Early Cannabinoid Exposure Influences Neuroendocrine and Reproductive Functions in Mice: II. Postnatal Effects[1]

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DALTERIO, S., R. STEGER, D. MAYFIELD AND A. BARTKE. *Early cannabinoid exposure influences neuroendocrine and reproductive functions in mice: II: Postnatal effects.* PHARMACOL BIOCHEM BEHAV **20**(1) 115–123, 1984.—Maternal exposure to Δ^9-tetrahydrocannabinol (THC), the major psychoactive component in marihuana, or to the non-psychoactive cannabinol (CBN) or cannabidiol (CBD) alters male reproductive functions and brain biogenic amines in male and female offspring. Postnatal exposure to THC or CBN reduced body weights, while testicular weights were lower in CBD-exposed mice. Testicular testosterone (T) levels were also lower in CBN- and CBD-exposed animals. Postnatal cannabinoid exposure reduced plasma luteinizing hormone (LH) levels in intact and castrated adults. Although basal T production *in vitro* was not affected by postnatal cannabinoid exposure, testes from CBD-exposed males were more responsive to gonadotropin stimulation. In contrast, *in vivo* responsiveness to intratesticular human chorionic gonadotropin (hCG) administration was significantly reduced in THC- and CBD-exposed males. Pituitary weights and their basal LH production *in vitro* was higher in THC- or CBN-exposed mice. Pituitaries from cannabinoid-exposed males were less responsive to LH releasing hormone (RH) stimulation, however, hypothalamic LHRH content was significantly higher in the THC-exposed males. Hypothalamic dopamine (DA) levels were significantly lower in CBN-exposed castrated mice, compared to castrated controls. The reduction in hypothalmic norepinephrine (NE) in THC- and CBN-exposed castrates after α-methylparatyrosine (α-MPT) was significantly less than that observed in control castrates. Hypothalamic DA levels were depleted to a greater extent in CBD-exposed males. Brain levels of serotonin (5-HT) and 5-HIAA were significantly higher in castrated, than in intact THC-exposed males. In ovariectomized CBN-exposed females, hypothalamic NE levels were lower, while the α-MPT-induced depletion of NE was less in CBD-exposed, compared to control females. Levels of 5-HT were lower only in THC-exposed females. Plasma levels of LH were significantly higher in CBN-exposed, while plasma levels of FSH were reduced in THC- and CBD females. Maternal exposure to psychoactive or non-psychoactive cannabinoids on the day of parturition results in long term alterations in neuroendocrine function in male and female offspring. It is possible that the observed alterations in biogenic amines may mediate the effects of cannabinoids on pituitary and gonadal function.

Biogenic amines   Cannabinol   Testosterone   Follicle-stimulating hormone   *In vitro* T production
Δ^9-Tetrahydrocannabinol   Cannabidiol   Luteinizing hormone   Hypothalamic LHRH
Postnatal cannabinoids

MARIHUANA, and its purified constituents, have been shown to alter androgen production *in vitro* [8] and *in vivo*, in both immature and adult animals [8, 9, 19] and in adult men (review in [3]). Since cannabinoids cross the placental barrier and are distributed into a wide variety of fetal tissues [3], it is not surprising that they also influence fetal T production. We have previously demonstrated that maternal exposure during mid-gestation, to either Δ^9-tetrahydrocannabinol (THC), the main psychoactive ingredient in marihuana, or to cannabinol (CBN), a relatively non-psychoactive component, significantly reduced testosterone (T) concentrations in male, but not in female, fetuses [9]. The influence of androgenic steroids in the establishment of sexual dimorphism of neuroendocrine regulatory mechanisms, such as pat-

terns of pituitary gonadotropin release, has been well documented [17, 20, 23]. In earlier studies, we demonstrated that combined pre- and postnatal maternal exposure to either THC or CBN results in long term alterations in pituitary-gonadal function, body weight regulation, responsivity to conspecific stimuli, and in adult copulatory activity in their male offspring [8]. However, these studies did not allow differentiation between the effects of in utero exposure and those of cannabinoid transfer via milk which occurs in lactating rodents and primates [3,8].

It is conceivable that the reported effects of prenatal cannabinoid exposure in human neonates, which include neurological changes [15], are related to cannabinoid-induced changes in neurotransmitters. Brain biochemical changes,

including alterations in RNA synthesis have been induced in neonatal rats prenatally-exposed to THC [18]. Prenatal stress has also been reported to reduce hypothalamic NE concentrations [21], as well as male copulatory behavior [6,7]. In addition, sex differences and neonatal castration or administration of gonadal steroids also influence brain catecholamine levels [6,7]. In addition, we have previously reported that male copulatory behavior was reduced differentially by THC or CBN [8]. Although the influences of neurotransmitters on sexual behavior are not clear [4], there have been indications that serotonin has an inhibitory effect on adult sexual behavior, while the catecholamines may be facilatory [16].

The present experiments were designed to further characterize the effects of perinatal exposure to either the psychoactive or non-psychoactive cannabinoids on the development of the hypothalamo-pituitary-gonadal (HPG) axis in mice. In particular, we have examined the effects of a single maternal exposure, on the day of parturition, to THC, CBN or to another nonpsychoactive component of marihuana, cannabidiol (CBD), on the subsequent development of neuroendocrine and reproductive functions in their offspring. These experiments were designed to determine whether postnatal cannabinoid exposure influenced pituitary release of gonadotropins *in vivo*, under basal conditions, or in response to castration or the administration of gonadal steroids, or *in vitro*, under basal conditions or in response to luteinizing hormone releasing hormone (LHRH). The present studies were also planned to determine if alterations in the concentrations and/or turnover of brain biogenic amines were related to neuroendocrine dysfunctions observed in animals exposed to cannabinoids early in development.

In addition, testicular responsiveness to exogeneous gonadotropins *in vivo* and *in vitro* was measured to determine whether previous findings of testicular dysfunction after early cannabinoid exposure reflected direct effects of these compounds on the developing gonad or were more likely the result of altered neuroendocrine parameters.

METHOD

Animals

Adult female mice were obtained from our colony of random-bred animals housed on a 14 hr light:10 hr dark lighting schedule and provided with commercial mouse chow and tap water ad lib. Within 12 hr of parturition, (the day of delivery), the dams received a single oral administration of either THC, CBN, or CBD at a dose of 50 mg/kg body weight or 20 μl sesame oil. At 21 days of age, the progeny were separated by sex and housed in groups of three or four per cage.

Male Mice

At 55 days of age, a group of males from each treatment were etherized and received an intratesticular injection of 2.5 mIU hCG (Follutein®, Squibb) into one testis and 10 μl saline into the contralateral testis. Thirty minutes later, the animals were castrated under ether anesthesia using a mid-ventral incision. This technique has been further described in a recent publication [14]. The testes obtained from these animals were weighed and homogenized in distilled water (9:1 wt/v) and stored frozen for the radioimmunoassay (RIA) determination of T as described [9].

The testes obtained from another group of mice at castration were weighed and incubated in Krebs-Ringer bicarbon-

ate buffer. Testosterone was measured by RIA, without extraction, as described in several recent reports from this laboratory [11,14]. One testis from each animal was incubated in the presence of 12.5 mIU hCG, while the contralateral testis was incubated in the absence of hCG, in order to assess basal T production *in vitro*.

In order to estimate T negative feedback, mice were injected one week post-castration with a single SC dose of T (20 μg) in sesame oil, and were bled by cardiac puncture under ether anesthesia one hour later. Plasma was stored frozen for the RIA determination of T, LH and FSH using the NIAMDD rat FSH kit and Niswender's anti-ovine LH, which have been previously validated for measurement of mouse gonadotropins [1]. At two weeks postcastration, catecholamine turnover was estimated by injecting about half the animals in each treatment group with 250 mg/kg α-MPT IP, while the remaining castrated males received saline. These animals were sacrificed by cervical dislocation one hour post-treatment, together with the intact males from each treatment group. Trunk blood was collected for RIA determination of LH and FSH, and the brain was rapidly removed and frozen on dry ice.

Gonadotropin samples were run in a single assay, the intra-assay coefficient of variation was 2.1% and 1.4% respectively, for LH and FSH. The intra-assay coefficient of variation for T was 8.1%.

Biogenic Amine Determinations and Hypothalamic LHRH

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 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.

Pituitary Weight, LH Content, In Vitro LH Production With or Without LHRH

Pituitaries were also removed at autopsy and the posterior lobe was dissected free and discarded. The anterior pituitary gland was placed in a 12×75 mm polypropylene culture tube and 1 ml of medium 199 plus bicarbonate (pH 7.3; Gibco Labs, Grand Island, NY) was added. The tubes were preincubated at 37°C in a Dubnoff Metabolic Incubator in an atmosphere of 5% CO$_2$:95% O$_2$. After 30 min, the medium was removed and discarded and fresh medium 199

TABLE 1

BODY AND TESTES WEIGHTS, TESTICULAR TESTOSTERONE (T) CONCENTRATIONS AND PLASMA LEVELS OF
T, LUTEINIZING HORMONE (LH) AND FOLLICLE-STIMULATING HORMONE (FSH) IN ADULT MALE MICE
POSTNATALLY-EXPOSED TO Δ^9-TETRAHYDROCANNABINOL (THC), CANNABINOL (CBN) OR CANNABIDIOL (CBD)

	OIL	THC	CBN	CBD
Body Weight (g)	37.9 ± 0.9 (19)	34.5 = 0.4 (12)*	33.2 ± 1.6 (10)*	34.1 ± 1.6 (14)
Testes weight (mg)	295 ± 9 (19)	292 ± 6.0 (12)	308 ± 12 (10)	260.0 ± 9 (14)*
Testicular T (ng/ml)	81 ± 15 (12)	104 ± 21 (12)	68 ± 10 (15)	67 ± 9 (16)
Plasma LH (ng/ml)				
intact	24 ± 4 (13)	9 ± 2 (14)†	7 ± 1 (8)†	7 ± 1 (16)†
castrated†	161 ± 31 (8)	119 ± 21 (6)†	96 ± 18 (7)†	86 ± 20 (5)†
castrated+T	111 ± 23 (10)	173 ± 51 (8)	99 ± 16 (9)	95 ± 30 (9)
castrated+α-MPT	141 ± 21 (3)	—‡	136 ± 16 (3)	222 ± 71 (4)
Plasma FSH (ng/ml)				
intact	1221 ± 47 (15)	987 ± 33 (16)†	1009 ± 107 (8)	1007 ± 40 (18)
castrated†	1842 ± 230 (8)	2168 ± 81 (8)	1653 ± 54 (8)	1676 ± 128 (6)
castrated+T	1585 ± 97 (10)	1843 ± 170 (9)	1764 ± 93 (9)	1673 ± 90 (9)
castrated+α-MPT	1817 ± 153 (5)	2074 ± 309 (5)	2108 ± 96 (3)	1912 ± 51 (4)

Castrated mice received 20 μg T in sesame oil one week post-castration and were bled by cardiac puncture one hour later. These same animals received either saline or α-methylparatyrosine (α-MTP; 250 mg/kg) IP and were sacrificed one hour later. Means ± SE (n).
*Significantly different from control (p<0.05) by analysis of variance and Duncan's test.
†Significantly diffferent from control (p<0.05) by Mann-Whitney U-test.
‡Sample volume was insufficient for LH determinations in this group.

was added. The tubes were incubated for one hour, at which time the medium was removed and frozen and new medium containing 10^8 M LHRH was added. One hour later the incubations were terminated and the pituitaries were weighed and sonicated in 0.5 ml of saline. The media and the pituitaries were subsequently assayed for LH [10]. Hypothalamic LHRH content was also measured in samples previously prepared for HPLC [25].

Female Mice

At adulthood (60–70 days of age), all female mice were ovariectomized under ether anesthesia using bilateral dorsal incisions. One week later, half the animals from each treatment group were injected IP with α-MPT (250 mg/kg), and the other half of the females received saline. One hour later the females were sacrificed by cervical dislocation. Trunk blood was collected for RIA determinations of plasma LH and FSH levels. Amine concentrations in hypothalamic blocks and the remaining brain tissue was also determined, as was hypothalamic LHRH content.

Statistical Analysis

Paired *t*-test was used to analyze the data obtained from direct intratesticular injections. Values which were not normally distributed or which did not exhibit homogeneity of variance were analyzed using the Mann-Whitney U-test. *In vitro* T production was analyzed using analysis of variance and Duncan's test [29].

RESULTS

Body and Organ Weights and Testicular T Levels

Body weights were reduced significantly in THC- and

CBN-exposed males, and tended to be lower in the CBD-exposed animals. Testicular weights were significantly decreased in CBD-exposed males, while comparable to controls in the THC- and CBN-exposed animals. Testicular T concentrations were significantly decreased in CBN- and CBD-exposed males (Table 1).

Plasma LH and FSH in Male Mice

Postnatal cannabinoid exposure significantly reduced plasma gonadotropins in intact adult males (Table 1).

Post-castration, plasma LH concentrations were significantly lower in the cannabinoid-exposed males compared to those in the castrated controls (p<0.05). Plasma FSH levels appeared higher in the castrated THC-exposed mice, while those in the CBN- and CBD-exposed animals appeared lower than those of control castrates, although these changes were not statistically significant. Plasma LH levels postcastration were increased approximately 13-fold in the cannabinoid-exposed males, while the control animals exhibited only a 7-fold increase compared to intact levels (p<0.05). Plasma FSH levels were also elevated to a significantly greater extent in THC-exposed castrates, i.e., 120% higher than THC-exposed intacts, while the levels of FSH in the plasma of control castrates were only 51% higher than those in the intact controls (p<0.02).

Administration of 20 μg T or 250 mg/kg α-MPT to castrated animals did not differentially affect LH and FSH levels in cannabinoid-exposed, compared to control males.

In Vitro T Production

Testosterone production in response to gonadotropic stimulation *in vitro* appeared to be higher in testes in all
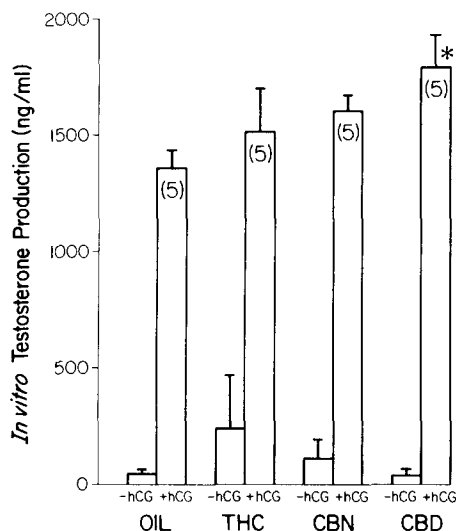
FIG. 1. Testosterone (T) production by decapsulated testes in adult male mice postnatally exposed to Δ^9-tetrahydrocannabinol (THC), cannabinol (CBN), or cannabidiol (CBD) and incubated 4 hr in the presence or absence of 12.5 mIU human chorionic gonadotropin (hCG, Follutein®, Squibb), using paired testes from the same animal Means±SE (n). *Significantly different from control ($p<0.05$) by analysis of variance.
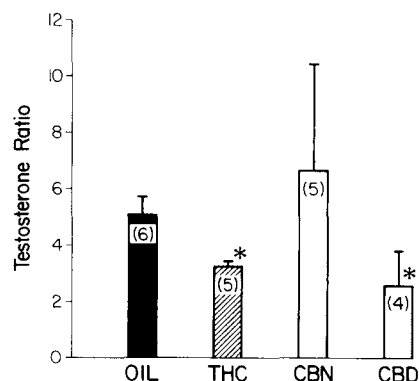

FIG. 2. The concentration of testosterone (T) in testes 30 min after intratesticular injections of 2.5 mIU hCG into one testis and saline (10 μl) into the contralateral testis of adult male mice postnatally-exposed to cannabinoids. Values are expressed as ratio of T in hCG-verus saline-injected testes. Means±SE (n). *Significantly different from control ($p<0.05$) by paired t-test.

cannabinoid-treated mice, although this effect was significant only in the CBD-exposed group (Fig. 1). Basal T production was not influenced by postnatal cannabinoid exposure.

Comparison of T production in testes incubated in the absence of hCG with that by the contralateral testis incubated in the presence of hCG (12.5 mIU/ml), suggests that testes from the THC animals appear less responsive, as indicated by a 74-fold increase in the THC-exposed, compared to a 112-fold increase in hCG-stimulated T production by decapsulated testes from OIL controls ($p<0.05$).

Testes from CBN-exposed males were comparable to control (110-fold increase), while testes from the CBD-exposed males showed a significantly greater response to hCG stimulation, i.e., 167-fold higher ($p<0.05$).

Testicular Responsiveness to Intratesticular Gonadotropin Injections

Testosterone ratios were significantly lower after intratesticular hCG administration in THC- and CBD-exposed mice, while that in CBN-exposed animals were comparable to controls (Fig. 2). The absolute concentrations of T in the saline-injected testes from the animals in the different treatment groups were not significantly different from each other.

Pituitary Weights, LH Content, in Vitro LH Production With or Without LHRH

Pituitary weights and their *in vitro* LH production was significantly increased in THC- and CBN-exposed animals (Table 2). The production of LH *in vitro* by pituitaries in response to LHRH was not different between cannabinoid-treated and control males. However, the THC- and CBN-exposed males were less responsive to LHRH in that LH production was increased only 195±13% in THC, and

218±42% in CBN, compared to 425±95% in the OIL controls ($p<0.05$).

Biogenic Amine Concentrations in Male Mice

The reduction in hypothalamic NE in THC- and CBN-exposed castrated males receiving α-MPT was significantly less than that in control castrates (Table 3). Dopamine levels were significantly lower in the hypothalamus of CBN-exposed castrated mice, compared to that of control castrates (Table 4), while hypothalamic DA levels were depleted to a greater extent in CBD-exposed males. The levels of 5-HT and 5-HIAA were significantly higher in castrated, compared to that in intact THC-exposed animals (Table 5).

Biogenic Amine Concentrations in Female Mice

In ovariectomized CBN-exposed female mice the levels of NE in hypothalamus was significantly lower than controls (Table 3), while the % reduction of NE after injection of α-MPT was significantly less in CBD-exposed females (Table 4). Depletion of brain DA after α-MPT was also significantly lower in the THC-exposed females. The levels of 5-HT were significantly lower in THC-exposed females, but the indoleamine levels were not affected by other treatments (Table 5).

Hypothalamic LHRH Content

The content of LHRH in hypothalamus of THC-exposed intact males was significantly higher than that in OIL-controls (Table 6), while postnatal exposure to CBN or CBD had no effect. Castration decreased hypothalamic LHRH content in all animals, but the THC-exposed males showed a marked reduction (↓54%) compared to a 23% decrease in castrated controls. Postnatal cannabinoid exposure did not affect LHRH content in female mice (Table 6).

Plasma LH and FSH in Ovariectomized Females

In CBN-exposed females, plasma LH levels were significantly increased, while those of THC- or CBD-exposed animals were comparable to controls (Fig. 3). In contrast, THC- and CBD-exposed animals had significantly lower levels of FSH compared to controls (Fig. 3).

TABLE 2

PITUITARY WEIGHTS FROM INTACT ADULT MALE MICE POSTNATALLY-EXPOSED TO THC, CBN OR CBD AND LH CONTENT AFTER INCUBATION IN MEDIUM 199 AND THE CONCENTRATION OF LH IN THE MEDIUM AFTER INCUBATION FOR ONE HOUR EACH IN THE ABSENCE OR PRESENCE OF LHRH

	OIL	THC	CBN	CBD
(n)	(5)	(9)	(6)	(8)
Pituitary weight (mg)	1.01 ± 0.09	1.45 ± 0.20*	1.44 ± 0.15*	1.28 ± 0.14
LH content (ng/mg)	20436 ± 2789	24907 ± 1186	25254 ± 1860	25241 ± 1157
LH, basal (ng/mg/hr)	1956 ± 339	3383 ± 298*	4030 ± 1036*	3320 ± 762
LH, + LHRH (ng/mg/hr)	6925 ± 595	6378 ± 480	8203 ± 225	6637 ± 792

Means ± SE.
*Significantly different ($p<0.05$) from control by Mann-Whitney U-test.

TABLE 3

THE CONCENTRATIONS (ng/g) AND TURNOVER (% REDUCTION AFTER α-MPT) NOREPINEPHRINE (NE) IN HYPOTHALAMUS AND REMAINING BRAIN IN ADULT MALE AND FEMALE MICE POSTNATALLY-EXPOSED TO Δ⁹-TETRAHYDROCANNABINOL (THC), CANNABINOL (CBN) OR CANNABIDIOL (CBD)

Treatment	Hypothalamus		Brain	
	NE	% Reduction	NE	% Reduction
Males				
Oil, Intact	2849 ± 213 (15)	—	800 ± 95 (11)	—
Castrate†	2880 ± 441 (8)	27 ± 12%	781 ± 107 (8)	25 ± 9%
THC, Intact	3014 ± 238 (16)	—	652 ± 42 (11)	—
Castrate†	2948 ± 441 (8)	18 ± 6%*	967 ± 229 (3)	18 ± 9%
CBN, Intact	3080 ± 305 (8)	—	815 ± 94 (5)	—
Castrate†	2729 ± 252 (8)	14 ± 6%*	826 ± 135 (5)	27 ± 8%
CBD, Intact	3099 ± 205 (7)	—	782 ± 64 (13)	—
Castrate†	3064 ± 189 (5)	15 ± 8%	855 ± 178 (4)	49 ± 2%
Females				
OIL	3430 ± 366 (9)	37 ± 5%	647 ± 119 (8)	32 ± 8%
THC	2713 ± 279 (15)	22 ± 10%	524 ± 72 (16)	0%*
CBN	2286 ± 194 (18)*	14 ± 6%	455 ± 65 (18)	12 ± 8%
CBD	2910 ± 238 (15)	12 ± 8%*	540 ± 91 (15)	16 ± 7%

Means ± S.E. (n).
*Significantly different ($p<0.05$) from control, by Mann-Whitney U-test.
†Animals were castrated two weeks prior to sacrifice.

TABLE 4

THE CONCENTRATIONS (ng/g) AND TURNOVER (% REDUCTION AFTER α-MPT) OF DOPAMINE (DA) IN HYPOTHALAMUS AND REMAINING BRAIN IN ADULT MALE AND FEMALE MICE POSTNATALLY-EXPOSED TO Δ⁹-TETRAHYDROCANNABINOL (THC), CANNABINOL (CBN) OR CANNABIDIOL (CBD)

	Hypothalamus		Brain	
Treatment	DA	% Reduction	DA	% Reduction
		Males		
Oil, Intact	1430 ± 173 (14)	—	2930 ± 219 (12)	—
Castrate†	1652 ± 196 (8)	15 ± 12%	2637 ± 356 (8)	6 ± 2%
THC, Intact	1577 ± 331 (16)	—	2893 ± 371 (12)	—
Castrate†	1584 ± 225 (7)	31 ± 13%	2968 ± 336 (4)	24 ± 4%*
CBN, Intact	1398 ± 205 (8)	—	3034 ± 346 (5)	—
Castrate†	997 ± 96 (8)*	33 ± 16%	2918 ± 353 (6)	18 ± 2%*
CBD, Intact	1513 ± 142 (17)	—	2645 ± 259 (15)	—
Castrate†	1444 ± 176 (5)	45 ± 6%*	2890 ± 278 (4)	9 ± 7%
		Females		
OIL	1265 ± 84 (9)	50 ± 5%	1658 ± 140 (8)	2 ± 1%
THC	1243 ± 84 (9)	50 ± 8%	1791 ± 137 (16)	18 ± 8%*
CBN	1344 ± 136 (19)	36 ± 9%	1671 ± 147 (19)	10 ± 4%
CBD	1658 ± 220 (15)	66 ± 4%	1788 ± 137 (15)	16 ± 7%*

Means ± SE (n).
*Significantly different ($p<0.05$) from control, by Mann-Whitney U-test.
†All animals were castrated two weeks prior to sacrifice.

TABLE 5

THE CONCENTRATIONS (ng/g) OF SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN HYPOTHALAMUS AND REMAINING BRAIN IN ADULT MALE AND FEMALE MICE POSTNATALLY-EXPOSED TO Δ⁹-TETRAHYDROCANNABINOL (THC), CANNABINOL (CBN) OR CANNABIDIOL (CBD)

	Hypothalamus		Brain	
Treatment	5-HT	5-HIAA	5-HT	5-HIAA
		Males		
Oil, Intact	3201 ± 487 (13)	1117 ± 287 (13)	804 ± 80 (12)	239 ± 27 (12)
Castrate†	2543 ± 396 (7)	906 ± 138 (7)	851 ± 117 (6)	251 ± 28 (6)
THC, Intact	2852 ± 395 (14)	836 ± 116 (8)	767 ± 57 (15)	232 ± 30 (15)
Castrate†	2924 ± 372 (8)	1065 ± 143 (8)	1212 ± 101 (7)*	423 ± 45 (7)*
CBN, Intact	2646 ± 288 (8)	849 ± 87 (8)	942 ± 86 (8)	301 ± 35 (8)
Castrate†	2414 ± 342 (8)	742 ± 86 (8)	916 ± 132 (7)	255 ± 33 (7)
CBD, Intact	3083 ± 269 (15)	1018 ± 126 (15)	930 ± 85 (18)	273 ± 35 (18)
Castrate†	2903 ± 437 (6)	1206 ± 153 (6)	942 ± 72 (6)	366 ± 80 (6)
		Females		
OIL	2382 ± 324 (9)	844 ± 126 (8)	852 ± 147 (7)	287 ± 36 (7)
THC	1823 ± 137 (16)*	797 ± 63 (16)	916 ± 115 (15)	362 ± 51 (15)
CBN	2448 ± 218 (19)	1023 ± 142 (20)	992 ± 142 (19)	361 ± 46 (19)
CBD	2020 ± 211 (13)	807 ± 84 (13)	704 ± 111 (14)	271 ± 48 (15)

Means ± SE (n).
*Significantly different ($p<0.05$) from control, by Mann-Whitney U-test.
†All animals were castrated two weeks prior to sacrifice.

TABLE 6

THE EFFECTS OF POSTNATAL CANNABINOID EXPOSURE ON
HYPOTHALAMIC LHRH CONTENT (ng) IN INTACT AND
CASTRATED MALES AND IN OVARIECTOMIZED (OVEX)
FEMALE MICE

Treatment	Intact Males	Castrated Males	OVEX Females
OIL	652 ± 60 (15)	501 ± 96 (8)	796 ± 94 (5)
THC	845 ± 60 (16)*	392 ± 85 (8)	791 ± 70 (5)
CBN	750 ± 90 (8)	521 ± 95 (8)	829 ± 36 (7)
CBD	791 ± 68 (18)	535 ± 142 (6)	851 ± 92 (6)

Means ± S.E. (n).
*Significantly different (p<0.05) from OIL controls.

FIG. 3. Plasma LH and FSH levels in ovariectomized female mice postnatally-exposed to cannabinoids. Means±SE (n). *Significantly different from control (p<0.05), by Mann-Whitney U-test.

DISCUSSION

The present findings indicate that postpartum maternal cannabinoid exposure results in long term alterations in endocrine and neurotransmitter function in their adult male and female offspring. The present findings, together with our earlier observations ([8]; Dalterio and Steger, unpublished), indicate that the functional integrity of the HPG axis, particularly that involving the regulation of feedback mechanisms, can be altered by pre-, post-, or combined perinatal cannabinoid exposure. However, the particular expression of the difference, e.g., increased or decreased testicular sensitivity to gonadotropic stimulation, may depend on the timing of maternal treatment.

Postnatal exposure to THC or CBN appears to have exerted similar effects on the development of the HPG axis in male mice, while CBD appeared to have few significant effects. Recently, we have observed that a single prenatal exposure, just prior to parturition, to the relatively non-psychoactive CBN or CBD produced comparable effects on several parameters involving HPG function in adult males, while exposure to the psychoactive THC during similar periods had few effects (Dalterio, unpublished). In contrast, after combined pre- and postnatal exposure THC and CBN both reduced adult copulatory behavior and disrupted HPG function during the pubertal period, but THC effects on the endocrine system persisted well into adulthood, while those of CBN did not [8].

It is therefore apparent that differences due to psychoactivity, or pharmacokinetic factors, such as metabolism rate, plasma distribution, or tolerance development, which are known to differ for various cannabinoids, may influence the consequences of early cannabinoid exposure. Certainly, in adult mice the cannabinoids used in this study alter endocrine parameters ([3, 8, 12], and Dalterio, unpublished).

Postnatal CBD exposure reduced testicular weights, while an increase in testes weights was observed after prenatal exposure (Dalterio, unpublished). In a previous study, prenatal cannabinoid exposure increased body weights in adult THC-, but had no effect in CBN-exposed mice [8]. However, in a more recent study, prenatal cannabinoid exposure did not affect adult body weight (Dalterio and Steger, unpublished). In the present study, postnatal cannabinoid exposure significantly reduced body weights. However, the present experiments were the first in which litters were not culled to retain only the male offspring. It is possible that reported effects of cannabinoids on milk production ob-
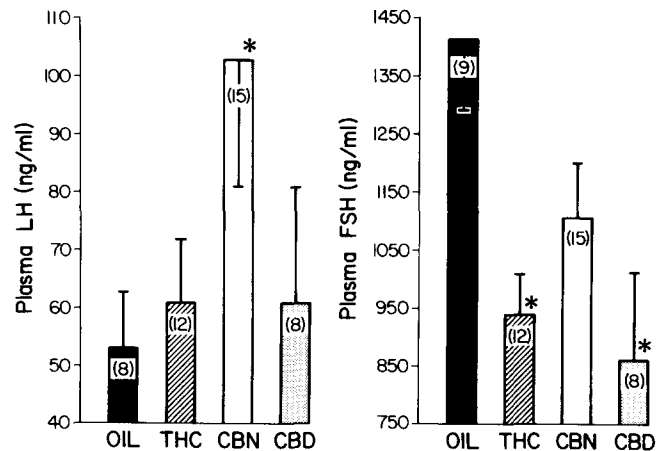
served after repeated treatment with cannabinoids [3,8] resulted from a single maternal treatment during the immediate postpartum period. However, it has been shown that nutritional factors can contribute to, but not totally account for, effects of cannabis exposure throughout pregnancy on development of rat pups [5]. Nonetheless, we are presently investigating the contribution of litter size to this phenomenon.

Alterations in responsiveness to gonadotropins in vivo or in vitro appears to reflect cannabinoid-induced changes in gonadotropin action at the target tissue, since basal T levels or production usually did not differ from that of the control males. In the present study, postnatal THC or CBN exposure appeared to decrease the ability of the testis to respond in vivo to intratesticular hCG injection, in terms of an increase in T production compared to basal levels. It is possible that cannabinoids alter testicular gonadotropin receptors, as has been reported for androgen receptors [22], influencing testicular steroidogenesis. Gonadotropin-stimulated T production in vitro was higher after postnatal exposure to CBN or CBD compared to controls. In previous studies, concomitant perinatal exposure to THC also attenuated the suppressive effects of perinatal ethanol on T production by decapsulated testes [14]. Although responsiveness to intratesticular hCG in vivo appeared enhanced in CBN-exposed animals, this effect was not significant, and, in contrast, testes from postnatally THC- and CBD-exposed mice were significantly less responsive to gonadotropins in vivo. In a previous report, prenatal THC treatment enhanced in vivo responsiveness to gonadotropins, although decreased basal T levels were observed (Dalterio and Steger, unpublished). However, postnatal THC exposure had no effect on basal T levels. These findings may also be related to our earlier observation that, just prior to puberty, male mice exposed to cannabinoids both pre- and postnatally were capable of responding to extended housing with an immature female with an increase in plasma T, as did the controls [8]. However, perinatally THC-exposed animals appeared least responsive to the stimulatory effects of female exposure on plasma T levels. Interestingly, female exposure appeared to normalize the elevated LH levels in these perinatally THC-exposed animals. In contrast, in the CBN-exposed males, there was a dramatic increase in plasma T levels with female

exposure, and indeed, the reduction in plasma LH levels observed in the all male housed, pubertal CBN-exposed mice was no longer evident [8].

It is possible that the present data may be related to our previous report that a single acute exposure to THC can result in a marked and rapid increase in plasma T and LH, which can persist for some time after a low dose, but which is followed by a significant reduction in both T and LH at higher doses [10]. Thus, placental transfer may represent a higher dose situation, while transfer via the milk, which represents about 1% of the THC maternal dose [8], may represent a low dose situation. It is, therefore, possible that the fetus or neonate is actually presented with a situation in which androgenic steroid production is influenced, but in opposite directions.

Pituitary-gonadal feedback also appears to be altered by cannabinoid exposure during early development. In the present study, gonadotropins were reduced in all intact cannabinoid-exposed animals. In other studies, perinatal and prenatal CBN exposure reduced plasma FSH levels ([8]; Dalterio and Steger, unpublished). However, combined pre-and postnatal THC resulted in marked increases in plasma LH levels (3-fold normal), yet plasma T concentrations were normal or slightly decreased. Castration resulted in differential endocrine effects in cannabinoid-exposed animals after prenatal (Dalterio and Steger, unpublished) and, in the present report, after postnatal exposure. In the present study, the increase in plasma LH and FSH postcastration was exaggerated in the THC-exposed animals, as we previously reported after perinatal exposure [13]. In postnatally-THC-exposed males, castration resulted in a marked increase in 5-HT and 5-HIAA levels in brain. Hypothalamic LHRH content was also reduced to a significantly greater extent in THC-exposed castrates, although levels in intact THC animals were significantly increased. In addition, castration revealed effects of postnatal cannabinoid exposure on the concentrations of biogenic amines, as we reported for prenatal cannabinoid exposure. It is possible, as previously suggested, that cannabinoid exposure results in a differential sensitivity to the stress associated with castration (Dalterio and Steger, unpublished). Certainly, it has been shown that after perinatal cannabinoid exposure, the presence of a female conspecific appears to evoke a stress response, as indicated by a reduction in body weights and an increase in the weights of the adrenals, while the opposite was true for the OIL-exposed males [8].

The observation that castration induced differential effects on brain neurotransmitter levels also in postnatally cannabinoid-exposed mice, as was observed after prenatal exposure, may be due to the interrelationships of gonadal steroids and the central nervous system biogenic amines [6, 7, 27]. 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, it is known that castration can cause significant changes in hypothalamic amine metabolism [6,27]. Alterations in the turnover and/or concentrations of biogenic amines suggest that changes in amine metabolism, or possibly neuron numbers, which are affected by early phenobarbital exposure [2], are also influenced by postnatal cannabinoid exposure, and that these changes become more apparent when the neuroendocrine axis reacts to the removal of steroid negative feedback. Based on our earlier observations, these findings seem to be consistent with cannabinoid-induced alterations in physiological responsivity to events which disturb homeostatic conditions.

The present findings indicate that the development of neurotransmitter function and pituitary gonadotropin release in female offspring are also affected by early cannabinoid exposure. Since it has been suggested that female sexual differentiation occurs largely independent of gonadal steroids, the present results in females further indicate that, in addition to possible direct effects of cannabinoids on testicular development, early exposure to these agents is likely to affect neuroendocrine function in offspring of either sex.

In summary, it is apparent that maternal exposure to a single dose of either psychoactive or nonpsychoactive cannabinoids on the day of parturition results in long term alterations in neuroendocrine and neurotransmitter function in both male and female offspring.

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