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 ED$_{50}$ value of about one-third mole-equivalent. The anti-PCP Fab also completely reversed the locomotor effects induced by two other 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 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.

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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 (McCarroll et al., 1981a). PCP use can also cause psychosis, catatonia, an acute brain syndrome or even death (McCarroll 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 arylecyclohexylamines and other drugs. The in vivo experiments included determination of the minimal effective Fab dose, the ED50 value for anti-PCP Fab inhibition of PCP-induced locomotor effects and the effectiveness of the therapy against several potent arylecyclohexylamines. 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. (Kalamazoo, MI) generously donated Dexoxadrol. Dizocilpine [MK-801 hydro- gen maleate] was obtained from Research Biochemical Interna- tional (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 frag- ments. The procedure for production of the monoclonal anti-PCP IgG (k light chain) from the hybridoma cell line mAb-6B5 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, Herndon, VA) supplemented with 10% fetal calf serum (Hyclone Laboratories Inc., Logan, UT) by cation exchange chromatography using an INDEXX-100 column (Pharmacia Biotech, Piscataway, 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 H2O, 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 approximately 400 ml/min. An in-line absorbance detector (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 H2O, 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 approximately 400 ml/min. An in-line absorbance detector (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 H2O, 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 approximately 400 ml/min. An in-line absorbance detector (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 H2O, 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 approximately 400 ml/min. An in-line absorbance detector (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 H2O, 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 approximately 400 ml/min. An in-line absorbance detector.
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 dose used in these experiments, because in preliminary experiments a bolus i.v. dose of 10 mg/kg of PCP killed one of two animals. The other animal exhibited labored breathing, was unable to move for approximately 90 min, and was then hyperactive for several hours.

The second series of experiments was designed to determine the anti-PCP Fab dose-response relationship for inhibition of 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 and 1.0 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 and 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 IC50 value was calculated from a 4 to 5-point standard curve after a logit-log transformation of the RIA data (Rodbard, 1974). Each IC50 value was reported as the average value from two separate determinations. This average IC50 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

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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 (McC lurkan et al., 1993), it was determined that the anti-PCP Fab Kd value (1.8 nM) is unaffected by the purification process and is essentially the same Kd 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 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 (+)-methamphet-amine, 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-[N-(1-phenylcyclohexyl)aminol])pentanoic acid. In these previous studies, rabbit antibodies were generated against a total of five PCP-like haptens to determine the molecular criteria for an immunological mimic of arylcyclohexylamine binding to the PCP receptor. The anti-5-[N-(1-phenylcyclohexyl)amino])pentanoic acid antibodies were the only antibodies that could distinguish between

<table>
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<tr>
<th>Drug</th>
<th>Relative Potency to PCP</th>
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<tr>
<td>PCP</td>
<td>1.0</td>
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<tr>
<td>PCE</td>
<td>1.0</td>
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<tr>
<td>TCP</td>
<td>1.0</td>
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<tr>
<td>THP</td>
<td>0.9</td>
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<tr>
<td>PCDMA</td>
<td>0.3</td>
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<tr>
<td>PCHP</td>
<td>0.3</td>
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<tr>
<td>4MePCP</td>
<td>0.1</td>
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<tr>
<td>PCM</td>
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<tr>
<td>TCP</td>
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<tr>
<td>tPPC</td>
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<tr>
<td>Ketamine</td>
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<tr>
<td>Diclophine</td>
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<tr>
<td>Dextroxdrol</td>
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<tr>
<td>(+)-N-allylnormetazocine</td>
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<td>(-)-N-allylnormetazocine</td>
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The drugs PCP through ketamine are arylcyclohexylamines. Diclophine, dextroxdrol, (+)-N-allylnormetazocine, and (-)-N-allylnormetazocine are noncompetitive NMDA antagonists. (+)-Methamphetamine and morphine are inactive at the NMDA receptor complex. Blank spaces for arylcyclohexylamines indicate that this drug was not studied. Relative potency data for receptor binding and behavioral studies for nonarylcyclohexylamines are not shown. "PCP RIA binding experiments for each drug were performed using the method of Owens et al. (1988). Relative potency to PCP = (RIA IC50 value for PCP)/(RIA IC50 value for test ligand). The relative potency data for the rat [3H]PCP receptor binding data and the rat behavioral discriminative stimulus studies are from Zukin and Zukin (1979) and Shannon (1981), respectively. The rat drug discrimination assay is based on the ability of rats to discriminate PCP-like drugs from a saline injection. Additional abbreviations used in this table are PHP, 1-(1-phenylcyclohexyl)piperidine; THP, 1-(1-[2-(2,2-diphenyl)cyclohexyl)pyrrolidin; PCDMA, N,N-dimethyl-1-phenylcyclohexylamine; PCHP, 1-(1-phenylcyclohexyl)-4-hydroxypiperidine; 4MePCP, 1-(1-phenylcyclohexyl)-4-methylpiperidine; PCM, 1-[1-[2-(2-phenylcyclohexyl)morpholine; TCP, 1-(1-[2-[2-(2,2-diphenyl)cyclohexyl)morpholine; IPP, trans-4-phenyl-4-(1-piperidinyl)cyclohexanol; ketamine, 2-(o-chlorphene- nyl)-2-(methylamino)cyclohexanone; dextroxdrol, d-2-(2,3-diphenyl-1,3-dioxolan-4-yl)piperidine.
tween 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 baseline.

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 baseline 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 baseline 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-

![Fig. 3. Dose-response relationship for the anti-PCP Fab inhibition of PCP-induced locomotor effects. The animals ($n = 4$) received seven treatments in a mixed-sequence, repeated-measures design. PCP was administered at 3 mg/kg in all cases, but the anti-PCP Fab doses were varied as shown on the x-axis. The treatments are represented by the following symbols: saline followed by saline treatment (△), PCP followed by saline treatment (○) and PCP followed by anti-PCP Fab (●). All values were calculated as a percentage of each animal’s response to the PCP followed by saline treatment (i.e., 100% response). Values for the total distance traveled (top panel) and total movement (middle panel) are plotted as the mean ± S.D. of the AUC$^{36-150}$. Values for duration of effects (bottom panel, mean ± S.D.) were calculated on the basis of the time needed for animal movement to return to baseline values. 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 PCP followed by saline treatment). Curves were fit to the data using a sigmoidal inhibitory pharmacodynamic model. The $E_{\text{max}}, E_{50}$, and the $\gamma$ values (upper right corner of each plot) were calculated from the curves.]
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_v 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 redistribute 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 with a high abuse potential. The one exception was ketamine, which was not recognized by the antibody. This lack of recognition was presumably due to interference by the chloride molecule (at the ortho position of the aromatic ring). The other arylcyclohexylamines used in this study do not contain chloride. Ketamine, a dissociative anesthetic agent, is the only arylcyclohexylamine with an approved medical use.

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 behavioral 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 locomotor 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-
ical applications of antibody-based therapy, such as radiolabelling of tumors and delivery of chemotherapeutic agents (Goldenberg, 1993). The biotechnology for producing large quantities of antibodies (such as the bioreactors used in these studies) continues to improve, and we think that in the near future it will be cost-effective to test this type of therapy in humans. Furthermore, the use of Fab fragments (as in the current study) is one way to reduce or prevent an antigenic response. In fact, in patients who receive ovine antidigoxin Fab (Digibind), allergic reactions to the antibody fragments are rare (Hickey et al., 1991; Kirkpatrick, 1991).

Another concern for using antibody-based therapy is the generation of antireceptor antibodies. Our mAb was generated against a hapten that was previously shown to mimic the binding properties of arylocylohexylamines to the PCP recognition site in the NMDA receptor complex (Owens et al., 1988). With repeated or long-term administration of this type of antibody, PCP-like antiparatype antibodies could be generated. If these antiparatype antibodies were to bind to PCP binding sites in the CNS, they could potentially produce drug-like effects. To address this point, we have generated anti-idiotype and antiparatype antibodies against this mAb. However, we have not been able to inhibit "HPCP binding to the PCP receptor with these antibodies (unpublished observations, S.M. Owens). Nevertheless, even if PCP-like antiparatype antibodies were generated in vivo, it is unlikely that these antibodies could cross the blood-brain barrier in sufficient quantities to produce effects.

There are significant potential advantages to developing antibody-based medications for treating the medical problems associated with drug abuse. For instance, it is a common practice for chemists in illicit drug laboratories to modify the chemical structures of popular drugs of abuse to avoid detection and to increase the potency of their drugs (e.g., fentanyl-like compounds). These clandestine drugs have been termed designer drugs. A carefully chosen class-selective mAb would likely be an effective treatment for these new drugs because, by analogy, it is a "designer mAb." Finally, it would be too costly to develop (and for hospitals to purchase) a highly specific antibody-based medication for each individual drug of abuse. Another advantage is that antibodies could potentially be a more effective therapy than a conventional receptor antagonist. This is because a receptor antagonist typically reverses the effects at only one site of action, whereas drugs like PCP have multiple sites of action within the CNS. By significantly increasing the protein binding in the vascular compartment and lowering the volume of distribution of the drug (Owens and Mayersohn, 1986; Valentine et al., 1994; Valentine and Owens, 1996), antibodies act as "pharmacokinetic antagonists" to remove the drug rapidly from the CNS. In other words, the antibodies antagonize the effects of the drug by completely altering the pharmacokinetic properties of the drug. Furthermore, because antibodies do not appear to cross the blood-brain barrier under normal conditions, and because they are usually highly selective for the drug or drug class, antibody therapy should not disrupt normal CNS homeostasis.

In conclusion, these studies demonstrated that a monoclonal Fab was extremely effective at reversing the CNS-mediated behavioral toxicity associated with a major class of abused drugs. These data also indicated that the underlying mechanism for Fab reversal of drug effects follows a predictable pharmacological dose-response relationship. Because PCP pharmacokinetics in humans follows the principles of a first-order process (Cook et al., 1982a; Cook et al., 1982b), these data suggest that the dose of Fab needed for reversal of drug effects could be accurately calculated on the basis of the PCP plasma concentration in patients. Finally, the pharmacological and immunological concepts in these studies could have broad applicability for use in treating adverse effects due to other drug classes.

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