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Pharmacodynamic mechanisms of monoclonal antibody-based antagonism of (+)-methamphetamine in rats

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Abstract

Our studies examined pharmacokinetic mechanisms involved in high-affinity (K_d ~ 11 nM) monoclonal antibody-based antagonism of (+)-methamphetamine-induced locomotor effects. Male rats received (+)-methamphetamine (0.3, 1, or 3 mg/kg i.v.) followed 30 min later by saline or anti-(+)-methamphetamine monoclonal antibody. All groups received a constant dose of monoclonal antibody that was equimolar in binding sites to the body burden of a 1 mg/kg i.v. (+)-methamphetamine dose 30 min after administration. The monoclonal antibody antagonized locomotor effects due to 0.3 and 1 mg/kg (+)-methamphetamine. In contrast, monoclonal antibody treatment increased locomotor activity due to 3 mg/kg (+)-methamphetamine. We also investigated the serum and brain pharmacokinetics of (+)-methamphetamine without and with the monoclonal antibody. Rats received (+)-methamphetamine (1 mg/kg i.v.) followed by saline or monoclonal antibody treatment at 30 min. The monoclonal antibody significantly increased serum methamphetamine concentrations and significantly decreased brain methamphetamine concentrations. These data indicate that anti-(+)-methamphetamine monoclonal antibody-induced pharmacodynamics are complex, but are related to time-dependent changes in (+)-methamphetamine brain distribution.

Keywords: Methamphetamine; Monoclonal antibody; Drug abuse; Pharmacokinetics; Behavior

1. Introduction

(+)-Methamphetamine (referred to hereafter as methamphetamine) abuse is a serious medical problem in the United States (Drug Abuse Warning Network, 1999). Excessive use of the drug can cause hyperactivity, agitation, and paranoia, and a psychotic state lasting from days to weeks (Beebe and Walley, 1995). Other complications include hyperthermia, seizures, hypertension, and cardiotoxicity (Derlet and Heischober, 1990; Beebe and Walley, 1995; Albertson et al., 1999). Recent studies suggest that signs of neurotoxicity (e.g., decreased dopamine transporter density) are present in chronic methamphetamine abusers (McCann et al., 1998; Ernst et al., 2000). In chronic methamphetamine users following a brief period of abstinence, the decrease in dopamine transporters correlates with a decrease in cognitive function (Volkow et al., 2001a); however, decreased cognitive deficits persist even when dopamine transporter levels recover (Volkow et al., 2001b).

Because there are no specific pharmacological therapies for excessive methamphetamine use, patients receive supportive care for their symptoms until the drug is eliminated by metabolic and renal pathways (Williams et al., 1973). Emergency care of these patients includes maintenance of ventilation, hydration, and electrolyte balance and control of body temperature (Beebe and Walley, 1995; Albertson et al., 1999). Treatment for seizures, agitation, or hypertensive crises may also be required (Derlet and Heischober, 1990; Beebe and Walley, 1995). Such treatments can aid in

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managing patients' symptoms, but they do not remove the causative agent, thus leaving patients susceptible to potential neurotoxic effects of methamphetamine.

The diversity of methamphetamine's effects in the central nervous system (e.g., Cho, 1990; Seiden et al., 1993; Geyer, 1996) has hindered the development of specific agonists or antagonists for use in the treatment of methamphetamine adverse effects. An alternative therapeutic approach is to treat patients with a high-affinity anti-methamphetamine monoclonal antibody. This antibody treatment could act as a "pharmacokinetic antagonist", potentially reversing methamphetamine effects by favorably altering distribution, metabolism, and elimination of the drug. In the current studies, we used a rat model to examine the ability of a high-affinity anti-methamphetamine monoclonal antibody to reverse the locomotor effects of a range of methamphetamine doses. Finally, we also conducted pharmacokinetic studies to help elucidate the pharmacodynamic mechanisms of antibody-based antagonism of methamphetamine-induced pharmacological effects in rats.

2. Materials and methods

2.1. Drugs and reagents

[^{3}H]-methamphetamine ((+)-[2′,6′-^{3}H(n)]methamphetamine; 23.5 Ci/mmol) labeled at two metabolically stable sites on the aromatic ring structure was obtained from the National Institute on Drug Abuse (Rockville, MD) after synthesis at the Research Triangle Institute (Research Triangle Park, NC). Methamphetamine, (+)- and (−)-amphetamine, (+)- and (−)-3,4-methylenedioxy-methamphetamine, and (+)-norpseudoephedrine were also obtained from the National Institute on Drug Abuse. (−)-Methamphetamine and (+)-norephedrine were obtained from Sigma (St. Louis, MO). All drug doses and concentrations are reported as the free base. All other reagents were purchased from Sigma unless otherwise noted.

2.2. Antigen synthesis

The (+)-P6-methamphetamine hapten (*S*-(+)-4-(5-carboxypentyl)methamphetamine HCl) was conjugated to bovine serum albumin by a two-step carbodiimide procedure (Davis and Preston, 1981). The starting hapten to bovine serum albumin molar ratio was 30:1. A complementary hapten–ovalbumin conjugate was synthesized in a similar manner for use in an enzyme-linked immunosorbent assay (ELISA) for testing antisera and monoclonal antibody production. After synthesis, the resulting drug–protein conjugate was dialyzed against deionized water over 24 h at 4 °C (Spectra/Por dialysis membrane, molecular weight cutoff 12,000–14,000 kDa; Spectrum Medical Industries, Houston, TX). The buffer was exchanged to phosphate-buffered saline (pH 7.5) by further dialysis over 24 h at 4 °C. The conjugates were then passed through a PD-10 Sephadex G-25 column to assure removal of all uncoupled hapten (Amersham Pharmacia Biotech, Piscataway, NJ). All conjugates were stored at −20 °C until needed.

2.3. Generation and characterization of anti-methamphetamine monoclonal antibodies

To generate anti-methamphetamine monoclonal antibodies, female BALB/c mice (Charles River Laboratories, Wilmington, MA) were immunized with 100 μg of the (+)-P6-methamphetamine–bovine serum albumin conjugate emulsified 1:1 (v/v) with TiterMax adjuvant (CytRx, Norcross, GA) and boosted monthly with 50 μg of the antigen. The initial immunization and subsequent boosts were administered in two 40-μl s.c. injections (one near each hindquarter). Blood samples were taken periodically by tail bleed to measure anti-methamphetamine antibody titers by an ELISA utilizing the (+)-P6-methamphetmaine–ovalbumin conjugate. The mouse with the highest anti-methamphetamine serum titer was chosen for monoclonal antibody production. Standard hybridoma technology, as described previously by our laboratory (Valentine et al., 1994), was utilized. Hybridoma cell lines were screened for anti-methamphetamine monoclonal antibody production by ELISA. An anti-methamphetamine secreting hybridoma cell line was chosen on the basis of cell line viability and antibody characteristics. The monoclonal antibody selected for these studies (mAb6H4) was determined to be an immunoglobin G1 with a κ light chain using a mouse-hybridoma isotyping kit (Boehringer Mannheim, Indianapolis, IN).

The cross-reactivity profile of mAb6H4 for methamphetamine and numerous structurally related and unrelated compounds was determined by radioimmunoassay in a manner similar to that described by Owens et al. (1988). An IC_{50} value was determined for each compound after fitting a sigmoidal curve to the data points.

2.4. Large-scale monoclonal antibody production and purification

To produce gram quantities of mAb6H4 for in vivo behavioral and pharmacokinetic studies, the hybridoma cell line was grown in a Cell-Pharm System 2500 hollow-fiber bioreactor (Unisyn Technologies, Hopkinton, MA) similar to the methods described by Valentine et al. (1996). Antibody purification was performed with an INdEX-100 cation exchange column (Amersham Pharmacia Biotech), as previously described by Hardin et al. (1998). The monoclonal antibodies were concentrated and the buffer was exchanged to 15 mM sodium phosphate containing 150 mM sodium chloride (pH 6.5) on a 500-ml stirred cell (Amicon, Beverly, MA) with a 30,000-kDa molecular weight cutoff cellulose membrane (Millipore, Bedford, MA). Final antibody purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Endotoxin units were quantified with a

Limulus Amebocyte Lysate kit (QCL-1000; BioWhittaker, Walkersville, MD) to ensure that they were within physiologically safe levels. The monoclonal antibody solution was stored at −80 °C until needed.

Before administration, the monoclonal antibody solution was ultracentrifuged at 100,000 × *g* for 90 min at 4 °C followed by 20-min centrifugation at 3300 × *g* and 4 °C to eliminate large molecular weight antibody complexes, which are known to be highly antigenic (Spiegelberg and Weigle, 1967). Final monoclonal antibody concentration was determined by ultraviolet spectrophotometry. Just prior to administration, the antibody preparations were warmed to 37 °C.

2.5. Animals

Male Sprague–Dawley rats with indwelling jugular vein catheters (Silastic medical-grade tubing, 0.020-in. inner diameter and 0.037-in. outer diameter; Dow Corning, Midland, MI) were obtained from Hilltop Laboratories (Scottsdale, PA). Catheter patency was maintained with a 0.2-ml saline flush followed by 0.05 ml of saline containing 25 U heparin every other morning. The rats were housed separately and fed each day with three food pellets, which resulted in weights between 270 and 300 g throughout the experiment. All animal experiments were conducted with the approval of 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* as adopted and promulgated by the National Institutes of Health.

2.6. Protocol for methamphetamine locomotor activity studies

Rats were habituated to the behavioral monitoring chambers before the start of the experimental protocol. This was accomplished by placing the rats in the chambers for a minimum of 3 h a day for 4–6 consecutive days. After the habituation phase, the rats were randomly divided into three methamphetamine dosing groups ($n = 6$ per group). Methamphetamine doses of 0.3, 1, and 3 mg/kg were chosen because they produce dose-dependent and reproducible increases in distance traveled and rearing, compared with saline treatment (Rivière et al., 1999). Use of higher doses was avoided because preliminary studies showed that a single i.v. dose of 5.6 mg/kg caused self-mutilation in drug-naïve rats and 10 mg/kg doses were sometimes lethal. The rats in each group were dosed every 3 days (days 1, 4, 7 and 10) throughout the experiment. All saline and methamphetamine injections were administered via the jugular catheter as 15 s i.v. infusions.

Changes in spontaneous locomotor activity were used as a measure of methamphetamine-induced behavioral effects. The parameters—distance traveled (in centimeters or meters), number of rearing events, and time spent moving (in seconds)—were individually quantified for each animal. The general protocol used to quantify these parameters was previously developed and validated in our laboratory (Hardin et al., 1998; Rivière et al., 1999). Briefly, animals were placed in polyethylene chambers (United States Plastic, Lima, OH) that contained a bedding of dark-gray clay. Animal behavior was videotaped, and the video images were digitized and quantitated in 4-min intervals using EthoVision software (Noldus Information Technology, Sterling, VA). The duration of drug action was calculated for each parameter starting at 30 min (time of treatment) until the locomotor activity had returned to baseline levels. Locomotor activity was considered to have returned to baseline when two consecutive 4-min intervals were less than or equal the mean + 1 S.D. of the 30-min behavioral baseline data observed just prior to drug administration.

For (+)-METH administration, we established conditions for multiple administration of (+)-METH (both dose and frequency of administration) that would lead to a stable behavioral baseline for reproducible results. Because of the behavioral sensitization known to occur after repeated amphetamine treatments (Robinson and Becker, 1986), two methamphetamine sessions were conducted prior to initiation of the experiment. The second pretreatment drug dose was used to determine the methamphetamine-induced locomotor activity baseline. On day 1, all groups received saline followed 30 min later by monoclonal antibody buffer (15 mM sodium phosphate, 150 mM sodium chloride, pH 6.5) to obtain baseline (non-drug-induced) behavior. Then on days 4 and 7, they received a pretreatment dose of either 0.3, 1.0, or 3.0 mg/kg methamphetamine (depending on dosing group) followed by buffer at 30 min.

The third and final dose of 0.3, 1, or 3 mg/kg methamphetamine was administered on the last day of the protocol. This dose was followed 30 min later by an i.v. injection of anti-methamphetamine monoclonal antibody (mAb6H4). The 30-min time point was chosen for treatment because previous behavioral and pharmacokinetic data from our laboratory have shown that locomotor activity and active metabolite concentrations (amphetamine) in tissues are near maximum at 30 min (Rivière et al., 1999, 2000). The amount of monoclonal antibody administered was 367 mg/kg, which is equimolar (assuming two binding sites per immunoglobin molecule) to the amount of methamphetamine left in the body 30 min after a 1 mg/kg methamphetamine dose. The amount of drug remaining was determined using data from Rivière et al. (1999) and by the equation: body burden = dose × e^{-kt} (Rowland and Tozer, 1995).

All control buffer and monoclonal antibody solutions were administered via the jugular venous catheter in an 8-ml volume at a rate of 2 ml/min. Three days after the end of the experimental protocol for the 1 mg/kg group, locomotor activity from a second saline administration was monitored to assure no changes had occurred in baseline activity.

2.7. Protocol for methamphetamine and amphetamine pharmacokinetic studies

For these studies, a 1 mg/ml methamphetamine dose, along with a 333 μCi/ml radioactive tracer dose of [^{3}H]-methamphetamine, was prepared in sterile saline. This allowed the administration of a 1 mg/kg methamphetamine dose plus a 333 μCi/kg [^{3}H]-methamphetamine dose (approximately 100 μCi per rat) by injection of 1 μl/g of rat body weight.

Rats were randomly divided into two groups. The first group (24 rats; $n = 3$ per time point) did not receive antibody (control group). Rats in this group were administered a 15 s i.v. injection of the methamphetamine/[^{3}H]-methamphetamine solution via the jugular venous catheter and were then placed in metabolism cages (Nalge Nunc International, Rochester, NY). At various predetermined times after injection the rats were sacrificed. For the early time points (1 and 5 min), the rats were anesthetized before drug injection so that an immediate laparotomy could be performed to obtain blood from the inferior vena cava, and samples could be collected at the appropriate time. At later time points (15 min onward), rats were anesthetized 5 min before the desired time of sacrifice. Ethyl ether was used for anesthesia to maintain hemodynamic stability. The rats were then decapitated and the brain was removed, rinsed with saline, weighed, and placed in liquid nitrogen within 3 min of decapitation. Hematocrit values were obtained for each animal. The serum and all collected tissues were stored at − 80 °C until needed for analysis.

The second group (15 rats; $n = 3$ per time point) was the monoclonal antibody-treatment group. All aspects of the experiment were as described for the control group, with the following exception. At 30 min following the methamphetamine dose, each rat was administered 367 mg/kg of the monoclonal antibody (equimolar to the body burden of methamphetamine in the rat at 30 min). The monoclonal antibody was given via the jugular venous catheter in an 8-ml volume at a rate of 2 ml/min. Because the experimental protocol was the same up until the monoclonal antibody was administered, the early time points (before 30 min) were not repeated in this group. The time points collected in this group were 38 min, 1, 2, 3, and 4.5 h.

2.8. Analysis of drug concentrations in tissue

Methamphetamine and amphetamine were extracted from serum and brain using a solid-phase extraction procedure. For serum analysis, guanidine hydrochloride was added to each serum sample to denature the proteins and eliminate drug–protein binding. The samples were vortexed and placed on a gentle shaker for 30 min. A 0.025 mg/ml methamphetamine/amphetamine internal standard (120 μl) was added, and each sample mixture was placed directly on solvent-conditioned Oasis HLB extraction cartridges (1 ml, 30 mg; Waters, Milford, MA). The internal standard was used for detection of methamphetamine and amphetamine peaks during high-performance liquid chromatography (HPLC) and to determine extraction efficiency. After sample application, the cartridges were centrifuged and then washed with water. For elution of both methamphetamine and amphetamine, the cartridges were transferred to siliconized test tubes, 1 ml of methanol was added, and 1 min of centrifugation at 230 × g followed. Then 1 ml of methanol/acetic acid (98:2) was added, and another 1-min centrifugation followed.

Brain tissues were homogenized for 30 s in 5 volumes of ice-cold water with a tissue homogenizer (Tekmar, Cincinnati, OH). Brain tissue was extracted essentially as described for serum except a protein precipitation step was included. Following the guanidine HCl step, 300 μl of a 10% zinc sulfate solution was added. The mixture was vortexed, placed on ice for 5 min, and then centrifuged at 12,500 × g for 5 min. Supernatants from the brain samples were applied to conditioned extraction cartridges and then centrifuged at 600 × g for 4 min. The brain pellets were washed with water and centrifuged again at 12,500 × g for 3 min. The supernatants were then added to their respective extraction columns and centrifuged at 600 × g for 4 min. The wash and elution steps were carried out as described for serum.

After elution, the brain and serum extracts were taken to dryness and resuspended in 120 μl of 7% acetonitrile and 93% water (the HPLC starting conditions). A Waters HPLC system, equipped with a pump controller, autoinjector, ultraviolet detector, and Millennium software, was used to separate methamphetamine and amphetamine for quantitation. The column was a Waters Symmetry Shield RP18 (3.5 μm, 4.5 × 75 mm), and the mobile phase was 7% acetonitrile/93% water with 0.1% trifluoroacetic acid. The run was isocratic for 15 min. Fractions (10 s) were collected, and the [^{3}H]-methamphetamine and [^{3}H]-amphetamine fractions were quantified by liquid scintillation spectrometry. The serum and brain drug concentrations were determined from the ratio of unlabeled methamphetamine to radiolabeled methamphetamine tracer, as previously described (Rivière et al., 1999).

2.9. Pharmacokinetic analysis

Brain concentrations were corrected for residual blood content (Valentine and Owens, 1996). When no monoclonal antibody was present, the whole-blood drug concentration was assumed to be equal to the serum drug concentration, as methamphetamine and amphetamine distribute about equally in red blood cells and serum (Rivière et al., 2000). When the monoclonal antibody was present, the drug was confined 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 minus hematocrit for each animal (Valentine and Owens, 1996).

To determine the distribution half-lives of methamphetamine and amphetamine in serum and brain, the average concentration-versus-time curves were analyzed by model-dependent methods using a nonlinear least-squares fitting routine. A curve was fit to the data points using a two- and three-compartment i.v. bolus model, with no weighting, $1/y$, or $1/y^2$ weighting. The best-fit line was chosen by visual inspection and analysis of the residuals. The terminal elimination half-life ($t_{1/2\lambda Z}$) was determined, where possible, from the terminal phase of the average concentration-versus-time profiles for methamphetamine and amphetamine with the use of model-independent analysis. AUCs for serum and brain were determined from 38 min (immediately after monoclonal antibody treatment) to 4.5 h (last measured time point). All pharmacokinetic analyses were performed using WinNonlin V3.0 (Pharsight, Mountain View, CA).

2.10. Statistical analysis

To assess the effect of the presence or absence of the high-affinity monoclonal antibody on methamphetamine-induced distance traveled and rearing events, an analysis of covariance was performed. Both methamphetamine dose and dose-squared of methamphetamine were used as covariates. Unequal coefficients of the covariates were modeled for each level of monoclonal antibody (without and with monoclonal antibody). Square-root transformations of the response variables distance traveled and rearing were used to satisfy the assumptions of normality of residuals and homogeneity of variance. Student's *t*-tests of the least-squared means (adjusted means) were carried out for the comparisons of interest. *P* values were adjusted with Bonferroni's correction when applicable. Analyses were performed with the SAS System V8.0 software (Cary, NC).

To determine whether a change in saline baseline activity occurred after exposure to multiple methamphetamine doses, saline-induced locomotor activity at the start of the 10-day study was compared with saline-induced locomotor activity after three 1 mg/kg methamphetamine doses using a paired Student's *t*-test. To assess monoclonal antibody-induced changes in methamphetamine and amphetamine 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 (Jandel Scientific, San Rafael, CA). A significance level of $P < 0.05$ was used for all statistical analyses.

3. Results

3.1. Anti-methamphetamine monoclonal antibody

Immunization with the (+)-P6-METH–bovine serum albumin conjugate generated a relatively high-affinity murine monoclonal antibody (mAb6H4; $K_d = 11$ nM). The mAb6H4 was highly specific for methamphetamine, having $< 0.1\%$ cross-reactivity with almost all compounds tested (Fig. 1). The one exception was the drug of abuse, methylenedioxymethamphetamine or "ecstasy". The mAb6H4 bound (+)-methylenedioxymethamphetamine with a slightly higher relative affinity than methamphetamine (9 versus 11 nM). The mAb6H4 was also stereospecific, having an approximately 100 times higher relative affinity for the plus forms of methamphetamine and amphetamine than the minus forms of these substances. In addition to the compounds shown in Fig. 1, we also tested (+)- and (-)-methylenedioxyamphetamine, (+)-norpseudoephedrine, L-phenylephrine, (+)-phenylpropanolamine, β-phenylethylamine, and tyramine. There was no significant cross-reactivity with any of these compounds.

3.2. Effect of the anti-methamphetamine mAb6H4 on locomotor activity

These studies determined the ability of a fixed mAb6H4 dose to antagonize the effects of methamphetamine at three doses. Behavioral effects were assessed by quantifying methamphetamine-induced locomotor activity (distance traveled, rearing, and time spent moving). The time-dependent pattern of time spent moving was very similar to the pattern of distance traveled, but it appeared to be a less sensitive measure and is thus not reported. Fig. 2 shows the time course of distance traveled after methamphetamine administration without and with mAb6H4 treatment. Changes in the duration of methamphetamine's

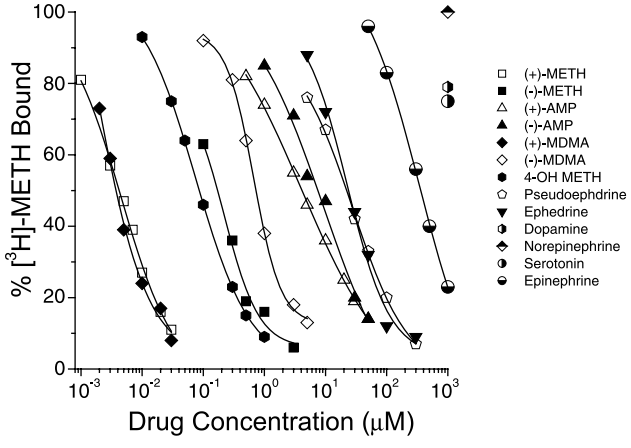

Fig. 1. Radioimmunoassay determination of anti-methamphetamine mAb6H4 cross-reactivity and dissociation constants for amphetamines and related compounds. Sigmoidal dose–response curves were fit to the datasets to allow the determination of the IC_{50} values (concentrations of unlabeled drug that caused a 50% inhibition of [^{3}H]-methamphetamine binding). The final K_d values were corrected for the 4.56 nM contribution of [^{3}H]-methamphetamine to the total methamphetamine concentration. (+)- and (−)-METH, (+)- and (−)-methamphetamine; (+)- and (−)-AMP, (+)- and (−)-amphetamine; (+)- and (−)-MDMA, (+)- and (−)methylenedioxymethamphetamine; 4-OH METH, 4-hydroxymethamphetamine.

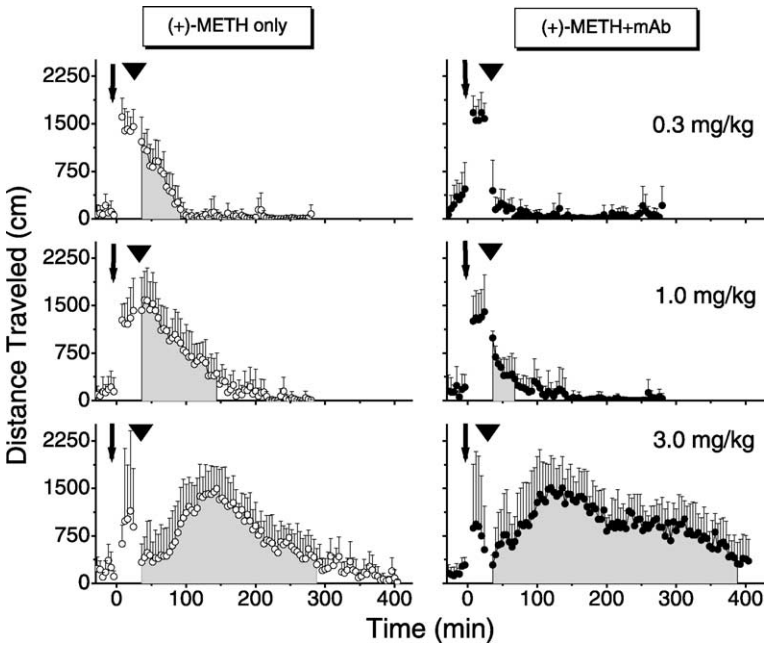

Fig. 2. Time course of methamphetamine-induced distance traveled in rats ($n=6$) with either buffer (left panel; open circles) or mAb6H4 (right panel; filled circles) treatment. The i.v. methamphetamine [(+)-METH] doses were 0.3, 1.0, and 3.0 mg/kg. The left arrow indicates the time of methamphetamine administration, and the arrowhead indicates the time of mAb6H4 administration. Shading indicates the duration of drug action above saline-induced (baseline) locomotor activity following buffer or mAb6H4 administration. The time needed to return to baseline was determined by statistical comparison of the behavior starting at $t=30$ min (time of treatment) with each animal's predosing behavior from -30 min to $t=0$.

action were also evaluated to measure the effects of mAb6H4 (Fig. 2). The duration of methamphetamine-induced locomotor effects following treatment (treatment = buffer versus mAb6H4) was approximately 1 h compared with 6 min for the 0.3 mg/kg dose and 2 h compared with 32 min for the 1 mg/kg dose. When mAb6H4 was administered to the 3 mg/kg group, the duration of drug action increased from 4 to 6 h (Fig. 2). The biphasic nature of the locomotor effects following the 3 mg/kg dose suggests that some stereotypy was present; however, we did not quantify this behavior.

Fig. 3 shows a summary of the methamphetamine-induced distance traveled and rearing events on the final experiment day for each dosing group. Statistical analysis of the locomotor activity showed an antibody-by-dose interaction for the locomotor effects. Thus, we examined the effect of mAb6H4 at each dose of methamphetamine. At the 0.3 and 1 mg/kg methamphetamine doses, the mAb6H4 significantly ($P<0.05$) reduced the locomotor effects (distance traveled and rearing events). In contrast, there was a significant increase in both distance traveled and rearing behavior when the animals received 3 mg/kg of methamphetamine followed by mAb6H4 (Fig. 3). At the end of the experimental protocol, the animals that received the 1 mg/kg doses of methamphetamine also received a second saline treatment followed by buffer. The saline-induced behavior was not significantly different from that obtained at the start of the study ($P<0.05$; data not shown).

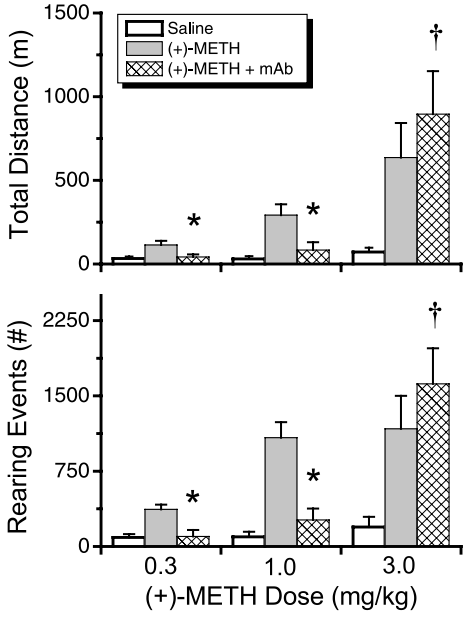

Fig. 3. Effect of anti-methamphetamine mAb6H4 on methamphetamine [(+)-METH] induced locomotor activity and rearing. The rats received saline followed 30 min later by buffer (open bars). Three days after a priming dose, rats received 0.3, 1.0, or 3.0 mg/kg of methamphetamine followed 30 min later by buffer (solid bars). For the final treatment (3 days later), rats received a methamphetamine dose (0.3, 1.0, or 3.0 mg/kg) followed at $t=30$ min by mAb6H4 (cross-hatched bars). These data are shown as the means + 1 S.D. ($n=6$ per group). * indicates a significant decrease in locomotor activity compared with methamphetamine; † indicates a significant increase in locomotor activity compared with methamphetamine ($P<0.05$).

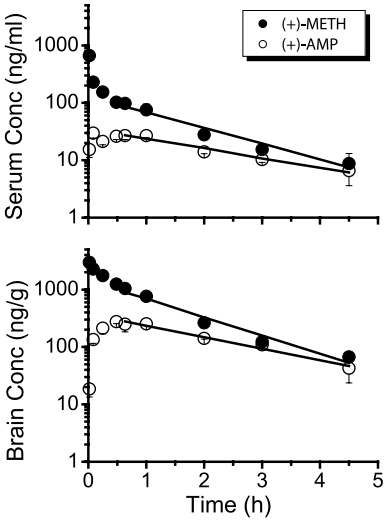

Fig. 4. Average concentration-versus-time profiles for methamphetamine [(+)-METH] and amphetamine [(+)-AMP] in serum (top panel) and brain (lower panel). The solid lines associated with the methamphetamine and amphetamine data show the linear-regression fit to the terminal log concentration-versus-time data as determined by model-independent analysis. All values are represented as the mean ± S.D. ($n = 3$ per time point).

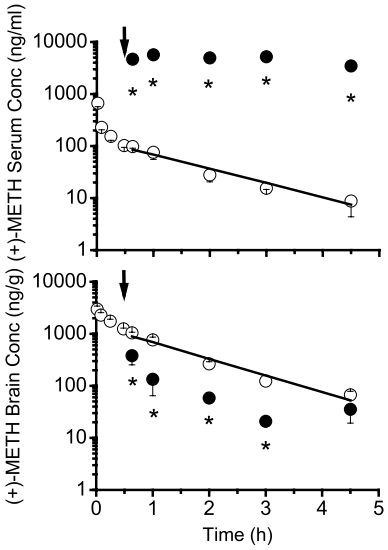

Fig. 5. Average concentration-versus-time profiles for methamphetamine [(+)-METH] with (filled circle) and without (open circle) mAb6H4 administered at $t = 30$ min in serum (top panel) and brain (lower panel). The solid lines associated with the data show the linear-regression fit to the terminal log concentration-versus-time data as determined by model-independent analysis. The arrow indicates the time of mAb6H4 administration. All values are represented as the mean ± S.D., $n = 3$ per time point. * indicates a significant difference from control ($P < 0.05$).

3.3. Pharmacokinetic profile of methamphetamine and amphetamine

The disposition of methamphetamine and its active metabolite, amphetamine, was determined after a 1 mg/kg i.v. methamphetamine dose. The results were similar to those of Rivière et al. (2000). In both serum and brain, the highest methamphetamine concentrations were achieved at the earliest measured time point (1 min) followed by a biexponential decline (Fig. 4). The metabolite amphetamine achieved apparent maximum concentrations in serum and brain at about 30 min (Fig. 4). Table 1 summarizes the pharmacokinetic values for both methamphetamine and amphetamine in both tissues.

3.4. Effect of mAb6H4 on methamphetamine and amphetamine pharmacokinetics

Administration of the high-affinity mAb6H4 30 min after methamphetamine administration led to a substantial change in the disposition of methamphetamine. Serum concentrations of methamphetamine were significantly higher, corresponding to lower concentrations in brain (Fig. 5). It was not possible to determine the complete pharmacokinetic profile for methamphetamine and amphetamine in the presence of the mAb6H4 since the $t_{1/2\lambda Z}$ for a mouse antibody in rats is approximately 8 days (Bazin-Redureau et al., 1997). Thus, to compare methamphetamine disposition without and with

Table 1
Pharmacokinetic parameters of methamphetamine and its metabolite amphetamine after a 1 mg/kg i.v. methamphetamine dose[a]

Tissue	Drug	$t_{1/2\lambda Z}$		$AUC_{38\ min}^{4.5\ h}$		AUC_{brain}/AUC_{serum}		Molar ratio of AMP to METH AUC[b]	
		Control (h)	Treated (h)	Control (ng h/ml or ng h/g)	Treated (ng h/ml or ng h/g)	Control	Treated	Control	Treated
Serum	METH	1.08	NC[c]	123	12,266	–	–	0.49	0.01
	AMP	1.8	NC	55	73	–	–		
Brain	METH	0.95	NC	1182	246	9.6	0.02	0.49	1.26
	AMP	1.5	NC	530	284	9.6	3.4		

[a] Data are shown from animals both without treatment (control) and with treatment (1 mol-equivalent dose of mAb6H4). All parameters were calculated by model-independent analysis.

[b] AMP, amphetamine; METH, methamphetamine. The molar ratio of AMP to METH AUC was calculated by dividing the nmol h/g or nmol h/ml AUC values.

[c] NC, not calculated due to inadequate sampling during the terminal phase.

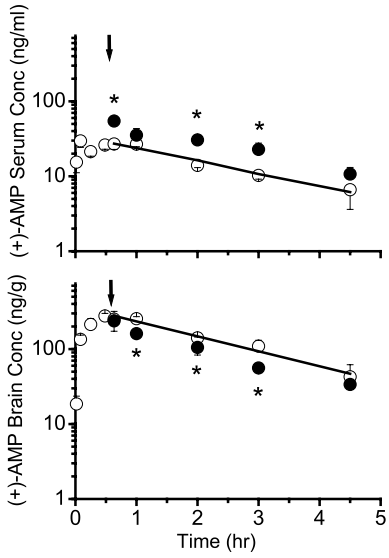

Fig. 6. Average concentration-versus-time profiles for amphetamine [(+)-AMP] with (filled circles) and without (open circles) mAb6H4 administration at $t = 30$ min in serum (top panel) and brain (lower panel). The solid lines associated with the data show the linear-regression fit to the terminal log concentration-versus-time data as determined by model-independent analysis. The arrow indicates the time of mAb6H4 administration. All values are represented as the mean ± S.D. ($n = 3$ per time point). * indicates a significant difference from control ($P < 0.05$).

mAb6H4, we used the $AUC_{38}^{4.5\ h}{}_{min}$. This time frame was chosen to correspond to the behavioral effects period for methamphetamine (see Fig. 2). The $AUC_{38}^{4.5\ h}{}_{min}$ value for the serum methamphetamine concentration-versus-time profile showed a >9000% increase, whereas the $AUC_{38}^{4.5\ h}{}_{min}$ for brain showed a >70% decrease. The methamphetamine AUC_{brain}-to-AUC_{serum} ratio was greatly decreased due to the large increase in serum concentrations (Table 1).

The mAb6H4 had a mild, but significant effect on serum and brain amphetamine concentrations at some of the time points, but the effect was not as large as that seen with methamphetamine (Fig. 6). Because of the mAb6H4's differential effects on methamphetamine and amphetamine, there were major changes in the molar ratio of their AUCs in both serum and brain (Table 1).

4. Discussion

These studies examined the pharmacodynamic mechanisms associated with monoclonal antibody-based antagonism of methamphetamine-induced effects. A fixed dose of the high-affinity methamphetamine antibody mAb6H4 effectively reduced the behavioral effects of methamphetamine when the molar dose was equal to or less than the number of mAb6H4 binding sites (Figs. 3 and 4). The mAb6H4 also substantially decreased the duration of action of methamphetamine at these doses (Fig. 3). However, when the drug dose was greater than the mAb6H4 binding capacity (3 mg/kg methamphetamine dose), methamphetamine-induced locomotor activity appeared to increase compared to a saline control treatment.

To help elucidate the mechanisms of action of the mAb6H4, we conducted pharmacokinetic studies using 1 mg/kg methamphetamine. Administration of mAb6H4 resulted in dramatically increased serum methamphetamine concentrations that remained consistently elevated throughout the duration of the experiment (Fig. 5). The increase in serum methamphetamine concentration was accompanied by an immediate decrease in brain methamphetamine concentration, which was sustained for at least 3 h. This reduction in brain concentration after mAb6H4 was consistent with the immediate reversal of the 1 mg/kg methamphetamine-induced locomotor effects after mAb6H4. However, by 4.5 h, the brain concentration appeared to rebound and was no longer significantly different from that of the control animals. By this time, however, the methamphetamine concentration in control and mAb6H4-treated animals appeared to be well below the threshold concentration associated with increased locomotor activity. We think that the initial decrease in methamphetamine brain concentration following mAb6H4 results from a rapid efflux of methamphetamine from the brain due to changes in distribution equilibrium mediated by high-affinity mAb6H4 binding in the serum. It is difficult to reconcile the increase in brain concentrations seen at 4.5 h without further investigations. However, these data are consistent with a similar dramatic decrease in phencyclidine brain concentrations after treatment with an anti-phencyclidine Fab fragments, which was followed by a rebound in phencyclidine brain concentrations at the later time points (Valentine and Owens, 1996). A possible explanation is that these late-occurring increases in concentration may be due to a re-equilibration of the drug, following a slower redistribution from other tissues.

Treatment with mAb6H4 also increased serum amphetamine concentrations and decreased brain amphetamine concentrations; however, the effect was much smaller than that observed for methamphetamine. This is not surprising because the mAb6H4 had little cross-reactivity with amphetamine in vitro. We think that the small increase in serum amphetamine concentration resulted from increased amounts of methamphetamine in the serum available for metabolism. To investigate this hypothesis, it would be necessary to determine the free concentrations of methamphetamine and its serum clearance. These parameters were not determined in the current study.

The 70% decrease in brain methamphetamine AUC appeared to correlate with the 70% decrease in behavioral effects following mAb6H4 treatment at the 1 mg/kg dose. We think amphetamine formation did not significantly contribute to methamphetamine's pharmacological effects at this dose, based on amphetamine and methamphetamine concentrations in the brain at the time the locomotor effects have subsided (see Figs. 2 and 4). However, at higher doses of methamphetamine, amphetamine would be expected to

reach higher concentrations in the brain, and could contribute to the overall locomotor activity effects. It is worth mentioning that the metabolite amphetamine may complicate design and preclinical testing of mAb6H4 medications. This is because in rats, the serum AUC for amphetamine constitutes about 40% of the serum AUC for methamphetamine (Rivière et al., 2000). In contrast, the same ratio for human serum is only about 15% (Cook et al., 1993). These differences in metabolism must be considered in the prediction of effects in humans based on rat data.

Although the anti-methamphetamine mAb6H4 antagonized locomotor-stimulating actions of the 0.3 and 1 mg/kg methamphetamine doses, the mAb6H4 did not similarly decrease the effects seen after 3 mg/kg. In fact, a modest but significant increase in the locomotor effects of methamphetamine was seen after mAb6H4 administration. This is in contrast to our previous findings where an anti-phencyclidine monoclonal antibody antagonized phencyclidine-induced effects even when the phencyclidine dose greatly exceeded the monoclonal antibody binding capacity (Hardin et al., 2002). While we do not fully understand the reasons for the increased locomotor activity at the highest methamphetamine-to-mAb6H4 ratio, several possible explanations exist. Because of the competitive interaction between methamphetamine-induced locomotor activity and stereotyped behaviors, the locomotor activity could have been maximal at doses between 1 and 3 mg/kg. This is commonly described as an inverted U-shaped dose–response curve. If this were the case, the mAb6H4 (administered at a dose equimolar to a 1 mg/kg methamphetamine dose) would have neutralized only part of the drug dose, thus shifting the dose–response curve back to the point of an apparent increase in locomotor activity. We tested this hypothesis by quantifying locomotor activity in rats after administering a 1.8 mg/kg methamphetamine dose, which was a half-log unit between the 1 and 3 mg/kg doses. These rats received the same dosing regimen as other experimental rats. The 1.8 mg/kg dose produced locomotor effects that were slightly higher, but not significantly different than the effects of the 1 mg/kg methamphetamine dose (data not shown). Thus, the dose effect curve for (+)-METH over the 0.3–3.0 mg/kg dose range for locomotor activity was linear and the increased activity following mAb6H4 treatment in the 3 mg/kg dose group did not appear to be explained by a simple shift to the left in the methamphetamine dose–response curve.

There is also a possible pharmacokinetic explanation for the increase in total locomotor activity. The mAb6H4 appeared to have facilitated the removal of methamphetamine from the central nervous system through high-affinity binding in serum (Fig. 5). This would have led to a decreased amount of methamphetamine in the central nervous system but prolonged availability of the drug due to decreases in clearance. Indeed, our previous studies have shown that the clearance of phencyclidine is significantly reduced in the presence of anti-phencyclidine mAb6H4 (Valentine et al., 1994; Proksch et al., 2000). In addition, the association and dissociation of methamphetamine with the mAb6H4 and its relationship to the influx and efflux of the drug in the central nervous system could also be factors. These factors could have lead to a slower input of drug into the brain for a prolonged duration. In an attempt to address this point, we determined the behavioral effects following a 3 mg/kg i.p. methamphetamine dose and compared these with a 3 mg/kg i.v. dose. We used the i.p. route because it provides a model of a slower drug input into the brain. These data showed that the i.p. route of administration significantly increased the total distance traveled compared with those of the i.v. route (650.7 ± 95.1 versus 460.2 ± 108.5 cm, respectively). While the duration of effects was increased following the i.p. route, the difference was not significant. A study by Cho et al. (1999) has demonstrated that the duration of behavioral effects resulting from a s.c. amphetamine dose was much longer than an i.v. dose, even though similar brain amphetamine concentrations were reached. These data suggest that the rate of drug entry into the central nervous system is an important factor in methamphetamine-induced behaviors. In addition, they support the idea that the mAb may indeed slow the input of methamphetamine while prolonging exposure. However, further experiments are necessary to examine the mechanism for the effects when the mAb:methamphetamine ratio is less than 1.

These studies suggest that passive immunization with a high-affinity anti-drug monoclonal antibody can significantly affect methamphetamine pharmacokinetics and pharmacological effects. Thus, treatment with anti-methamphetamine mAb6H4 may be useful for treatment of human methamphetamine users. However, further studies of the complex mechanisms involved at higher methamphetamine-to-mAb6H4 ratios must be carried out to improve the pharmacological properties of the antibody (e.g., affinity, specificity, and capacity). We think that a mAb6H4 with increased affinity for methamphetamine, and possibly enhanced cross-reactivity with amphetamine (or a cocktail of an anti-methamphetamine and an anti-amphetamine monoclonal antibodies), could offer significant improvements in the effectiveness of the therapy.

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