ABSTRACT:

These studies examined the effects of a high-affinity anti-(+)-methamphetamine monoclonal antibody (mAb; $K_D = 11 \text{ nM}$) on (+)-methamphetamine ([(+)-METH]) and (+)-amphetamine [(+)-AMP] serum and tissue disposition and serum protein binding following i.v. (+)-METH administration. Male Sprague-Dawley rats were pretreated with a buffer solution (control rats) or with anti-(+)-METH mAb (equimolar in binding sites to the (+)-METH dose). The next day, both groups received a 1 mg/kg i.v. (+)-METH dose. At various time points after (+)-METH administration, rats were sacrificed ($n = 3$ per time point), and serum and tissues were collected. (+)-METH serum protein binding was increased from $-5\%$ in controls to $-88$ to $99\%$ in the mAb-treated rats. The (+)-METH area under the concentration versus time curves from 0 to 4.5 h (AUC$_{0-4.5}$ h) in mAb-treated rats showed an increase of $>6600\%$ for serum and a decrease of $>60\%$ for brain, compared with buffer-treated controls. Differential effects of anti-METH mAb on (+)-METH concentrations were observed in other tissues. For example, in the liver, anti-(+)-METH mAb caused significant increases in (+)-METH concentrations. The AUC$_{0-4.5}$ h for (+)-AMP, a pharmacologically active metabolite, was decreased by $-50\%$ in all tissues examined. These data show that pretreatment with an anti-(+)-METH mAb can significantly alter the disposition of (+)-METH and (+)-AMP in rats. Since the mAb has no significant cross-reactivity with (+)-AMP, the data suggest that the mAb reduced (+)-METH metabolic clearance through high-affinity binding to (+)-METH. Finally, rapidly equilibrating tissues, like the brain, appear to be preferentially protected by the mAb.

Pharmacotherapies for drug abuse treatment and rehabilitation are generally limited to drug receptor agonists and antagonists (Kreek et al., 2002). Although monoclonal antibodies (mAbs$^2$) and antigen binding fragments (Fabs) have been used clinically to reverse toxicity resulting from digoxin and snake venom for some time (Sullivan, 1986), their usefulness for drug abuse treatment is still at the stage of preclinical investigation. In addition to treating drug overdose, investigators are exploring passive immunization as a mechanism to reduce or prevent drug effects and/or toxicity (e.g., Malin et al., 2001; Hardin et al., 2002). Pretreatment with a highly specific anti-drug mAb could potentially block or suppress the effects of the drug, which could aid the patient in discontinuing use of the drug. In addition, mAb pretreatment could prevent or reduce toxicity associated with excessive drug exposure.

There are other potential advantages of mAb treatment over conventional small molecule receptor agonists and antagonists. First, mAbs target the drug rather than the receptor; therefore they should have no intrinsic effect on receptor activity. Second, mAbs have a relatively small volume of distribution (Vd) and do not readily penetrate tissues (Bazin-Redureau et al., 1997). Through high-affinity binding, they are thus able to reverse toxicity by altering disposition of the drug and preventing the drug from reaching its site of action. Finally, mAbs have a relatively long half-life ($>21$ days in humans; Knapp and Colburn, 1990), which would allow long intervals between treatments and thus improve compliance of patients in drug abuse rehabilitation programs.

Previous studies, from our laboratory and others, have examined the use of mAbs in overdose treatment scenarios (i.e., administration of mAb after a high dose of drug). Our laboratory has shown that administration of anti-drug mAb or Fab reverses drug-induced behavioral effects and alters drug pharmacokinetics in rat models of (+)-methamphetamine [(+)-METH] and phencyclidine (PCP) overdose (e.g., Valentine et al., 1996; Proksch et al., 2000; Byrnes-Blake et al., 2003). The mAb “pretreatment” or “protection” model for reduction or prevention of drug effects has not been examined as thoroughly. We have shown that pretreatment with an anti-PCP mAb will substantially reduce behavioral effects resulting from repeated PCP chal-
lenges for at least 2 weeks (Hardin et al., 2002). However, to our knowledge, there are no studies that examine the effect of (passive) anti-drug mAb pretreatment on drug disposition in serum and tissues. Since these anti-drug mAbs act as pharmacokinetic antagonists of drug effects, a full understanding of the time-dependent dispositional changes in multiple tissues is needed to optimize treatment strategies and to better understand the mechanism of action.

The purpose of the current study was to characterize changes in (+)-METH and (+)-AMP pharmacokinetics induced by pretreatment with anti- (+)-METH mAb. For this study, rats were pretreated with a high-affinity anti- (+)-METH mAb (κG = 11 nM). This mAb is highly specific for (+)-METH and has little cross-reactivity with (+)-AMP. The day after mAb treatment, the rats were administered (+)-METH (1 mg/kg i.v.). Serum and tissue concentrations of (+)-METH and its metabolite, (+)-AMP, as well as (+)-METH serum protein binding, were determined.

## Materials and Methods

### Drugs and Reagents.

(+)-Methamphetamine HCl and (+)-amphetamine sulfate were obtained from the National Institute on Drug Abuse (Rockville, MD). [3H]-(+)-METH [(+)-Z.6'-H]methamphetamine; 23.5 Ci/mmol] was obtained from the Research Triangle Institute (Research Triangle Park, NC), as a gift from the National Institute on Drug Abuse. The tritium labels on the [3H]-(+)-METH are incorporated at two metabolically stable sites. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

### Large-Scale mAb Production and Purification.

Production of the high-affinity (κG = 11 nM) anti- (+)-METH mouse mAb (designated mAb6H4, IgG2 isotype with a κ light chain) has been described previously (Byrnes-Blake et al., 2003). mAb purification was performed using a cation exchange IMEX-100 column (Pharmacia, Peapack, NJ) as described by Hardin et al. (1998).

After purification, anti- (+)-METH mAb was concentrated to ~30 mg/ml and the buffer exchanged to 15 mM sodium phosphate, 150 mM sodium chloride (pH 6.5). The final antibody product was characterized by UV absorbance, SDS-polyacrylamide gel electrophoresis, and with a Limulus Ameboocyte Lysate kit (QCL-1000; Cambrex Corp., East Rutherford, NJ) to determine concentration, ensure purity, and assure the absence of endotoxins, respectively.

Before being administered to animals, the mAb preparation was ultrafiltered (100,000) for 90 min at 4°C to eliminate potential antigenic antibody complexes (Spiegelberg and Weigle, 1967), dilute to the appropriate concentration with vehicle, and then warmed to 37°C.

### Animals.

Male Sprague-Dawley rats, each with a single indwelling jugular venous catheter, were obtained from Hilborn Laboratory Animals, Inc. (Scottsdale, PA). Catheter patency was maintained by a saline flush, followed by 25 units of heparin. The rats were housed separately and their weight was maintained between 270 and 300 g throughout the experiment. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences and were in accordance with the Guide for the Care and Use of Laboratory Animals promulgated by the National Institutes of Health.

(+)-METH and (+)-AMP Pharmacokinetic Experiments. For the mAb-treated group (21 rats; n = 3 per time point), rats were pretreated with 503 mg/kg mAb6H4 [equimolar in binding sites to a 1 mg/kg (+)-METH dose]. The mAb6H4 was administered in a total volume of 8 ml at an infusion rate of 2 ml/min. Immediately after mAb administration, the catheters were flushed with saline to ensure that the mAb remaining in the catheter was injected into the bloodstream. The following day (17 h later), rats received a 15-μl i.v. injection of 1 mg/kg (+)-METH with a [3H]-(+)-METH tracer (333 μCi/kg tracer) via the jugular venous catheter. This dosing time was chosen to allow the mAb to fully distribute before the (+)-METH challenge, and to simulate the use of the therapy as a protective antagonist against (+)-METH effects. The control animals (without mAb) received 8 ml of mAb vehicle followed 17 h later by the i.v. (+)-METH[3H]-METH.

Blood and tissues were collected at predetermined time points (1, 5, 15, and 38 min; 1, 2, 3, and 4.5 h) after (+)-METH administration. This sampling period was chosen because it encompasses the entire pharmacological effect period for the 1 mg/kg (+)-METH dose used in this study (i.e., ~2.5 h of increased locomotor activity; Rivière et al., 1999; Byrnes-Blake et al., 2003).

For the early time points (1 and 5 min), the rats were anesthetized with ethyl ether before (+)-METH injection so that blood and brain tissue could be collected rapidly at the appropriate time. At the later time points, rats were anesthetized with ethyl ether 5 min before the desired time of sacrifice. Ethyl ether was used for maintenance of hemodynamic stability. After laparotomy, blood was collected from the inferior vena cava and the rats were immediately decapitated. The brain, right testis, right liver lobe, kidney, and spleen were harvested, rinsed with saline, weighed, and frozen in liquid nitrogen within 3 min. Whole blood hemocrits were determined for each rat for use in pharmacokinetic calculations. The blood was allowed to clot at room temperature for 1 h and then centrifuged at 230 g for 10 min to obtain serum. All samples were stored at −80°C until analysis.

### Analysis of Drug Concentrations in Serum and Tissue.

Tissues were homogenized in 5 volumes of ice-cold water; (+)-METH and (+)-AMP were extracted from serum and homogenized brain, testis, liver, and spleen tissue samples using a solid-phase extraction procedure as described by Byrnes-Blake et al. (2003). For kidney samples, a liquid-liquid extraction procedure was used. This procedure consisted of mixing 200 μl of kidney homogenate with 200 μl of 1 M NaClO4, A 70:30 (v/v) hexane/ethyl acetate mixture (3.2 ml) was then added. The samples were then incubated on a rotary device for 15 min and centrifuged at 700 g, and the organic layer was transferred to a clean siliconized test tube. The solvent extracts were taken to dryness under nitrogen stream and resuspended in 120 μl of 10% acetonitrile/90% H2O. The extraction recoveries for serum and brain were 84 to 96% and were 72 to 84% for testis, 68 to 92% for liver, 64 to 88% for spleen, and 68 to 80% for kidney. Recoveries were independent of concentration. In addition, the denaturation and precipitation of protein with guanidine and ZnSO4, respectively (Byrnes-Blake et al., 2003), decreases the effect of the mAb on (+)-METH and (+)-AMP recovery. (+)-METH and (+)-AMP dpm recovered were determined by liquid scintillation spectrometry following high-pressure liquid chromatography separation on a Waters Symmetry Shield RP18 column (3.5-μm, 4.5 × 75 mm) using an isocratic 10% acetonitrile/90% H2O mobile phase as previously described (Byrnes-Blake et al., 2003). The serum and tissue drug concentrations were determined by converting the dpm of (+)-METH or (+)-AMP to milligrams, using the ratio of unlabeled methamphetamine to radiolabeled methamphetamine tracer. This value is then corrected for percentage recovery and dilution factor. Tissue drug concentrations were then corrected for blood content in the tissues as described by Valentine and Owens (1996). Briefly, when no monoclonal antibody was present, the whole-blood drug concentration was assumed to be equal to the serum drug concentration, since methamphetamine and amphetamine distribute about equally in red blood cells and serum (Riviere et al., 2000). When the monoclonal antibody was present, the drug was confined mostly to the serum (rather than in the red blood cells) through high-affinity monoclonal antibody binding. The methamphetamine or amphetamine concentration in whole blood for this calculation was determined by multiplying the serum drug concentration by 1 − hematocrit for each animal (Valentine and Owens, 1996).

(+)-METH Serum Protein Binding. (+)-METH serum protein binding in the control and mAb-treated rats was determined by equilibrium dialysis. Aliquots of serum samples (700 μl) from the pharmacokinetic studies were placed in one side of a 2-ml Teflon dialysis cell, divided with a 3500 molecular weight cut-off dialysis membrane. In the other side, 700 μl of 0.13 M sodium phosphate buffer (pH 7.4) was added. The dialysis cells were incubated with constant rotation in a 37°C water bath for 5 h. After incubation, the serum and buffer were removed from each side of the dialysis chamber. The serum samples were then extracted as previously described (Byrnes-Blake et al., 2003) and analyzed by high-pressure liquid chromatography and liquid scintillation spectrometry as described above. The fraction of unbound (+)-METH was calculated by dividing the unbound (+)-METH dpm in the buffer side by the total (+)-METH dpm in the serum side. (+)-AMP protein binding could not be determined due to technical difficulties.

### Pharmacokinetic Analysis.

Pharmacokinetic analyses of (+)-METH and (+)-AMP concentration-time data were conducted by means of both model-dependent and model-independent methods using WinNonlin software (Pharsight, Mountain View, CA). For model-dependent pharmacokinetic analysis, bi- and tri-exponential curves with a first-order input function were fit to the
average concentration-time data sets using a 1/y and 1/y^2 (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 assessment of precision with coefficients of variation for each parameter. Because drug bound to mAb takes on the pharmacokinetic profile of the mAb (Proksch et al., 2000), and the elimination half-life (t1/2,mAb) of mAb in rats is ~8 days (Bazin-Redureau et al., 1997), some tissue pharmacokinetic parameters for (+)-METH and (+)-AMP could not accurately be determined due to the limited time period of sampling. We would have needed to collect samples for 4 to 7 days (e.g., 16–28 days) to accurately estimate t1/2,mAb. Therefore, to make comparisons between control and mAb-treated animals, area under the tissue concentration versus time curve (AUC) was determined from t = 0 [time of (+)-METH administration] to 4.5 h (the last measured time point, AUC4.5 h).

Statistical Analysis. To assess mAb-induced changes in (+)-METH and (+)-AMP serum or tissue concentrations at each time point, a Student’s two-tailed t test was used. All t tests were conducted with SigmaStat V1.0 software (SPSS Inc., Chicago, IL), and a significance level of p < 0.05 was used for these tests. The Bailer method was used for calculating AUC4.5 h and its standard error, based on the experimental data (Nedelman et al., 1995). Additionally, the t test prescribed by the method was conducted to compare control and mAb groups for each of the 12 combinations of tissue and drug. Since 12 tests were done, a Bonferroni significance was declared if the p value was less than 0.002 (0.05/12).

Results

Effect of Anti-(+)-METH mAb on Serum Pharmacokinetics.

The control (no mAb treatment) (+)-METH concentration-time data shown in Fig. 1 have been previously published (Byrnes-Blake et al., 2003); however, the current mAb6H4 studies were carried out in parallel with the control studies. Administration of the anti-(+)-METH mAb, 17 h before (+)-METH administration, led to significantly higher total concentrations of (+)-METH in the serum compared with controls throughout the entire 4.5-h experiment (Fig. 1, upper panel). A small but statistically significant increase in serum (+)-AMP serum concentration at the 2-, 3-, and 4.5-h time points was also observed (Fig. 1, lower panel). The t1/2,mAb values of (+)-METH and (+)-AMP in serum and tissue of control rats were consistent with our previous studies (data not shown; Riviere et al., 2000). The t1/2,mAb of (+)-METH and (+)-AMP in the mAb-treated group could not be accurately estimated due to an insufficient sampling time (as discussed under Materials and Methods). However, even with a limited sampling period, it is clear that the t1/2,mAb of (+)-METH is substantially increased in the mAb-treated group. The value for the serum (+)-METH AUC0.5 h was significantly increased (>6600%) in the presence of mAb, whereas there was no change in serum (+)-AMP AUC0.5 h when compared with controls (Table 1).

Serum protein binding estimates revealed that pretreatment with mAb6H4 led to significantly increased serum protein binding compared with controls (Fig. 2, upper panel). In the controls, (+)-METH binding remained constant and very low over time (~1–10%). In the mAb-treated rats, (+)-METH binding increased from 88% to 99% bound over the first three time points, and then remained constant at high levels. Free concentrations of (+)-METH (determined by multiplying average fraction unbound by total serum concentrations) in mAb-treated rats were not dramatically different from those in control rats, with significant decreases only at the 15- and 38-min time points (Fig. 2, lower panel). Although we did not have a complete dataset for the protein binding, the free (+)-METH AUC0.5 h in mAb-treated rats was estimated with the assumption that the percentage bound at 270 min was the same as 180 min. With this assumption, the free (+)-METH AUC0.5 h was estimated to be 241 ng · hr/ml, which was not significantly different from the control (+)-METH AUC0.5 h. It is important to note that the serum protein binding data were derived under equilibrium conditions in vitro and may not accurately reflect serum protein binding in vivo. Thus, the bound and unbound serum (+)-METH concentrations are only an estimation of in vivo values.

Effect of Anti-(+)-METH mAb on Tissue (+)-METH and (+)-AMP Disposition. The significant increase in (+)-METH serum concentrations was accompanied by a significant decrease in (+)-METH brain concentrations (Fig. 3, upper panel). The mAb also led to significantly lower (+)-AMP concentrations in the brain compared with controls (Fig. 3, lower panel). In addition, the value for the brain (+)-METH and (+)-AMP AUC0.5 h was significantly decreased in mAb-treated rats compared with controls (Table 1).

Table 1 and Fig. 4 summarize the pharmacokinetics of (+)-METH and (+)-AMP in selected tissues from control and mAb-treated rats. The serum and tissue (+)-METH and (+)-AMP data from control rats are in good agreement with previous studies from our laboratory (Riviere et al., 2000). Like the serum, in the presence of mAbs, liver and testis (+)-METH t1/2,mAb appeared to increase substantially but could not be accurately estimated due to an insufficient sampling time. Similar to serum, liver and testis (+)-METH AUC0.5 h values were increased in mAb-treated rats; however, in testis the increase was not significant. The kidney data were similar to those of the brain; that is, (+)-METH and (+)-AMP AUC0.5 h values were significantly decreased in mAb-treated rats compared with controls. (+)-METH
Thus, even though the mAb has a high affinity for (+)-METH, it did not significantly decrease brain (+)-METH concentrations at the earliest time points (i.e., during the first 15 min; Fig. 3).

Due to the differential effects of the mAbs on (+)-METH and (+)-AMP in serum, tests, and spleen, there were major changes in the molar ratio of AUC_{METH} to AUC_{AMP} (Table 2). In tissues from mAb-pretreated rats in which (+)-METH and (+)-AMP followed the kinetics of a one-compartment model with first-order elimination, there was no effect on the ratio of (+)-METH to (+)-AMP compared with controls. The tissue-to-serum ratio of (+)-METH and (+)-AMP was dramatically altered in tissues from mAb-pretreated rats. In control rats, the ratio of tissue (+)-METH to serum (+)-METH bound to anti- (+)-METH mAb was 1. Nevertheless, at higher (+)-METH doses [in which (+)-METH/mAb is 1. Nevertheless, at higher (+)-METH concentrations and AUC_{0–4.5 h} for (+)-METH administration, and at this time point, (+)-METH brain concentrations began to decrease significantly compared with controls. Although the capacity and K_d value of the mAb are very important considerations for these studies, the K_d value is derived under equilibrium conditions in buffered solutions. It is important to note that the experiments in the current study were not conducted at equilibrium. In reality, drug concentrations are rapidly changing due to mAb binding, along with drug distribution, metabolism, and elimination. Thus, the in vivo association and dissociation with the mAb may be different from those predicted by in vitro studies.

Pretreatment with mAb resulted in >95% binding of (+)-METH in the serum after the first 15 min, which resulted in a 6600% increase in serum total (+)-METH concentrations. However, the estimated free concentration of (+)-METH in the serum did not appear to be greatly affected by mAb. Nevertheless, (+)-METH tissue concentrations and AUC_{0–4.5 h} for (+)-METH were significantly decreased in some tissues (Table 1; Figs. 3 and 4). At this time, we do not have an adequate explanation for these findings; however, we have observed analogous results with phencyclidine and anti-phencyclidine Fab and mAb (Valentine and Owens, 1996; Proksch et al., 2000). Clearly, further studies are needed to understand these findings.

Because (+)-METH bound to anti-(+)-METH mAb takes on the pharmacokinetic profile of the mAb, the half-life of (+)-METH appeared to increase in the mAb-pretreated rats. Thus, the 0- to 4.5-h time period measured is only a fraction of the total AUC (0–infinity) in the mAb-pretreated rats. Therefore, it is possible that as free (+)-METH re-equilibrates in the body, concentrations of (+)-METH in the tissues may rebound over time. However, based on our behavioral studies, which were conducted for 8 h (Byrnes-Blake et al., 2003), we have no reason to believe this occurs when the molar ratio of (+)-METH/mAb is 1. Nevertheless, at higher (+)-METH doses [in which (+)-METH exceeds mAb binding sites], the mAb may result in prolonged exposure to (+)-METH.

The tissue data indicate that there are differential effects of the mAb on tissue (+)-METH concentrations and AUC_{0–4.5 h}. In brain and kidney, pretreatment with mAb decreased (+)-METH AUC_{0–4.5 h} by about 50%, whereas mAb pretreatment decreased spleen METH AUC_{0–4.5 h} by 20% (not significant) and had little effect on testis (+)-METH AUC_{0–4.5 h}. The ratios of (+)-METH tissue AUC_{0–4.5 h} to

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* Significant differences from control values (p < 0.05).
* Values (± S.E.) were calculated using Bailer’s method.
* Control rats were treated with vehicle 17 h before (+)-METH (1 mg/kg).
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AUC_{0–4.5 h} in testis and spleen of mAb-treated rats was not significantly different from the control values; however, the (+)-AMP AUC_{0–4.5 h} for the spleen was significantly decreased.

**Discussion**

This study examined the effects of anti-(+)-METH mAb pretreatment on the pharmacokinetics of (+)-METH. Based on previous studies with an anti-phencyclidine mAb, we hypothesized that the highly specific, high-affinity anti-(+)-METH mAb would have dramatic but differential effects on (+)-METH serum and tissue distribution. We think our hypothesis was confirmed; however, some of the changes in (+)-METH disposition cannot be explained based solely on pharmacokinetic principles, and may be due to the mAb itself.

Although the anti-(+)-METH mAb dramatically decreased brain AUC_{0–4.5 h}; it did not significantly decrease brain (+)-METH concentrations at the earliest time points (i.e., during the first 15 min; Fig. 3). Thus, even though the mAb has a high affinity for (+)-METH, it did not block the initial distribution of (+)-METH into the brain. This suggests that the association rate of (+)-METH with the anti-(+)-METH-mAb was not fast enough to prevent this early penetration of (+)-METH into the brain. The serum protein binding data support this hypothesis (Fig. 2). Maximum serum protein binding was not reached until 15 min after (+)-METH administration, and at this time point, (+)-METH brain concentrations began to decrease significantly compared with controls.

The tissue data indicate that there are differential effects of the mAb on tissue (+)-METH concentrations and AUC_{0–4.5 h}. In brain and kidney, pretreatment with mAb decreased (+)-METH AUC_{0–4.5 h} by about 50%, whereas mAb pretreatment decreased spleen METH AUC_{0–4.5 h} by 20% (not significant) and had little effect on testis (+)-METH AUC_{0–4.5 h}. The ratios of (+)-METH tissue AUC_{0–4.5 h} to

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For the same reason, a few points represent an estimated free (+)-METH serum AUC_{0-5\, h} (values in parentheses in Table 1) also suggest differential effects in the tissues. We believe these differences are partly a function of the tissue-specific distribution properties of (+)-METH under control conditions (without mAb). For example, in control animals, maximum tissue concentrations of (+)-METH were achieved at the earliest (0.5-min) time point in the brain and kidney. However, in testis and spleen, maximum (+)-METH concentrations were not reached until the 38-min time point. This suggests that (+)-METH distribution in brain and kidney is rapid and blood flow-limited, and that testis and spleen (+)-METH are slower to equilibrate and appear to have membrane-limited distribution. Thus, it appears that organs in which (+)-METH distribution is limited only by blood flow “benefit” more from the anti- (+)-METH mAb effects than organs in which (+)-METH distribution is restricted by tissue membrane penetration. In line with this idea, differences in blood clearance by organs due to changes in protein binding may also contribute to these effects. In control animals, protein binding is negligible, but in mAb-treated rats, it is a significant factor. Thus, brain and kidney clearance changes from a nonrestrictive type to a restrictive type of clearance in the presence of the mAb, which leads to reduced organ concentrations. Regardless of the mechanism, the anti- (+)-METH mAb is effective at reducing (+)-METH concentrations in target tissues and at reversing (+)-METH behavioral effects in a rat (+)-METH overdose model (Byrnes-Blake et al., 2003).

These differential effects of the mAb on tissue (+)-METH concentrations and AUC_{0-5\, h} may also involve differential tissue penetration of the mAb itself. Physiology-based pharmacokinetic models of antibody distribution have shown differences in antibody tissue levels (Baxter et al., 1994, 1995). Interestingly, another study of antibody distribution showed that antibody penetration into the brain is lowest compared with other tissues (Hong et al., 1999), and others have described receptor-mediated efflux of antibody molecules from brain to blood (Zhang and Pardridge, 2001; Schlachetzki et al., 2002). These differences in tissue antibody distribution may also contribute to the observed differences of the anti-METH mAb on (+)-METH tissue concentrations. Regardless of the mechanism, our studies are in agreement with previous studies that show differential effects of anti-drug mAb on tissue drug distribution (Pentel et al., 1991; Valentine and Owens, 1996; Ragusi et al., 1998; Proksch et al., 2000).

In contrast to other tissues from mAb-treated rats in which there were decreases or no significant change, the liver (+)-METH AUC...
was increased compared with controls. Because the liver is involved in uptake and clearance of IgG complexes from the circulation (Man- nink and Arend, 1971; Skough et al., 1985; Kosugi et al., 1992), the increase in (+)-METH liver concentration could in part be due to uptake of (+)-METH-mAb complex. The uptake of this bound complex (and subsequent transfer to lysosomes) may prevent or delay metabolism of (+)-METH, resulting in increased liver (+)-METH concentrations. From the pharmacokinetic analysis we know that pretreatment with anti-(+)-METH mAb reduced the (+)-METH Vd from 5.5 l/kg to 0.23 l/kg; thus, the mAb appears to limit the apparent space in which (+)-METH can distribute. Consequently, another factor that could contribute to the increased liver concentration is the decreased Vd of (+)-METH in the presence of mAb, if the liver is part of the central compartment. Other investigators have also reported increased liver concentrations of drugs in rats following treatment with anti-drug mouse mAbs (Pentel et al., 1991; Ragusi et al., 1998).

TABLE 2

Changes in tissue (+)-AMP to (+)-METH AUC molar ratios in control and mAb-treated rats.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Molar Ratio of (+)-AMP to (+)-METH AUC&lt;sub&gt;0-4.5 h&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total serum</td>
<td>0.34</td>
</tr>
<tr>
<td>Unbound serum</td>
<td>0.32</td>
</tr>
<tr>
<td>Brain</td>
<td>0.39</td>
</tr>
<tr>
<td>Liver</td>
<td>0.30</td>
</tr>
<tr>
<td>Testis</td>
<td>0.27</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.31</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.34</td>
</tr>
</tbody>
</table>

<sup>a</sup> The molar ratio of (+)-AMP to (+)-METH AUC was calculated by dividing the nmol·h/g or nmol·h/ml AUC<sub>4.5 h</sub> values.

<sup>b</sup> Control rats were treated with vehicle 17 h before a 1 mg/kg i.v. (+)-METH dose.

<sup>c</sup> Rats were treated with anti-(+)-METH mAb6H4 17 h before a 1 mg/kg i.v. (+)-METH dose.
mAb-treated rats, it seems reasonable that the (+)-AMP serum concentration was not dramatically different. Indeed, the ratio of serum (+)-AMP to free (+)-METH did not change in mAb-treated rats compared with control rats. However, there were significant decreases in (+)-AMP concentration and AUC0–5 h in almost all of the tissues examined (Fig. 4; Table 1). Based on our pharmacokinetic analysis, the apparent Vd for (+)-AMP decreased by 50% in the mAb-treated rats compared with control rats. This decrease in (+)-AMP Vd correlates well with the observed ∼50% decrease in (+)-AMP AUC0–5 h observed in the tissues. Thus, it appears that changes in (+)-METH disposition limit the formation of (+)-AMP. However, the changes in (+)-METH and (+)-AMP concentrations are difficult to reconcile, and further studies will be necessary to elucidate the mechanisms for these effects.

In conclusion, these studies demonstrate that pretreatment with an anti-(+)-METH mAb can dramatically alter the disposition (both distribution and metabolism) of (+)-METH through high-affinity binding in the serum. The alterations in (+)-METH disposition resulted in significant changes in the pharmacokinetics of a pharmacologically active (+)-METH metabolite, (+)-AMP. However, some of the effects could not be explained with pharmacokinetic principles; thus, further studies are necessary to address these issues. The effects of the mAb appeared to be tissue-specific; i.e., more dramatic reductions in (+)-METH concentrations were observed in rapidly equilibrating tissues, such as the brain. Although the anti-(+)-METH mAb significantly decreased brain concentrations and AUC0–5 h, (+)-METH did not appear to associate with the mAb rapidly enough to prevent the initial distribution of (+)-METH into the brain. Thus, this mAb may not be entirely effective in decreasing the initial (+)-METH effects. Nevertheless, the overall decreases in brain concentrations suggest that at the correct dose, the mAb could provide reductions in at least some of the neurotoxic effects of (+)-METH, such as adverse behavioral effects.

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References