Anti-Phencyclidine Monoclonal Antibodies Provide Long-Term Reductions in Brain Phencyclidine Concentrations during Chronic Phencyclidine Administration in Rats

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ABSTRACT

These studies examined the hypothesis that a single large dose of monoclonal anti-phencyclidine (PCP) antibody could provide long-term reductions in brain PCP concentrations despite continuous PCP administration. PCP (18 mg/kg/day, s.c.) was infused to steady-state (24 h) and then a mole-equivalent dose of a short-acting anti-PCP antigen-binding fragment (Fab) or a long-acting anti-PCP IgG was administered i.v. The PCP infusion continued for up to 27 days, even though the binding capacity of the single dose of antibody used should have been saturated within the first day. At selected time points after antibody administration, brain, testis, and serum PCP concentrations were measured. Serum PCP concentrations rapidly increased ~100- and 300-fold after Fab or IgG administration, respectively. Based on the antibody-bound PCP concentrations in serum, the functional elimination half-life \((t_{1/2}\text{Z})\) values for PCP-Fab and PCP-IgG complexes were 9.4 h and 15.4 days, respectively. Fab and IgG administration produced a complete removal of PCP from the brain within 15 min. Although brain PCP concentrations were significantly decreased for only 4 h in Fab-treated animals, IgG administration resulted in significant decreases in brain PCP concentrations lasting for at least 27 days. In contrast, testis PCP concentrations were not substantially affected by antibody administration, suggesting that redistribution of PCP from the testis is too slow to benefit from a limited dose of antibody. These results indicate that anti-PCP IgG can preferentially protect the brain for ~4 weeks after IgG administration, even when the antibody binding capacity should have been saturated with continuously administered PCP.

Previous studies using a rat model of acute phencyclidine (PCP) overdose have demonstrated that administration of high-affinity \((K_d = 1.8 \text{ nM})\) anti-PCP monoclonal antibody fragments (anti-PCP Fab) causes a rapid and effective redistribution of PCP out of the brain and other tissues in the rat (Valentine and Owens, 1996). This redistribution of PCP after anti-PCP Fab administration also produces a rapid recovery from the behavioral toxicity produced by PCP-like drugs in rats (Valentine et al., 1996; Hardin et al., 1998).

Rapid reduction of brain concentrations in an acute medical crisis is not the only potential medical application for antibody-based therapies in the treatment of substance abuse. Indeed, there is a significant need for medications to reduce drug craving and recidivism among recovering drug abusers. Several animal studies have examined the utility of antibody-based therapies for blocking or inhibiting the rapid penetration of drugs into the brain and the subsequent behavioral effects. For example, active immunization with drug-protein conjugates has been suggested for treating heroin, cocaine and nicotine abuse (Bonese et al., 1974; Carrera et al., 1995; Fox et al., 1996; Hieda et al., 1997). The results from these animal studies demonstrate that active immunization can alter the serum pharmacokinetics of the drugs, decrease brain drug concentrations, and decrease drug-induced effects. However, because the amount of antibody that is generated after active immunization is slow to increase and the antibody concentrations vary significantly over time, it would be difficult to control the timing and level of protection with active immunization against drugs of abuse. Furthermore, the relationship between the limited amounts of antibody present and the total body burden of drug administered is not understood.

Other investigators find that increasing the metabolic degradation of cocaine decreases the terminal elimination half-life \((t_{1/2}\text{Z})\) and the behavioral effects (Carmona et al., 1998; Mets et al., 1998). Indeed, catalytic antibodies generated against transition-state analogs in the cocaine-metabolic

ABBREVIATIONS: PCP, phencyclidine; Fab, antigen-binding fragment; \(C_{SS}\), concentration at steady state; \(\lambda_e\), terminal elimination rate constant; RIA, radioimmunoassay; \(t_{1/2,2}\), terminal elimination half-life; \(V_{dSS}\), volume of distribution at steady state.

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pathway are effective in increasing the in vivo metabolism of cocaine, reducing cocaine self-administration, and reducing the lethality of cocaine in mice (Mets et al., 1998). Assuming the rate of entry of drugs of abuse into the brain is an important factor in the addiction liability (Russell and Feyerabend, 1978; Verebey and Godl, 1988; Henningfield and Keenan, 1993), it is not clear how the high $K_{m}$ value of this catalytic antibody (~220 μM) acts to reduce the rapid entry of cocaine into the brain during self-administration. Indeed, little is known about optimizing the in vivo interplay between functional capacity, $K_{m}$ values (for enzymatic degradation), or $K_{d}$ values (for antibody binding) and maximum therapeutic benefits from antibody-based medications.

Another potential medical approach for treating recovery from chronic addiction is the prophylactic use of large doses of high-affinity monoclonal anti-drug antibodies. The biological half-life of i.v. IgG is ~21 days in humans (Knapp and Colburn, 1990), making sustained protection, with infrequent dosing, potentially feasible. Data from previous studies suggests that a single dose of anti-PCP IgG administered to rats is effective in reducing PCP-induced behavioral effects for ~2 weeks (Hardin et al., 1999). This duration of effect is consistent with the reported half-life of a monoclonal IgG in rats ($t_{1/2} = 8$ days; (Bazin-Redureau et al., 1997). Despite the apparent effectiveness of using high-affinity antibodies as long-term antagonists, the complex pharmacokinetic and pharmacodynamic mechanisms are poorly understood. For example, a limiting factor in the use of antibody-based medications to treat drug addiction would be the total drug-binding capacity of the administered antibody dose. Given an adequate supply of drug, a person could overcome the protective effects of the antibody, potentially resulting in unexpected adverse effects, including overdose or death.

The current studies were designed to examine the protective effects of a single large dose of anti-PCP Fab and anti-PCP IgG in the presence of continuous PCP administration. To accomplish this goal, the effects of anti-PCP Fab and anti-PCP IgG administration on brain, testis, and serum PCP concentrations, along with PCP serum protein binding, were studied during a continuous s.c. infusion of PCP. Fab and IgG were used as prototypic short-acting ($t_{1/2} = 7.5$ h; McClurkan et al., 1993) and long-acting ($t_{1/2} = 8.4$ days; Bazin-Redureau et al., 1997) antagonists, respectively. The testis was chosen as a control tissue for the brain because both have blood-tissue barriers that prevent the penetration of Fab and IgG. Furthermore, previous studies in our lab showed that the brain and testis represent organs with rapid and slow PCP equilibration, respectively (Valentine and Owens, 1996).

Materials and Methods

Drugs and Chemicals. [3H]PCP (1-(1-phenyl-[3H]ethyl)cyclohexyl)piperidine) and PCP HCl (1-(1-phenylcyclohexyl)piperidine hydrochloride) were obtained from the National Institute on Drug Abuse (Rockville, MD). The [3H]PCP (15.3 Ci/mmol) was used as a standard for determining PCP concentrations in tissue extracts by radioimmunoassay (RIA) and for PCP protein binding experiments with equilibrium dialysis. All PCP concentrations were calculated as the free base. Sodium sulfate, sodium azide, and BSA were purchased from Sigma (St. Louis, MO). All other chemicals were obtained from Fisher Scientific (Springfield, NJ), unless otherwise stated.

Production and Purification of Monoclonal Anti-PCP IgG and Fab. Gram quantities of monoclonal anti-PCP IgG were produced from the hybridoma cell line Mab6B5 in a Cell-Pharm System II hollow fiber bioreactor (Unisyn Technologies, Tusin, CA). The details of the anti-PCP IgG production are described elsewhere (McClurkan et al., 1993; Valentine et al., 1994; Hardin et al., 1998). Papain digestion of the monoclonal anti-PCP IgG was used to obtain anti-PCP Fab, which was purified as described by Hardin et al. (1998). After final purification, anti-PCP Fab and IgG were concentrated to ~40 and 60 mg/ml, respectively, in buffer containing 15 mM phosphate, 10% sucrose, and 0.15 M NaCl (pH 6.5).

Animals. Adult male Sprague-Dawley rats (270–300 g) were purchased from Hilltop Laboratory Animals (Scottsdale, PA). Animals were housed with an indwelling cannula (Dow Corning silastic tubing, 0.020-inch inside diameter; 0.037-inch outside diameter) placed in the right external jugular vein. Before shipping, the cannula was placed in the subdermal space for protection during shipping. On arrival, the cannulas were removed from the subdermal space and kept patent with heparinized saline (25 U every other day). Animals were allowed at least 1 week to acclimate to their new environment before use and were fed enough food on a daily basis to maintain their body weight at ~300 g. All animal experiments in these studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Protocol for Pharmacokinetic Studies. Osmotic minipumps (Alza, Palo Alto, CA) were implanted s.c. on the back of rats under ether anesthesia. The pumps were filled before implantation with PCP dissolved in sterile saline (2 ml per pump) at a concentration that would result in a continuous infusion of PCP at the desired dose. PCP was s.c. infused from the pumps at a rate of 18 mg/kg/day (4 μg/mg/min). The pumps delivered ~10.0 μl/day (7-day pumps) or 5.0 μl/day (14-day pumps) for the duration of the experiment.

Animals received anti-PCP Fab or anti-PCP IgG at 24 h after the start of the PCP infusion. Because the $t_{1/2}$ of PCP in the rat is 3.9 h (Valentine et al., 1994), the animals were assumed to have achieved steady-state PCP concentrations (i.e., 4 to 7 $t_{1/2}$, 15.6 to 27.3 h). The dose of Fab and IgG binding sites was equivalent on a mole basis (1 mol-equivalent) to the amount of PCP in the animal at steady state. The amount of PCP (mol. wt. = 243 g/mol) at steady-state was calculated by multiplying the concentration of PCP at steady-state ($C_{SS} = 183$ ng/ml), by the volume of distribution at steady-state (Vdss = 27 L/kg; pharmacokinetic values from Wessinger and Owens 1991). The corresponding mole-equivalent dose of antibody was calculated using a molecular mass of 50 kDa for Fab and 150 kDa for IgG. In addition, the mole-equivalent IgG dose was corrected for the presence of two binding sites per IgG molecule. The final mole-equivalent Fab dose was 1.92 μg/kg, whereas the IgG dose was 1.53 μg/kg. The Fab and IgG were prepared at concentrations of 40 mg/ml and 60 mg/ml, respectively, making the final injection volume ~7 ml for each antibody solution. Anti-PCP Fab or IgG was administered via the jugular venous cannula at a rate of ~0.5 μl/min. In all experiments, the start of the antibody infusion was considered time zero.

Anti-PCP Fab-treated animals ($n = 3$ per time point) were sacrificed at 15 min, 1, 2, 4, 8, and 24 h after the start of the Fab infusion. Anti-PCP IgG-treated animals ($n = 3$ per time point) were sacrificed at 15 min, 1, 2, 4, 8, 24 h, 6, 13, and 27 days after the start of IgG infusion. Because the osmotic pumps used in this study had a maximum pumping duration of 14 days, animals treated with anti-PCP IgG on day zero and sacrificed on day 27 underwent an additional procedure on day 14 to implant a new osmotic minipump. Animals sacrificed at 15 min (i.e., immediately after antibody infusion) were anesthetized immediately before the antibody infusion, and anesthesia was maintained until decapitation. In addition, the i.v. antibody infusion for the animals sacrificed at 15 min was made using a syringe infusion pump (Medfusion Systems, Norcross, GA) to provide the most reliable and consistent infusion rate. For all other time
points, the i.v. antibody infusion was made by hand using a 10-ml syringe.

At the predetermined sampling times, animals were anesthetized with ethyl ether and blood was collected from the inferior vena cava. Animals were then quickly sacrificed by decapitation and tissues (brain and right testis) were removed, rinsed with water, weighed, and frozen in liquid nitrogen. The total time required from the start of blood collection until the tissues were frozen was always <3 min. Blood samples were first allowed to clot and then centrifuged to obtain serum. All serum and tissue samples were stored at ~80°C until analyzed.

Analysis of Biological Samples. Tissue samples were homogenized in four volumes of ice-cold distilled water using a SDT Tissumizer (Tekmar, Cincinnati, OH). Aliquots (300 μl) of serum or tissue homogenates were alkalized with 150 μl of 2 N NaOH and extracted twice with 500 μl of hexane for 1 h. The samples were then back-extracted into water by adding 300 μl of 0.1 N HCl to the hexane fractions and then mixed for 1 h. The aqueous layer was alkalized with 150 μl of 2 N NaOH after discarding the hexane layer and again extracted twice with 500 μl of hexane. The hexane fractions were transferred to siliconized test tubes, brought to dryness by vacuum centrifugation, and resuspended in 300 μl of normal sheep serum. Extraction efficiency was determined with blank serum, testis, and brain homogenates spiked with a known amount of [3H]PCP. These controls were extracted along with samples for RIA analysis. The percentage of recovery in the spiked control samples was calculated from liquid scintillation spectrometry analysis of the amount of radioactivity in each specimen after extraction.

PCP concentrations in 10-μl aliquots of tissue and serum extracts were determined by RIA using a high-affinity goat anti-PCP serum that does not significantly cross-react with PCP metabolites (Owens et al., 1982; Owens, 1985). Aliquots (300 μl) of serum or tissue homogenates were alkalized with 150 μl of 2 N NaOH and extracted twice with 500 μl of hexane. The hexane fractions were transferred to siliconized test tubes, brought to dryness by vacuum centrifugation, and resuspended in 300 μl of normal sheep serum. Extraction efficiency was determined with blank serum, testis, and brain homogenates spiked with a known amount of [3H]PCP. These controls were extracted along with samples for RIA analysis. The percentage of recovery in the spiked control samples was calculated from liquid scintillation spectrometry analysis of the amount of radioactivity in each specimen after extraction.

PCP concentrations in 10-μl aliquots of tissue and serum extracts were determined by RIA using a high-affinity goat anti-PCP serum that does not significantly cross-react with PCP metabolites (Owens et al., 1982; Owens, 1985). The goat anti-PCP serum was diluted 1:5000 for use in the assay. [3H]PCP (15.3 Ci/mmol) was used as the radioligand, whereas 1% donkey anti-goat IgG (Scantibodies, Santee, CA) was used to precipitate the primary antibody for separation of bound from free [3H]PCP. Aliquots were diluted in normal sheep serum as needed to obtain concentrations that were within the working range of the assay (i.e., 2 to 100 ng/ml). PCP standards for analysis of PCP concentrations in serum and tissue extracts were also prepared in normal sheep serum.

Determination of PCP Serum Protein Binding. PCP protein binding in serum was determined by equilibrium dialysis as previously described (Valentine and Owens, 1996). Briefly, dialysis discs with a molecular weight cutoff of 3500 (Spectrum Medical Industries, Los Angeles, CA) were placed in Teflon dialysis cells that were engineered at the University of Arkansas for Medical Sciences (Little Rock, AR). Serum aliquots (120 μl) were spiked with a tracer amount of [3H]PCP (about 100,000 dpm) and placed in one side of the equilibrium dialysis chamber. Phosphate buffer (120 μl, 0.13 M, pH 7.4) was added to the opposite side of the dialysis chamber. The dialysis cells were incubated overnight using constant rotation in a 37°C water bath. Serum and buffer samples were removed from the dialysis chamber, and the [3H]PCP concentrations were determined in each side by liquid scintillation spectrometry. The fraction of unbound [3H]PCP was calculated by dividing the unbound dpm in the buffer side by the total dpm in the serum side.

Pharmacokinetic Analysis. PCP concentrations in brain and testis were corrected for the residual blood content in each tissue as previously described (Valentine and Owens, 1996). At each sample time point, the average PCP concentration in serum, brain, and testis from three rats was used for analysis. The functional $f_{1/2,z}$ of the PCP-Fab and PCP-IgG complexes in serum were determined by measuring bound PCP concentrations in serum using equilibrium dialysis. A linear regression line was then fit to the terminal bound concentration versus time data points. For the purpose of this study, the functional $f_{1/2,z}$ in serum was defined as the half-life for the terminal phase decline in antibody-bound PCP concentrations in the serum.

Results

General Experimental Strategy. One of the goals of this study was to determine the limits of immunotherapy when substantial amounts of drug are administered after the antibody dose is given. The PCP infusion of 18 mg/kg/day and the 1 mol-equivalent antibody dose were carefully chosen to ensure that the antibody capacity would quickly become saturated. For example, the 18 mg/kg/day PCP infusion used in this study equates to 240 μg/h of PCP in a 300-g rat. On a molar basis, 240 μg of PCP is equivalent to 49 mg of Fab (or 148 μg of IgG) or ~15% of the injected Fab or IgG binding sites. In other words, about 15% of the steady-state PCP body burden was replaced every hour.

We previously published that a 10-day i.v. or s.c. infusion of PCP results in the same steady-state concentrations in serum from 1 day (at steady state) through 10 days of continuous infusion (Wessinger and Owens, 1991). These studies fully validate the infusion model and prove there are no time-dependent changes in the serum pharmacokinetics. In preliminary studies, we found PCP brain concentrations to be constant and not statistically different after 1 to 4 days of an 18 mg/kg/day PCP infusion (e.g., 1 and 4 day brain concentrations were 866 ± 151 and 993 ± 207 ng/g, respectively, n = 4 per group). Although we did not check at later time points such as 2 to 4 weeks, we still think these representative serum and brain concentration time points suggest the pharmacokinetics of PCP (in the absence of antibody) are constant throughout an entire month-long dosing period.

Effect of Anti-PCP Fab on Serum and Tissue PCP Concentrations. PCP infusions of 18 mg/kg/day resulted in a total serum PCP steady-state concentration ($C_{SS}$) of 183 ± 7 ng/ml (Fig. 1). A previous steady-state infusion study from this laboratory shows a $C_{SS}$ of 82 ± 10 ng/ml during infusion of 8.7 mg/kg/day PCP (Wessinger and Owens, 1991). Based on the previous study, we would predict a $C_{SS}$ of ~170 ng/ml from the PCP infusion (18 mg/kg/day) used in the current study. Consequently, the current results are in excellent agreement with previously published data. At steady state, before Fab administration, serum PCP was ~54% bound to proteins and 46% unbound (Fig. 2). This result is also consistent with published results showing PCP is 47% unbound in rat serum (Valentine et al., 1996). Anti-PCP Fab infusion (1.02 g/kg over 15 min) produced a 100-fold increase in total serum PCP concentrations by the end of the Fab administration (Fig. 1). There was also a dramatic shift in the PCP protein binding after Fab administration, with ~95% of the serum PCP bound for at least 8 h after Fab administration (Fig. 2). Consequently, the plot of bound PCP concentration versus time was essentially superimposable on the plot of total PCP concentration versus time (Fig. 1). Although free PCP concentrations in the serum also increased for ~2 h after Fab administration, this increase accounted for <10% of the 100-fold increase in total serum PCP concentrations.

Because the 100-fold increase in protein-bound serum PCP
concentrations was due to high-affinity Fab binding and because PCP in the serum remained highly protein bound for the duration of the experiment, the subsequent decline in bound PCP concentrations appeared to be due to elimination of anti-PCP Fab. The monoexponential decline in bound (and total) PCP serum concentrations from 4 to 24 h was consistent with first-order elimination of Fab. Therefore, a $t_{1/2,2}$ for the monoeponential phase of the protein-bound serum PCP concentration versus time curve was determined. Using the protein-bound serum PCP concentrations from 4 to 24 h, the functional half-life of the PCP-Fab complex was determined to be 9.4 h (Fig. 1). However, absolute accuracy of this value should be considered with caution because it was only calculated from three data points.

Figure 3 shows the effect of Fab administration on brain and testis PCP concentrations. Fab removed nearly 100% of the PCP that was in the brain at steady state, and maintained this depletion for at least 2 h. Brain PCP concentrations returned to their original steady-state level by 4 h after Fab administration, and remained at steady state for the duration of the experiment. In contrast, Fab administration had no effect on testis concentrations at the first time point measured (15 min). Testis concentrations appeared to be decreased $\sim 30\%$ at 1 and 2 h after Fab administration; however, this effect was not statistically significant.

**Effect of Anti-PCP IgG Administration on Serum and Tissue PCP Concentrations.** Figure 4 shows the effect of anti-PCP IgG administration on total and protein-bound serum PCP concentrations. Total serum PCP concentrations increased 137-fold by the end of the IgG infusion, and continued to increase to concentrations $\sim 300$-fold above $C_{SS}$ from 2 to 4 h after anti-PCP IgG administration.

Serum PCP was 54% bound to protein before IgG administration, but was $\sim 95\%$ bound to protein from 15 min to 27 days after IgG administration (Fig. 5). Consequently, the bound PCP serum concentrations were essentially superimposable on total serum PCP concentrations (Fig. 4). Any increase in free PCP concentrations after IgG administration...
The goal of these studies was to characterize the effects of anti-PCP Fab and anti-PCP IgG on serum and tissue PCP concentrations during chronic PCP infusions. Despite major differences in the experimental design, the 100-fold increase in serum PCP concentrations after the Fab administration was consistent with two previous studies in which a single i.v. bolus dose of PCP (1.0 mg/kg) was administered 2 h before a 1 mol-equivalent dose of Fab (Valentine et al., 1994; Valentine and Owens, 1996). In addition, the finding of the current studies that peak serum PCP concentrations after IgG administration were approximately three times higher than peak serum PCP concentrations after Fab administration (Figs. 1 and 4) was consistent with previously reported findings.

Although serum Fab or IgG concentrations were not measured directly, functional $t_{1/2,2}$ values for the Fab-PCP and IgG-PCP complexes were determined by measuring protein-bound PCP concentrations in the serum. Previous studies
have demonstrated that the affinity for PCP binding to the Fab fragment ($K_d = 1.8$ nM) or IgG ($K_d = 1.3$ nM) is several thousand times higher than the affinity for binding of PCP to serum proteins (McClurkan et al., 1993). Furthermore, because the PCP in the serum was so highly protein-bound at all time points after antibody administration, and because the antibody binding sites were essentially saturated with PCP (see Figs. 2 and 5), the rate of decline in protein-bound PCP complex determined in the current experiments. We also know the biological $t_{1/2}$ of anti-PCP $[^{3}H]$Fab is unaffected by the presence of PCP (i.e., 7.8 h; Proksch et al., 1998). Other studies report monoclonal and polyclonal Fab $t_{1/2}$ values from 1.3 to 16.3 h in rats (Arend and Silverblatt, 1975; Pentel et al., 1988; Sabouraud et al., 1992; Bazin-Redureau et al., 1997). In contrast, the functional $t_{1/2}$ of IgG-PCP determined in these studies (15.4 days) was much longer than any previous values for IgG in the rat (i.e., $t_{1/2}$ values of 2.1 to 8.1 days; Arend and Silverblatt, 1975; Bazin-Redureau et al., 1997). We think this was due to the ability of the anti-PCP IgG to maintain PCP in the serum in a highly protein-bound form (>90%) for at least 27 days after IgG administration.

Previous studies show that ~60% of the anti-PCP Fab dose is eliminated in the first 3 h after i.v. bolus administration, with a $t_{1/2}$ of ~30 min. The remaining 40% of the Fab dose is eliminated more slowly with a $t_{1/2}$ of 7.8 h (Proksch et al., 1998). Thus, the short duration of brain protection from PCP found after Fab administration (<4 h; Fig. 3) was most likely due to a combination of rapid renal elimination of the Fab (60% would have been eliminated by 4 h) and the continuous PCP infusion (15% of the PCP steady-state body burden is replaced every hour). In contrast, because intact IgG is eliminated much more slowly, the longer duration of complete PCP removal from the brain observed after IgG administration (<8 h; Fig. 6) probably reflects the time required for equilibration of the antibody-PCP binding, the saturation of antibody, and the infusion of additional PCP into the system.

In the IgG experiments, brain PCP concentrations remained significantly below steady-state levels for at least 27 days (Fig. 6). The exact mechanism for this finding was unclear, and it was surprising because free PCP concentrations in serum were not different before and after antibody administration. Our previous studies of a single i.v. bolus dose of PCP followed by a single i.v. bolus dose of Fab find that the free serum PCP concentrations are unaffected by antibody administration, and yet the PCP is substantially removed from the brain (Valentine and Owens, 1996), and PCP-induced behavioral effects are rapidly reversed (Valentine et al., 1996; Hardin et al., 1998). These previous and current results appear inconsistent with a basic rule of pharmacology, which states that the free serum drug concentration is the driving force for drug distribution and the prediction of pharmacological effects. However, there is a possible explanation. PCP appears to be nonrestrictively cleared from the brain but not the testis under normal conditions (Valentine and Owens, 1996). Thus, under normal conditions (i.e., in the absence of anti-PCP IgG), most of the PCP in the cerebral blood is removed with each pass through the brain and protein binding would not affect the clearance. In contrast, in the presence of the long-acting, high-affinity anti-PCP IgG, protein binding is a significant factor, and PCP clearance is converted from a nonrestrictive to a restrictive-type clearance. Thus, only the free fraction is removed with each pass through the brain. However, we realize that more studies will be needed to fully understand these important findings and their implications for medication development.

In the current studies, it appears that the antibody is capable of sequestering significant amounts of PCP in the blood even though on a mole basis it should not be capable of binding all of the PCP that is present in the body. This interpretation is consistent with the long functional $t_{1/2}$ of the IgG-PCP complex and the prolonged serum protein binding of PCP. These pharmacokinetic findings are supported by the pharmacodynamic studies of Hardin et al. (1999) showing that anti-PCP IgG can protect against the behavioral effects of repeated, high-dose challenges of PCP over a 13-day period.

In previous studies, Valentine and Owens (1996) show that when a mole-equivalent dose of anti-PCP Fab is administered after an i.v. bolus dose of PCP, the Fab can effectively redistribute PCP out of the testis. In the current studies, testis PCP concentrations were not significantly affected by Fab or IgG administration (Figs. 3 and 6). We think that redistribution of PCP from the testis is too slow to benefit from the antibody binding before the antibody became saturated with newly infused drug. The results from other PCP tissue distribution studies in our lab are consistent with this hypothesis (Valentine and Owens, 1996). These data show that distribution of PCP into testis is slow relative to distribution of PCP into the brain. From a pharmacokinetic standpoint, these data suggest that PCP distribution and redistribution from the brain are blood flow limited, whereas in the testis these processes are diffusion or membrane limited.

A significant implication of the current studies is that mole-equivalent doses of antibody to the total body-burden of drug may not be needed to significantly reduce drug-induced behavioral effects. Because PCP equilibration between brain and serum appeared to be faster than equilibration between the serum and other tissues like the testis, the brain benefits more from the protective effects of the antibody. In other words, binding of PCP by the antibody was on a first-come, first-served basis, and the PCP in the brain appeared to reach the antibody faster than PCP from other tissues. This descriptive explanation of the mechanism may help to account for the observation that brain PCP concentrations were decreased even 27 days after a single dose of IgG. Previous behavioral studies support this finding. For instance, Hardin et al. (1998) find that Fab doses as low as 0.18 mol-equivalent to the PCP dose significantly reduce PCP-induced behavioral effects. In addition, Hardin et al. (1999) find that a single large anti-PCP IgG dose can significantly reduce PCP-induced effects even with repeated PCP doses for at least 13 days.

In summary, these studies showed that administration of anti-PCP Fab or anti-PCP IgG produced a dramatic, complete removal of PCP out of the brain for 2 to 4 h during a continuous infusion of PCP. Although repenetration of PCP
into the brain occurred within several hours after antibody administration. PCP was sequestered in the serum through high-affinity binding with a functional $t_{1/2, Axel}$ value of 9.4 h and 15.4 days for Fab and IgG, respectively. In addition, the removal of PCP from the brain appeared to occur in preference to more slowly equilibrating tissues such as the testis. This could have important clinical implications because the brain is clearly the organ that is most directly involved in the pharmacologic effects of drugs of abuse. Finally, these studies suggest that high-affinity, anti-drug IgG could act as a long-acting antagonist to provide long-term protection from drug effects in the central nervous system.

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References


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