Pharmacodynamics of a Monoclonal Antiphencyclidine Fab with Broad Selectivity for Phencyclidine-Like Drugs

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ABSTRACT
The development of treatment strategies for drug intoxication has been hindered in part by the lack of clinically useful antagonists. Consequently, the major goal of these studies was to determine whether a monoclonal antibody Fab fragment (of IgG) could be used as an effective drug class-selective antagonist and to understand better the dose-response relationships for reversing CNS drug toxicity. Changes in drug-induced locomotor effects in a rat model were used to assess the ability of the antiphencyclidine (anti-PCP) Fab to reverse the behavioral effects of PCP and other potent arylcyclohexylamines. In experiments to determine the pharmacodynamics of Fab-induced antagonism of behavioral effects, the Fab completely reversed all PCP-induced locomotor effects in a Fab dose-dependent manner with a minimal effective dose of 0.18 mole-equivalents of Fab and an ED50 value of about one-third mole-equivalent. The anti-PCP Fab also completely reversed the locomotor effects induced by two structurally related potent analogs of PCP: 1-[1-(2-thienyl)cyclohexyl]piperidine and N-ethyl-1-phenylcyclohexylamine. In addition, pharmacological and immunological selectivity was further tested by treatment of the behavioral effects induced by the structurally unrelated locomotor stimulant (+)-methamphetamine. The antibody did not effectively reverse the effects of methamphetamine-induced locomotor activity. These results indicate that antibody-based medications can be developed to treat toxicity caused by classes of drugs as well as by individual drugs.

Treatment of substance abuse is a particularly difficult problem, because sites of action are in the CNS and a wide range of structurally related drugs are often abused. For those drugs that act primarily at a single, specific CNS site, it is sometimes possible to use an agonist or antagonist to treat addiction or drug overdose. However, antagonists are not available for most drugs, and the medical use of an agonist or antagonist can potentially disrupt normal CNS homeostasis (Heishman et al., 1989; Howlett and Rees, 1986). To add to the problem, many drugs of abuse produce their effects through multiple mechanisms and binding sites in the CNS. This makes it even more difficult to develop antagonists.

PCP and other arylcyclohexylamines are examples of structurally related drugs of abuse that activate multiple systems in the brain and have no known antagonists. For example, PCP is a noncompetitive antagonist at the NMDA receptor complex (Lodge and Anis, 1982), but it also acts on the dopaminergic (Vignon et al., 1982; Chaudieu et al., 1989) and serotonergic systems (Hori et al., 1996). Whereas some arylcyclohexylamines produce their effects primarily through the NMDA receptor complex (Vignon et al., 1983; Johnson et al., 1988) or the dopamine transporter (Vignon et al., 1988; Maurice et al., 1991), other members of this drug class produce their effects through both of these binding sites, as well as other sites in the CNS (e.g., PCP). Consequently, it would be advantageous to have a medical strategy for treating the multiple effects of these drugs. One such strategy is to develop an antibody-based medication capable of recognizing the common structural features of this broad class of drugs. This medical strategy targets the drugs rather than the receptors.

Previous studies have shown that an anti-PCP monoclonal Fab fragment (the antigen binding fragment of IgG) can remove PCP from the brain (Valentine and Owens, 1996) and can reverse PCP-induced locomotor activity in rats at low doses of PCP (Valentine et al., 1996). In these previous studies, however, the quantity of available antibody was a limiting factor. Consequently, we could not examine the efficacy of the therapy at high PCP doses. In addition, we were unable to test a full range of Fab doses to understand better the pharmacodynamics of less than completely neutralizing antibody-based medications.

ABBREVIATIONS: AUC, area under the behavioral response vs. time curve; Fab, the antigen binding fragment of IgG; mAb, monoclonal antibody; Mes, 2-[N-morpholino]ethanesulfonic acid; mol-eq, mole-equivalents; NMDA, N-methyl-D-aspartate; PCE, N-ethyl-1-phenylcyclohexylamine; PCP, phencyclidine; RIA, radioimmunoassay; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine; [3H]PCP, 1-[1-(phenyl-3,4-3H)]cyclohexyl]piperidine.

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doses. A treatment for PCP abuse in humans is needed, because intoxicated individuals can endanger themselves as well as other persons during periods of drug-induced violent or irrational behavior (McCarron et al., 1981a). PCP use can also cause psychosis, catatonia, an acute brain syndrome or even death (McCarron et al., 1981a; McCarron et al., 1981b; Miller et al., 1988). Furthermore, the behavioral effects of PCP intoxication resemble a schizophrenic psychosis (Rosenbaum et al., 1959; Jentsch et al., 1997) and can last for several days.

The current studies were conducted in a rat model of human behavioral toxicity that allowed extensive examination of the pharmacological properties of an antibody-based therapy for PCP-like drugs. In addition, improvements in our ability to produce large quantities of antibody permitted us to perform more thorough pharmacodynamic studies. The in vitro experiments included structure-activity studies of the antibody using a large series of arylcyclohexylamines and other drugs. The in vivo experiments included determination of the minimal effective Fab dose, the E_{D_{50}} value for anti-PCP Fab inhibition of PCP-induced locomotor effects and the effectiveness of the therapy against several potent arylcyclohexylamines. Finally, these studies also suggest that a single carefully selected mAb can be used to reverse the effects of several structurally related drugs of abuse.

**Materials and Methods**

**Drugs and reagents.** [3H]PCP (50 Ci/mmol) was purchased from New England Nuclear (Boston, MA). The Upjohn Co. (Kalazoo, MI) generously donated Dexoxadrol. Dizocilpine [±MK-501 hydrogen maleate] was obtained from Research Biochemicals International (Natick, MA). All other drugs were obtained from the National Institute on Drug Abuse (Rockville, MD). All drug concentrations were calculated as the free base. All reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

**Large-scale production and purification of mAb Fab fragments.** The procedure for production of the monoclonal anti-PCP IgG (k light chain) from the hybridoma cell line mAb-685 in a Cell-Pharm System II hollow fiber bioreactor (Unisyn Technologies, Inc., Hopkinton, MA) is described elsewhere (Valentine et al., 1996). The IgG was purified at room temperature from the bioreactor tissue culture media (Dulbecco's modified Eagle's medium, Mediatech, Inc., Manville, NJ) packed with 1.0 liter of SP Sepharose Big Beads (Pharmacia Biotech). The unpurified antibody in bioreactor tissue culture media (typically 20–30 g of IgG in 20 liters of tissue culture media) was diluted 1:5 (v/v) in deionized H_{2}O, and the pH was adjusted to 6.0 using concentrated HCl. This solution (approximately 100 liters) was then pumped through the cation exchange media at a flow rate of about 400 ml/min. An in-line absorbance detector (Pharmacia Bio-tech) was connected downstream of the column to monitor the absorbance of the eluent at 280 nm. After the application of the IgG, the column was washed with 50 mM 2-[N-morpholino]ethanesulfonic acid (Mes) buffer (pH 6.0) until the absorbance returned to base line. The IgG was then eluted from the column using 0.15 M NaCl in 50 mM Mes (pH 6.0) buffer at a flow rate of approximately 400 ml/min. The final concentration of anti-PCP IgG was about 7 mg/ml in a volume of 3 to 4 liters.

The anti-PCP Fab was produced from the purified IgG using papa pin digestion as described by McClurkan et al. (1993). After papain digestion, the Fab was concentrated and the buffer was exchanged to 25 mM Mes (pH 7.0) using a concentrator equipped with a spiral membrane ultrafiltration cartridge (Amicon, Beverly, MA). The Fab was purified from undigested IgG and Fc fragments by anion exchange chromatography using DEAE Streamline (Pharma- cia Biotech) and 25 mM Mes (pH 7.0) as the buffer. The purified Fab was concentrated and the buffer was exchanged to sterile saline (pH 7.4). The purity of the anti-PCP Fab was determined by SDS-PAGE, and the concentration of the purified product was determined by spectrophotometry.

The anti-PCP Fab was administered to the rats by an i.v. route in sterile saline at approximately 100 mg/ml. The dose of Fab administered in each experiment was calculated on the basis of stoichiometric moles of Fab. For example, if a 300-g rat received a 3 mg/kg dose of Fab (MW 24,000) and it was treated with a 1.0 mol-equivalent dose of anti-PCP Fab (50 KD), then the anti-PCP Fab dose was 185 mg.

**Characterization of the anti-PCP mAb.** The ligand binding studies of the mAb IgG (in tissue culture media) were conducted in a [3H]PCP RIA similar to the method of Owens et al. (1988). A 100-μl aliquot of [3H]PCP (30,000–40,000 decays per min) in RIA buffer (50 mM Tris adjusted to pH 7.6, 0.15 M NaCl, 0.1% BSA, 0.2% NaNO_{3}) was added to duplicate 50 × 14 mm polypropylene tubes, followed by the appropriate concentration of test ligand. Then a 100-μl aliquot of diluted mAb in tissue culture media was added. The mAb had been titrated in RIA buffer to a concentration that would bind 15% to 20% of the [3H]PCP. To nonspecific binding tubes, 100 μl of tissue culture media was added (at the same dilution as the mAb-containing tubes). The reaction mixture was incubated overnight at 4°C to 8°C after vortex mixing. Next, 1 ml of cold RIA buffer containing 5% goat anti-mouse IgG serum (v/v; Pel-Freez Biologicals, Rogers, AR) and 5% polyethylene glycol 8000 (J.T. Baker Chemical Co., Phillipsburg, NJ) was added. The tubes were incubated for 15 min in the cold, followed by centrifugation at 4°C to 8°C for 15 min at 2000 × g to precipitate the antibody-bound radioactivity. The supernatant fluid was aspirated, and the pellet was resuspended in the same tube using 2 ml of scintillation fluid (Liquiscint, National Diagnostics, Manville, NJ). The tube was placed in a 7-ml scintillation vial, and the concentration of [3H]PCP was determined by liquid scintillation spectrometry.

**Animals.** All animal experiments were carried out 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. Adult male Sprague-Dawley rats were purchased with a single cannula (silastic 0.51 mm I.D. × 0.94 mm O.D.) implanted in their right jugular vein (Hilltop Lab Animals, Scottsdale, PA). For shipping purposes, the cannula was enclosed in a s.c. pocket. The day after arrival from the vendor, the cannulas were externalized, flushed with sterile saline and filled with heparinized saline (500 U/ml). The cannulas were flushed and filled 3 to 4 times a week to maintain patency. All drug and Fab treatments were given i.v. via the cannula. Each animal's food intake was monitored to maintain a constant weight of approximately 300 g. The animals were allowed at least 2 weeks to acclimate to their new environment before treatments. During the acclimation period, we thoroughly conditioned the animals to the experimental environment by handling them and placing them in the testing chambers on multiple occasions.

**Protocols for behavioral experiments.** Each experiment was conducted between 0700 and 1330 h (during the light phase of the light-dark cycle). Experiments were carried out in open-top polypropylene chambers (60 × 45 × 40 cm; I × W × H). Bedding of gray clay gravel was used to provide a nonreflective, contrasting background and a neutral odor. The experiments were recorded with a closed-circuit television camera located above the chambers. The camera was connected to a monitor and a S-VHS recorder. Two animals could be filmed in two separate chambers (one animal in each chamber) at the same time. For experiments, the animals were placed in the chambers 60 min before saline or drug administration. Saline or...
drug was administered at time 0 (zero) for all groups. Saline or anti-PCP Fab treatment was given 30 min after drug administration, except for the first series of experiments, which were designed to determine the dose-response relationship for PCP-induced locomotor effects.

The animals were removed from the chambers for saline, drug or anti-PCP Fab administration. Only one animal was removed from a chamber at a time. Because both animals could be administered treatments in less than 6 min, the time period when the animals were out of the chambers for treatments was not included in the analysis (i.e., the time periods 0–6 min for saline or drug administration and 30–36 min for saline or anti-PCP Fab treatment). Data for behavioral analysis were collected for a total of 4.5 h beginning 30 min before drug administration and ending 4.0 h after saline or drug administration.

Each of the experiments was carried out in a mixed-sequence, repeated-measures design. The animals were randomly assigned to one of four experimental groups. The first series of experiments was designed to determine the dose-response relationships for PCP-induced locomotor effects. In this series of experiments, the animals \((n = 3–4)\) received four treatments: saline, 1.0 mg/kg PCP, 3.0 mg/kg PCP and 6.0 mg/kg PCP. The 6 mg/kg dose of PCP was the highest designed to determine the dose-response relationship for PCP-induced locomotor effects. The animals \((n = 4)\) in this experimental group received seven treatments in a mixed-sequence, repeated-measures design: saline followed by saline, 3.0 mg/kg of PCP followed by saline and 3.0 mg/kg of PCP followed by one of five doses of anti-PCP Fab \((0.1, 0.18, 0.32, 0.56 \text{ and } 1.0 \text{ mol-eq})\). For a 300-g rat, these doses of Fab were approximately 19, 33, 60, 104 and 185 mg, respectively.

The third series of experiments was designed to test the hypothesis that the anti-PCP Fab could be used as an arylocyclohexylamine class-selective antagonist. PCP, TCP and PCE were chosen as prototypic ligands because they are the most potent arylocyclohexylamines in pharmacological assays (table 1), and the anti-PCP Fab binds with high affinity to each of them (table 2). For these experiments, PCP, TCP and PCE were administered as 3.0 mg/kg doses. Each animal in this group \((n = 4)\) received the following seven treatments in a mixed-sequence, repeated-measures design: saline followed by saline, PCP followed by saline, PCP followed by anti-PCP Fab, TCP followed by saline, TCP followed by anti-PCP Fab, PCE followed by saline and PCE followed by anti-PCP Fab. The anti-PCP Fab was administered as a 1.0 mol-eq dose. After a 3 mg/kg dose of PCP (MW 243), TCP (MW 249) or PCE (MW 203), the 1.0 mol-eq dose of anti-PCP Fab for a 300-g rat would be 185, 181 or 222 mg, respectively.

The fourth series of experiments was used as a control to determine whether the anti-PCP Fab was effective against a structurally unrelated stimulant drug. (+) Methamphetamine was chosen for this control because it increases locomotor activity and is a common drug of abuse. The animals in this group \((n = 3)\) received four treatments in a mixed-sequence, repeated-measures design: saline followed by saline, 3.0 mg/kg of PCP followed by saline (for comparison purposes), 1.0 mg/kg of methamphetamine followed by saline and 1.0 mg/kg of methamphetamine followed by a 1.0 mol-eq dose of anti-PCP Fab. Although the animals in these experiments received four treatments, only the data from the methamphetamine treatments were used for statistical comparison.

Behavioral analysis. Analysis of the videotaped experiments was conducted off-line after completion of each experiment using the EthoVision system (Noldus Information Technology, Inc., Sterling, VA). The EthoVision software (versions 1.7–1.8) was run on a personal computer equipped with frame-grabber technology (TARGA +, Release 4.0, Truevision, Inc., Indianapolis, IN) and connected to a S-VHS recorder. A video image-sampling rate of 3.0 samples/s resulted in 48,600 samples during the 4.5-h period of data collection. For each sample, the \(x\) - and \(y\)-coordinates of the animal in the chamber and the size of the animal’s image were recorded. Although several behavioral parameters (such as rearing, meandering, sinuosity and angular velocity) were considered for use during preliminary behavioral studies, the parameters distance traveled and total movement were found to be the most useful measures for arylocyclohexylamine (PCP, TCP and PCE) intoxication in rats. In the experiments with methamphetamine, animal rearing was also used as an indicator of drug effects. The results of all behavioral analyses were reported in 2-min cumulative intervals.

An erosion filter (set at a value of 1) in the EthoVision software was used to increase the ability to discriminate between the animal and inanimate objects (such as feces) in the chamber. The erosion filter was also used to eliminate the animal’s tail from the image. This allowed the center of the rat’s body to be calculated as the center of the object. Preliminary studies showed that the erosion filter increased the accuracy of the video tracking.

Distance traveled was reported as the total distance traveled, in centimeters, for each 2-min time interval. Our preliminary experiments and the studies of PCP in rats by Sams-Dodd (1995, 1996) suggested that a step-down sampling rate of six and a minimal distance-traveled threshold of zero centimeters were an optimal setting for the determination of distance traveled. These criteria were optimized to reduce the effects of body wobble and analytical noise.

The movement parameter was reported as the total time, in seconds, that the animal spent moving during each 2-min interval. The animal was considered to have started moving when it had exceeded a velocity of 15 cm/s and to have stopped moving when its velocity decreased below 5 cm/s. These software specifications were set to ensure that actual locomotion was being measured, and they were confirmed by comparing visual observations with the computer analysis.

Animal rearing was detected by measuring changes in the size of the animal’s body surface image. Because the camera was mounted above the chamber, when the animal stood on all four legs, the size of the image was greater than when the animal was rearing (i.e., standing only on its hind legs). The animal was considered to be rearing when the size of its image for one sample had changed a minimum of 15% from the mean of the previous five samples. Rearing was reported as the number of rearing events per 2-min interval. In preliminary studies, there was no statistical difference between the number of rearing events counted by a manual rater and the number determined by the EthoVision program. In addition, rearing was found to be a useful measure for the effects of methamphetamine intoxication, but arylocyclohexylamines did not produce significant rearing behavior. Therefore, rearing was measured only for the experiments that examined methamphetamine-induced behavior.

Data and statistical analysis. For each of the drugs in table 1, a \(IC_{50}\) value was calculated from a 4 to 5-point standard curve after a logit-log transformation of the RIA data (Rodbard, 1974). Each \(IC_{50}\) value was reported as the average value from two separate determinations. This average \(IC_{50}\) value for each drug was used to calculate its relative potency to PCP.

The 30-min period (–30 to 0 min) before saline or drug administration was used to determine the base-line response for each behavioral experiment. This base-line response was then used as an objective measure for determining the duration of drug-induced immobility (or deep anesthesia) for the higher doses of PCP (3 and 6 mg/kg PCP) and for determining the conclusion of all drug-induced effects at the end of the hyperactive phase. The duration of animal immobility was determined by analysis of the movement parameter (in 2-min intervals) from the time of drug administration until the first two consecutive 2-min intervals that exceeded the mean + 1 S.D. of the base-line response period. In addition, we considered all drug-induced locomotor effects to be over when two consecutive 2-min intervals for the movement parameter were equal to or below normal.
the mean + 1 S.D. of the 30-min base-line response period. Using these criteria, we reported the total duration of drug effects (mean ± S.D.) for the movement parameter. Although the duration of effects was calculated for both distance traveled and movement, we reported only the duration of effects on the movement parameter because the results were similar.

We assessed the overall response after each treatment for each behavioral parameter (distance traveled and movement) by calculating the AUC for a fixed period for each set of experiments. This fixed period was chosen as the longest time needed for all animals to recover from drug-induced effects. For instance, in the experiments to determine the PCP dose-effect curves, we calculated the AUC for all animals from 6 min (the time when the animals were back in the chambers after saline or drug administration) until the time needed for all animals to recover after a 6 mg/kg dose of PCP (the highest dose of PCP). Because all other sets of experiments were used to assess anti-PCP Fab treatment effects, we did not include the first 30 min of behavior (0–30 min) in the AUC calculation. For these experiments, the AUC was calculated from 36 min (the time when both animals had been returned to the chambers after treatments) until the time-point corresponding to the longest duration of drug effects in each experimental group.

The data from the behavioral experiments examining the effectiveness of the anti-PCP Fab against methamphetamine were analyzed using a paired t test (P < .05). The data from all other behavioral experiments were analyzed using a one-way, repeated-measures ANOVA. When the F value was significant (P < .05), a post-hoc pairwise multiple comparison analysis was conducted using a Student-Newman-Keuls test. For the experiments designed to determine the dose-response relationship for the anti-PCP Fab inhibition of PCP-induced locomotor effects, all values were calculated as a percentage of each animal's response to PCP followed by a saline treatment at 30 to 36 min (i.e., 100% response).

To determine the dose-response relationship of PCP-induced locomotor effects, a logistical dose-response equation of the form

\[ y = \frac{(A_1 - A_2)}{1 + \left(\frac{XXy}{p}\right)} + A_2 \]

was fit to the averaged response data (Origin, Microcal Software, Inc., Northampton, MA). \(A_1\) and \(A_2\) are the asymptotic minimum and maximum response values, respectively; \(p\) is the slope parameter for the sigmoidal curve; \(X\) is the X value at the inflection point of the curve. For determination of the dose-response relationship for the anti-PCP Fab inhibition of PCP-induced locomotor effects, a sigmoidal inhibitory dose-response curve was fit to the average data from each type of behavioral response (WinNonlin, Scientific Consultants, Inc., Apex, NC). The equation for this calculation was

\[ \text{Effect} = E_{\text{max}} \times (1 - (R^2/R^* + ED_{50}^2)) \]

where \(E_{\text{max}}\) is the maximum effect after PCP, \(ED_{50}\) is the dose of anti-PCP Fab that produced a 50% inhibition of the maximum effect, \(R\) is response and \(y\) is a shape factor that accommodates the curve.

### Results

**Large-scale production, purification and characterization of the anti-PCP mAb Fab fragments.** The overall recovery of anti-PCP Fab binding sites from the IgG (two Fab binding sites per IgG molecule) in tissue culture media was approximately 80%. The purity of the anti-PCP Fab was approximately 90% as determined by SDS-PAGE and densitometry. In a previous study (McCulrkan et al., 1993), it was determined that the anti-PCP Fab \(K_d\) value (1.8 nM) is unaffected by the purification process and is essentially the same \(K_d\) value as for the native IgG (1.3 nM). Furthermore, we have found no decrease in binding activity or solubility after long-term storage of the IgG or Fab at –80°C.

The high affinity of the antibody for PCP and the RIA binding specificity data suggested that the anti-PCP Fab would be an effective treatment for many of the more potent arylcyclohexylamines (table 1). Therefore, we decided to test the effectiveness of the Fab against three of the most behaviorally potent arylcyclohexylamines (PCP, TCP and PCE). This also provided us with drugs with a 10-fold range of cross-reactivity (affinity) in the RIA. We also decided to test the structurally unrelated locomotor stimulant (+) methamphetamine, which did not cross-react with the antibody.

As pointed out in the introduction, arylcyclohexylamines can also produce effects through other sites of action in the CNS. Nevertheless, we generated our mAb against a unique hapten that was previously shown to contain the pharmacologically active features needed to immunologically mimic arylcyclohexylamine binding to the PCP receptor (Owens et al., 1988). This hapten was 5-[(1-phenylcyclohexyl)aminophenyl]pentanoic acid. In these previous studies, rabbit antibodies were generated against a total of five PCP-like haptons to determine the molecular criteria for an immunological mimic of arylcyclohexylamine binding to the PCP receptor. The anti-5-[(1-phenylcyclohexyl)amino]pentanoic acid antibodies were the only antibodies that could distinguish between...
between pharmacologically active forms of arylcyclohexylamines.

**Dose-response relationship of PCP-induced locomotor effects.** These experiments were conducted to determine whether the behavioral parameters we had chosen (distance traveled and movement) were dose-dependent for PCP. There was a dose-dependent increase in PCP-induced locomotor effects as measured by these parameters (figs. 1 and 2). For this series of experiments, the AUC was calculated from 6 min (the time when the animals were returned to the chambers after saline or drug administration) until 226 min (the longest duration of effects for an animal in this experimental group). The duration of PCP-induced locomotor effects lasted 50.5 ± 8.1 min after the 1 mg/kg dose, 115.3 ± 13.3 min after the 3 mg/kg dose and 184.0 ± 34.0 min after the 6 mg/kg dose. After i.v. administration of the 1 mg/kg PCP dose, the animals became immediately hyperactive. In contrast, the animals were completely immobile immediately after the 3 and 6 mg/kg doses of PCP, except for tremors and head weaving. In a preliminary study, it was determined that this period of immobility was characterized by the inability of the animals to respond to a painful stimulus (results not shown). The painful stimulus was an ear pinch administered every 2 min for 1 h. This informal experiment suggested that the animals were under the anesthetic effects of PCP. This immobility stage lasted 18.7 ± 10.3 min for the 3 mg/kg PCP dose and 36.3 ± 16.3 min for the 6 mg/kg PCP dose. After emerging from the immobility stage, the animals were severely ataxic. As the ataxia decreased, the animals became extremely hyperactive. Eventually, the hyperactivity decreased until the activity returned to base line.

**Fig. 1.** Animal movement vs. time after saline or one of three doses of PCP in a representative animal. These experiments were designed to determine the dose-response relationship of PCP-induced locomotor effects. Movement (the time the animal spent moving during each 2-min interval) was recorded continuously. The animal was placed in the chamber 1 h before drug administration (−60 min). At time zero (0), the animal received an i.v. bolus dose of saline, of 1 mg/kg PCP, of 3 mg/kg PCP or of 6 mg/kg PCP. The time when the animal was removed from the chamber for saline or drug administration (0–6 min) was not plotted. The arrow indicates the time of saline or drug administration. For ease of comparison between doses, the AUC (the longest duration of effects in one of the animals at 6 mg/kg) is shaded. The AUC for each animal after each treatment was used to calculate the PCP dose-response curves shown in figure 2. The behavioral parameter “distance traveled” (not shown) produced similar results.

**Fig. 2.** PCP dose-response curves for total distance traveled (top panel), total movement (middle panel), and duration of effects (bottom panel). These experiments provided the preliminary PCP dose-response data for the validation of our behavioral model of arylcyclohexylamine-induced effects. Values are mean ± S.D. of the AUC for total distance traveled and total movement (see fig. 1). Duration of effects (mean ± S.D.) was calculated on the basis of the time required for the movement to return to base-line levels (“Materials and Methods”). The animals received four treatments in a mixed-sequence, repeated-measures design (n = 4, except for the 3 mg/kg dose, where n = 3). The control (saline) results are represented as the hollow circle, and the PCP treatments are represented as solid circles. A logistical dose-response curve (solid line) was fit to each data set. A one-way, repeated-measures ANOVA followed by a Student-Newman-Keuls test was used to evaluate the difference between treatments (*P < .05 compared with saline control). All doses of PCP were also different from each other.

**Dose-response relationship for the anti-PCP Fab inhibition of PCP-induced locomotor effects.** The goal of these experiments was to determine the dose of anti-PCP Fab required to reverse the effects of PCP-induced locomotor effects. For this series of experiments, the AUC was measured from 36 min (the time when the animals were returned to the
chambers after saline or anti-PCP Fab treatment) until 150 min (the longest PCP-induced effect in the animals). The anti-PCP Fab decreased total distance traveled, total movement and duration of effects in a dose-dependent manner (fig. 3). The minimal effective dose for the anti-PCP Fab to reverse the measured behavioral effects was 0.18 mol-eq ($P < .05$).

**Effectiveness of the anti-PCP Fab against PCP, TCP and PCE.** These experiments were used to determine whether the anti-PCP Fab was an effective treatment for several potent, prototypic members of the arylcyclohexylamine drug class (tables 1 and 2). For these experiments, the AUC was calculated from 36 min until 194 min. As expected, the effects from the three arylcyclohexylamines followed the known pharmacological potencies of these drugs (PCP < TCP < PCE) (table 1; fig. 5). The anti-PCP Fab treatment markedly decreased the locomotor activity induced by PCP, TCP and PCE to levels that were not statistically different from base-line control treatments (saline administration followed by saline treatment) (figs. 4 and 5). The duration of the locomotor effects induced by PCP, TCP and PCE were dramatically decreased by the anti-PCP Fab treatment (table 3).

As previously described for PCP at 3 and 6 mg/kg, a period of immobility followed TCP (3 mg/kg) and PCE (3 mg/kg) administration. This immobility stage after administration of TCP and PCE lasted approximately 30 to 40 min. As figure 4 shows, there was no animal response during the initial periods after drug administration. A precise calculation of the duration of the immobility stage was not possible in these experiments, because the animals were handled during their removal from the chambers for saline or Fab treatments 30 to 36 min after drug administration. When the animals returned to base-line levels of activity after anti-PCP Fab treatment, they quickly exhibited normal behaviors such as calmly sitting in the chamber, grooming and sleeping.

**Effectiveness of the anti-PCP Fab against methamphetamine.** These experiments were conducted to determine the selectivity of the anti-PCP Fab therapy. Methamphetamine was chosen as a control drug, because it belongs to a drug class different from PCP but produces some of the same locomotor effects. The dose of methamphetamine (1 mg/kg) used for these studies produced a period of hyperactivity that was similar in duration to the period of hyperactivity produced by a 3 mg/kg dose of PCP (results not shown). Animal rearing was used as an additional behavioral param-

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**Table 2**

<table>
<thead>
<tr>
<th>Arylcyclohexylamine</th>
<th>Structure</th>
<th>Anti-PCP Fab binding ($K_d$, nM)</th>
<th>PCP receptor binding ($K_d$, nM)</th>
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<tr>
<td>PCP</td>
<td>![PCP structure]</td>
<td>1.8</td>
<td>150</td>
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<tr>
<td>TCP</td>
<td>![TCP structure]</td>
<td>16.4</td>
<td>104</td>
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<tr>
<td>PCE</td>
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<td>91</td>
</tr>
</tbody>
</table>

The $K_d$ value for $[^3H]PCP$ mAb binding is from McClurkan et al. (1993) and was determined by equilibrium dialysis. The $K_d$ values for TCP and PCE were estimated by dividing the $K_d$ for PCP by the relative potency values for each ligand from table 1. The PCP receptor binding $K_d$ value was determined by Scatchard analysis of $[^3H]PCP$ binding in the hippocampus (Zukin and Zukin, 1979). The relative $K_d$ values for TCP and PCE receptor binding were estimated by dividing the $K_d$ for $[^3H]PCP$ by the relative potency values for each ligand from table 1.
eter for this experimental group, because in preliminary experiments it was determined to be a dose-dependent effect of methamphetamine. However, PCP did not produce an increase in rearing behavior (results not shown), as judged by comparing the rearing behavior in animals administered PCP (followed by a saline treatment) and in animals administered saline (followed by a saline treatment).

For these experiments, the AUC was calculated from 36 min until 184 min. Although the effects of anti-PCP Fab on methamphetamine-induced behavior were not statistically significant, there appeared to be small decreases in behavioral effects after anti-PCP Fab treatment. These percentage decreases for total distance traveled, total movement and rearing were 19%, 25% and 20%, respectively (fig. 6).

**Discussion**

A major goal of these studies was to determine whether a mAb could be developed for treating the CNS toxicity due to a chemical class of structurally related drugs, instead of a single drug. To accomplish this goal, we needed to generate a mAb that recognized the pharmacologically active features needed for binding of arylcyclohexylamines to CNS sites of action. For most arylcyclohexylamines, the primary site of action is the PCP recognition site, which is in the ion channel associated with the NMDA receptor complex (Lodge and Anis, 1982; Vignon et al., 1983; Johnson et al., 1988).

To determine whether this mAb recognized the most pharmacologically potent forms of arylcyclohexylamines, we stud-
ied the relative potencies of binding of PCP and other arylcyclohexylamines in a $[^3$H]PCP RIA. In general, the mAb recognized the most pharmacologically potent forms of arylcyclohexylamines (PCP, TCP, PCE, PHP, THP and PCHP) (table 1). However, the actual antibody affinity constants ($K_a$ or $1/K_D$ values) for these compounds were significantly higher (see representative values for PCP, TCP and PCE in table 2) than the actual affinity constant for the CNS receptor. We considered the higher-affinity binding a necessity because the mAb must regenerate these drugs from the CNS. In addition, the mAb showed virtually no recognition for other drugs. These in vitro data suggested that the antibody would be an effective antagonist for most of the potent arylcyclohexylamines, many of which are already Schedule I drugs. These in vitro data suggest that the antibody would be an effective antagonist for most of the potent arylcyclohexylamines, many of which are already Schedule I drugs.

The first series of behavioral experiments determined the dose-response relationship for PCP-induced locomotor effects in a rat model of human behavioral toxicity (figs. 1 and 2). These measures were used because behavioral problems account for some of the major medical problems associated with PCP toxicity in humans (McCarron et al., 1981a; McCarron et al., 1981b) and because PCP effects could be monitored in freely moving animals in a noninvasive manner (Valentine et al., 1996; Sams-Dodd, 1995). After considering these data, we chose a PCP dose of 3 mg/kg for use in all other studies because it produced profound effects with sufficient duration for our purposes. Furthermore, on the basis of the actual PCP serum concentrations in emergency room patients (Walberg et al., 1983) and the average pharmacokinetics values for PCP in humans (Cook et al., 1982a; Cook et al., 1982b), we calculated that 99% of these patients had less than 3 mg/kg in their body. Therefore, a 3 mg/kg dose in the rat provided a good model of the maximal dose of PCP found in patients in an emergency room situation.

To understand better the pharmacological principles of the therapy, we conducted a full dose-response study of anti-PCP Fab reversal of CNS-mediated behavioral effects. We also determined the minimal effective dose of Fab and the ED$_{50}$ value for reversal of effects. As far as we know, this is the first reported full dose-response study of the pharmacodynamic relationship of antibody and drug interactions. The highest dose of anti-PCP Fab administered in these experiments was 1.0 mol-eq to the PCP dose, because in a previous study we showed that a 3.0 mol-eq dose of anti-PCP Fab was no more effective than a 1.0 mol-eq dose (Valentine et al., 1996). The minimal effective dose for the anti-PCP Fab inhibition of PCP-induced locomotor effects ranged from 0.1 to 0.18 mol-eq (fig. 3). The ED$_{50}$ values for the reversal of the behavioral effects ranged from 0.32 to 0.51 mol-eq. These data suggest that reductions in PCP-induced responses are directly related to the dose of anti-PCP Fab, and significant changes in PCP-induced behavioral effects can be achieved with less than 0.2 mol-eq of Fab. It is important to point out that this mAb Fab follows many of the established dose-response relationships for pharmacological antagonists.

To determine whether our anti-PCP Fab would be effective against a broad range of arylcyclohexylamines, we studied three potent, prototypic ligands (PCP, TCP and PCE). The anti-PCP Fab was equally effective at reversing the locomo-

tor effects of PCP, TCP and PCE (table 3 and figs. 4 and 5), although there were significant differences between receptor and antibody affinities for the drugs (table 2). Consequently, both in vitro and in vivo data demonstrated that the anti-

PCP Fab was an effective therapy for toxicity caused by PCP and other related arylcyclohexylamines.

In this and a previous study, we have examined aspects of the in vivo selectivity of the anti-PCP Fab. Although dizocilpine is a more potent NMDA antagonist than PCP, it is structurally unrelated to PCP, and the anti-PCP Fab is not effective at inhibiting dizocilpine-induced locomotor activity (Valentine et al., 1996). In the same study (Valentine et al., 1996), we found that a nonspecific Fab prepared from polyclonal human IgG had no effect on PCP-induced locomotor activity. In the current study, methamphetamine was chosen as an additional control, because it induces locomotor activity but is structurally unrelated to PCP. The antibody did not show a significant cross-reactivity with methamphetamine in the in vitro studies of the mAb specificity (table 1). Furthermore, the anti-PCP Fab did not have a statistically significant effect on methamphetamine-induced behavior (fig. 6). Nevertheless, the anti-PCP Fab appeared to produce a small decrease in methamphetamine-induced effects. Although we do not know the reason for these effects, the increased protein load resulting from administration of the anti-PCP Fab may have produced some nonspecific pharmacokinetic changes. However, the overall effects were negligible compared with the anti-PCP Fab’s reversal of the effects of PCP-like drugs (fig. 5).

We think these studies represent a logical step in our attempts to scale up the development of large-scale production and testing of antibody-based therapy for drug abuse. However, there are advantages and disadvantages that need to be considered for the optimal use of immunotherapeutic agents in humans. On the basis of our calculations, the quantities of antidrug antibodies that will be needed for this type of therapy are significantly greater than for other med-

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**Fig. 6.** The effectiveness of the anti-PCP Fab against (+)methamphetamine as measured by total distance traveled, total movement and rearing. The rats (n = 3) received four treatments in a mixed-sequence, repeated-measures design. The animals received methamphetamine (1 mg/kg) followed 30 min later by saline (hatched bars) or a 1.0 mol-eq dose of anti-PCP Fab (solid bars). Values are mean ± S.D. of the AUC$^{36}_{24}$. A paired t test was used to evaluate the difference between treatments ($^*P < .05$).


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