GESTATIONAL EXPOSURE TO PHENCYCLIDINE (PCP) IN RATS DECREASES PCP BINDING SITES IN TERM FETAL BRAIN

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Abstract—Pregnant Sprague-Dawley rats were treated with 5 mg/kg body weight of phencyclidine (PCP) injected at 1 ml/kg subcutaneously on three consecutive days at four different stages of gestation. Within 10–30 min after treatment, dams showed some lack of motor coordination and became lethargic. On gestational day 21, all rats were killed by decapitation and brains were dissected and stored from mother and fetus for biochemical analysis. PCP, dopamine and mescaline cholinergic receptor binding was measured in membranes prepared from maternal and fetal whole brain. Neurotransmitter concentrations were also measured in the fetal brain homogenates. There was a significant decrease in PCP binding sites in fetal but not maternal brains after maternal PCP injection at gestational days 12–14, 15–17 and 18–20, but not at 9–11 days. Dopamine and mescaline cholinergic receptor binding was not significantly altered in fetal or maternal brain when compared with vehicle control animals. The whole brain dopamine, 3,4-dihydroxyphenylacetic acid, serotonin, and 5-hydroxyindoleacetic acid concentrations did not show significant change in any group studied. These data indicate that gestational exposure to PCP decreases high affinity binding of PCP in term fetal brain at doses which do not alter maternal PCP receptor binding.

Key words: Phencyclidine, PCP receptors, Fetal brain, Gestational exposure, Neurotransmitter.

Phencyclidine (PCP, 'angel dust'), [1-(1-phenyl-cyclohexyl)piperidine] is a widely abused psychoactive drug which is known to interact with several different neurotransmitter systems. PCP and its related analogs inhibit the reuptake and also enhance the release of dopamine, norepinephrine and serotonin both in vitro and in vivo. Kaufman et al. reported the use of PCP among pregnant women resulted in the birth of neonates exhibiting behavioral abnormalities including temporary rigidity and irritability. Analysis of blood and urine collected from these neonates revealed the presence of PCP. Recently, Ahmad et al. reported the presence of PCP in serum and brain of rat pups from mothers dosed with PCP during pregnancy. They reported that the levels of PCP in pups' brains were much higher than in the corresponding maternal brains. Several investigators have demonstrated the presence of PCP binding sites in adult and fetal rat brain homogenates and these have been further localized in brain slices by autoradiographic studies. The main purpose of the present study was to evaluate the effect of prenatal PCP exposure on fetal and maternal PCP binding sites in brain. Other specific neurotransmitter receptors and neurotransmitter concentrations in fetal and maternal brain were also studied.

EXPERIMENTAL PROCEDURES

Pregnant Sprague-Dawley rats weighing 235–250 g were used in this study. Animals were divided into four groups of six animals each. They were housed individually in a controlled environment (12 hr light:dark cycle, 20 ± 2°C and 50 ± 2% relative humidity). Each of the rats in the four experimental groups were administered 5 mg PCP/kg body weight (dissolved in 10% methanol in 0.9% saline and injected at 1 ml/kg) injected subcutaneously into the scruff of the neck on three consecutive days. Gestational days of injection were 9, 10 and 11 (group 1), 12, 13 and 14 (group 2), 15, 16 and 17 (group 3), and 18, 19 and 20 (group 4). Eight dams received corresponding injections of the methanol-saline vehicle (control group). Two control rats were injected at each of the times corresponding to each experimental group. On gestational day 21 all

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mothers and Caesarean-derived fetuses were killed by decapitation; maternal and fetal brains were collected and rapidly frozen after weighing. Fetal weights were also recorded. Fetal brain tissue collected from the progeny of each mother was pooled and treated as \( n = 1 \).

**Receptor binding**

**Membrane preparation.** For assay of the PCP and neurotransmitter receptor binding sites, membranes were prepared from whole brain minus cerebellum and brain stem by homogenization of tissue in 20 volumes (w/v) of 0.32 M sucrose followed by centrifugation (50,000 \( g \) for 10 min). The pellet was rehomogenized in distilled deionized water (pH adjusted to 7.4) and centrifuged (50,000 \( g \) for 10 min). The pellet was resuspended in 50 mM Tris–HCl (pH 7.4) buffer and centrifuged. This step was repeated in order to aid in the washing out of any residual ligand. The final pellet was then resuspended in the incubation buffer (50 mM Tris–HCl containing 2.5 mM CaCl\(_2\), 1 mM MgCl\(_2\), 5 mM KCl, 120 mM NaCl, 0.1% ascorbate and 10 \( \mu \)M pargyline, pH 7.4) at a concentration of 50 mg (original wet weight equivalent)/ml. In order to determine if residual brain levels of PCP were removed during the membrane preparation, control gestational day 21 fetal brain homogenate was spiked with \(^{3}H\)-PCP and prepared as stated in Experimental Procedures. Less than 0.2% of \(^{3}H\) remained, indicating that this procedure is suitable for removing residual PCP.

\[^{3}H\]-Phencyclidine binding

For PCP binding sites, we followed the method of Zukin and Zukin\(^{32}\) with minor modifications. Membrane preparations were preincubated at 4°C for 15 min, centrifuged at 50,000 \( g \) for 10 min, and the pellet was resuspended in incubation buffer. Aliquots (100 \( \mu l \)) of each membrane suspension were incubated with 5.0 nM \(^{3}H\)-PCP (47.6 Ci/mmol, New England Nuclear, Boston, MA). Incubations were carried out in triplicate for 45 min at 4°C in a total volume of 1 ml. A parallel incubation was performed in the presence of 5.0 \( \mu \)M PCP. After incubation, samples were diluted with 5.0 ml of ice-cold 50 mM Tris–HCl buffer and rapidly filtered under vacuum through Whatman GF/C glass fiber filters (Whatman Inc., Clifton, NJ) using a Brandel Cell Harvester. The filters were washed twice with 5 ml cold Tris–HCl buffer. This separation and washing procedure generally took less than 20 sec. The filters were air dried and placed into scintillation fluid (Isolab Inc., Akron, OH). Total radioactivity was quantified by liquid scintillation spectrometry (Tracor Mark III, Elk Grove Village, IL). Specific binding was calculated as the difference between the amount of \(^{3}H\)-PCP alone (total binding) and that in the presence of 5.0 \( \mu \)M PCP (non-specific binding). PCP binding sites demonstrated saturability and specificity at 5.0 nM as reported by other investigators\(^{18\ldots32}\) and as confirmed in our laboratory.

\[^{3}H\]-Quinuclidinyl benzilate (QNB) binding

Muscarinic cholinergic receptor binding was assayed by incubating 100 \( \mu l \) of the membrane preparation with 1.0 nM \(^{3}H\)-quinuclidinyl benzilate (QNB, 33.2 Ci/mmol, New England Nuclear, Boston, MA). Incubations were carried out in triplicate for 60 min at 37°C in a total volume of 1 ml. Parallel incubations were performed in the presence of 1 \( \mu \)M atropine. After incubation, the same procedure was followed as that for PCP binding. Specific binding was calculated by subtracting the binding occurring in the presence of 1 \( \mu \)M atropine from the total binding.

\[^{3}H\]-Spiroperidol binding

Dopamine receptor binding was assayed by incubating 100 \( \mu l \) of the membrane preparation with 1.0 nM \(^{3}H\)-spiroperidol (24.2 Ci/mmol, New England Nuclear, Boston, MA). Incubations were carried out in triplicate for 20 min at 37°C in a total volume of 1 ml. Parallel incubations were performed in the presence of 1 \( \mu \)M (+) butaclamol. After incubation, the same procedure was followed as that for PCP binding. Specific binding was calculated by subtracting the binding occurring in the presence of 1 \( \mu \)M (+) butaclamol from the total binding. The methods used were essentially the same as other filtration binding methods.\(^{31}\) However, it was necessary to establish the basic binding characteristics of the ligand used. The saturability, specificity and reversibility of \(^{3}H\)-QNB and \(^{3}H\)-spiroperidol binding have also been previously delineated.\(^{3}\).
Neurotransmitter and metabolite assay

The neurotransmitters dopamine (DA), serotonin (5-HT) and their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were resolved by high performance liquid chromatography (HPLC) and quantified by an electrochemical detection method. Briefly, each brain tissue was weighed and diluted with a measured volume (10 v/w) of 0.2 N perchloric acid containing 250 ng/ml of the internal standard 3,4-dihydroxybenzylamine (DHBK). Brain tissue was then disrupted by ultrasonication, centrifuged (1000 g, 5 min), and 150 µl of the supernatant was removed and filtered through a 0.2 µm microfilter [MF-1 microcentrifuge filter, Bioanalytic System (BAS), W. Lafayette, IN]. Aliquots of 25 µl, representing 10 mg of brain tissue, were injected directly onto the HPLC system for separation of the neurotransmitters and their metabolites.

The analytical system included a Waters Associates M-6000A pump (Milford, MA), a Rheodyne® 7125 injector (Rainin Instrument, Woburn, MA), a Biophase® ODS, 5 µm (250 x 4.6 mm) analytical column (BAS), a LC-4A amperometric detector and LC-17 oxidative flow cell consisting of a glassy carbon electrode (TL-5) vs Ag-AgCl reference electrode maintained at a potential of 0.65 V (BAS). The mobile phase consisted of 0.15 M monochloroacetate, pH 3.0, 4.5% acetonitrile and 0.5 mM octyl sodium sulfate as an ion pairing reagent. Chromatograms were recorded and integrated on a Hewlett-Packard 3380A integrator (Hewlett-Packard, Avondale, PA). The endogenous biogenic amine concentrations were calculated using a standard curve for each amine. The standard curves were generated by determining in triplicate the ratio between three different known amounts of each amine and a constant amount of DHBA. Concentrations of DA, DOPAC, 5-HT and 5-HIAA were all determined in each sample.

Protein determination

Aliquots of the membrane preparations were used for the determination of protein content by the method of Lowry et al., using bovine serum albumin as the standard.

Statistical analysis

Data were evaluated by one-way analysis of variance followed, where appropriate, by Student's t-test. A value of P<0.05 was taken as significant.

RESULTS AND DISCUSSION

PCP, DA and muscarinic cholinergic receptor binding sites were measured in fetal whole brain after prenatal exposure to PCP. The specific binding intensity of ligands for these receptors in gestational day 21 fetal rat brain is shown in Fig. 1. There was a significant reduction of PCP binding sites in fetal brain after maternal exposure to PCP during gestational days (GD) 12-14, 15-17 and 18-20 (groups 2, 3 and 4). This reduction in PCP binding (approximately 40%) was similar over GD 12-20 but was not apparent when PCP exposure occurred earlier in gestation (GD 9-11). Dopaminergic and cholinergic binding did not show any significant changes at any time point studied.

![Fig. 1. Receptor binding in gestational day 21 whole fetal rat brain after prenatal PCP treatment. *indicates PCP binding which is significantly reduced from control values (P<0.05).](image-url)
The ontogeny of the PCP receptor has been characterized in the rat by Sircar and Zukin. Displaceable PCP binding was observed from GD 13, and $B_{\text{max}}$ values increased to above adult levels just prior to birth. The down-regulation of PCP binding after gestational exposure to PCP may be associated with the ontogeny of the PCP receptor, since no effect was observed in the GD 9–11 exposure group (group 1). In the present study, fetal PCP binding was measured late in gestation (day 21) when full stereospecific PCP binding has been reported. Although Scatchard analysis was not performed in this study, a decrease in PCP binding is hypothesized to represent a decrease in $B_{\text{max}}$ because the apparent $K_d$ of PCP binding does not change during development or after chronic exposure to PCP in the adult.

Table 1 shows the maternal brain receptor binding data from dams killed on GD 21 after treatment with PCP at different times during gestation. Neither PCP nor neurotransmitter receptor binding sites showed significant changes at any time point studied. There are several reports in the literature describing the significant changes in DA concentrations, DA metabolism, tyrosine hydroxylase activity and also in muscarinic cholinergic receptor regulation after acute, subacute or chronic treatment with PCP. However, doses of 30 mg/kg for acute and at least 10 mg/kg for subacute and chronic exposure were required to affect a significant alteration of these parameters associated with the DA system. In our study, we used only 5 mg/kg for 3 days at different gestational stages. While no changes were found in the DA or muscarinic cholinergic receptor or associated neurotransmitter systems, a selective alteration of PCP binding was observed in fetal but not maternal brain (Fig. 1).

Table 1. Receptor binding in female rat brain after treatment with phencyclidine (PCP)

| Gestational days injected | PCP receptor | Dopamine receptor | Muscarinic cholinergic receptors |
|--------------------------|--------------|-------------------|--------------------------------|
| Control group            | 7.0 ± 1.9    | 85 ± 3            | 559 ± 24                       |
| Group 1                  | 9.2 ± 3.9    | 73 ± 10           | 588 ± 35                       |
| Group 2                  | 9.7 ± 2.2    | 77 ± 4            | 542 ± 25                       |
| Group 3                  | 6.8 ± 1.1    | 86 ± 2            | 575 ± 19                       |
| Group 4                  | 7.1 ± 1.1    | 81 ± 2            | 508 ± 13                       |

Each value expressed as fmoles radioligand bound/mg protein ± S.E.M. and is derived from 6 to 8 individual animals. Animals were treated with 5 mg PCP/kg body weight daily for three consecutive gestational days. See text for details.

Recently, we have found that another psychoactive drug, imipramine, produced no significant changes in adrenergic or muscarinic cholinergic binding on postnatal day 1 or 21 when administered prenatally at 5 or 10 mg/kg daily through days 8–20 of gestation. In contrast, reserpine given daily at 0.375 or 0.75 mg/kg on days 12–15 of gestation, produced significant changes in DA receptor binding and in DA concentrations on postnatal days 1 and 21. The present study, we also measured the concentration of DA, DOPAC, 5-HT and 5-HIAA in fetal brain and did not find any significant changes in any of their concentrations in response to gestational exposure to PCP (Table 2).

Relatively large doses of PCP may be required to produce changes in the dopaminergic system. Such doses may mediate the acute psychotic effects noted after PCP exposure. The relatively low

Table 2. Neurotransmitter concentrations in gestational day 21 whole fetal rat brain after prenatal treatment with phencyclidine (PCP)

| Gestational days injected | Dopamine | DOPAC  | Serotonin | 5-HIAA |
|--------------------------|----------|--------|-----------|--------|
| Control group            | 23.5 ± 2.2 | 4.4 ± 0.6 | 17.9 ± 3.6 | 18.5 ± 1.6 |
| Group 1                  | 26.8 ± 6.3 | 5.4 ± 1.4 | 20.3 ± 4.3 | 26.6 ± 1.9 |
| Group 2                  | 25.4 ± 4.0 | 4.1 ± 0.9 | 15.5 ± 5.2 | 20.2 ± 2.0 |
| Group 3                  | 26.4 ± 4.4 | 5.1 ± 1.3 | 21.8 ± 4.5 | 23.9 ± 2.6 |
| Group 4                  | 23.9 ± 4.1 | 3.6 ± 0.1 | 18.0 ± 2.1 | 22.4 ± 2.6 |

Each value expressed as ng/100 mg wet weight ± S.E.M. and is derived from 6 to 8 individual mothers. Mothers were treated with 5 mg PCP/kg body weight daily for three consecutive gestational days.
doses used in the present study, seem to produce prolonged changes in the binding capacity of the PCP receptor system in the developing pups. Studies are underway to determine the degree of persistence of this apparent down-regulation and to inquire as to whether behavioral correlations with this neurochemical change can be observed.

These data indicate, for the first time, that fetal brain PCP binding is down-regulated by gestational PCP exposure. Since the low doses used in this study failed to alter maternal PCP binding, the data suggest that the developing fetus is more susceptible than the adult rat to such effects of PCP. This fetal sensitivity may be due to the maturational stage of the PCP receptor system at this time, as noted by Smith et al.2 The authors wish to thank Ms. Barbara Jacks for efficient and helpful secretarial assistance in preparing this manuscript. This work was partly supported by NIH Grant ES-04071.

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