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In vitro effect of Δ^9-tetrahydrocannabinol to stimulate somatostatin release and block that of luteinizing hormone-releasing hormone by suppression of the release of prostaglandin E_2

(*in vitro* hypothalamic incubation/dopamine/norepinephrine/prostaglandin E_2 synthesis and release)

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ABSTRACT **Previous *in vivo* studies have shown that Δ^9-tetrahydrocannabinol (THC), the principal active ingredient in marijuana, can suppress both luteinizing hormone (LH) and growth hormone (GH) secretion after its injection into the third ventricle of conscious male rats. The present studies were designed to determine the mechanism of these effects. Various doses of THC were incubated with either stalk median eminence fragments (MEs) or mediobasal hypothalamic (MBH) fragments *in vitro*. Although THC (10 nM) did not alter basal release of LH-releasing hormone (LHRH) from MEs *in vitro*, it completely blocked the stimulatory action of dopamine or norepinephrine on LHRH release. The effective doses to block LHRH release were associated with a blockade of synthesis and release of prostaglandin E_2 (PGE_2) from MBH *in vitro*. In contrast to the suppressive effect of THC on LHRH release, somatostatin release from MEs was enhanced in a dose-related manner with a minimal effective dose of 1 nM. Since PGE_2 suppresses somatostatin release, this enhancement may also be related to the suppressive effect of THC on PGE_2 synthesis and release. We speculate that these actions are mediated by the recently discovered THC receptors in the tissue. The results indicate that the suppressive effect of THC on LH release is mediated by a blockade of LHRH release, whereas the suppressive effect of the compound on growth hormone release is mediated, at least in part, by a stimulation of somatostatin release.**

Intensive investigations in lower animals and humans have indicated that Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana, has important effects on pituitary hormone secretion. Studies in intact male rats have demonstrated that THC injected into the third cerebral ventricle lowers plasma growth hormone (GH) and luteinizing hormone (LH) levels (1, 2). The principal site of action of THC to inhibit the release of GH and LH seems to be hypothalamic, since THC did not alter the release, content, or responsiveness of pituitary hormones to GH-releasing hormone and LHRH from cultured anterior pituitary cells (1, 2). The central site of action of THC is also supported by the finding that *in vivo* administration of THC elevated the LHRH content in the mediobasal hypothalamus (MBH) (1, 3) at a time when plasma LH levels were lowered.

It is known that the release of LHRH is stimulated by noradrenergic terminals in the MBH that release prostaglandin E_2 (PGE_2). This in turn stimulates the release of LHRH. PGE_2 increases LHRH release into peripheral (4, 5) and hypophyseal portal blood (6) and stimulates the release of LHRH from hypothalamic fragments (7). We hypothesized that the suppression of LH release by THC may be caused by a reduction of noradrenergic stimulation of PGE_2 and LHRH release since THC was shown to reduce norepinephrine turnover in the MBH (3). The release of GH, which is also suppressed by THC, is controlled by GH-releasing hormone and somatostatin (SRIF), and SRIF release is inhibited by PGE_2 (8).

Consequently, the present experiments were performed to determine the mechanisms by which THC affects the release of LHRH and SRIF from median eminence fragments (MEs) *in vitro*. The effect of THC was evaluated under both basal conditions and during stimulation by catecholamines. Measurements of release of PGE_2 and $PGF_{2\alpha}$ and the metabolism of [^{14}C]arachidonic acid in the MBH were also performed in the presence and absence of THC.

MATERIALS AND METHODS

Experimental Animals. Male (Sprague–Dawley) rats (230–270 g) (Holtzman, Madison, WI) were used as tissue donors. They were housed in group cages (10 rats per cage) under controlled conditions of light (14 hr of light/10 hr of dark) and temperature (24 ± 1°C); rat chow and water were provided ad libitum.

Experimental Procedures for Measurement of SRIF and LHRH. The rats were decapitated, their brains were removed, and MEs were dissected free under a stereoscopic microscope according to a described procedure (9). The tissue sample included only the ME and the proximal stump of the pituitary stalk. The MEs were incubated in Krebs–Ringer bicarbonate glucose buffer (pH 7.4) (KRBG) in an atmosphere of 95% O_2/5% CO_2 with constant shaking at 60 cycles per min at 37°C. The ethanol solution of THC of >95% purity was provided by the National Institute of Drug Abuse. Immediately before use, the alcohol was evaporated under N_2, and the residue was redissolved in a volume of ethanol such that the final ethanol concentration in the medium was 0.01%. The same concentration of ethanol was added to media of control flasks. Each flask contained one ME fragment in 500 μl of medium. In all cases, tissues were preincubated for 30 min, after which the medium was replaced by fresh medium containing different concentrations of THC (10 nM to 10 pM) and/or dopamine (50 μM) and/or norepinephrine (50 μM). Both dopamine and norepinephrine were obtained from Sigma. PGE_2 (Sigma) was added in one experiment at a concentration of 2.8×10^{-6} M to flasks also containing THC. Incubation was then carried out for 30 min.

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Abbreviations: THC, Δ^9-tetrahydrocannabinol; GH, growth hormone; LH, luteinizing hormone; LHRH, LH-releasing hormone; MBH, mediobasal hypothalamus; PG, prostaglandin; SRIF, somatostatin; ME, median eminence fragment.

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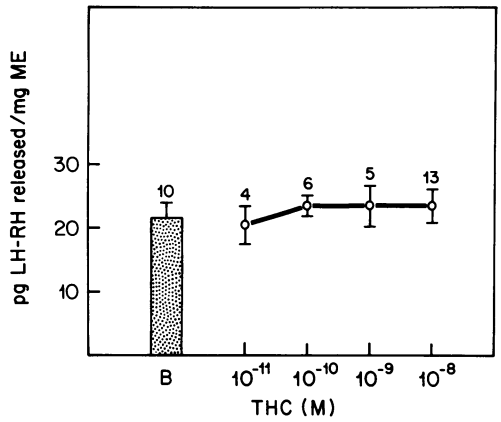

FIG. 1. Failure of THC to modify release of LHRH from MEs incubated *in vitro*. In this and other figures, bars (B) indicate basal release; vertical lines indicate SE; numbers above mean represent number of flasks incubated.

At the end of the incubations, the media were transferred to tubes (10 × 75 mm) and centrifuged at low speed at 4°C. Aliquots were assayed for SRIF or LHRH.

RIA of SRIF and LHRH. The amount of SRIF released into the incubation medium was determined by RIA according to a procedure previously described (9) with minor modifications. The highly specific antiserum (R-IIC) was generously provided by Louis De Palatis (Dow Chemical, Midland, MI). All results were expressed as pg of SRIF released per mg of incubated tissue.

LHRH was assayed as described (7) with antisera no. R11373 kindly provided by V. D. Ramirez (University of Illinois). The sensitivity of the assay was 0.6 pg per tube and the curve was linear up to 100 pg of LHRH.

Determination of Endogenous PG Release from the MBH. After removal of the brain, the MBH was dissected as described (7). The MBHs were incubated in KRBG as described above for MEs. Each flask contained one MBH in 2 ml of KRBG. In all cases, tissues were preincubated for 30 min, after which the medium was replaced by fresh medium containing different concentrations of THC (10–100 nM) and incubated for 1 hr. At the end of the incubation period, tissues were removed and the incubation media were stored frozen (−20°C) until PG determinations were performed. For PG extraction, the medium was thawed and extracted three times with 1 vol of ethyl acetate. Pooled ethyl acetate extracts were dried under a nitrogen atmosphere and the residue was resuspended in 1 ml of phosphate gelatin buffer (pH 7.4). Aliquots of the media (100 μl) were removed for RIAs of PGE_2 and $PGF_{2\alpha}$. The antisera were kindly provided by Harold Behrman (Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT). The sensitivities of the RIA for PGE_2 and $PGF_{2\alpha}$ were 15 and 25 pg, respectively. The assays were performed following the protocol described (10). In brief, incubations of samples overnight at 5°C were followed by separation of the bound and free PG by dextran-coated charcoal. The intra- and interassay coefficients of variation were 10% and 12%, respectively. Triplicate results were obtained for each biological sample assayed. The tritiated PGE_2 and $PGF_{2\alpha}$ were purchased from New England Nuclear. Standard curves were obtained with authentic PGE_2 or $PGF_{2\alpha}$ (Sigma) in the range of 12.5–6000 pg and only the linear part of the curve was used. The results are expressed as ng per mg of protein.

Metabolism of [^{14}C]Arachidonic Acid by MBH. After preincubation of MBHs for 30 min in KRBG as described above, they were incubated for 1 hr in 2 ml of medium containing 0.25 μCi of [^{14}C]arachidonic acid (New England Nuclear; 52.9 Ci/mol) with or without addition of THC (10–100 nM).

At the end of the incubation period, tissues were removed and the remaining incubation medium was acidified to pH 3.0 with 1.0 M HCl. The arachidonic acid metabolites were extracted two times with 2 ml of ethyl acetate. Pooled ethyl acetate extracts were dried under nitrogen. The residue was suspended in chloroform/methanol (2:1; vol/vol) and applied to silica gel thin-layer chromatography (TLC) plates. Prior to application of extracts, reference compounds 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, thromboxane B_2, and PGE_2 were placed on the plates. The plates were developed in a solvent system of benzene/dioxane/glacial acetic acid (60:30:3.0; vol/vol/vol). The position of authentic PGs was visualized by spraying the dried plates with 10% phosphomolybdic acid in ethanol, followed by heating at 110°C for 10 min; the plates were scraped off and ^{14}C radioactivity was measured by liquid scintillating counting. Average R_f values were 0.30 for 6-keto-

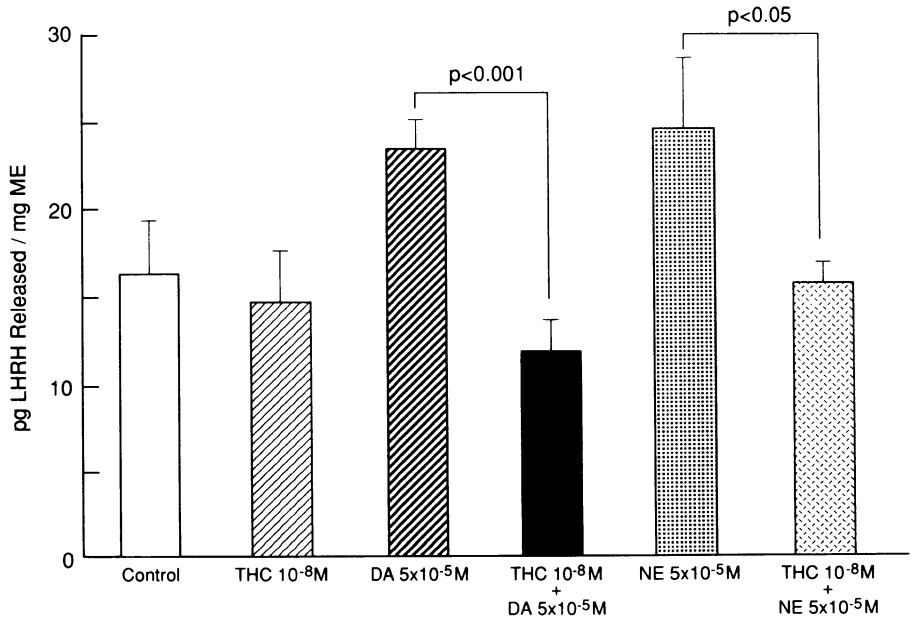

FIG. 2. Ability of THC to block catecholaminergic stimulation of LHRH release from MEs incubated *in vitro*. DA, dopamine; NE, norepinephrine. MEs were incubated either with THC, dopamine, or norepinephrine alone or with THC plus dopamine or norepinephrine.

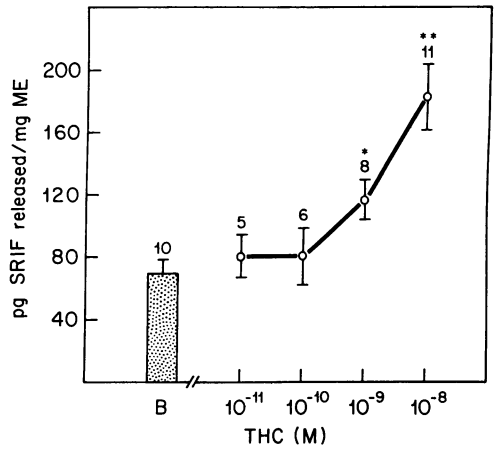

FIG. 3. Stimulation by THC of SRIF release from MEs incubated *in vitro*. *, $P < 0.05$ vs. control; **, $P < 0.01$ vs. control.

$PGF_{1\alpha}$, 0.35 for $PGF_{2\alpha}$, 0.47 for PGE_2, 0.57 for thromboxane B_2, and 0.80 for arachidonic acid. Results were expressed as percentage conversion of [^{14}C]arachidonic acid per mg of MBH over 60 min.

Statistics. Data were analyzed by one-way analysis of variance and the Student–Newman–Keuls multiple comparison test for unequal replicates. Differences with P values <0.05 were considered significant.

RESULTS

Effect of THC on Release of LHRH from ME Fragments. There was no effect of various concentrations of THC on the basal release of LHRH from ME fragments (Fig. 1). As reported (7), both dopamine (50 μM) and norepinephrine (50 μM) stimulated the release of LHRH from ME fragments. This release was completely inhibited by THC (10 nM) (Fig. 2). On the other hand, when PGE_2 (2.8×10^{-6} M) was added to the medium in the presence of THC (10 nM), the release of LHRH from ME fragments was significantly enhanced {THC alone = 15.9 ± 3.9 [mean ± SEM; no. of flasks (n) = 4] vs. THC + PGE_2 = 33.1 ± 2.6 pg per mg of ME, [n = 10]; $P < 0.01$}.

Effect of THC on Release of SRIF from ME Fragments. In contrast to the lack of effect on basal release of LHRH by various doses of THC, THC evoked a dose-related stimulation of SRIF release from ME fragments with a minimal effective dose of 1 nM (Fig. 3).

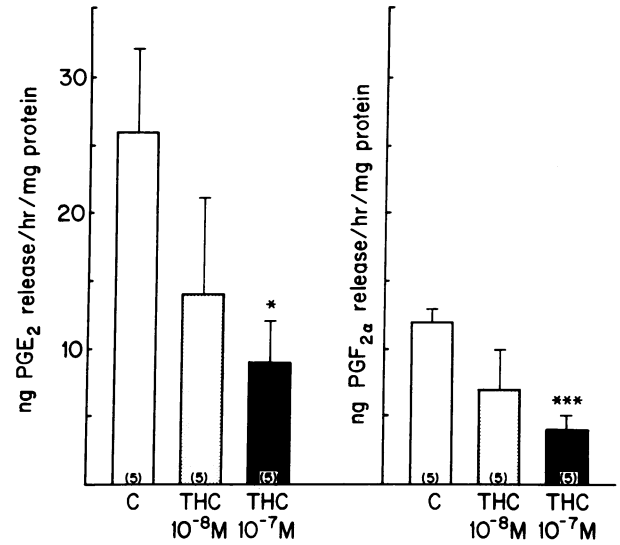

FIG. 4. Effect of THC on the release of PGE_2 and $PGF_{2\alpha}$ from MEs. *, $P < 0.05$ vs. control (C); ***, $P < 0.001$ vs. control. Number of flasks is indicated in parentheses.

Effect of THC on Metabolism of Arachidonic Acid and Release of PGs from Hypothalamic Fragments. The release of both PGE_2 and $PGF_{2\alpha}$ from ME fragments was suppressed by THC and the suppression reached significance at a concentration of 100 nM (Fig. 4).

THC also blocked the conversion of arachidonic acid into all metabolites studied, which included 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, thromboxane B_2, and PGE_2. The effect was nearly complete even at the lowest concentration tested (10 nM THC) (Fig. 5).

DISCUSSION

Previous *in vivo* studies have shown that the intraventricular injection of THC can suppress the secretion of both LH (1) and GH (2). The present *in vitro* studies show that the compound suppresses catecholamine-stimulated LHRH release while stimulating the resting release of SRIF in a dose-related fashion. Therefore, at least part of the suppressive action of THC on the release of LH and GH may be mediated by suppression of LHRH release plus stimulation of SRIF release. It will be of interest to determine whether the

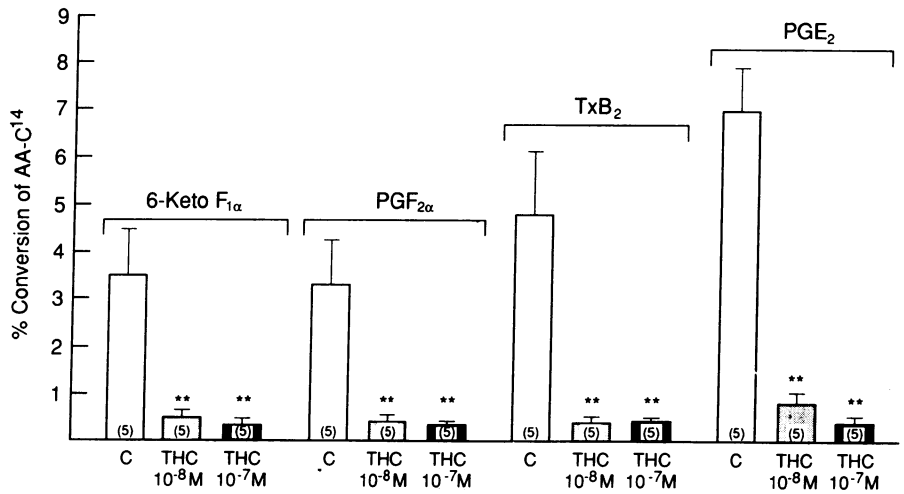

FIG. 5. Effect of THC on the conversion of [^{14}C]arachidonic acid (AA-C^{14}) to various metabolites: 6-Keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, thromboxane B_2 (TxB_2), and PGE_2; C, control. **, $P < 0.01$ vs. control. Number of flasks is indicated in parentheses.

compound also inhibits the release of GH-releasing hormone *in vitro*.

In earlier studies, intravenous administration of THC at doses that produced marked behavioral changes, including catatonia sedation, plus hypothermia produced a significant reduction of PGE_2-like material in the hypothalamus but not in other brain areas, which suggested that the mechanism by which THC alters hypothalamic hormone release might be via a decrease of hypothalamic PG synthesis (11, 12). The current studies provide evidence that the mechanism for the suppression of LHRH release is the suppression of not only the synthesis of PGs but also their release. On the basis of previous studies it appears that the release of LHRH provoked by catecholamines is brought about by liberation of PGE_2 (7), and the synthesis and release of this PG were suppressed by THC. Furthermore, the addition of PGE_2 in the presence of THC could reverse the inhibition of catecholamine-induced LHRH release, which indicates that the tissue was responsive and places the THC block at the prostaglandin step in catecholamine-induced LHRH release. On the other hand, PGE_2 was inhibitory to dopamine-induced somatostatin release (8). Therefore, inhibition of PGE_2 release could account for the stimulation of somatostatin release induced by THC. The concentrations of THC to induce these effects *in vitro* compare favorably with those that probably existed in hypothalamic tissue in previous *in vivo* studies (1–3).

Recent studies indicate that the brain and hypothalamus contain THC receptors (13, 14). Presumably, the action of the compound to inhibit PG synthesis and release may be a receptor-mediated event. Thus, these studies provide important insights into the mechanism by which THC suppresses the release of both LH and growth hormone by hypothalamic action. It is possible that these studies may provide a mechanism of action of THC that is widespread throughout the nervous system and could account for many of its actions on the brain.

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