

A Single Dose of Monoclonal Anti-Phencyclidine IgG Offers Long-Term Reductions in Phencyclidine Behavioral Effects in Rats

J. SHANE HARDIN,¹ WILLIAM D. WESSINGER, GALEN R. WENGER, JOEL W. PROKSCH,²
ELIZABETH M. LAURENZANA, and S. MICHAEL OWENS

Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

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ABSTRACT

These studies tested the hypothesis that a single dose of high-affinity anti-phencyclidine monoclonal antibody (anti-PCP mAb) provides long-term protection against behavioral effects of repeated PCP administration in rats. Rats were treated with saline, nonspecific bovine IgG (NS-IgG), or anti-PCP mAb (1.0 g/kg). The next morning, the rats were challenged with escalating i.v. doses of PCP (0.32, 0.56, and 1.0 mg/kg) at 90-min intervals. This regimen was repeated every 3 days for 2 weeks. In the saline and NS-IgG control groups, PCP yielded reproducible and linear dose-dependent effects that remained constant during the experiment. In contrast, the anti-PCP mAb treatment blocked PCP effects on

day 1, and sustained significant ($P < 0.05$) reductions in drug effects for the entire 2-week experiment. Brain PCP concentrations (determined at study termination) were reduced by ~55%, whereas serum concentrations were increased over 4000% compared with controls. Thus, a single dose of antibody medication provided long-term reductions in drug effects and brain concentrations, beyond the expected capacity of the drug-antibody interaction. These data challenge current concepts about in vivo dose dependence and unimolecular interaction between antibody binding sites and small molecules and establish that neuroprotection by mAbs may have an unique mechanism of action.

Drugs of abuse like phencyclidine (PCP) and methamphetamine produce their adverse effects through multiple mechanisms that involve several sites of action in the central nervous system (CNS; Vignon et al., 1982; Chaudieu et al., 1989; Cho, 1990). These complex actions have hindered the development of medications that are based on selective action at single sites in the CNS. To add to the problem, it is extremely difficult to find drug treatments for substance abuse that are not associated with serious side effects (e.g., abuse potential).

As an alternative therapeutic strategy, drug-specific antibodies have been used to target the drug rather than the site(s) of action. The antibody medication acts as a pharmacokinetic antagonist to neutralize the drug effects, along with producing significant changes in drug distribution, metabolism, and elimination. The changes in drug disposition resulting from high-affinity antibody binding and the subse-

quent reductions in brain concentrations provide the major beneficial effects. These immunological treatments are of two types: active immunization with drug-protein conjugates (Fox et al., 1996; Carrera et al., 2000) or passive immunization with laboratory generated antibodies (usually monoclonal; Valentine et al., 1996; Hardin et al., 1998; Carrera et al., 2000).

Passive administration with drug-specific, high-affinity mAb could have important therapeutic advantages over active immunization. First, the pharmacological properties of a mAb medication can be carefully selected and designed for optimal affinity and specificity. Second, the structure and function of mAbs are consistent and uniform from batch to batch, and if human (or humanized) mAb are used for the treatment of human diseases, the possibility of allergic type reactions is greatly reduced or prevented. Third, the dose of antibody can be precisely controlled, and patients can be offered immediate immunological protection against drug effects without waiting weeks or months for a response to an active immunization protocol.

A significant hindrance to the use of mAbs is the theoretically high doses of antibody that would be needed to neutralize or significantly reduce drug effects. This is a particularly serious hurdle for medical situations like drug overdose or binge usage. Indeed, it is often assumed that successful

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¹ Current address: Department of Emergency Medicine, Pitt County Memorial Hospital, Greenville, NC 27835.

² Current address: Molecumetics, 2023 120th Avenue NE, Bellevue, WA 98005.

ABBREVIATIONS: mAb, monoclonal antibody; PCP, phencyclidine; NS-IgG, nonspecific bovine IgG; CNS, central nervous system.

treatment would require an equimolar dose of antibody binding sites to the molar dose of drug. Using this assumption, it is further conjectured that the drug user could also easily surmount the antibody binding capacity by simply increasing the drug dose. However, these mechanistic assumptions are not based on actual *in vivo* experimental data because only limited data are available on the use of mAbs for the treatment of adverse drug effects. Also, most studies have failed to adequately control for drug-dependent factors like pharmacokinetic properties or important antibody-dependent factors like affinity constants.

To address some of these issues, we previously determined that a single dose of anti-PCP mAb ($K_d = 1.3$ nM) offers long-term reductions in PCP brain concentrations in a rat model of extreme PCP usage (18 mg/kg/day for 28 days; Proksch et al., 2000a). The studies showed the mAb produced significant reductions in rat brain PCP concentrations for at least 1 month. These long-term reductions persisted even though the antibody binding capacity was purposely saturated during the 1st day of treatment, and the PCP infusion continued at a rate of 15% replacement of the body burden per hour. These results demonstrate that antibody dose, the apparent antibody binding capacity, and the biological $t_{1/2}$ (i.e., 8 days; Bazin-Redureau et al., 1997) of the mAb are not good predictors of these remarkable effects.

In the current study, we investigated the ability of this same anti-PCP mAb to protect against PCP-induced behavioral effects for a 2-week time period. After a single administration of anti-PCP mAb, rats were challenged every 3 days with escalating *i.v.* doses (0.32, 0.56, and 1.0 mg/kg) of PCP over the course of 2 weeks (i.e., 13 days). This experimental design was used to mimic the extreme conditions of frequent and repeated *i.v.* drug abuse in humans and to assess the time-dependent pharmacological protection by the mAb. Serum and brain PCP concentrations in the presence and absence of anti-PCP mAb were also determined at the end of the 2-week period.

Materials and Methods

Drugs and Reagents. PCP hydrochloride was obtained from the Research Triangle Institute (Research Triangle Park, NC) as a gift from the National Institute on Drug Abuse (Rockville, MD). All drug concentrations were calculated as the free base form. Unless otherwise stated, all reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Large-Scale Production and Purification of Mouse Monoclonal Anti-PCP mAb and Polyclonal Nonspecific Bovine IgG. The production of the anti-PCP mAb (IgG1, κ -light chain, K_d 1.3 nM) from hybridoma cell line mAb-6B5 is described in a previous publication (Valentine et al., 1996). Polyclonal bovine IgG (Pel-Freez Biologicals, Rogers, AR) was used as the nonspecific IgG (NS-IgG) control treatment for these studies. Although the purity of the NS-IgG was reasonably high, it was subjected to the same purification process as the anti-PCP mAb (Hardin et al., 1998) to assure consistency in the protein formulations. After purification, the buffer was exchanged to sterile saline. The purified IgG was then concentrated to about 40 mg/ml (determined by spectrophotometry) using an Amicon ultrafiltration cell (Millipore Corporation, Bedford, MA). At this concentration, the anti-PCP mAb and the NS-IgG were fully soluble. The purity of the anti-PCP mAb and the NS-IgG were both greater than 90% as determined by SDS-PAGE. To decrease the possibility of immune complexes, all antibody solutions were ultracentrifuged at 100,000g for 1 h just before use. This procedure prevents and or

significantly reduces the potential antigenic response when IgG is used across species (Spiegelberg and Weigle, 1967; Sedlacek et al., 1987).

Animals. Adult male Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA) were purchased with a single cannula (silastic 0.020-inch inner diameter \times 0.037-inch outer diameter) implanted in their right jugular vein by the vendor. Maintenance of the cannula was as described by Hardin et al. (1998). During preliminary studies, we determined that we could consistently keep the intravenous cannulae patent for about 5 to 6 weeks past the cannulation procedures.

Over the course of the experiments, the food intake of each rat was adjusted to maintain a body weight of approximately 300 g. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as promulgated and adopted by the National Institutes of Health. All animal protocols were approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee.

Experimental Protocols. Basic procedures and equipment for the behavioral studies have previously been described (Hardin et al., 1998). The animals were habituated to the testing chamber for approximately 1 week until exploratory behavior was minimal. To optimize the experimental design and reproducibility of the studies, extensive preliminary experiments were conducted. During these experiments, we noted a significant variability in the activity of individual rats with and without PCP. However, the rats with the greatest drug-free activity (i.e., normal baseline activity) were always the highest responders to PCP-induced effects and the rats with the lowest drug-free activity were always the lowest responders to PCP-induced effects. Consequently, the experimental protocol was designed to allow each animal to serve as its own control for PCP-induced effects.

To establish pretreatment drug responses for each rat, the animals were challenged with a single dose of PCP on two separate occasions (see Fig. 1 for the overall summary of the experimental design). For each experiment, the rat was placed in the behavioral chamber 60 min before drug administration to allow for stabilization of animal baseline behavioral activity. At time 0 (zero), the animals received an *i.v.* bolus dose of 1.0 mg/kg PCP, and the behavior was recorded for the next 90 min. The time when the animals were out of the chambers for drug administration (0–4 min) was not included in the behavioral analysis. Because preliminary studies showed the locomotor activity after the first dose was significantly ($P < 0.05$) lower than the effects after the second and all subsequent doses, the

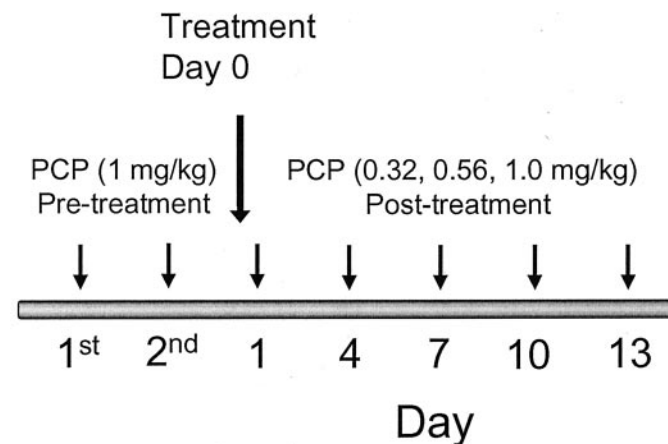


Fig. 1. Timeline for the experiments examining the ability of the anti-PCP mAb to protect against PCP intoxication. Before treatment, the rats were challenged with 1.0 mg/kg PCP on two separate occasions. On day 0 (zero), the rats received the following treatments: saline, 1000 mg/kg NS-IgG, and 1000 mg/kg anti-PCP mAb. Beginning on day 1 and continuing until day 13, the animals were challenged with escalating doses of PCP (0.32, 0.56, and 1.0 mg/kg).

behavioral response to the second dose of PCP was then used as the 100% response value for normalizing all subsequent PCP-induced behavioral responses.

At the start of the actual experiments, each rat was randomly assigned to one of three treatment groups ($n = 7/\text{group}$). The control groups consisted of animals that received either saline or NS-IgG at 1000 mg/kg. The third group of rats was given a treatment of anti-PCP mAb (1000 mg/kg). On day 0 after the two pretreatment PCP challenges, the rats were administered either saline or IgG (anti-PCP or NS). All treatments (including the saline control) were administered in a volume of approximately 8 ml at a rate of 1 to 2 ml/min. This fluid volume (21 ml/kg) was chosen based on human rates of saline administration and because it has been safely administered to rats in several studies in our laboratory (Hardin et al., 1998; Proksch et al., 2000a). The treatments were administered the afternoon before the start of the challenge of escalating PCP doses.

Beginning on day 1 and continuing until day 13, the animals were challenged with multiple doses of PCP every 3rd day. This cumulative PCP dose design allowed for the characterization of a dose-response curve within each experimental day (Wenger, 1980). The PCP doses and time intervals between doses were optimized during the preliminary experiments, which showed an i.v. PCP dose of 0.32 mg/kg as the lowest dose that induced a reproducible locomotor effect in all rats. Moreover, it was found that PCP doses higher than 1 mg/kg (e.g., 3 mg/kg) often produced the rapid onset of immobility (deep anesthesia) followed later by ataxia and high levels of locomotor activity. To reduce the complexity of PCP-induced behaviors, we used PCP doses that led to maximum locomotor activity without immobility and ataxia. Thus, PCP doses of 0.32, 0.56, and 1.0 mg/kg were selected for the cumulative dose-response curve.

For each experiment, the rat was placed in the chamber 60 min before the first dose of PCP to establish baseline behavior. The first dose (0.32 mg/kg) was administered at time 0. The second dose (0.56 mg/kg) and the last dose (1.0 mg/kg) were given at times 90 and 180 min, respectively. The 90-min interval was sufficient to allow for the PCP-induced locomotor effects to return to baseline before the next dose and before the end of each day's experiment. The experiments were terminated each day at 270 min. The animals were removed from the behavioral chambers for drug administration from 0 to 4 min, 90 to 94 min, and 180 to 184 min. These 4-min time intervals were not included in the behavioral data analysis.

Behavioral Analysis. The videotaped results for each experiment were analyzed using EthoVision software (version 1.9, Noldus Information Technology, Inc., Sterling, VA). In previous studies (Hardin et al., 1998), we determined that the digitized data derived from this computer analysis provided reliable measurements of the dose-dependent behavioral effects (distance traveled and time spent moving) produced by PCP. During preliminary experiments we optimized the settings for the EthoVision video tracking and motion analysis system, which were somewhat different from our previous settings. This included a sampling rate of three video images per second, no step-down sampling option, and a minimal distance traveled threshold of 0 cm for the distance traveled parameter. For the time spent moving parameter, the rat was considered to have started moving when its velocity had exceeded 15 cm/s and to have stopped moving when its velocity decreased below 5 cm/s. To assure the accuracy of the computer system analysis, we also spot-checked the data by visual comparison of the rat's behavior on the videotapes with data generated by the computer analysis.

For both distance traveled and time spent moving, the results of the analyses were reported in two-min cumulative intervals and in 86-min cumulative intervals (i.e., 90-min sessions minus 4 min out of the test chamber). This 86-min cumulative interval was used to determine the response to each PCP challenge. By using these experimental protocols, we were able to develop a three-point dose-response curve (0.32, 0.56 and 1.0 mg/kg) for each treatment group, which could then be used for statistical comparison across treatment groups.

Serum and Brain PCP Concentrations. After the final behavioral determination (day 13), rats were anesthetized with ethyl ether and blood was collected from the inferior vena cava. Animals were then quickly sacrificed by decapitation and the brain was removed, rinsed with saline and frozen in liquid nitrogen. Blood samples were allowed to clot and serum was collected after centrifugation. Serum and brain samples were stored at -80°C until analysis. PCP concentrations in brain and serum were determined by radioimmunoassay (Proksch et al., 2000a).

Data and Statistical Analysis. For the comparison of the first and second pretreatment PCP challenges, all of the animals were considered as a single group ($n = 21$) and a paired t test was used to determine statistical significance. For the between-treatment group, comparison of the second pretreatment PCP challenges for the three treatment groups (saline, anti-PCP, and bovine IgG), a one-way analysis of variance was used. This comparison was used to assure that the random assignment of rats to treatment groups had not inadvertently created significant differences or a bias between the groups.

The data for the post-treatment PCP challenges were analyzed using a two-way, repeated-measures analysis of variance, followed by a Student-Newman-Keuls test. Because two behavioral parameters (distance traveled and time spent moving) were used to measure PCP-induced effects, an effect was considered to be substantial only when it was statistically significant for both parameters. Statistical significance was considered achieved at a level of $P < 0.05$. The data for PCP brain and serum concentration in control (NS-IgG) and anti-PCP IgG were compared using a Student's t test.

Results

General Experimental Observations. We first determined the largest dose of pooled human polyclonal immunoglobulins that can be safely administered to humans because we wanted to study the duration of action of a single large dose of mAb in our rat model. In previous human clinical studies, polyclonal IgG at doses of 1000 mg/kg result in relatively few and minor side effects (Achiron et al., 1998). In fact, high i.v. doses are safely administered even during pregnancy (Gibson et al., 1989; Achiron et al., 1996; Porter et al., 1997). These studies suggest that most of the side effects in humans result from problems with the antibody formulation and purity. For this study we used a relatively pure formulation of monoclonal antibodies that provided a homogeneous protein-based medication from a single source. These extremely high i.v. doses of IgG were well tolerated by the animals, without any observable side effects. To our knowledge, the doses (on a per kilogram weight basis) used in this study and our previous study (Proksch et al., 2000a) are the highest doses of mAb given to animals.

A second part of our experimental strategy was to administer a sufficient number of antibody binding sites to neutralize at least the first few i.v. doses of PCP and then to determine whether the antibody could offer protection from PCP effects after the binding sites were theoretically saturated. By our calculations, the binding sites of our 1000 mg/kg dose of monoclonal antibody should have been sufficient to neutralize 3.24 mg/kg PCP. Because we administered a cumulative dose of 1.88 mg/kg PCP on each day, the binding capacity could have been theoretically saturated during the 2nd day of dosing. This assumes there was no loss of drug from the previous PCP administration, which is probably a somewhat inaccurate assumption because we had no models of predicting the in vivo rate of loss of drug and/or antibody under

these complex conditions. However, our previous study suggested the half-life of PCP is dramatically increased in the presence of mAb. Thus it is unlikely that substantial amounts of PCP from the first administration (day 1) were eliminated at the time of the second administration (day 4).

Establishment of Pretreatment Baseline Values for PCP-Induced Locomotor Response. The total distance traveled during the first and second pretreatments was 203.7 ± 66.9 and 267.6 ± 50.3 m (mean \pm S.D.), respectively. The total time spent moving during the first and second pretreatments was 17.8 ± 6.1 and 22.4 ± 4.3 min, respectively. Thus, for both distance traveled and time spent moving, the rat responses to the second PCP pretreatment were greater than to the first PCP pretreatment. In addition, the variance in the response was less after the second PCP pretreatment.

A second analysis of these data after the animals were randomly assigned to their individual treatment groups ($n = 7$ /group for the saline, NS-IgG, and anti-PCP mAb groups), showed there was no statistical difference between the groups in their responses to the second PCP pretreatment. The individual values for distance traveled and time spent moving (respectively) for each group were: 243.3 ± 67.4 and 20.1 ± 5.4 for saline, 281.5 ± 39.9 and 23.6 ± 2.05 for NS-IgG, and 277.9 ± 35.7 and 23.6 ± 4.2 for anti-PCP mAb. Thus, by the second PCP administration results were more stable and reproducible.

Effect of Saline and NS-IgG Controls on PCP-Induced Locomotor Activity. During and after the infusions of the saline, NS-IgG, and anti-PCP mAb treatments on day 0, the rats seemed to tolerate the treatments very well with no apparent indications of adverse effects at any point during the 2-week experiment. For the animals in the saline and NS-IgG control groups, the post-treatment challenges with the escalating doses of PCP (0.32, 0.56, and 1.0 mg/kg) resulted in dose-dependent locomotor responses, as measured by total distance traveled and total time spent moving. A representative plot for the total distance traveled from one animal in the saline-treated group during the testing session on day 1 is shown in Fig. 2. On this same figure, we also show a PCP serum concentration versus time curve that was simulated using previously determined PCP pharmacokinetic serum parameters for the rat (Valentine et al., 1994) with the following conditions: three i.v. bolus doses of PCP (0.32, 0.56, and 1.0 mg/kg) separated by 90 min with a two-compartment pharmacokinetic model using a $1/y^2$ weighting function. To construct this PCP concentration-time curve, we used the pharmacokinetic software package WinNonlin (Pharsight, Mountain View, CA).

Based on our analysis of the data (see Fig. 2), we were able to conclude that the locomotor activity always returned to baseline before the next dose. Second, there were still significant amounts of drug in the rats at the time of each new injection (i.e., 0.56 and 1.0 mg/kg). Because the terminal elimination half-life of PCP in the rat is 3.9 h (Valentine et al., 1994), it can be calculated that in the control animals after 90 min (the interval between drug administrations) about 77% of the previous dose was remaining. Consequently, in the control animals, the cumulative doses after each injection were actually about 0.32, 0.8, and 1.6 mg/kg of PCP. Because behavioral response was calculated as a percentage of the PCP response (y-axis) after the second pre-

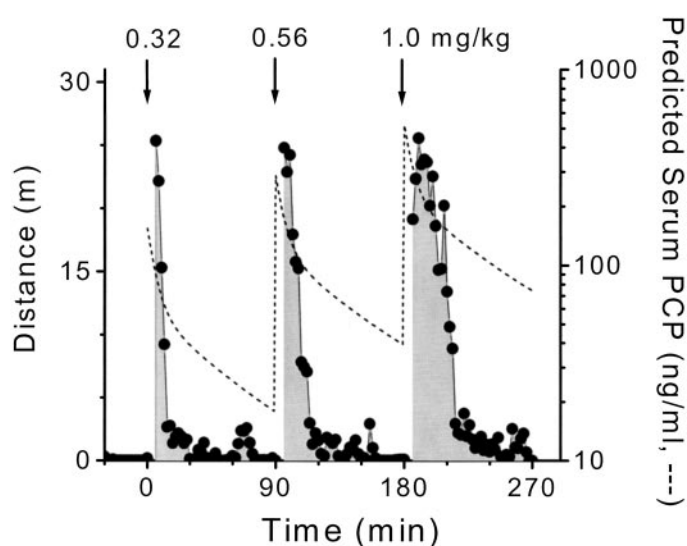


Fig. 2. Distance traveled versus time in an animal from the saline control group after repeated challenges of PCP (left axis). The dashed line shows the simulated PCP serum concentrations after administration of the 0.32, 0.56, and 1.0 mg/kg doses (right axis). The arrows indicate the times and doses of PCP.

treatment PCP challenge (1 mg/kg), the responses to the 1.0 mg/kg doses were greater than 100% due to the higher cumulative doses in the saline- and NS-IgG-treated animals (see Figs. 3 and 4).

For the control groups (saline and NS-IgG), the response to each dose of PCP did not significantly change over the 13-day course of the experiment (Tables 1 and 2; Fig. 3). Furthermore, the slopes of the dose-response curves also did not seem to change over the course of the experiment for either control group. Because data from the distance traveled and time spent moving showed similar dose- and time-dependent changes, we only showed plots from total distance traveled. There were no differences in the locomotor responses (for either distance traveled or time spent moving) between the

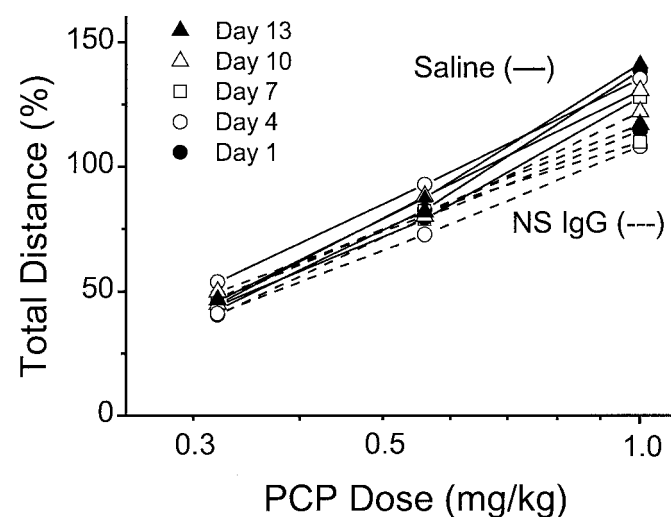


Fig. 3. Mean values for total distance traveled by the control groups (saline and NS IgG) for days 1 through 13 of the post-treatment challenges. Values are presented as percent control of the pretreatment PCP dose (1 mg/kg). For clarity, error bars are not shown. There were no statistical differences between the saline and NS IgG groups for any of the days. All values are the mean \pm S.D. ($n = 7$ rats/time point).

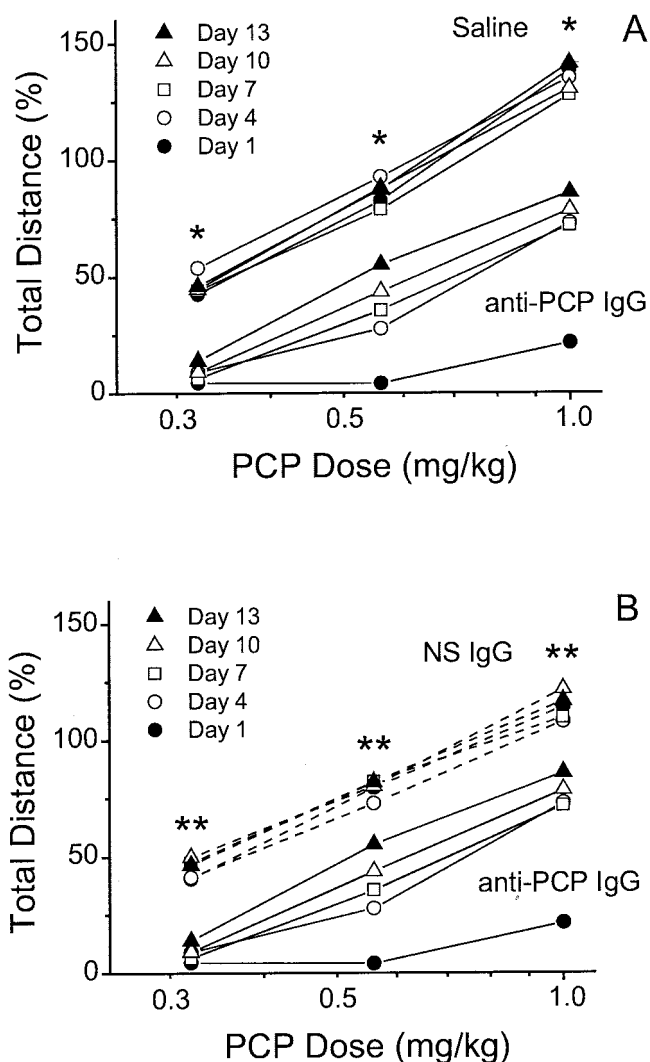


Fig. 4. Total distance traveled (mean values) for the post-treatment PCP challenges for the saline (A) and NS-IgG (B) control groups compared with the anti-PCP mAb treatment group (*, $P < 0.05$ for days 1–13; **, $P < 0.05$ for days 1–10). Values shown are percentage of control of the pretreatment PCP dose (1 mg/kg). For clarity, error bars are not shown. On day 13, the results for the two highest doses (0.56 and 1.0 mg/kg) of anti-PCP IgG were not statistically different from NS-IgG control values.

saline and NS-IgG control groups for any dose on any test day with one exception. On day 4 for the 0.56 mg/kg dose, the time spent moving for the NS-IgG group was significantly lower ($P < 0.05$) than the matched saline treatment group. Because this was the only time-point and dose-point that showed a change and because the distance traveled parameter was not different, we did not consider this to be a substantial difference.

Effects of Anti-PCP mAb on PCP-Induced Effects. On the 1st day of testing, the anti-PCP mAb completely blocked PCP-induced effects at the two lowest doses, and the effects were minimal at the 1.0 mg/kg dose (Table 1; Fig. 4). The anti-PCP mAb-induced reductions in response over the next four sessions (days 4–13) were not as great as the 1st day but were still significantly ($P < 0.05$) lower than either of the control groups (Fig. 4; Tables 1 and 2). On closer examination, a time-dependent small incremental increase in the

response to the post-treatment PCP challenges was observed (Fig. 4; Tables 1 and 2).

The anti-PCP mAb caused long-lasting, statistically significant reductions in both total distance traveled and total time spent moving compared with both saline and NS-IgG controls (Tables 1 and 2, respectively). When comparing locomotor effects (both distance traveled and time spent moving), the PCP-induced responses in the anti-PCP mAb treatment group were significantly lower than the saline treatment group for all doses on all days ($P < 0.05$). Furthermore, the anti-PCP mAb treatment group was also significantly lower ($P < 0.05$) than the NS-IgG group. Only on day 13 at the two highest doses (0.56 and 1.0 mg/kg) were the results not statistically lower. However, there was one data point exception. For the total distance traveled parameter, the response to the 1.0 mg/kg PCP dose for the anti-PCP mAb treatment group was not different from the NS-IgG group for day 7. Because this was the only exception and the time spent moving parameter for this same point was statistically different, we did not consider this a substantial difference.

Effects of Anti-PCP mAb on Serum and Brain PCP Concentrations. Rats from the NS-IgG and anti-PCP mAb ($n = 4$ /group) were sacrificed on the final day (day 13) after determination of the PCP cumulative dose-effect curve to quantify PCP in the serum and brain. Compared with the nonspecific IgG group, anti-PCP mAb significantly increased serum PCP concentrations (Fig. 5). In addition, brain PCP concentrations were reduced, although this decrease was not significant (Fig. 5).

Discussion

Using a rat model of excessive PCP use in humans, we demonstrated that a single dose of an anti-PCP mAb provided remarkable, long-term protection against frequent and repeated PCP challenges. Pretreatment with the anti-PCP mAb was effective at antagonizing the locomotor effects of PCP across a wide range of doses (0.32–1.0 mg/kg) over a 2-week period compared with a saline control treatment group (Tables 1 and 2; Fig. 4).

Because most of the PCP in the anti-PCP mAb treatment group was probably highly bound to the antibody, the PCP in these rats would have taken on the pharmacokinetics of the antibody and thus be restricted to a much smaller volume of distribution. Based on previous pharmacokinetic studies of PCP and the PCP-anti-PCP mAb complex (Proksch et al., 2000a), we would expect the mAb to significantly slow PCP clearance. Consequently, the cumulative body burden of PCP (bound plus free) for the anti-PCP mAb group was likely much higher than the cumulative body burden for the two control groups. Despite this higher body burden, the mAb was still able to protect against repeated PCP administration.

The distribution and elimination half-lives of mouse monoclonal IgG in rats are 8 h and 8 days, respectively (Bazin-Redureau et al., 1997). Assuming similar kinetics for our anti-PCP mAb, on day 1 the anti-PCP mAb would still be in the distribution phase and only a small percentage would have been eliminated. Thus, ample binding capacity probably accounts for the almost complete blockade of PCP-induced effects by the anti-PCP mAb group on day 1 (Fig. 4). Binding

TABLE 1
Distance traveled in PCP-treated rats following saline, NS-IgG, or anti-PCP mAb

PCP Dose	Treatment	Distance Traveled (Percentage of Control)				
		Days after Treatment				
		1	4	7	10	13
0.32 mg/kg	Saline	42.7 ± 17.7	53.8 ± 12.7	44.2 ± 16.0	44.7 ± 20.6	46.1 ± 18.7
	NS-IgG	40.6 ± 8.5	41.1 ± 9.6	47.3 ± 13.6	49.8 ± 23.1	46.5 ± 16.4
	Anti-PCP mAb	4.6 ± 3.0	9.1 ± 5.4	6.4 ± 2.5	9.3 ± 3.5	13.9 ± 7.1
0.56 mg/kg	Saline	82.6 ± 16.8	92.8 ± 13.0	78.9 ± 10.9	88.1 ± 12.8	87.4 ± 20.6
	NS-IgG	79.5 ± 15.1	72.8 ± 20.8	82.1 ± 21.3	80.2 ± 32.1	81.9 ± 24.3
	Anti-PCP mAb	4.2 ± 2.8	27.8 ± 13.1	35.6 ± 6.0	43.6 ± 10.9	55.2 ± 10.9
1.0 mg/kg	Saline	138.6 ± 17.3	135.2 ± 24.4	127.8 ± 24.7	130.5 ± 36.1	141.3 ± 31.6
	NS-IgG	114.4 ± 18.9	108.0 ± 20.2	109.8 ± 21.6	121.9 ± 40.2	116.8 ± 25.9
	Anti-PCP mAb	21.6 ± 8.4	73.0 ± 28.5	71.9 ± 17.5	78.5 ± 13.3	86.0 ± 14.9

TABLE 2
Time spent moving in PCP-treated rats following saline, NS-IgG, or anti-PCP mAb

PCP Dose	Treatment	Time Spent Moving (Percentage of Control)				
		Days after Treatment				
		1	4	7	10	13
0.32 mg/kg	Saline	38.8 ± 19.1	47.7 ± 11.6	40.2 ± 12.7	41.9 ± 19.7	41.3 ± 14.1
	NS-IgG	38.8 ± 10.3	37.0 ± 9.8	40.8 ± 12.3	41.3 ± 15.7	41.2 ± 12.3
	Anti-PCP mAb	2.4 ± 1.6	5.7 ± 3.5	4.4 ± 2.8	7.4 ± 4.4	12.3 ± 7.8
0.56 mg/kg	Saline	78.7 ± 18.7	90.6 ± 15.4	74.9 ± 14.7	76.4 ± 13.3	81.8 ± 19.4
	NS-IgG	76.1 ± 11.2	63.2 ± 13.8	71.2 ± 12.5	69.5 ± 27.0	71.7 ± 15.3
	Anti-PCP mAb	2.6 ± 2.4	25.2 ± 12.6	34.3 ± 5.0	41.9 ± 13.6	50.8 ± 13.9
1.0 mg/kg	Saline	137.8 ± 14.7	137.1 ± 26.3	129.0 ± 24.5	129.9 ± 28.0	137.5 ± 34.2
	NS-IgG	114.9 ± 17.1	108.0 ± 22.2	102.5 ± 18.8	110.1 ± 24.5	109.1 ± 22.8
	Anti-PCP mAb	20.8 ± 8.5	65.1 ± 16.9	66.5 ± 14.4	75.5 ± 15.4	81.3 ± 18.2

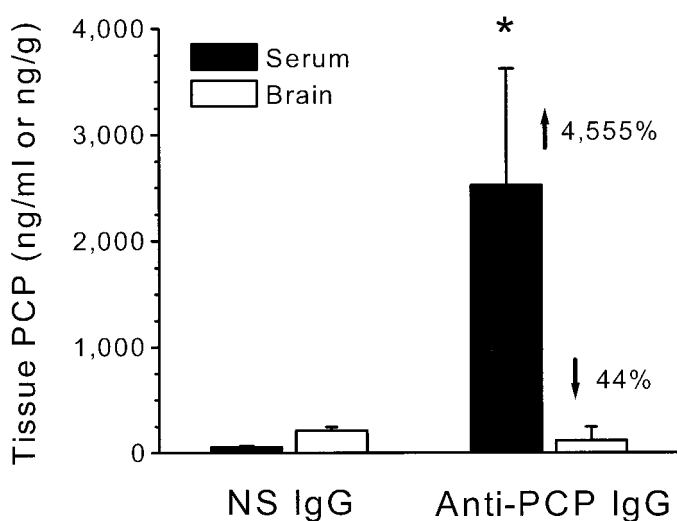


Fig. 5. PCP concentrations in serum and brain from NS-IgG and anti-PCP mAb groups. Serum and brain were obtained 2 h after the final PCP dose on day 13. PCP concentrations were determined using a specific radioimmunoassay after hexane extraction of serum samples or brain homogenates. *, $P < 0.05$ compared with NS IgG group using Student's t test. All values are the mean \pm S.D. ($n = 4$ rats/group).

capacity does not explain the fairly constant 40 to 50% reduction in effects on all subsequent days.

Administration of mouse mAb to rats could result in the production anti-mouse antibodies, which could adversely affect (decrease) the function and disposition of our anti-PCP mAb. To reduce (or prevent) an antigenic response against the mAb in rats, we eliminated large molecular weight mAb complexes in our formulations by ultracentrifugation.

Spiegelberg and Weigle (1967) have shown that these large molecular weight complexes are a main cause of antigenicity when immunoglobulins from one species are administered to a different species. Our data from this study and our previous study (Proksch et al., 2000a) show that the elimination half-life of the anti-PCP mAb may be even longer than previously reported values for mouse IgG in rats (8 days; Bazin-Redureau et al., 1997). Furthermore, we have not observed any clinical indications of an immune response in any of the rats that received mouse mAb.

The changes in PCP pharmacokinetics support the behavioral findings. Serum and brain concentrations determined at the time of sacrifice (2 h after the final PCP dose) suggested that anti-PCP mAb was still significantly affecting PCP distribution 14 days after mAb administration (or 13 days after the start of the PCP dosing). In the presence of NS-IgG, brain PCP concentrations on day 13 were about 3 times higher than serum PCP concentrations; however, in the presence of anti-PCP mAb, serum PCP concentrations exceeded brain concentrations by about 25-fold and brain concentrations were reduced by about 45% (Fig. 5). These long-lasting effects were much greater than expected based simply on mAb binding capacity, but they are in agreement with our previous studies that showed the functional half-life of the PCP-anti-PCP mAb complex is about 15 days (Proksch et al., 2000a). These previous studies also showed that a single dose of anti-PCP mAb produced significant reductions in brain PCP concentration during a continuous, high dose infusion of PCP. The decreased brain concentrations were observed for up to 1 month, even though the binding capacity of the mAb should have been rapidly saturated by the continuous PCP infusion.

The time-dependence of the behavioral protective effects showed remarkable parallels with our previous PCP brain pharmacokinetic studies (Proksch et al., 2000a). During early time points (on day 1 of the current study and in the first few hours of the pharmacokinetic study), the mAb almost completely blocked PCP behavioral effects and reduced brain concentrations to immeasurable levels, respectively. At representative times points 1 and 2 weeks later, the behavioral effects were still reduced by 56 and 61%, respectively, and PCP brain concentrations were still reduced by 43 and 53%, respectively. The high degree of consistency in the results from these new behavioral studies and previous PCP pharmacokinetic studies suggest a common mechanism for the prolonged protective effects of mAb.

We think several factors are involved in the mechanism of action of the anti-PCP mAb. Although the overall changes in PCP pharmacokinetics are the result of the summation of the PCP-mAb interaction in individual organs, the behavioral effects are primarily driven by changes in PCP brain concentrations. Given that the affinity of the anti-PCP mAb is constant, the degree of PCP-mAb interaction in each organ is dependent on a number of factors. These include the rate at which the PCP can redistribute, the amount of PCP, and the apparent volume of the drug-mAb interaction (i.e., molar concentration) in each organ, and the free fraction of mAb in each organ. From our previous studies (Proksch et al., 2000a), we think the mAb inactivates the drug by substantially reducing the volume of distribution of PCP from 6.4 l/kg to a volume that is approximately equal to the volume of distribution of the mAb IgG (0.13 l/kg; Bazin-Redureau et al., 1997). Without anti-PCP mAb treatment, the brain clearance (i.e., uptake) of PCP seems to be a nonrestrictive type clearance; that is, virtually all PCP in the blood is cleared with each pass through the brain. This type of clearance is dependent only on blood flow because dissociation of PCP from plasma proteins and partitioning into the brain are extremely rapid events (Valentine et al., 1994; Proksch et al., 2000b). In the presence of anti-PCP mAb, the brain clearance of PCP is changed to a restrictive type, in which only the free fraction of PCP can enter the brain. Thus, when mAb binding capacity is not a limiting factor (as on day 1 of the current experiment), most of the PCP is prevented from entering the CNS and no significant pharmacological effects are produced (Tables 1 and 2; Fig. 4). However, once mAb binding capacity becomes limited (after day 1), a new condition is established in which the unbound mAb and brain-specific factors become major determinants of the beneficial effects.

Based on previous brain pharmacokinetic studies of the mAb functional capacity (Proksch et al., 2000a), the unoccupied mAb binding capacity (i.e., free fraction) in the plasma seems relatively constant at ~3 to 7% over a 1-month period. This occurred even though the infusion rate was sufficient to replace 15% of the PCP steady-state body burden each hour. We think that the mAb temporarily becomes more fully occupied in the vascular compartment in the brain. For this to occur: 1) PCP would have to freely pass across the blood-organ barrier; 2) the affinity of the mAb must be high enough to rapidly prevent the PCP from re-entering the CNS; and 3) the volume of the organ plasma compartment would have to be very small relative to other organs (i.e., high molar concentration of PCP mAb). These conditions allow for the unoccupied mAb binding sites in the organ plasma compart-

ment to temporarily achieve a higher molar concentration and thereby reduce movement of PCP back into the tissue. For the brain, these criteria are met. The rat brain plasma volume is relatively small (Khor et al., 1991), and the rate and extent of PCP distribution varies among organs with the brain being the most rapidly equilibrating (and re-equilibrating) organ (Valentine et al., 1994; Proksch et al., 2000b). Thus, with each pass through the brain, the available mAb binding sites in the serum are sufficient to reduce PCP movement across the blood brain barrier, which significantly reduces brain concentrations. We do not think this occurs to the same extent in most other organs.

Based on an 8-day half-life for mouse mAb in rats (Bazin-Redureau et al., 1997) and a 24-day half-life for passively administered exogenous human IgG in humans (Knapp and Colburn, 1990), it is reasonable that the duration of protective effects of a humanized or fully human anti-PCP antibody would be easily extrapolated for 2 months or more in humans. However, the cost of a 1000 mg/kg dose in humans is currently prohibitive. We had previously assumed that a dose of anti-PCP mAb that was equivalent to the molar amount of PCP in the body would be required to offer protection against the effects of PCP (Hardin et al., 1998). The results of these new behavioral studies and our previous brain pharmacokinetic study (Proksch et al., 2000a) suggest that the amount of mAb is not the only determinant of its neutralizing effects and that mole-equivalent doses of mAb are not required to produce significant and long-lasting protective effects. Studies to characterize the anti-PCP mAb dose-response relationships are currently underway. Additional studies to examine the role of affinity in the protective effects of anti-PCP mAb are also being conducted.

In conclusion, our studies established that a single dose of anti-PCP mAb was extremely effective at protecting against PCP locomotor effects up to 14 days after administration and suggest that monoclonal antibodies can function as long-acting selective antagonists to protect against some of the harmful effects of drug abuse. These data challenge the current ideas and concepts about the in vivo dose dependence and the unimolecular interaction between antibody binding sites and small molecules, and they establish that neuroprotection of the brain from chemicals by mAb may have a unique mechanism of action. Because PCP is a prototype for drugs that have been difficult to treat due to complex actions at multiple sites within the brain, these studies may provide a new approach for treating a wide range of CNS acting drugs or chemicals.

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Address correspondence to: Dr. S. Michael Owens, Department of Pharmacology and Toxicology, College of Medicine, Slot 611, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, AR 72205. E-mail: owenssamuelm@uams.edu
