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TOXIC EFFECTS OF PHENCYCLIDINE ON DEVELOPING CHICK EMBRYO BRAIN

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SUMMARY

We studied the effects of Phencyclidine (PCP, Angel Dust) on the developing chick embryo brain. In Group-1, the eggs were injected with PCP on the 7th day of incubation and the embryo brains were studied on the 10th day. In Group-2, eggs were injected twice; first on the 7th day and then on the 10th day of incubation. Group-2 brains were then studied on the 16th day of incubation. PCP significantly depressed the development of embryo brains. Cerebral hemisphere weight, total protein and total DNA were significantly lower on day 10 of incubation in Group-1. Similar results were observed in Group-2. Concomitantly, the concentration of brain serotonin at day 10 was also significantly reduced when PCP was injected into the eggs on the 7th day of incubation. Since serotonin has been reported to influence development of the chick embryo brain, the present finding of the effect of PCP on brain development might be a secondary phenomenon. The possible implications of the effects of PCP on human brain development are also discussed.

Phencyclidine or Angel Dust because of its selective and hallucinogenic effects has become a major drug of abuse among adolescents, young adults and women of child bearing age (2). Ethnographic studies have revealed that PCP ranged third in frequency of drug abuse after marijuana and alcohol (3). Although the general effects of PCP have been frequently reported, the effects during pregnancy as related to infant outcome have not been well studied (4). Moreover, since a high proportion of PCP addicts use additional drugs (5), animal studies are needed to identify effects of PCP alone.

PCP crosses the placental barrier and is detectable in the urine of neonates exposed in utero. When pregnant pigs and mice were exposed to PCP, a tenfold increase of PCP in piglet plasma (6) and fetal mice tissue (2) was detected. Because of its excessive abuse and easy availability to a potential embryo, the teratogenic effects of PCP have been postulated. When high doses of PCP were administered during pregnancy, a significant number of gross anomalies were observed in rats (7). Dysmorphic features have also been observed in human

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infants (4). In an other study (8) of two infants exposed to PCP during gestation, one was born with microcephaly. Except for the above, the authers are unaware of additional research showing the effects of PCP on the fetal development of the human brain.

The present study was undertaken to obtain information on the effects of PCP on the embryonal brain development, using chick embryo as a model. PCP has been reported to affect the concentration of biogenic amines found in the brain (9) which have been reported to be growth promoting and regulating factors for the chick embryo brain (10). It appears that brain development effects may be more severe in magnitude than those found elsewhere in the body.

MATERIALS AND METHODS

The eggs and their handling were essentially as described in (11). Phencyclidine (Applied Science Div., Riviera Beach, Florida) was administered as a soluition in normal saline (10 mg/ml). Fertile eggs (White Leghorn Strain K137, from 10 month old hens) were supplied by Pace Setter Products (Altaloma, California). To avoid seasonal variations (12), the eggs laid in the same month were used. In both experimental and control groups the eggs were maintained in a Jamesway incubator, type 252, at 37.5 C ± 0.1 C. The PCP was introduced into the embryo by dropping the membrane (13). On day 7 of incubation the eggs were candled to locate the embryo. Two holes, one over the air space and another over the embryo, were made in the shell. The air from the air space was then aspirated by holding rubber tubing under vacuum against the hole. A new air space was thus created over the embryo. An aqueous solution of PCP was then introduced through the hole above the embryo, care being taken that the needle did not injure the embryo. Both holes were then sealed with wax and the eggs placed in the incubator.

EXPERIMENT-A (Table-1). Two different doses of PCP, 0.3 mg and 0.6 mg/egg were introduced on the 7th day of incubation and the embryo brain was dissected at 10th day of incubation. The brain weight was weighed, the serotonin (5-HT) was measured, and the 5-HT concentration calculated.

EXPERIMENT-B (Table 2 and 3). PCP was introduced either on the 7th day of incubation (Group 1) or on the 7th and 10th day of incubation (Group 2). Body weight, cerebral hemisphere weight, total DNA and total protein were measured at 10th and 16th day of incubation; the protein/DNA ratio was also calculated.

Science it has been established that injection of eggs with up to 0.2 ml of saline did not affect the embryonic development, the controls were not injected with saline (11, 14, 15). After completion of incubation for the length of time indicated, the eggs were opened, the embryo weighed and decapitated. The cerebral hemispheres were dissected out, weighed individually and stored at -15 C untill processed for analysis.

DNA and Protein Determination. Cerebral hemispheres were homogenized individually. Total DNA in each cerebral hemishpere was determined by diphenylamine colorimetric method (16) modified by Zamenhof et al (17). Protein content was determined by the method of Lowry et al (18).

Serotonin Determination. Serotonin was extracted from the cerebral hemisphere (19) and 0.25 ml of sample extract was used for determination. O-Phthaldialdehyde (OPT) reaction of Maickel and Miller (20) with reagent modification of Schlumpf, et al. (21) was used for the fluorometric determination of serotonin. To 0.25 ml of extract, 0.3 ml of the OPT reagent (20 mg/ 100 ml

conc. HCl) was added. The fluorescence was developed by heating in boiling water bath for 10 minutes. The mixture was washed with chloroform (22) and the fluorescence was measured in an Aminco-Bowman Spectrophotofluorometer at an activation wavelength of 360 mμ, using quartz microcuvettes.

RESULTS AND DISCUSSION

When various doses of PCP were injected into the eggs on the 7th day of incubation, embryonic brain weight and serotonin content were significantly depressed at the 10th day of incubation (Table 1). A dose of 0.6 mg PCP/egg produced a more severe effect than 0.3 mg/egg.

TABLE 1

Effect of various doses of Phencyclidine (PCP) injected into the Eggs on 7th day of Incubation, on Chick Embryo Cerebral Hemispheres at day 10th of Incubation.

Group	PCP/ Egg	Cerebral Hemispheres wet weight (mg)	Serotonin	
			Total (ng)	Concentration ng/g wet weight
Control	0.0	55.9 ± 1.07 (14)	28.8 ± 0.91 (14)	441.4 ± 9.3 (14)
Experiment 1	0.3	52.3 ± 1.08* (14)	25.1 ± 1.06* (14)	413.2 ± 9.8* (14)
Experiment 2	0.6	51.8 ± 1.32* (15)	23.9 ± 1.10* (13)	405.3 ± 10.1* (13)

In parentheses = Number of eggs
Data are presented ± S.E.
* $P<0.05$ that the value differs significantly from corresponding control (Student's two tailed t-test)

Conflicting findings have been reported by previous investigators using a variety of experimental animals. Tonge and Leonard (9) reported that 10 mg/kg of PCP caused an increase in serotonin concentration in Source A adult rat brain and a decrease in animals obtained from Source B. In an other study it is reported that 5 mg/kg of PCP administered to adult guinea pigs did not effect the concentration of serotonin in the brain, 30 minutes after administration of the drug (23). In both these studies the effects of PCP have been investigated in adult animals of various species. The effect of PCP on the level of neurotransmitters in the developing embryo brain has not been studied.

In our work when 0.6 mg PCP/ egg was introduced on the 7th day of incubation, the weight, total protein and total DNA of the cerebral hemishpere tissue were significantly depressed. The weight of the body and protein/DNA which is an index of cell size, remained unaffected (Table 2). When PCP was injected on days 7 and 10, the effect on the embryonal brain development was more pronounced on the 16th day of incubation (Table 3). In the chick embryo

brain PCP depresses serotonin, which has been reported to be a growth-promoting factor in the chick embryo brain (13). This depression is evidenced by a

TABLE 2

Effect of PCP injected into Eggs (0.6 mg PCP/Egg) on day 7th of Incubation, on Embryonic Development on day 10th (Group 1).

	Body Weight (g)	Cerebral Hemispheres			
		Total Weight (mg)	Total DNA (μg)	Total Protein (mg)	Protein/DNA
Control	1.97±0.034 (19)	55.3±1.09 (19)	149.0±2.6 (16)	2.84±0.073 (16)	18.8±0.34 (14)
Group 1	1.92±0.031 (19)	52.4±1.13* (19)	142.0±3.1* (15)	2.58±0.070* (16)	18.5±0.54 (15)

In parentheses = Number of eggs.
Data are presented + S.E.
* $P<0.05$ that the values differ significantly from corresponding control (Student's two tailed t test).

concomitant reduction in total DNA and protein in the embyonic brain. These findings suggest that the effect on the growth of the chick embryo brain may be the result of a reduced concentration of embryonic serotonin. A reduction in the total amount of protein and DNA suggests that PCP indeed depresses cellular growth of the developing chick embryo brain. It is of parallel interest that one out of two infants born to mothers who used PCP during gestation, was microcephalic (8).

TABLE 3

Effect of PCP injected on day 7th and 10th (0.6 mg PCP/Egg), on Embryonic Development at day 16th (Group 2).

	Body Weight (g)	Cerebral Hemispheres			
		Total Weight (mg)	Total DNA (μg)	Total Protein (mg)	Protein/DNA
Control	16.56 ±0.215 (18)	309.5±3.1 (18)	384.0±5.7 (18)	15.8±0.31 (17)	41.6±0.25 (17)
Group 2	15.86 ±0.313 (17)	300.1±3.36* (17)	365.0±4.7* (17)	14.7±0.31* (17)	40.7±0.45 (17)

In parentheses = Number of Eggs.
Data are presented + S.E. * $P<0.05$ that the values differ significantly from corresponding control (Student's two tailed t test).

The results indicate that even if teratogenic effects of PCP during embryonic development may not be physically apparent on the body in general, teratogenic effects at cellular level are present in the brain. More work on this subject is needed to ascertain how PCP affects mammals and if it is the cause of abnormal behavior in infants exposed to PCP abuse during gestation.

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