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Phencyclidine Pharmacokinetics and Concentration-Response Relationships in the Pigeon

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OWENS, S. M., D. E. McMILLAN, W. C. HARDWICK AND W. D. WESSINGER. *Phencyclidine pharmacokinetics and concentration-response relationships in the pigeon.* PHARMACOL BIOCHEM BEHAV **35**(4) 797–801, 1990.—Phencyclidine (PCP) pharmacokinetics and drug discrimination were examined in pigeons (n = 6 in both groups) after intramuscular doses of 1.48 mg/kg. PCP absorption was rapid with maximum measured plasma concentrations ranging from 559 to 1450 ng/ml at 10–30 min after dosing, which corresponded to the time of maximum PCP stimulus effects in the drug discrimination studies. The terminal elimination half-life was 0.88 hr (harmonic mean). Average values for the volume of distribution and total body clearance were 1.6 l/kg and 18.2 ml/min/kg, respectively. In the behavioral studies, pigeons discriminated PCP-like effects from about 2 min to 2 hr after dosing. An average value for response on the PCP-appropriate key and for PCP concentration at each time point from 2 min to 2 hr was calculated from the individual subject data. Least-squares linear regression analysis of these data showed a highly significant relationship between the ability to discriminate PCP and log PCP concentration ($y = 103x - 219$, $r^2 = .810$, $p<0.005$). This analysis suggests PCP concentration is a good predictor of behavioral efficacy.

Phencyclidine Pigeons Pharmacokinetics Kinetics of response Intramuscular dosing Drug discrimination

THE study of phencyclidine (PCP) and similar compounds in drug discrimination studies has made important contributions to our understanding of the pharmacological effects and mechanism of action of PCP-like drugs. Indeed, the strong quantitative correlation between relative potency data from PCP drug discrimination studies and [^{3}H]PCP receptor binding studies (4, 10, 15) provides critical evidence for establishing that binding of compounds to the PCP receptor produces pharmacological effects.

The first study to investigate the kinetics of the pharmacological effects of PCP (2) showed that the duration of PCP-induced anaesthesia in monkeys was directly related to the size of the intravenous dose. In a more recent report on the relationship between PCP concentration and pharmacological response, McMillan *et al.* (4) showed correlation between PCP concentration and discriminative stimulus effects of PCP after cumulative and noncumulative intramuscular (IM) doses of PCP in pigeons. However, these data did not provide a complete description of the pharmacokinetics or response profile of PCP because the first blood specimen was not collected until 15 min after the start of the experiment and only three additional blood specimens were collected over the next 90 min. Inasmuch as the terminal elimination half-life ($t_{1/2}$) of PCP in pigeons is about 2 hr after intravenous doses (7), a 90-min blood collection period covers less than one $t_{1/2}$ of the drug.

PCP pharmacokinetics have been determined in a wide variety of animals after intravenous doses (6,7). Using these interspecies pharmacokinetic data, Owens *et al.* (7) showed that PCP pharmacokinetic parameters such as half-life, total body clearance and volume of distribution can be mathematically correlated with animal body weight, regardless of the species (i.e., man, monkey, dog, rat, mouse and pigeon). However, these studies did not characterize PCP concentration-time profiles after routes of administration such as IM or intraperitoneal, which are the usual ways of administering PCP in behavioral experiments [e.g., (4, 5, 13)].

Therefore, neither the pharmacokinetics nor the mathematical relationship between PCP concentration and effect have been adequately described after nonintravenous routes of administration. These data would be useful in better understanding how PCP pharmacokinetics and changes in PCP plasma concentration over time can affect the animals' ability to discriminate PCP-like discriminative stimulus effects. The purpose of our studies was to characterize PCP pharmacokinetics and drug discrimination in pigeons after IM dosing. These data were then used to determine the relationship between PCP concentration and PCP discrimination in the pigeon.

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METHOD

Pharmacokinetic Studies

Six male White Carneaux pigeons (Palmetto Pigeon Plant, Sumter, SC) were used for each study. PCP hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD). A 1.48 mg/kg dose (expressed as the free base) of PCP in sterile saline (0.1 ml/100 g body weight) was administered as a bolus into the breast muscle. Blood samples were collected from a brachial vein through an indwelling catheter inserted before beginning the study. The times of blood collection were 1, 2, 5, 10, 15, 20, 30 min and 1, 2, 4, 6 and 8 hr. No more than 3.5 ml of whole blood was obtained from each bird during the entire 8-hr pharmacokinetic study. The catheter was kept patent by occasional flushing with heparinized saline. All of the animals had received PCP in the past but were drug free at the time of the study.

Analysis of Samples

PCP concentrations in plasma samples were determined in duplicate using a radioimmunoassay [RIA; (7,9)]. Briefly, the RIA used high affinity goat anti-PCP serum, an [^{125}I]PCP radioligand and a rabbit anti-goat solid phase second antibody separation method. PCP plasma concentrations were calculated from a PCP standard curve, using the logit-log transformation of the average counts of duplicate RIA tubes (12). Since the radioimmunoassay procedure was highly sensitive, only 1–20 μl of plasma per time point was required for analysis. This RIA is specific for PCP and does not crossreact significantly with known PCP metabolites (7,8).

Behavioral Studies

Because the collection of blood specimens from pigeons during drug discrimination studies would have disrupted the behavioral measurements, we used a separate group of animals for the behavioral studies. Six male White Carneaux pigeons with a long history of training to discriminate PCP (1.0 mg PCP HCl/kg or 0.87 mg PCP free base/kg) from saline were used. Throughout the experiments all birds were maintained at 80% of their free-feeding weights (525–703 g).

Behavioral Apparatus

A standard pigeon operant test chamber, housed inside a sound- and light-attenuating enclosure (Ralph Gerbrands Co., Arlington, MA) was used in all behavioral experiments. The operant chamber was equipped with three response keys which could be transilluminated by lights of various colors. A relay mounted in the chamber provided auditory feedback for responses on the side keys. Two DC houselights illuminated the chamber except during feed cycles, when the feeder opening was illuminated by separate lights. Experiments were programmed and data recorded by a TRS-80 Model IV microcomputer (Tandy Corp., Fort Worth, TX).

Behavioral Testing

Pigeons had been trained to peck a white center key to turn it off and light two side keys, one with a green light and one with a red light. Five responses on either side key turned off the side keys and relighted the center key. The position of the red and green side keys changed randomly each time they were lighted. Completion of 10 of these 5 response sequences on the "correct" key produced food during training. The red key was designated as correct if PCP was administered 10 min before the session, while the green key was designated correct if saline was administered 10 min before the session (5-sec access to grain). Birds were trained five days per week.

During the determination of the time course for the discriminative stimulus properties of PCP, a modified procedure was used. Birds were injected IM with a 1.48 mg/kg dose of PCP in saline in a volume of 0.1 ml/100 g body weight. This was the same dose of PCP used for the pharmacokinetic studies. The bird was then placed in the operant chamber and the session was initiated 1 min after the injection. The birds received access to food after the first 5 responses on either the red or green key, whichever occurred first during the 30 sec trials. If the bird did not make 5 responses on one of the two side keys within 30 sec, the trial was terminated. Additional trials were initiated at 2, 5, 10, 20, 30 min and 1, 2 and 4 hr after the injection. The birds were removed from and then returned to the test chamber just prior to each trial. The time points for collection of behavioral data correspond to the first 4 hr of time points of blood collection in the pharmacokinetic studies. If birds failed to respond on either key during several trials, their data were not included in the average values.

Data Analysis

We chose to use model-independent methods (1) for the pharmacokinetic analysis of the IM data since the majority of PCP pharmacokinetic studies have used this method of data analysis (6,7). For the model-independent area method, a least-squares linear regression equation was fitted to the terminal log-linear plasma PCP concentration-time data to compute an estimate of the terminal elimination rate constant (λn) and $t_{1/2}$ ($t_{1/2} = 0.693/\lambda n$). Average values for the terminal elimination $t_{1/2}$ for the six birds were calculated as the harmonic mean (11). The area under the PCP concentration-time curve (AUC) from the time of dosing to the last measured time point (tn) was calculated by the linear trapezoidal rule. The remaining area to time infinity was determined from the last measured concentration (Cn) and λn using the following equation:

$$AUC_{tn}^{\infty} = \frac{Cn}{\lambda n}$$

The sum of the above areas gave the total AUC. Total body clearance (CLs) and the apparent volume of distribution (V_β) were determined by:

$$Cls = \frac{dose}{AUC_0^{\infty}}$$

$$V_\beta = \frac{CLs}{\lambda n}$$

Model-dependent, nonlinear least-squares regression analysis (RSTRIP; Micromath, Inc., Salt Lake City, UT) was also performed on the IV data of Owens *et al.* (7) to determine the distribution half-life for use in interpreting the IM data in the current study. A two-compartment model with $1/y^2$ weighting was found to provide the best-fit to the data sets. Nonlinear regression analysis was also used to fit a curve to the representative plasma concentration-time data shown in Fig. 1 and the average response- and concentration-time data in Fig. 2. A least-squares linear regression equation was fitted to the average percentage response on the PCP key-log PCP concentration data. Only values from 2

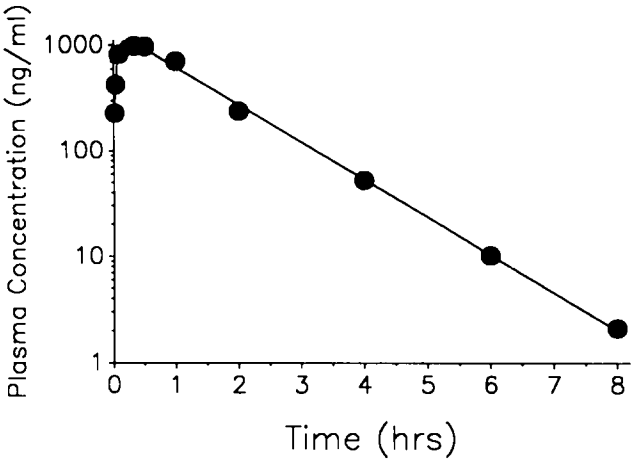

FIG. 1. Representative PCP plasma concentration-time curve after IM dosing of a male pigeon with 1.48 mg PCP/kg. The solid line represents a nonlinear regression curve fit to the data. The pharmacokinetic parameters calculated for this pigeon (P1) are shown in Table 1.

min to 2 hr were included in this analysis because this was the time period during which the birds were able to discriminate the PCP stimulus.

RESULTS

Figure 1 shows a representative PCP plasma concentration-time curve from one of the pigeons (pigeon P1 in Table 1). Peak concentrations were reached in 10–30 min in all the birds. The concentration-time profiles of four of the birds appeared to be best described as a simple absorption and terminal elimination phase as is seen in Fig. 1. The other two birds appeared to have a short distribution phase just after the peak concentration, but before entering the terminal elimination phase. By 1–2 hr after dosing all of the birds were clearly in the terminal elimination phase. Table 1 summarizes PCP pharmacokinetic parameters in the six pigeons after IM dosing.

Figure 2 compares the average percentage of responses on the PCP key with the average log concentration at the same time

TABLE 1

PHARMACOKINETIC PARAMETERS OF PCP IN THE PIGEON AFTER AN IM ADMINISTRATION OF THE DRUG

Pigeon	Body Weight kg	$t_{1/2}$ hr	V_β l/kg	CLs ml/min/kg
P1	0.545	0.88	1.1	14.8
P2	0.436	1.0	0.96	11.1
P3	0.505	1.1	1.4	15.4
P4	0.432	0.83	1.7	23.1
P5	0.578	1.9	3.2	19.6
P6	0.420	0.48	1.1	25.3
Mean	0.486	0.88*	1.6	18.2
(±SD)	(0.066)		(0.84)	(5.4)

*Calculated as the harmonic mean (11).

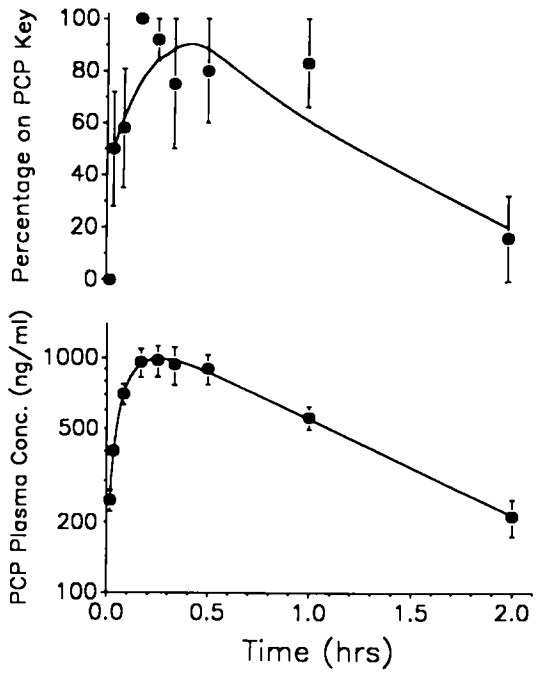

FIG. 2. Mean percent response on the PCP key (upper panel, ±SE, n = 6) and mean PCP plasma concentration (lower panel, ±SE, n = 6) as a function of time. The nonlinear regression least-squares two-compartment model fit to these data sets (represented by the solid lines) predicted the times for maximal response and concentration were 24.6 min and 15.6 min, respectively. In both studies, pigeons were administered a 1.48 mg/kg intramuscular dose of PCP. If birds failed to respond on either key during several trials, their data were not included in the averaged values for mean percentage response on the PCP-appropriate key. See Fig. 3 for the linear regression analysis of these data.

points in the two sets of pigeons (n = 6 in each group). The two-compartment model fit to these data sets (Fig. 2) predicted the time for maximal response and concentration would have occurred at approximately 24.6 min and 15.6 min, respectively. Therefore, the highest percentage of responses on the PCP-appropriate key occurred at about the same time as the highest PCP concentrations. By 2 hr after PCP administration responding occurred primarily on the saline-appropriate key.

Figure 3 shows the correlation between the percentage of responses on the PCP-appropriate key and log PCP concentration. These variables were significantly ($p<0.005$) and positively correlated. Extrapolation of the regression line to 0% and 100% response on the PCP concentration axis predicted responding on the PCP key is unlikely to occur at plasma concentrations below 134 ng/ml and likely to be maximal at a concentration of 1251 ng/ml.

DISCUSSION

Peak concentrations of PCP were achieved 10–30 min after the IM dose in the pigeons. Two of the six birds showed considerably lower maximum plasma concentrations of PCP (P5 = 559 ng/ml and P6 = 657 ng/ml compared to 1240, 1290, 1447, 1450 ng/ml for the other four birds). Also, individual variations in pharmacokinetic values (Table 1) for $t_{1/2}$ (e.g., 1.9 and 0.48 hr), V_β (e.g., 3.2 l/kg) and CLs (e.g., 11.1 and 25.3 ml/min/kg) were significant enough to suggest that pharmacokinetic factors could

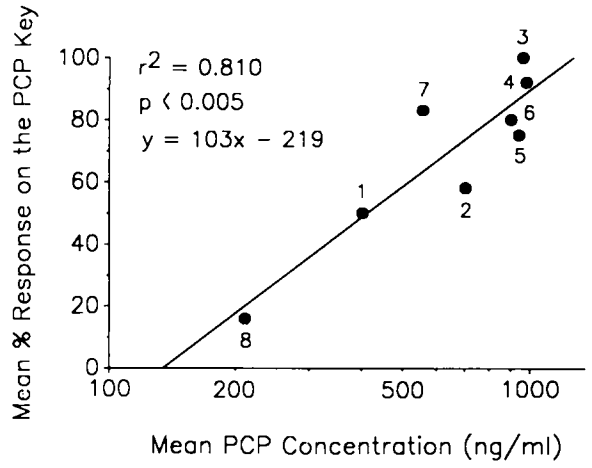

FIG. 3. Relationship between mean percent response on the PCP key and PCP plasma concentration in pigeons from 2 min to 2 hr after dosing. Values for the apparent minimum concentration and the apparent maximum concentration were 134 ng/ml and 1251 ng/ml, respectively. These values were obtained by extrapolation of the line to 0% and 100% response, respectively. The number (1–8) by each data point corresponds to the order of collection of the data from 2 min to 2 hr after dosing (see Fig. 2). Note that the data points go progressively up then down the same general straight line.

be a potential source of variation in the magnitude and duration of the behavioral effects in individual animals. Indeed, some of the variation in the ability of the birds to discriminate PCP (upper panel of Fig. 2) could have been due to differences in plasma concentrations.

As can be seen in the respresentative plasma concentration-time curve shown in Fig. 1, absorption was rapid. Based on a model-dependent pharmacokinetic analysis of previously reported PCP concentration-time data from pigeons after IV administration (7), the distribution half-life for PCP ranged from 7–27 min (harmonic mean = 12 min). Therefore, PCP distribution was essentially complete in 0.5–2 hr after IV administration (i.e., about four distribution half-lives). Since PCP peak concentrations occurred at 10–30 min after IM doses and the concentration-time data was linear by 1–2 hr, it appears that the PCP absorption rate was faster than the distribution rate; but, it was not sufficiently different to allow an accurate calculation of the absorption rate constant.

When the PCP IM pharmacokinetic data from this study were compared with the PCP IV pharmacokinetic data from a previous study conducted in this laboratory (7), some important differences are noted. The terminal elimination $t_{1/2}$ for PCP after IM dosing was much shorter than after IV dosing (0.88 hr versus 2.1 hr after IV dosing). On the average, the apparent volume of distribution was about four times smaller after the IM administration (1.6 l/kg versus 6.8 l/kg). The systemic clearance after IM dosing was about one-half the value found after IV dosing (18.2 ml/min/kg versus 34.5 ml/min/kg). If there was incomplete absorption or a significant delay in the absorption, this could have produced some of these relative changes. Based on the apparent rapid absorption and linear terminal elimination phase after IM dosing, we would not have predicted these effects. It is also possible that PCP produced somewhat different physiological effects, which could have affected the pharmacokinetics. For instance, a decreased blood flow to the liver (compared to physiological effects after an IV administration) could have resulted in a decreased metabolic clearance. Woodworth *et al.* (14) have shown that, at least in the dog, PCP hepatic clearance approaches a condition of nonrestrictive clearance and would therefore be dependent to some degree on hepatic blood flow. We do not know how the drug was eliminated in the pigeon (e.g., metabolism and/or excretion), however, in mammals the major route of elimination is metabolism (6) and PCP pharmacokinetic parameters for the pigeon correlate quite nicely with data from these mammals after correcting for body weight and applying appropriate species scaling factors (7). Regardless of the mechanism, administration of PCP into the pigeons breast muscle tends to decrease the terminal elimination $t_{1/2}$, volume of distribution and systemic clearance compared to an IV dose.

A direct relationship was found between average values for the percentage of responses on the PCP-appropriate key and the log PCP concentration in plasma (Figs. 2 and 3). The shape of the response-time curve for PCP as a discriminative stimulus (Fig . 2, upper panel) and the log concentration-time curve (Fig. 2, lower panel) for PCP in plasma were very similar. Since none of the pigeons responded on the PCP-appropriate key after the first 2 hr and the pharmacokinetic data indicated that by this time absorption and distribution were complete, it would appear that the animals did not recognize the effects of PCP for very long during the terminal elimination phase. Although some of our response measurements were outside what should have been the most linear portion of the response-log concentration curve (i.e., below 20% and above 80% ; Fig. 3), the least-squares linear regression model still showed a good fit to the data. As can be seen in Fig. 3, the eight average response-concentration data points go progressively up, then down the same general straight line. Therefore, we observed no hysteresis-like effect such as is seen when response lags significantly behind peak plasma concentrations. In fact, the nonlinear regression curve fits to the two data sets (Fig. 2) were very similar in shape and the predicted times for maximal response on the PCP key and concentration were only 9 min apart (i.e., 24.6 min and 15.6 min, respectively). Overall, these data indicated there was a direct relationship between PCP concentration and selection of the PCP-appropriate key in drug discrimination.

Based on the 0% and 100% response intercepts on the concentration axes in Fig. 3, the apparent minimum and maximum concentrations which elicited PCP-appropriate responding in the pigeons (Fig. 3) were 134 and 1251 ng/ml, respectively. This minimum value was slightly less than the value of about 150–200 ng/ml which McMillan *et al.* (5) report as the approximate minimum concentration for discrimination of a dose of PCP from a saline injection after noncumulative dosing procedures. In the study by McMillan *et al.* (5), pigeons were trained to discriminate 1.5 mg of PCP HCl/kg (1.3 mg PCP free base/kg) from saline, compared to the training dose of 1.0 mg of PCP HCl/kg (0.87 mg PCP free base/kg) used in the present study. The lower minimum generalized concentration is consistent with the general observation that lower training doses confer increased sensitivity to discriminative stimulus properties of drugs. In this earlier report the determination of the minimum generalized concentration was based on an empirical evaluation of the concentration-response data from four time points (i.e., 15, 30, 60 and 120 min) and not a mathematical evaluation of the entire concentration-response time course (i.e., our study used eight time points from 2 min to 2 hr; see Figs. 2 and 3).

In summary, PCP IM pharmacokinetic parameters in the pigeons were fairly reproducible but different from those found after IV administration of the drug. The response-log concentration data suggested that PCP concentration was a good predictor of simultaneously occurring drug discrimination. Since PCP is known to interact with its central nervous system receptor in a reversible fashion (15), PCP kinetics of response for drug discrimination can be classified as a simple, directly reversible pharmacological response.

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