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Relationship Between the Development of Behavioral Tolerance and the Biodisposition of Phencyclidine in Mice[1]

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FREEMAN, A. S., B. R. MARTIN AND R. L. BALSTER. *Relationship between the development of behavioral tolerance and the biodisposition of phencyclidine in mice.* PHARMACOL BIOCHEM BEHAV **20**(3) 373–377, 1984.—Mice trained on a differential reinforcement of low rate 10-sec schedule (DRL 10) were treated daily with either IP saline or 10 mg/kg of phencyclidine hydrochloride (PCP). After 21 consecutive days of treatment, dose-effect determinations for PCP were obtained in both groups. Chronic treatment with PCP resulted in approximately 1.5-fold development of tolerance to the PCP-induced reduction of reinforcement rate. Following completion of the dose-effect determinations, the mice were treated for an additional 13 days with either saline or PCP (10 mg/kg, IP). On the fourteenth day, the biodisposition of ^{3}H-PCP·HCl (10 mg/kg, IP) was studied in both groups. The ratio of the brain levels of PCP in the saline-trained animals to that in the PCP-trained animals was 1.3 to 1 which accounted in large part for the development of tolerance. It appears that dispositional factors are involved in the development of tolerance of mice to the disruptive effects of PCP on DRL performance.

Phencyclidine Behavioral tolerance Reinforcement rate

PHENCYCLIDINE (PCP), originally developed as an intravenous surgical anesthetic, has become a major drug of abuse in recent years [17]. The subjective effects of PCP in humans may include confusion, irritability, apathy, disorientation and euphoria. It has been reported that chronic daily users of the drug require escalation of dosage in order to obtain the desired psychological high [4]. Cessation of use results in a withdrawal syndrome which includes PCP craving, depression, confusion and irritability suggesting that physical dependence may develop [20].

Several studies have been conducted in an attempt to determine the degree to which tolerance to PCP can be induced in laboratory animals by repeated administration. A variety of injection regimens have been employed to maximize tolerance development but generally only modest degrees of tolerance have been obtained. A 4.6-fold tolerance developed to the effects of PCP on the rate at which rhesus monkeys responded under FI 9-min FR 1 chain schedule of food reinforcement [2] while a 1.7-fold tolerance occurred in squirrel monkeys under a similar schedule [3]. In rhesus monkeys, a nearly 2-fold tolerance developed to the effects of orally self-administered PCP on subsequent self-administration of saccharin solution [5]. Tolerance developed to the effects of PCP on the response rate of rats under a FI 1-min schedule of water reinforcement (1.8-fold) [26] and on rotarod performance of rats (1.7-fold) [18] and mice (1.2–1.6-fold) [8]. A 2.8-fold tolerance to the effects of PCP on the milk intake of rats was obtained by Woolverton *et al.* [27] and complete tolerance developed to the response rate-decreasing effects of PCP in rats under VI 60-sec schedule of water reinforcement [14]. In the latter two studies, the observed tolerance was attributed to pharmacological variables and not to behavioral compensatory mechanisms. Partial to complete tolerance developed to the response rate-decreasing effects of PCP in rats responding under FR 30 [23] and FR 10 [19] schedules of food reinforcement. First dose behavioral tolerance (which was not augmented upon continued treatment) to the rate-depressant action of PCP has been reported to occur in rats responding under a FR 4 schedule of milk reinforcement [21]. Tolerance has also been reported to the ataxia, locomotor stimulation [6,25] and stereotypy [25] produced by PCP in rats and to the duration of anesthesia in non-human primates [12].

On the other hand, increases in stereotypy ratings with chronic PCP treatment have been reported for rats [6,24] and stumptail macaques [22]. Similarly, increased sensitivity to the effects of PCP on rotarod performance, conditioned avoidance responding and locomotor activity occurred in rats following repeated treatment [19]. It must be emphasized that the above studies differ widely in the dosage and length of PCP treatment employed and, of course, in the species and specific behaviors studied.

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The contribution of biodispositional factors to the development of tolerance to PCP has not been throughly investigated. Several attempts to correlate plasma and brain levels of PCP with tolerance development have not produced conclusive evidence of a biodispositional component of the observed tolerance [9, 15, 27], although the continuous exposure of mice to PCP via implanted osmotic minipumps led to complete tolerance to the disruptive effects of the drug on motor coordination [16]. In this case, the tolerance was accompanied by enhanced hepatic enzyme activities as well as a decreased brain half-life of PCP. The aim of the present study was to further characterize the relationship between tolerance development to effects on schedule-controlled behavior and the biodisposition of PCP.

METHOD

Behavioral Procedure

Adult male ICR mice (Flow Laboratories, Dublin, VA) weighed 28–35 g at the beginning of the experiment. The animals were maintained at a relatively constant weight by daily feeding of Ralston-Purina Chow 5001 (approximately 3 g). They weighed 35–39 g at the end of the experiment. The animals were housed individually in standard plastic mouse cages (18×20×13 cm) in a room maintained on a 12-hr light-dark cycle at 23°C. Water was available ad lib in the home cages. Experimental sessions were conducted during weekday mornings using an apparatus described by Balster and Baird [1].

A week after arriving from the supplier, the mice were individually housed for a week before restricted feedings were initiated. Food access was gradually reduced over a 2-week period during which lever press training was initiated. The subjects were shaped to lever press for milk using a continuous reinforcement schedule. After the subjects were trained to lever press and adapted to the food deprivation regimen, schedule-controlled responding was shaped. The mice were trained to respond on a differential reinforcement of low rate (DRL) 10-sec schedule, where interresponse times greater than 10 sec were reinforced. The animals were given at least two months of training on the DRL schedule before drug injections were given. Saline injections (IP) were administered near the end of this period to habituate the animals to the injection routine. Experimental sessions of 30 min were conducted Monday through Friday. A stimulus light was illuminated during the session and was momentarily extinguished with each lever press. Injections were given 5 min before the session and subjects were placed in the chambers immediately after the injection.

After DRL responding was initiated, response and reinforcement rates were recorded daily. When each subject's performance reached stability, the mice were divided into two groups (N=9/group) matched for reinforcement rate. Group 1 continued to receive daily IP injections of saline while Group 2 received 10 mg/kg PCP·HCl (obtained from the National Institute on Drug Abuse). All animals received injections 7 days a week while experimental sessions continued to be held Monday through Friday. Twenty-one days later, dose-effect curves for PCP were obtained in both groups. The doses of PCP were 1, 3, 10 and 20 mg/kg and were administered in ascending order.

For dose-effect determinations, both groups of animals received test doses of PCP on Tuesdays and Thursdays. Group 1 received saline and Group 2 received PCP (10 mg/kg) for the rest of the week and for 12 days following completion of the dose-effect determinations. Vehicle (saline) injections were tested as a drug dose (0 mg/kg). Data were collected in the form of response rates and reinforcement rates as well as interresponse time (IRT) distributions. For IRT distributions, 12 two-sec bins were used with responses occuring with IRT's greater than 22 sec counted in the twelfth bin.

Biodisposition Studies

On the 13th day following completion of the dose effect determination, all animals received 10 mg/kg of ^{3}H-PCP·HCl (obtained from the National Institute on Drug Abuse). They were decapitated upon completion of the test session 35 min after injection. Blood from the cervical wound was collected in heparinized glass tubes and centrifuged at 1000 g for 20 min to obtain plasma. Liver, lung, brain and epididymal fat were removed, blotted dry and individually weighed. Liver and brain were homogenized with a polytron (Brinkman Instruments, Westbury, NY) in 5 volumes of 0.5 N HCl containing 0.5 mg PCP·HCl/ml. Lung and fat were homogenized in 2 ml of the same solution.

Aliquots (0.2 ml) of the homogenates were added to oxidation cups, allowed to dry and combusted to ^3H_2O in a Packard Tricarb sample oxidizer (efficiency>95%) for determination of total radioactivity by liquid scintillation spectrometry (counting efficiency established by external standardization). Plasma samples (0.1 ml) were added to scintillation vials and counted in aqueous counting scintillant (Amersham, Arlington Heights, IL).

^{3}H-PCP was selectively extracted from the tissue homogenates by a modification of the method of Misra *et al.* [13] as described by Martin [10]. Plasma (0.5 ml) was added to 2.0 ml of 0.5 N HCl containing 1 mg of PCP·HCl. The diluted plasma and 1–2 ml of tissue homogenates were adjusted to pH 9.5 with 2 N ammonium hydroxide plus 1 ml of 2% (w/v) potassium phosphate (dibasic). Fifteen ml of hexane was added to each sample which was then shaken gently for 15 min and subsequently centrifuged (10 min of 3000 g) to separate the phases. Eight ml of the organic layer was transferred to scintillation vials and radioactivity counted in 10 ml of toluene containing 0.6% diphenyloxazole and 0.01% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene. *In vitro* extraction efficiencies were determined with tissue homogenates spiked with ^{3}H-PCP·HCl. Over 85% of ^{3}H-PCP was recovered from tissue blanks by the procedure described above. The selectivity of the extraction was verified by subjecting hexane extracts of tissues from ^{3}H-PCP·HCl-treated mice to TLC analysis [11]. Greater than 90% of the radioactivity in the extracts corresponded to PCP. The radioactivity in each sample that corresponded to ^{3}H-PCP was subtracted from the total radioactivity to obtain radioactivity corresponding to metabolites. These data were then converted to nmoles by dividing the metabolite radioactivity by the specific activity of ^{3}H-PCP·HCl.

RESULTS

Average control response rates ranged between 6 and 8 per min during saline treatment. Of the maximum possible reinforcement rate of 6 per min, averages between 2.3 and 2.9 were generally obtained. These results are consistent with those previously reported for the control DRL performance of male ICR mice [1].

The effects of PCP on the average rate of reinforcement of Group 1 (saline-treated) and Group 2 (PCP-treated) were

next determined and are shown in Fig. 1A. Dose-related decreases were observed in both groups of mice. Using a t-test for differences, comparing reinforcement rates at each dose to average control performance, the effects of 3 and 10 mg/kg were significant ($p<0.05$) in both groups while almost complete loss of reinforcers occurred with 20 mg/kg. Linear regression lines were fit to these data by least squares [7]. The dose ± the 95% confidence limits of PCP required to produce a 50% confidence limits of PCP required to produce a 50% decrease in reinforcement rate from vehicle control values was also estimated for each group [7]. For Group 1 this dose (with 95% confidence limits) was 3.7 mg/kg (2.1–5.6) and for Group 2 it was 5.4 mg/kg (4.2–8.7) indicating a 1.5-fold development of tolerance to the effects of PCP on reinforcement rate.

The effects of PCP on the response rates are shown in Fig. 1B. Large increases in responding were obtained with 3 mg/kg in both groups. Whereas the mean response rate of Group 1 decreased dramatically following 10 mg/kg, the responding of Group 2 was maintained at high levels indicating tolerance to the response rate decreasing effects of PCP. Responding in both groups was almost completely disrupted by 20 mg/kg.

Interresponse time (IRT) distributions, presented in Fig. 2, show the percentage of responses for each subject in each bin averaged for all subjects responding at greater than 0.5 responses per min for each of the doses. The number of subjects whose data were averaged for each dose is indicated in the figure. Control IRT distributions after saline administration to both groups were typical bimodal distributions for DRL responding. For the saline-treated groups, 1 and 3 mg/kg of PCP produced a shift toward shorter IRT's without increases in long IRT's. At 10 mg/kg, increases in both the first and last bin were observed indicating long pauses. For the PCP-trained animals, no change in the IRT distribution occurred with 1 mg/kg but a shift toward shorter IRT's was seen with 3 mg/kg. A large increase in the first bin occurred following 10 mg/kg without a concomitant increase in the last bin.

The first injection of PCP (10 mg/kg) during the repeated dosage regimen in Group 2 resulted in a decrease in the average reinforcement rate to less than 1 per min. The average response rate in this group was similar to control values but was the result of great individual variation. Two animals responded much more rapidly than normal while the others' rates were severely reduced. During the 21-day injection period no tolerance to the loss of reinforcement developed. This was reflected in continued disruption of DRL responding. As the 21-day period progressed several animals exhibited large increases in responding compared to control while others responded at low rates.

On the final test day all mice received 10 mg/kg ^{3}H-PCP·HCl and were decapitated immediately following the session (35 min after injection). On this day the average reinforcement rates for Group 1 (1.0 per min) was not significantly less than for Group 2 (1.2 per min). Similarly, average response rates did not differ significantly between the groups. The tissue levels of ^{3}H-PCP and ^{3}H-metabolites are shown in Table 1. Levels of ^{3}H-PCP were lower in the brain, liver and plasma of mice in Group 2 compared to Group 1 and the difference reached statistical significance in liver and plasma. Levels of ^{3}H-metabolites were also lower in the brain, liver, lung and plasma of the mice in Group 2, the difference reaching statistical significance only in brain. Extremely high levels of radioactivity were measured in the

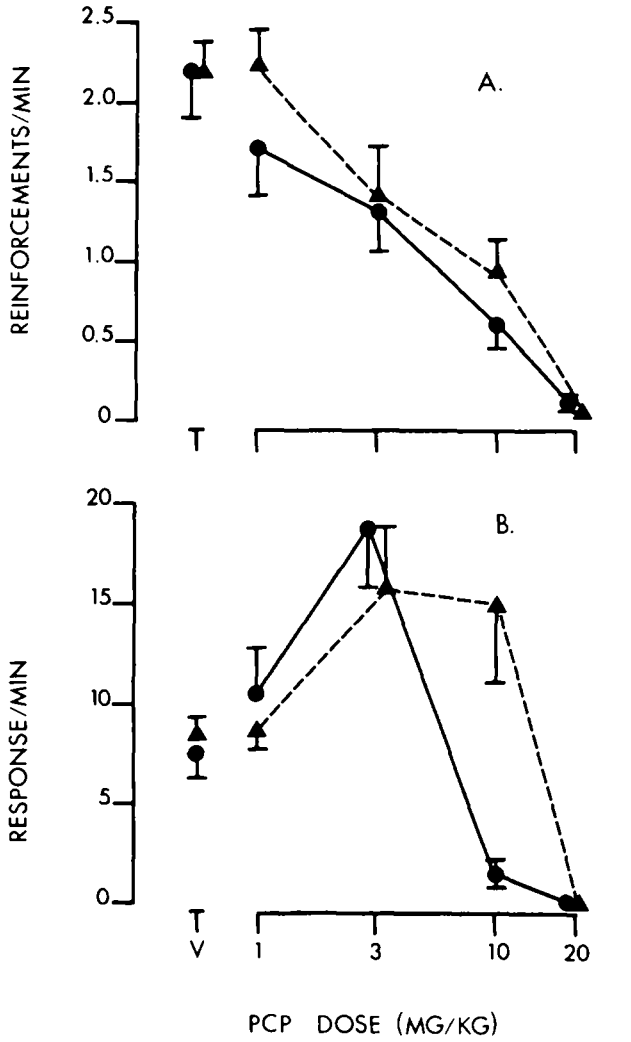

FIG. 1. The effects of PCP on reinforcement rate (Panel A) and response rate (Panel B) for DRL performance of saline-treated (●) and PCP-treated (▲) mice. Values represent the mean ± 1 standard error for 9 mice. The points at "V" represent the results of vehicle (saline) administration.

epididymal fat of both groups. The proximity of the IP injection site to the fat taken together with the lipophilicity of PCP is the most likely explanation for the high levels detected there.

DISCUSSION

The results of the dose-effect determinations showed that a 1.5-fold tolerance developed to the effects of PCP on the reduction of reinforcement rate in mice which received daily injections of 10 mg/kg PCP. Tolerance was no longer revealed by comparison of the average response and reinforcement rates of the groups obtained on the last day of testing when all animals received 10 mg/kg ^{3}H-PCP·HCl. Therefore, it appeared that behavioral tolerance developed only to doses of PCP lower than the repeated treatment dose of 10 mg/kg. Alternatively, it is possible that the exposure of saline-trained animals to PCP during the dose-effect determination affected the response to PCP on the final day of

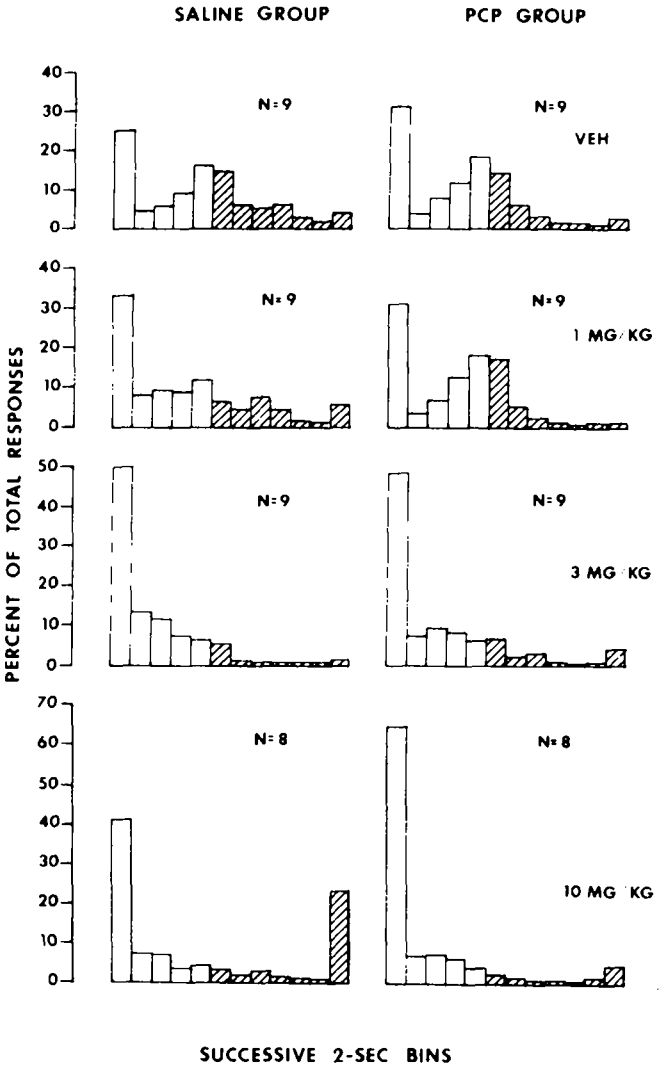

FIG. 2. The effects of PCP on interresponse times (IRT's) of mice responding on a DRL 10-sec schedule. Left panels are saline-treated subjects and right panels are PCP-treated subjects. The shaded portion of the histograms are reinforced IRT's.

testing. Nevertheless, tolerance development was clearly provided by the 21-day dosing regimen as revealed by the shift in the dose-effect curve for reinforcement rate, the lack of response-rate decreasing effects of 10 mg/kg in the PCP group (Group 2), and the lack of effect of 1 mg/kg PCP on the IRT distribution in this group compared to the saline group (Group 1). The low degree of tolerance is consistent with previous reports of roughly 2-fold tolerance to various behavioral effects of PCP in rodents [18, 25, 27].

TABLE 1

DISPOSITION OF ^{3}H-PCP AND ITS METABOLITES IN MICE TREATED CHRONICALLY WITH EITHER SALINE OR PCP

Tissue	^{3}H-PCP*	^{3}H-Metabolites*
Group 1: Saline-Treated		
Brain	4.8 ± 0.6	2.4 ± 0.2
Liver	14.6 ± 1.1	64.7 ± 4.9
Lung	7.2 ± 1.4	26.6 ± 2.6
Fat	147.1 ± 38.5	121.0 ± 30.5
Plasma	1.5 ± 0.1	8.6 ± 1.2
Group 2: PCP-Treated		
Brain	3.6 ± 0.4	1.9 ± 0.2†
Liver	9.9 ± 1.0†	55.0 ± 3.8
Lung	7.1 ± 0.8	21.9 ± 1.5
Fat	247.6 ± 67.7	130.4 ± 26.5
Plasma	1.1 ± 0.1†	7.7 ± 0.6

*nmoles/g or ml of tissue.
†Significantly different from saline-treated animals at $p < 0.05$ by Student's t-test.

Comparison of the disposition of PCP in the two groups revealed lower levels in brain, liver and plasma of the PCP-treated group. However, the lower PCP levels were not accounted for by increased levels of metabolites as metabolite concentrations were also lower in the PCP group. The ratio of the brain levels of PCP in Group 1 animals to that of Group 2 animals was 1.3 to 1 which was similar to the level of tolerance development. This suggests that the observed tolerance may have been due to dispositional factors resulting in reduced brain levels of PCP. The finding that the levels of PCP and metabolites were both reduced in Group 2 following administration of ^{3}H-PCP·HCl raised the question of how the biological fate of PCP is modified by chronic treatment. It is possible that the low levels observed could be accounted for by the concomitant increases in tissues not studied. On the other hand, the reduced levels may be due to an increase in excretion of PCP prior or subsequent to metabolism. Employing continuous PCP infusion via minipumps, Nabeshima *et al.* [16] have obtained evidence for the involvement of dispositional components to the observed tolerance of mice to the disruptive effects of PCP on simple motor performance. In that study, a reduction in the brain half-life of PCP as well as increased activities of hepatic biotransformational enzymes was associated with tolerance development.

The present study supports the notion that dispositional factors play a major role in the development of tolerance to the behavioral effects of PCP.

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