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The Effects of Δ^9-Tetrahydrocannabinol on Serum Thyrotropin Levels in the Rat

CECILIA J. HILLARD, NEIL E. FARBER,
THAD C. HAGEN* AND ALAN S. BLOOM

*Departments of Pharmacology and Toxicology and *Medicine, Medical College of Wisconsin
P.O. Box 26509, Milwaukee, WI 53226
and *Department of Medicine, Wood V.A. Medical Center, Wood, WI 53193*

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HILLARD, C. J., N. E. FARBER, T. C. HAGEN AND A. S. BLOOM. *The effects of Δ^9-tetrahydrocannabinol on serum thyrotropin levels in the rat.* PHARMACOL BIOCHEM BEHAV **20**(4)547–550, 1984.—The effects of acute treatment with Δ^9-tetrahydrocannabinol (Δ^9-THC) on serum levels of thyrotropin (TSH) and the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) were determined in the rat. Intraperitoneal doses of Δ^9-THC greater than 3 mg/kg reduced serum TSH levels to less than 10% of control. The ED50 for Δ^9-THC was approximately 0.3 mg/kg. After a 10 mg/kg dose of Δ^9-THC, the maximum decrease in serum TSH occurred at one hour. Both serum T_3 and serum T_4 levels were decreased by a single 10 mg/kg Δ^9-THC injection with maximal decreases at 6 hr post-injection. The effects of Δ^9-THC on the ability of thyrotropin releasing hormone (TRH) to increase serum TSH and T_3 were determined. TRH produced a 10-fold increase in serum TSH levels and this increase was unaffected by Δ^9-THC pretreatment. Serum T_3 levels were slightly increased by TRH and this increase was also unaffected by Δ^9-THC. These findings indicate that acute treatment with Δ^9-THC results in a decrease in circulating TSH, T_3 and T_4 levels but has no effect on the pituitary or thyroid response to exogenous TRH.

Δ^9-Tetrahydrocannabinol Serum thyrotropin Serum triiodothyronine Serum thyroxine

THE interactions of marihuana and its principal psychoactive constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC) with neuroendocrine systems have received considerable scientific and social scrutiny. Interference with normal endocrine function is one of the risks to public health posed by marihuana abuse. This risk is potentiated by the demographics of marihuana use. A significant portion of the teenaged population are using marihuana during puberty and during the primary reproductive period when disturbances in the endocrine systems could have important consequences [11]. These concerns have stimulated investigations of the effects of marihuana and Δ^9-THC on endocrine function and regulation.

In 1965, Miras [8] first reported that the administration of cannabis resin to rats depressed the uptake of radioactive iodine into the thyroid gland. Lomax [7] confirmed the inhibitory effect of marihuana on thyroid function and tenatively identified the hypothalamus as the site of action. In that study, injection of a crude marihuana extract containing 25 mg/kg Δ^9-THC inhibited the release of radioiodine from the thyroid gland in rats. This inhibition could be reversed by the administration of thyroid stimulating hormone (TSH), suggesting that a decrease in circulating TSH levels was the primary cause of the inhibition. Furthermore, bilateral electrolytic lesions in the region of the medial mammillary nuclei of the hypothalamus abolished the decrease in thyroid function while lesions in rostral areas of the hypothalamus had no effect on marihuana-induced thyroid inhibition. These findings suggest that marihuana acts at the caudal hypothalamus to either reduce thyrotropin-releasing hormone (TRH) release or to stimulate the release of an inhibitor of pituitary TSH release.

The studies described here were carried out to test the hypothesis that Δ^9-THC inhibits thyroid function by primarily reducing circulating TSH levels. Furthermore, investigations were begun to determine the site of Δ^9-THC action by assessing the effects of Δ^9-THC on circulating T_3 and T_4 levels and on thyrotropin releasing hormone (TRH) stimulation of TSH release.

METHOD

Drugs

Δ^9-Tetrahydrocannabinol (Δ^9-THC) was generously supplied by the National Institute of Drug Abuse. Δ^9-THC was administered in an Emulphor (GAF, New York, NY) -ethanol vehicle [3]. The stock Δ^9-THC solution (200 mg/ml in 100% ethanol) was added to an equal volume of Emulphor and the suspension was diluted to the desired concentration with saline.

Thyrotropin-releasing hormone (TRH) was obtained as an injectable solution (Thypinone®, Abbott Laboratories, North Chicago, IL) and was diluted to the desired concentration with saline.

Experimental

Male Sprague-Dawley outbred albino rats (King Animal Labs, Madison, WI) weighing 180–250 g were used in these studies. Before the experimental period, the rats were

housed 7–9 in a cage and had continuous access to food and water.

Δ^9-THC or equivalent Emulphor vehicle were administered by intraperitoneal injection in 0.1 ml saline/100 g body weight. In the TRH-challenge experiments, rats were injected intravenously with 0.1 ml of TRH or saline through a tail vein one half hour before sacrifice. All rats were sacrificed by decapitation between 1400 and 1600 hr, trunk blood was collected and placed on ice. Serum was separated and stored at −17°C until assay. Control (untreated) rats were sacrificed as quickly as possible at various times throughout the experimental session.

Radioimmunoassays

TSH radioimmunoassay. Serum TSH was determined using materials distributed by NIAMDD consisting of rabbit anti-rat TSH serum-5, rat TSH-I-1 and standard rat TSH-RP-1 (0.22 U/mg). Rat TSH-I-1 was iodinated using a chloramine T method, iodinated TSH was separated using Sephadex G-75. The assay was carried out in 0.01 M phosphate-buffered saline (pH 7.6) containing 0.01% thimersol, 1% bovine serum albumin and 0.025 M EDTA. A second antibody, goat antirabbit IgG in normal rabbit serum, was used to separate bound and free ^{125}I-TSH. All incubations were carried out at room temperature. Twenty-four hours following addition of the second antibody, samples were centrifuged at 3,000 rpm, the supernatant was aspirated and pellets were counted. Standards and samples were assayed in triplicate. The sensitivity of the assay was 4 ng/tube. Neither Δ^9-THC nor vehicle had any effect on the TSH assay.

Triiodothyronine (T_3) and thyroxine (T_4) radioimmunoassay. Total serum T_3 and total serum T_4 levels were measured using commercial assay kits (Diagnostic Products, Los Angeles, CA). Samples and standards were assayed in duplicate. T_3 assay sensitivity was 0.6 ng/tube and T_4 assay sensitivity was 75 ng/tube.

Data Analysis

Serum TSH data from the Δ^9-THC dose response study were analyzed using one-way analysis of variance. Comparisons between the control group (untreated animals) and each treatment group were made using Dunnett's modification of the *t*-test [17]. Emulphor vehicle concentrations equivalent to that administered with 0.1, 1.0, 3.0, 10.0 and 30.0 mg/kg Δ^9-THC were found to have no overall effect on serum TSH ($F=0.3$, $p=0.7$). Therefore, the pooled data has been reported as a single vehicle treatment and used as the vehicle group in subsequent analyses. Serum TSH, serum T_3 and serum T_4 data from time course study were analyzed using two-way analysis of variance followed by Duncan's Multiple Range test to make comparisons between vehicle and Δ^9-THC treated animals at each time point [5]. Serum TSH data from the TRH-challenge study were analyzed using two-way analysis of variance followed by Dunnett's modification of the *t*-test [17].

RESULTS

The effects of one hour pretreatment with various doses of Δ^9-THC on basal serum TSH levels were determined (Fig. 1). Δ^9-THC produced a dose-related decrease in serum TSH levels with statistically significant reductions at doses greater than 3.0 mg/kg. Serum TSH levels in animals receiving 0.3 mg/kg Δ^9-THC were reduced to 50% of control while levels in animals receiving doses of 3.0 mg/kg or greater were less than 10% of control. Emulphor vehicle alone had no effect on serum TSH levels at doses equivalent to those administered with Δ^9-THC.

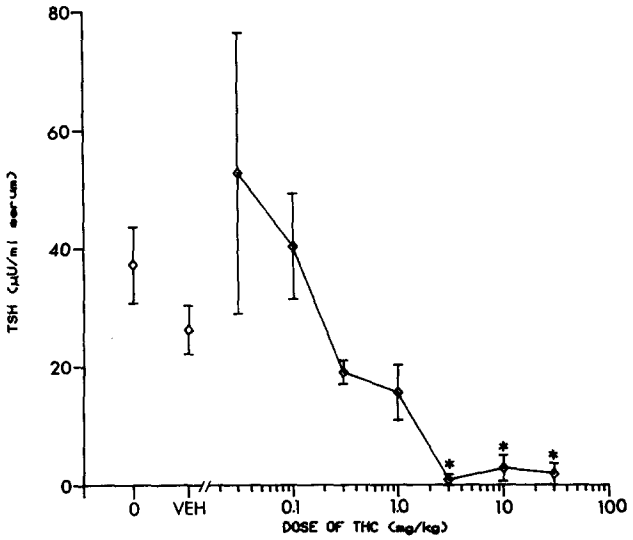

FIG. 1. Effects of Δ^9-THC on basal serum TSH levels. Δ^9-THC or vehicle were administered by IP injection one hour before sacrifice. Each Δ^9-THC dose is the mean of 6 animals, untreated control ("0") is the mean of 12 animals, vehicle ("VEH") is the mean of 30 animals and represents pooled data from five emulphor vehicle doses corresponding to 30, 10, 3, 1.0 and 0.1 mg/kg Δ^9-THC. Each mean is shown ±S.E.M. *$p<0.001$ compared to untreated control.

The time course of the effect of a single injection of 10 mg/kg Δ^9-THC on serum TSH was determined (Fig. 2). Serum TSH levels were lower in Δ^9-THC treated animals than in vehicle treated animals with statistically significant reductions at 0.25, 0.5, 1.0 and 3.0 hr. The maximal decrease in serum TSH levels occurred one hour following Δ^9-THC administration. Although not statistically significant, serum TSH levels were still lower in Δ^9-THC treated animals 24 hours following Δ^9-THC injection. The vehicle treated animals demonstrated a slight but statistically insignificant decrease in serum TSH levels after injection that reached a nadir at one hour and returned to control levels by 3 hours following injection.

Serum thyroid hormones were also measured. Serum T_3 levels were significantly reduced at 3 and 6 hours following Δ^9-THC administration (Fig. 3). Serum T_4 levels were also reduced following Δ^9-THC administration with a statistically significant reduction seen 6 hours after Δ^9-THC administration (Fig. 4). Emulphor vehicle had no effect on either serum T_3 or serum T_4 levels at any time.

The effects of Δ^9-THC on the TSH and T_3 response to exogenous TRH were determined (Table 1). Rats were pretreated with either Δ^9-THC or vehicle then injected with either TRH or saline. Consistent with the results reported in Fig. 1, Δ^9-THC produced a significant reduction in serum TSH in animals injected with saline. However, there was no difference in the TSH response to TRH between animals pretreated with vehicle and animals pretreated with Δ^9-THC. Similarly, Δ^9-THC had no effect on the TRH-induced increase in serum T_3 levels.

DISCUSSION

We have demonstrated that acute treatment of rats with

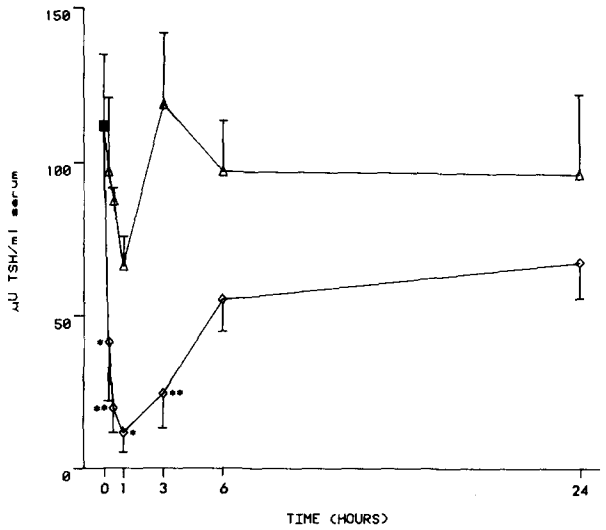

FIG. 2. Time course of the effect of Δ⁹-THC on basal serum TSH levels. Ten mg/kg Δ⁹-THC (◇) or equivalent vehicle (△) were administered by IP injection. TSH level in untreated control animals is shown at time 0 (■). Each point is the mean of 5–6 animals, vertical lines represent S.E.M. *$p<0.05$ compared to vehicle treated animals at the same time point. **$p<0.001$ compared to vehicle treated animals at the same time point.

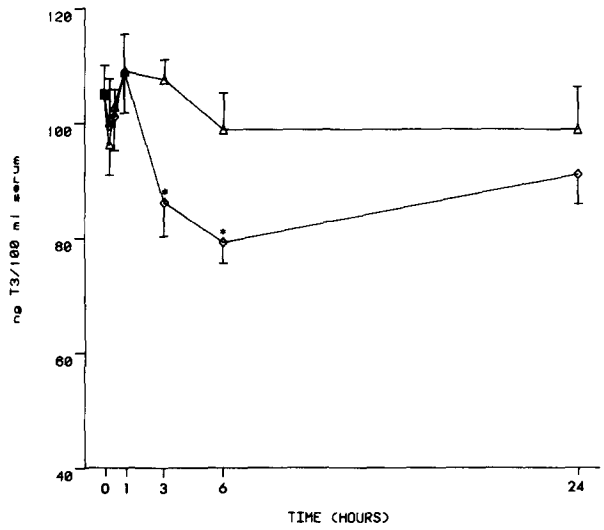

FIG. 3. Time course of the effect of Δ⁹-THC on serum T_3 levels. Ten mg/kg Δ⁹-THC (◇) or equivalent vehicle (△) were administered by IP injection. Serum T_3 level in untreated control animals is shown at time 0 (■). Each point is the mean of 5–6 animals, vertical lines represent S.E.M. *$p<0.05$ compared to vehicle treated animals at the same time point.

Δ⁹-THC produces a dose-related decrease in circulating TSH levels. TSH levels are reduced as soon as 15 minutes after Δ⁹-THC injection and remain depressed for at least three hours after treatment. The Δ⁹-THC-induced decrease in serum TSH levels is followed temporally by a decrease in serum T_3 and serum T_4 levels. These findings provide direct evidence that the inhibition of thyroid function by Δ⁹-THC seen by earlier investigators was a result of a decrease in circulating TSH levels. The studies presented here also confirm and extend the findings of Nazar and coworkers who reported a decrease in serum thyroxine 6 hours after Δ⁹-THC injection [10]. Rosenkrantz and Esber [14] also found that serum thyroid hormones were still reduced after 14 days of treatment with marihuana smoke or Δ⁹-THC.

Throughout these studies, we have seen a consistent although not statistically significant decrease in TSH levels at one hour after vehicle injection. TSH levels return to control levels by 3 hours after vehicle injection and no changes in serum T_3 or serum T_4 levels are seen. Similar decreases in circulating TSH levels have been reported after restraint [9] and injection [4] stresses. Since the degree and duration of the depression of TSH levels after Δ⁹-THC is greater than after vehicle alone, it is unlikely that Δ⁹-THC is acting as a nonspecific stressor.

In the present study, TRH was found to stimulate TSH release from the pituitary and increase serum TSH levels one-half hour following treatment. In response to the increased TSH levels, serum T_3 levels were also slightly elevated in rats treated with TRH, although the peak increase in serum T_3 levels had probably not occurred at one-half hour after TRH injection. Pretreatment of the rats with Δ⁹-THC had no effect on either the TSH or T_3 responses to TRH. These findings suggest that neither the ability of the pituitary to respond to TRH nor the responsiveness of the thyroid to TSH are directly affected by Δ⁹-THC. These results give some insights into the possible sites of Δ⁹-THC inhibition of

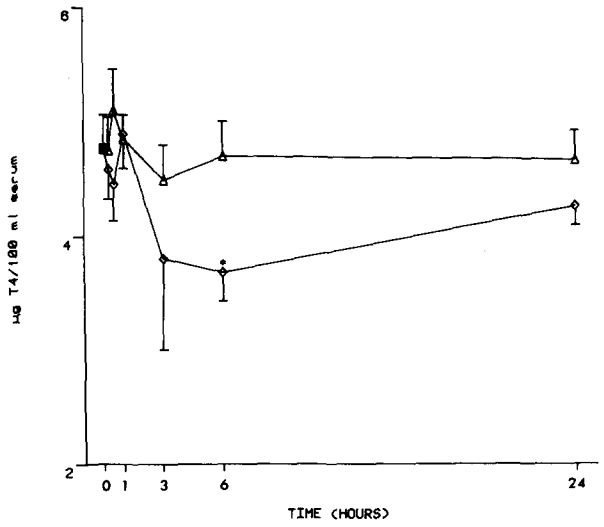

FIG. 4. Time course of the effect of Δ⁹-THC on serum T_4 levels. Ten mg/kg Δ⁹-THC (◇) or equivalent vehicle (△) were administered by IP injection. Serum T_4 level in untreated control animals is shown at time 0 (■). Each point is the mean of 5–6 animals, vertical lines represent S.E.M. *$p<0.05$ compared to vehicle treated animals at the same time point.

TSH release. Since circulating T_3 and T_4 levels are not increased at any time after Δ⁹-THC, Δ⁹-THC is probably not acting to increase the inhibitory feedback of the thyroid hormones on TSH release. Secondly, since the TSH response to exogenous TRH is unaffected by Δ⁹-THC, it is unlikely that Δ⁹-THC acts at the pituitary to block the action of TRH. The simplest explanation is Δ⁹-THC inhibition of TRH release, either directly or through one of the neurotransmitter systems which control TRH release. However,

TABLE 1

EFFECT OF Δ^9-THC ON TRH-INDUCED INCREASES IN SERUM TSH AND T_3 LEVELS

	Serum TSH (μU/ml)		Serum T_3 (ng/100 ml)	
Treatment†	Vehicle‡	Δ^9-THC§	Vehicle	Δ^9-THC
Saline	32.1 (6.6)¶	7.0* (2.2)	89.8 (10.7)	87.0 (10.8)
TRH (5 μg)	275.5 (19.8)	232.3 (22.0)	106.6 (17.1)	100.7 (13.2)

*$p<0.01$ compared to saline/vehicle group.

†Saline or TRH were administered by IV injection 0.5 hr before sacrifice.

‡Vehicle equivalent to that administered with 10 mg/kg Δ^9-THC was administered by IP injection one hr before sarifice.

§10 mg/kg Δ^9-THC was administered by IP injection one hour before sacrifice.

¶S.E.M.

effects of Δ^9-THC on inhibitory inputs to the pituitary cannot be eliminated.

Δ^9-THC has been shown to have effects on several other pituitary hormones. Δ^9-THC has been found to produce lowered plasma gonadotropin levels and to decrease ovarian and testicular weights [11]. It has been suggested that the effects of Δ^9-THC on the reproductive system are indirect and due to a decrease in hypothalamic LHRH release [16]. Plasma prolactin levels are also reduced by Δ^9-THC. The decrease in prolactin has been shown to be reversed by both cyproheptadine [6] and by TRH [15]. These findings suggest a hypothalamic effect of Δ^9-THC, perhaps a reduction in the release of a prolactin releasing factor. Therefore, there is evidence in other endocrine systems for an inhibitory influence of Δ^9-THC on the release of pituitary hormones. Furthermore, it appears that the site of these inhibitory effects is at the level of the hypothalamus.

TRH is widely distributed throughout the brain and, in addition to its endocrinological effects, it has been shown to be involved in many other biological processes. For example, TRH potentiates the behavioral excitation induced by administration of pargyline and l-dopa in mice [12] and has been found to antagonize pentobarbital sedation and hypothermia [13]. Both of these effects are independent of the ability of TRH to release TSH or prolactin. Recent studies have demonstrated that TRH, injected intracerebrally, antagonizes Δ^9-THC-induced hypothermia [1] and Δ^9-THC-induced sedation and analgesia [2] at TRH doses that have no effect on body temperature. Although the neuroendocrine and neurotransmitter functions of TRH are anatomically and functionally distinct, there may be similarities between these neuronal systems, including an antagonistic influence by Δ^9-THC.

These preliminary studies demonstrate that Δ^9-THC reduces basal serum TSH levels in rats which is followed in time by a decrease in serum T_3 and serum T_4. However, Δ^9-THC has no effect on the TSH response to exogenous TRH. These findings suggest that Δ^9-THC acts either to potentiate an inhibitory input to the pituitary thyrotropes or to inhibit TRH release.

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