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ENDOCRINE EFFECTS OF CHRONIC ADMINISTRATION OF PSYCHOACTIVE DRUGS TO PREPUBERAL MALE RATS. I: Δ^9-TETRAHYDROCANNABINOL

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Summary

The endocrine effects of the chronic administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) to prepuberal animals were studied by injecting intraperitoneally three times a week for a month either 1 mg/kg or 10 mg/kg of the psychoactive drug or the vehicle to groups of Sprague-Dawley male rats starting at 21 days of age. At the end of the experiment the animals were autopsied in the morning, 30 to 45 min. after the last injection. Animals injected with 10 mg/kg had smaller tails and prostates than controls. Plasma levels of growth hormone (GH) and luteinizing hormone (LH) were decreased in both groups of experimental rats, while pituitary levels remained unmodified. Plasma levels of follicle stimulating hormone (FSH) were not affected by the drug. Brain levels of noradrenaline (NA), dopamine (DA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were assayed in the 10 mg/kg experimental group and found comparable to those of the control group. These data indicate that Δ^9-THC, the psychoactive ingredient of marihuana, when administered chronically to developing animals can inhibit the secretion of GH and LH and suppress prostate growth.

Since the recent identification of Δ^9-tetrahydrocannabinol (Δ^9-THC) as the psychoactive ingredient of marihuana (1), a few reports have appeared on the endocrine effects of its acute administration to animals (2,4). Very little is however known about the endocrine effects of its chronic, long term administration to developing animals. The present work was undertaken to study the endocrine effects of long term administration of Δ^9-THC to prepuberal male rats.

Materials and Methods

Experiment 1: Groups of 21-day old male Sprague-Dawley rats were injected intraperitoneally three times a week for a month with either 1 mg/kg of Δ^9-THC or with vehicle (40% propylene glycol and 60% rat serum: volume of injection 1 ml/kg). An intermittent schedule of administration was chosen in order to prevent the development of tolerance. Δ^9-THC was obtained from the Dept. of National Health and Welfare, Ottawa, Canada (lots No. 69961 and 75518). The animals had free access to water and rat pellets, but the amount of food ingested was recorded daily. The housing quarters had a constant temperature of 22°C and a light-dark cycle of 14-10 hr. starting at 0500. At the end of the experiment the rats were sacrificed by decapitation between 1000 and 1200 hr. in order to obviate to the diurnal variations of plasma growth hormone (GH) levels (5). Rats were decapitated 30 to 45 min. after the last injection since peak changes in monoamines and hormone levels are reported to occur at that moment. Handling of the animals was limited to a minimum and decapitations were performed in a room separate from living quarters within 2 min. after removal from the cages. Body weight, tail lenght and the weights of brain, anterior pituitary, adrenals, testes, prostate and seminal vesicles were recorded. Trunk blood was collected in heparinized tubes. Plasmas were then separated and stored at -20°C until determination of : GH levels with the radioimmunoassay method of Schalch and Reichlin (6); luteinizing hormone (LH) levels with the radioimmunoassay method of Niswender et al. (7) using NIAMD-Rat-LH-RP1 as standard preparation; and follicle stimulating hormone (FSH) levels with the radioimmunoassay method of Parlow et al. (8), using NIAMD-Rat-FSH-RP1 as standard preparation. Anterior pituitaries were homogenized in 5 ml of saline and the supernatant kept at -20°C until determination of GH, LH and FSH levels. All hormone determinations were performed in single batches.

Experiment 2: In this experiment, which was performed 2 months later, the protocol of Experiment 1 was followed with some modifications. The

amount of Δ^9-THC injected was increased to 10 mg/kg. This dose was chosen since, according to Holtzman et al. (16), it can increase 5-HT and decrease NA brain levels, while the lower dose (1 mg/kg) is ineffective. At the end of the experiment the brains were stored at -20°C for no more than two weeks until determination of the levels of serotonin (5-HT), noradrenaline (NA), dopamine (DA) and 5-hydroxyindoleacetic acid (5-HIAA). This was performed in the same sample, after extraction in acidified butanol (9), by the method of Miller et al. (10) for 5-HT and 5-HIAA and by the method of Ansell and Beeson (9) for NA and DA.

Fluorescence values were read in an Aminco-Bowman spectrophotofluorometer.

Statistical analysis. Statistical evaluation of the data was performed with the Student "t" test.

Results

The administration of 1 or 10 mg/kg of Δ^9-THC three times a week did not significantly modify food intake. The effects on body weight, tail length and organ weights are shown in Table 1. The only statistical significant modifications were a decrease in tail length and prostate weight following the administration of 10 mg/kg of Δ^9-THC. However, a small decrease in prostate weight occurred with 1 mg/kg. As shown in figure 1, plasma levels of GH were decreased in both groups of experimental animals; however, due to a very large spread of values, statistical significance was reached only in animals treated with 1 mg/kg. No modification of pituitary levels of GH was observed.

Plasma levels of LH were also significantly reduced in both groups of experimental animals as shown in figure 2. Pituitary levels of LH increased slightly in treated rats. Plasma levels and pituitary content of FSH were not modified in treated animals, but a significant decrease in pituitary

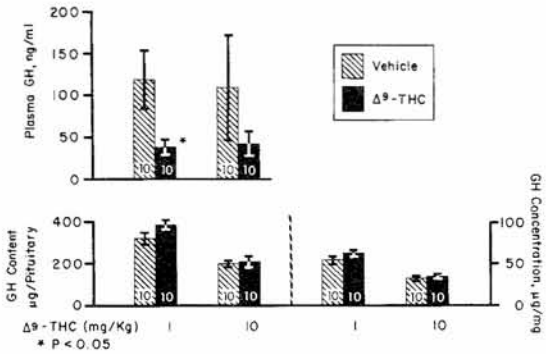
TABLE 1

Body Weight, Tail Length and Endocrine Organ Weights of Male Rats Injected Intraperitoneally Three Times a Week for a Month with Δ^9-tetrahydrocannabinol (Δ^9-THC) or vehicle. Treatment Started at 21 days of Age.

GROUPS[1]	BODY	TAIL	PITUITARY	BRAIN	ADRENALS	PROSTATE	SEMINAL VESICLES	TESTES
	g	cm			mg			
Δ^9-THC (10) 1 mg/kg	197 ± 3[2]	17.0 ± 0.2	6.2 ± 0.2	1300 ± 20	35.8 ± 2.2	170 ± 10	196 ± 11	2310 ± 70
Vehicle (10)	212 ± 3	16.9 ± 0.3	5.8 ± 0.2	1350 ± 30	39.2 ± 1.8	221 ± 28	208 ± 22	2460 ± 60
Δ^9-THC (10) 10 mg/kg	224 ± 4	17.0 ± 2.2[3]	6.9 ± 0.2	1280 ± 20	38.9 ± 1.6	153 ± 7[3]	228 ± 15	2320 ± 40
Vehicle (10)	221 ± 5	17.8 ± 0.2	7.1 ± 0.2	1310 ± 10	41.4 ± 1.9	184 ± 9	191 ± 11	2410 ± 80

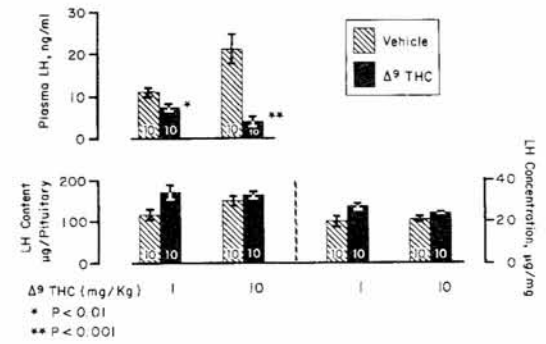
1) Number of rats in parentheses
2) Values are means ± SE
3) $P < 0.02$ versus vehicle

FIGURE 1



Effect of Δ^9-THC on plasma and pituitary GH levels.

FIGURE 2



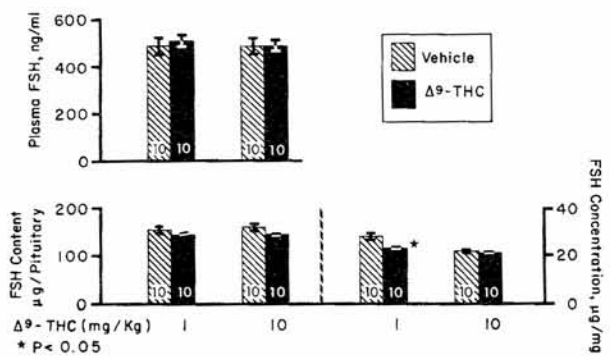
Effect of Δ^9-THC on plasma and pituitary LH levels.

concentration was observed with 1 mg/kg (Fig. 3).

Plasma and pituitary levels of the two gonadotropins and of GH in the controls were not different from those found in adult male rats injected with saline (unpublished data). This excludes the possibility that the effects observed in the experimental animals were due to diluent, 40% propylene glycol, a CNS depressant, acting synergistically with Δ^9-THC. As shown in Table 2, no statistically significant modification of brain

monoamine content was observed in the group of rats treated with 10 mg/kg. The values of 5-HIAA, 5-HT, NA and DA were similar to the values we have observed at that time of the day in normal, untreated (5) or in saline injected adult male rats (unpublished data).

FIGURE 3



Effect of Δ^9-THC on plasma and pituitary FSH levels.

TABLE 2

Brain content of 5-hydroxyindoleacetic acid (5-HIAA), serotonin (5-HT), noradrenaline (NA) and dopamine (DA) in male rats injected intraperitoneally three times a week for a month with 10 mg/kg of Δ^9-tetrahydrocannabinol (Δ^9-THC) or vehicle. Treatment started at 21 days of age.

GROUPS[1]		5-HIAA	5-HT	NA	DA
		ng/g			
Δ^9-THC	(10)	568 ± 49[2]	607 ± 38	462 ± 26	1016 ± 97
Vehicle	(10)	505 ± 28	633 ± 58	483 ± 19	964 ± 45

1) Number of rats in parentheses.
2) Values are means ± SE.

Discussion

The chronic administration of Δ^9-THC, the psychoactive principle of marihuana (1), to prepuberal male rats had definite deleterious effects on some endocrine functions. Plasma levels of GH and LH were reduced by both low and high dosage of the drug. This appears to be due to an inhibition of the secretion, since pituitary levels of both hormones increased slightly. An inhibition of GH secretion has also been observed by Kokka and Garcia 30 to 120 min. after the administration of 5-20 ug of Δ^9-THC to adult male rats (11). A decrease in circulating LH levels, 45 min. following the acute intravenous administration of 5 mg/kg of Δ^9-THC, has also been observed by Marks in ovariectomized rats (3). Niz et al. have reported that Δ^9-THC blocks the cyclic surge of LH and ovulation in normal rats (4).

Growth parameters were not strikingly modified by Δ^9-THC, and only the tail length was decreased in the 10 mg/kg group. This is in contrast with reports by Manning et al. (12) and by Sjöden et al. (13), who observed a decrease in body weight. These authors, however, administered the drug daily for respectively 30 and 19 days, and observed a decrease in food intake. It is thus likely that decreased protein intake was responsible for the abnormal body growth, although a daily inhibition of GH secretion may have contributed to this effect. Mean food intake was not significantly modified in our experimental animals, and GH secretion was suppressed only intermittently. This can explain the very modest inhibition of growth even in our animals treated with the highest dosage of Δ^9-THC.

Prostate, an LH dependent gland (14), was slightly smaller in the 1 mg/kg experimental group, and significantly reduced in the 10 mg/kg group, while seminal vesicles, which are also LH dependent structures, remained unmodified in both groups. This discrepancy could be due to a greater sensitivity of the prostate to the intermittent inhibition of LH secretion. Testis weight, a good index of FSH secretion, was not modified by Δ^9-THC. This is an

indirect confirmation of the selective effect of the drug on pituitary gonadotropins. Another hypothesis can be advanced to explain the lack of effect of Δ^9-THC on FSH secretion. In effect, this could be due to development of tolerance, specifically to the inhibitory effect of Δ^9-THC on FSH, in spite of the intermittent administration of the drug. Ling et al. (15) have not observed any gonadal modification in their treated animals; these authors, however, administered the drug for only four days. Dixit et al. have recently reported that daily administration of cannabis extract produced a complete arrest of spermatogenesis in mice (16).

The mechanism of the psychoactive action of Δ^9-THC is still unknown, but some authors have suggested that it is exerted through a modification of the metabolism of brain biogenic amines, although the results are rather contradictory (17,19). This is very interesting in view of the role that biogenic amines play in the control of anterior pituitary hormone secretion (20). We could find no modification of brain monoamines levels in our experimental animals, and our data are thus in accord with those of Gallagher et al. (21) and of Leonard (22). The turnover of 5-HT seems also to be unaffected since the brain level of its metabolite, 5-HIAA, was found to be normal. However, subtle or transient modifications of monoamine levels and/or turnover in specific brain areas such as hypothalamus, amygdala or hippocampus may have been missed. Since dose-response studies were not performed it is also possible that the dose utilized was inadequate to produce significant modifications in developing animals.

The recent observation that Δ^9-THC can activate the pituitary-adrenal system (2, Kokka and Garcia, personal communication) allows another hypothesis on the mechanism of the inhibitory effect of this drug on GH secretion. It is in effect well established the stress inhibits rat GH secretion (6,23). An inverse relationship between ACTH and GH secretions has also been postulated by Kokka et al. in the rat (24).

Although no data can be found in the literature on the endocrine effects

of chronic marihuana intoxication in prepuberal human subjects, a recent paper by Kolodny et al. (25) has shown that the gonadal function can be modified also in humans possibly through inhibition of gonadotropin secretion.

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