Disposition of Methamphetamine and Its Metabolite Amphetamine in Brain and Other Tissues in Rats after Intravenous Administration

GILLES J. RIVIÈRE, W. BROOKS GENTRY, and S. MICHAEL OWENS

Departments of Pharmacology and Toxicology (G.J.R., W.B.G., S.M.O.) and Anesthesiology (W.B.G.), College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

Accepted for publication November 13, 1999. This paper is available online at http://www.jpet.org

ABSTRACT

These studies characterized the concentration-time profile of (+)-methamphetamine [(+)-METH] and its metabolite (+)-amphetamine [(+)-AMP] in the brain and five other tissues after (+)-METH administration. Male Sprague-Dawley rats received a pharmacologically active (+)-METH i.v. bolus dose (1.0 mg/kg) or a nonpharmacologically active s.c. infusion (20 h at 1.2 mg/kg/day). Tissues (n = 3 per time point) were collected for more than four elimination half-lives in the i.v. group, or at a single steady-state time point (20 h) in the s.c. group. Based on data from the area under the concentration-time curves after i.v. dosing, the rank order of (+)-METH tissue accumulation was kidney > spleen > brain > liver > heart > serum with terminal elimination half-life values ranging from 53 to 66 min. (+)-METH concentrations were highest at the first measured time point (2 min) in all tissues except the spleen, which peaked at 10 min. The brain-to-serum concentration ratio rose from 7:1 at 2 min to a peak of 13:1 at 20 min before equilibrating to a constant value of 8:1 at 2 h. Following s.c. (+)-METH dosing, the (+)-METH brain-to-serum concentration ratio was the same as the equilibrated ratio following i.v. dosing. (+)-AMP concentrations peaked at 20 min in all tissues before decaying with terminal elimination half-life values ranging from 68 to 75 min. Analysis of the area under the concentration-time curve molar amounts of (+)-AMP and (+)-METH showed that (+)-AMP accounted for approximately one-third of the drug tissue exposure over time. Thus, these data indicate the importance of both (+)-METH and (+)-AMP in pharmacological effects following i.v. (+)-METH administration.

Many habitual drug abusers self-administer their drugs via smoking or i.v. injection to achieve more rapid onset of effects (Farre and Cami, 1991). Thus, for drugs of abuse such as methamphetamine [(+)-METH] this may be an important factor in the abuse potential. For instance, Oldendorf (1992) postulates that the shorter the interval between intake and drug effects, the greater the propensity for a more severe addiction. However, the data to support this hypothesis are incomplete and the mechanisms are poorly understood concerning the relationship between rate of administration and onset of effects.

To adequately characterize the impact of rapid (+)-METH administration (i.e., i.v. or smoking), it is necessary to understand the complete time course of (+)-METH and its active metabolites at important sites of action. However, given the inability to continuously measure concentrations directly at the sites of action, most investigators choose to measure venous and/or arterial concentrations as surrogate markers. Nevertheless, these blood concentrations do not necessarily reflect the complex pattern of real-time changes in tissue drug concentrations.

Pharmacological studies of (+)-METH are further complicated by the formation of an active metabolite, (+)-amphetamine [(+)-AMP] with a potentially unique tissue concentration-time profile. Melega et al. (1995) studied selected aspects of brain and serum concentrations of (+)-METH or (+)-AMP after i.v. administration of each drug separately, and studied brain concentrations of the (+)-METH metabolite (+)-AMP following i.v. (+)-METH administration. These investigators found that the highest (+)-METH concentrations in the striatum occur at their first measured time point (5 min). In addition, the (+)-METH brain-to-serum ratio at this time point was 10:1 and it remained constant for the short duration of their study (1 h). They also found that (+)-AMP concentrations increased in the striatum from the moment of (+)-METH administration to peak at 20 to 30 min, after which the (+)-AMP concentrations remained constant.
for the duration of sampling in the striatum (60 min). However, these data do not provide an adequate description of the pharmacokinetic processes (i.e., complete concentration versus time curves for more than four elimination half-lives) for (+)-METH and (+)-AMP in the brain and other pharmacologically important tissues such as the heart, liver, and kidney.

The purpose of this study was to characterize the concentration versus time profile of (+)-METH and its metabolite (+)-AMP in brain and other important tissues after acute i.v. injection of (+)-METH in rats. The other tissues were the heart, kidney, liver, serum, and spleen. These data were then used to better understand the time course of concentrations, accumulation, metabolism, and elimination of (+)-METH and (+)-AMP in rat tissues. To better understand the significance of drug and metabolite partitioning following acute dosing, we also characterized (+)-METH and (+)-AMP partitioning after chronic, steady-state dosing following s.c. (+)-METH administration for 20 h. Finally, these pharmacokinetic data for (+)-METH and (+)-AMP were used to better understand the potential contribution of this parent drug and metabolite to the overall pharmacological effects.

Materials and Methods

Drugs and Chemicals. (+)-METH, (+)-AMP, and [3H](-)-METH were obtained from the National Institute on Drug Abuse (Rockville, MD). Norephedrine was obtained from Sigma Chemical Co. (St. Louis, MO). The [3H](-)-METH ([(2R,6'S)-2,6,22H])(-)-methamphetamine; 25 Ci/mmol, 1.01 mCi/ml) was synthesized with radioslabel at positions 2 and 6 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. All drug doses and concentrations are expressed as the free base form. All other reagents used in these studies were obtained from Fisher Scientific (Springfield, NJ).

Animals. Male Sprague-Dawley rats (250–300 g; n = 3 per time point) with a single surgically implanted jugular venous cannula were obtained from Hilltop Laboratory Animals (Scottsdale, PA). The rats were shipped with the cannula imbedded in the s.c. space between the scapulae. The day after delivery, the rats were anesthetized with ethyl ether, and the cannulas were exposed. Each cannula was flushed after exposure with 200 µl of saline and 50 µl of heparin (50 U) to avoid clotting of the cannulas. Rats were then allowed to recover for 1 week during which the cannulas were flushed with heparinized saline every 2 days. 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 adopted and promulgated by the National Institutes of Health. The University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee (Little Rock, AR) also approved all animal protocols.

Tissue Distribution Experiments. Rats were placed in metabolism cages the day before the experiment with free access to water. At the beginning of each experiment, 1.0 mg/kg of (+)-METH diluted in saline containing 80 µCi of [3H](-)-METH (as a tracer) was injected as a 15-s bolus dose into the jugular venous cannula. The volume of injection was 1.0 ml/kg. At predetermined time points (2, 5, 10, 20, and 40 min; 1, 2, 3, 4, and 5.5 h) after the injection, rats were anesthetized with ethyl ether. A laparotomy was performed and blood was drawn from the inferior vena cava. The animal was decapitated and the brain, heart, left hepatic lobe, left kidney, and spleen were removed rapidly. For early time points (2–10 min), rats were anesthetized with ethyl ether before drug administration to facilitate removal of blood and tissues on time. Ethyl ether was used because it maintains hemodynamic stability. Three rats were used per time point. Tissues were rinsed with saline, weighed, quick frozen in liquid nitrogen, and stored at −80°C until analysis. All tissues were removed in 3 min or less. The blood was allowed to clot for 1 h at room temperature, after which it was centrifuged to collect serum. The serum was stored at −80°C until analysis.

In a study of (+)-METH and (+)-AMP steady-state tissue distribution after a (+)-METH infusion, three rats were implanted with s.c. osmotic minipumps (Alza Corporation, Palo Alto, CA), which deliver a constant flow rate over 24 h (9.2 µl/h). The rats were anesthetized with ether and, after shaving and ethanol skin preparation, an incision (2 cm) was made across the lower back. The underlying tissues were dissected bluntly to the fascia overlying the back muscles, and a pocket was formed with blunt dissection cephalad from the incision. The loaded pump was inserted into the pocket, and the incision closed. Rats received a total dose of 1.0 mg/kg of (+)-METH diluted in sterile saline containing 80 µCi of [3H](-)-METH over a 20-h period. Blood and tissues were harvested 20 h after pump implantation as described above. Because the t1/2 z (+)-METH is 63 min (Rivière et al., 1999), the animals would have achieved steady state at 4–7 t1/2 z (i.e., 4–7 h).

Tissue Analysis. For extraction of (+)-METH and (+)-AMP, tissues were homogenized after addition of 4 volumes (v/v) of ice-cold water with a tissue homogenizer (Tekmar Company, Cincinnati, OH). (+)-METH and (+)-AMP were extracted from the tissue homogenates with a two-step procedure, which consisted of an extraction step with hexane, and a back extraction step with HCl (0.1 N) (Rivière et al., 1999). (+)-METH and (+)-AMP concentrations were then determined with the HPLC procedure described previously (Rivière et al., 1999). (+)-METH and (+)-AMP tissue concentrations were corrected for blood content with the following equation: C TISSUE = [C TISSUE (C B × V T ) + (1 − V B)] where C TOT is the (+)-METH or (+)-AMP total tissue concentration, C TISSUE is the concentration of (+)-METH or (+)-AMP determined in tissue, C B is the concentration in blood [(+)-METH and (+)-AMP are equally distributed in blood and serum], and V B is the volume fraction of blood contained in each tissue (Tripplet et al., 1985).

Pharmacokinetic Analysis. The tissue average concentration versus time profiles for (+)-METH and (+)-AMP were analyzed with model-independent pharmacokinetic methods. Area under the serum concentration versus time curve (AUC) values were calculated for the averaged data with the log-trapezoidal rule from time zero to the last experimental data point. AUC values were then extrapolated from the last experimental data point to infinity by calculating C TOT X = [C TISSUE (C B × V T ) + (1 − V B)] where C TOT = C TISSUE (C B × V T ) + (1 − V B) and Z is the terminal rate constant. The terminal half-life was estimated with data from the terminal elimination phase for each animal [from 1 to 5.5 h and from 3 to 5.5 h after the injection for (+)-METH and (+)-AMP concentration-time curves, respectively]. In addition, the brain and serum average concentration versus time profiles for (+)-METH were analyzed with model-dependent methods. The nonlinear least-squares fitting routine of the software WinNonlin (Scientific Consulting, Inc., Cary, NC) was used for the model-dependent analysis. Two- and three-compartment models were fit to the averaged concentration versus time data with 1/y or 1/y2 weighting, where y is the predicted concentration. The best fit line was selected with visual inspection of the line, analysis of the residuals and a statistical F test for selecting between the alternative equations (Boxenbaum et al., 1974).

Results

General Experimental Strategy. The 1.0 mg/kg i.v. bolus dose of (+)-METH used in this study was chosen based on our previous study of (+)-METH dose-response relationships (Rivière et al., 1999). This previous study of rat spontaneous motor activity and serum pharmacokinetics of (+)-METH...
and its metabolite (+)-AMP showed that effects lasted 2 to 3 h and the pharmacokinetics of (+)-METH was not different following a 10-fold range of doses (0.1–1.0 mg/kg) (Rivière et al., 1999). In addition, this 1.0-mg/kg dose was chosen because it is in the range of doses that are reportedly used by humans on a milligram per kilogram basis (Cho, 1990; Beebe and Walley, 1995). Conversely, the dose used for the s.c. infusions (i.e., 1.2 mg/kg/day) was chosen because it does not produce pharmacological effects. This allowed us to compare (+)-METH and (+)-AMP tissue partitioning at steady-state concentrations following s.c. doses with the tissue partitioning after i.v. doses. We were particularly interested in the time course of tissue partitioning during and after the period of pharmacological activity.

To more accurately determine brain tissue concentrations, we first measured whole-brain concentrations and then subtracted the amount of drug in the blood content in the brain based on inferior vena cava venous concentrations. We used this approach in part because it is known that many rapidly acting drugs exhibit a high arterial-to-venous concentration gradient especially during the first few minutes after i.v. administration. For instance, Tucker and Boas (1971) noted a 10:1 peak in arterial versus venous concentrations for lidocaine immediately after i.v. injection in humans, and Gourlay and Benowitz (1997) noted a temporary 2:1 peak for nicotine at the end of a 30-min i.v. infusion in humans. However, in both studies the arterial-to-venous differential decreased quickly over the next 10 min. Because the volume percentage of blood in the brain is so small in the human and the rat (4 and 3%, respectively; Birnbaum et al., 1994) compared with the total volume of brain tissue, temporarily elevated drug concentrations in the arterial blood are unlikely to contribute to the significantly elevated brain/serum ratios found in this study. Indeed, we calculated that if concentrations of blood in the brain were actually 10 times greater than our measured venous concentrations, this would only result in a 10% decrease in our calculated brain concentrations.

**Pharmacokinetics of (+)-METH and (+)-AMP in Serum after i.v. Dosing.** The time course of (+)-METH concentrations in serum demonstrated a biexponential decline that was best described by a two-compartment pharmacokinetic model with 1/2 weighting (Fig. 1). The fitted curves were used to estimate the distribution half-life values (1/2 values) of (+)-METH in the serum (and brain), to help determine the starting point of the terminal elimination phase, and to check the accuracy of the model-independent pharmacokinetic analysis in serum. The calculated pharmacokinetic values determined by model-independent and model-dependent methods did not vary by >10% (data not shown). Based on visual inspection of the graphs of the concentration-time data for (+)-METH and (+)-AMP, we observed that the 3-h time point appeared to be consistently above the general trend of the exponential decay of the concentration-time data in all tissues. In addition, when we compared this trend with our previous pharmacokinetic studies in serum (Rivière et al., 1999), we did not see aberrant data at this time point to suggest a reason (e.g., physiological) for an increase in concentration at ~3 h. Thus, we considered the data from the 3-h time point in all tissues to be outliers and did not include them in our pharmacokinetic analysis. We did include this time point in our graphs for completeness (Fig. 1).

The serum (+)-METH pharmacokinetic parameters calculated in this study were determined with model-independent analysis of averaged concentration versus time data from three animals at each time point. Consequently, it was not surprising that the serum pharmacokinetic parameters were somewhat different from our previous study in which serum pharmacokinetic values were determined from multiple samples in individual animals with the same dose (Rivière et al., 1999). Data from the previous study showed the Vdss was 9.0 l/kg (versus 4.9 l/kg in the current study), the Clf was 126 ml/min/kg (versus 76.7 ml/min/kg), and the t1/2Z was 63.0 min (versus 54.2 min). Because the analytical methods were the same and many aspects of the experimental protocol were similar between the two studies, we do not think the differences were due to analytical errors. The differences more likely resulted from the use of averaged serum concentration-time data to determine pharmacokinetic parameters in the current study compared with the use of multiple serum samples from individual animals to calculate pharmacokinetic values in the previous study. We observed similar pharmacokinetic differences when we analyzed averaged serum concentrations from groups of animals versus multiple serum samples from individual animals in a previous study of phencyclidine (i.e., the Vdss and Clf were lower with averaged serum concentration-time data; Valentine et al., 1994; Valentine and Owens, 1996). Although we think multiple samples from an individual animal yield the most accurate re-
sults for serum pharmacokinetics, this approach cannot be used when quantifying tissue (i.e., organ) pharmacokinetics.

The time course of (+)-AMP concentrations in serum showed that the highest concentration of (+)-AMP was detected at the 20-min time point after the injection of (+)-METH (Fig. 1). The serum \( t_{1/2,z} \) of (+)-AMP was 74.9 min, which appeared to be greater than the 54.2 min \( t_{1/2,z} \) calculated for (+)-METH (Table 1). In our previous serum pharmacokinetic studies of (+)-METH and (+)-AMP in individual animals, the (+)-AMP \( t_{1/2,z} \) also was found to be longer (and statistically different, \( P < .05 \)) than the (+)-METH \( t_{1/2,z} \) following the same dose of (+)-METH used in the current study (Rivière et al., 1999).

**Pharmacokinetics of (+)-METH and (+)-AMP in Brain and Other Tissues after i.v. Dosing.** In all tissues except the spleen, the (+)-METH concentrations were highest at the first measured time point (2 min), and the concentration versus time curves showed biexponential declines. In the spleen, the maximum (+)-METH concentration occurred at the 10-min time point (Fig. 1). As in the other tissues, (+)-AMP concentrations increased to a maximum value at 20 min before decreasing. The \( t_{1/2,z} \) values of (+)-AMP were from 3.8 min longer in the liver to 20.7 min longer in the serum and kidney than the (+)-METH \( t_{1/2,z} \) values (Table 1).

**Pharmacokinetics of (+)-METH and (+)-AMP during s.c. Infusion.** Because we wanted to determine steady-state brain-to-serum partitioning after a chronic infusion of a non-pharmacologically active (+)-METH dose, we chronically infused rats at a rate of 1.2 mg/kg/day for a total dose of 1.0 mg/kg at the time of sacrifice. Although this was the same total dose as used in the acute i.v. administration, when infused over the 20-h period, it did not produce apparently pharmacologically apparent differences.

Based on the \( t_{1/2,z} \) values in serum for (+)-METH (i.e., 54.2 min) and (+)-AMP (i.e., 74.9 min; Table 1), we estimated that steady-state concentrations would be reached by \(-4--9\) h (i.e., \(4-7 t_{1/2,z}\)) after starting the s.c. infusion. However, for convenience, we chose to infuse the animals for a 20-h period. The brain:serum (+)-METH concentration ratio at 20 h was similar to that calculated with concentrations determined in the terminal elimination phase starting at 2 h after the i.v. bolus injection (9.3:1 for s.c. infusion versus 8.4:1 for i.v. bolus). We observed the same results for (+)-AMP during pharmacologically active (+)-METH and (+)-AMP partitioning values were similar to the values during the terminal elimination phase starting at 2 h. However, the absolute magnitude of the value varied depending on the tissues.

**Discussion**

In this study, we characterized the concentration history of (+)-METH and its metabolite (+)-AMP in the brain and other tissues following i.v. bolus administration of (+)-METH (Fig. 1). These data showed that (+)-METH distributes rapidly into the brain after i.v. bolus dosing. Indeed, the high (+)-METH concentrations observed in the brain immediately after (+)-METH administration suggested that there is essentially no hindrance to passage of (+)-METH at the blood-brain barrier. In addition, the (+)-METH brain concentrations were 7 times the serum concentrations from the first measured time point (2 min) through the remainder of the study. The extremely rapid partitioning of (+)-METH into the brain is consistent with its physicochemical properties (Cho, 1990). It is a small (mol. wt. 149.2), lipid-soluble molecule (octanol/water partition coefficient of 2.3; Brodin et al., 1976) that would be expected to distribute extensively and rapidly into high lipid-content tissues (e.g., brain) that have high blood flow-to-tissue volume ratios. Furthermore, the partitioning could be affected by (+)-METH and (+)-AMP binding to active sites in the central nervous system, and by drug transporters at the blood-brain barrier. For instance, P-glycoprotein is an important efflux pump for some drugs and carrier-mediated transport into the brain via a choline transporter has been reported for other lipophilic drugs such as lidocaine and propranolol (Pardridge et al., 1984; Yamazaki et al., 1994).

An important finding of the current study was that the concentration-time profile in the brain was not readily predictable from the concentration-time profile in the serum, or from the physicochemical properties of (+)-METH (Fig. 2). Indeed, if the rate and direction of (+)-METH movement across the blood-brain barrier were only dependent on cerebral blood flow and (+)-METH lipid solubility, the concentration-time courses of (+)-METH in the brain and serum should be parallel. However, we found that the concentration-time courses did not decrease in parallel in the two tissues during the first hour after drug administration. This was primarily because brain concentrations did not decrease as rapidly as serum concentrations for at least 1 h (Fig. 2). This also is demonstrated by the different \( t_{1/2,z} \) values for the brain and serum (11.8 versus 2.3 min, respectively), and by the appar-

**TABLE 1**

Pharmacokinetic parameters of (+)-METH and its metabolite (+)-AMP in tissues after an i.v. bolus injection of (+)-METH (1.0 mg/kg). All parameters were calculated by model-independent analysis.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( t_{1/2,z} )</th>
<th>AUC</th>
<th>AUC/Serum AUC</th>
<th>Molar Ratio of (+)-AMP to (+)-METH AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>METH</td>
<td>AMP</td>
<td>METH</td>
<td>AMP</td>
</tr>
<tr>
<td>Serum</td>
<td>54.2</td>
<td>74.9</td>
<td>205</td>
<td>88</td>
</tr>
<tr>
<td>Brain</td>
<td>53.1</td>
<td>70.7</td>
<td>2085</td>
<td>736</td>
</tr>
<tr>
<td>Heart</td>
<td>54.3</td>
<td>67.8</td>
<td>1033</td>
<td>446</td>
</tr>
<tr>
<td>Liver</td>
<td>65.9</td>
<td>69.7</td>
<td>1928</td>
<td>851</td>
</tr>
<tr>
<td>Kidney</td>
<td>52.6</td>
<td>73.3</td>
<td>7597</td>
<td>2799</td>
</tr>
<tr>
<td>Spleen</td>
<td>56.1</td>
<td>71.4</td>
<td>3054</td>
<td>1120</td>
</tr>
</tbody>
</table>

* The molar ratio of (+)-AMP to (+)-METH AUC was calculated by dividing the nmol/h/g or nmol/h/ml AUC values for (+)-AMP by the nmol/h/g or nmol/h/ml AUC values for (+)-METH.
Fig. 2. Average concentration versus time profiles for (+)-METH in the brain (●) and serum (○) (n = 3 rats per time point) following i.v. dosing. The solid line represents a best-fit line determined by model-dependent analysis. The inset shows the brain-to-serum ratios for METH, which allows a better visual representation of the apparent early (first 2 h) dysequilibrium between the brain and serum METH concentrations. All values are represented as the mean ± S.D. The inset shows the average (+)-METH brain-to-serum concentration ratios.

The consistently elevated (+)-AMP concentrations in all tissues above serum concentrations indicated that, like (+)-METH, (+)-AMP distributes extensively into these tissues. Analysis of the molar ratio of the (+)-AMP to (+)-METH AUC showed that (+)-AMP accounted for 39 to 48% of the (+)-METH AUC (Table 1). This has important implications for the overall pharmacological effects of i.v. (+)-METH because these data suggest that approximately one-third of the molar drug concentration in the tissues is due to (+)-AMP. Because (+)-AMP concentrations increased in tissues at rates similar to the rate of rise of (+)-AMP concentrations in the serum, the (+)-AMP formation rate and not the (+)-AMP
elimination rate appears to be the limiting factor in determining (+)-AMP tissue concentrations. In addition, during the terminal elimination phases, tissue/serum partitioning of (+)-METH and (+)-AMP were similar.

In conclusion, (+)-METH distributes very rapidly to all of the tissues in this study except the spleen. Furthermore, the relationship of (+)-METH and (+)-AMP brain and serum concentrations is complex. It also appears that time-dependent brain-to-serum partitioning of (+)-METH could be related to the time course of behavioral effects. Finally, based on molar amounts of (+)-AMP and (+)-METH in tissues, it appears that (+)-AMP could make a significant contribution to the pharmacological effects after i.v. administration of (+)-METH. Consequently, these data suggest that medical treatments for (+)-METH abuse must consider the effects of both (+)-METH and (+)-AMP.

Acknowledgments

We thank Melinda Gunnell and Yingni Che for their excellent technical assistance.

References


Send reprint requests to: Dr. S. Michael Owens, Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, 4301 W. Markham, Slot 611, Little Rock, AR 72205. E-mail: owenssamuelm@exchange.uams.edu or Dr. W. Brooks Gentry, Department of Anesthesiology, University of Arkansas for Medical Sciences, 4301 W. Markham, Slot 515, Little Rock, AR 72205. E-mail: gentrywilliamb@exchange.uams.edu