefficient in peanut oil as compared to d-MPH. Nonetheless, the CNS results help to shed light on the locomotor activity profiles obtained in previous experiments. The ability of these drugs to penetrate the blood-brain barrier and to remain within the brain seems to be a crucial factor in maintaining in vivo activity. The high levels of d-Br MPH in the CNS throughout the 180-min period provide evidence that its longer duration of locomotor inducing activity are the result of a maintenance of above-threshold levels at the site of action. Differences in maximal locomotor activity at 30 min for d-MPH and d-OCH3 MPH coincide with the higher brain concentration of the former. At 120 min, the locomotor activity of rats treated with d-MPH and d-OCH3 MPH returned to baseline; this was coincident with a 4- to 14-fold decrease in the CNS levels of both drugs.

Metabolism of MPH by cleavage of the methyl ester group abolishes the psychostimulant properties of the drug. In an attempt to understand the role of deesterification in the organ distribution patterns of the three compounds, a study was undertaken to assess their relative resistance to plasma esterases. The half-life of d-Br MPH in rat plasma relative to the other two drugs was significantly longer, and this relative resistance to metabolism may have been responsible for its higher circulating plasma and tissue concentrations. The results for d-OCH3 MPH as compared to d-MPH were not consistent with the notion that resistance to esterases leads to higher overall drug levels. Although the methoxy derivative had a longer half-life in plasma in vitro, its in vivo plasma pharmacokinetic profile was not significantly different from that of d-MPH. Other metabolic factors, including liver oxidation, may be contributing. One final note on the results of the degradation study was the surprisingly longer half-life of d-Br MPH in plasma relative to buffer. In light of this compound’s higher plasma protein binding, it was felt that the amount of drug available to plasma esterases was limited. The presence of plasma proteins may have provided an additional storage site for d-Br MPH and thus imparted a certain level of resistance to degradation in rat plasma.

In conclusion, we report the first comparative pharmacokinetic and tissue distribution study of the d-threo enantiomers of MPH and its p-bromine and p-methoxy analogs in rats. This study was initiated to obtain preliminary pharmacokinetic data that might aid further clinical exploration of these drugs. In addition, we hoped that it would provide possible explanations for the discrepancies between in vitro and in vivo potencies of these drugs. For d-Br MPH, the cumulative consequence of a slower elimination from plasma and favorable CNS penetrability and retention along with high intrinsic potency at the dopamine and norepinephrine transporter systems seems to lead to an extended duration of in vivo locomotor activity. Discrepant in vitro and in vivo activities of MPH and its methoxy derivative may be the result of lower brain levels of d-OCH3 MPH relative to d-MPH. Related studies including lipid solubility, plasma protein binding, and plasma stability lend further support to some of the pharmacokinetic and tissue distribution results. The data above would strongly support further exploration of d-Br MPH as a clinical candidate for a once daily dose treatment of attention deficit hyperactivity disorder and as an in vivo probe for imaging of CNS dopaminergic activity.

References


