Pharmacology Biochemistry & Behavior, Vol. 37, pp. 299–302. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Effects of Psychoactive and Nonpsychoactive Cannabinoids on the Hypothalamic-Pituitary Axis of the Adult Male Rat[1]

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Received 23 April 1990

STEGER, R. W., L. L. MURPHY, A. BARTKE AND M. S. SMITH. *Effects of psychoactive and nonpsychoactive cannabinoids on the hypothalamic-pituitary axis of the adult male rat.* PHARMACOL BIOCHEM BEHAV **37**(2) 299–302, 1990.—The acute dose-response effects of delta-9-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) on gonadotropin and testosterone (T) secretion and on hypothalamic norepinephrine (NE) metabolism were tested in adult male rats. THC and CBN both produced an acute suppression of plasma-luteinizing hormone (LH) and T levels and median eminence NE turnover although a dose-response relationship could not be demonstrated. CBD had no significant effect on any of these parameters and none of these cannabinoids had any effect on plasma follicle-stimulating hormone levels or median eminence LH-releasing hormone (LHRH) content. Except for the highest dose of CBN, none of the in vivo cannabinoid treatments significantly altered in vitro LH secretion although there was a trend towards decreased LH secretion. These results suggest that the decrease in LH secretion in THC- or CBN-treated rats is due to reductions in NE stimulation of LHRH release rather than to changes in LHRH synthesis or pituitary LHRH response.

Tetrahydrocannabinol Cannabinol Cannabidiol Luteinizing hormone Testosterone Norepinephrine Hypothalamus Pituitary

MARIHUANA, and its major psychoactive constituent, delta-9-tetrahydrocannabinol (THC), can alter endocrine and gonadal function in a number of species (2, 3, 7, 12, 17, 21, 23, 25). Administration of THC can suppress circulating testosterone (T) levels in male mice and rats (5,6). This appears to be due to inhibition of pituitary gonadotropin secretion (2, 6, 7, 12) and a direct inhibitory effect on testicular steroidogenesis (4–6, 9). The effects of THC to inhibit LH release are probably not due to direct actions of THC on the pituitary since in vitro LH secretion and the pituitary response to LHRH stimulation are not inhibited by THC treatment (2, 8, 15, 17, 21). Likewise, THC does not inhibit basal or norepinephrine (NE)-stimulated LHRH release in vitro in adult male mice (8), although it does inhibit hypothalamic NE metabolism in both adult mice and rats [(21,23); Steger, unpublished observations]. Thus, THC-induced inhibition of pituitary LH secretion appears to be the result of reduced LHRH secretion which may be secondary to reduced hypothalamic noradrenergic activity.

Much less is known about the effects of cannabinol (CBN) and cannabidiol (CBD) on endocrine and gonadal function even though these compounds make up a significant proportion of the cannabinoids in extracts of marihuana (24). Both CBN and CBD inhibit in vitro T production by mouse testicular tissue (5) and chronic treatment of male mice with CBN can lead to suppression of plasma LH, FSH and T levels (3). In the male rat, low doses (0.5 mg/kg) of CBN or CBD did not affect LH levels but did alter the time course of THC action to suppress plasma LH levels (14).

The present experiment was designed to determine the acute effects of THC, CBN and CBD on the hypothalamic-pituitary-gonadal axis of adult male rats. We have examined the effects of a wide range of cannabinoid doses on plasma LH, FSH and T levels and on hypothalamic LHRH content and norepinephrine metabolism. We have also examined the effects of these in vivo treatments on pituitary function in vitro.

METHOD

Adult male Sprague-Dawley rats purchased from Harlan

[1]Supported by NIDA grants DA 03875 and DA 05452 and NIH grant HD 14643.

Sprague-Dawley (Indianapolis, IN) were housed under constant environmental conditions (12-hr light/12-hr dark; 23 ± 1°C) with food and water provided ad lib. Rats received a single oral dose of either THC, CBN or CBD at 0.1, 1.0 and 10.0 mg/kg for each drug or the sesame oil vehicle. The cannabinoids were administered in a volume of 0.1 ml.

One hour after cannabinoid administration, animals were sacrificed by decapitation and trunk blood was collected. This time was chosen from a previous study demonstrating the time course of THC action on plasma LH levels (14). One hour prior to sacrifice and immediately following cannabinoid administration, half of the rats were injected with alpha methyl-p-tyrosine, a tyrosine hydroxylase inhibitor (aMPT; 250 mg/kg IP) for determination of norepinephrine turnover rates in the median eminence. At the time of autopsy, the brain was quickly removed and the median eminence was dissected free and frozen (21).

Prior to assay, the ME were sonicated in 0.1 M $HClO_4$ containing the internal standard for the catecholamine assay (dihydroxybenzylamine) and 1 mM sodium bisulfite. Median eminence supernatants were separated by high performance liquid chromatography (HPLC) and quantitated by electrochemical detection as previously described (21). Catecholamine turnover rates were estimated using the formula $K = k[CA]_0$ where $[CA]_0$ equals the mean catecholamine concentration at zero time (uninjected controls), and the rate constant, k, represents the $-\log$ of the slope of the line describing the decline of NE or DA concentration during the one hour following the blockade of tyrosine hydroxylase with aMPT (21).

Pituitaries were removed at autopsy from the non-aMPT-treated rats and the posterior lobe separated and discarded. The anterior pituitaries were hemisected and one hemipituitary from each rat was placed in a separate 12 × 75 mm glass culture tubes. The other hemipituitary was used in a separate experiment that will not be reported here. One ml of Medium 199 (M199) + bicarbonate (pH 7.4) was added and the glands were preincubated at 37°C in a Dubnoff Metabolic Incubator. The tubes were maintained in an atmosphere of 5% CO_2:95% O_2. After 60 min, fresh M199 was added and the pituitaries were incubated for an additional 60 min for the determination of basal LH release. At this time the medium was removed and saved and M199 containing 10^{-8} M LHRH was added. Tubes were incubated for 60 additional minutes at which time all media were frozen and the hemipituitaries were removed, weighed and then sonicated in 1 ml of saline for subsequent measurements of LH.

Plasma levels of LH, FSH and T and media levels of LH were measured by RIA as described previously (18,19) and expressed in terms of ng of LH-RP2 and FSH-RP1 reference preparations. Content of LHRH was measured in aliquots of the ME homogenate as previously described (19). All samples for a specific hormone were assayed in the same assay.

The effects of drug treatment and dose on hormone levels and NE turnover were evaluated using 2-way and 1-way analysis of variance. Student-Neuman-Keul's tests were used to determine which mean values were significantly different. Mean values between groups were considered significantly different when the p value was <0.05.

RESULTS

Administration of THC or CBN resulted in a significant reduction of plasma LH levels measured in peripheral blood collected 60 min later (Fig. 1). However, within the range of doses tested (0.1–10 mg/kg), there was no significant effect of the dose

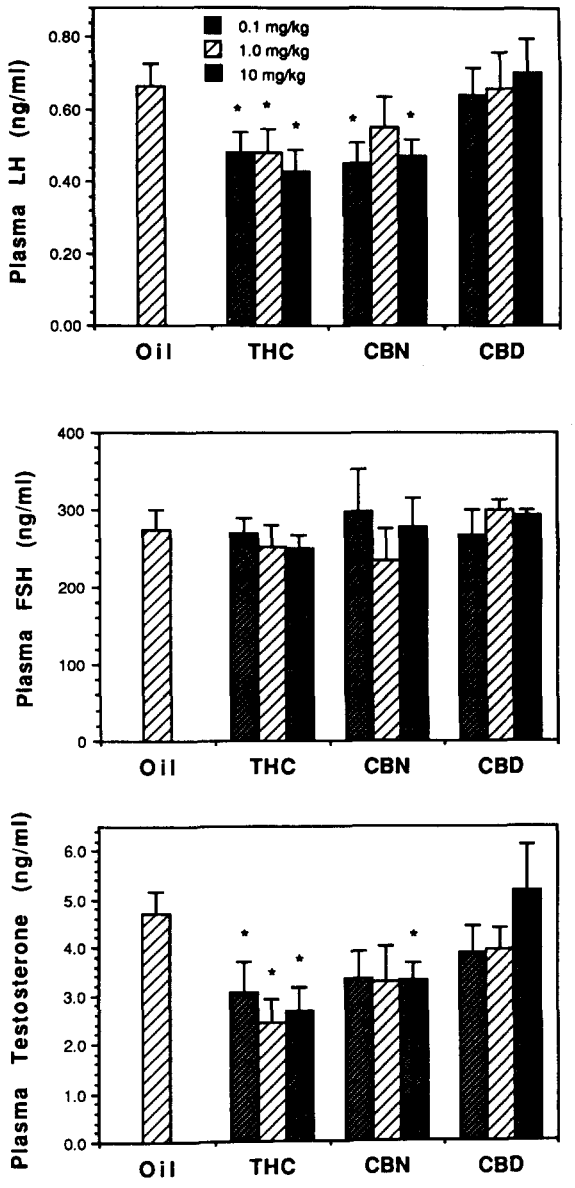

FIG. 1. Plasma LH, FSH and T levels in male rats treated with various doses of cannabinoids or the sesame oil vehicle. Cannabinoids were orally administered 60 min prior to autopsy and plasma collection. Values represent the mean ± SEM of seven to eight rats. The asterisks denote statistical significance ($p<0.05$).

(dose effect; $p>0.7$: interaction; $p>0.8$). There was no significant effect of CBD treatment on LH levels. None of the cannabinoids influenced plasma FSH levels. All doses of THC resulted in a significant reduction of plasma T levels but again, a dose-response relationship could not be demonstrated (dose effect; $p>0.6$: interaction; $p>0.6$). All three doses of CBN caused a comparable reduction in plasma T levels, but the effect was only significant for the 10 mg/kg dose because of a large variability of results for the two lower doses of CBN. Similar to what was seen for LH, CBD administration had no significant effect on T levels.

None of the treatments altered LHRH content of the median

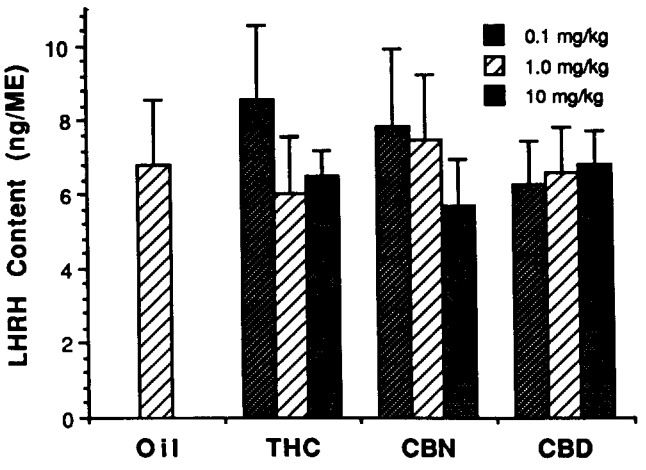

FIG. 2. Median eminence LHRH content of the animals described in Fig. 1. Values represent the mean ± SEM of seven to eight rats. The asterisks denote statistical significance ($p<0.05$).

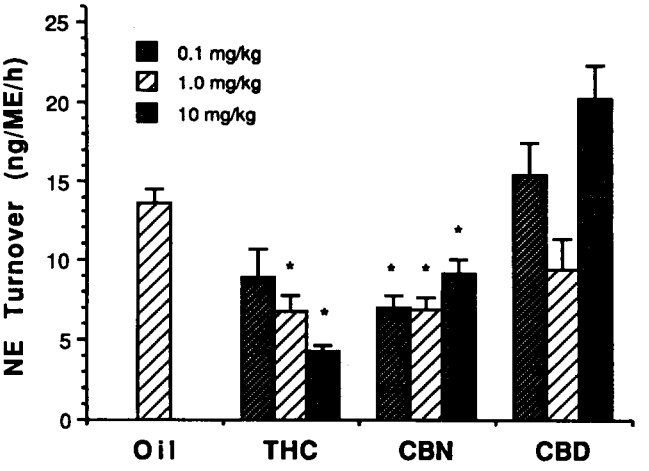

FIG. 3. Median eminence norepinephrine turnover in cannabinoid-treated rats. NE turnover, an index of noradrenergic neuronal activity, was calculated from the decline of NE content after inhibition of tyrosine hydroxylase with aMPT. Values represent the mean ± SEM of six to eight rats. The asterisks denote statistical significance ($p<0.05$).

eminence (Fig. 2). Norepinephrine turnover in the median eminence was suppressed by all three doses of THC, but the effect was only significant for the 1 and 10 mg/kg dose (Fig. 3). All three doses of CBN resulted in a significant attenuation of ME NE turnover, while CBD had no such effect.

All of the cannabinoids tended to decrease the release of LH from incubated pituitaries under basal conditions, but the only significant effect was seen with the highest dose (10 mg/kg) of CBN (Table 1). Treatment with LHRH in vitro led to a significant stimulation of LH secretion in all groups, but the magnitude of the increase did not differ with cannabinoid treatment. Pituitary LH concentration measured at the end of the incubation period did not vary between the groups.

TABLE 1

IN VITRO LH SECRETION BY PITUITARIES FROM ADULT MALE RATS TREATED WITH VARIOUS DOSES OF THC, CBN OR CBD 60 MINUTES PRIOR TO SACRIFICE

	Basal LH (ng/mg)	10^{-8} M LHRH (ng/mg)	Pit LH (μg/mg)
Vehicle	540 ± 114	922 ± 172	14.0 ± 2.1
THC (mg/kg)			
0.1	404 ± 53	856 ± 143	14.6 ± 2.1
1.0	430 ± 49	754 ± 93	16.3 ± 1.6
10.0	453 ± 114	692 ± 173	14.3 ± 2.7
CBN (mg/kg)			
0.1	364 ± 44	561 ± 90	16.5 ± 2.5
1.0	375 ± 36	619 ± 54	16.5 ± 1.1
10.0	321 ± 42*	642 ± 88	15.8 ± 1.6
CBD (mg/kg)			
0.1	413 ± 59	619 ± 74	17.9 ± 2.2
1.0	408 ± 27	717 ± 62	15.5 ± 1.4
10.0	430 ± 40	748 ± 116	15.1 ± 1.3

Values are reported as the mean ± SEM (n = 8–12). The asterisk denotes statistical significance ($p<0.05$) vs. vehicle treatment.

DISCUSSION

Results of this study demonstrate that administration of a single dose of CBN, a nonpsychoactive constituent of marihuana, can significantly lower circulating levels of both LH and T in adult male rats. Moreover, the present results strongly suggest that inhibitory effects of both CBN and THC on pituitary LH release are due at least in part, to suppression of noradrenergic transmission within the hypothalamus by these cannabinoids.

The inhibitory effects of THC on plasma LH and T levels observed in the present study were anticipated from previous findings of other investigators (2, 12, 17, 25) and from our own studies in this and in other species (7, 8, 21). The acute effect of CBN on these parameters is novel and contrasts with earlier findings in male mice (3,6). In contrast to the effects of CBN, the same doses of CBD did not affect either LH or T levels. None of the cannabinoids had any significant effects on circulating FSH levels.

Despite the significant effects of THC and CBN on plasma LH and T levels there was no clear dose-response relationship. Previous studies have also been unable to show a dose-response relationship for the effects of THC on the magnitude of plasma LH levels, although it appears that the duration of the inhibitory effect of THC might be dose dependent [(25); Murphy *et al.*, unpublished]. We have no explanation why the low (0.1 mg/kg) and the high (10.0 mg/kg) dose of CBN caused a decrease in LH and NE turnover, while the intermediate (1.0 mg/kg) dose decreased NE but had no effect on LH levels. However, this observation is in agreement with a recent study in which we demonstrated that a 0.5 mg/kg dose of CBN also decreased NE turnover while not significantly affecting LH levels (14).

The reduction in LH levels does not appear to be entirely due to a direct effect of THC or CBN on the pituitary since LH secretion in vitro and the in vitro response to LHRH were only minimally affected by exposure to these cannabinoids in vivo. It could perhaps be argued that absence of inhibitory effects under these conditions may have been due to the fact that CBN and THC were not present in the incubation media. However, previous

studies have shown that in vitro exposure to THC does not reduce pituitary LH secretion (8).

Numerous reports have established the stimulatory role of median eminence noradrenergic terminals on LHRH release (1,10). We have observed close associations between the changes in ME-NE turnover rate and plasma LH levels in three different rodent species in situations as diverse as long- and short-term responses to photoperiod (22), acute responses of males to female conspecifics (20) and experimentally induced hyperprolactinemia (20) or diabetes (19). The ability of THC and CBN but not CBD to reduce ME-NE turnover directly reflects the actions of these cannabinoids on LH release in the present study and strongly suggests that reduced LH secretion was secondary to reduced noradrenergic activity. In support of these findings is our recent report that hypothalamic NE injections reverse the LH inhibitory effects of THC in castrate, testosterone-implanted male rats (13).

It is of interest that FSH levels were not reduced after THC or CBN treatment despite significant reductions in LH levels and a hypothesized decrease in hypothalamic LHRH release. These results may be explained by a longer half-life of FSH in the circulation, a longer latency to diminished pituitary secretion after LHRH withdrawal or the existence of a separate FSH releasing factor (11) whose secretion is not diminished by cannabinoid treatment. It is also possible that a reduction in FSH secretion due to diminished LHRH release could have been counteracted by a direct effect of THC or CBN on testicular Sertoli cells to reduce testicular inhibin release. We have recently found that cannabinoids have direct effects on Sertoli cells to alter in vitro release of lactate and androgen binding protein [(16); Murphy *et al.*, unpublished].

In conclusion, CBN shares the ability of THC to produce acute suppression of plasma LH and T levels in adult male rats after a single administration. Furthermore, both drugs appear to influence pituitary function by altering the activity of noradrenergic neurons whose terminals lie within the median eminence.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Jeanie Rathert and Ms. Danelle Glassburg for excellent technical assistance. The authors also thank the Hormone Distribution Branch, NIDDK, NIH, for supplying the materials used in the rat pituitary hormone RIA's and the NIDA for providing the cannabinoids. The gift of T antiserum (GDN S250) from Dr. G. D. Niswender is gratefully acknowledged.

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