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Effects of Acute and Subchronic Δ^9-Tetrahydrocannabinol Administration on the Plasma Catecholamine, β-Endorphin, and Corticosterone Levels and Splenic Natural Killer Cell Activity in Rats (42195)

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Abstract. The effect of acute (1 day) or subchronic (25 days) treatment with Δ^9-tetrahydrocannabinol (THC), the major psychoactive constituent of marihuana, on plasma norepinephrine (NE), epinephrine (E), corticosterone, β-endorphin (β-end), and splenic natural killer (NK) cell activity of the rat was studied. Groups of animals received subcutaneously, either THC in corn oil + saline (3 mg THC/kg); oil + saline; or THC + naloxone (2 mg naloxone/kg and 3 mg THC/kg). Acute injection of THC with or without naloxone did not significantly change plasma levels of NE, E corticosterone, β-end, or the NK cell activity. However, subchronic treatment with THC significantly reduced plasma levels of NE, E, corticosterone, and NK cell activity, compared to controls. The plasma β-end levels were significantly elevated in the THC-treated animals. In the THC + naloxone group of animals, the plasma hormone levels (corticosterone and β-end) were similar to control levels and the NK cell activity was significantly higher than in THC-treated animals. These results indicate that subchronic exposure to THC results in suppression of splenic NK cell activity. The interaction of THC with the endogenous opiate system appears to be a contributing factor leading to the NK cell suppression in rats. A direct suppressive action of THC or its metabolites on the NK cell is not ruled out by this study.

The major psychoactive constituent of marihuana, Δ^9-tetrahydrocannabinol (THC), has been shown to depress various aspects of the immune system. The effects of THC on the immune system include lowering of humoral immune response to primary antigenic stimulation (1–3), impaired cellular immunity (4–6), impaired lymphocyte rosette formation (7), and inhibition of macrophage migration (8). However, the effects of subchronic exposure to THC on the natural killer (NK) cell are not known. The ability of NK cells to lyse many tumor cell lines without prior immunization implicates these lymphoid cells as a first line of defense against neoplasia. In recent years, the endocrine system has gained considerable importance as the major control system of immune function. The adrenal corticosteroids (9, 10), plasma catecholamines (11), and the endogenous opiate peptides (12–15) have all been shown to have significant modulatory effects on the immune system. Recent evidence indicates that THC significantly increases plasma corticosterone (16, 17) and brain enkephalin levels in rats exposed to an acute dose of THC (16). Although no reports exist on the effects of THC on plasma catecholamines, suppression of brain tissue catecholamine levels has been shown in rats exposed to THC (18). This study reports the effect of acute and chronic exposure to THC on the plasma catecholamine, β-endorphin, corticosterone levels, and splenic NK cell activity in the rat.

Material and Methods. Male Sprague–Dawley rats (Charles River, Wilmington, Mass.) of approximately 230 g body wt were used in this study. Δ^9-Tetrahydrocannabinol (obtained from the National Institute on Drug Abuse) was prepared for injection by evaporating the alcohol under a stream of nitrogen and reconstituting the residue in corn oil (16). Groups of rats were injected subcutaneously with 50 μl of THC in oil (3 mg THC/kg body wt) and naloxone, a specific opiate antagonist (2 mg/kg body wt, in saline); or vehicle and saline; or THC and saline.

In the acute experiment, rats were decapitated 24 hr after the only injection. In the subchronic experiment, rats were treated daily for

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25 days and decapitated 24 hr after the last injection. Trunk blood and spleen were collected following decapitation. Plasma was separated and kept at −70°C for subsequent analyses of catecholamines, corticosterone, and β-endorphin. Spleens were kept in sterile cold RPMI media for use in NK cell assay (RPMI 1640, GIBCO Laboratories, with 1% glutamine, 1% penicillin-streptomycin, 5×10^{-5} *M* β-mercaptoethanol, 25 m*M* Hepes buffer, and 20% heat inactivated pooled human serum).

Preparation of effector cells. Spleen cell suspensions were obtained by teasing the splenic pieces with the frosted ends of sterile glass slides into RPMI media and filtering through a nylon mesh. The lymphocytes were isolated by Ficoll–Hypague gradient centrifugation. The cells were washed three times with RPMI media and finally suspended in RPMI media at a concentration of 8×10^6 cells/ml. The NK cell activity was measured in a standard 4-hr chromium release assay using YAC-1 lymphoma cells as targets.

Labeling of target cells. YAC-1 lymphoma cells were labeled with isotope by incubation in 0.5 ml of RPMI media containing 100 μCi Na 51 chromate (^{51}Cr) for 45 min at 37°C. The labeled cells were washed three times with RPMI media and then suspended in the same media at a concentration of 1×10^5 cells/ml.

^{51}Cr release cytotoxicity assay. Effector cell suspensions (8×10^6 cells/ml) were serially diluted to 4×10^6 and 2×10^6 cells/ml with RPMI media. One hundred microliters of each effector cell dilution were pipetted into microtiter wells (Corning plastics) in triplicate. Target cell suspension (100 μl, containing 1×10^5 cells/ml) was added to each well. These mixtures gave final effector:target cell ratios of 80:1, 40:1, and 20:1. The plates were centrifuged at 200*g* for 5 min and then incubated at 37°C for 4 hr in 5% CO_2 in air. At the end of incubation, the plates were centrifuged at 100*g* for 10 min. A 100-μl aliquot of the supernatant was removed and counted in a gamma radiation counter. The spontaneous release (SR) of ^{51}Cr was determined by culturing the labeled target cells in RPMI media without effector cells. Maximum release (MR) was obtained from target cells lysed with 25 μl of concentrated HCl. The percentage of specific cytotoxicity was calculated by

$$\text{specific percentage cytotoxicity} = \frac{\text{test CPM} - \text{SR CPM}}{\text{MR CPM} - \text{SR CPM}} \times 100.$$

Percentage specific release is plotted against effector–target cell ratio for each sample. These curves are then analyzed by an exponential fit equation as described by Pross *et al.* (19).

Hormone and catecholamine analyses. Plasma corticosterone was measured by specific radioimmunoassay (16) using antisera kindly supplied by Dr. G. D. Niswender of Colorado State University. β-Endorphin radioimmunoassay has been described in detail previously (20). The plasma norepinephrine (NE) and epinephrine (E) levels were estimated by high-performance liquid chromatography (HPLC). The HPLC procedure has been described in detail elsewhere (21). The data were tested for statistical significance by analysis of variance and Duncan's multiple range test (22).

Results. Single injection of THC did not have any significant effect on the plasma levels (expressed as ng/ml; $\bar{x} \pm$ SEM) of NE (5.1 ± 0.4), E (7.2 ± 0.4), corticosterone (67.5 ± 5.4), and β-endorphin (0.26 ± 0.03) when compared with the levels of NE (4.5 ± 0.6), E (7.9 ± 0.3, corticosterone (73.4 ± 4.3), and β-endorphin (0.24 ± 0.03) of control animals. Also, a single injection of THC did not influence the NK cell activity when compared with control animals.

Subchronic treatment with THC resulted in significant changes in the plasma hormone levels (Table I) as well as the activity of NK cells (Table II). Compared to controls, the plasma NE, E, and corticosterone levels were significantly lower and the plasma β-endorphin levels were significantly higher in subchronically THC-treated animals. Rats treated with THC + naloxone showed no significant change in plasma corticosterone and β-endorphin levels when compared to control animals. However, plasma E levels were slightly higher in these rats compared to THC-treated animals, but lower than the control levels. The plasma NE levels remained low in THC + naloxone and THC + saline group of animals.

The NK cell activity was significantly reduced at all effector:target cell ratios after subchronic THC treatment. In the rats treated

TABLE I. EFFECT OF CHRONIC Δ^9-THC TREATMENT ON PLASMA HORMONES

Group	*n*	NE[a]	E[a]	Corticosterone[a]	β-Endorphin[b]
Control	8	5.53 ± 0.44	9.16 ± 0.66	64.0 ± 13.5	735 ± 103
THC	8	3.69 ± 0.21*	6.84 ± 0.30*	34.7 ± 4.5*	1112 ± 107*
THC + naloxone	8	3.15 ± 0.27*	7.81 ± 0.40*	64.0 ± 13.5	844 ± 118

[a] Expressed as ng/ml plasma (mean ± SEM).
[b] Expressed as pg/ml plasma (mean ± SEM).
* $P < 0.05$ compared to corresponding control values.

with naloxone along with THC, the NK cell activity was lower than that of control rats but significantly higher than that of THC + saline-treated groups of animals at all effector:target cell ratios tested (Table II).

Discussion. This study indicates that subchronic treatment with THC suppresses NK cell activity as well as the stress-related hormones, NE, E and corticosterone but increases plasma β-endorphin levels. A single injection of THC did not have any significant effect on the above parameters.

The most significant finding of this study is the association of elevated β-endorphin levels with suppressed NK cell activity in rats exposed to subchronic THC treatment. Physiological and psychological stress factors which release endogenous opiate peptides have been shown to increase susceptibility to infection and to suppress the immune system (23, 24). Opioid-mediated footshock stress also has been shown to suppress both lymphocyte proliferation and natural killer cell cytotoxicity in rats (15, 25). Although other studies suggest that endorphins and enkephalins may be immunostimulants (26, 27), these were single exposure *in vitro* experiments and not comparable to our study. Naloxone, when given simultaneously with THC, reversed some of the effects of THC, most notably the effect of THC on the plasma levels of corticosterone and β-endorphin. The significantly higher NK cell activity in the THC + naloxone-treated group compared to the THC-treated group also lends credence to the thesis that the suppression of the NK cell activity in rats subchronically exposed to THC may be mediated in part by an increase in the endogenous opiate activity. In addition to their distribution in the brain, β-endorphin and methonine enkephalin also occur in the anterior pituitary (28–29). The pituitary is also the primary source of circulating plasma β-endorphin (29). Autoradiographic studies indicate accumulation of THC in selective brain areas and pituitary (30). It is possible that the THC acts directly on the pituitary to increase plasma β-endorphin levels. Recently THC has been shown to bind selectively to mu receptors [(31), also our unpublished observation]. Since lymphocytes have been shown to possess surface opiate receptors (32), it is also quite likely that THC

TABLE II. EFFECT OF CHRONIC Δ^9-THC TREATMENT ON THE NATURAL KILLER CELL ACTIVITY[a]

		Effector: target cell ratio		
Group	*n*	80:1	40:1	20:1
Control	6	47.65 ± 1.4	31.57 ± 1.34	16.29 ± 1.00
THC	6	38.71 ± 0.86**	19.65 ± 1.41**	7.97 ± 0.71**
THC + naloxone	6	44.61 ± 1.72	25.83 ± 1.24*	13.27 ± 0.85*

Note. In rats exposed to a single dose of THC, the control ranges of NK cell activities were 52.02 ± 2.45 (80:1 effector:target ratio); 32.98 ± 1.76 (40:1); 20.75 ± 1.34 (20:1), expressed as specific percentage ^{51}Cr release.
[a] Expressed as specific percentage ^{51}Cr release (mean ± SEM).
* $P < 0.01$ compared to corresponding control and THC groups.
** $P < 0.005$ compared to corresponding control and THC + naloxone groups.

may act directly on the NK cell to suppress its activity.

It is interesting to note that the NK cell activity of the naloxone-THC-treated animals was significantly lower at 40:1 and 20:1 effector:target cell ratios than that of the control group of animals (Table II). Two possibilities should be considered here to explain this phenomenon. Due to the relatively long half-life of THC, the dose used and the frequency of naloxone injection may have been suboptimal to counter the effects of THC completely. Alternatively, because of its lipophilic nature, THC may gain entry into the NK cell and directly suppress its activity by interfering with the intracellular metabolic processes. Two lines of evidence support the latter possibility. First, there is substantial concentration of THC in the spleen after administration of the drug. At 1 hr postadministration, THC concentration in the spleen is higher than any other tissue except fat (33). Second, interaction of THC with membrane transport enzymes has been shown to impair macromolecular synthesis and replication of lymphocytes (34, 35). In light of the available evidence, the inhibitory effects of subchronic exposure to THC on NK cell activity are most likely due to interactions of direct actions of THC on lymphocytes and indirect influences of hormones such as β-endorphin.

The decreased plasma NE and E levels in subchronically THC-treated rats indicates a suppressed sympatho-adrenomedullary system in these animals. It is significant to note that although the catecholamines (11) and corticosterone (9, 10) have been shown to be immunosuppressive, the decrease in NK cell activity was not accomplished by elevated plasma catecholamine and corticosterone levels in the subchronically THC-treated animals. Our previous work (unpublished) indicates that subchronic THC exposure generally results in low levels of plasma corticosterone levels, irrespective of the time of sampling after the last injection. It is possible that subchronic THC treatment induces tolerance in the pituitary–adrenal axis.

In contrast to previous reports (16, 17, 36), we failed to demonstrate elevated corticosterone levels after an acute injection of THC. Recently, we observed a significant increase in plasma corticosterone levels at 4 hr but not at 16 hr after a single THC injection. Although the time of blood collection after the single THC injection appears to determine the corticosterone levels, other factors may also be involved. Perhaps, the existence of a 24-hr circadian corticosterone rhythm may have masked the effects of the THC injection.

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ERRATUM

Volume **179,** Number 4 (1985), in the article "Prolactin Responses to Chronic Exercise in Males," by F. L. Kaufman, D. E. Mills, R. L. Hughson, and G. T. Peake, pages 546–548: On page 547, left column, the last two lines of the second paragraph under Results were inadvertently omitted. For the readers' convenience the complete paragraph is reprinted below.

RESULTS

Pre-exercise plasma PRL levels on day 1 were within the normal range reported for healthy men (12) and did not change significantly from this value on either days 5 or 10 of exercise.

Acute exercise on day 1 significantly increased plasma PRL (Figure 1) from 8.6 ± 1.1 ng/ml to 14.1 ± 3.0 ng/ml ($p<0.025$). However, chronic exercise at a similar workload failed to elicit PRL increases on subsequent test days, so that the day 1 PRL response was significantly greater than those observed on days 5 and 10 ($p<0.03$).