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Delta-9-Tetrahydrocannabinol During Pregnancy in the Rat: Effects on Development of RNA, DNA, and Protein in Offspring Brain[1]

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MORGAN, B., S. C. BRAKE, D. E. HUTCHINGS, N. MILLER AND Z. GAMAGARIS. *Delta-9-tetrahydrocannabinol during pregnancy in the rat: Effects on development of RNA, DNA, and protein in offspring brain.* PHARMACOL BIOCHEM BEHAV 31(2) 365–369, 1988.—Either 15 or 50 mg/kg of delta-9-tetrahydrocannabinol (THC) was administered from Day 2 through Day 22 of gestation. Pair-fed and nontreated groups served as controls and all treated and control litters were fostered at birth to untreated dams. To determine the effects of THC on offspring brain development, DNA, RNA and protein values were determined at 7, 14, and 21 days of postnatal age. DNA and RNA levels appeared unaffected by THC but brain protein levels of the 50 mg/kg offspring were significantly lower than in the other groups at Day 7 and 14. This suggests that the high THC dose inhibited protein synthesis for at least the first 14 days of life. Subsequently, protein levels of the 50 mg/kg offspring increased rapidly so that there were no differences between any of the groups at 21 days of age. These findings for developing CNS parallel the delayed rate of somatic growth previously reported from our laboratory and suggest a transitory rather than a permanent effect of THC on both somatic and brain growth. We also found that THC produces a significant dose-related increase in the sex-ratio of live male-to-female offspring, a finding we have reported previously.

Delta-9-THC Rat Prenatal Postnatal brain growth RNA DNA Protein Female embryolethality

IN previous work from our laboratory, we studied the effects of prenatal exposure to delta-9-tetrahydrocannabinol (THC) on somatic growth in rat offspring (8). Our results along with studies from other laboratories (2) suggest differential dose-response effects on rate of body growth: Low doses produce relatively short-term growth inhibition followed by rapid catch-up whereas high doses produced a more prolonged period of delayed growth with relatively slow catch-up. The present experiment was carried out to extend the studies of prenatal THC to effects on postnatal brain growth. For this we analyzed offspring brains at 7, 14 and 21 days of age for DNA, RNA and protein content.

A second problem addressed in this study is the severe inhibition of both food and water intake produced by THC administration in the rat. In prenatal studies, this inhibition confounds primary THC effects in the offspring with effects of maternal undernutrition and dehydration (1, 3, 8). In our earlier studies we initiated THC administration on gestation Day 8 (3,8). This resulted in an 80% reduction in both food and water intake on the first day, although tolerance developed and intake returned to about 10–15% below nontreated dams over the following 4–5 days. The maximal inhibition of food and water intake occurred during the earliest development of embryonic central nervous system (CNS), on gestation Days 9–11. Thus, we suggested that the behavioral deficit found among the THC-treated and pair-fed controls was mediated by the severe maternal undernutrition during early CNS development. In the present study, THC treatment was initiated on the day after conception so that the severe THC-induced undernutrition/dehydration would be

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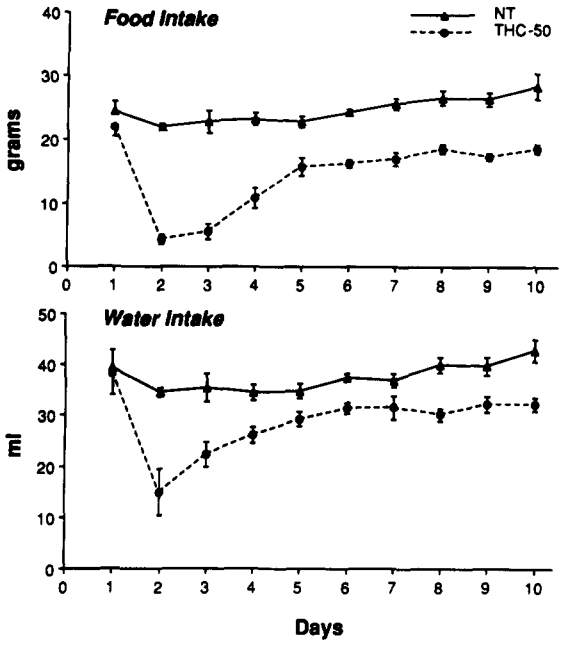

FIG. 1. Mean food and water intake for the nontreated controls and THC-50-treated dams during the first 10 days of gestation.

confined to the preimplantation period (i.e., Day 1 to approximately Day 6), a period generally found to be refractory to teratogenic effects [for discussion, see (7)].

METHOD

Animals and Timing of Pregnancy

Individual nulliparous Wistar females weighing 175–199 g (Hilltop Lab Animals, Inc., Scottdale, PA) were paired with males of the same strain in hanging wire cages. Females, as well as the pans beneath the cages, were examined in the early afternoon for the presence of sperm plugs. The date of finding a plug was designated Day 1 of gestation. Gravid dams were then randomly assigned to either one of two dose-level THC groups or to one of two control groups housed in plastic cages on wood chips. All dams except those in the pair-feeding condition had continuous access to Purina Lab Chow and water.

Drug Administration and Control Groups

Beginning on Day 2 of gestation, either 15 or 50 mg/kg of THC suspended in sesame oil was administered to two groups of gravid dams (THC-15; THC-50) once daily by gastric intubation. Both dose-level groups received daily drug administration through Day 22 of gestation, approximately 24 hr prior to expected parturition. One control group received the vehicle and, in addition, was pair-fed (PF) for food and water to the 50 mg/kg THC group. A nontreated (NT) control group was left undisturbed throughout pregnancy.

Pair-Feeding

Pair-feeding to the THC-50 group for both food and water intake was carried out using a yoked design. On gestation Day 1 through 22, food and water intake was measured daily for each dam in the THC-50 group. Food was placed in stainless steel hanging food dispensers and water in 8 oz bottles. Food and water containers were weighed daily and consumption was recorded.

Each THC-50 dam was yoked to a pair-fed control dam matched by body weight (±5 g). On Days 2 through 22 of gestation, 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. To minimize spillage, rat chow was placed in conical shaped ceramic food dishes that sat on the cage floor. In addition, the pair-fed controls were administered the vehicle in the same volume as the treated dams.

Fostering

Within 1–5 hr after birth, all treated and control offspring were sexed and weighed. Litters were culled when necessary to 10 pups; litters containing less than 8 pups were also sexed and weighed but excluded from further testing. Offspring were then fostered to normal dams of the same strain that had delivered approximately 24 hr earlier. All experimental and control dams were sacrificed to determine the number of implantation sites.

Brain Assays

On PND's 7, 14, and 21, 3 pups were sacrificed from 10 NT, 7 PF, 8 THC-15, and 9 THC-50 litters. Pups were decapitated, brains excised without cerebellum and immediately frozen and stored at −70 degrees C. On the days of sacrifice, age-matched pups were added to each litter in order to maintain a constant litter size. For the assays, three brains collected from each sacrifice day were pooled by litter. Tissue was homogenized in distilled water and analyzed for total protein, DNA, and RNA. DNA and RNA were extracted from the homogenate using Klemperer's procedure (9), and assayed colorimeterically by the methods of Burton (4) and Munro and Fleck (14) respectively. Protein was measured using the Lowry method (10).

Statistical Analysis

ANOVA or repeated-measures ANOVA were performed on most of the measures. When necessary, Scheffe's tests or Student's *t*-test ($p<0.05$) were used to compare groups following overall ANOVA. The chi-square statistic was used to analyze sex-ratio data. Except for the calculation of mortality and sex-ratio, the litter served as the unit of analysis for all measures taken on offspring.

RESULTS

Maternal and Offspring Effects

There were no maternal deaths among any of the THC-treated or control groups. The food and water intake of the THC-50 dams was initially reduced, particularly on the first 2–3 days after the beginning of drug treatment, and subsequently showed partial recovery. Figure 1 shows the intake data for NT control and THC-50 dams for the first 10 days of gestation. ANOVA revealed that the difference between the groups was highly significant for both food, $F(9,135)=11.83$, $p<0.001$, and water, $F(9,135)=2.46$, $p<0.01$. From gestation days 11 to term, mean intake was aymptotic for both the NT controls (food=29.1 g; water=48.7 ml) and the THC-50 dams

TABLE 1
MATERNAL AND OFFSPRING EFFECTS

	NT	PF	THC-15	THC-50
Litters	10	7	8	9
Mean Maternal Weight Gain (g)	168.7 ± 7.12	125.9 ± 6.95	130.3 ± 2.92	106.7 ± 7.03
Mean Implantation Sites	14	15	14	14
Resorptions	7.97%	3.77%	1.74%	7.81%
Perinatal Mortality	1.45%	0.94%	0.87%	6.25%
Total Offspring Mortality	9.42%	4.72%	2.61%	14.06%
Number Born Live				
Male	51 (41%)	47 (47%)	59 (53%)	67 (61%)
Female	74 (59%)	54 (53%)	53 (47%)	43 (39%)
Mean Liter Size	13	14	14	12
Mean Birth-Weight (g)				
Male	7.26 ± 0.17	6.38 ± 0.12	6.68 ± 0.17	6.60 ± 0.12
Female	6.80 ± 0.14	6.10 ± 0.09	6.31 ± 0.12	6.18 ± 0.15

(food=19.9 g; water=37.5 ml). Table 1 shows that the PF, THC-15, and THC-50 dams failed to gain as much body weight from conception on term compared with the NT, a difference that was highly significant, $F(3,29)=18.01$, $p<0.001$.

Table 1 also shows that the mean implantation sites were nearly identical across all groups whereas the percentage of resorptions was rather variable. Compared with the PF and THC-15 animals, percent resorptions were higher in the NT and THC-50 animals but differences among the groups were not statistically significant. Similarly, the percentage of perinatal mortality was in the range of 1% for the NT, PF and THC-15 groups and 6% in the THC-50s but did not differ significantly. However, ANOVA revealed a significant effect of treatment for percent total offspring mortality, $F(3,30)=3.10$, $p<0.05$. Post hoc analyses indicates that this effect was due largely to the high percent mortality in the THC-50 group; mean percent scores did not differ significantly among the NT, PF and THC-15 groups but did among the PF, THC-15 and THC-50 groups [Scheffe $F(2,30)=4.33$, $p<0.03$].

Overall, birthweights of the males were higher than the females, $F(1,60)=11.36$, $p<0.001$. And while the birthweights of the PF, THC-15 and THC-50 pups were significantly lower than the birthweights of the NT pups, $F(3,60)=9.97$, $p<0.001$, there were no differences between the PF controls and the THC groups.

Additionally, there was a dose-related increase in the proportion of viable male offspring at birth. Fifty-three percent of the THC-15 and 61% of the THC-50 offspring were males. Using the PF controls as the "expected" proportion of males offspring (47%), the proportion of males born in the THC-50 group is higher than expected for both the THC-50, chi-square(8)=57.9, $p<0.001$, and the THC-15, chi-square(7)=33.4, $p<0.001$, groups.

Brain Analysis

Figure 2 shows that there was an increase in brain DNA, RNA and protein with increasing age in all groups, $F(2,64)=39.87$, $p<0.001$; $F(2,64)=77.41$, $p<0.001$ and, $F(2,73)=211.29$, $p<0.001$ respectively. There were no differences among groups at any time for values of DNA or RNA. However, ANOVA revealed that the brains of the nontreated, THC-15 and pair-fed groups all had significantly greater amounts of total protein than the THC-50 group, $F(3,73)=3.54$, $p<0.01$. Post hoc tests revealed that this difference occurred both on days 7 ($t=6.52$, $p<0.05$) and 14 ($t=4.79$, $p<0.05$). The difference disappeared with age [Group × Age interaction, $F(6,146)=2.68$, $p<0.02$], so that by Day 21, all of the treated and control groups yielded the same brain protein content.

DISCUSSION

The present findings with respect to maternal weight gain, offspring birthweight and the dose-related increase in the proportion of male with a corresponding decrease in the proportion of female offspring, replicate our previous observations (8). This provides further evidence that THC exerts a selectively embryolethal effect in females and the effect appears to be independent of maternal undernutrition.

The nontreated total offspring mortality in the present study exceeded 9%, and is somewhat higher than 3–5% value obtained over several years in our laboratory for nontreated offspring. In addition, the nontreated yielded 41% live males, a percentage that normally ranges from 47–53%. Although we have no explanation for these differences, nontreated data currently being collected in our laboratory approximate the historical values and we suggest that the deviations obtained here probably represent random biological variation.

In order to determine if prenatal exposure to THC affects

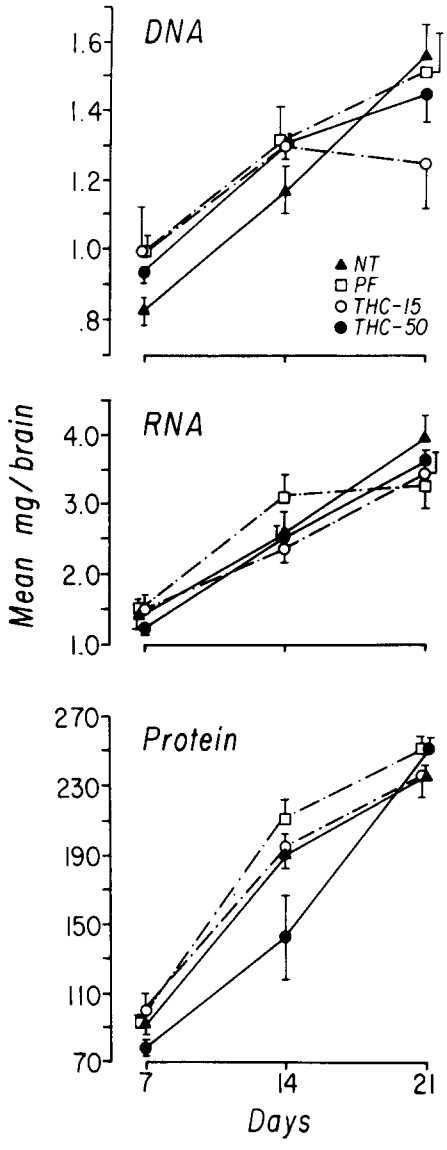

FIG. 2. Mean DNA, RNA, and protein values for the THC-exposed and control offspring. The number of pools of tissues ranged from 4 to 8 for each of the brain measures.

offspring brain growth and development, DNA and protein were assayed as estimates of brain cell number and cell size (6,15). Studies by Morgan and Dickerson (12) and Winick (15) and others (16) have described reductions in these variables after prenatal nutritional insults. Similar reductions in RNA occur in animals exposed to prenatal undernutrition (15,16).

In this study no differences were observed among the nontreated and the pair-fed pups with respect to DNA and RNA, indicating that the reduced food intake of the mother during gestation had no effect on nucleic acid synthesis in the brain. Furthermore, there were no differences in RNA and DNA levels between the THC-treated pups and the pair-fed group, suggesting that RNA or DNA synthesis was not affected by THC.

Protein, however, was affected by THC. As with the nucleic acids, there were no differences between the values for pair-fed and nontreated animals, indicating that the nutritional deficit in the dam was not great enough to impair protein synthesis. Neither was the 15 mg/kg dose of THC sufficient to affect protein synthesis as pups in this group showed similar protein accumulation to the NT and pair-fed groups. However, the THC-50 pups were significantly affected. Brain protein levels were significantly lower than in the other groups at Day 7 and 14, suggesting that the higher dose reduced protein synthesis for at least the first 14 days of life. Subsequently, THC-50 pups rapidly caught up, increasing their brain protein by 43% in the next seven days compared with only 18% in the pair-fed controls.

Protein synthesis in the brain correlates with growth of axons and dendrites and the formation of synaptic connections between cells. Thus, the 50 mg/kg dose of THC appears to have inhibited proliferation of neural processes during the first 14 days of life. This heightened synthesis parallels a similar phenomenon seen in severely malnourished children who have been nutritionally rehabilitated (5).

In our findings for somatic growth (8), we described a dose-response relationship for THC; 15 mg/kg produced short-term growth inhibition followed by rapid catch-up whereas 50 mg/kg produced a prolonged period of delayed growth followed by gradual catch-up. In the present study the 15 mg/kg dose had no observable effect on brain growth. That the lower dose was without affect on brain parallels similar studies of maternal undernutrition that find offspring CNS to be more resistant to growth deficits than other developing organ systems (15).

We have found only one other report of the effects of prenatal administration of THC on rat brain growth and development. Luthra (11) reported a significant reduction in brain RNA, DNA, and protein measured at birth and seven days of age following oral administration of 5 and 10 mg/kg of THC. However, direct comparison between the studies is not possible because the experimental design and protocol are not described adequately; method of brain dissection is not indicated, no data with respect to maternal body weight gain or nutritional intake are provided, surrogate fostering was not utilized, and it is not clear how the 16 animals analyzed in each group were selected. Therefore, Luthra's conclusion that THC retards brain growth and development is questionable.

In summary, the observation that there were no differences in RNA, DNA and protein between the NT and PF controls suggests that confining the severe maternal nutrition/dehydration to the preimplantation period spared offspring brain from growth inhibition. The decreased brain protein synthesis among the THC-50 animals followed by catch-up parallels the delayed rate of somatic growth previously reported by our laboratory (8) and suggests a transitory rather than a permanent effect of THC on both somatic and brain growth. As suggested previously (8), these effects may be mediated either by the persistence of pharmacologically active amounts of the compound in neonatal tissue and/or inhibition of a central neuroendocrine pathway affecting growth. An unanswered question is whether or not brains that have undergone a transitory period of THC-induced growth inhibition followed by catch-up may be functionally impaired.

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