Pharmacology Biochemistry & Behavior, Vol. 25, pp. 117–121, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Relationship of Plasma Phencyclidine Levels to Phencyclidine Discrimination in the Pigeon

D. E. McMILLAN, W. C. HARDWICK

Department of Pharmacology and Interdisciplinary Toxicology
University of Arkansas for Medical Sciences, Little Rock, AR

D. J. CANNON AND L. COUCH

John L. McClellan Memorial Veterans Administration Medical Center, North Little Rock, AR

Received 5 August 1985

McMILLAN, D. E., W. C. HARDWICK, D. J. CANNON AND L. COUCH. *Relationship of plasma phencyclidine levels to phencyclidine discrimination in the pigeon.* PHARMACOL BIOCHEM BEHAV **25**(1) 117–121, 1986.—Plasma phencyclidine levels were determined in pigeons trained to discriminate 1.5 mg/kg phencyclidine from saline under a second-order schedule using a color-tracking procedure. With both cumulative and non-cumulative dosing procedures, pigeons reliably discriminated plasma phencyclidine levels above 200 ng/ml. When the time course of phencyclidine discrimination was determined and compared with the time course of phencyclidine levels in plasma in a different group of birds, a similar relationship between discrimination and plasma phencyclidine was generally observed. Plasma phencyclidine levels did not correlate well with position responding observed in some birds after lower phencyclidine doses.

Plasma phencyclidine Phencyclidine Drug discrimination Pigeon Color tracking

DURING the past 5 years we have performed a series of experiments in pigeons trained to discriminate phencyclidine from saline using a color tracking procedure under second order schedules [4–6]. In most of these experiments we have used variations of the cumulative-dosing procedure described by Wenger [7] to study response generalization from the training dose to other doses of phencyclidine.

In performing cumulative-dosing studies in the absence of empirical pharmacokinetic data, one of two assumptions can be made. One can assume that when second, third and fourth doses in a sequence are given, all of the previously administered doses are still available. Conversely, one could assume that at the time that later doses in a series are given, all of the previous doses have disappeared, so that the repeated doses are independent. The first alternative seems unlikely, as distribution, metabolism, and excretion are proceeding continuously and the net result may be difficult to predict. The second alternative also seems unlikely, since with injections spaced only 10 to 15 min apart in our experiments, it is highly unlikely that drug elimination is proceeding rapidly enough to eliminate the drug in this short time period. However, pharmacokinetic data are generally not available for the pigeon and we know of no pharmacokinetic data on cumulative dosing in the pigeon. The purpose of the present experiments was to collect data comparing phencyclidine blood levels with cumulative and non-cumulative dosing procedures and to correlate these blood levels with phencyclidine discrimination under similar experimental conditions. From such data it might be possible to make statements about the phencyclidine stimulus in the pigeon in terms of plasma levels rather than dose administered.

METHOD

Subjects

The same male White Carneaux Pigeons (n=5) used in previous studies in this series [3–6] were used in both behavior studies and studies on phencyclidine levels in plasma after cumulative and non-cumulative dosing. In addition, 4 other pigeons subjected to training to key peck for food, but not drugged previously, were employed to study the time course of phencyclidine in plasma. All pigeons were deprived of food and maintained at 80% of their free-feeding weights (525–703 g) throughout all experiments.

Behavioral Apparatus

A Model G7313 sound- and light-attenuating enclosure (Ralph Gerbrands Co.) was used in all behavioral experiments. The chamber was equipped with three response keys which could be transilluminated by lights of various colors. The force required to operate the keys was 0.05 N. A relay mounted in the chamber provided auditory feedback for responses on the side keys. Two DC houselights illuminated

the chamber except during the feed cycle, when the feeder opening was illuminated by separate lights. Experiments were programmed and data recorded by a TRS-80 Model IV microcomputer (Radio Shack).

Behavioral Procedure

All pigeons used in the behavioral experiments had been trained to peck a white center key once to turn it off and to light the side keys, one with a red light and one with a green light. Completion of 5 responses (FR 5) on a lighted side key extinguished the lights behind both side keys and relighted the center key, after which a response again was required to light the side keys. Completion of 10 FR 5s on the correct side key produced food. Key pecks on the red side key were correct if 1.5 mg/kg phencyclidine had been administered before the session and key pecks on the green side key were correct if saline had been administered before the session. Position of the red and green colors on the side keys varied randomly each time that the side keys were lighted. Using the terminology of Kelleher [2], this is a second order FR 10 (FR 5) schedule. Birds received these training sessions Monday through Wednesday and a cumulative dose-effect curve was determined on Thursday or Friday.

Under the cumulative dosing procedure, the first injection was given, and after a 10-min delay, a bird was tested for stimulus control with food being delivered whenever the schedule requirement was met (10 FR 5s) on either key. Immediately after food delivery or after 10 min without delivery of food, whichever occurred first, the bird was removed from the cage and injected with the second dose. Doses administered to each bird were 0.3, 0.26, 0.44 and 0.7 mg/kg to produce cumulative doses of 0.3, 0.56, 1.0 and 1.7 mg/kg. As a control experiment, a series of four cumulative saline injections were administered and behavior was determined after each injection in a manner identical to that used for experiments on cumulative phencyclidine dosing.

In a separate series of experiments, single intramuscular doses of 0.3, 0.56, 1.0 and 1.7 mg/kg were given non-cumulatively to each bird 10 min before the behavioral session. Two birds received these doses in an ascending series and two in a descending series. During two additional sessions, saline was injected instead of phencyclidine as a control. Non-cumulative injections were administered to each bird twice weekly during these experiments.

Phencyclidine was used as the hydrochloride salt and all doses refer to the salt. Injections were intramuscular in a volume of 1.0 ml/kg.

Analysis of Plasma Phencyclidine Levels

Drugs and chemicals were obtained as follows: phencyclidine hydrochloride from Research Triangle Institute (Research Triangle Park, NC) as approved by the National Institute on Drug Abuse; ketamine hydrochloride from the U.S. Pharmacopeia Convention (Rockville, MD); reagent grade sodium hydroxide, sodium borate and hydrochloric acid from Fisher Scientific (Pittsburgh, PA); HPLC grade n-heptane from Baker Chemical Co. (Phillipsburgh, NJ); and nanograde dichloromethane from Mallinckrodt, Inc. (Paris, KY).

Blood was drawn from two groups of pigeons. One group of 4 birds (see the Subjects section) was used to measure the time course of 1.5 mg/kg phencyclidine in plasma. These birds were drug naive, but they had been food deprived and

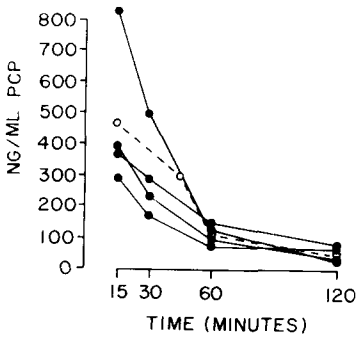

FIG. 1. Time course of phencyclidine in pigeon plasma following intramuscular injection. Abscissa: time in minutes. Ordinate: ng/ml phencyclidine in plasma. Filled circles show data from individual birds and the unfilled circles show mean data.

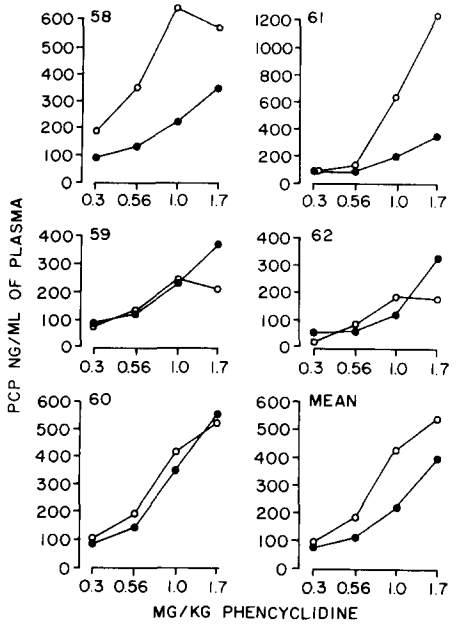

FIG. 2. Comparison of phencyclidine plasma levels with cumulative (filled points) and non-cumulative dosing (unfilled points). Abscissa: mg/kg phencyclidine, log scale. Ordinate: ng/ml phencyclidine in plasma. Numbers in each frame show data from individual birds. Mean data are shown in the lower right frame. Blood samples were taken 10 min after intramuscular injection of phencyclidine to coincide with the beginning of the behavioral test sessions.

undergone behavioral training, although they had not been used to study the discriminative stimulus properties of phencyclidine. The second group of 5 birds were the same birds used to study the discriminative stimulus properties of phencyclidine. Blood was drawn from these birds according to the same schedules described for cumulative and non-cumulative dosing for behavioral measurements; however, blood sampling and behavioral measurements were not done on the same day since it appeared likely that repeated blood sampling would have affected the behavior. Therefore, the same protocol was used for blood sampling and behavioral measurements, but the experiments were performed on different days.

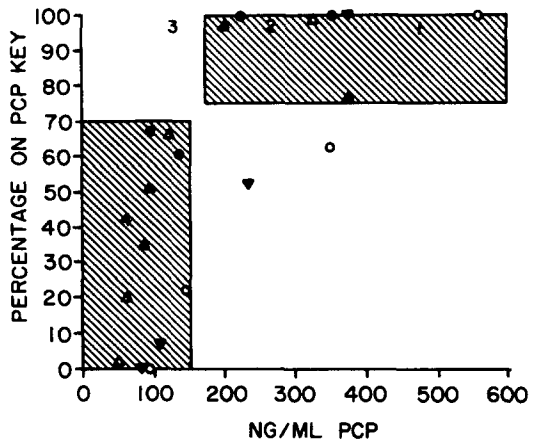

FIG. 3. Responding on the phencyclidine key as a function of plasma phencyclidine level during cumulative dosing (symbols) and at different time points of after a single dose (numbers). Ordinate: percentage of total responses on the phencyclidine (red) key. Abscissa: ng/ml phencyclidine in plasma. Each symbol represents a data point from a single dose in an individual pigeon as follows: ●, No. 58; ▼, No. 59; ○, No. 60; ▲, No. 61; △, No. 62. Numbers represent mean percentage of responses on the phencyclidine key plotted against mean phencyclidine plasma level in the group of birds not used in the behavioral studies at 15 min (1), 30 min (2), 60 min (3) and 120 min (4) after administration of 1.5 mg/kg phencyclidine.

Approximately 1.0 ml of blood was drawn from the brachial vein into a heparinized syringe. The whole blood was centrifuged and the plasma was collected and frozen for later analysis by a modification of the procedure of Kammerer *et al.* [1]. To 50 μl of sample, 50 μl (95 pmol) of ketamine, 20 μl 10 N NaOH and 1.5 ml heptane were added to capped polypropylene tubes. The tubes were shaken for 15 min followed by centrifugation at 3000 rpm for 2 min. A 1.3 ml aliquot of the heptane layer was transferred to a clean polypropylene tube containing 200 μl of 0.1 M HCl and the tubes shaken for 15 min and then centrifuged at 3000 rpm for 2 min. The organic phase was aspirated and 180 μl of the acid phase was transferred to a polypropylene microtube containing 25 μl of 0.2 M sodium borate in 2.5 M NaOH and 50 μl dichloromethane. The capped tubes were vortexed for 30 sec and then centrifuged. The aqueous phase was aspirated and 2 μl of organic phase was injected into a Hewlett-Packard 5993B GC-MS analyser with splitless injection. A 0.2 mm × 12.5 m capillary column of crosslinked methyl silicone (Hewlett Packard) was used. The initial temperature was 150°C, and after 1 min it was increased at 15°C/min for 5 min to 210°C. The injection port temperature was 210°C. Identification of peaks was by selected ion monitoring. Five ions were monitored during each run; 91, 200 and 243 for phencyclidine and 180 and 209 for ketamine. The base peak ratio 200/180 was used in all calculations. A standard curve from 0.4 to 3.4 pmol phencyclidine with 3.8 pmol of ketamine was run daily. With every run, control phencyclidine samples in drug-free serum were analyzed. Standard curves were linear with a correlation coefficient of 0.999. Recoveries of phencyclidine ranged from 75–100%. The detection limit for phencyclidine was 0.2 pmol per injection.

RESULTS

Figure 1 shows the time course of phencyclidine in plasma from 15 min after intramuscular injection to 2 hr after injection. The highest phencyclidine levels in plasma were observed 15 min after administration and declined rapidly during the next 45 min. By two hr after administration, the phencyclidine levels in plasma had dropped to less than one third of the levels observed at 15 min after injection.

Figure 2 shows a comparison between blood levels achieved with cumulative dosing (filled points) and non-cumulative dosing (unfilled points). For birds 59, 62 and 60 quite similar dose-dependent blood levels were generated under the two procedures. For birds 58 and 61, the non-cumulative dosing procedure resulted in higher plasma levels, especially at the higher dose levels. The mean curve for non-cumulative dosing was slightly higher than that for cumulative due to these latter two birds.

In Figure 3, the percentage of total responses on the phencyclidine-associated key (red key) has been plotted against the plasma phencyclidine level in the same birds under the cumulative-dosing procedure, using identical protocols in the same birds to generate the behavioral measurements and the plasma phencyclidine levels. If an arbitrary criterion of 75% responding on the phencyclidine key is adopted, so that the bird must respond on the red key 75% of the time before the birds can be considered to have discriminated phencyclidine, an orderly relationship between plasma phencyclidine level and discrimination of the phencyclidine stimulus evolves. In only 2 of 9 instances with phencyclidine plasma levels above 200 ng/ml, did a bird fail to respond more than 75% of the time on the phencyclidine key and in both of these instances the bird responded on the phencyclidine key more than 50% of the time. In no instance did birds

TABLE 1

PERCENTAGE OF RESPONSES ON THE PHENCYCLIDINE KEY AFTER CUMULATIVE AND NON-CUMULATIVE ADMINISTRATION OF SALINE

Bird	Cumulative	Non-Cumulative
58	12	0
	0	2
	5	7
	7	—
59	0	0
	1	0
	0	—
	0	—
60	1	0
	12	0
	5	—
	22	—
61	11	0
	15	0
	34	—
	8	—
62	0	2
	0	0
	3	—
	0	—

Only two or three sessions were devoted to non-cumulative saline administration.

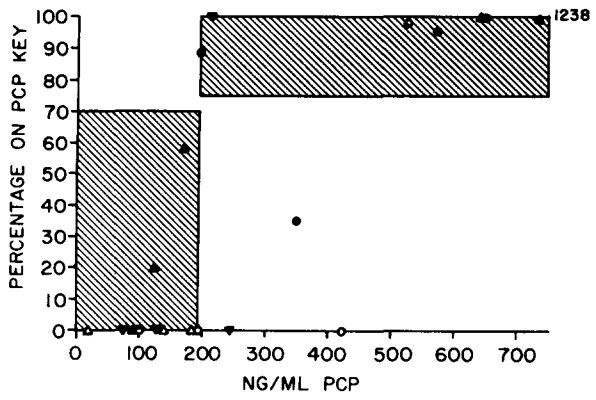

FIG. 4. Responding on the phencyclidine key as a function of plasma level during non-cumulative dosing. Ordinate: percentage of total responses on the phencyclidine (red) key. Abscissa: ng/ml phencyclidine in plasma. Symbols as in Fig. 3.

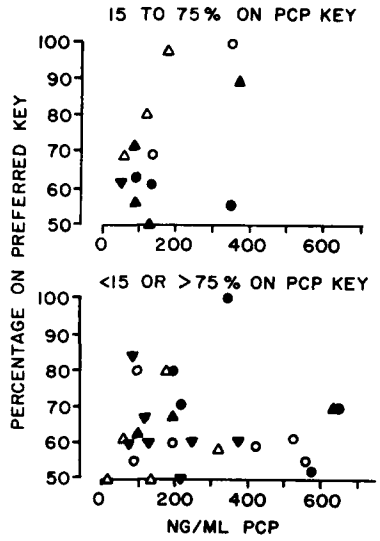

FIG. 5. Responding on the preferred key (defined as that key as which half or more of the total responses occurred) as a function of phencyclidine blood level. Ordinate: percentage of total responses on the preferred key. Abscissa: ng/ml phencyclidine in plasma. Symbols as in Fig. 3. Data are for both cumulative and non-cumulative dosing.

with plasma phencyclidine levels below 150 ng/ml respond on the phencyclidine key more than 75% of the time. Thus, it appears that under the cumulative dosing procedure pigeons can reliably discriminate a plasma phencyclidine concentration somewhere between 150 and 200 ng/ml. Under conditions where saline is administered according to a similar cumulative dosing schedule, responding on the phencyclidine key rarely rises above 15% (Table 1).

We also determined the time course for phencyclidine discrimination in these same birds following a single 1.5 mg/kg dose. Using an identical protocol with respect to the timing of injections and drawing blood samples we also measured the time course of plasma phencyclidine levels but in a different group of birds (see Fig. 1). Figure 3 includes data on the time courses of phencyclidine in plasma and the time course of phencyclidine discrimination. Since different groups of birds were used for the behavioral and the plasma observations, only means for the two groups of birds can be plotted. At 15 and 30 min after phencyclidine administration, the pigeons in the behavioral experiments were discriminating the phencyclidine stimulus and the plasma phencyclidine levels in the group of birds drugged according to the same protocol were above 200 ng/ml. At 1 hr after phencyclidine administration, the plasma level had fallen below 200 ng/ml, but the birds in the behavioral experiment were continuing to respond on the phencyclidine key. By 2 hr after administration, the phencyclidine level had fallen well below 100 ng/ml and the birds were no longer responding on the phencyclidine key. Thus, 3 of 4 points derived from time course data fit the pattern established for cumulative dosing, despite the fact that different birds were used to determine phencyclidine discrimination and phencyclidine plasma levels.

Figure 4 shows the relationship between phencyclidine plasma levels and phencyclidine discrimination in the same group of birds under non-cumulative dosing procedures. Although there are several exceptions (3 out of 20 observations), the relationship established between phencyclidine plasma level and phencyclidine discrimination using cumulative dosing procedures also seems to hold for non-cumulative dosing. The data for non-cumulative dosing suggests that 200 ng/ml marks the approximate plasma level above which phencyclidine discrimination is reliably observed, with plasma levels below 200 ng/ml rarely being discriminated.

We have shown previously [6,7] that at intermediate doses of phencyclidine, pigeons frequently adopt position responses, which results in responding to both key colors rather than tracking the location of either color. In the present experiments, we adopted a criterion of 75% responses on the phencyclidine associated key as an indication of control by the phencyclidine stimulus, although following administration of saline it was unusual for more than 15% of the responses to occur on the phencyclidine key (Table 1). Therefore, it was of interest to determine if responding on the phencyclidine key between 15 and 75% of the time was associated with position responding and if responding at a position also could be related systematically to plasma phencyclidine levels. Figure 5 shows a plot of percentage of responses on the preferred key against plasma phencyclidine level both when birds were responding 15 to 75% of the time on the phencyclidine key (weak stimulus control) and when the birds were responding less than 15% of the time or more than 75% of the time on the phencyclidine key (strong stimulus control). There did not appear to be any systematic relationship between plasma phencyclidine level and position responding.

DISCUSSION

In previous experiments we have shown an orderly relationship between phencyclidine dose and phencyclidine discrimination in the pigeon using both cumulative and non-cumulative dosing procedures [5]. The present experiments, as expected, showed that a similar orderly relationship exists between the phencyclidine dose and the plasma phencyclidine level, thereby permitting us to relate plasma phencyclidine levels to phencyclidine discrimination in the pigeon.

The data were remarkably consistent. Whether the data

on plasma levels were derived from cumulative dosing, non-cumulative dosing, or from the time course of phencyclidine following the administration of a single dose, the data indicated that phencyclidine was reliably discriminated at plasma levels above 200 ng/ml and rarely discriminated at plasma levels below that value. Although the 200 ng/ml plasma level might be considered as a discrimination threshold based on the criterion adopted for the present experiments (75% or more responses on the phencyclidine key), it is clear that something was happening at lower phencyclidine plasma levels. When saline was given either with cumulative or non-cumulative dosing, responding on the phencyclidine key rarely exceeded 15%. Figure 3 shows that responding on the phencyclidine key between 15 and 75% of the time was not infrequent at plasma levels below 200 ng/ml, even though the birds did not meet the arbitrary criterion for phencyclidine discrimination.

In drug discrimination experiments it is always difficult to interpret data when animals make both drug-associated and non-drug-associated responses during a given discrimination test. We have argued previously [5,6] that discriminative control by phencyclidine in the pigeon can be influenced by factors such as schedule bias and position preferences. These same factors may have modified stimulus control at phencyclidine plasma levels below 200 ng/ml in the present studies. However, we were not able to relate plasma phencyclidine levels to position responding by the pigeon in the present studies.

Absorption of phencyclidine in the pigeon following intramuscular administration is clearly quite rapid. We observed highest levels in plasma at 15 min after administration and higher levels may have occurred even sooner, but in the time course studies, no measurements were made prior to that time. The shape of the time course curves for phencyclidine in plasma suggests that at 15 min after phencyclidine administration, the drug is still in a distribution phase. Because of the limited number of points determined, half lives for distribution and elimination cannot be calculated from our data, but the limited data available suggest a rapid distribution of phencyclidine following intramuscular administration in the pigeon and an elimination half life of a few hours.

ACKNOWLEDGEMENTS

Supported by the National Institute on Drug Abuse, Grant DA 02251. We wish to thank the National Institute on Drug Abuse for supplies of phencyclidine, Brenda Selby for typing the manuscript, and G. R. Wenger, W. D. Wessinger and S. M. Owens for helpful comments on the manuscript.

REFERENCES

1. Kammeror, R. C., D. E. Stefano and D. Schmitz. A gas liquid chromatography assay for phencyclidine and its metabolites. *J Anal Toxicol* **4:** 293–298, 1980.
2. Kelleher, R. T. Conditioned reinforcement in second order schedules. *J Exp Anal Behav* **9:** 475–485, 1966.
3. McMillan, D. E. Generalization of the discriminative stimulus properties of phencyclidine to other drugs in the pigeon using color tracking under second order schedules. *Psychopharmacology (Berlin)* **78:** 131–134, 1982.
4. McMillan, D. E., D. A. Cole-Fullenwider, W. C. Hardick and G. R. Wenger. Phencyclidine discrimination in the pigeon using color tracking under second-order schedules. *J Exp Anal Behav* **37:** 143–147, 1982.
5. McMillan, D. E. and G. R. Wenger. Effects of barbiturate and other sedative hypnotics in pigeons trained to discriminate phencyclidine from saline. *J Exp Anal Behav* **40:** 133–142, 1983.
6. McMillan, D. E. and G. R. Wenger. Bias of phencyclidine discrimination by the schedule of reinforcement. *J Exp Anal Behav* **42:** 51–66, 1984.
7. Wenger, G. R. Cumulative dose-response curves in behavioral pharmacology. *Pharmacol Biochem Behav* **13:** 647–651, 1980.