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Direct and Pituitary-Mediated Effects of Δ^9-THC and Cannabinol on the Testis[1]

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DALTERIO, S., A. BARTKE, C. ROBERSON, D. WATSON AND S. BURSTEIN. *Direct and pituitary-mediated effects of Δ^9-THC and cannabinol on the testis.* PHARMAC. BIOCHEM. BEHAV. 8(6) 673–678, 1978. – In mouse testes incubated with hCG, addition of Δ^9-tetrahydrocannabinol (THC) resulted in a significant inhibition in the accumulation of testosterone (T) and progesterone. The concentrations of 17α-OH progesterone, androstenedione, and estradiol were not changed. The inhibition of T production in this in vitro system by THC was dependent upon the presence of hCG in the medium, suggesting that THC may interfere with gonadotropin stimulation of testicular steroidogenesis. In contrast, suppression of T secretion by cannabinol (CBN) also occurred in the absence of hCG. In the in vivo studies, administration of a single oral dose of THC to adult male mice resulted in a reduction in plasma T, LH and FSH levels, as well as an increase in the concentration of esterified cholesterol in the testis. In contrast, a single dose of CBN produced no significant changes in either plasma T or gonadotropin levels. Treatment with THC, but not with CBN, resulted in a pronounced reduction in the level of copulatory activity in adult male mice. It appears that the THC-induced reduction in plasma T levels observed in vivo is due to an inhibition of pituitary LH release, and to a direct effect on the testicular responsiveness to LH stimulation. The reduction in copulatory behavior observed after acute exposure to THC may be secondary to a reduction in peripheral T concentration.

Δ^9-tetrahydrocannabinol Cannabinol Testis function Testosterone Sexual behavior
Luteinizing hormone Follicle-stimulating hormone Prostaglandins

ADMINISTRATION of marihuana or THC to men or experimental animals has been reported to cause a decrease in plasma testosterone (T) levels [22], spermatogenesis [14], fertility [30], and several androgen-dependent behaviors, including sexual potency in men [22], copulatory behavior in rats [11], and intra- and interspecies aggression in rats and mice [12,15]. These findings strongly suggest that marihuana can decrease T production by the testis, but the mechanism of this action is poorly understood. Reports that THC can reduce plasma LH levels [25], and responsiveness of the pituitary to LHRH stimulation [42] suggest pituitary-mediated effects of marihuana on testicular steroidogenesis. On the other hand, experiments in vitro have indicated that THC can exert a variety of direct effects on the testis including changes in sperm head proteins, reduced synthesis of proteins, lipids and nucleic acids in testicular homogenates [20]; suppression of enzymatic activity in rat testis microsomes [24]; interference with certain protein markers in testicular cells [35]; and inhibition of T production by decapsulated mouse testes [13]. It is of interest that constituents of marihuana considered devoid of psychoactivity, namely cannabinol (CBN) and cannabidiol, are also capable of directly influencing testicular function [13, 20, 24].

Reports that THC is estrogenic [37] and that several cannabinoids can inhibit prostaglandin (PG) synthesis in various tissues [8] suggest other possible mechanisms of marihuana action on the testis. Estrogens can inhibit gonadotropin release [27] and were also claimed to exert direct effects on the function of the testis [10,33]. Both PG's and inhibitors of PG synthesis can affect testicular function [1] and PG's may be involved in mediating the effects of gonadotropins on their target cells [23]. Prostaglandins influence the release of gonadotropins [1], probably by a direct action on the hypothalamus [29].

The present studies were aimed at obtaining more information on the mechanism of action of cannabinoids on the testis. We have examined the direct effects of THC on the production of several steroids and PG's by mouse testes in vitro, determined whether the previously demonstrated inhibition of T production by THC and CBN in this system [13] can be demonstrated in the absence of gonadotropins, and compared acute effects of THC and CBN on plasma LH, FSH, and testosterone in mice. We have also attempted to correlate the changes in the endocrine function of the testis with alterations in copulatory activity in mice treated with cannabinoids.

METHOD

Adult male mice (2–3 months old) were obtained from random-bred stock raised at the Worcester Foundation. The

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animals were housed on a 14 hr L–10 hr D light schedule and maintained on tap water and Purina Mouse Breeder Chow ad lib.

For the in vitro studies, adult male mice were killed by cervical dislocation, the testes were immediately removed, decapsulated, and incubated in Krebs-Ringer bicarbonate-buffer, glucose (1 mg/ml), and human chorionic gonadotropin (hCG, Follutein, Squibb; 12.5 mIU/ml unless indicated otherwise) [3, 16, 44]. The THC or CBN at the various doses were introduced into the incubation medium in a 20 μl volume of ethanol at the onset of the incubation. Control flasks received ethanol alone. In separate studies we have determined that this volume of ethanol does not affect T production in this system [3]. In these experiments cannabinoid-containing and control incubations were individually paired by using testes removed from the same animal [3,13]. Testosterone, estradiol, progesterone, 17α-OH-progesterone, androstenedione, and prostaglandins E and F were measured by radioimmunoassay [4, 36, 40, 41, 43]. Significance of the differences was calculated by a *t* test for paired comparisons.

In all in vivo experiments, control and treated animals were matched by age, body weight and litter. The THC, at doses of either 50 or 100 mg/kg body weight, or CBN, at 50 mg/kg body weight, were administered by oral feeding in 20 μl sesame oil. Pharmacologically, intragastric doses of 2–50 mg THC/kg body weight in rats correspond to amounts of marihuana or hashish commonly used by humans [32]. The approximately 10 fold difference in body weight between rats and mice, and the associated difference in metabolic rate justifies the use of higher doses in the latter species. Control animals were given sesame oil alone. Sesame oil was selected as the vehicle on the basis of evidence that this compound is non-toxic and does not interact with cannabinoids [27]. The oral route was chosen for these acute experiments in view of its applicability to human studies [19], and evidence on the preferability of this route over parenteral administration [31]. Four hr after the last treatment the mice were anesthetized with ether and blood was collected by cardiac puncture. The concentration of T in the plasma was determined by radioimmunoassay [4]. Plasma LH and FSH were measured using NIAMDD kits for rat LH and FSH, which have already been validated for measuring mouse gonadotropins [6]. Testes were homogenized in acetone:ethanol (1:1), and stored at −20°C for determination of free and esterified cholesterol after chromatographic separation [39]. In additional animals, copulatory behavior was assessed after acute treatment with THC or CBN. Four hr prior to testing, the animals received the cannabinoid or oil and were then housed individually. Ovariectomized females were brought into behavioral estrus by two injections (SC) of 25 μg estradiol benzoate, 24 hr apart, followed 24 hr later by 500 μg progesterone, approximately seven hr before testing. The stimulus females were introduced into the homecage of the male and the regular lid replaced by a clear plastic lid to facilitate behavioral observation. During a one hour test session the following measures were recorded: (i) mount latency – time from the introduction of the female to the first mount, with or without intromission by the male, (ii) intromission latency – the time from the introduction of the female to the first mount with intromission by the male, and (iii) ejaculatory latency – time from the first intromission to the beginning of the ejaculatory reflex [28]. The number of mounts and intromissions were also recorded. Statistical analysis of the data employed Friedman's two-way analysis of variance for matched samples [37], and Chi-square test for heterogeneity.

RESULTS

Studies In Vitro

Addition of 25 μg THC per ml of incubation medium resulted in a significant inhibition of progesterone and testosterone production by decapsulated mouse testes (Table 1). There were no changes in the accumulation of 17α-OH-progesterone, androstenedione, or estradiol. In an additional study, both THC, at 12.5 μg/ml and CBN, at 25 μg/ml resulted in a significant reduction in the production of prostaglandins E and F in this incubation system (Table 2). Further experiments determined that the inhibition of T production by THC in vitro occurs in the presence but not in the absence of hCG (Table 3). However, the CBN-induced suppression of T production occurred both in the presence and in the absence of hCG.

Studies In Vivo

In the in vivo studies (Table 4), the acute oral administration of 50 mg THC/kg resulted in a significant decline in plasma levels of T, LH and FSH, and a significant increase in the concentration of esterified cholesterol in the testis. There was no change in the level of free cholesterol. At 100 mg/kg body weight, THC treatment produced a significant decrease in plasma T. The apparent declines in peripheral LH and FSH levels were not significant. There was no change in the concentration of free or esterified cholesterol in the testis. In contrast, the acute administration of CBN, a non-psychoactive constituent of marihuana, produced no significant alteration in either plasma T concentration, or in peripheral gonadotropin levels.

In tests for copulatory activity, a single oral dose of 50 or 100 mg THC/kg resulted in a complete absence of copulatory activity, although the animals did exhibit some pre-copulatory behaviors, such as genital sniffing and grooming. In contrast, the copulatory pattern observed after acute exposure to CBN (Table 5) was comparable to that of the control animals.

DISCUSSION

The results of the present in vitro studies, together with the earlier findings that both CBN and THC inhibit the production of T in decapsulated mouse testes [13] suggest some possible mechanisms for the action of cannabinoids. The observation that blockage of T synthesis by THC was not associated with accumulation of several of the T precursors (progesterone, 17α-OH-progesterone, androstenedione), together with the evidence that THC in this system does not affect the metabolism of ^{3}H-pregnenolone, an early precursor in the formation of testosterone [9] suggests that THC effects must be limited to early stages of steroidogenesis, before the formation of pregnenolone. Comparable findings were reported for the adrenal [45]. However, this suggestion is not entirely compatible with the observation that release of progesterone, 17α-OH-progesterone, androstenedione, and estradiol did not decline in proportion to the THC-induced suppression of T production. We have no explanation for this discrepancy.

The findings that the THC-induced suppression of T

TABLE 1

THE EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC; 25 μg/ml) ON TESTICULAR STEROIDOGENESIS IN AN IN VITRO INCUBATION SYSTEM IN THE PRESENCE OF hCG (12.5 mIU/ml) (MEANS ± SE; N = 8)

	hCG	hCG + THC
Progesterone (ng/ml)	0.54 ± 0.06	0.36 ± 0.04*
17α-OH-progesterone (ng/ml)	0.36 ± 0.07	0.37 ± 0.04
Androstenedione (ng/ml)	15.6 ± 3.7	9.1 ± 1.5
Testosterone (ng/ml)‡	517 ± 58	71 ± 16†
Estradiol (pg/ml)	19.0 ± 3.6	17.6 ± 3.8

*$p<0.05$ †$p<0.001$ ‡Data from Dalterio *et al.* [13]

TABLE 2

PROSTAGLANDIN PRODUCTION BY DECAPSULATED MOUSE TESTES INCUBATED FOR 4 HR WITH THC OR CANNABINOL (CBN) IN THE PRESENCE OF 12.5 mIU hCG/ml (MEANS ± SE)

	N	PGE (ng/ml)	PGF (ng/ml)
hCG (control)	11	1.27 ± 0.18	3.16 ± 0.23
hCG + THC (12.5 μg/ml)	11	0.50 ± 0.05*	1.31 ± 0.10*
hCG (control)	9	1.30 ± 0.12	3.16 ± 0.15
hCG + CBN (25 μg/ml)	9	0.73 ± 0.07*	1.78 ± 0.11*

*Significantly different from controls: $p<0.01$

TABLE 3

EFFECTS OF THC AND CBN ON TESTOSTERONE PRODUCTION BY MOUSE TESTES IN VITRO IN THE PRESENCE OF 12.5 mIU hCG/ml AND IN THE ABSENCE OF hCG. RESULTS REPRESENT THE MEAN CONCENTRATION OF TESTOSTERONE IN THE MEDIUM AFTER 4 HR INCUBATION (ng/ml) ± SE

	N	Controls	THC (25 μg/ml)	p	N	Control	CBN (25 μg/ml)	p
Without hCG	6	71 ± 19	70 ± 17	NS	7	82 ± 17	45 ± 11	<0.05
With hCG (12.5 mIU/ml)*	8	517 ± 58	71 ± 16	<0.001	9	368 ± 27	99 ± 10	<0.001

*Taken from Dalterio *et al.* [13]

TABLE 4

PLASMA TESTOSTERONE (T), LH, FSH, AND TESTICULAR CHOLESTEROL IN ADULT MALE MICE 4 HR AFTER A SINGLE ORAL DOSE OF THC OR CBN (MEANS ± SE)

	Controls (20 μl oil)	THC (50 mg/kg)	Controls (20 μl oil)	THC (100 mg/kg)	Controls (20 μl oil)	CBN (50 mg/kg)
No. of mice	23	23	23	23	15	15
Plasma T (ng/ml)	4.38 ± 0.96	0.97 ± 0.61†	4.60 ± 1.30	1.85 ± 0.58*	7.13 ± 2.42	4.02 ± 1.22
Plasma LH (ng/ml)	38.5 ± 4.5	23.0 ± 1.4‡	28.4 ± 1.3	24.3 ± 2.4	35.7 ± 7.1	37.4 ± 7.6
Plasma FSH (ng/ml)	1271 ± 99	1084 ± 68*	1412 ± 82	1296 ± 66	1098 ± 61	1030 ± 91
Esterified cholesterol (μg/mg)	0.79 ± 0.08§	1.00 ± 0.09§	0.74 ± 0.11¶	0.71 ± 0.07¶	–	–
Free cholesterol (μg/mg)	1.42 ± 0.10§	1.50 ± 0.11§	1.50 ± 0.08¶	1.49 ± 0.04¶	–	–

*$p<0.05$ †$p<0.01$ ‡$p<0.001$ §Data based on 15 animals ¶Data based on 14 animals

production is dependent upon the presence of hCG in the medium suggests that THC interferes with the action of gonadotropins on testicular steroidogenesis. It is impossible to determine from the present data which step of LH (hCG) action on testicular steroidogenesis is inhibited by cannabinoids.

The inhibition of prostaglandin synthesis by both THC and CBN suggests that changes in testicular prostaglandin levels may mediate the effects of cannabinoids on the testis. If prostaglandins are involved in the mechanism of LH action on testicular steroidogenesis, as has been suggested [23], then the blockade of prostaglandin synthesis by cannabinoids could have attributed to the observed decline in T levels. A reduction in PG synthesis may also contribute to the observed effects of THC on the level of copulatory activity, since PG's modulate neural activity in the lateral preoptic region in the hypothalamus involved in sexual behavior and gonadotropin secretion [29]. High levels of exogenous prostaglandins can inhibit testicular T production [3], but production of endogenous prostaglandins may well be necessary for the steroidogenic response of the testes to LH (hCG). The precise relationship

TABLE 5

COPULATORY BEHAVIOR DURING A ONE HR OBSERVATION PERIOD IN MALE MICE TREATED WITH A SINGLE ORAL DOSE OF Δ^9-TETRAHYDROCANNABINOL (THC), OR CANNABINOL (CBN) (MEANS ± SE)

	Proportion of Animals Mounting	Latency to Mount (min)	No. of Mounts	Intromission Latency (min)	No. of Intromissions	Ejaculatory Latency (min)
Oil	5/5	10.4 ± 2.0	10 ± 3.6	43.0 ± 10.4	3.0 ± 2.0	56.2 ± 3.8
THC (50 mg/kg)	0/5*	>60	0	>60	0	>60
Oil	8/8	14.5 ± 2.0	16.8 ± 4.1	18.2 ± 2.5	13.6 ± 3.3	>60
THC (100 mg/kg)	0/8*	>60	0	>60	0	>60
Oil	5/5	8.8 ± 2.0	12.0 ± 2.9	25.0 ± 9.3	12.6 ± 5.2	>60
CBN (50 mg/kg)	5/5	10.6 ± 4.0	12.2 ± 5.0	27.2 ± 11.5	13.8 ± 6.4	>60

*Significantly different from controls: $p<0.01$ (Chi-square df = 1)

between the reduction in steroidogenesis and in prostaglandin synthesis cannot be elucidated from the present findings.

It is often difficult to extrapolate from the in vitro situation to results obtained from the in vivo studies. The results of the present in vivo experiments suggest that the reduction in plasma T levels observed after acute treatment with THC is due, at least in part, to the inhibition of pituitary LH release. The reduction in LH levels could also account for the accumulation of esterified cholesterol in the testes [2]. However, in view of the in vitro data, a direct effect of THC on testicular steroidogenesis must also be considered. Therefore, it seems likely that the decline in plasma T levels observed 4 hr after the administration of THC in vivo was due to a combination of reduced release of LH from the pituitary and impaired responsiveness of the testis to LH stimulation.

The ability of THC to reduce the concentration of FSH in the plasma has not been previously described, except for a report that FSH levels were lower in heavy than in moderate marihuana cigarette smokers [22]. The reduction in peripheral FSH levels by THC may contribute to the decrease in plasma T concentration [17,21] and also suggests a possible mechanism for the impairment of spermatogenesis by chronic exposure to cannabinoids in mice [14] and in men [30]. The dramatic reduction in copulatory activity observed after acute exposure to THC may be secondary to the suppression of hypothalamo-pituitary-gonadal axis.

The apparently normal testicular function after acute exposure to CBN in vivo was unexpected. In the in vitro system both THC and CBN inhibited T production to levels observed without hCG [13]. Thus, both cannabinoids were found to completely prevent the effects of hCG on steroidogenesis in this system. In addition, CBN suppressed T production also in the absence of hCG. Possibly, THC and CBN affect testicular function by different mechanisms. THC decreases LH release and prevents gonadotropin stimulation of testicular steroidogenesis in vitro. CBN does not reduce LH release, but inhibits T production in the presence and in the absence of gonadotropin in vitro. In vivo, both compounds can reduce plasma T levels [13]. The differences in vivo could be due to pharmacokinetic factors since THC and CBN are not identical in this respect [26].

The results of these studies suggest a possible mechanism for the action of THC on testis function, but the action of cannabinoids on the pituitary-gonadal axis is still difficult to elucidate. It is possible that cannabinoids alter the metabolism of testosterone by their action on hepatic enzymes [24]. The weak but measurable estrogenic activity of THC [38] may explain the suppression of pituitary LH release, and perhaps contribute to the direct inhibitory effects on the testis. However, in this incubation system estradiol inhibited T production only at an extremely high dose of 50 μg/ml [5].

It can therefore be concluded that THC and CBN may affect the testis by a variety of mechanisms. The striking correlation between the changes in plasma T and in sexual behavior after administration of cannabinoids in this study, as well as in chronic experiments (Dalterio, unpublished) suggests that the effects of marihuana on androgen-dependent behaviors, such as copulatory activity and aggression, may be due, at least in part, to an alteration in the endocrine function of the testis.

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