Spontaneous Locomotor Activity and Pharmacokinetics of Intravenous Methamphetamine and Its Metabolite Amphetamine in the Rat

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ABSTRACT

The purpose of these studies was to better understand the behavioral effects and pharmacokinetics of an i.v. bolus dose of (+)-methamphetamine [(+]\text{METH}) in a rat model of (+)-METH abuse. We characterized the behavioral effects after increasing (+)-METH doses (0.1, 0.3, and 1.0 mg/kg) and the pharmacokinetics of (+)-METH (and its metabolite (+)-amphetamine [(+]\text{AMP})) at the lowest and highest of these doses in adult male Sprague-Dawley rats. The doses and route of administration were selected to mimic aspects of human use on a dose/body weight basis. Although the 0.1 mg/kg dose did not cause statistically significant increases in locomotor activity compared with saline controls, the higher doses (0.3 and 1.0 mg/kg) caused statistically significant increases in locomotor activity (p < .05), which lasted for up to 3 h at the highest dose. After the 1.0 mg/kg dose, the volume of distribution at steady state was 9.0 liters/kg, the total clearance was 126 ml/min/kg, and the average distribution and elimination half-lives were 9.2 and 63.0 min, respectively. Because the pharmacokinetic values after the 0.1 mg/kg dose were not different from those after the 1.0 mg/kg dose, the pharmacokinetics of (+)-METH were considered to be independent of the dose over this 10-fold range. (+)-AMP serum concentrations after the 1.0 mg/kg dose peaked from 10 to 30 min, and exhibited a \( T_{1/2z} \) of 98.5 min. The statistically longer \( T_{1/2z} \) of (+)-AMP (p < .05) suggested that the (+)-AMP terminal elimination rate and not the (+)-AMP metabolic formation rate is the rate-limiting step in (+)-AMP elimination following i.v. (+)-METH dosing.

Rapid i.v. injection or smoking of the active form of methamphetamine [(+]\text{METH})] are the preferred routes of administration by many human drug abusers. This is because the onset of pharmacologic effects is much more rapid and intense than when (+)-METH is taken orally or by other nonparenteral routes of administration (Cho, 1990). Indeed, the initial self-reported “flash” or intensely pleasurable feeling may occur even before an i.v. injection is complete (Cox and Smart, 1970), which could partially explain the increasing preference of many drug abusers for methods that allow them to control the rate of drug input (Hall and Hando, 1993).

Previous reports suggest self-administration of (+)-METH i.v. doses range from 10 to 50 mg (Cho, 1990; Beebe and Walley, 1995), or ~0.1 to 0.8 mg/kg for an average 70-kg adult. In studies of the central nervous system (CNS) and hemodynamic effects of (+)-METH in humans, Mendelson et al. (1995) found that i.v. bolus doses of 30 mg (or ~0.45 mg/kg in their patients) result in immediate effects with peak intoxication ratings reported as early as 10 min after drug administration. Doses in this range result in CNS and hemodynamic effects that last for ~2 h (Cook et al., 1993; Mendelson et al., 1995).

Because the administration of high i.v. doses to humans could produce neurotoxicity and because ethical considerations limit the repeated administration of this highly addicting substance to humans (Fumagalli et al., 1998), animal models that mimic human use of (+)-METH are needed. Unfortunately, most investigators use doses and/or routes of administration that do not accurately mimic the time course of behavioral effects and/or the time course of the metabolic profile observed in humans after i.v. or smoked (+)-METH. For instance, most animal studies use extravascular routes of (+)-METH administration (e.g., i.p. or s.c.) (Sakai et al., 1983; Yamada et al., 1986; Segal and Kuczenski, 1997). Although these routes are more convenient and technically less difficult for repeated drug administration, the onset of effects

Abbreviations: (+)-METH, (+)-methamphetamine; CNS, central nervous system; (+)-AMP, (+)-amphetamine; AUC, area under the serum concentration-versus-time curve; Cl\text{NR}, nonrenal clearance; Cl\text{R}, renal clearance; Cl\text{T}, total body clearance; \( \lambda_{\text{z}} \), terminal elimination rate constant; \( Vd_{\text{SS}} \), volume of distribution at steady state; \( T_{1/2z,1} \), distribution half-life; \( T_{1/2z,2} \), terminal elimination half-life.
and time courses of these nonvascular routes of administration do not accurately mimic the intense rush of drug effects, which is preferred by many human (+)-METH users (Sakai et al., 1983; Yamada et al., 1986). In addition, animal models that use i.p. administration are complicated because of significant liver first-pass effects and subsequent alterations in the time course of the (+)-METH and (+)-amphetamine ([+]AMP) metabolic profile (Sakai et al., 1983). As a result, only very limited data are available concerning the behavioral effects and pharmacokinetics of (+)-METH and its metabolites after rapid drug input (i.e., i.v. or smoking).

(+)-METH concentration- and effect-time relationships in animal models after doses and routes of administration used by humans have not been adequately characterized. The most complete study of (+)-METH pharmacokinetics in rats after i.v. dosing is by Hutchaleelaha and Mayersohn (1996). This study was designed to investigate the potential therapeutic benefits of intragastric charcoal administration on the disposition of METH after i.v. administration. In their study, an extremely large dose (15 mg/kg i.v.) of a racemic mixture of the active and inactive (+)-METH and (−)-METH forms of the drug was used. However, the potential impact of using a racemic mixture of the individual METH forms on the distribution, metabolism, and elimination of the active (+)-METH is not addressed. In another pharmacokinetic study, Melega et al. (1995) report changes in brain and serum concentrations during the first hour after i.v. administration of a 1.0 mg/kg dose of (+)-METH in a study of the changes in (+)-METH-induced striatal dopamine concentrations. However, the tissue and serum collection period was too short to adequately calculate pharmacokinetic parameters. In addition, neither of these studies provided information about the behavioral effects of the doses used in these experiments.

The current studies were conducted to better understand the pharmacodynamic and pharmacokinetic properties of (+)-METH after rapid i.v. administration of (+)-METH in an animal model designed to mimic selected aspects of human (+)-METH use. To accomplish this goal, we evaluated the time course of selected behavioral effects of (+)-METH after i.v. bolus doses (0.1, 0.3, and 1 mg/kg) in rats and then determined the pharmacokinetics of (+)-METH after the lowest and highest of these doses. In addition, we determined the time course of the formation and elimination of the major (+)-METH metabolite, (+)-AMP. The doses and route of administration were selected to mimic the range of doses and a major route of administration used by human drug abusers.

Materials and Methods

Drugs and Chemicals. (+)-METH, (+)-AMP, and (+)-[^3H]METH were obtained from the National Institute on Drug Abuse (Rockville, MD). The tritiated (+)-METH ([+]-2,6-[3H]methylamphetamine, 23.5 Ci/mmol) was synthesized with the radiolabel at the 2 and 6 positions of the aromatic ring, which are metabolically stable sites. The synthesis was performed by the Research Triangle Institute (Research Triangle Park, NC) for the National Institute on Drug Abuse. Norephedrine was obtained from Sigma Chemical Co. (St. Louis, MO). All drug doses and concentrations were expressed as the free base form. Trifluoroacetic acid was obtained from Pierce Chemical Co. (Rockford, IL). All other reagents used in these studies were purchased from Fisher Scientific Co. (Fairlawn, NJ), unless otherwise specified.

Animals. Male Sprague-Dawley rats (250–300 g) with surgically implanted vascular cannulas were obtained from Hilltop Lab Animals, Inc. (Scottsdale, PA). Rats used for behavioral experiments had a single surgically implanted cannula in the jugular vein. Rats used for pharmacokinetic experiments had two cannulas (one in the external jugular vein and one in the femoral artery) for administration of drugs and collection of blood. The day after delivery, rats were anesthetized with ethyl ether, and the cannulas were exposed and flushed with 200 µl of saline and 50 µl of heparin (50 U) to avoid clotting of the cannulas. Rats were then allowed to acclimate to their environment for 1 week, during which time each cannula was flushed every 2 days with heparinized saline. Rats were maintained in an animal care facility with a 12-h light/dark cycle (7:00 AM–7:00 PM) and a mean temperature of 22°C. All experiments performed in these studies were in accordance with the Guide for the Care and Use of Laboratory Animals, as promulgated and adopted by the National Institutes of Health. All animal protocols were approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee (Little Rock, AR) before starting the experiments.

Behavioral Experiments. Behavioral experiments were carried out in open-top polyethylene chambers (60 x 45 x 40 cm). Gray gravel bedding was added to the bottom of each chamber to provide an absorbent, nonreflective, contrasting background with a neutral odor. Spontaneous behavior of the rats was recorded by a video camera located above the chambers. The camera was connected to a monitor and to a S-VHS recorder. Beginning 1 week before the experiments, the rats were placed in the chambers every day to allow habituation. On the day of an experiment, the rats were placed in the chambers 1 h before the administration of saline or drug. Four different i.v. treatments were administered on separate occasions: saline (1 ml/kg), 0.1, 0.3, or 1.0 mg/kg of (+)-METH (in 1 ml/kg sterile saline) with a repeated-measures, mixed sequence design (n = 6 per group). Saline or (+)-METH was administered beginning at time zero as a 15-s bolus dose via the jugular cannula followed by an equal volume of saline to flush the cannula. The behavioral effects were recorded over a 3.5-h (210-min) period.

Analysis of behavior was conducted as described by Hardin et al. (1998) with EthoVision software (Noldus Information Technology, Inc., Sterling, VA). This computer software allowed us to quantify both (+)-METH-induced rearing behavior and distance traveled during each experiment. The number of times each animal reared and the total distance each animal moved were accumulated over consecutive 4-min time intervals. Computer analysis of the videotaped experiments was conducted off-line after the completion of each experiment.

Pharmacokinetic Experiments. Rats were placed in metabolism cages (Nalgene Co., Nalgene Labware Div., Rochester, NY) the day before the experiment, with free access to water. On the day of the experiment, either 0.1 mg/kg (n = 4) or 1.0 mg/kg (n = 5) (+)-METH containing 100 µCi of tritiated (+)-METH (as a tracer) was injected as a 15-s bolus dose into the femoral artery. Injection into the femoral artery and sampling via the jugular vein was done because the arterial line was found to be more prone to occlusion in preliminary studies. Because intravascular mixing of drug in the blood is so rapid in rats (<10 s [Ebling et al., 1994]), we assumed that venous concentrations would not be substantially different from arterial concentrations at 1 min and all subsequent time points. The animals suffered no untoward effects from the femoral arterial injections. Blood samples were collected at 1, 2, 5, 10, 20, 40, 60, 150, 240, and 330 min after the injection at total volumes ranging from 100 µl at the early time points to 500 µl at the later time. The volume of blood collected during the entire experiment was controlled not to exceed 10% of the total blood volume. After collection of each blood sample, an equal volume of sterile saline was administered to maintain a constant blood volume. The blood was allowed to clot at room temperature, and the serum was collected after centrifugation. The samples were frozen at −80°C until analysis. Urine was collected.
from the metabolic cages as soon as possible and kept at −80°C until analysis. At the end of the experiment, rats were sacrificed with CO₂.

**Analysis of Biological Samples.** The extraction procedure for (+)-METH and (+)-AMP from serum and urine was based on the method described by Burchfield et al. (1991). Briefly, serum or urine samples (30–200 μl for serum and 100 μl for urine) in 10 × 75 mm siliconized test tubes were mixed with 10 μl of nonradioactive standards [(+)-METH, (+)-AMP, and norephedrine at 1 mg/ml diluted in saline], NaOH (10 N; final concentration 3.3 N) was added, followed by 1 ml of hexane. The contents of the tubes were then gently mixed for 1 h. After removal of the hexane phase, the samples were again extracted with a fresh volume (1 ml) of hexane. Then, 200 μl of 0.1 N HCl was added to the pooled hexane extracts and gently mixed for 2 h to back extract the (+)-METH and (+)-AMP. We did not use a back extraction of (+)-METH and (+)-AMP into hexane followed by an evaporation of the organic phase because it is known that (+)-METH and its metabolites are volatilized under these conditions (Cheung et al., 1997).

The HPLC procedure consisted of the injection of 100 μl of the HCl-extracted phase into a μ-Bondapak C₁₈ column (300 × 3.9 mm i.d.; Waters Associates, Millipore Corp., Milford, MA). Separation of (+)-METH and metabolites on the HPLC column was achieved with a convex gradient of acetonitrile and water with 0.1% trifluoroacetic acid (from 10 to 20% acetonitrile over a 10-min period). The flow rate was 1 ml/min and the UV detector absorbance was 215 nm. (+)-METH, (+)-AMP, and norephedrine were fully separated (Fig. 1) under these conditions, which allowed HPLC fraction collection into scintillation vials based on predetermined elution time periods. Twenty milliliters of scintillation fluid was added to each vial, and the radioactivity in each sample was quantified with liquid scintillation spectrometry (Packard Instrument Co., Inc., Meriden, CT). Analytical recoveries and identities of metabolic products were determined by comparison with an exact amount of authentic external standards injected at the start of each analytical run. The (+)-METH and (+)-AMP recoveries were calculated by the areas of the peaks obtained by monitoring the absorbance of the authentic standards at 215 nm. The concentrations of (+)-METH and (+)-AMP (and [³H]METH and [³H]AMP) from the i.v. administration of (+)-METH were not detectable by UV absorbance. The analytical recovery of (+)-METH and (+)-AMP through the complete procedure (extraction and HPLC analysis) was at least 90%.

**(+)-METH Serum Protein-Binding Studies.** Serum from drug-free rats was used for protein-binding studies. The serum samples were spiked with 0.2 or 200 ng/ml (final concentrations) (+)-METH, along with a tracer dose of (+)-[³H]METH (20,000 dpm). Teflon equilibrium dialysis cells (engineered at the University of Arkansas for Medical Sciences) were filled with 100 μl of serum on one side and with 100 μl of phosphate buffer (0.13 M; pH 7.3) on the other. A 12- to 14-kDa cut-off membrane ( Spectrum Scientific Corp., Houston, TX) separated the two sides of the cells. Equilibrium was reached after incubation of cells (with constant turning) overnight at 37°C in a water bath. The serum and buffer were removed from each side of the chamber, and the (+)-[³H]METH concentration was determined by liquid scintillation spectrometry.

**Pharmacokinetic Data Analysis.** Model-dependent pharmacokinetic analysis of (+)-METH concentration-time data was performed with a nonlinear least-squares curve-fitting routine ( WinNonlin, Scientific Consulting Inc., Cary, NC). Biexponential and triexponential curves were fit to the concentration-time data sets with a 1/y and 1/y² (where y is the predicted concentration) weighting function. The best-fit line was selected after visual inspection of the fit of each curve to the data, analysis of the residuals, and a statistical F test for selecting between alternative equations (Boxenbaum et al., 1974). Pharmacokinetic parameters were calculated with the following equations:

\[
V_d_{ss} = (D/AUMC)/AUC_0^{5/2}
\]

\[
Cl_{f} = D/AUC_0^{*}
\]

\[
Cl_{ux} = Fu - Cl_{lx}
\]

\[
Cl_{NR} = Cl_{f} - Cl_{ux}
\]

where \(V_d_{ss}\) is the volume of distribution at steady state, \(D\) is the injected dose, \(AUMC\) is the area under the first moment curve, \(AUC_0\) is the area under the serum concentration versus time curve from time zero to infinity, \(Cl_{f}\) is the total body clearance, \(Cl_{ux}\) is the renal clearance, \(Fu\) is the fraction of the intact dose of (+)-METH in the urine, and \(Cl_{NR}\) is the nonrenal clearance.

Pharmacokinetic analysis of (+)-AMP concentrations resulting from the administration of (+)-METH was conducted with model-dependent and model-independent analysis. The maximum serum (+)-AMP concentrations (\(C_{max}\)) and the time to maximum serum (+)-AMP concentrations (\(T_{max}\)) were estimated for each animal by fitting a monoexponential model with a first-order input function to each data set with 1/y and 1/y² weighting. The best-fit line was determined visually. The \(T_{1/2a}\) was determined with linear regression analysis of the data points in the terminal elimination phase of the (+)-AMP concentration history ( WinNonlin; Scientific Consulting Inc.).

**Statistics.** All values are expressed as means ± S.D. Statistical comparisons of locomotor activity were performed with a Kruskal-Wallis one-way analysis of variance on ranks. When significant differences were found (p < .05), a post hoc pairwise multiple comparison was conducted with a Student-Newman-Keuls test. Values for the harmonic mean and “pseudo” standard deviation of the \(T_{1/2a}\) were calculated as previously described (Lam et al., 1985). Significance of pharmacokinetic data were analyzed with a two-tailed unpaired Student’s t test. The level of significance was set at p < .05.

**Results**

**Behavioral Experiments.** We observed a dose-dependent increase in behavioral response with increasing (+)-METH doses (Figs. 2–4). The locomotor activity appeared to increase very mildly after the 0.1 mg/kg dose compared with
saline treatment (Figs. 2 and 3); however, the behavioral effects (distance traveled and rearing events) were not statistically different from those of the saline treatment (Fig. 4). The number of rearing events following the 0.3 mg/kg dose was statistically different from that of all other groups ($p < .05$). The distance traveled after the 0.3 mg/kg dose was different from those of the saline treatment and 1.0 mg/kg dose groups ($p < .05$; Fig. 4), but not different from the 0.1 mg/kg dose group. The distance traveled and the number of rearing events increased significantly with the 1.0 mg/kg dose compared with all other doses ($p < .05$; Fig. 4).

We attempted to define the time needed to return to baseline behavior based on statistical comparison of data from the baseline, pre-(+)-METH spontaneous locomotor activity (i.e., −30 to 0 min). However, this approach did not yield satisfactory results because there appeared to be a generalized increase in locomotor activity even after the major (+)-METH-induced effects had subsided (i.e., a noisy posteffects baseline). In previous studies of phencyclidine-induced locomotor activity (Hardin et al., 1998), we were able to clearly define a reproducible termination of effects based on statistical comparison of pre- and postbaseline response data. This apparent loss of baseline after (+)-METH administration was observed for both rearing events and distance traveled. Nevertheless, visual assessment of the duration of effects after the 1.0 mg/kg (+)-METH dose showed that (+)-METH-induced behavior appeared to return to near-baseline values after $\sim 175$ min (Figs. 2 and 3).

(+)-METH Pharmacokinetic Experiments. With radiolabeled (+)-METH, the lower level of reproducible quantitation was $\sim 0.2$ ng/ml of (+)-METH or (+)-AMP in the serum, whereas HPLC separation of unlabeled drug combined with absorbance detection at 214 nm has a limit of detection of $\sim 20$ ng/ml (Li et al., 1997). Even with the increased sensitivity for (+)-METH and metabolites, the only metabolite we detected was (+)-AMP. We also were able to study the pharmacokinetics of (+)-METH following very low doses (e.g., 0.1 mg/kg). This allowed us to determine the pharmacokinetic dose dependence of (+)-METH over a 10-fold range of doses, which is within the range of doses taken by humans on a milligram per kilogram basis. The serum concentration versus time curves for the low and high (+)-METH doses were best described by a two-compartment model with distinct distribution and terminal
elusion phases (Fig. 5). The pharmacokinetic parameters determined with the two i.v. doses of (+)-METH were not statistically different (Table 1); thus, the serum pharmacokinetics of (+)-METH appeared to be independent of dose over a 10-fold range of doses. From the (+)-METH protein-binding experiments, we found that ~10% of serum (+)-METH was protein bound at a representative very low (0.2 ng/ml) and high (200 ng/ml) serum (+)-METH concentration, and these values were not statistically different (10.6 ± 3.5 and 8.2 ± 1.6%, respectively). These relatively low values for protein binding indicated that (+)-METH protein binding will not be a significant factor in the pharmacokinetics or pharmacodynamics of (+)-METH in rats.

Although the fraction of the (+)-METH dose in the urine and the renal clearance of (+)-METH following the 1.0 mg/kg dose appeared to be slightly higher than that after the 0.1 mg/kg dose, the values were not statistically different (Table 1). Nevertheless, we wondered if the slightly greater values after the higher (+)-METH dose could have reflected a (+)-METH-induced increase in renal blood flow or changes in urine pH due to increased locomotor activity (Figs. 2–4).

The total clearance for (+)-METH averaged ~130 ml/min/kg after both doses. Because total body clearance appeared to exceed hepatic blood flow in the rat (i.e., 72–95 ml/min/kg; Birnie and Grayson, 1952), these in vivo data suggest that the liver, kidney, and possibly other clearing organs are involved in (+)-METH elimination. This high total body clearance resulted in a rapid elimination half-life (~63–75 min), which was independent of the dose over our 10-fold range of doses (Table 1).

(+)-AMP Pharmacokinetic Experiments. The pharmacokinetic parameters of the metabolite (+)-AMP are shown in Table 2. As with (+)-METH, the pharmacokinetics of (+)-AMP appeared to be independent of dose. Indeed, $T_{max}$, $T_{1/2\alpha}$, and percentage of dose appearing in the urine were not significantly different for the 0.1 and 1.0 mg/kg dose. In addition, $C_{max}$ and AUC after the 1.0 mg/kg dose were 7.1 and 8.7 times greater, respectively, than with the 0.1 mg/kg dose. Thus, the magnitude of change in these values was also consistent with a 10-fold difference in the doses.

The $T_{1/2\alpha}$ for (+)-AMP did not differ between the low and high (+)-METH doses. However, when we compared the values for $T_{1/2\alpha}$ (+)-AMP to those of (+)-METH at the low and high doses, we found the values were 19 and 56% greater, respectively. Statistical comparison of the $\lambda_1$ values for (+)-AMP and (+)-METH at the low and high doses showed that only the (+)-AMP and (+)-METH $\lambda_1$ values at the high dose were statistically different from each other (p < .05). Thus, at least at the 1.0 mg/kg dose, metabolite elimination (and not metabolite formation) was the rate-limiting step in the clearance of (+)-AMP. This difference can be seen in Fig. 5 by comparing the slopes of the best-fit lines to the terminal elimination phases of (+)-AMP and (+)-METH concentration-time data in each of the individual animals.

### Discussion

The major goal of these studies was to characterize the spontaneous locomotor effects and pharmacokinetics of (+)-

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**Table 1**

<table>
<thead>
<tr>
<th>(+)-METH Dose</th>
<th>0.1 mg/kg$^b$</th>
<th>1.0 mg/kg$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2\alpha}$ (min)</td>
<td>15.8 ± 6.1</td>
<td>9.2 ± 5.7</td>
</tr>
<tr>
<td>$T_{1/2\alpha}$ (min)</td>
<td>75.2 ± 9.8$^c$</td>
<td>63.0 ± 14.0</td>
</tr>
<tr>
<td>AUC$^0$ (ng-min/ml)</td>
<td>760 ± 120</td>
<td>8120 ± 1400</td>
</tr>
<tr>
<td>$V_{d}$ (l/kg)</td>
<td>9.3 ± 1.3</td>
<td>9.0 ± 2.0</td>
</tr>
<tr>
<td>$C_{p}$ (ml/min/kg)</td>
<td>132 ± 18.8</td>
<td>126 ± 20.2</td>
</tr>
<tr>
<td>$C_{l}$ (ml/min/kg)</td>
<td>122 ± 19.0</td>
<td>108 ± 15.6</td>
</tr>
<tr>
<td>$C_{l}$ (l/min/kg)</td>
<td>11.5 ± 7.1</td>
<td>19.2 ± 14.6</td>
</tr>
<tr>
<td>Dose in urine (%)</td>
<td>9.2 ± 4.8</td>
<td>12.8 ± 8.1</td>
</tr>
</tbody>
</table>

$^a$ Statistical comparison of AUC$^0$ values at the two doses was conducted after dividing the AUC$^0$ values by dose of (+)-METH administered.

$^b$ $n$ = 4 for the 0.1 mg/kg dose and $n$ = 5 for the 1.0 mg/kg dose. All results are expressed as means ± S.D.

$^c$ Harmonic mean and "pseudo" standard deviation (Lam et al., 1985).

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**Table 2**

<table>
<thead>
<tr>
<th>(+)-AMP dose</th>
<th>0.1 mg/kg$^b$</th>
<th>1.0 mg/kg$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2\alpha}$ (min)</td>
<td>89.3 ± 9.0$^c$</td>
<td>98.5 ± 11.9</td>
</tr>
<tr>
<td>AUC$^0$ (ng-min/ml)</td>
<td>325 ± 70</td>
<td>2840 ± 1300</td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>2.6 ± 0.3</td>
<td>18.5 ± 5.7</td>
</tr>
<tr>
<td>$T_{max}$ (min)</td>
<td>25.5 ± 15.2</td>
<td>22.9 ± 17.8</td>
</tr>
<tr>
<td>Dose in urine (%)</td>
<td>2.9 ± 0.8</td>
<td>3.3 ± 1.2</td>
</tr>
</tbody>
</table>

$^a$ Statistical comparison of AUC$^0$ and $C_{max}$ values at the two doses was conducted after dividing the AUC$^0$ and $C_{max}$ values by dose of (+)-METH administered.

$^b$ $n$ = 4 for the 0.1 mg/kg dose and $n$ = 5 for the 1.0 mg/kg dose. All results are expressed as means ± S.D.

$^c$ Harmonic mean and "pseudo" standard deviation (Lam et al., 1985).
METH in rats after i.v. bolus doses. High levels of spontaneous locomotor activity (a measure of CNS effects) occurred immediately (Figs. 2 and 3). Mendelson et al. (1995) show that significant increases in self-reported intoxication ratings, heart rate, and blood pressure occur immediately after i.v. bolus doses of (+)-METH in humans. Thus, our locomotor activity results in the rat are consistent with the immediate onset of subjective and physiological effects in humans following i.v. (+)-METH.

We were concerned that stereotyped behaviors may have interfered with the time of onset and the magnitude of locomotor activity. Segal and Kuczenski (1997) report that s.c. administration of 4.2 mg/kg (+)-METH in rats (versus 1.0 mg/kg i.v. in our study) does not result in spontaneous locomotor effects for over an hour, even though the duration of locomotor effects in both studies was similar (~210 versus 175 min, respectively). To address this concern, we had two observers independently rate selected (+)-METH-induced stereotyped behaviors (sniffing, head weaving, gnawing, and licking) by viewing the films of three of the rats in the behavior studies. The raters analyzed the stereotyped behaviors after the saline treatment and the 1.0 mg/kg (+)-METH dose. Although a very significant amount of (+)-METH-induced sniffing occurred (with very little of the other behaviors), the number of sniffing events appeared to be relatively constant throughout the period of (+)-METH-induced locomotor effects. Therefore, our analysis of the behavioral films does not support the hypothesis that time-dependent stereotyped behaviors at the highest dose interfered with locomotor activity. However, more studies of the comparison of effects after different routes of administration need to be conducted to elucidate these apparent differences.

Visual assessment of the graphs for distance traveled and rearing events showed that maximum values occurred from 28 to 34 min after the 1.0 mg/kg dose, whereas maximum values occurred at 6 to 14 min after the 0.3 mg/kg dose (Figs. 2 and 3). This apparent dose-dependent delay is misleading because individual rearing events appeared to last longer at the 1.0 mg/kg dose (~5 s) than at the lower doses (~1 s) for 20 to 30 min after dosing. The increased time spent in rearing appeared to decrease the total number of rearing events and the available time for movement. After the high dose, the duration of rearing events decreased over time. Thus, the distance traveled and number of rearing events did not reach a maximum until after these higher dose effects on rearing began to wane.

Comparison of the time course of behavioral effects with the serum (+)-METH concentrations after the 1.0 mg/kg dose showed there was no apparent correlation, and the locomotor effects lasted well into the terminal elimination phase. The serum pharmacokinetic values in this study (Table 1) were different from those in a previous study of (+)-METH and (-)-METH pharmacokinetics following a very large (15 mg/kg) dose of a racemic mixture administered to male Sprague-Dawley rats (Hutchaleelaha and Mayersohn, 1996). The observed T1/2ss for (+)-METH in the previous study was ~50% greater than in our studies (90 versus 63 min, respectively), and the values for Vdss and Clp were approximately one-third of ours (2.9 versus 9.3 liters/kg and 48.7 versus 133 ml/min/kg).

Assuming there were no analytical errors in either study, the differences in values could be due to dose-dependent physiological differences and/or a pharmacokinetic interaction between the (+)-METH and (-)-METH following the administration of a racemic mixture in the previous study. (+)-METH is known to cause profound dose-dependent hemodynamic stimulation in humans (Cho, 1990; Mendelson et al., 1995) that leads to increases in heart rate, blood pressure, and cardiac output. Although increases in blood flow to clearing organs such as the liver could produce increases in clearance, the overall impact on drug disposition at higher doses is difficult to predict because there also would be organ-specific differences in vascular resistance to blood flow. In addition, Hutchaleelaha and Mayersohn (1996) did not study the possibility of pharmacokinetic drug interactions between the (+)-METH and (-)-METH after administration of racemic METH. Regardless of the reasons for the differences in pharmacokinetic parameters between the two studies, our studies are easier to interpret because we know the range of effects that result from our doses, and because we administered only the (+)-enantiomer of METH.

Melega et al. (1995) also conducted an i.v. (+)-METH pharmacokinetic study as an aid to understanding the effects of (+)-METH on striatal dopamine concentrations. They report a 49-min T1/2 for the (+)-METH concentration versus time profile in plasma following a 1.0 mg/kg dose. This value is suspect because Melega et al. (1995) only used the (+)-METH concentration-time data from the first hour after drug administration and they assumed the (+)-METH pharmacokinetics would be best described by a one-compartment model. Our data clearly show that during the first hour, both distribution and elimination are occurring (Fig. 5). Thus, their T1/2 value is a hybrid value comprised of distribution and elimination processes.

We also quantified the concentration versus time profile of the primary (+)-METH metabolite, (+)-AMP. The T1/2ss of (+)-AMP was longer (89–98 min) than the T1/2ss for (+)-AMP determined after the i.v. administration of a racemic mixture of (+)/(−)-AMP in male Sprague-Dawley rats (67 min; Hutchaleelaha et al., 1994). Although they also report (+)/(−)-AMP concentrations after administration of a racemic mixture of METH in another of their studies (Hutchaleelaha and Mayersohn, 1996), they did not report any pharmacokinetic values for (+)-AMP. Because the T1/2ss for (+)-AMP was significantly longer than the T1/2ss for (+)-METH at the 1.0 mg/kg dose, the terminal elimination rate of (+)-AMP (and not the metabolite formation rate) appears to be the rate-limiting step in the elimination of (+)-AMP after i.v. (+)-METH. These data suggest that with frequent, repeated administration of (+)-METH (e.g., once every T1/2ss), the concentrations of (+)-AMP would exceed those of (+)-METH after several doses in the rat. Although we did not find reports of pharmacokinetic data concerning formation and elimination rates of (+)-AMP after i.v. (+)-METH administration in humans, the potential for the accumulation of this potent psychoactive metabolite could be an important pharmacological consideration in situations of repeated use such as occurs in “speed runs” or binges and for consideration in the development of therapeutic strategies for medical intervention.

Because a goal of these studies was to characterize an animal model of human (+)-METH use, we also compared selected aspects of the pharmacokinetics in rats and humans. Both Cook et al. (1993) and Mendelson et al. (1995) have
studied i.v. (+)-METH pharmacokinetics in humans and find similar values. For simplicity, we will compare our data with the earlier of the two studies. In summary, the pharmacokinetic values are as follows for the rat and human, respectively: Vdss, 9.0 versus 3.7 liters/kg; ClT, 126 versus 3.2 ml/min/kg; and T1/2α, 63 min versus 13.1 h. When the pharmacokinetic parameters are considered in terms of physiologic, anatomic, and allometric differences between these species, some interesting observations about the appropriateness of the rat as a model of human use can be made. For instance, the Vdss only differs by a factor of 2.4, which suggests that the rat is a reasonable model of the apparent high volume of distribution of (+)-METH in humans. However, the ClT is significantly different between the two species because (+)-METH ClT in the rat exceeds hepatic blood flow (72–95 ml/min/kg; Birnie and Grayson, 1952), whereas in the human ClT is substantially lower than hepatic blood flow (19 ml/min/kg in a 70-kg human; Pang and Rowland, 1977). Furthermore, renal elimination of unchanged drug is a much greater capacity for metabolic elimination in the rat. Although ClT and T1/2α differences between the two species are greater than would be expected based on allometric scaling factors, the rat can still be a useful model if these differences are accounted for. For instance, the usefulness of the rat as a model of human use can be made. For simplicity, we will compare our data with the earlier of the two studies. In summary, the pharmacokinetic values are as follows for the rat and human, respectively: Vdss, 9.0 versus 3.7 liters/kg; ClT, 126 versus 3.2 ml/min/kg; and T1/2α, 63 min versus 13.1 h. When the pharmacokinetic parameters are considered in terms of physiologic, anatomic, and allometric differences between these species, some interesting observations about the appropriateness of the rat as a model of human use can be made. For instance, the Vdss only differs by a factor of 2.4, which suggests that the rat is a reasonable model of the apparent high volume of distribution of (+)-METH in humans. However, the ClT is significantly different between the two species because (+)-METH ClT in the rat exceeds hepatic blood flow (72–95 ml/min/kg; Birnie and Grayson, 1952), whereas in the human ClT is substantially lower than hepatic blood flow (19 ml/min/kg in a 70-kg human; Pang and Rowland, 1977). Furthermore, renal elimination of unchanged drug is a much greater capacity for metabolic elimination in the rat. Although ClT and T1/2α, differences between the two species are greater than would be expected based on allometric scaling factors, the rat can still be a useful model if these differences are fully considered in the experimental design.

In conclusion, we found that (+)-METH pharmacokinetics do not differ over a 10-fold range of doses in the rat, which includes a dose that produced essentially no pharmacological response (0.1 mg/kg) and a dose that produced profound pharmacological response (1 mg/kg). In addition, we found that spontaneous locomotor effects peaked soon after i.v. bolus administration, which is consistent with the time course of human effects. Finally, we found that (+)-AMP elimination is slower than (+)-METH elimination following a 1.0 mg/kg dose. Thus, accumulation of (+)-AMP with repeated administration of (+)-METH could lead to greatly increased (+)-AMP concentrations that could presumably affect the magnitude and spectrum of effects observed after repeated i.v. (+)-METH dosing in rats.

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References


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