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Prenatal Phencyclidine in Rats: Effects on Apomorphine-Induced Climbing

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FICO, T. A., A. N. BANKS AND D. E. HUTCHINGS. *Prenatal phencyclidine in rats: Effects on apomorphine-induced climbing.* PHARMACOL BIOCHEM BEHAV **35**(1) 93–97, 1990.—Either 5 or 10 mg/kg of phencyclidine (PCP) in saline was administered by subcutaneous injection to gravid dams during the last two weeks of gestation. A pair-fed control group was administered the vehicle alone and allowed to eat and drink only the amount consumed by the 10 mg/kg group on the same gestation days. A nontreated control group was left undisturbed during pregnancy. All treated and control litters were fostered at birth to untreated dams. Among the dams receiving 10 mg/kg of PCP, food and water intake was initially reduced to 33–43% of nontreated controls, but then returned to control levels. Surprisingly, after 3 days of drug administration, water intake of PCP-treated dams exceeded that of the nontreated dams by approximately 15%. Compared with the nontreated dams, both PCP groups and pair-fed control dams gained significantly less body weight from conception to term. PCP had no significant effect on number of implantation sites or number of live births, however, PCP produced an apparent selective embryolethal effect on males and body weight reduction in all groups at birth. Prenatal PCP did not alter the sensitivity to apomorphine-induced climbing behavior during the second postnatal week. These results are discussed with respect to published animal and clinical studies of PCP exposure during pregnancy.

Rat Phencyclidine PCP Apomorphine-induced climbing Dopamine receptors

WE recently reported on neurobehavioral effects in mouse offspring following prenatal administration of phencyclidine (PCP) during two critical periods (6,7). Despite rapid transfer of PCP across placenta and significant concentrations reaching fetal brain, no effects on aggressive behavior, response to PCP-induced motor activity and ataxia or ^{3}H-PCP binding to brain membranes were observed postnatally. We did find, however, a dose-related increase in perinatal mortality among male offspring.

Although pharmacological research has shown that PCP affects many neurotransmitter systems within the central nervous system, there is a large body of data linking PCP behavioral effects to PCP-dopamine interaction (9,19). Because prenatal exposure to haloperidol, a dopaminergic antagonist, attenuates responses to apomorphine-induced behaviors (23), prenatal exposure to a dopamine agonist such as PCP might be expected to result in an increased responsiveness to apomorphine. To further examine possible effects of prenatal PCP specifically on postnatal dopaminergic function, the present study utilized apomorphine-induced wall-climbing measured at 7–10 days of age. Although this phenomenon does occur in the mouse (22), it has been more thoroughly described in the immature rat. Thus, two dose levels of PCP were administered during the last two weeks of gestation in the rat. One control group was nontreated and another pair-fed to the highest PCP dose. In addition, all experimental and control litters were fostered at birth to surrogate dams.

METHOD

Animals and Timing of Pregnancy

Upon their arrival in the lab, Wistar rats (Hilltop Lab Animals, Inc., Scottdale, PA) were housed in single sex groups of 2–3 in plastic cages for a minimum of one week before breeding. Nulliparous females, 200–225 g, were then paired with males of the same strain in wire cages. Females, as well as the pans beneath the cages, were examined in the late morning for the presence of sperm plugs. The day a plug is found is designated gestation day 1 or G1. Gravid dams were individually housed in plastic cages and randomly assigned to either one of two dose-level groups or one of two control groups. All dams except those in the pair-feeding condition had continuous access to food and water.

Drug Administration and Control Groups

Beginning on G8 and continuing daily through G22, either saline, 5 (PCP5) or 10 (PCP10) mg/kg of phencyclidine was administered. Dams were given subcutaneous (SC) injections in a volume which corresponded to 1 ml/kg body weight (vehicle, 0.5% and 1.0% PCP solutions). One control group (PF) received

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the vehicle and was pair-fed to the food and water intake of the PCP10 group. A nontreated control group (NT) was left undisturbed throughout pregnancy.

Pair-Feeding

Pair-feeding was carried out using a yoked design. Food and water intake of each dam were measured daily from gestation day 7–22 to determine the amount of food and water consumed during the previous 24 hr. A PF control dam was yoked to a PCP10 dam of similar body weight (±5 g). On gestation days 8–22, each pair-fed dam was given access to the same amount of food and water consumed by their yoked drug-treated dam on the same gestation day.

Fostering

Pups were removed from their mother within 5 hours of parturition (designated as postnatal day 0 or PND0). Number of live births, sex and birth weight were recorded. Litters were culled when necessary to 10 pups and those containing less than eight were sexed and weighed, but excluded from further testing. Experimental and control dams were sacrificed to determine the number of implantation sites. All treated and control litters were surrogate fostered to an untreated dam that had given birth within the previous 48 hr.

Apomorphine-Induced Wall Climbing

Litters were assessed for apomorphine-induced wall climbing between PND 7–10 in a sound-attenuated test room. The temperature of the room was recorded at the beginning of each test. The mean temperature (±SD) was 28.0±0.6°C. The test apparatus consisted of a clear, bottomless, 19.5×19.5×17.5 cm Plexiglas box resting on a screen mesh floor. On the test day, apomorphine solutions were freshly prepared by dissolving it in vehicle (0.9% saline containing 0.1% ascorbic acid). Eight pups from each litter were randomly chosen and received a SC injection of either vehicle, 0.05, 0.1 or 1.0 mg/kg apomorphine so that there were two pups (usually one of each sex) at each dosage level.

Immediately after the injection, pups were placed in the center of the test box and the 20-min test recorded on video tape. At a later date, the tests were scored by an investigator unaware of the experimental conditions. A 'climb' was defined as an animal having both forepaws off the floor for a duration greater than 1 sec. Latency to first climb, duration of wall climbing and total number of wall climbs were recorded.

Statistics

Data analysis was carried out on an IBM XT using SYSTAT and SPSS. Nonparametric tests were used to analyze maternal and offspring effects that were not normally distributed. ANOVA or repeated measures ANOVA were performed on most measures and the litter served as the unit of analysis. For the apomorphine-induced climbing variables, an initial ANOVA was run to determine the effect of sex. Since there was no significant effect of sex on the number of climbs and total climbing time, the data from both pups at each dose level within a litter were combined. The analysis was followed by Duncan's multiple range test.

Materials

Phencyclidine hydrochloride was generously supplied by the National Institute of Drug Abuse. Apomorphine hydrochloride was obtained from Sigma Chemical Co.

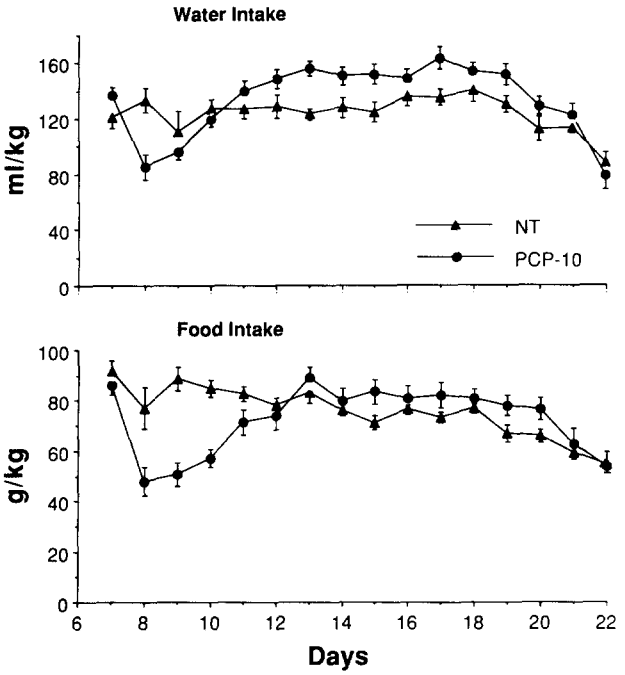

FIG. 1. Mean (±SEM) food (g/kg body weight/day) and water (ml/kg body weight/day) intake for 8 nontreated and 13 PCP10 dams from gestation day 7 through 22.

RESULTS

Maternal and Offspring Effects

There were no maternal deaths among any of the PCP or control groups. However, other toxic effects were observed in the dams exposed to PCP: Fig. 1 shows that the food intake of the PCP10 dams was reduced by 33–43% on the first 3 days of dosing compared to the NT dams. Subsequently, tolerance to the anoretic effects of the drug developed and food intake returned to the NT level. By comparison, water intake was initially reduced by 35%, but after day 3 of drug administration the PCP10 dams consumed approximately 15% more water than did the NT dams. A one-way repeated measure analysis of variance (ANOVA) revealed a significant effect of treatment, $F(15,285)=4.78$, $p<0.0001$, and a univariate F-test showed that the PCP10 group differed reliably from the NT group on G13–15 and G17. Table 1 shows that PF, PCP5 and PCP10 failed to gain as much body weight from conception to term compared with NT dams. A one-way ANOVA revealed a significant treatment effect, $F(3,39)=9.18$, $p<0.0001$, on maternal weight gain.

Table 1 also shows that mean implantation sites ranged from 14.6–15.9 and the percentage of resorptions did not differ across groups. However, the percentage of perinatal mortality (i.e., nonviable offspring observed at parturition) was 0 to 1.4% for the NT, PF and PCP5 groups, but 4% for the PCP10 group. Total perinatal mortality was calculated by adding resorptions (calculated to be number of implantation sites minus number of pups born) and perinatal deaths and dividing by total implantation sites. Thus, the total mortality was the highest among the PCP10 litters, although this did not reach statistical significance. In addition, there was a decrease in the percentage of viable males at birth in the PCP10 group; 43% of the PCP10 offspring were male. Using 48% as the expected percentage of males in a litter (the value

TABLE 1

MATERNAL AND OFFSPRING EFFECTS (MEAN ± SEM)

	NT	PF	PCP-5	PCP-10
Litters	12	9	11	13
Mean Maternal Wt. Gain (g)	188.7 ± 5.5	151.3 ± 11.2*	159.3 ± 5.8*	140.2 ± 4.8*
Mean Implantation Sites	15.8 ± 0.5	15.8 ± 0.8	15.9 ± 0.5	14.6 ± 0.4
% Resorptions	5.08	2.99	3.24	2.54
% Perinatal Mortality	0	1.39	0.60	3.92
Total Offspring Mortality (%)	5.08	4.38	3.84	6.46
Number Born Live				
Male	88 (49%)	65 (48%)	88 (52%)	76 (43%)
Female	92 (51%)	71 (52%)	81 (48%)	101 (57%)
Mean Litter Size	15.0 ± 0.7	15.1 ± 0.9	15.4 ± 0.7	13.6 ± 0.4
Mean Birth Weight (g)				
Male†	7.42 ± 0.35	6.44 ± 0.18*	6.57 ± 0.14*	6.34 ± 0.20*
Female	6.97 ± 0.33	6.06 ± 0.16*	6.22 ± 0.20*	6.00 ± 0.18*

*$p<0.05$, significantly different from NT.
†$p<0.05$, significantly different from females.

obtained from the PF group) the chi-squared analysis shows that the percentage of males observed in the PCP10 group was less than expected, $\chi^2(12)=31.1$, $p<0.05$.

Overall, birth weights of the males were higher than the females, $F(1,7)=3.45$, $p<0.02$. Table 1 shows a dose-related decrease in the birth weights of the offspring. A significant effect was obtained for treatment in males, $F(3,41)=4.33$, $p<0.01$, and females, $F(3,41)=3.67$, $p<0.02$. The birth weights of the male and female PCP-treated and PF pups were significantly lower than the NT group.

Apomorphine-Induced Wall Climbing

Percentage of rat pups exhibiting climbing behavior in response to apomorphine is given in Fig. 2 In general, apomorphine-induced climbing increased in frequency with increasing dose of apomorphine. Kruskal-Wallis ANOVA revealed no significant effect of prenatal PCP on percentage of pups exhibiting this behavior. A three-way ANOVA (APO × PCP × postnatal test day) revealed a significant effect of apomorphine on total climbing time, $F(3,116)=8.64$, $p=0.0001$, time per climb, $F(3,116)=3.47$, $p=0.019$, and latency to first climb, $F(3,116)=10.08$, $p=0.0001$. As the dose of apomorphine increased, latency to first climb decreased and total climbing time increased. However, there was no significant effect of prenatal PCP or postnatal test day on any of the climbing variables measured.

DISCUSSION

We have been unable to find other animal studies of prenatal PCP administration that measured effects on maternal food and water intake. Thus, the observation of a significant increase in water intake following an initial 3-day decrement was surprising. However, cocaine, another indirect dopamine agonist, yielded a similar, but less potent effect on maternal water intake in both pregnant (16) and nonpregnant rats (4). By comparison, delta-9-tetrahydrocannabinol at doses that depress the central nervous system, consistently inhibits both food and water intake in rats throughout pregnancy [e.g., see (14)]. Since we are unaware of any evidence to indicate that PCP either increases body tempera-

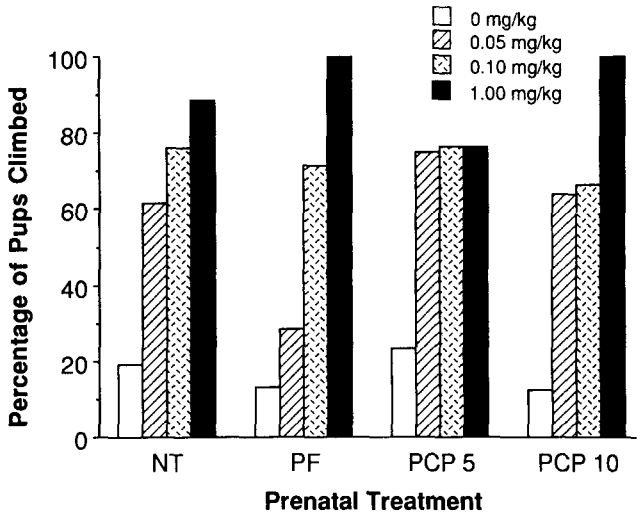

FIG. 2. Percentage of 7–10-day rat pups climbing in response to apomorphine (doses in key).

ture, acts as a diuretic or alters electrolytes, we suggest that the increased fluid intake produced by PCP is probably secondary to the increased motor activity induced by PCP (1,3).

Whereas the percentage of male offspring among the NT, PF and PCP5 groups approximated 50%, only 43% of the PCP10 offspring were males. Resorptions were not affected by drug treatment, but perinatal mortality was increased, though not significantly, at the highest dose. This suggests that the reduction in male offspring in the PCP10 group took place during the perinatal period, a finding that corroborates our previous report (6). Previous studies of prenatally administered PCP have reported modest dose-related reductions on birth weight (6, 13, 18) compared with nontreated controls. The present study, the first to utilize a pair-fed control group, found that the reduced birth weight appeared to be secondary to maternal undernutrition, rather than a primary effect of PCP.

Apomorphine is a specific dopaminergic agonist and elicits effects by a direct interaction with dopamine receptors (5,20). Shalby and Spear (23) demonstrated that apomorphine-induced behaviors are sensitive measures of dopamine receptor function; animals prenatally exposed to haloperidol, a dopaminergic antagonist, showed attenuated responses to apomorphine-induced behaviors. In the present study, no differences in responsiveness to postnatal apomorphine were observed between the treated and control offspring suggesting that the functional status of dopamine receptors in the second postnatal week is not altered by prenatal exposure to PCP.

Although PCP has been reported to be developmentally toxic in humans, corroborating animal research has not been reported [for review, see (8)]. PCP has been reported to produce embryolethality and dysmorphogenesis in both rat (17) and mouse (18), but only at doses that approximated the maternal LD40. A number of laboratories have reported that subteratogenic doses of PCP produce mild maternal toxicity, but no significant neurobehavioral deficits in the offspring (6, 7, 12, 13). Recently, Nabeshima *et al.* (20) reported that prenatal administration of 10 mg/kg/day PCP to rats produced a significant decrease in birth weights and transient sex-dependent effects on neurobehavioral development, but no gross morphological abnormalities. Unfortunately, fostering was not employed, thus, it is not clear whether the postnatal effects were produced prenatally or may have been mediated by altered postnatal maternal-pup interactions.

The first clinical reports of PCP determined drug use during pregnancy solely by self-report (10, 21, 24), a notoriously unreliable method. Although the infants were found to be difficult to console, jittery, hyperresponsive to stimuli and showed rapid state changes, it should be noted that these symptoms are not unique to PCP, but common to many prenatal abuse compounds [e.g., see (15)]. One case study did (10) report dysmorphogenesis, but subsequent studies have not found PCP to be teratogenic in humans.

Chasnoff *et al.* (2) reported on seven infants whose mothers use PCP prior to and during pregnancy and were all urine-positive at the time of delivery. Neonatal examination revealed serious neurobehavioral effects that included rapid and severe state changes ranging from lethargy to irritability with flapping tremors, facial grimacing and hyperacusis. However, what the authors consider 'classic' signs of withdrawal—fine tremors, sweating, vomiting and voracious suckling—were not observed. At three months of age, Bayley scales revealed no differences between the PCP infants and drug-free controls.

In a follow-up prospective study, Golden *et al.* (11), using a urine toxicology screen of over 2000 women, found one hundred eighty-eight subjects had used PCP during their pregnancy. Infants tested at one and three days of age were found to have significantly decreased attention and depressed reflexes compared with controls. Because of the high frequency of polydrug abuse in the population, the authors were unable to rule out possible interaction with other abuse compounds.

Because of methodological differences, these two clinical studies are difficult to compare. Nevertheless, judging by the clinical description, the infants studied by Golden *et al.* (11) did not appear to be as severely affected as the infants in the Chasnoff (2) study. These early neonatal effects, however, are poor predictors of later outcome and without clinical follow-up studies, little can be said about possible risk of long-term neurobehavioral effects.

To summarize the animal studies, prenatal PCP has been found to produce both dysmorphogenesis and neurobehavioral effects, but only at doses that approached the maternal LD50. Of those studies that examined doses that were pharmacologically active, but of relatively low maternal toxicity, neither dysmorphogenesis nor neurobehavioral effects have been found in mouse or rat offspring. The present study further examined for possible effects on the dopaminergic system and similarly found no effect. Thus, maternal toxicity may be an important component for PCP to produce developmental toxicity.

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