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# THE EFFECTS OF 9-ENE-TETRAHYDROCANNABINOL ON HORMONE RELEASE AND IMMUNE FUNCTION

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**Summary**—We investigated effects of 9-ene-tetrahydrocannabinol (THC) on endocrine and immunological function. Seventeen male volunteers entered into a double blind, randomized study to receive oral THC (10 mg t.i.d. for 3 days and on the morning of the fourth day) or placebo, after at least 2 weeks of abstinence. Plasma prolactin, ACTH, cortisol, luteinizing hormone and testosterone were not altered during or after THC, compared with baseline concentrations. Tests of lymphocyte function showed no differences compared to baseline between THC and placebo groups. As the relatively low dosing regimen of THC (10 mg t.i.d.) resulted in no alterations, another group of 6 men were administered higher doses of THC by inhalation (18 mg/marijuana cigarette) following the same dosing regimen. No endocrine or immunological alterations were observed. When the subjects were grouped according to their history of THC use prior to admission, heavy THC users had lower prolactin concentrations than light users. No differences were observed in concentrations of other hormones or in tests of immune function.

## INTRODUCTION

The continued dissemination of the acquired immunodeficiency syndrome epidemic and the relatively poor prognosis of drug abusers infected with human immunodeficiency virus, has prompted new interest in the role of drugs of abuse as immunomodulators [1–3]. Marijuana, the active component of which is 9-ene-tetrahydrocannabinol (THC), is an appropriate candidate for study of immunomodulating effects because it is widely used [4, 5], may have glucocorticoid-like properties [6] and has been reported to affect the immune system [7, 8]. The mechanisms of these effects are not clear but they may be secondary to the steroid-like effects of THC, or due to promiscuous or nonspecific effects of high doses of THC.

Many perturbations of immunologic functions have been attributed to THC (see recent reviews by Munson and Fehr[7], Hollister[8], and others [9, 10]), but the results are inconclusive in spite of a wide range of test paradigms that have been used. In *in vivo* studies, the duration of which range from testing of immune function after single doses to testing in subjects who have used THC for years, the effects of THC on almost every known immune function test have been examined. Its effect on lymphocyte subsets has not been examined until recently. No changes in subsets were found in chronic THC smokers compared with tobacco smokers and nonsmokers [11]. Overall the observed trend in the effects of THC on immune function have been toward immunosuppression. It has been postulated that glucocorticoid or other endocrine mediators may be involved in modulating immune functions in response to THC.

To date, very few studies have examined endocrine and immune responses to THC in the same subjects. Patel *et al.*[12] found that acute administration of THC to rats changed neither plasma norepinephrine, epinephrine, corticosterone or $\beta$-endorphin, nor natural killer cell activity. Treatment with THC for 25 days decreased plasma concentrations of norepinephrine, epinephrine and corticosterone, but elevated plasma $\beta$-endorphin. Natural killer cell activity was also decreased in these animals. We have, in turn, examined whether short-term administration

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of THC affected differently immune parameters and the rhythmic secretion of prolactin and cortisol in the same subjects. To examine more chronic effects of THC, endocrine and immune function tests were examined when subjects who were either light or heavy users by self-reports, were admitted to the research ward.

## EXPERIMENTAL

### *Subjects*

Male volunteers were recruited through newspaper advertisements. They presented with histories of occasional or heavy (at least 3 marijuana cigarettes/day for 6 months) THC use and may have also used alcohol, caffeine and/or nicotine. Some subjects reported using cocaine intra-nasally sporadically, but stated that they had not used it in the 6 months prior to study admission. All urine tests prior to admission were negative for cocaine metabolite, but all subjects tested positive for cannabinoids. The results of physical examinations, routine biochemistry and psychological screen examinations were within normal limits for all subjects. Of the 17 subjects randomly assigned to receive either orally administered THC or placebo, 12 subjects completed the protocol. Another group of 6 subjects were given THC by inhalation.

### *Protocol*

The experimental protocol is illustrated in Fig. 1. Subjects resided on the closed research ward of the Addiction Research Center. Week 1 of the protocol commenced at least one week after urine tests (Roche Diagnostics, Abuscreen) were negative for cannabinoids. During the first week of the protocol, 40 ml blood was drawn at 0800 h on days 1 and 5 for immune function tests and at 0800, 1200, 1600, 2000 and 0200 h for measurement of hormone concentrations (baseline assessments). Blood for the assessment of hormones was collected into EDTA and after plasma separation, 50 $\mu$l Trasylol per 450 $\mu$l plasma was added prior to storage at $-70°C$. During the second week THC (Marinol 10 mg p.o., Roxane Labs, Inc. Columbus, Ohio, U.S.A.) or 1 marijuana cigarette (NIDA, THC cigarettes containing approx. 18 mg/1.2 g cigarette) [13] was administered 3 times daily for 3 days and once on the fourth day (D4; D1 designated as first day of THC). Plasma levels of THC and 11-OH-THC in oral and inhaled THC have been described previously [14]. Blood was drawn daily during this week for hormone measurements at 0800, 1200, 1600, 2000 and 0200 h. Blood for immune assays was drawn on D4 and D5. During the third week, blood was drawn on D8, D10 and D12 for both immune function and hormone measurement at the same times of day as above.

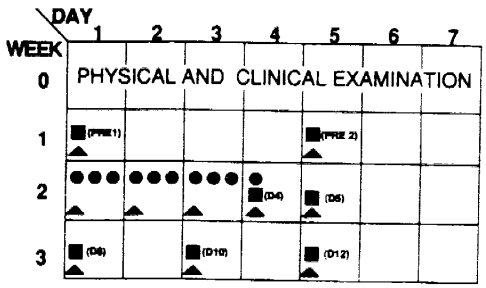


Fig. 1. The study protocol to investigate the temporal relationships between effects on endocrine and immunological parameters during THC administration was conducted over at least 4 weeks. Subjects rested on the research ward until at least 1 week after urine testing by radioimmunoassay was negative for cannabinoids. Blood was drawn at 0800, 1200, 1600, 2000 and 0200 h (▲) for assay of hormone concentrations in plasma. Immune function tests were carried out in blood drawn at 0800 h (■). THC (10 mg p.o. t.i.d. or an 18 mg marijuana cigarette inhaled t.i.d.) was administered at 0900, 1200 and 1500 h (●) on D1, 2, 3 and at 0900 h on D4. D1 was designated as the first day of THC administration.

### *Immune function tests*

Tests included white cell and differential counts, T and B cell and T cell subsets quantitation using fluorescein tagged antibodies to $CD_3$, $CD_4$, $CD_8$, and $CD_{19}$ (Becton-Dickinson), *in vitro* lymphocyte proliferation responses to the mitogens phytohemagglutinin (PHA), pokeweed (PWM) and conconavalin *A* (Con *A*), antibody-dependent cellular cytotoxicity (ADCC) and natural killer cell lytic activity, as previously described [15–19].

### *Hormone measurements*

Plasmas were assayed in duplicate for PRL, ACTH, corticosterone, LH, and testosterone by commercially available radioimmunoassays (Radioassay Systems Laboratories, Carson, Calif., U.S.A.). Intra-assay variability in the immunoassays ranged from 0.5 to 6.6%; interassay variability ranged from 11.2 to 17.0%.

### *Statistics*

Data were analyzed by repeated measures ANOVA, using BMDP.2V. Missing data were estimated using BMDP AM. *Post hoc* Duncan's multiple range tests were used to distinguish significant differences among study days.

## RESULTS

### *General*

All subjects, whether given THC orally or by inhalation, reported subjective effects, usually described as elevated mood or as a "high". All showed temporary mild alterations in pulse rate and/or blood pressure. The study reported here is ongoing.

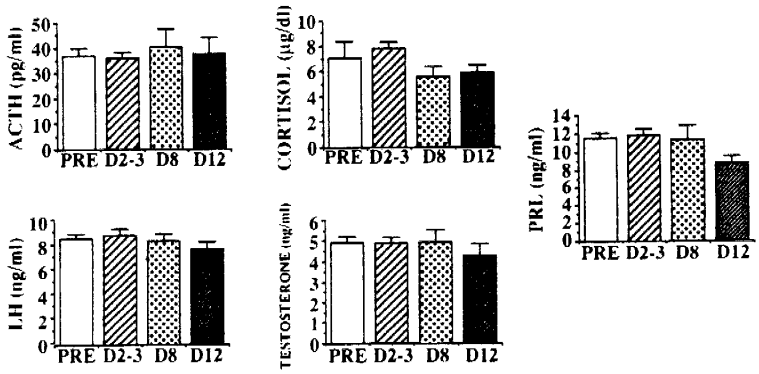


Fig. 2. Twenty-four hours mean hormone concentrations in plasma for study days (D) 2–3 were compared with the average concentrations prior to and after (D8, 12) athe administration of THC (10 mg p.o. t.i.d.), $n = 8$). Blood samples were drawn at 0200, 0800, 1200, 1600 and 2000 h. In plasma no differences were observed between the THC and placebo groups or between pre- and post-THC concentrations of hormones for each of the hormones measured throughout the study. When one marijuana cigarette (18 mg THC) was inhaled t.i.d., similar results were achieved in 6 subjects.

*Endocrine effects*

Concentrations of each hormone in plasma drawn at 0800, 1200, 1600, 2000, 0200 h were averaged to obtain a 24-h mean for each hormone for each subject. No differences were observed in the 24-h mean plasma concentrations of PRL, LH, ACTH, cortisol and testosterone for subjects treated with oral THC throughout the study (Fig. 2), nor were differences found in subjects who inhaled THC (data not shown). In view of the glucocorticoid-like effects of THC, it was surprising that no changes were found in cortisol concentrations through the day, either in terms of rhythmicity compared with the group that received placebo only (Fig. 3) or in terms of dose of THC administered per day (data not shown).

Plasma PRL concentrations were lower in subjects given 10 mg THC p.o. t.i.d. than in those given the placebo, $P < 0.01$ (Fig. 3). However, the placebo group had more subjects who reported only occasional use of marijuana prior to admission suggesting that heavy THC use may have played a causative role in the lower PRL concentrations. We, therefore, compared hormone concentrations in subjects who used marijuana heavily (3 marijuana cigarettes/day for at least 6 months; $n = 12$) with those who reported light or occasional use ($n = 11$). Prolactin

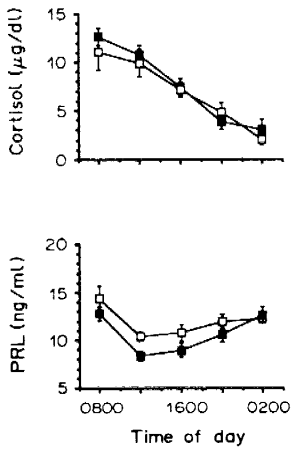


Fig. 3. Concentrations of plasma cortisol (upper panel) and prolactin (lower panel) on D3 of the protocol, in subjects administered 10 mg THC p.o. t.i.d. (■, $n = 7$) and subjects administered placebo (□, $n = 5$). Blood was withdrawn from indwelling catheters at the times of day indicated.

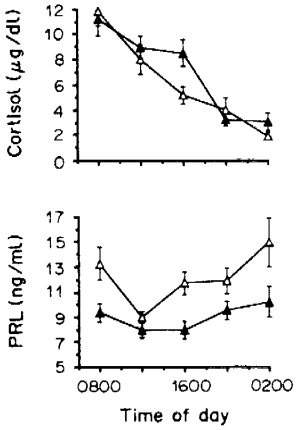


Fig. 4. Concentrations in plasma cortisol (upper panel) and prolactin (lower panel) following admission for 23 subjects reporting heavy use of THC prior to admission (▲, $n = 11$) and light users (△, $n = 12$). Indwelling intravenous catheters were placed at least 1 hr prior to the first withdrawal of blood on the day following admission to the research ward. Prolactin levels were significantly lower in the heavy users ($F = 7.59$, $P < 0.012$, $df$ 1,21).

Table 1. White blood cell (WBC) counts and percent of lymphocyte subset representation in 6 subjects following the administration of 10 mg THC p.o. t.i.d. for 3 days (D1-3) and after a single dose on D4. Results are expressed as mean ± SE

| Study day | WBC total | Lymphs | $CD3^+$ | $CD4^+$ | $CD8^+$ | $CD4^+/CD8^+$ | $CD19^+$ |
|---|---|---|---|---|---|---|---|
| Pre | 7783 ± 797 | 34.5 ± 2.3 | 76.2 ± 2.9 | 49.7 ± 2.8 | 31.5 ± 3.9 | 1.7 ± 0.2 | 13.5 ± 2.7 |
| D4 | 6917 ± 750 | 41.2 ± 6.8 | 73.5 ± 3.0 | 49.2 ± 3.3 | 29.7 ± 3.9 | 1.8 ± 0.3 | 12.5 ± 2.3 |
| D5 | 7017 ± 1109 | 36.7 ± 4.9 | 73.7 ± 2.6 | 49.7 ± 3.1 | 30.0 ± 4.4 | 1.8 ± 0.3 | 13.7 ± 2.9 |
| D12 | 7783 ± 606 | 36.3 ± 4.4 | 72.5 ± 3.0 | 46.2 ± 2.5 | 31.3 ± 4.2 | 1.6 ± 0.2 | 14.5 ± 3.0 |

was significantly lower (Fig. 4; $F = 7.59$, $P < 0.01$, $df$ 1, 21) in the heavy users on admission but also during the study (data not shown). No differences in ACTH, cortisol, LH or testosterone were observed between these groups.

*Immune function tests*

No significant alterations in immune function tests were observed. No changes in mitogen-induced cell proliferation were observed using lymphocytes withdrawn during the time of THC administration or at time of admission between subjects self-reporting heavy or light THC use.

No changes in peripheral blood lymphocyte subset representations for 6 subjects given THC p.o. were observed after 4 days of THC administration (D4), compared with the percentage representation pre or post THC (Table 1) or after THC inhalation (data not shown). There were no significant differences between habitually heavy and light users of THC (Table 2).

No significant differences were found in natural killer cell activity or in ADCC between any of the study groups (Fig. 5).

## DISCUSSION

The time-dependent effects of acute THC administration on hormone concentrations and immune function were investigated. Novel findings included a decrease in plasma prolactin concentrations that may be a consequence of chronic use of THC. No other alterations in hormone concentrations were observed either during THC administration or in chronic THC users compared with occasional acute users, nor were alterations in assays of immune function in lymphocytes observed.

The significance of the decreased prolactin levels is unclear but they may related to increased host susceptibility to disease or to an altered host response to immunological challenge reported in heavy THC users. Prolactin or prolactin-like substances appear to modulate some aspects of immune functions [20, 21]. Decreased resistance to pathogens occurs in hosts treated with THC. Guinea-pigs inoculated intravaginally with herpes simplex virus type 2 were treated with 4 and 10 mg/kg THC intermittently over 18 days [22]. THC-treated animals had more severe lesions then the virus-inoculated, vehicle-treated controls. Cumulative mortalities were correlated positively with increasing doses of THC. In mice treated with 100 mg/kg THC and then inoculated with herpes simplex virus type 2, greater severity of genital lesions, higher mortalities and higher mean titers of virus shed from the vagina were observed in THC-treated mice than in controls [23]. In 13 or 14 human marijuana smokers, fungi were identified in sputum cultures [24] and fungal precipitins were more common, leading these authors to conclude that marijuana conferred a risk of fungal infections and other immunologic lung disorders.

Using a different approach, THC decreased the signs of experimental encephalomyelitis in rats and guinea-pigs if the THC was administered either at the time of inoculation or after inoculation [25]. Overall, these *in vivo* studies would suggest that THC reduces host immune function. Further, the immunosuppressive effects of THC have been suggested to be therapeutically useful [26]. Because the concentrations of ACTH and cortisol did not vary between groups or within subjects over the course of this study, it seems that alterations in plasma concentrations or perturbation of their normal diurnal rhythms are unlikely to contribute to the postulated THC-dependent alterations in host susceptibility.

The present study did not support the suggestions of others that THC has glucocorticoid-like actions [6, 27]; such postulated actions could explain some of

Table 2. White blood cell (WBC) counts and percent of lymphocyte subset representation in 6 heavy and 6 light THC users (by history) on admission to the closed research ward. Results are expressed as mean ± SE

| Usage | WBC total | Lymphs | $CD3^+$ | $CD4^+$ | $CD8^+$ | $CD4^+/CD8^+$ | $CD19^+$ |
|---|---|---|---|---|---|---|---|
| Light | 8333 ± 1500 | 9.38 ± 3.83 | 74.17 ± 3.14 | 53.83 ± 3.21 | 26.33 ± 1.99 | 2.07 ± 0.23 | 17.17 ± 3.37 |
| Heavy | 7660 ± 1228 | 17.27 ± 7.05 | 71.17 ± 5.91 | 45.83 ± 4.47 | 35.67 ± 4.90 | 1.42 ± 0.23 | 10.83 ± 2.34 |

the reported immunosuppressive effects. However, from available evidence, it is difficult to explain the mechanism of the hypothesized glucocorticoid effects. In rats and mice, THC (2–50 mg/kg) acutely increased corticosterone levels [28–30], but adaptation to repeated THC administration seemed to occur [28, 29]. The increase was not observed in hypophysectomized animals [31]. Others found that the acute effect of THC was centrally mediated, because no increase of plasma corticosterone was observed in rats with hypothalamic deafferentation [32]. Basal plasma concentrations of ACTH and corticosterone in rats chronically treated with THC (4–8 mg/kg i.p. 5 days/week for 8 months) were similar to those of vehicle-treated rats, but these hormones increased markedly over controls 12 min (but not 2 min) after placement in a restraint cage [6]. In rodents, THC may elevate glucocorticoids acutely, however, this effect is not maintained during long-term administration [28, 29]. This is apparently not a stress response because most stressors result in elevations of both corticosterone and prolactin. However, in animal studies, corticosterone is elevated with acute THC administration, but prolactin is decreased. Alternatively, after chronic administration, rats had an increased ACTH and corticosterone response to restraint [6]; prolactin was not measured.

In humans, evidence for THC-induced alterations in the hypothalamo–pituitary–adrenal axis is less clear. Plasma cortisol did not change after acute oral administration of THC [33] but has been shown to increase during the first hour after smoking THC cigarettes [34]. A depressed cortisol response to insulin-induced hypoglycemia was found in subjects who had used THC chronically [35]. Concentrations of ACTH and cortisol in our subjects were all taken at rest.

THC may bind to one or more steroid receptors. THC competed for binding to estrogen receptors in two studies [36, 37] but not in another [38] where THC and 17$\beta$-estradiol apparently bound to different macromolecules. Decreases in testosterone concentrations may also occur after both chronic [39] and acute [40] administration of THC, but these findings have not received universal support [34, 41–44]. Finally, delta-9-THC competed with [$^3$H]dexamethasone for similar binding sites in rat hippocampal tissue[45]. Studies in animals support the hypothesis that THC suppresses LH via an estradiol-dependent mechanism [46]. Acute exposure of female subjects to smoked marijuana depressed luteinizing hormone with no concomitant changes in estradiol and progesterone [47].

Contrary to the hypothesized steroid actions of THC, Howlett *et al.* showed that THC inhibits adenylate cyclase activity [48, 49] through the inhibitory guanyl nucleotide binding protein ($G_i$) [50]. They demonstrated that the binding of [$^3$H]HCP 55940, a potent THC analog, defines a specific site for which THC and other analogs compete that is in relative potency to their pharmacological analgesic properties [51]. The pharmacological inhibition constants in these experiments are in the nanomolar range, which is appropriate for THC effects.

THC, a highly lipid soluble molecule, may have non-specific effects on membranes at high concentrations and also may have nonspecific glucocorticoid effects at high doses. Thus, immunogenicity could be altered at sufficiently high doses. Cannabinoids accumulate in adipose tissue and when those stores are saturated, as may occur with heavy THC use, higher levels of THC may be achieved by plasma that may lead to neuroendocrine-immune alterations. In the present study we intentionally used low, but psychoactive, dosages of THC to enable us to examine the primary THC-specific effects.

Earlier reviews [7, 8] indicated that proliferative activity in response to nonspecific mitogen simulators, was either depressed or unaffected by various THC dosing regimes. Many studies were conducted with large single doses of THC or in heavy habitual users. More recently, Klein *et al.*[52], observed that the addition of $2–3 \times 10^{-5}$ M THC to mouse lymphocyte cultures inhibited proliferation. However, such high concentrations of THC may provoke nonspecific effects, because the affinity of THC for its receptor is in the nanomolar range [51]. The lymphoproliferative response to

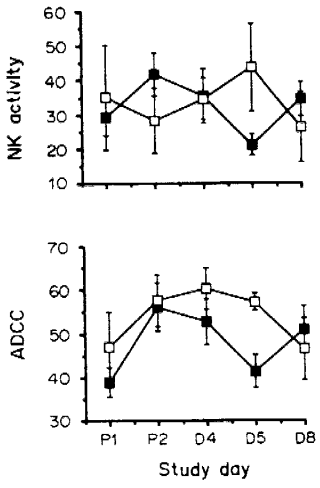


Fig. 5. Natural killer cell activity and antibody dependent cell cytotoxicity (ADCC) were not different throughout the study in the subjects administered THC orally ($n$ = 13 and 8 for NKA and ADCC, respectively, ■) compared with subjects administered placebo ($n$ = 3, □). Natural killer mediated lysis was obtained at a 12.5:1 effector to target cell ratio using $^{51}$Cr-labeled K562 target cells. ADCC activity was measured at a 2.5:1 E:T using antibody coated $^{51}$Cr-labelled chicken red blood cells as targets. P1 and P2 are the means ± SE of the pre-THC values and demonstrate the marked variability in these assays.

phytohemagglutinin and con *A* was the same in volunteers with histories of heavy usage of marijuana with and without concommitant tobacco smoking when compared with tobacco smokers or non-smokers [11]. Cellular chemotaxis was different between the groups [53].

Natural killer cell activity measures the ability of a subset of lymphocytes to kill a tumor cell line *in vitro*. Lymphocytes from healthy blood donors exposed *in vitro* to THC (1–20 $\mu$g/ml) showed natural killer cell lytic activity was suppressed at 5–10 $\mu$g/ml and abolished at THC concentrations of 20 $\mu$g/ml [54]. Decreased natural killer cell activity was observed in rats treated with 3 mg/kg THC daily for 25 days [12], but no changes were seen after acute treatment.

We conclude that THC may act through specific receptors or via nonspecific membrane perturbation that can modify immune-endocrine responses [10]. THC may also act through prostanoid mechanisms, particularly with regard to its nociceptive [55] and cataleptic [56] actions. Furthermore, these mechanisms may operate singly or in concert to account for the highly varied results of previous studies of THC-induced modulation of the immune and endocrine systems. We were unable to demonstrate alterations in tests of lymphocyte function on exposing subjects to psychoactive doses of THC. This is the first report of lymphocyte subset investigations in humans following controlled administration of THC, and no alterations were observed. Since no significant alterations were observed either in the acutely treated subjects or in those reporting heavy THC use, we suggest that at least some of the previously described immunosuppressive effects of THC may be nonspecific effects secondary to very high doses of THC used for study.

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