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Effects of Δ^9-THC and Castration on Behavior and Plasma Hormone Levels in Male Mice

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DALTERIO, S. L., D. L. MAYFIELD, S. D. MICHAEL, B. T. MACMILLAN AND A. BARTKE. *Effects of Δ^9-THC and castration on behavior and plasma hormone levels in male mice.* PHARMACOL BIOCHEM BEHAV **18**(1) 81–86, 1983.—Gonadectomy resulted in a rapid increase in plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels, but had no consistent effects on plasma prolactin (PRL) and growth hormone (GH) levels. In castrated males, oral administration of THC (50 mg/kg) significantly increased plasma LH levels within hours following surgery and again from 3 to several weeks post-castration, while THC treatment decreased LH levels between 1 day and 2 weeks post-castration. Administration of THC to 12-hour sham castrates significantly increased plasma LH levels. Plasma FSH, PRL and GH levels were either reduced or unchanged by THC. Administration of THC significantly reduced levels of gonadotropins, PRL and GH in intact males. In additional studies, we examined the influence of THC on the negative feedback response of the anterior pituitary to gonadal steroids. In testosterone propionate (TP)-treated castrated males, concomitant administration of THC increased plasma testosterone (T) and LH at 20 min, while plasma FSH levels were elevated after 60 min. In contrast, in intact TP-treated mice, concomitant THC exposure reduced plasma T levels except at 60 min, when plasma LH levels were significantly increased. Testosterone replacement failed to restore copulatory behavior in castrated mice given a single dose (50 mg/kg) of THC. In fact, acute THC administration to these TP-treated castrates resulted in marked sedation, which was not observed in intact mice given the same dose of THC in an earlier study. The present findings indicate that the effects of acute THC treatment on pituitary gonadotropin release is dependent upon the time after castration. Furthermore, THC administration can suppress copulatory behavior even in animals whose peripheral T levels have been maintained. Effects of THC on plasma androgen levels in animals injected with TP suggest that THC can alter the metabolism or target tissue response to gonadal steroids.

Oral Δ^9-THC Luteinizing hormone Follicle stimulating hormone Prolactin Growth hormone
Testosterone Castration effects Copulatory behavior

MARIHUANA and its major psychoactive constituent, Δ^9-tetrahydrocannabinol (THC), alter male reproductive functions in laboratory animals and in humans [4]. Reductions in testicular weights [29] impaired spermatogenesis [12] and suppression of plasma levels of testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH) and prolactin (PRL) have been reported [9, 17, 18, 21]. Although the findings in the human have been questioned [23], the ability of THC to depress pituitary function in a variety of laboratory animals is well documented [4]. However, the mechanism of cannabinoid action in suppressing hypothalamic-pituitary-gonadal function remains to be elucidated.

The present studies were undertaken to elucidate the effects of THC on the regulation of anterior pituitary function by endogenous and exogenous steroids in male mice. For this purpose, we examined the time course of changes in plasma LH, FSH, PRL and GH levels and the effects of administering a single dose of THC at different intervals after gonadectomy. Since it is well-documented that exogenous administration of gonadal steroids exerts negative feedback effects on pituitary LH release [20,26], it was of interest to examine the effects of THC treatment on pituitary gonadotropin release in the presence of exogenous steroids. Therefore, we determined the effects of THC on plasma T, LH and FSH levels in intact and in castrated male mice injected with TP. Levels of copulatory behavior were also assessed in a group of castrated male mice after TP replacement in order to determine whether a single exposure to THC was capable of abolishing sexual behavior in animals with normal to high

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plasma T, since we had reported previously that, in intact mice, acute THC exposure significantly reduced copulatory behavior and plasma T levels [8].

METHOD

Animals

Adult male mice (60–80 days old) were obtained from our colony of random-bred animals. Animals were housed since weaning in groups of 4 in a room with a 14 hr L:10 hr D lighting schedule and provided Wayne Breeder Blox and tap water ad lib.

Procedures

Mice were castrated using a mid-ventral incision under ether anesthesia between 0800–1000 hrs. The sham-castrated animals were anesthetized and an incision was made, followed by probing and suturing. The THC (50 mg/kg body weight) was administered by oral feeding in 20 μl sesame oil, using a blunted 23 gauge syringe needle, as described previously [8].

This dose would correspond to about 4 mg/kg orally in the human user or 3 marihuana cigarettes of 1% THC based on a body surface conversion factor of 12 for mouse [29]. In our previous studies [8–11] this dose of THC produced behavioral intoxication for about 5 hours, a period of time quite comparable to effects of a single marihuana smoking episode by a human user.

Blood was obtained by cardiac puncture under light ether anesthesia between 0800–1000 hrs, with the exception of the 12 hr post-castration sample, which was collected between 2000–2200 hrs. The samples were collected at intervals ranging from 12 hrs to 7 months after gonadectomy. The THC was administered as a single oral dose 1.5 hrs before blood collection, in order to provide optimal timing for the expression of depressive effects of THC on plasma T and LH levels [10]. Control animals received identical amounts of vehicle. We have determined that bleeding under ether anesthesia does not influence PRL in mice [9]. Plasma was stored frozen for the radioimmunoassay determinations of LH, FSH, PRL and GH. In the additional studies in intact mice and in males 3 weeks post-castration, blood was collected at different intervals after administration of TP (125 μg) and/or THC (50 mg/kg) for measurement of T, LH and FSH at 20, 40, 60 and 90 min post-treatment. For behavioral testing, the stimulus females were ovariectomized and brought into behavioral estrus with 25 μg estradiol benzoate per day by SC injection for two days and a single 500 μg SC injection of progesterone 24 hours later. The females were used for behavioral testing 7 hours after receiving progesterone [13]; they were introduced into a clean cage with the male and the cage cover was replaced with a clear plastic lid to facilitate observations. The following test measures (adapted from [22]) were recorded during a one-hour test session: (1) mount latency—time from the introduction of the female to the first mount, with or without intromission by the male; (2) intromission latency—time from introduction of the female to the first mount with intromission; (3) ejaculatory latency—time from the first intromission to the beginning of the ejaculatory reflex. The number of mounts and intromissions was also recorded. The latency for an animal not exhibiting behavior was considered as 60 minutes for statistical purposes.

Hormone Assays

Testosterone was measured as described previously [8].

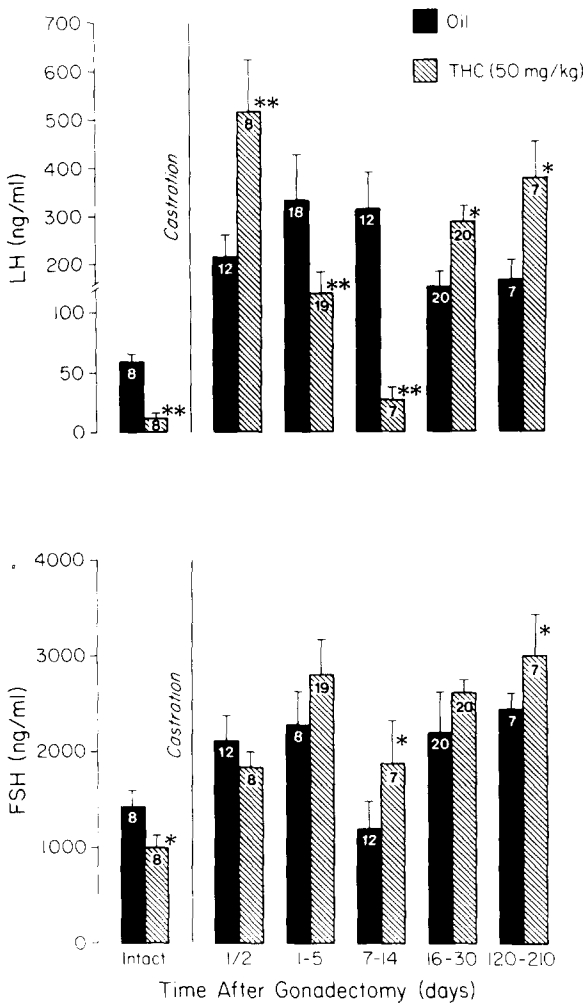

FIG. 1. The effect of gonadectomy and Δ^9-tetrahydrocannabinol (THC) on plasma levels of LH (top) and FSH (bottom). The THC was administered by oral feeding 1.5 hr before blood sampling. (Means±SE). *Significantly different from control ($p<0.05$) by analysis of variance and Duncan's test (Winer, [38]. **$p<0.01$.

Gonadotropins were measured using the NIAMDD kits for rat LH and FSH, which have already been validated for measuring mouse gonadotropins [2]. The intra- and inter-assay coefficients of variation were, respectively, 2.1% and 15.1% for LH, 1.4% and 13.2% for FSH, and 8.1% and 13% for T.

Prolactin and GH were measured by homologous assays using the materials and protocol of Sinha *et al.* [30,31]. For both hormones, determinations were made on duplicate 20 μl aliquots of plasma. The coefficient of variation and the sensitivity were 2.1% and 2 ng/ml for PRL and 3.4% and 2 ng/ml for GH, as described previously [24].

Statistics

The data were evaluated by analysis of variance and Duncan's test for multiple comparisons Student's *t*-test or Chi-square [38].

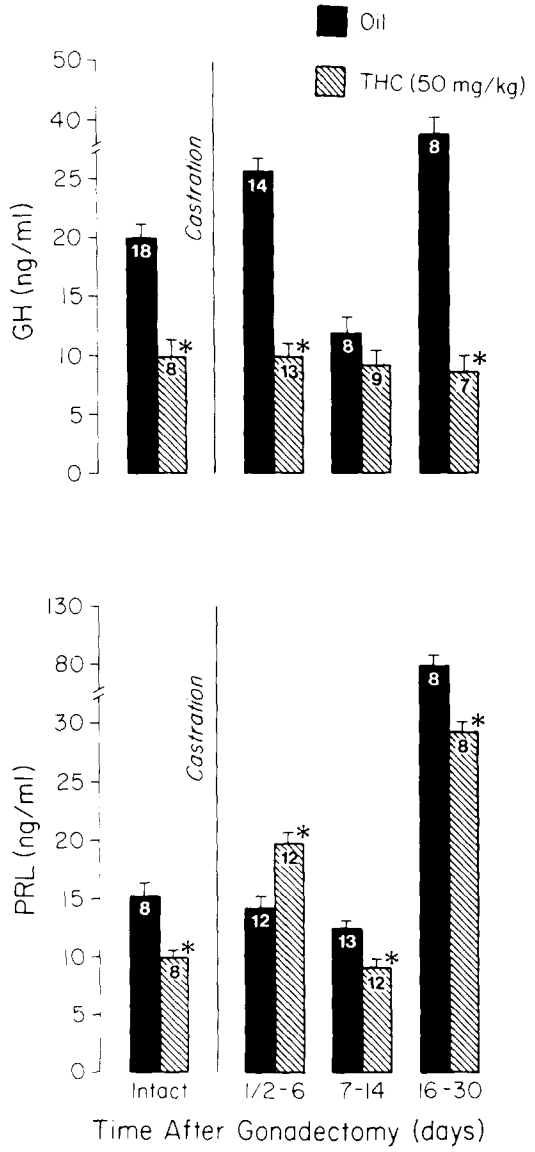

FIG. 2. The effect of castration and Δ^9-tetrahydrocannabinol (THC) on plasma levels of growth hormone (GH), top, and prolactin (PRL), bottom. The THC was administered 1.5 hr before blood collection. (Means±SE). *Significantly different from control ($p<0.05$) by analysis of variance and Duncan's test (Winer [38]).

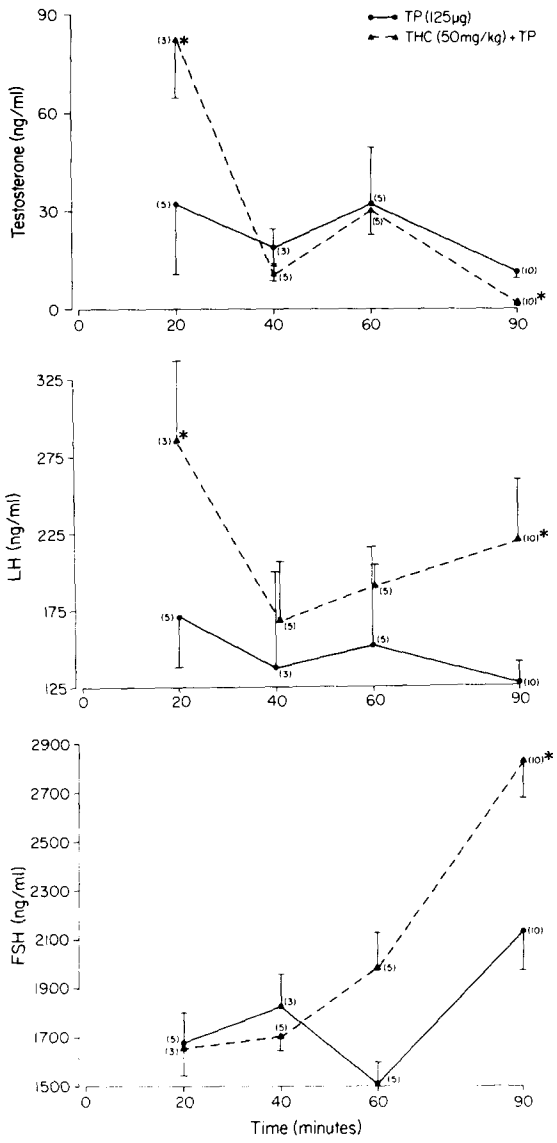

FIG. 3. Plasma testosterone, LH and FSH levels in castrated† male mice at the times indicated after receiving Δ^9-tetrahydrocannabinol (THC, 50 mg/kg) and/or testosterone propionate (TP, 125 μg SC). (Means±SE). *Significantly different from TP-treated mice ($p<0.05$), using Student's t-test. †Three weeks post-castration.

RESULTS

Effects of THC on Pituitary Gonadotropin Release in Castrated Mice

Administration of THC 12 hrs after castration produced a significant increase in plasma LH levels which were already elevated by gonadectomy (Fig. 1, top). Administration of THC to 12-hour sham-castrates resulted in a significant increase in plasma LH levels within 90 min, 120±28 (n=10) vs 15±4 ng/ml, (n=10) $p<0.01$. At other sampling intervals, the response of the sham-castrated animals to THC administration did not differ from that of intact animals. With increasing time intervals after castration, administration of THC caused either a significant further elevation or reduction in plasma LH levels from their increased post-castration levels (Fig. 1, top).

Plasma levels of FSH were increased by approximately 2-fold as a result of castration. Exposure to THC significantly increased peripheral FSH levels at 1–2 weeks and again at 4–7 months (Fig. 1, bottom).

Castration alone appeared to have no consistent effect on plasma PRL or GH concentrations. The GH concentrations were significantly ($p<0.05$) reduced 7–14 days post-gonadectomy, but were higher than those in intacts shortly after surgery and again after several months. Administration of THC significantly reduced these elevated GH levels to values considerably lower than those observed in intact animals (Fig. 2, top).

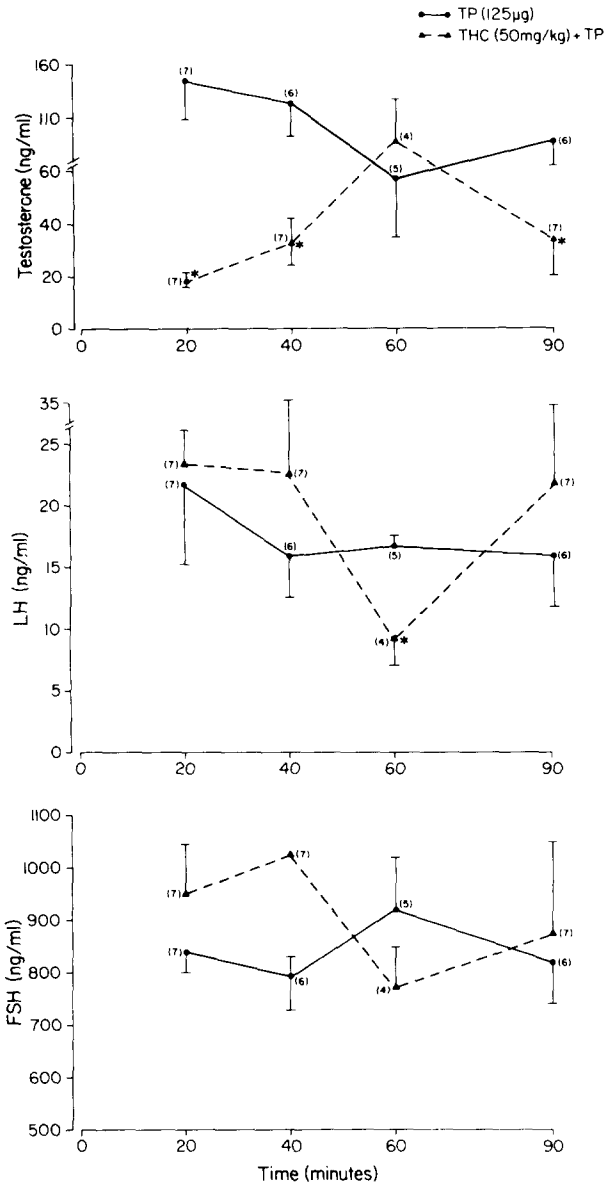

FIG. 4. Plasma testosterone, LH and FSH levels in intact male mice at the times indicated after receiving Δ^9-tetrahydrocannabinol (THC, 50 mg/kg) and/or testosterone propionate (TP, 125 μg). (Means±SE). *Significantly different from TP-treated controls ($p<0.01$), using Student's *t*-test.

Prolactin levels were not influenced by castration until several weeks post-surgery, when significantly ($p<0.05$) higher levels than those in intact animals were observed. Administration of THC increased plasma PRL levels in castrated males within 6 days after castration, while reducing PRL levels in castrates thereafter (Fig. 2, bottom).

Administration of THC to intact male mice significantly reduced plasma LH, FSH, GH and PRL (Figs. 1,2).

Interaction of THC and TP on Plasma Hormone Levels and Sexual Behavior

Twenty minutes after administration of THC and TP (125 μg) to castrated mice, levels of plasma T and LH were significantly higher than T and LH levels observed 20 min after administration of TP alone. However, at 40 and 60 min, plasma T and LH levels in THC + TP-treated animals were comparable to those measured in males treated with TP only, while at 90 min, plasma T levels were reduced in THC + TP treated males, although plasma LH levels were elevated (Fig. 3). At 60 and 90 minutes after treatment, plasma FSH levels were higher in males given THC + TP than in those given TP alone, while no differences were observed at 20 and at 40 min.

In intact mice treated with 125 μg TP, concomitant administration of THC significantly reduced plasma T levels after 20, 40 and 90 min, but not after 60 min, while plasma LH levels were significantly reduced at only 60 min. Plasma FSH levels were not significantly affected by treatment with THC, but the pattern of the response appeared similar to that for LH (Fig. 4).

In TP-treated castrates a single dose of THC (50 mg/kg) produced marked sedative effects and the maintenance of peripheral T levels could not reverse the suppressive effects of acute THC exposure on copulatory activity (Table 1).

TABLE 1

EFFECTS OF ACUTE TREATMENT WITH Δ^9-TETRAHYDROCANNABINOL (THC; 50 mg/kg) ON COPULATORY BEHAVIOR DURING A ONE-HOUR PERIOD IN CASTRATED MALE MICE RECEIVING 500 μg TESTOSTERONE PROPIONATE ON ALTERNATE DAYS FOR ONE WEEK

	OIL	THC
Proportion of animals mounting	5/9	0/9*
Latency to mount (min)	13.3 ± 2.1†	>60
Number of mounts	9.0 ± 4.0†	0
Intromission latency (min)	34.6 ± 10.5†	>60
Proportion of animals ejaculating	2/9‡	0/9

Means ± SE.
*Significantly different from controls, $\chi^2=6.92$, $p<0.01$.
†Data based only on those animals that mounted.
‡Both mice ejaculated before 30 min.

DISCUSSION

The pattern of plasma gonadotropins post-gonadectomy in this study are in essential agreement with the data obtained in rats [3,15] and in mice [1,32]. However, in the gonadectomized mouse, effects of THC on anterior pituitary hormone release appear dependent upon the intervals between gonadectomy and THC exposure. Thus, administration of THC can either increase or decrease plasma concentrations of LH, FSH and PRL in castrated mice, while plasma GH levels are depressed at various times post-gonadectomy.

Previous reports indicate that THC is a potent inhibitor of pituitary LH release in intact male mice [8] and Rhesus monkeys [33]. In the present studies, the effects of THC on LH in castrated male mice differed, depending on the time after gonadectomy. It has been suggested that the pituitary gonadotropin releasing hormone receptors may undergo time-

dependent changes after castration [6], and that an increased amount of exogenous T (compared to intact levels) is required to reduce the elevated post-castration levels of LH [35]. It has also been reported [28] that, in castrated male Rhesus monkeys, estrogen administration suppresses LH at 1–3 days, but increases LH concentrations in plasma afterwards. Thus, duration of castration influences the responsiveness of the hypothalamic-pituitary-gonadal axis to feedback inhibition by gonadal steroids, and apparently to effects of THC administration.

Administration of THC is capable of stimulating as well as inhibiting plasma LH levels in both intact and castrated mice. In the present paper, we report that treatment with THC produces even further elevation of the already high post-castration plasma LH levels at 12 hours and again at 3 weeks after surgery. Although it is conceivable that THC treatment has differential effects in surgically-stressed animals, we have observed stimulation as well as inhibition of plasma LH levels also in the intact animal.

We observed that THC (5 or 50 mg/kg) is capable of producing rapid (within 10 min) almost simultaneous elevations in plasma levels of LH and T [10], followed by a significant reduction in levels of both hormones which from earlier studies [8] persists for up to four hours. However, if a lower dose of THC is used (0.5 mg/kg), the increased plasma T and LH are maintained for at least one hour [10].

The present results also indicate that the presence of THC may influence the metabolism of gonadal steroids. Although metabolic clearance rate of T was not actually measured, the results of this study are compatible with the hypothesis that cannabinoid-induced changes in steroid production may alter hepatic function with resultant effects on androgen metabolism or distribution [19]. Sex differences in the activity of hepatic mixed function oxidases are well-known in rats [5,14] and may also occur in mice [36]. In the present report, THC exposure appeared to have modified the metabolism of T, as well as the response of the pituitary to negative feedback inhibition by this steroid. Thus, it is conceivable that the depression of T by THC in intact male mice was probably partly due to THC-induced increases in T metabolism, since the testicular contribution to these high T levels induced by TP administration was probably very small, and LH levels were not depressed. Therefore, the THC-induced alterations in negative feedback regulation of pituitary gonadotropin release by sex steroids may also be partially related to changes in hepatic function.

It is conceivable that altered sensitivity to THC may reflect changes in drug metabolism consequent to gonadectomy which may influence the dose effectiveness. This suggestion is consistent with our observation that a single dose of THC (50 mg/kg) produced marked sedation in TP-treated castrated mice, a finding not observed in intact mice treated with this same dose of THC [8].

Gonadal steroids, depending upon the circumstances, can exert positive and negative feedback effects on pituitary gonadotropin release [28,35]. The fact that THC appears to behave in a similar fashion does not necessarily imply a similar mechanism of action. However, the evidence that cannabinoids can interact with androgen receptor [27] and the persistent ability of THC to produce effects similar to that of estrogens [34], although a controversial issue [25,37], suggest that further investigation of these issues is warranted.

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