Pharmacology Biochemistry & Behavior, Vol. 20, pp. 107–113, 1984. © Ankho International Inc. Printed in the U.S.A.

Early Cannabinoid Exposure Influences Neuroendocrine and Reproductive Functions in Male Mice: I. Prenatal Exposure

S. DALTERIO†, R. STEGER,* D. MAYFIELD* AND A. BARTKE*

*Departments of Pharmacology† and Obstetrics and Gynecology**
The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

Received 17 June 1983

DALTERIO, S., R. STEGER, D. MAYFIELD AND A. BARTKE. *Early cannabinoid exposure influences neuroendocrine and reproductive functions in male mice: I. Prenatal exposure.* PHARMACOL BIOCHEM BEHAV 20(1) 107–113, 1984.—Maternal exposure to Δ^9-tetrahydrocannabinol (THC), the major psychoactive constituent in marihuana, or to the non-psychoactive cannabinol (CBN) or cannabidiol (CBD) alters endocrine functions and concentrations of brain biogenic amines in their male offspring. Prenatal CBN exposure on day 18 of gestation resulted in decreased plasma FSH levels, testicular testosterone (T) concentrations, and seminal vesicles weights, but increased plasma levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) post-castration in adulthood. Prenatal exposure to THC significantly enhanced the responsiveness of the testes to intratesticular LH injection *in vivo* and tended to increase human chorionic gonadotropin (hCG)-stimulated T production by decapsulated testes *in vitro*. In the CBN-exposed mice, hCG-stimulated T production was enhanced, while CBD exposure had no effect. Prenatal THC exposure altered the negative feedback effects of exogenous gonadal steroids in castrated adults, with lower plasma T and FSH levels after 20 μg T than in castrated controls. In contrast, CBD-exposed mice had higher levels of LH in plasma post-castration. In CBN-exposed adults, two weeks post-castration the concentration of norepinephrine (NE) and dopamine (DA) in hypothalamus and remaining brain were reduced, while levels of serotonin (5-HT) and its metabolite, 5-HIAA, were elevated compared to that in castrated OIL-controls. Prenatal CBD-exposure also reduced NE and elevated 5-HT and 5-HIAA, but did not affect DA levels post-castration. Concentrations of brain biogenic amines were not influenced by prenatal THC exposure in the present study. A single prenatal exposure to psychoactive or non-psychoactive components of marihuana results in long term alterations in the function of the hypothalamo-pituitary-gonadal axis. Changes in the concentrations of brain biogenic amines may be related to these effects of prenatal cannabinoids on endocrine function in adult male mice.

Δ^9-Tetrahydrocannabinol Cannabidiol Testosterone Follicle-stimulating hormone
Intratesticular injections Cannabinol Biogenic amines Luteinizing hormone *In vitro* T production
Prenatal cannabinoids

THE early hormonal milieu of the developing organism is critical for sexual differentiation of a variety of neurochemical, physiological and behavioral characteristics [16,20]. Marihuana, and its purified constituents, have been reported to exert a wide range of effects on the function of the endocrine system. In adult males, both men and laboratory animals, administration of Δ^9-tetrahydrocannabinol (THC), the major psychoactive component in marihuana, can alter plasma levels of testosterone (T), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [3].

Cannabinoids cross the placental barrier and are distributed into a wide variety of fetal tissues [3]. Biochemical changes, including alterations in brain RNA synthesis, have been induced in neonatal rats as a result of prenatal THC exposure [18]. We recently reported that treatment of female mice during the mid-portion of gestation with THC or cannabinol (CBN), a relatively non-psychoactive constituent of marihuana, resulted in a significant reduction in the concentration of T in their male, but not female, fetuses [8]. It is, therefore, possible that cannabinoids interfere with the well-documented androgen-dependent sexual differentiation during critical prenatal periods of development [16]. Androgen production by the fetal testis plays a critical organizational role in the establishment of the male reproductive structures and in the development of male patterns of sexual behavior. Neonatal castration [23], or stress [21,28] are well known to produce alterations in the steroidogenic function of the testis, resulting in long term consequences for sexual differentiation.

We have previously reported that exposure of female mice to THC or CBN on the last day of gestation and for the first six days post-partum produced long term alterations in body weight regulation, pituitary-gonadal function, responsivity to stimuli from female conspecifics, as well as in adult copulatory behavior [7].

The present experiments were designed to characterize

TABLE 1

PLASMA LEVELS OF TESTOSTERONE (T), LUTEINIZING HORMONE (LH), OR FOLLICLE-STIMULATING HORMONE (FSH) IN ADULT MALE MICE PRENATALLY EXPOSED TO CANNABINOIDS

Treatment	Plasma T (ng/ml)	Plasma LH (ng/ml)	Plasma FSH (ng/ml)
Oil			
intact	16.5 ± 4.1 (5)	23.7 ± 4.1 (7)	959 ± 59 (9)
castrated†	—	82.0 ± 5.0 (12)	2102 ± 125 (12)
THC			
intact	9.4 ± 2.6 (3)	26.5 ± 7.6 (5)	885 ± 78 (3)
castrated†	—	80.0 ± 10.0 (17)	2073 ± 119 (17)
CBN			
intact	7.8 ± 2.7 (4)	16.4 ± 7.5 (5)	663 ± 76 (5)*
castrated†	—	121.0 ± 6.0 (8)*	2326 ± 61 (15)*
CBD			
intact	10.5 ± 4.9 (6)	41.5 ± 15.3 (7)	919 ± 47 (7)
castrated†	—	95.0 ± 9.0 (15)	1898 ± 64 (12)

Means ± SE (n).
*Significantly different from controls ($p<0.05$) by Mann-Whitney U-Test.
†Animals were castrated two-weeks prior to sacrifice.

the effects of prenatal exposure to THC, CBN, or to another non-psychoactive component of marihuana, cannabidiol (CBD). These effects of prenatal exposure are compared to those previously reported for combined pre- and postnatal exposure. In the present study we examined plasma levels of T and gonadotropins, and weights of testes and seminal vesicles. We also measured testicular responsiveness to gonadotropins, *in vivo* or *in vitro*, in adult male mice that were prenatally exposed to cannabinoids. We also examined the pituitary responses to castration and to the administration of exogeneous gonadal steroids. In addition, the concentrations of brain biogenic amines in hypothalamus and in the remaining brain tissue were measured to determine if alterations in biogenic amines were related to changes in neuroendocrine function in these adult male mice.

METHOD

Animals

Primiparous female mice obtained from our colony of randomly bred animals were housed individually with an adult male and checked for the presence of a copulatory plug (considered day 1 of pregnancy). On day 18 of gestation the females received a single oral dose of 50 mg THC, CBN, or CBD per kg body weight or 20 μl of sesame oil by oral administration. Since female mice in our colony deliver between 19 and 21 days post-mating, females in each treatment group received cannabinoids or oil on one of the last four days of gestation. On the first day post-partum, litters were culled to five or six male pups; the offspring were weaned at 21 days of age and housed in groups of three until adulthood (60–80 days).

Effects of Exogenous Androgens in Castrates

Half of the animals in each treatment group were castrated for studies of the negative feedback effects of exogeneous androgen. While the animals were under ether anesthesia, the testes were removed, weighed, homogenized in distilled water (9:1 w/v) and stored frozen for the radioimmunoassay determination of T, as described previously [7–13]. One week later, castrated males received 20 μg of free T in a 50 μl volume of sesame oil by SC injection and the animals were bled by cardiac puncture under ether anesthesia one hour after T injection. A small volume of blood (200 μl) was taken so that the animals were allowed to survive for an additional week.

Biogenic Amine Determinations

Two weeks post-castration, the males were sacrificed by cervical dislocation, along with intact males from the same treatment groups, trunk blood was collected and the plasma stored frozen for the RIA determination of T, LH, and FSH, and the brains were removed and stored frozen for measurement of amine concentrations.

Prior to the amine assay, the brains were partially thawed and the hypothalamus was dissected free. The hypothalamus consisted of a tissue block 2.0 mm deep extending from the rostral margin of the mammillary body to the caudal border of the optic chiasm and laterally to the hypothalamic sulci. The hypothalamic block and the remaining brain tissue were weighed and sonicated in 0.1 N $HClO_4$ containing 3-methoxy-4-hydroxyphenylethanol (MOPET), as a standard for the indoleamine assay, dihydroxybenzylamine (DHBA), as an internal standard for the catecholamine assay, and 1.0 mM sodium metabisulfite.

Indoleamines were separated by high performance liquid chromatography (HPLC) and quantitated by electrochemistry [25,26]. Standards were run concurrently, and serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were calculated by comparison of peak heights with those of the standards. Values were corrected for recovery of the internal standard which averaged 97.3±1.2%. The intra-assay coefficient of variation was 5.6% for 5-HT, and 7.2% for 5-HIAA.

Catecholamines were prepared for chromatography as

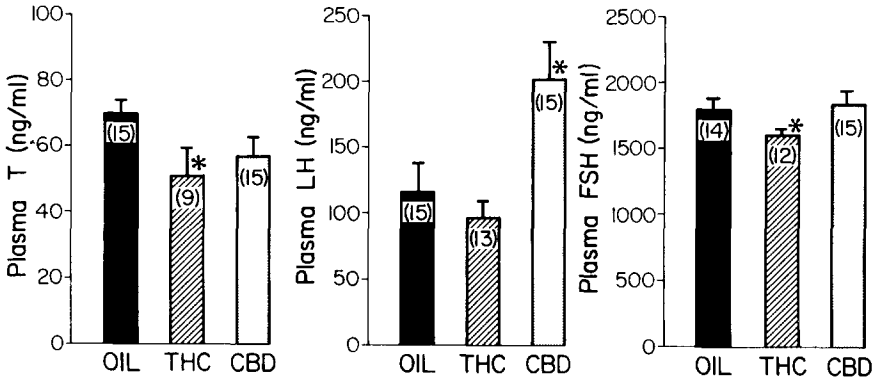

FIG. 1. Effect of exogeneous administration of 20 μg testosterone (T) in sesame oil on plasma levels of T, LH and FSH one hour later in castrated adult male mice prenatally-exposed to Δ^9-tetrahydrocannabinol (THC), or cannabidiol (CBD). Means±SE (n). *Significantly different from control ($p<0.05$) by Mann-Whitney U-test.

previously described [25]. Norepinephrine (NE), dopamine (DA) and DHBA were separated by HPLC and quantitated by electrochemistry. The recovery of DHBA averaged 82.3±1.1% and the intra-assay coefficient of variation was 6.1% for NE and 6.7% for DA.

Testicular Responsiveness to Gonadotropins

For determinations of testicular responsiveness to gonadotropins *in vivo*, adult mice were lightly anesthetized with ether and 10 ng LH was injected into one testis through the scrotal skin using a 27-gauge needle. The contralateral testis received 10 μl saline. The animals were sacrificed by cervical dislocation 30 min later, and T levels were measured in the testicular homogenate. The values are expressed as the ratio of T in the LH- versus that in the saline-injected testis. This intratesticular injection technique is described in further detail in two recent publications from this laboratory [10,13].

Plasma Hormone Levels

At the time of sacrifice, trunk blood was collected for radioimmunoassay (RIA) determination of plasma T, LH and FSH levels. Testosterone was measured without chromatographic separation [7–13]. Gonadotropins were measured using the NIAMDD kits for rat FSH and Niswender's antiovine LH, which have been previously validated for the measurement of mouse gonadotropins [1], and the results expressed in terms of NIAMDD RP-1 standards. The samples for each hormone were run in a single assay and the intra-assay coefficients of variation were, respectively, 2.1% for LH, 1.4% for FSH and 8.1% for T.

In Vitro *T Production*

Testicular responsivity to gonadotropins *in vitro* was determined using decapsulated testes incubated in Krebs-Ringer bicarbonate buffer containing glucose (1 mg/ml) and 12.5 mIU/ml human chorionic gonadotropin (hCG; Follutein®, Squibb). The accumulation of T in the media was determined after a 4 hr incubation by RIA, without extraction, as described previously [10].

Statistics

Data was analyzed by a two-way analysis of variance and in all cases except that noted in Table 3 there were no significant differences between groups due to the time of prenatal treatment relative to delivery. Therefore, results were combined for further statistical analysis and presentation.

Student's *t*-test was used to determine the significance of the differences between two treatment groups, and analysis of variance was used with Duncan's test for multiple comparisons for three or more groups [29].

For values which were not normally distributed nonparametric tests were used.

RESULTS

Plasma T, LH and FSH

Prenatal exposure to CBN resulted in a significant decrease in the levels of FSH in plasma, and, an apparent, although not statistically significant, reduction in plasma T levels (Table 1). Prenatal exposure to THC or CBD did not significantly affect plasma hormone levels, although plasma LH levels tended to be increased in the CBD-exposed males.

Post-castration, plasma levels of LH and FSH were significantly higher in CBN-exposed males, compared to those in castrated controls (Table 1).

In an additional experiment, plasma T and FSH levels after injection of 20 μg T were significantly lower in castrated THC-exposed animals, than in castrated controls (Fig. 1). In contrast, plasma LH levels measured after T injection were higher in the CBD-exposed castrates than in the THC-treated or control animals (Fig. 1).

Biogenic Amine Concentrations

Castration did not influence catecholamine levels in hypothalamic or remaining brain tissue in the OIL-exposed control males (Tables 2 and 3). However, serotonin and 5-HIAA concentrations were significantly lower in castrated, compared to intact control males. The amine concentrations in THC-exposed animals were not different from those in the controls, in terms of absolute values or the response to castration. In contrast, in castrated CBN- and CBD-exposed males, the concentrations of NE in hypothalamus and remaining brain were reduced in comparison to castrated controls, as well as intact CBN- or CBD-exposed animals (Tables 1 and 2). The concentration of DA was also lower in

TABLE 2

THE CONCENTRATIONS (ng/g) OF NOREPINEPHRINE (NE), DOPAMINE (DA), SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN BRAIN TISSUE FROM ANIMALS PRENATALLY EXPOSED TO Δ^9-TETRAHYDROCANNABINOL (THC), CANNABINOL (CBN), OR CANNABIDIOL (CBD)

Treatment	NE	DA	5-HT	5-HIAA
Oil				
intact	374 ± 18 (20)*	1268 ± 77 (20)*	1064 ± 85 (20)*	238 ± 13 (20)*
castrated‡	398 ± 12 (12)*	1288 ± 81 (12)*	579 ± 21 (11)†	181 ± 6 (11)†
THC				
intact	387 ± 22 (13)*	1410 ± 65 (11)*	747 ± 82 (13)*†	251 ± 10 (13)*
castrated‡	398 ± 12 (18)*	1345 ± 39 (18)*	583 ± 46 (18)†	194 ± 5 (18)†
CBN				
intact	364 ± 26 (8)*	996 ± 34 (8)†	1243 ± 74 (13)*	250 ± 13 (12)*
castrated‡	254 ± 29 (14)†	793 ± 97 (14)†	1024 ± 60 (14)*	267 ± 9 (14)*
CBD				
intact	372 ± 7 (13)*	976 ± 43 (10)†	1201 ± 70 (14)*	252 ± 8 (14)*
castrated‡	247 ± 20 (14)†	748 ± 60 (14)†	1010 ± 28 (14)*	249 ± 4 (14)*

Means ± SE (n).

*†Values with the same superscript not significantly different by analysis of variance.

‡All animals were castrated two weeks prior to sacrifice.

N.B. The brain tissue includes the remaining tissue after the removal of the hypothalamic block (see text for details).

TABLE 3

THE CONCENTRATIONS (ng/g) OF NE, DA, 5-HT AND 5-HIAA IN THE HYPOTHALAMUS OF ADULT MALE MICE PRENATALLY EXPOSED TO THC, CBN, OR CBD

Treatment	NE	DA	5-HT	5-HIAA
Oil				
intact	1325 ± 87 (20)*	1391 ± 130 (20)*	2436 ± 169 (19)*	563 ± 32 (19)*
castrated‡	1371 ± 101 (12)*	1244 ± 189 (12)*	1308 ± 52 (12)†	359 ± 17 (12)†
THC				
intact	1496 ± 82 (14)*	1321 ± 157 (14)*	1850 ± 139 (14)*	488 ± 39 (13)*
castrated‡	1320 ± 54 (18)*	1161 ± 83 (18)*	1274 ± 54 (18)†	377 ± 13 (18)†
CBN				
intact	1034 ± 39 (13)*	1068 ± 136 (15)*	2982 ± 182 (15)*	568 ± 34 (13)*
castrated‡	849 ± 76 (23)†	1006 ± 103 (14)*	2247 ± 206 (14)*	504 ± 45 (14)*
CBD				
intact	988 ± 167 (15)*	1201 ± 146 (13)*	2639 ± 129 (13)*	536 ± 12 (14)*
castrated‡				
Day 2:	800 ± 72 (9)†	1134 ± 146 (13)*	2191 ± 144 (14)*	477 ± 33 (14)*
Day 3:	1225 ± 256 (5)*			

Means ± SE (n).

*†Values with the same superscript not significantly different by analysis of variance.

‡All animals were castrated two weeks prior to sacrifice.

N.B. The brain tissue includes the remaining tissue after the removal of the hypothalamic block (see text for details).

TABLE 4

TESTICULAR TESTOSTERONE (T) CONCENTRATIONS AND WEIGHTS OF THE TESTES AND SEMINAL VESICLES IN ADULT MALE MICE PRENATALLY EXPOSED TO THC, CBN, OR CBD

	Testicular Testosterone Concentrations (ng/ml)	Testes Weights (mg)	Seminal Vesicles (mg)
OIL	138 ± 22 (12)	278 ± 7 (12)	258 ± 13 (12)
THC	112 ± 15 (17)	296 ± 9 (18)	219 ± 28 (18)
CBN	69 ± 17 (14)*	268 ± 12 (14)	164 ± 16 (14)*
CBD	126 ± 21 (14)	310 ± 8 (14)*	308 ± 12 (14)*

Means ± SE (n).
*Significant different from controls ($p<0.05$) by analysis of variance.

brain of CBN-exposed males, and appeared lower in hypothalamus, although this was not statistically significant. In contrast, the levels of hypothalamic and brain 5-HT and 5-HIAA were significantly higher in castrated CBN- and CBD-exposed mice, in comparison to castrate controls. The levels of hypothalamic 5-HT also appeared elevated in CBN-exposed intact males compared to those in the intact OIL-controls, although the difference was not significant. There were no significant differences in the weights of the brain or the hypothalamic tissue blocks, or in body weight among the animals in the different treatment groups (data not shown).

Testicular Responsiveness to Gonadotropins

Prenatal THC exposure significantly enhanced the responsivity of the testis to *in vivo* intratesticular LH administration. The ratio of T, i.e., the concentration of T in LH- versus saline-injected testes, was significantly higher in the THC-exposed animals (Fig. 2). However, testicular T concentrations under basal conditions, i.e., in the saline-injected testis, were significantly reduced in the testes from the THC-exposed males (20±1 vs. 66±12 ng/ml; $p<0.05$), but the absolute T concentrations in LH-injected testes did not differ between THC- and OIL-exposed males (146±36 vs. 180±28 ng/ml). Testicular weights were also not different in these two groups, 327±21 (n=6) vs. 338±7 mg (n=10).

Organ Weights and Testicular T Levels

Testicular T concentrations and seminal vesicles weights were reduced in adult male mice exposed to CBN, although testicular weights were not affected. In contrast, prenatal CBD exposure resulted in an increase in seminal vesicles weights (Table 4). Exposure to THC did not influence these parameters.

In Vitro *T Production*

There was a significant increase in T production by decapsulated testes obtained from mice prenatally exposed to CBN (Fig. 3, left). Prenatal THC exposure tended to enhance testicular responsiveness to hCG *in vitro*, but this apparent effect was not significant. In this group of animals the weights of the testes were significantly increased (Fig. 3, right).

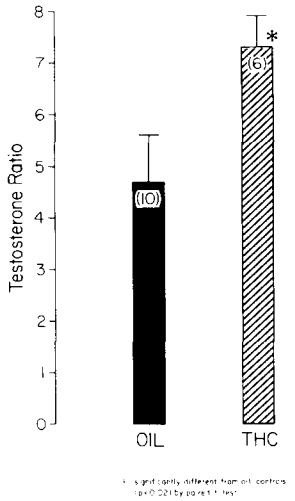

FIG. 2. Effect of intratesticular injection of LH (10 ng) into one testis and 10 μl saline into the contralateral testis of adult mice prenatally exposed to Δ^9-tetrahydrocannabinol (THC). Values are expressed as the ratio of the T concentration in the LH- versus that in the saline-injected testis (absolute values in the Text). Means±SE (n).

DISCUSSION

The present findings indicate that prenatal exposure to cannabinoids results in alterations in endocrine function and brain amine levels in adulthood. The day on which the cannabinoids were administered relative to the day of delivery did not appear to affect the parameters measured. Cannabinoid exposure occurred on the 18th day after the presence of the copulatory plug, and although the lengths of gestation varied among females, there were no significant differences due to treatment.

In the present study, prenatal CBN exposure resulted in a significant reduction in plasma FSH levels in adulthood, and plasma LH levels also tended to be lower than controls. These findings are consistent with the effect of perinatal [7] and postnatal (Dalterio, unpublished) CBN exposure. Thus, exposure to CBN appears to have a consistent suppressive effect on pituitary gonadotropin release.

We have previously reported that the concentration of LH in the plasma of male mice exposed to THC both pre-

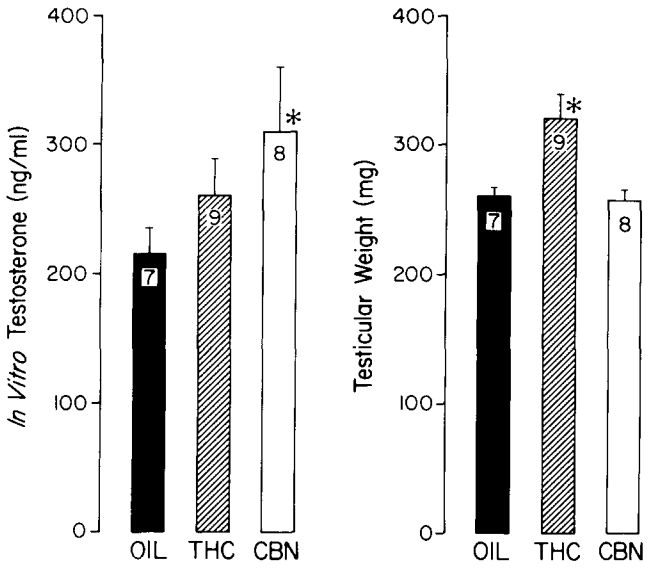

FIG. 3. *In Vitro* testosterone (T) production by decapsulated testes (left) and testicular weights (right) in adult mice prenatally exposed to Δ^9-tetrahydrocannabinol (THC) or cannabinol (CBN). The testes were incubated in the presence of 12.5 mIU human chorionic gonadotropin (hCG) and T was measured in the media after a 4 hr incubation. Means±SE (n). *Significantly different from OIL ($p<0.05$) by Mann-Whitney U-test.

and postnatally was significantly elevated (almost 3 fold normal) beginning at about 35 days of age through adulthood, although plasma levels of T were normal or low normal. Thus, testicular responsiveness to increased levels of endogenous gonadotropin appeared reduced in these animals, while in the present study testicular responsiveness to exogeneous gonadotropins appears enhanced. This increase in responsiveness may be related to the fact that, under basal conditions, the concentration of T in the testes of THC-exposed mice was significantly lower than that of the controls. Since plasma LH levels were comparable to control values, it is difficult to understand the reasons for the decreased testicular T levels. Our results indicate that testes of THC-exposed mice are able to respond to exogeneous gonadotropin to an extent at least comparable to that of the controls under similar levels of stimulation. It would seem, therefore, that critical time periods exist for cannabinoid-induced alterations of pituitary-testicular interrelationships. In view of the biphasic effects of THC on androgen production *in vivo* [9], and *in vitro* [10], and the existence of critical periods for the actions of androgenic steroids on several target tissues [16], it is also possible that a single THC dose may have opposite effects on the same parameter. At present, we can only conclude that early exposure to THC is capable of altering the sensitivity of the testis to gonadotropic stimulation.

Cannabinoids appear capable of disrupting pituitary-gonadal feedback regulation. In the present study, administration of T post-castration resulted in lower T and FSH levels in the THC-exposed than in control mice, while the animals exposed to CBN had elevated plasma LH levels. It is possible that early cannabinoid exposure may have affected hepatic enzyme activity resulting in altered steroid metabolism and/or clearance, as has been reported after cannabinoid administration in adult males [19]. However, we have reported that pituitary gonadotropin release post-castration is influenced by perinatal THC exposure [12], and, in this report, by prenatal CBN treatment. Therefore, it is likely that changes in pituitary sensitivity to steroids may also result from exposure to cannabinoids early in development. These changes may be related to the effects of cannabinoids on steroid binding in the target tissues [22].

The relatively non-psychoactive CBN and CBD altered brain and hypothalamic concentrations of NE, DA, 5-HT, and 5-HIAA, as well as testicular T concentrations and seminal vesicles weights, while prenatal THC exposure did not influence these parameters. However, we have recently observed that exposure to THC during mid-gestation alters brain amine levels in adult mice (Dalterio and Steger, unpublished observations).

The effects of prenatal cannabinoid exposure on brain amine levels were more evident after castration. Although we do not know the precise mechanism by which castration differentially affected cannabinoid-exposed animals, it is known that castration can cause significant changes in hypothalamic amine metabolism. For example, castration stimulates NE turnover in adult rats [27], and neonatal castration alters DA levels in the adult [5]. Hypothalamic 5-HT turnover is decreased after castration, but returns to intact control levels within a week of surgery [27]. In the present experiment, amine turnover was not measured, but changes in amine levels suggest that amine metabolism or possibly neuron numbers, which are affected by early exposure to phenobarbital [2], were influenced by prenatal cannabinoid exposure, and that these changes were more apparent when the neuroendocrine axis reacted to the removal of steroid negative feedback.

It is possible that cannabinoid exposure altered the responsiveness to the surgical stress associated with castration. We have previously reported that adult male mice treated with cannabinoids exhibit a differential hormonal response to stressful stimuli [11], and pre-pubertal male mice perinatally exposed to cannabinoids appear to respond to individual housing with a female as a stressful situation, as indicated by reductions in body and seminal vesicles weights, and increased adrenal weights [7]. Indeed, it has been reported that prenatal stress reduces hypothalamic NE concentrations [21] as well as male copulatory behavior [28], and dysfunction of the hypothalamo-noradrenergic system has been reported in prenatally androgen-sterilized rats [17].

The observation that castration induced differential effects on brain neurotransmitter levels in prenatally cannabinoid-exposed mice may be due to the interrelationships of gonadal steroids and the central nervous system biogenic amines [6]. The participation of biogenic amines in androgen-dependent brain sexual differentiation has been suggested, with feminization of male neuroendocrine centers associated with suppression of catecholamines and serotonin synthesis [24]. At present, the precise mechanism by which castration appears to reveal effects of prenatal cannabinoid exposure on central nervous system neurotransmitters remains unclear. However, based on our earlier observations these findings seem to be consistent with cannabinoid-induced alterations in physiological responsivity to events which disturb homeostatic conditions.

It is conceivable that the reported effects of prenatal cannabinoid exposure on human neonates, which include neurological changes [14], are related to cannabinoid-induced changes in neurotransmitters. We have previously reported that male copulatory behavior was reduced differentially by

THC or CBN [7]. Although the influences of neurotransmitters on sexual behavior are not clear, there have been indications that serotonin has an inhibitory effect on adult sexual behavior, while the catecholamines may be facilatory [15]. This would appear consistent with the present findings that, in animals exposed to CBN prenatally, serotonin levels were increased, while those of NE were reduced, because we have previously observed that perinatal CBN exposure significantly altered adult sexual performance [7]. Thus, it is conceivable that changes in behavioral responsivity to stimuli from conspecifics may be related to cannabinoid-induced alterations in brain neurotransmitters during critical periods of sexual differentiation.

In summary, it is evident that maternal exposure to either the psychoactive or the non-psychoactive components of marihuana can produce long term alterations in neuroendocrine and reproductive functions in their male offspring. Furthermore, it is apparent that some effects of cannabinoids on the endocrine system may not become apparent until maturational or environmental factors require physiological responses.

ACKNOWLEDGEMENTS

This work was supported by a grant from NIH (5 R 23 HD 16329) and a grant to the Radioimmunoassay Core in the Department of OB-GYN (P 30 HD 10202).

REFERENCES

1. Beamer, W. G., S. M. Murr and I. I. Geschwind. Radioimmunoassay of mouse luteinizing and follicle stimulating hormones. *Endocrinology* **90:** 823–826, 1972.
2. Bergman, A., J. J. Feigenbaum and J. Yanai. Neuronal losses in mice following both prenatal and neonatal exposure to phenobarbital. *Acta Anat* **114:** 185–192, 1982.
3. Bloch, E., B. Thysen, G. A. Morrill, E. Gardner and G. Fujimoto. Effects of cannabinoids on reproduction and development. *Vit Horm* **36:** 203–258, 1978.
4. Bradshaw, W. G., M. S. Erskine and M. J. Baum. Dissociation of the effects of gonadal steroids on brain serotonin metabolism and sexual behavior in the male rat. *Neuroendocrinology* **34:** 38–45, 1982.
5. Crowley, W. R., T. L. O'Donohue and D. M. Jacobowitz. Sex differences in catecholamine content in discrete brain nuclei of the rat: Effect of neonatal castration or testosterone treatment. *Acta Endocrinol* **89:** 20–28, 1978.
6. Demarest, K. T., D. W. McKay, G. D. Riegle and K. E. Moore. Sexual differences in tuberoinfundibular dopamine nerve activity induced by neonatal androgen exposure. *Neuroendocrinology* **32:** 108–113, 1981.
7. Dalterio, S. L. Perinatal or adult exposure to cannabinoids alters male reproductive functions in mice. *Pharmacol Biochem Behav* **12:** 143–153, 1980.
8. Dalterio, S. and A. Bartke. Fetal testosterone in mice: Effects of gestational age and cannabinoid exposure. *J Endocrinol* **91:** 509–514, 1981.
9. Dalterio, S., A. Bartke and D. Mayfield. Delta-9-tetrahydrocannabinol increases plasma testosterone levels in mice. *Science* **231:** 581–583, 1981.
10. Dalterio, S., A. Bartke and D. Mayfield. Cannabinoids stimulate and inhibit testosterone production in vitro and in vivo. *Life Sci* **32:** 605–612, 1983.
11. Dalterio, S., S. D. Michael, B. T. Macmillan and A. Bartke. Differential effects of cannabinoid exposure and stress on plasma prolactin, groth hormone and corticosterone levels in male mice. *Life Sci* **28:** 761–766, 1981.
12. Dalterio, S., A. Bartke and C. Sweeney. Interactive effects of ethanol and Δ^9-tetrahydrocannabinol on endocrine functions in male mice. *J Androl* **2:** 87–93, 1981.
13. Dalterio, S., A. Bartke, A. Brodie and D. Mayfield. Effects of testosterone, estradiol, aromatase inhibitor, gonadotropin and prolactin on the response of mouse testes to acute gonadotropin stimulation. *J Steroid Biochem* **18:** 391–396, 1983.
14. Fried, P. A. Marihuana use by pregnant women: Neurobehavioral effects in neonates. *Drug Alcohol Depend* **6:** 415–424, 1980.
15. Gessa, G. L. and A. Tagliamonte. Role of brain serotonin and dopamine in male sexual behavior. In: *Sexual Behavior: Pharmacology and Biochemistry*, edited by M. Sandler and G. L. Gessa. New York: Raven, 1975, pp. 117–132.
16. Gorski, R. A., R. E. Harlan and L. W. Christensen. Perinatal hormonal exposure and the development of neuroendocrine regulatory processes. *J Toxicol Environ Health* **3:** 97–121, 1977.
17. Lookingland, K. J., P. M. Wise and C. A. Barraclough. Failure of the hypothalamo-noradrenergic system to function in adult androgen-sterilized rats. *Biol Reprod* **27:** 268–281, 1982.
18. Luthra, Y. K. Brain biochemical alterations in neonate of dams treated orally with Δ^9-tetrahydrocannabinol during gestation and lactation. In: *Marihuana: Biochemical Effects*, edited by G. G. Nahas and W. D. M. Paton. New York: Pergamon, 1979, pp. 531–537.
19. Maskarinec, M. P., G. Shipley, M. Novotny, D. J. Brown and R. P. Farney. Different effects of synthetic Δ^9-THC and cannabis extract on steroid metabolism in male rats. *Experientia* **34:** 88–89, 1978.
20. McEwen, B. S. Sexual maturation and differentiation: The role of gonadal steroids. *Prog Brain Res* **48:** 291–308, 1978.
21. Moyer, J. A., L. R. Herrenkohl and D. M. Jacobowitz. Effects of stress during pregnancy on catecholamines in discrete brain regions. *Brain Res* **121:** 385–393, 1978.
22. Purohit, V., B. S. Ahluwalia and R. A. Vigersky. Marihuana inhibits dihydrotestosterone binding to the androgen receptor. *Endocrinology* **107:** 848–850, 1980.
23. Quadagno, D. M., H. G. Wolfe, G. Kan Wha Ho and B. D. Goldman. Influence of neonatal castration or neonatal antigonadotropin treatment on fertility, phallus development and male sexual behavior. *Fertil Steril* **26:** 939–944, 1975.
24. Reznikov, A. G., N. D. Nosenko and L. P. Demkiv. New evidence for participation of biogenic amines in androgen-dependent sexual differentiation of hypothalamic control of gonadotropin secretion in rats. *Endokrinologie* **73:** 11–19, 1979.
25. Steger, R. W., A. Bartke and B. D. Goldman. Alterations in neuroendocrine function during photoperiod induced testicular atrophy and recrudescence in the Golden hamster. *Biol Reprod* **26:** 437–444, 1982.
26. Steinlechner, S., R. W. Steger, T. S. King and R. J. Reiter. Diurnal variation in the serotonin content and turnover in the pineal gland of the Syrian hamster. *Neurosci Lett* **35:** 167–172, 1983.
27. Van de Kar, L., J. Levine and L. S. Van Order III. Serotonin in hypothalamic nuclei: Increased content after castration of male rats. *Neuroendocrinology* **27:** 186–192, 1978.
28. Ward, I. Prenatal stress feminizes and demasculinizes the behavior of male rats. *Science* **175:** 82–84, 1972.
29. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.