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Acute Δ^9-Tetrahydrocannabinol Exposure: Effects on Hypothalamic-Pituitary-Testicular Activity in Mice

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DALTERIO, S , R STEGER, J PELUSO AND L DE PAOLO *Acute Δ^9-tetrahydrocannabinol exposure Effects on hypothalamic-pituitary-testicular activity in mice* PHARMACOL BIOCHEM BEHAV **26**(3) 533–537, 1987 —The endocrine functions of the median eminence, pituitary and testes were examined in male mice after exposure to Δ^9-tetrahydrocannabinol (THC) either *in vivo* or *in vitro*. The secretion of luteinizing hormone-releasing hormone (LHRH) under basal conditions, and in the presence of norepinephrine (NE, 60 μM), was significantly enhanced in median eminence fragments obtained 1 hr post-treatment with THC (50 mg/kg), while addition of THC (250 ng/ml) to the incubation media enhanced clonidine, as well as NE-stimulated LHRH release, but did not affect basal LHRH release *In vitro* exposure to THC also enhanced LHRH-stimulated LH release by pituitaries, but did not affect basal secretion rates *In vivo* THC exposure tended to enhance pituitary responsiveness to LHRH, although this effect was not statistically significant In testicular perifusions, addition of THC at a concentration of 250 ng/ml completely blocked hCG-stimulated T secretion within 30 min The suppressive effects of a lower dose of THC, 25 ng/ml, required 60 min to inhibit T production, an effect which persisted for 60–80 min post-THC These findings indicate that THC exposure enhances responsivity at neuroendocrine target sites, but attenuates gonadotropin-stimulated testicular steroidogenesis

Δ^9-Tetrahydrocannabinol Median eminence Pituitary *In vitro* LH production *In vitro* T production NE-stimulated LHRH release Luteinizing hormone Luteinizing hormone-releasing hormone Clonidine

MARIHUANA, and its major psychoactive constitutent, Δ^9-tetrahydrocannabinol (THC), alters neuroendocrine and gonadal functions in a wide variety of laboratory animals (review in [2]) It has been shown that THC suppresses pituitary gonadotropin release in males [7,20] and females [28, 33, 34], and influences feedback effects of gonadal steroids [10,29]. In addition, THC treatment alters hypothalamic luteinizing-hormone releasing hormone (LHRH) levels in rats [6, 19, 26] Administration of THC to adult males also suppresses plasma levels of testosterone (T) [7,21], and there is considerable evidence that cannabinoids can directly affect testicular steroidogenesis [4, 7, 15, 17]. The neuroendocrine actions of cannabinoids may be related to alterations in catecholamine concentrations and/or metabolism, since involvement of noradrenergic and dopaminergic pathways in neuroendocrine regulation have been well-documented [1,18]. However, the effects of THC on brain biogenic amines have not been consistent, and reports of increased and decresed levels of norepinephrine (NE), and either stimulation or inhibition of NE or dopamine (DA) release by hypothalamic synaptosomes have been reported after cannabinoid exposure [16,25].

In the present study, the effects of THC administration either *in vivo* or *in vitro* on hormone production and responsiveness to trophic factors by isolated median eminence fragments and pituitary glands were determined. In an additional study, the effect of *in vitro* THC exposure on gonadotropin-stimulated testicular T production was also assessed

METHOD

Median Eminence Incubations

The median eminence was obtained from adult male mice treated with THC (50 mg/kg) or vehicle, using 9 mice per group, and each median eminence was individually incubated in a Dubnoff metabolic shaker under 95% O_2:5% CO_2 in 500 μl Krebs-Ringer bicarbonate buffer, glucose (1

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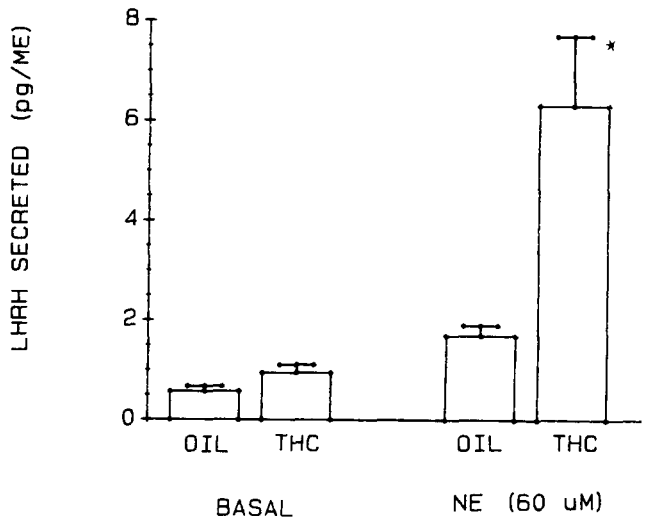

FIG 1 Luteinizing hormone-releasing hormone (LHRH) secretion by median eminence (ME) fragments incubated for 30 min each under basal conditions or in the presence of NE (60 μM) The MEs were obtained from mice treated 1 hr previously with Δ^9-tetrahydrocannabinol (THC, 50 mg/kg) or oil Means±S E (n=9/group) *Significantly different ($p<0$ 05) from OIL-controls also stimulated with NE, by ANOVA

TABLE 1

SECRETION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) BY MEDIAN EMINENCE FRAGMENTS (pg/ME) OBTAINED FROM UNTREATED MALE MICE 20 MIN AFTER EXPOSURE TO Δ^9-TETRAHYDROCANNABINOL (THC, 250 ng/ml) OR ETHANOL (ETOH, 2% v/v), AT EXPOSURE TO NOREPINEPHRINE (NE, 60 μM), AND 20 MIN LATER (EXPERIMENT I), OR 30 MIN AFTER CLONIDINE (10 μM) EXPOSURE (EXPERIMENT II)

	Treatments	
	ETOH	THC
	Experiment I	
Basal	4 39 ± 0 52 (4)	3 83 ± 0 45 (4)
At NE exposure	4 09 ± 0 44 (4)	3 55 ± 0 57 (4)
20 min post-NE	3 91 ± 0 39 (4)	6 51 ± 0 7 (4)*
	Experiment II	
Basal	2 15 ± 0 26 (4)	1 87 ± 0 20 (5)
Clonidine	2 36 ± 0 28 (4)	2 95 ± 0 56 (5)*

*Significantly different ($p<0$ 05) from ETOH-controls by ANOVA and Student Newman-Keuls test

mg/ml), and ascorbic acid (0 06 μM), at 37°C Tissues were pre-incubated for 30 min and media discarded. After 30 min incubation the media was removed and stored frozen for subsequent measurement of LHRH Fresh media containing NE, at a 60 μM concentration, was added to the median eminence fragments, and after an additional 30 min incubation, the media was separated from the tissue, and each were stored frozen for subsequent measurement of LHRH content. All samples for LHRH were run in a single assay

In additional experiments, the median eminence from untreated male mice were incubated as described, and THC (250 ng) was added in 10 μl ethanol (ETOH) to the media and ETOH alone was used in controls The content of LHRH in media was determined, first at 20 min post-THC or ETOH, again at the time of exposure to 60 μM NE, and 20 min after NE exposure. The content of LHRH in control and THC-exposed tissue was also measured. In a separate study, basal LHRH release was measured 30 min after addition of THC or ETOH at the same concentration described above, and at 30 min after exposure to clonidine (10 μM) (Table 1)

The content of LHRH in median eminence or media were determined by radioimmunoassay as described by Negro-Vilar *et al.* [24] The synthetic decapeptide used for radioiodination and standards was obtained from Peninsula Labs (Belmont, CA). The antibody used, CRR 11 B73, was provided by Dr V D. Ramirez, University of Illinois at Urbana-Champaign At an initial dilution of 1 50,000, the lower limit of sensitivity of the antiserum, as defined by the ability to displace 10–15% of the labeled hormone, is 1–2 pg/tube. This assay has also been described in recent publications [13,31]

Pituitary Gland Perfusion

Whole pituitaries were obtained from adult male mice 1 hr after receiving oral THC (50 mg/kg) or vehicle (25 μl sesame oil) Tissues were placed individually into microchambers (APS-10 Controller and microchamber culture module, Endotronics, Coon Rapids, MN), perifused with Medium 199 and bicarbonate under an atmosphere of 95% O_2 5% CO_2 Fractions were collected at 15 min intervals during a 60 min baseline period, then at 10 min intervals during an additional 60 min period, during which 3 pulses (10 min duration each), of LHRH (10^{-8} M), were introduced at 20 min intervals During an additional 60 min perifusion, post-LHRH exposure samples were collected also at 10 min intervals The flow rate to the microchamber was 9 6 ml/hr. Two runs each, using six THC-treated animals and six controls, were conducted as described Each perifusate was measured individually and average secretion rates were calculated and expressed as ng LH secreted/mg pituitary/10 min

Using this perifusion system in another study, pituitaries were obtained from untreated adult male mice, and baseline LH production determined during a 60 min period. A single pulse of LHRH (10^{-8} M) was added, along with ethanol (ETOH) or THC (250 ng/mg) in ETOH, and the secretion rate of LH was determined during an additional 30 min period. The content of LH in the samples was determined by a radioimmunoassay using anti-ovine LH, provided by Dr G Niswender, Colorado State University, which we have used previously for measurement of mouse LH [9,11] The samples were run in a single assay and the intra-assay coefficient of variation was 2.1%

Testes Perifusion

One testis each was, alternating left and right, obtained from adult untreated male mice, decapsulated and placed into the microchamber unit and perifused with Krebs-Ringer bicarbonate buffer, glucose (1 mg/ml) and hCG (12 5 mIU/ml), under a 95% O_2:5% CO_2 atmosphere The basal production of T during a 60 min period was determined, after which THC, at doses of 25 or 250 ng/ml, was added to the media. Samples were collected at 10 min intervals for an additional 90 min Data is expressed as percent change in T

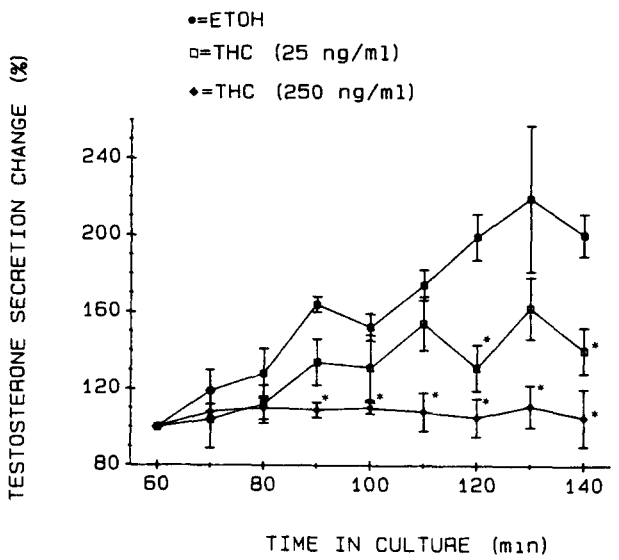

FIG 2 Effect of Δ⁹-tetrahydrocannabinol (THC) at 25 or 250 ng on testosterone (T) production, expressed as percent change compared to baseline rates, in the presence of human chorionic gonadotropin (hCG, 12 5 mIU/ml) *Significantly different ($p<0$ 05) from controls by a repeated measures ANOVA, $F(2)=60$ 98, $p<0$ 01, and Student Newman-Keuls test

compared to baseline Testosterone was measured by a radioimmunoassay without extraction, as described in several prior publications [7–10] The intra- and inter-assay coefficients of variation were 8 1% and 12.2%, respectively

Statistics

Data were analyzed using analysis of variance (ANOVA), and multiple comparisons were made using Student Newman Keuls test.

RESULTS

Median Eminence Secretion of LHRH

Basal LHRH release by median eminence fragments obtained from male mice previously treated with THC was comparable to OIL-control values (Fig. 1) Addition of NE (60 μM) significantly stimulated LHRH secretion by tissues obtained from either THC-treated ($p<0.02$) or control mice ($p<0.05$) However, in control mice, the increase was approximately 3-fold greater than basal release, while after THC-treatment, the increase was almost 7-fold higher than that of basal LHRH secretion (Fig 1). There were no significant differences in the content of LHRH between tissues obtained from THC- or vehicle-treated mice after incubation (253±34 (9) vs. 243±51 (9) pg/ME)

The NE-stimulated release of LHRH was also significantly enhanced after 20 min in median eminence perifusions exposed to THC (250 ng/ml) *in vitro*, compared to that in controls (Table 1)

Pituitary LH Secretion

Using a perifusion system in which LHRH (10^{-8} M) pulses were introduced at 70, 90 and 110 min, pituitaries from THC-treated mice tended to secrete more LH in response to LHRH pulses, but these average secretion rates

TABLE 2

SECRETION RATE OF LUTEINIZING HORMONE (LH) EXPRESSED AS ng/mg/10 MIN BY PITUITARIES UNDER PRE-TREATMENT CONDITIONS, AFTER ADDITION OF THC (250 ng/ml) OR ETHANOL (ETOH, 10 μl/ml), AND AFTER STIMULATION WITH LHRH (10^{-8} M)

	Treatment	
	ETOH	THC
Pre-Treatment	101 7 ± 6 6	96 9 ± 10 4
Treatment	86 9 ± 6 1	105 7 ± 14 2
LHRH (10^{-8} M)	166 0 ± 20 3	241 1 ± 26 7*

Means ± S E (n=6/group)
*Significantly different ($p<0$ 05) from ETOH-treated animals by ANOVA

were not significantly different from controls (100±9 vs. 81±5 ng/mg/10 min, $p>0.05$). Basal LH release over a 60 min period was comparable in pituitaries from THC-exposed and OIL-control mice (46±5 vs 47±7 ng/mg/10 min; n=6 mice/group)

Also using a perifusion system, the addition of THC (250 ng/ml) *in vitro* to media did not affect the basal secretion rate of LH by pituitaries. Responsiveness to LHRH (10^{-8} M) was significantly enhanced in both THC- and vehicle-exposed tissue ($p<0$ 05), but the increase was 150% in THC-exposed tissue, and only 60% in ETOH-exposed tissue.

Testicular T Production In Vitro

Addition of media containing THC, at a concentration of 250 ng/ml, resulted in a significant suppression of hCG-stimulated T production within 30 min of exposure, and this effect persisted for another 50 minutes (Fig. 2). A lower dose of THC (25 ng/ml), did not influence T production until 60 min after exposure, with T secretion significantly lower than that produced by control testes exposed to the ETOH vehicle after 60 and 80 min (Fig. 2)

DISCUSSION

Exposure to THC enhances the responsivity of neuroendocrine sites, i.e., median eminence or pituitary, to adrenergic agonists or LHRH, respectively. The results obtained after *in vitro* exposure to THC were comparable to those obtained after *in vivo* THC treatment. In addition *in vitro* exposure to THC produced a time and dose-dependent reduction in gonadotropin-stimulated T production by isolated testes.

The enhanced responsivity at neuroendocrine sites resulting from THC exposure is surprising. In the present studies, the responsivity of the median eminence to NE, as well as that of the pituitary to LHRH, was significantly enhanced by THC exposure Other investigators have observed that repeated exposure to THC in adult rats increased hypothalamic LHRH content, but did not influence pituitary responsiveness to LHRH *in vitro* or *in vivo* [26] However, animals were sacrificed 24 hr after the last treatment. Therefore, it is conceivable that neuroendocrine responsiveness was returning to normal, or that tolerance development had occurred as a result of the 7-day treatment regimen.

It is difficult to explain the lack of response by ETOH-exposed MEs to NE. Exposure to THC either *in vivo* or *in vitro* enhanced ME responsiveness to noradrenergic stimulation The MEs from mice receiving sesame oil per os exhibited the expected increase in LHRH after NE exposure *in vitro* However, MEs from untreated mice exposed to ethanol (2% v/v) *in vitro* failed to respond to NE or clonidine with enhanced LHRH release However, MEs exposed to THC in ethanol did exhibit significant increases in LHRH-stimulated compared to basal secretion rates. The vehicle (ethanol) may have influenced responsiveness to NE or clonidine, but did not significantly affect basal LHRH release into media. Unfortunately, the highly lipophylic THC is not readily soluable in aqueous media. Other vehicles used to deliver THC also produced effects [3], and ethanol was one of the few that did not affect basal activity. Clearly, these potential confounding effects of vehicle make interpretation of results difficult. However, it is very interesting to note that THC appears capable of reversing these effects of ethanol It is still difficult to determine the relationship between the present findings and the well-documented THC-induced decreases in plasma LH [6, 20, 28, 34]. It is conceivable that cannabinoids may affect catecholamine receptor binding or peptide metabolism after LHRH release from ME nerve terminals Certainly, the suppressive effects of THC on gonadotropin release do not appear to be due to an inability of the LHRH neuron to respond to NE stimulation

In vivo THC treatment tended to enhance LHRH pituitary responsiveness, but only direct *in vitro* exposure to THC significantly affected this parameter. It is possible that direct addition of THC resulted in higher or more sustained tissue concentrations. It is also conceivable that by 1 hr post-THC treatment the effect on pituitary had already occurred and we are presently investigating this possibility Pituitaries obtained from mice receiving either THC or sesame oil exhibited self-priming effects in responses to repeated pulses of LHRH Although the pituitaries from THC-treated mice secreted slightly more LH at each pulse, the incremental increase in LH release after each successive LH pulse was quite similar in treated and controls Indeed, the overall release of LH was also not significantly different It is not clear whether the differences in basal LH secretion rates by pituitaries obtained from untreated male mice and those receiving oral administration of sesame oil or THC are due to the variability in hormone levels which are characteristic in mice or the result of handling, drug or vehicle administration

It has been reported that treatment with phenobarbital enhances subsequent pituitary responsiveness to LHRH [22]. In contrast, morphine, which blocks the steroid-induced LH surge, does not affect pituitary responsiveness to LHRH [32]. In another study in adult mice, we observed that concomitant exposure to low doses of THC and LHRH resulted in higher levels of LH than that produced by LHRH alone [9]. Interestingly, exposure to a novel female not only abolished the suppressive effects of THC (50 mg/kg) on plasma LH, but plasma LH levels were higher in THC-treated than in control males after exposure to female-related stimuli [9]. Certainly THC also affects gonadal steroids [7,8] and androgens and estrogen levels can influence pituitary responses to LHRH [12, 14, 23].

The findings that THC suppressed hCG-stimulated, but not basal T production, is consistent with previous reports from this laboratory concerning THC effects on plasma T levels and on testicular T content and/or *in vitro* T production [7–10]. Since it has been reported that THC does not affect gonadotropin binding by isolated Leydig cells [4], and we have previously demonstrated that the inhibitory effects of THC occur prior to the formation of pregnenolone [7], the actions of THC probably occur during the early events activated by hCG receptor binding. Cannabinoids inhibit cholesterol esterase activity in testis [4], and the mobilization of cholesterol from mitochondrial stores is an early event in LH/hCG receptor activation [5] However, at present the mechanism(s) by which cannabinoids influence testicular steroidogenesis remain to be determined

It is conceivable that THC may influence functions at each level of the HPG axis, and this possibility must be considered in evaluating THC effects on HPG feedback regulation Indeed, we have observed that the THC-induced biphasic effects on plasma T levels occur almost simultaneously with those for plasma LH concentrations [8]. We have also recently observed that a single dose of THC alters catecholamine concentrations and/or turnover in median eminence, medial preoptic area, and medial basal hypothalamus (Dalterio and Steger, unpublished). However, we have not yet determined whether these neurotransmitter actions of THC mediate its neuroendocrine effects In a previous study in mice treated with the non-psychoactive cannabinol, changes in noradrenergic activity in the median eminence appeared to correlate with changes in plasma LH levels [11], and, in female rats, THC-induced alterations in brain biogenic amines correlate with those on gonadotropins and prolactin [29] The present data are consistent with THC-associated interference with neurotransmitter modulation of neuroendocrine functions. However, whether these alterations in neuroendocrine target sensitivity are the result of cannabinoid actions on neurotransmitter activity, or are related to direct THC actions on neuroendocrine or gonadal target sites still remain to be determined

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