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Evidence for a Direct Anterior Pituitary Site of Delta-9-Tetrahydrocannabinol Action

LAURA L. MURPHY, SEAN C. NEWTON, JAYSHREE DHALI AND DAN CHÁVEZ*

*Department of Physiology and the *Department of Anatomy*
Southern Illinois University School of Medicine, Carbondale, IL 62901

MURPHY, L. L., S. C. NEWTON, J. DHALI AND D. CHÁVEZ. *Evidence for a direct anterior pituitary site of delta-9-tetrahydrocannabinol action.* PHARMACOL BIOCHEM BEHAV **40**(3) 603–607, 1991.—The effects of delta-9-tetrahydrocannabinol (THC), alone and in the presence of estradiol (E), on several estrogen-sensitive parameters in the immature female rat were examined, and it was demonstrated that THC administration antagonized the stimulatory effects of E on anterior pituitary weight and on both the secretion and pituitary content of prolactin. In the current study, the anterior pituitary gland was examined as a potential site of THC action in the ability of this cannabinoid to antagonize E-induced stimulation of pituitary function. A stimulatory dose of E (1 nM) significantly elevated prolactin levels in pituitary cells derived from either immature or retired breeder animals. Whereas THC (1 μM) alone had no effect on prolactin levels when compared to controls, THC completely prevented the E-induced increase in media prolactin levels. Moreover, THC blocked the ability of E to desensitize pituitary cells to the inhibitory influence of dopamine. Together with the findings that THC inhibited E-induced stimulation of total RNA synthesis in pituitary cell cultures, these data strongly suggest that THC antagonizes the stimulatory effect of E on the pituitary by a direct action at the adenohypophyseal level.

Delta-9-tetrahydrocannabinol Estradiol Anterior pituitary Prolactin Dopamine RNA synthesis

DELTA-9-TETRAHYDROCANNABINOL (THC), the principal psychoactive cannabinoid in marijuana, significantly alters many reproductive parameters in both female and male laboratory animals and in humans. In early studies, it was suggested that cannabis may contain estrogenic- or antiestrogenic-like activity which could explain the reproductive effects of this drug (Fig. 1). Cannabinoids and, in particular, THC, exhibit a number of estrogen-like actions, including stimulation of mammary gland development (10) and uterine weight (26) in the female and suppression of testicular, seminal vesicle, and prostate weights in the male (20). However, in other studies, administration of THC has been shown to block the estrogen-induced rise in serum prolactin at the time of the preovulatory gonadotropin surge in intact animals (2), and the surge of prolactin induced by estrogen priming in ovariectomized rats (11). Moreover, THC treatment reversed the ability of a low dose of estradiol (E) to facilitate or a high E dose to inhibit the release of luteinizing hormone (15). Together, these latter findings are compatible with the hypothesis that cannabinoids may act systemically as estrogen antagonists.

Estradiol is an important physiological regulator of prolactin production and acts directly on the pituitary gland to stimulate prolactin synthesis (17) and modulate the pituitary response to hypothalamic prolactin-releasing (5) and inhibitory factors (8). In contrast, THC is primarily inhibitory to prolactin release (18), and studies have suggested that THC may elicit its inhibitory effects via a central nervous system site of action (23). The effects of THC, alone and in the presence of E, on several estrogen-sensitive parameters in the immature female rat were examined, and it was demonstrated that THC administration antagonized the stimulatory effects of E on anterior pituitary weight and on both the secretion and pituitary content of prolactin (19), whereas THC alone had no effect on these parameters. Moreover, the ability of THC to antagonize E action did not appear to be mediated by THC-induced alterations in hypothalamic dopaminergic mechanisms (19). Therefore, the aim of the present study was to examine the anterior pituitary as a potential site of THC action in its ability to antagonize E-induced stimulation of prolactin synthesis and release.

METHOD

Pituitary Cell Culture

Pituitary glands were obtained from immature (26 days old) or retired breeder randomly cycling female rats (Harlan Sprague-Dawley, Inc.; Indianapolis, IN). Monodispersed cells were obtained according to previously published procedures (24) with some modifications. Briefly, pituitary glands were dissected free of the posterior lobe, and the anterior portion of the gland was minced under sterile conditions. Anterior pituitary tissue was placed in a flask containing 10 ml Dulbecco's modified Eagle medium-F12 (DMEM/F12), antibiotics, including 10,000 U/ml penicillin, 10 mg/ml streptomycin, and 25 μg/ml Amphotericin, 0.1% of both bovine serum albumin and trypsin (1:250). The contents were incubated for either 60 min (immature rat-derived tissue) or 120 min (retired breeder rat-derived tissue) in a Dubnoff shaking water bath at 37°C under a steady stream of 95% oxygen:5% carbon dioxide. Pituitary fragments were gently agi-

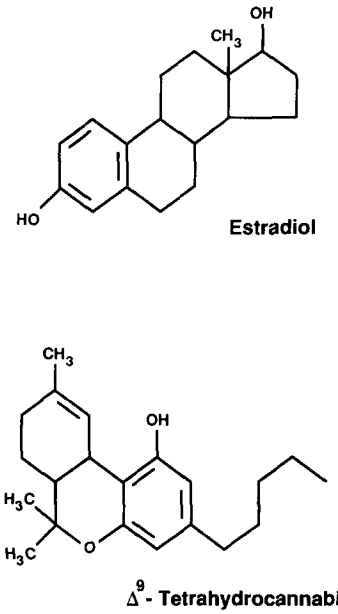

FIG. 1. Chemical structure of estradiol and THC.

tated by repeated aspiration with a siliconized Pasteur pipet every 30 or 45 min for immature or retired breeder rat-derived tissue, respectively. Dispersed cells were centrifuged at $200 \times g$ for 15 min, and the pellet was resuspended at a concentration of approximately 2×10^5 cells/ml in DMEM/F12 containing antibiotics, 5% fetal calf serum and 5% horse serum prior to plating. The cells were plated in 35-mm tissue culture dishes (Corning) previously coated with poly-L-lysine and allowed to incubate for 72 h in a water-jacketed tissue culture incubator (Forma) maintained at 37°C under 95% room air and 5% carbon dioxide at saturated humidity. The culture medium was replaced every 36 h. All culture materials were obtained from Sigma Co. (St. Louis, MO).

Experimental Paradigms

After the initial 72-h incubation, the plating medium was aspirated and each dish was washed twice with serum-free DMEM/F12 before the addition of medium containing either estradiol-17β (E; 1 nM), THC (1 μM), E+THC or neither of these substances. Cells were allowed to incubate in this treatment medium for 40 h. The E was first dissolved in absolute ethanol at a concentration of 10 mM. THC of greater than 95% purity was provided in an ethanol solution by the National Institute on Drug Abuse. Serial dilutions of each solution were made in DMEM/F12, resulting in a final concentration of ethanol not greater than 0.001%. Thus control dishes were treated with media containing 0.001% ethanol. After the 40-h incubation period, medium was harvested and stored at −20°C until assayed for prolactin. At this time, the dispersed pituitary cells from the retired breeder rats were washed twice with fresh DMEM/F12. Some of the culture dishes were further incubated in medium with or without dopamine (0.1 μM) for an additional 3 h. Medium was then harvested and stored for prolactin determination. In other dispersed cell cultures, the relative rates of RNA synthesis were determined as described below.

Incorporation of ^{3}H-Uridine

RNA synthesis was determined by measuring ^{3}H-uridine incorporation according to previously described procedures (4) modified for dispersed pituitary cells. Briefly, cells were cultured for 3 h in the presence of 200 μCi/ml of (5,6-^{3}H) uridine (New England Nuclear; Wilmington, DE) which had a specific activity >40 Ci/mmol. The plated cells were washed once with 0.1 M phosphate buffered saline (PBS; pH 7.4), after which 0.25 ml of a bovine serum albumin solution (0.4% in PBS) and 0.25 ml of 20 M urea containing 2% sodium dodecyl sulfate were added to each dish. After 20 min at room temperature, 0.5 ml of ice-cold 0.6 N sodium perchlorate was added to each dish followed by 2 ml of ice-cold 10% trichloroacetic acid (TCA). Each sample was applied onto a glass fiber filter (Whatman GF/A) under continued vacuum. Filters were then transferred to glass scintillation vials, and radioactivity was estimated using a Packard 1500 tricarb scintillation counter.

Prolactin Radioimmunoassay

Individual media samples were assayed for prolactin by use of a radioimmunoassay kit supplied by the NIADDK. The assay sensitivity was 0.02 ng/tube and the inter- and intraassay coefficients of variation were 10.1% and 4.0%, respectively, as determined by the measurement of prolactin in 25- and 50-μl aliquots of a rat plasma pool. Results were expressed in terms of the rat prolactin RP-3 reference preparation per milliliter of media.

Statistics

The results were subjected to one-way analysis of variance followed by the Newman-Keuls post hoc test to determine the statistical significance of differences between treatment means.

RESULTS

After a 40-h incubation period, media prolactin levels were significantly elevated in pituitary cell cultures exposed to a stimulatory dose of E (1 nM) when compared to controls (Fig. 2). The prolactin response to E stimulation was greater in immature rat-derived cells (53% increase) than in pituitary cells derived from retired breeder rats (19% increase). Incubation with THC (1 μM) alone had no effect on prolactin levels as compared with controls. In contrast, when THC was coincubated with E, the drug completely blocked the E-induced increase in media prolactin levels from both immature and retired breeder rat-derived cells.

Anterior pituitary glands from retired breeder rats were much larger than pituitary glands from immature rats (12.2 ± 0.6 mg vs. 2.9 ± 0.2 mg) and the cell yield, which included an enriched population of lactotrophs, was considerably greater. Thus subsequent studies were performed using pituitary cells derived from retired breeder rats. In one study, the effects of dopamine (10^{-7} M) on media prolactin levels after 3 h of incubation were investigated in cells that had been preincubated with either E, THC or E+THC for 40 hr (Fig. 3). Media prolactin levels were significantly increased in cell cultures pretreated with E and exposed to dopamine when compared to controls treated with dopamine. This suggests that the ability of dopamine to inhibit prolactin release was significantly lessened in cells preincubated with E. Whereas THC itself did not affect dopamine-induced inhibition of prolactin release into the media, coincubation of THC with E did prevent the ability of E preincubation to attenuate the inhibitory effects of dopamine on prolactin release.

Estradiol has a potent stimulatory effect on pituitary growth and hormone synthesis. In order to determine if THC directly

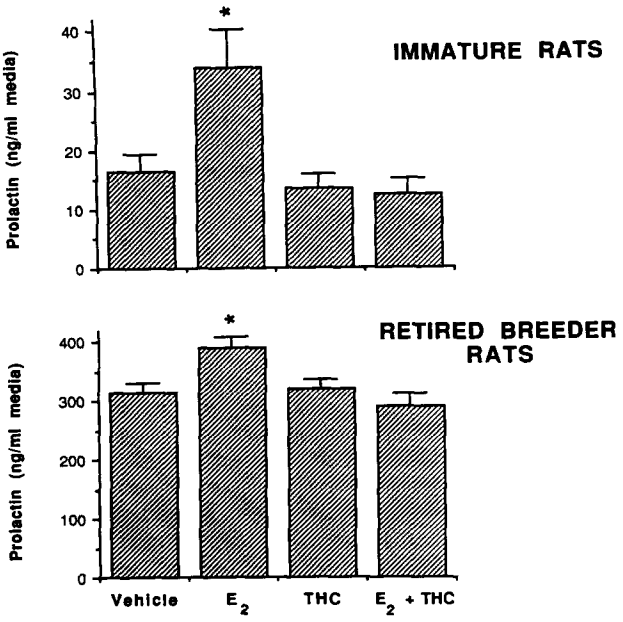

FIG. 2. Media prolactin levels from anterior pituitary cell cultures derived from either immature (top graph) or retired breeder female rats (bottom graph) and incubated with vehicle, estradiol (E; 1 nM), THC (1 μM) or E+THC for 40 h. *Denotes significance of $p<0.05$ when compared to vehicle control.

interferes with the ability of E to stimulate the pituitary gland, the relative rates of RNA synthesis in pituitary cells exposed to E, THC or E+THC were determined. A 40-h incubation with E significantly increased the amount of ^{3}H-uridine incorporation into pituitary cells (Fig. 4). Incorporation of ^{3}H-uridine was unaffected by THC treatment when compared to controls. However, the E-induced increase in uridine incorporation was completely prevented by concomitant E+THC treatment.

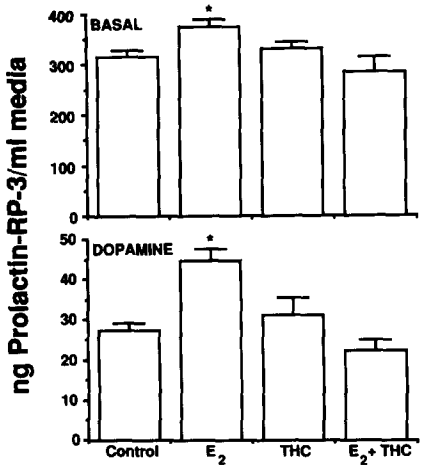

FIG. 3. Media prolactin levels from monodispersed anterior pituitary cell cultures derived from retired breeder female rats. Cultures were treated with vehicle, E (1 nM), THC (1 μM) or E+THC for 40 h and subsequent incubation for 3 h in media with or without dopamine (10^{-7} M). *Denotes significance of $p<0.05$ when compared to vehicle control.

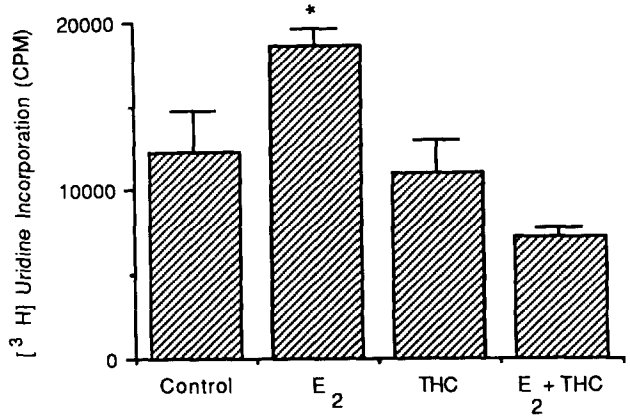

FIG. 4. ^{3}H-uridine incorporation (cpm) by anterior pituitary cells incubated with vehicle, E (1 nM), THC (1 μM) or E+THC for 40 h. *Denotes significance of $p<0.05$ when compared to vehicle control.

DISCUSSION

In an earlier study, it was demonstrated that in vivo treatment with THC inhibited the stimulatory effect of E on anterior pituitary weight and on the pituitary concentration and secretion of prolactin (19). Moreover, E-induced changes in hypothalamic dopaminergic activity were not antagonized by concomitant THC treatment, thus suggesting some other mechanism or site of THC action. The present findings strongly indicate that THC blocks the ability of E to enhance pituitary prolactin release by a direct action on the pituitary. When anterior pituitary cells were cultured from either immature or retired breeder female rats and were exposed to a similar paradigm of concomitant THC and E treatment as used for the in vivo studies, THC completely blocked the ability of E to stimulate prolactin release from either cell culture model. Although concomitant THC treatment is able to antagonize E action at the pituitary level, THC itself has no measurable effect on pituitary function in vitro or in vivo in the absence of E. This finding is in accordance with those of other investigators who have been unable to demonstrate a direct effect of cannabinoids on the unstimulated pituitary gland (11,23). It is interesting that the prolactin response to E was much greater from cells derived from immature female rats versus the retired breeder rats. However, it is known that the hypothalamo-pituitary axis of immature rats is much more sensitive to an E assault than the neuroendocrine system of the adult animal (28). The basal levels of prolactin in cultures derived from retired breeders were much greater than the basal levels of prolactin obtained from immature rats. This was most likely indicative of the hyperplasia and hypertrophy of lactotrophs which has occurred in the multiparous rat due to high fluctuating E levels (13). Because the retired breeder rat provided this enriched population of lactotrophs, cell cultures derived primarily from retired breeders were utilized for the remainder of the studies.

Estradiol increases the secretion of prolactin from the anterior pituitary gland by disrupting the tonic inhibition exerted on lactotrophs by dopamine, one of the most important physiological prolactin-inhibiting factors (14). It has been shown that estradiol can modulate hypothalamic dopaminergic activity (9) as

well as alter the sensitivity of the anterior pituitary gland to dopamine (22). It has been clearly demonstrated in earlier studies that THC does not antagonize the ability of E to decrease hypothalamic dopamine turnover rates (19), an index of hypothalamic dopaminergic activity. However, the present findings indicate that THC may interfere with E-induced desensitization of the anterior pituitary cells to dopamine by a direct pituitary site of action. The ability of E to attenuate dopamine inhibition of prolactin secretion from retired breeder rat pituitary cells was prevented by THC treatment. Although a possible mechanism by which E may act to desensitize the pituitary to dopamine involves an E-induced reduction in dopaminergic D_2 receptors (21), recent results have indicated that THC does not antagonize the ability of E to modulate dopamine D_2 receptors (19). An alternative possibility is that THC modifies E effects on membrane enzymes and cell product modulators. Estradiol increases cAMP production and binding in the pituitary (29) and attenuates dopamine-induced decreases in cAMP production (8). Moreover, THC has been shown to alter adenylate cyclase activity and intracellular cAMP concentrations (16). Thus the possibility that THC attenuates E-induced changes in the intracellular second messenger systems warrants further consideration.

The ability of THC, a highly lipid soluble compound, to interact with cell membranes and alter E uptake into the cell cannot be disregarded. Studies indicate that THC can alter steroid uptake and subcellular distribution, thus potentially altering nuclear steroid concentration and macromolecular synthesis (3). Examining ^{3}H-uridine incorporation into anterior pituitary cells, as an index of pituitary RNA synthesis, it was demonstrated that E significantly increased total RNA synthesis. Although THC has been shown to limit nucleotide uptake and transcriptional processes in vitro in other studies (27), there was found to be no effect of treatment with THC alone on uridine incorporation by pituitary tissue when compared to controls. However, coincubation with THC completely prevented E-stimulated uridine incorporation into cells. These results suggest that THC may interfere with the stimulatory effects of E on nuclear events leading to increased protein synthesis (i.e., prolactin synthesis) and pituitary growth (7,13) by a direct action on the pituitary. Whether THC attenuates the E response by limiting E uptake into the cell or nucleus or competes for specific E receptors, consequently limiting the binding of E and resulting in an attenuated E response, is unclear. Some investigators have been unable to demonstrate a competition of THC with E for binding to cytoplasmic uterine or hypothalamic binding sites (25).

Lastly, whereas E has been shown to modulate prolactin releasing factors (i.e., endogenous opiate peptides, TRH, VIP) which may play a role in the stimulation of prolactin by E (5), THC has been demonstrated to interfere with the action of endogenous opiates (12) and the secretion of TRH (1), which could lead to a suppression of prolactin secretion. Although the current data are clearly suggestive of a direct pituitary site of THC action in the ability of this cannabinoid to attenuate pituitary effects of E, there is also evidence to suggest that THC may act within the central nervous system to antagonize E action (6). Additional studies are needed to answer these and other questions concerning the site and mechanisms of THC action in its ability to antagonize E action.

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