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Prenatally Administered Delta-9-Tetrahydrocannabinol Temporarily Inhibits the Developing Hypothalamo-Pituitary System in Rats

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WENGER, T., D. CROIX, G. TRAMU AND J. LEONARDELLI. *Prenatally administered delta-9-tetrahydrocannabinol temporarily inhibits the developing hypothalamo-pituitary system in rats.* PHARMACOL BIOCHEM BEHAV **40**(3) 599–602, 1991.—We have reported earlier that Δ^9-tetrahydrocannabinol (THC) if injected over the 3rd week of pregnancy with a daily dose of 1 μg/kg b.wt., caused a significant prolongation of pregnancy and 42% of stillbirths, although no teratological effects were observed. In the present study, we investigated the postnatal development of hypothalamo-hypophyseal-gonadal axis of the above mentioned rats' living litters. The rats were killed every 5th day between the delivery (D0) and the 20th postnatal day (D20). The weight of the body and the gonads was measured and hormonal parameters were registered by RIAs. The body weight was lower in treated animals, with a higher degree in males. The weight of the gonads also decreased. Serum testosterone levels were lower in rats from D0 till D10, while at D15 and D20 no differences were recorded between controls and treated rats. No significant differences were observed in serum estrogen levels during the investigated period. Serum progesterone decreased up to D10. Pituitary luteinizing hormone (LH) diminished postpartum in both sexes up to D5. From D0 till D5, serum LH increased in males while it decreased in females. Hypothalamic luteinizing hormone releasing hormone decreased up to D15 in both sexes, less markedly in females than in males. The neuropeptide Y content of the hypothalamus was also decreased in early postnatal age. We concluded that a very low dose of prenatally administered THC caused transitory inhibition of the developing hypothalamo-pituitary-gonadal axis, i.e., postnatal changes were evoked in the neuroendocrine system. By the 20th postnatal day this effect of THC seemed to be eliminated.

THC Prenatal Reproductive system Rat

PREGNANCY follows an orderly sequence of critical phases influencing parturition as well as maturation of several organ systems in the fetus. Both fetal and maternal endocrine factors are of importance in the mechanisms that maintain the pregnancy and the normal embryological development (7).

About 14–15% of pregnant women in the USA use marihuana to some extent (1). Δ^9-Tetrahydrocannabinol (THC), the main psychoactive component of marihuana (6), is transmitted through the placenta (3), representing a potential risk factor that may affect pregnancy and/or fetal development.

Our laboratory has been studying the effects of THC on reproduction for several years (9, 11, 12). Recently we found that prenatal administration of a low dose of THC in pregnant rats produced a significant prolongation of gestation period together with a high percent of stillbirth (10). We assumed that these effects might be related to the prenatal accumulation of THC in both dams and fetuses.

In the present study, we have examined the changes in postnatal development of reproductive hormones in both sexes of the litters of THC-treated pregnant rats.

METHOD

Animals

Virgin CFY (Sprague-Dawley derivates) rats weighing 200–240 g were paired with males of the same strain. The day of finding spermatozoa in the vaginal smears was designated as Day 1 of gestation. The animals were under controlled condition of temperature (20 ± 2°C) and light (on 0500, off 1900 h) and were provided with water and food (rat pellets) ad lib.

THC Administration

In all experiments, the animals received 1 μg THC per kg body weight/day in the form of a 10^{-6} M THC solution in-

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TABLE 1

EFFECTS OF PRENATALLY ADMINISTERED THC* ON BODY WEIGHT OF YOUNG, PREMATURE RATS

Days of Age	Males		Females	
	Control	Treated	Control	Treated
0	6.73 ± 0.10 (21)	6.14 ± 0.1[a] (18)	6.62 ± 0.07 (17)	6.14 ± 0.15[c] (21)
5	11.75 ± 0.27 (25)	9.70 ± 0.23[a] (24)	11.58 ± 0.49 (22)	12.71 ± 0.23 (24)
10	24.50 ± 0.45 (20)	22.64 ± 0.71[b] (21)	21.49 ± 0.52 (24)	19.50 ± 0.73 (22)
15	31.28 ± 0.44 (15)	24.30 ± 0.73[a] (19)	30.18 ± 0.66 (26)	27.18 ± 1.08[c] (12)
20	45.26 ± 0.51 (18)	40.21 ± 0.82[a] (21)	41.17 ± 0.51 (18)	40.23 ± 0.52 (24)

Number of animals in parentheses; b.wt. in g ± SEM; *1 μg/kg b.wt. THC/day over the 3rd week of pregnancy; [a]$p<0.001$; [b]$p<0.05$; [c]$p<0.01$.

traperitoneally. Due to the very low concentration, the drug could be dissolved in watery solutions according to Harvey (4). The controls received only physiological saline (the solvent). Rats were grouped as follows: controls injection of solvent; daily injections of THC during the entire pregnancy; daily injections of THC during the 1st, 2nd or 3rd week of pregnancy, respectively.

The present study includes offspring from controls and the litters from dams that have received THC during the 3rd week of gestation, because in our previous study it was demonstrated that THC affected the pregnancy only if administered during the 3rd week (10).

Sample Collections

The progeny of altogether 52 controls and 96 treated dams were followed. Data were recorded every 5th day beginning from the day of delivery (D0) up to the 20th postnatal day (D20). After weighing the rats, the animals were decapitated, the mediobasal hypothalamus (MBH) together with the suprachiasmatic region (the two latter are further commonly referred as MBH) and pituitaries were quickly dissected out and subsequently frozen in liquid nitrogen. The trunk blood was collected, allowed to clot at 4°C and centrifuged. The sera were stored. All samples were frozen until further hormonal assay.

The gonads of both sexes were weighed and frozen.

Hypothalamic luteinizing hormone releasing hormone (LHRH) and neuropeptide-Y (NPY), pituitary luteinizing hormone (LH), serum LH were assayed as described before (12). Testosterone (T) (in males only), progesterone (P) and 17-β-E2 (E) (the latter in females only) were measured with kits supplied by BioMerieux Laboratories (France). The hormone levels were measured by RIA. To avoid interassay variations, all samples were measured in the same procedure for each hormone.

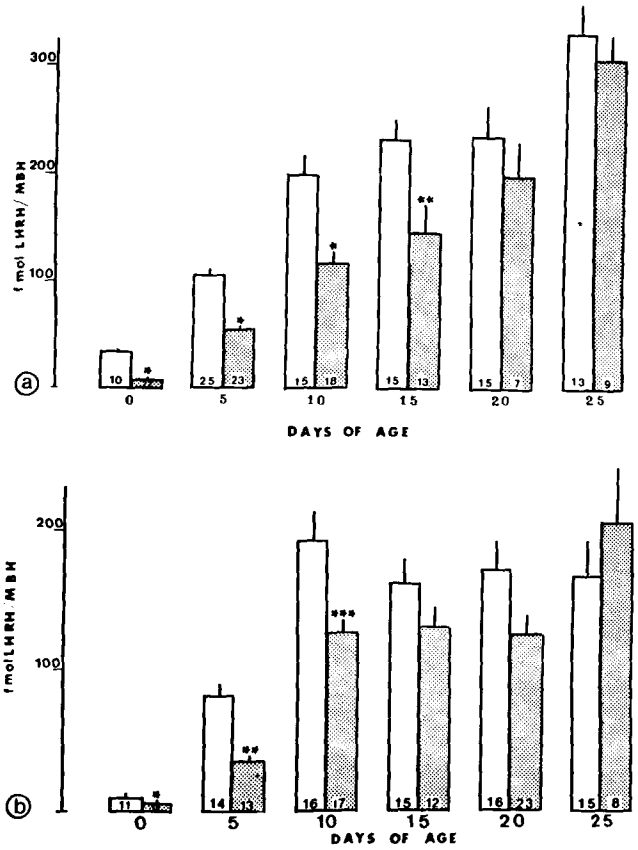

FIG. 1. The effects of prenatally administered THC on the LHRH content of mediobasal hypothalamus (MBH) in offspring (fmol LHRH/MBH ± SEM). (a) males, (b) females. Number in bars represents the number of animals investigated. (a) Open bars: controls; dotted bars: treated (1 μg/kg b.wt./day over the third week of pregnancy). *$p<0.005$, **$p<0.0025$. (b) Open bars: controls; dotted bars: treated (1 μg/kg b.wt. THC/day over the third week of pregnancy). *$p<0.025$, **$p<0.0025$, ***$p<0.01$.

TABLE 2

EFFECTS OF PRENATALLY ADMINISTERED THC* ON SERUM TESTOSTERONE LEVELS IN OFFSPRING

Days of Age	Control	Treated
0	5.06 ± 0.47 (18)	1.98 ± 0.56[a] (22)
5	2.08 ± 0.30 (21)	1.04 ± 0.31[b] (19)
10	1.43 ± 0.22 (20)	1.24 ± 0.20 (19)
15	1.08 ± 0.26 (18)	1.41 ± 0.37 (14)
20	1.53 ± 0.44 (10)	1.39 ± 0.14 (14)

Results are expressed in pmol/ml ± SEM; number of animals in parentheses; *1 μg/kg b.wt./day over the 3rd week of pregnancy; [a]$p<0.0025$; [b]$p<0.025$.

Statistical Analysis

Data were presented as the mean ± SEM and were analyzed using Student's *t*-test and ANOVA. $p<0.05$ was considered as significant.

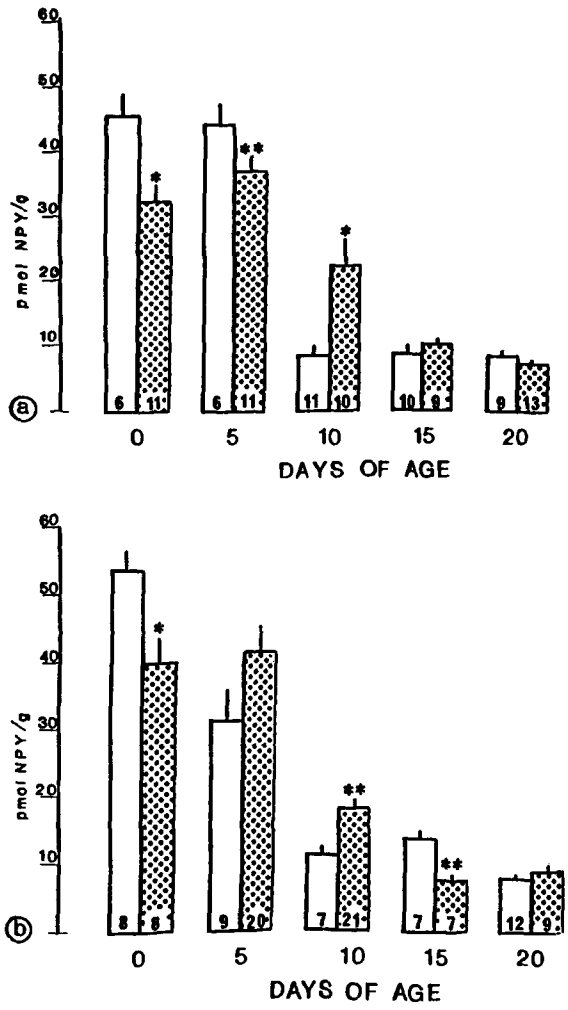

FIG. 2. The effects of prenatally administered THC on the NPY content of mediobasal hypothalamus in offspring (pmol NPY/gMBH ± SEM). (a) males, (b) females. Number in bars represents the number of animals investigated. (a) Open bars: controls; dotted bars: treated (1 μg/kg b.wt. THC/day over the third week of pregnancy). *$p<0.0025$, **$p<0.05$. (b) Open bars: controls; Dotted bars: treated (1 μg/kg b.wt. THC/day over the third week of pregnancy). *$p<0.0025$, **$p<0.025$.

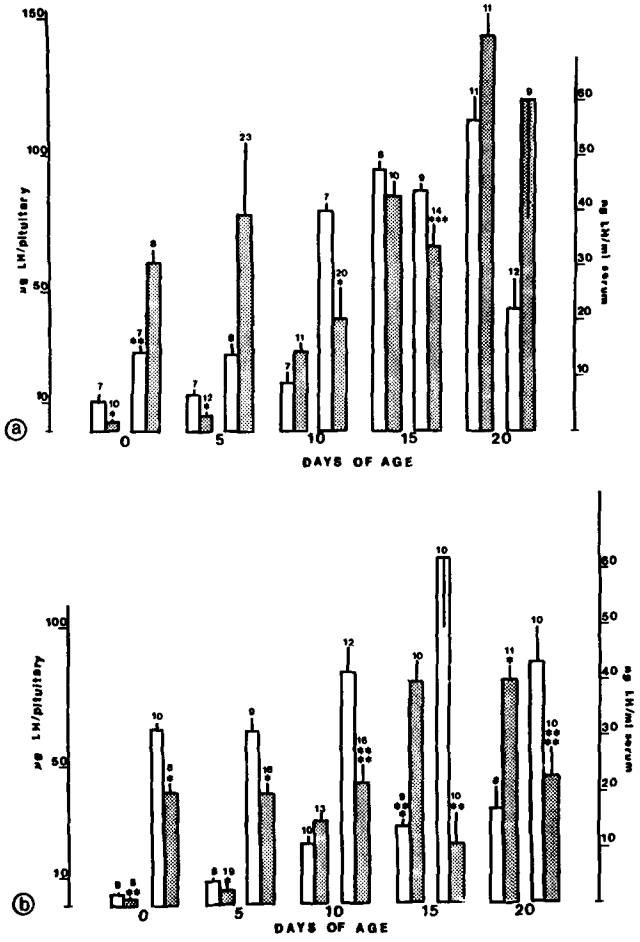

FIG. 3. The effects of prenatally administered THC on the LH content in pituitary (μg LH/pituitary ± SEM; left pairs of bars of each day) and serum (ng LH/ml serum; right pairs of bars of each day) in offspring. (a) males, (b) females. Number above the bars represents the number of animals investigated. (a) Open bars: controls; dotted bars: treated. *$p<0.001$ vs. controls, **$p<0.001$ vs. treated, ***$p<0.01$ vs. controls. (b) Open bars: controls; dotted bars: treated. *$p<0.001$ vs. controls, **$p<0.01$ vs. controls, ***$p<0.001$ vs. treated, ****$p<0.02$ vs. controls.

RESULTS

Table 1 shows the mean weight gain of newborn rats. The treated young animals of both sexes weighed less than the controls. The weight of males was less ($p<0.001$ vs. controls) than that of females ($p<0.01$ vs. controls). At D20, no differences were recorded between the treated and the control females, although male rats of that age were still significantly smaller in body weight ($p<0.001$).

The weight of the two testes or ovaries proved to be less in treated animals than in the controls, and the difference did not change all over the examined period (data not shown).

MBH LHRH decreased till D15 in both sexes; however, the males exhibited a more pronounced decrease (Fig. 1). NPY content (Fig. 2) was lower on D0 and D5. In females, differences were observed only at birth. By D20, neither LHRH nor NPY levels exhibited any difference in comparison with the controls.

The LH content in the pituitary was decreased only at D0 and D5 of the treated animals of both sexes (Fig. 3). Serum LH in females was lower all over the examined period, although in the pituitary no significant differences were recorded as from D10. The higher LH level in treated males from early postnatal period returned to the control level by D20. Serum T was significantly decreased in treated males in comparison to the controls, with the controls only in D0 and D5 (Table 2). No considerable differences in serum E levels were recorded after prenatal THC treatment. Serum P decreased in treated rats up to D10 (data not shown).

DISCUSSION

The present findings show that prenatal THC administration resulted in a decrease of birth weight of both body and gonads in rat offspring. Of particular interest were the alterations in hypothalamo-pituitary-gonadal axis. Dalterio et al. (2) have suggested that prenatal exposure of THC in mice produced long-

term alterations in pituitary gonadal functions. Our results showed that when the THC dose was low enough, the effects caused only temporary inhibition. It is possible that the observed alterations in neuroendocrine parameters were mediated by affecting biogenic amines, as it was postulated elsewhere (8). Further studies are needed to elucidate this problem.

Hutchings et al. (5) found a significant increase of the proportion of male offspring of THC-treated mothers. In our experimental conditions, the males were more affected by the prenatal THC administration than females. Hutchings et al. administered a much higher dose (50 mg/kg) then those used in our experiments. The different results observed might be attributed to the differences in doses. It seems reasonable to assume that while the females are affected more strongly by the higher doses than by lower ones, the responsiveness of the males may be less dose dependent.

Multiple doses of THC in the dams may result in the increase of maternal plasma concentration of THC or its metabolites. THC is readily transmitted through the placenta and it can be found in a relatively high concentration in the fetus (3). All the above mentioned effects may be mediated by either the persistence of active THC or by its metabolites in neonatal tissue. The direct inhibition of pathways regulating the development of neuroendocrine system cannot be excluded either.

The effects of THC if administered at a very low dose to pregnant rats were transitory. The highest amount of THC might be found in the newborns (D0). After delivery, no more THC gets access to the litters. The THC present in the offspring's body may decrease consecutively. The decrease of pharmacologically active THC in the postnatal period may be the most important element in these temporary effects.

If our assumption based on the results obtained here in rats is correct, it might also be extrapolated to other species, e.g., the human.

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