EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL ON SERUM PROLACTIN IN FEMALE RATS BEARING CNS LESIONS: IMPLICATIONS FOR SITE OF DRUG ACTION

Claude L. Hughes, Jr.[1]
John W. Everett
Lee Tyrey[2]

Departments of Obstetrics and Gynecology and Anatomy
Duke University Medical Center
Durham, North Carolina

I. INTRODUCTION

Delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent of marijuana (1), acutely suppresses serum prolactin (PRL) concentrations in male (2,3) and ovariectomized (4) or proestrous (5) female rats. The PRL-suppressive effect of THC appears to depend upon a central nervous system (CNS) site of action, since THC failed to alter serum PRL in hypophysectomized pituitary-autografted rats (4), block the TRH-induced PRL rise in rhesus monkeys (6), or reduce PRL secretion from rat pituitary glands incubated *in vitro* (4). To investigate possible CNS sites of THC action, we administered THC to young adult female rats that had been previously subjected to hypothalamic or limbic system lesions.

II. MATERIALS AND METHODS

Two to four month-old female rats of the Charles River Crl:CD(SD) BR strain (180-260 g BW) were maintained in air-conditioned quarters with 14 hours of light daily (0500 h-

[1]Supported in part by NIH training grant GM-07046.
[2]Supported by NIDA grant DA-02006.

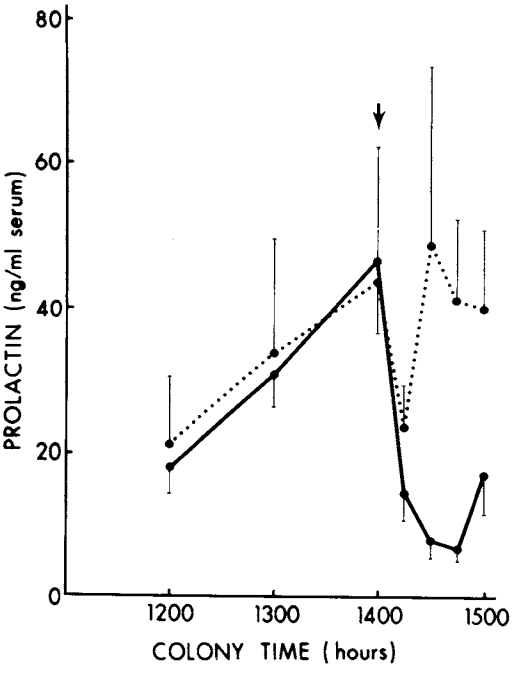


FIGURE 1. Serum PRL concentrations (mean +/- SE) on diestrus-1 in sham-operated rats. Vehicle alone (n = 5; .....) or 0.5 mg THC/kg BW (n = 4:_____) was injected iv at 1400 h. Within the THC group, PRL levels at 1415 h, 1430 h and 1445 h were significantly reduced ($p < 0.05$) relative to the level at 1400 h. Post-treatment PRL levels in the THC group were significantly lower than both the pre-treatment levels within the THC group ($p < 0.05$) and the post-treatment levels in the control group ($p < 0.01$). Administration of vehicle was without effect ($p < 0.05$).

1900 h) and with Purina Laboratory Chow and water available ad libitum. Daily vaginal smears were recorded and only rats demonstrating at least two consecutive 4- or 5-day estrous cycles immediately before use were selected for lesioning.

CNS lesions were placed in animals under ether anesthesia with the aid of a small animal stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). In order to control for possible nonspecific effects of surgery, control animals were subjected to sham operations performed with a slender scalpel blade (number 11) oriented in a frontal plane. The blade was lowered bilaterally 1.9 mm from the midline to a point 3.5 mm above and 6.7 mm anterior to ear bar zero (EBZ). Animals were

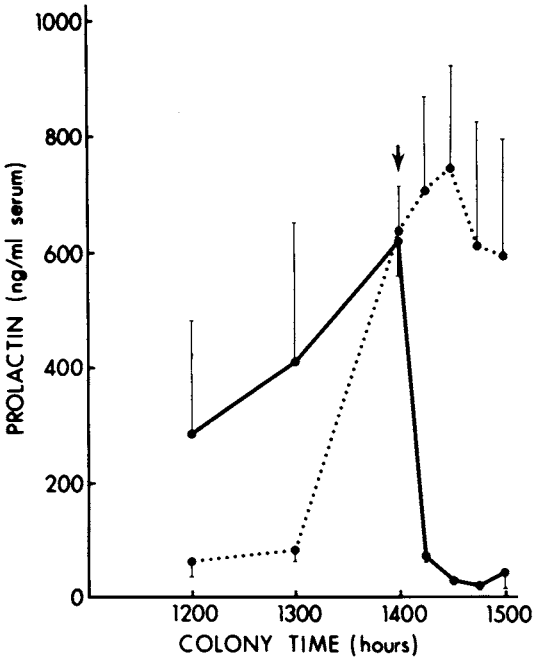


FIGURE 2. Serum PRL concentrations (mean +/- SE) on proestrus in sham-operated rats. Vehicle alone (n = 5; .....) or 0.5 mg THC/kg BW (n = 4; _____) was injected iv at 1400 h. Post-treatment PRL levels in the THC group were significantly lower than pre-treatment levels within the THC group ($p < 0.01$) and post-treatment levels in the vehicle group ($p < 0.01$). Administration of vehicle was without effect ($p < 0.05$).

used 23 to 59 days after sham-operation.

Complete hypothalamic deafferentations (CHD) were performed with a spring-loaded, bayonet-shaped knife which had a vertical blade of 1.9 mm and a 1.3 mm radius of swing. With the head of the animal mounted in the stereotaxic apparatus with the incisor bar adjusted to a point 5 mm below the horizontal plane passing through the earbars, the blade tip was oriented rostrally at a position 7.7 mm anterior to EBZ, and the knife was lowered in the midline to the base of the skull. The blade was rotated counterclockwise through a full 360°, and then through an additional 90° to insure a complete cut rostrally. The blade was then returned to the starting position and the knife was withdrawn. The placement of the cut was designed to put its most rostral extension just caudal to

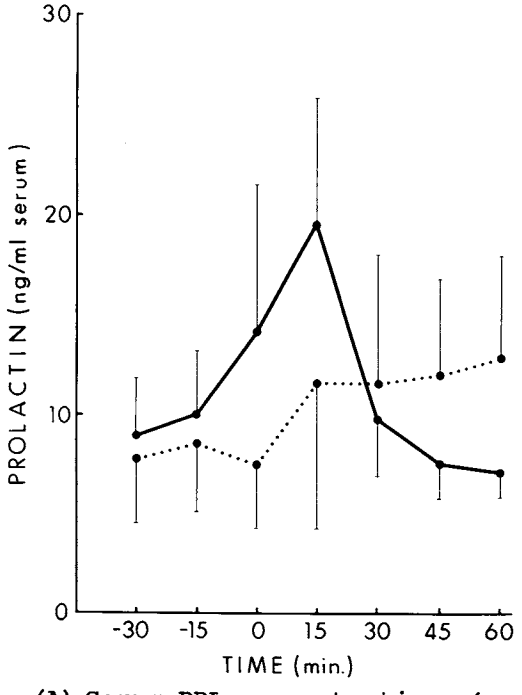
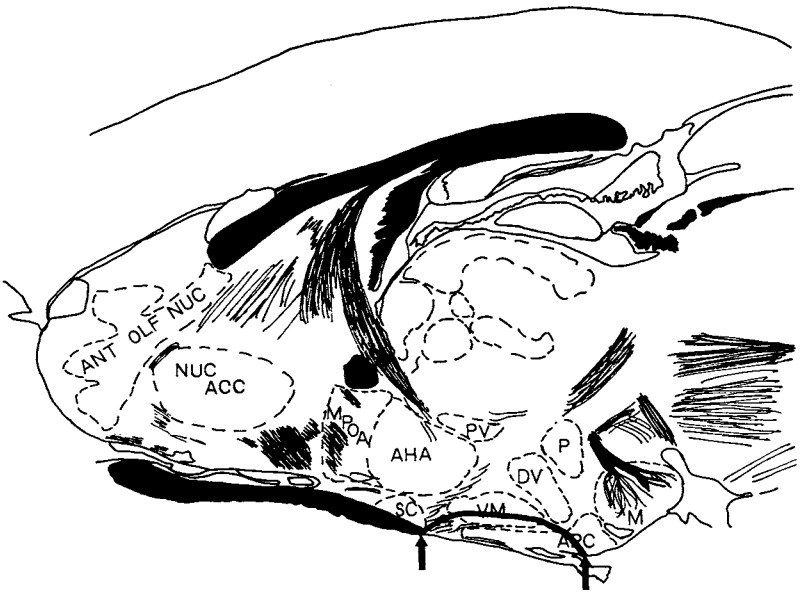


FIGURE 3. (A) Serum PRL concentrations (mean +/- SE) in CHD rats. Vehicle alone (n = 2; .....) or 0.5 mg THC/kg BW (n = 6; _____) was injected iv at t = 0 min. Statistical comparisons revealed no significant differences between pre- and post-treatment levels within or between groups at any sampling interval ($p < 0.05$). (B) A parasagittal sketch of the approximate location of lesions in CHD rats.

the suprachiasmatic nuclei. Animals were used 31 to 37 days later.

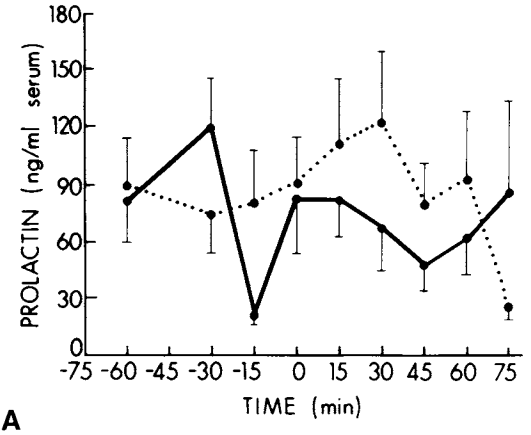
Frontal cuts at the level of the suprachiasmatic nuclei (frontal suprachiasmatic cuts; FSC) were produced with two different knifes. Cuts confined to the frontal plane were produced with a spring-loaded 4 mm straight-edged knife. With the incisor bar fixed 4 mm below the earbars, the blade was oriented in a frontal plane 6.4 anterior to EBZ. Centered over the midline, the blade was lowered through the brain to the base of the skull, and immediately withdrawn. Frontal cuts with arcing posterolateral extensions were produced with the same bayonet-shaped knife used for CHD. With the head oriented as for the straight frontal cuts, the blade tip was positioned rostrally 8.1 mm anterior to EBZ and lowered in the midline to the base of the skull. A sweeping frontal cut was produced by rotating the blade 90° counterclockwise, and then



back through 180° to the opposite side. The blade was then returned to the starting point and withdrawn. Animals were used 22 to 56 days after lesioning.

Cuts just rostral to the suprachiasmatic nuclei (presuprachiasmatic cuts; PSC) were produced with the same bayonet-shaped knife used for CHD. The head of the rat was mounted in the stereotaxic apparatus with the incisor bar 5 mm below the earbars. With the tip of the knife oriented rostrally, the knife was lowered to the base of the skull in the midline 9.5 mm rostral to EBZ. The knife was rotated 90° counterclockwise, then 180° clockwise, before it was returned to the starting point and withdrawn. These rats exhibited a reduction in food and water intake over the first 5 to 15 days post-operatively. Consequently, they were fed by gavage until food and water intake improved. Each rat was given 1.5 to 2.0 ml of a soy protein formula ("Isomil", Ross Laboratories, Columbus, Ohio) 3 or 4 times daily during this recovery interval. Animals were used 21 to 48 days after lesioning.

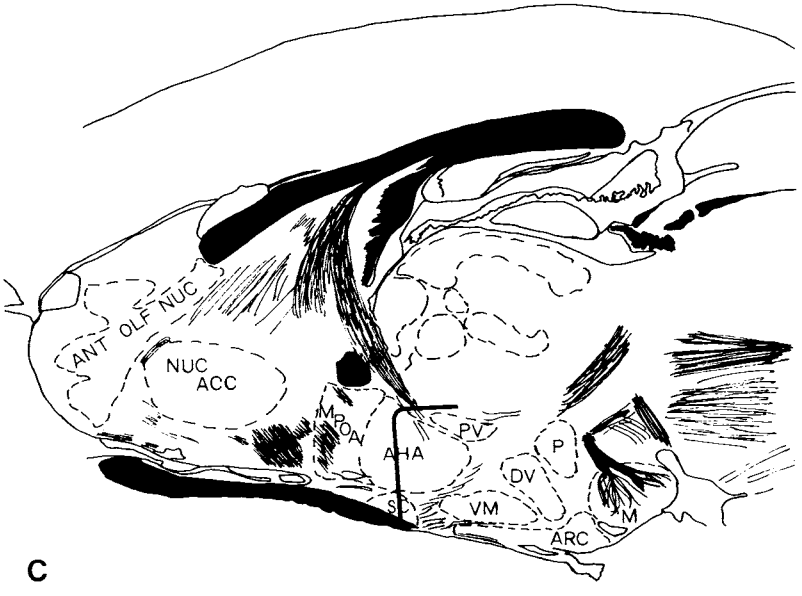
Lateral cortico-hypothalamic tract sections (LCHTS) were performed with a semi-cylindrical knife. The knife radius was 2.5 mm and a notch of 1.0 mm width and 4.0 mm height was located at the midpoint of the curved knife edge. With the incisor bar 3 mm below EBZ, the knife was positioned, concavity posterior, such that its posterior edges were located 7.9 mm anterior to EBZ and then lowered in the midline to the base



A

B

FIGURE 4. (A) Serum PRL concentrations (mean +/- SE) in FSC rats. Vehicle alone (n = 12; .....) or 0.5 mg THC/kg BW (n = 12; _____) was injected iv at t = 0 min. There were no significant differences between pre- and post-treatment levels within or between groups ($p < 0.050$). (B) A parasagittal sketch of the approximate location of frontal cuts produced with the straight edged knife. (C) A parasagittal sketch of the approximate location of frontal cuts produced with the bayonet-shaped knife.



C

of the skull. The central notch in the blade allowed preservation of the anterior cerebral arteries. Animals were used 18 to 34 days later.

Amgdalofugal projections to caudal septal and rostral preoptic areas, identifiable as the diagonal band of Broca, were sectioned (DBBS) with a 2.2 mm straight-edged knife. With the incisor bar fixed 4 mm below EBZ, the blade was oriented in the sagittal plane 1.3 mm to either side of the midline and lowered bilaterally to the base of the skull. The caudal edge of the knife was located 8.6 mm anterior to EBZ. Animals were used 22 to 31 days later.

Stria terminalis sections (STS) were performed bilaterally with a 4 mm straight-edged knife oriented in the frontal plane 6.2 mm anterior to EBZ. With the incisor bar 4 mm below EBZ, the blade was centered 3.0 mm to either side of the midline and lowered to a point 2.0 mm above EBZ. Animals were used 28 to 33 days later.

Dorsal longitudinal fascilculus sections (DLFS) were performed with a 2.2 mm straight-edged knife. With the incisor bar 4 mm below EBZ and the blade oriented in the frontal plane 3.4 mm anterior to EBZ and centered over the midline, the knife was lowered to a point 1.5 mm above EBZ. Animals were used 23 to 56 days later.

An ethanol solution of THC of greater than 95% purity was provided by the National Institute on Drug Abuse. Prior to use, the alcohol was evaporated under nitrogen at 45-50°C and

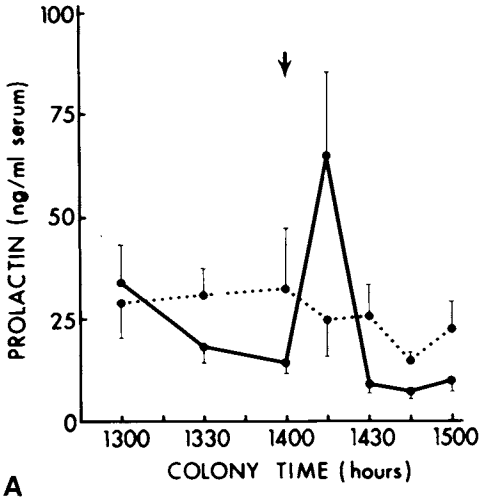


FIGURE 5. (A) Serum PRL concentrations (mean +/- SE) in PSC rats. Vehicle alone (n = 8; .....) or 0.5 mg THC/kg BW (n = 9; _____) was injected iv at 1400 h. PRL levels within the vehicle group did not differ significantly ($p < 0.05$) over the sampling interval. Within THC group, the PRL level at 1415 h was significantly increased ($p < 0.05$) relative to that at 1400 h, while those from 1430 h through 1500 h did notdiffer significantly ($p < 0.05$) from the pre-treatment level. PRL in the THC group at 1415 h was elevated ($p < 0.01$) above that in the vehicle group, but no between group differences were detected at other times. (B) Sketch of the approximate location of lesions in PSC rats.

the residue was emulsified in a vehicle consisting of 10% propylene glycol and 1% Tween-80 in 0.9% saline. The appropriate THC dose was always administered in a volume of0.5 ml/kg BW, with several animals receiving vehicle alone.

The intravenous (iv) administration of THC or vehicle and serial sampling of blood were carried out in unrestrained, fully alert rats by means of intra-atrial cannulae. Cannulae were filled with heparinized saline (10 U/ml) and implanted under ether anesthesia 2-3 hours prior to drawing the initial blood sample. Seven blood samples (0.4 ml each) were typically drawn every 15 min with 0.2 ml replacement with heparinized saline to minimize plasma volume changes and to maintain cannula patency. Blood samples were allowed to clot at room temperature for approximately one hour and then were centrifuged at 1500 x g for 15 min at 4°C. Sera were aspirated and stored at -20°C for later radioimmunoassay.

B

Upon completion of the experiment, rats were perfused through the ventral aorta with 0.9% saline and then with 10% formalin in saline. Brains were later removed and embedded in paraffin. Serial sections (18 microns) were cut and stained with Luxol fast blue and neutral red for microscopic examination to determine the location and extent of all lesions. Sketches of selected sections were prepared by direct projection.

Serum PRL concentrations were determined by a double antibody radioimmunoassay utilizing materials provided by the NIADDK. Aliquots of sera (100 mcl or less) were assayedin duplicate and the mean result expressed in terms of the NIADDK rat PRL reference RRP-1. Within and among assay coefficients of variation for the measurement of PRL in aliquots of a rat serum pool containing 15.0 ng PRL.ml were 4.5% and 10.1%, respectively. The assay sensitivity was 0.6 ng PRL/ml serum, and no value fell below that level in these studies.

Serum PRL levels were subjected to one-way analysis of variance with partitioning of the sums of squares for planned between group comparisons, compared by the Student's t-test or paired t-test, as appropriate.

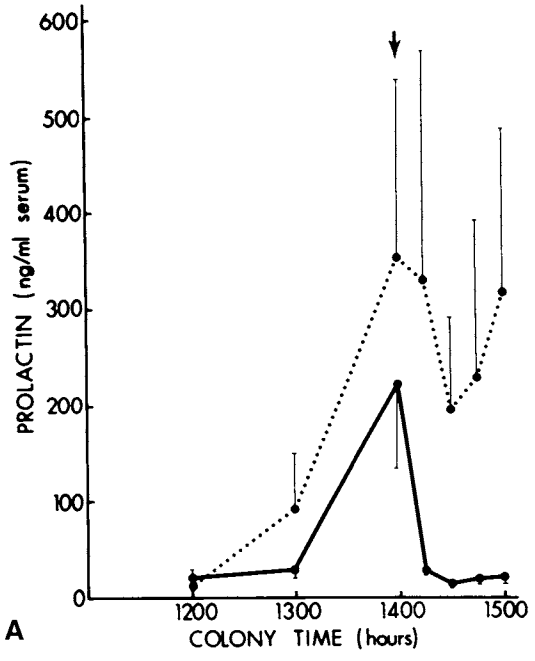
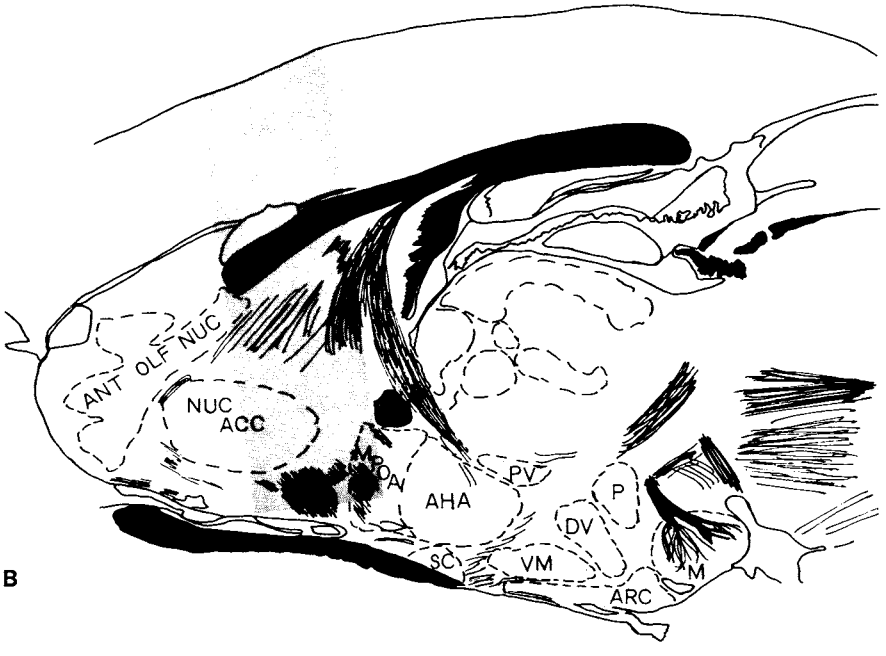


FIGURE 6. (A) Serum PRL concentrations (mean +/- SE) on proestrus in LCHTS rats. Vehicle alone (n = 4; .....) or 0.5 mg THC/kg BW (n = 4; _____) was injected iv at 1400 h. Post-treatment PRL levels in the THC group were significantly reduced from 1415 through 1500 h relative to that at 1400 h ($p < 0.05$). Post-treatment PRL levels in the THC group were also significantly lower than those in the vehicle group ($p < 0.01$). Vehicle administration did not alter serum PRL levels ($p < 0.05$). Both groups show the expected proestrus PRL rise prior to treatment. (B) Sketch of the approximate rostrocaudal extent of lesions in LCHTS rats.

III. RESULTS

A. Sham-Operated Rats

Rats subjected to sham-operation suffered limited CNS damage in a region approximately 1 mm in width which extended vertically through the dorsal cortex to reach approximately 1 mm into the underlying corpus striatum. These rats had regular 4- or 5-day estrous cycles post-operatively and were treated with 0.5 mg THC/kg BW or vehicle at 1400 h on the day of either diestrus-1 or proestrus. As shown, in Fig. 1, mean serum PRL in diestrus rats ranged from approximately 20 to 50



ng/ml prior to treatment. After treatment with THC, PRL was decreased within 15 min and remained suppressed 45 min after injection, relative to either post-treatment levels in the vehicle group ($p < 0.01$) or to the immediate pre-treatment level within the THC group ($p < 0.05$). Vehicle injection was without effect.

Mean serum PRL concentrations in proestrus rats averaged approximately 600 ng/ml at the time of THC or vehicle injection at 1400 h (Fig. 2). PRL levels in the vehicle-treated group remained in the range of 600 to 800 ng/ml in samples drawn during the hour following treatment, while over the same interval serum PRL in the THC-treated rats was suppressed to below 100 ng/ml ($p < 0.05$).

B. Complete Hypothalamic Deafferentation

CHD rats showing predominantly leukocytic vaginal smears following deafferentation were treated with either vehicle alone or 0.5 mg THC/kg BW. Mean serum PRL in these animals ranged from 5 to 20 ng/ml throughout the sampling interval (Fig. 3A) and neither vehicle nor THC injection altered serum PRL levels. An apparent small increase in serum PRL in the THC group 15 min following injection was not statistically

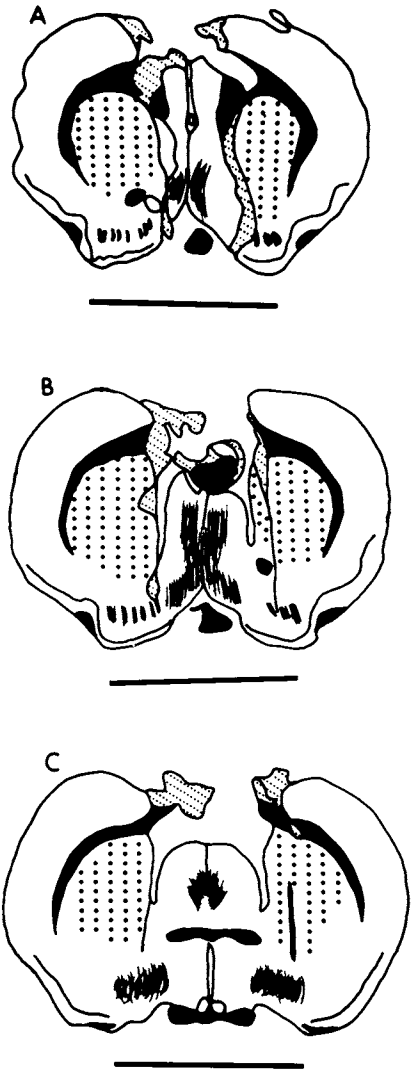


FIGURE 7. Projection sketches of frontal sections of a representative brain from a LCHTS rat at the (A) mid-nucleus accumbens level, (B) caudal nucleus accumbens level and (C) rostral preoptic area level. The fine stippling indicates the region of gliosis and the bar beneath each section provides a 4 mm reference scale. The coarse stippling indicates the extent of the corpus striatum at the several levels.

significant ($p < 0.05$). The location and extent of the CHD lesions are shown in Fig. 3B, with the surviving mediobasal "island" of tissue approximately delineated by the arcing line which extends from the caudal edge of the suprachiasmatic nuclei to a point just caudal to the separation of the infundibular stem from the basal hypothalamus. The surviving isolated brain region thus included predominantly the arcuate nucleus and the lower portions of the ventromedial nuclei.

C. Frontal Suprachiasmatic Cuts

FSC rats showing predominantly cornified vaginal smears were treated with vehicle or 0.5 mg THC/kg BW. Mean serum PRL levels varied widely and ranged from 20 to 123 ng/ml serum during the sampling interval (Fig. 4A). PRL concentrations in individual animals in both vehicle and THC groups showed wide variation from sample to sample with no apparent correlation with experimental manipulations. Neither vehicle nor THC produced statistically detectable alterations in serum PRL during the post-treatment interval. Fig. 4B illustrates the approximate location of the cut produced with the straight edged knife (4 rats group), and Fig. 4C that for the bayonet-shaped knife (8 rats group). FSC by either technique interrupted connections between the mediobasal hypothalamus and rostral structures at a mid-to-caudal level of the suprachiasmatic nuclei. There were no differences in the response to treatment between these two subgroups.

D. Presuprachiasmatic Cuts

PSC rats showed variations in postsurgical vaginal smear patterns, but did not show regular cyclicity. Smears were predominantly cornified with occasional leukocytic types. PSC rats were treated on a day showing a leukocytic smear which was immediately preceded by one or more days of cornified smears. Vehicle alone did not alter serum PRL (Fig. 5A), but THC treatment (0.5 mg/kg BW) at 1400 h increased the mean PRL concentration 15 min later ($p < 0.05$) to a level which was significantly greater than the concurrent level in the vehicle group ($p < 0.01$). Subsequent PRL levels in the THC group did not differ from the pretreatment level within that group or from post-treatment levels in the vehicle group. The location and extent of the PSC lesions are illustrated in Fig. 5B. These lesions consisted of an arcing cut from a level just anterior to the anterior hypothalamic area andsuprachiasmatic nuclei to a more caudal and lateral level at approximately the caudal edge of the suprachiasmatic nuclei.

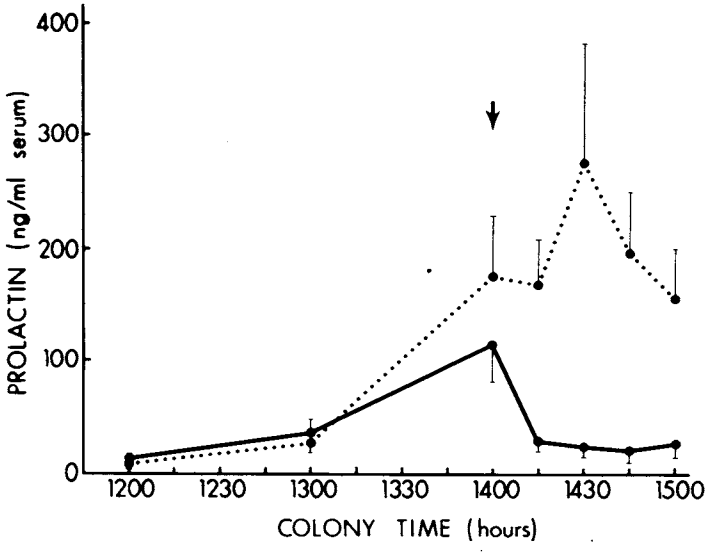
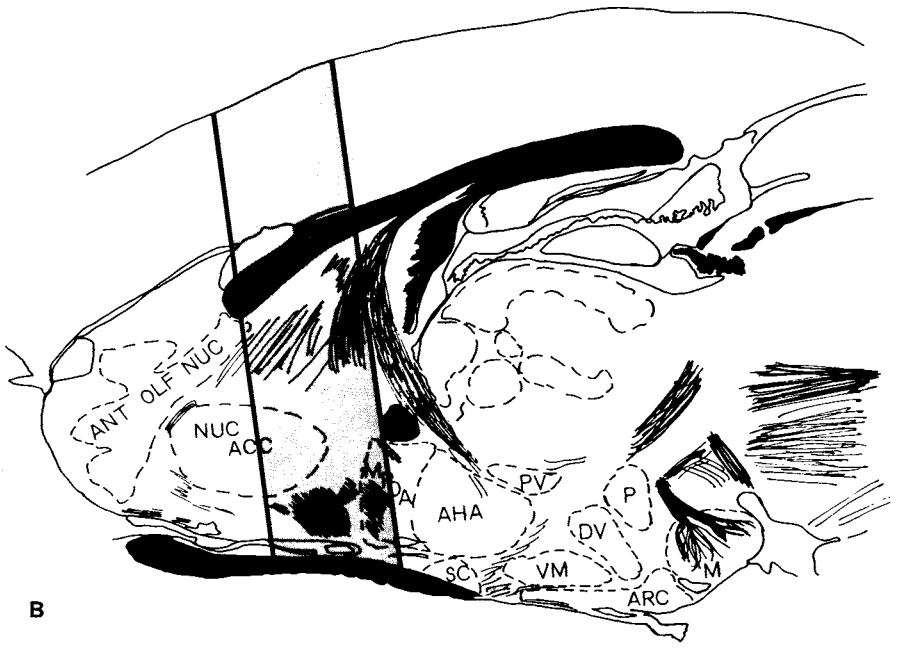


FIGURE 8. (A) Serum PRL concentrations (mean +/- SE) on proestrus in DBBS rats. Vehicle alone (n = 7; .....) or 0.5 mg THC/kg BW (n = 8;_____) was injected iv at 1400 h. Post-treatment PRL levels in the THC group were significantly lower than both the 1400 h level in the THC group ($p < 0.05$) and the post-treatment levels in the vehicle group ($p < 0.01$). Vehicle administration did not alter serum PRL levels ($p < 0.05$). (B) Sketch of the approximate rostrocaudal extent of lesions in DBBS rats.

E. Lateral Corticohypothalamic Tract Sections

LCHTS rats had regular 4- or 5-day estrous cycles, and vehicle alone or 0.5 mg THC/kg BW was injected at 1400 h on a day of proestrus. Although vehicle administration did not significantly alter serum PRL (Fig. 6A), THC administration markedly suppressed PRL concentrations at all post-treatment times sampled, both with respect to the 1400 h level in the THC group, ($p < 0.05$) and the post-treatment levels in the vehicle group ($p < 0.01$). Both groups showed the expected proestrus rise in serum PRL prior to treatment. Figs. 6B and 7 illustrate the approximate location and extent of lesions in LCHTS rats. These lesions were bilateral arcs which extended from the mid-region of the nucleus accumbens caudally and laterally to a level immediately anterior to the transverse portion of the anterior commissure. The most ventral portions of the diagonal band of Broca were preserved in these animals.



F. Diagonal Band of Broca Sections

DBBS rats showed regular 4- or 5-day estrous cycles and were treated on a day of proestrus by the injection of either vehicle or 0.5 mg THC/kg BW at 1400 h. While the vehicle alone did not alter serum PRL concentrations (Fig.8A), THC significantly suppressed serum PRL levels relative to both the 1400 h level in the THC group ($p < 0.05$) and the post-treatment PRL levels in the vehicle group ($p < 0.01$). Both groups show the proestrus PRL rise. Figs.8B and 9 illustrate the approximate location and extent of lesions in DBBS rats. These lesions have a rostrocaudal extent which approximates those in the LCHTS rats. In the DBBS rats, however, the linear bilateral lesions extended completely to the base of the brain and thus transected ventral connections between the amygdalae and the more medial portions of the septal and preoptic areas.

G. Stria Terminalis Sections

STS rats showed regular 4- or 5-day estrous cycles and were injected with vehicle or 0.5 mg THC/kg BW at 1400 h on a

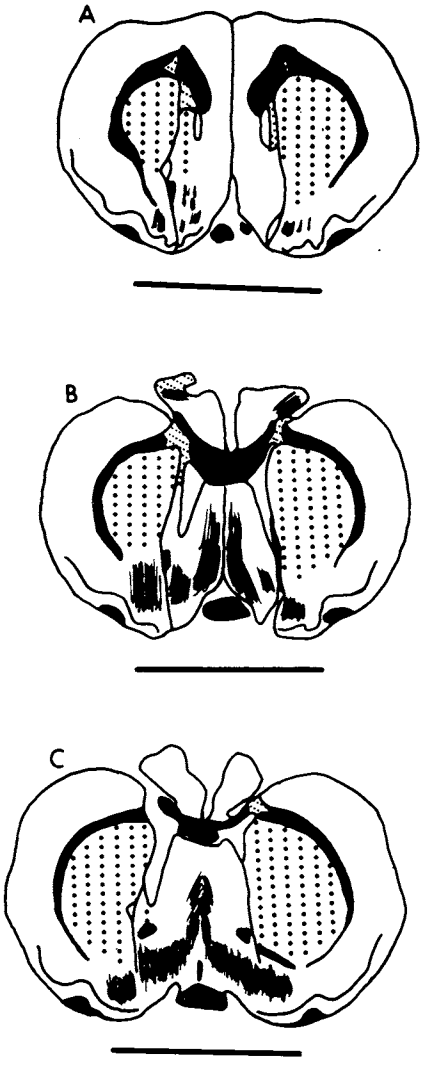


FIGURE 9. Projection sketches of frontal sections of a representative brain from a DBBS rat at the (A) mid-nucleus accumbens level, (B) caudal nucleus accumbens level and (C) rostral preoptic area level. The fine stippling indicates the region of gliosis and the bar beneath each section provides a 4 mm reference scale. The coarse stippling indicates the extent of the corpus striatum at the several levels.

day of proestrus. While vehicle alone did not alter serum PRL (Fig. 10A), post-treatment PRL levels in the THC group were

significantly lower than both the THC group pretreatment level ($p < 0.01$) and the post-treatment levels in the vehicle group ($p < 0.01$). Figs. 10B and 10C illustrate the approximate location and extent of lesions in STS rats. These bilateral lesions severed the stria terminalis and destroyed a portion of the nearby corpus striatum.

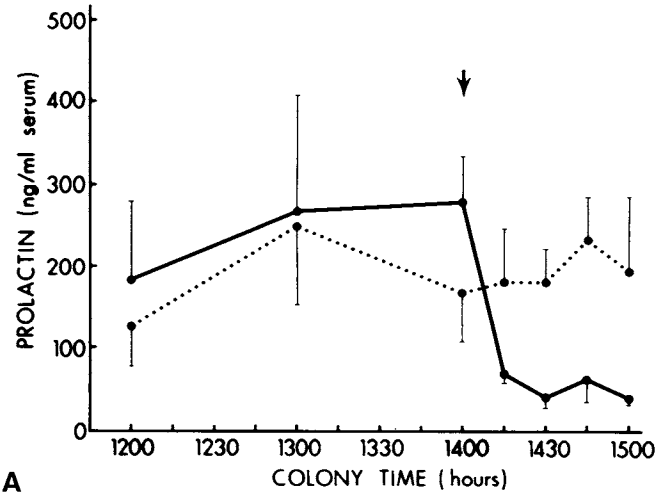
H. Dorsal Longitudinal Fasciculus Sections

DLFS rats showed regular 4- or 5-days estrous cycles and were treated with either vehicle alone or 0.5 mg THC/kg BW at 1400 h on a day of proestrus. Although vehicle was without significant effect (Fig. 11A), serum PRL was suppressed at all post-treatment times in the THC group relative to both the THC group pretreatment level at 1400 h ($p < 0.05$) and the post-treatment PRL levels in the vehicle group ($p > 0.01$). Figs. 11B and 11C illustrate the approximate location and extent of lesions in the DLFS rats. These lesions damaged the periventricular region and adjacent midline brain tissue at the level of the posterior commissure and the caudal portion of the mamillary complex. The midline periventricular connections between the hypothalamus and the midbrain were thus disrupted, but the more ventrolateral connections by way of the medial forebrain bundle and associated fiber systems remained intact.

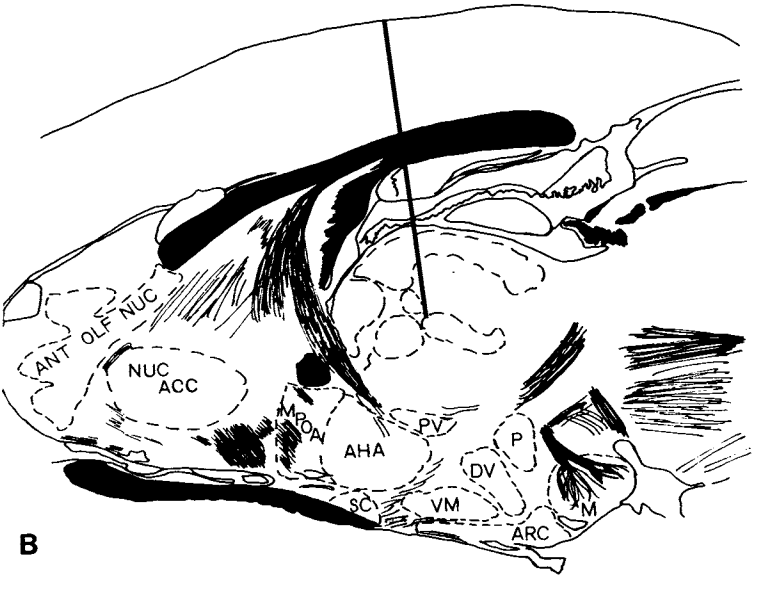
IV. DISCUSSION

Since the current evidence indicates that the site of THC action in the suppression of PRL is within the CNS (4,6), we studied the effects of THC on serum PRL in rats previously subjected to hypothalamic or limbic system lesions in order to more precisely define potential CNS loci of THC action. Experiments with sham-operated rats demonstrated a PRL suppression following THC treatment which was similar to that previously reported for male (2) and ovariectomized female rats, with suppression detectable within 15 min and persistence for at least 1 h after a dose of 0.5 mg THC/kg BW. These results suggested that the stress of stereotaxic CNS surgery would not in itself compromise the expression of THC action on PRL secretion.

Because the mediobasal hypothalamus (MBH) is widely accepted as the major brain region involved in the regulation of PRL release from the anterior pituitary, the lack of effect of THC on PRL secretion in rats previously subjected to CHD provides important information. These data suggest that THC does not suppress PRL secretion by action at a site within the

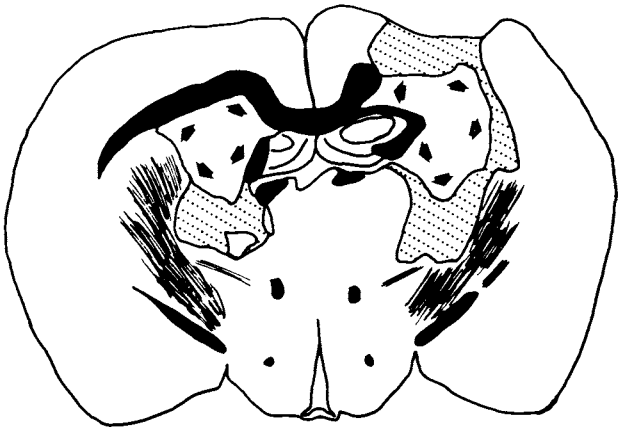


A



B

MBH, and therefore, presumably not by action on those elements most directly involved in PRL regulation. While it is possible that the low pretreatment PRL levels in CHD animals many have compromised our ability to detect a decrease in serum PRL which may have resulted from THC action within the MBH, the observations with the FSC animals provide some reassurance against this point. The ineffectiveness of THC in FSC rats, in which pretreatment PRL was not low, implies that the sup-



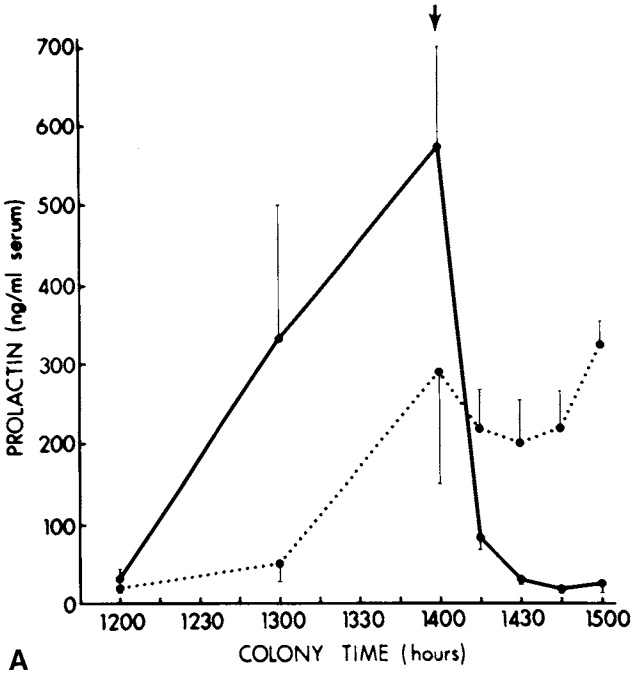
C

FIGURE 10. (A) Serum PRL concentrations (mean +/- SE) on proestrus in STS rats. Vehicle alone (n = 5; .....) or 0.5 mg THC/kg BW (n = 5; _____) was injected iv at 1400 h. Post-treatment PRL levels in the THC groups were significantly lower than both pretreatment levels within the THC group ($p < 0.01$) and post-treatment levels in the vehicle group ($p < 0.01$). Vehicle administration did not alter serum PRL levels ($p < 0.05$). (B) Sketch of the approximate location of lesions in STS rats. (C) Projection sketch of a frontal section of a representative brain from a STS rat at the level of the arcuate nucleus and median eminence. The stippling indicates the region of gliosis, the arrow indicate tissue-free spaces and the bar beneath the section provides a 4 mm reference scale.

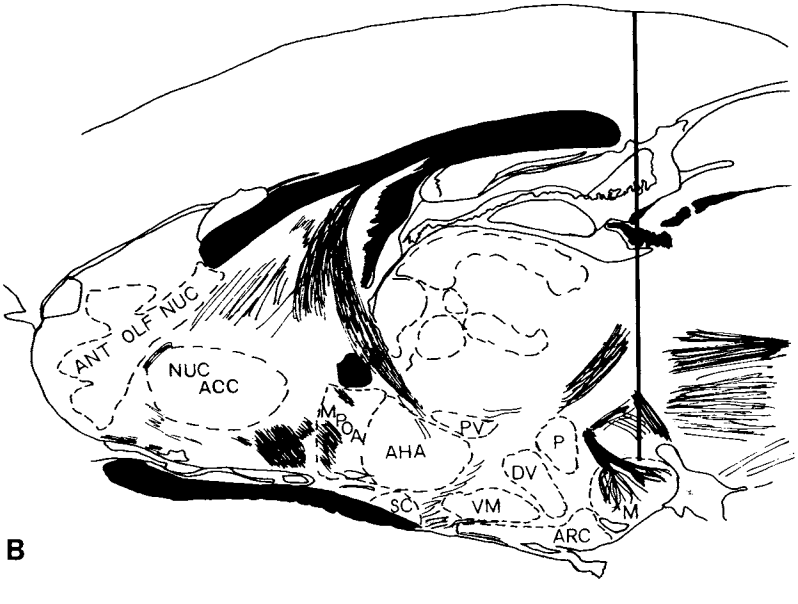
pression of PRL by THC depends either upon the anterior hypothalamic region destroyed by these lesions, or upon the rostral connections which pass through this region into the MBH.

The fact that THC failed to suppress PRL levels in animals having FSC lesions produced with two different knife types which caused different local damage, and also failed to suppress serum PRL in PSC rats in which the lesions were located more rostrally near the junction of the medial preoptic and anterior hypothalamic areas, argues rather strongly in favor of the disruption of some critical fiber pathway. The remarkable brief rise in serum PRL in PSC rats remain unexplained, but may represent the unmasking of a THC-sensitive PRL-stimulatory mechanism; however further exploration of the possibility is necessary.

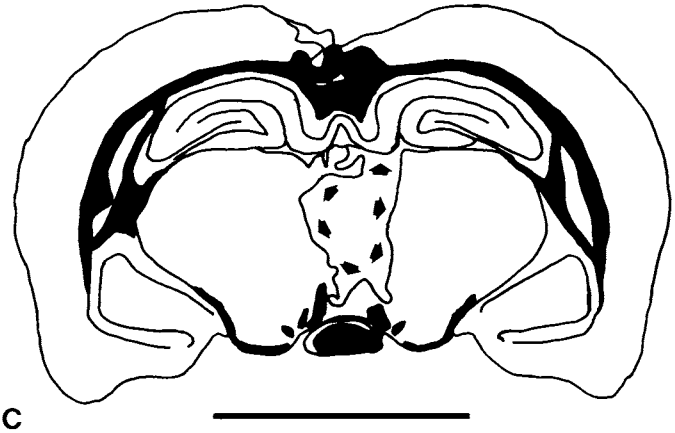
THC action at a site outside of the MBH with communication of its PRL suppressive effects over rostral afferent pathways into the MBH would be the interpretation most consistent with


PROLACTIN (ng/ml serum)
700
600
500
400
300
200
100
0
1200
1230
1300
1330
1400
1430
1500
COLONY TIME (hours)

A


ANT OLF NUC
NUC ACC
MPOA
AHA
PV
P
DV
SC
VM
ARC
M

B



C

FIGURE 11. (A) Serum PRL concentrations (mean +/- SE) on proestrus in DLFS rats. Vehicle alone (n = 6; .....) or 0.5 mg THC/kg BW (n = 7; _____) was injected iv at 1400 h. PRL levels were significantly decreased at all post-treatment times in the THC group relative to that at 1400 h ($p < 0.05$). Post-treatment PRL levels in the THC group were also significantly lower than those in the vehicle group ($p < 0.01$). Vehicle administration did not alter serum PRL levels ($p < 0.05$). (B) Sketch of the approximate location of lesions in DLFS rats. (C) Projection sketch of a frontal section of a representative brain from a DLFS rat at the level of the caudal portion of the mamillary nuclei. The bar beneath the section provides a 4 mm reference scale. The arrows indicate the margins of the lesion, a midline tissue-free space.

the data obtained from CHD and frontal cut animals. However, no indication is provided by these data as to whether the pertinent structures for THC action reside within the immediately rostral preoptic area or at other more distant sites. While earlier workers (7) found little regional localization of ^{3}H-THC within the rat brain after intraperitoneal injection of the labeled compound, Yashpal and Henry (8) more recently reported that after intravenous administration ^{3}H-THC was localized to the amygdala, striatum, cerebellum, and periaqueductal grey, but, notably, not to a hypothalamus or mesencephalic reticular formation, Thus an extra-hypothalamic site remote from the MBH for THC action appears quite tenable, and therefore, the effects of THC on serum PRL were determined in rats previously subjected to destruction of several extra-hypothalamic pathways believed to be functionally pertinent to the control of PRL secretion.

Septal lesions which interrupted the presumptive PRL-regulatory pathway from the orbitofrontal neocortex to the preop-

tic area by way of the lateral corticohypothalamic tract (9) failed to prevent the suppression of PRL after THC administration. In addition, although amygdaloid lesions can influence PRL secretion (10), lesioning of the ventral projections from the amygdala to the medial preoptic via the diagonal band of Broca, or sectioning of the dorsal stria terminalis pathway failed to prevent THC suppression of PRL secretion. Finally, midline lesions of the periventricular structures at the diencephalic-mesencephalic junction which included the dorsal longitudinal fasciculus interrupted the ascending PRL-regulatory pathway described by Tindal and Knaggs (9), but did not alter the suppressive effect of THC. These data suggest that the THC-induced suppression of serum PRL does not depend upon a site of action within the orbitofrontal neocortex or amygdala, and that if THC acts at a site in the midbrain, the essential rostral projections to the hypothalamus do not pass via fiber systems which are midline at the level of the diencephalic-mesencephalic junction.

In summary, the current data suggests that THC acts at a site or sites outside of the MBH to suppress PRL secretion in the rat, but that suppression does not appear to dependon hypothalamic projections from the orbitofrontal neocortex, amygdala, and a midbrain periventricular areas which have been implicated in the control of PRL secretion. Other extra-hypothalamic sites of potential importance in the preoptic, septal, hippocampal, mesencephalic and dorsal thalamic regions remain to be evaluated as to their possible role in the mediation of the PRL-suppressive action of THC.

ACKNOWLEDGMENTS

The data reported herein were submited by C.L.H. in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Duke University. The authors thank the National Institute of Arthritis, Diabetes and Digestive and Kidney Disease for the gift of materials used in the prolactin radioimmunoassay, and the National Institute on Drug Abuse for the gift of THC.

REFERENCES

1. Mechoulam, R., Marijuana Chemistry, Science 168:1159 (1970).
2. Kramer, J., and Ben-David, M., Suppression of prolactin secretion by acute administration of delta-9-THC in rats,

Proc. Soc. Exper. Biol. Med. 147:482 (1974).
3. Kramer, J., and Ben-David, M., Prolactin suppression by (-)-delta-9-tetrahydrocannabinol (THC): involvement of serotonergic and dopaminergic pathways, Endocrinol. 103:452 (1978).
4. Hughes, C. L., Everett, J. W., and Tyrey, L., Delta-9-Tetrahydrocannabinol suppression of prolactin secretion in the rat: lack of direct pituitary effect, Endocrinol. 109:876 (1981).
5. Ayalon, D., Nir, I., Cordova, T., Bauminger, S., Puder, M., Naor, Z., Kashi, R., Zor, U., Harell, A., and Lindner, H. R., Acute effects of delta-1-tetrahydrocannabinol on the hypothalamo-pituitary-ovarian axis in the rat, Neuroendocrinol. 23:31 (1977).
6. Asch, R. H., Smith, C. G., Siler-Kohdr, T. M., and Pauerstein, C. J., Acute decreases in serum prolactin concentrations caused by delta-9-tetrahydrocannabinol in non-human primates, Fert. Steril. 32:571 (1979).
7. Layman, J. M., and Milton, A. S., Distribution of tritium labelled delta-1-tetrahydrocannabinol in the rat brain following intraperitoneal administration, Brit. J. Pharmacol. 42:308 (1971).
8. Yashpal, K., and Henry, J. L., Delta-9-tetrahydrocannabinol: central distribution after intravenous administration in the awake rat, Fed. Proc. 38:1402 (ABS) (1979).
9. Tindal. J. S., and Knaggs, G. S., Pathways in the forebrain of the rat concerned with the release of prolactin, Brain Res. 119:211 (1977).
10. Peters, J. A., and Gala, R. R., The effect of corticomedial amygdaloid lesions on prolactin secretion in male and female rats, Endocrinol. 106:1665 (1980).