

## Cannabinoid ligand–receptor signaling in the mouse uterus

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Communicated by Robert L. Sinsheimer, University of California, Santa Barbara, CA, January 30, 1995 (received for review December 6, 1994)

**ABSTRACT** Using RNA (Northern) blot hybridization and reverse transcription–PCR, we demonstrate that the brain-type cannabinoid receptor (CB1-R) mRNA, but not the spleen-type cannabinoid receptor (CB2-R) mRNA, is expressed in the mouse uterus and that this organ has the capacity to synthesize the putative endogenous cannabinoid ligand, anandamide (arachidonyl ethanolamide). The psychoactive cannabinoid component of marijuana— $\Delta^9$ -tetrahydrocannabinol (THC)—or anandamide, but not the inactive and nonpsychoactive cannabidiol (CBD), inhibited forskolin-stimulated cyclic AMP formation in the mouse uterus, which was prevented by pertussis toxin pretreatment. These results suggest that uterine CB1-R is coupled to inhibitory guanine nucleotide-binding protein and is biologically active. Autoradiographic studies identified ligand binding sites ( $[^3\text{H}]$ anandamide) in the uterine epithelium and stromal cells, suggesting that these cells are perhaps the targets for cannabinoid action. Scatchard analysis of the binding of  $[^3\text{H}]$ WIN 55212-2, another cannabinoid receptor ligand, showed a single class of high-affinity binding sites in the endometrium with an apparent  $K_d$  of 2.4 nM and  $B_{\text{max}}$  of  $5.4 \times 10^9$  molecules per mg of protein. The gene encoding lactoferrin is an estrogen-responsive gene in the mouse uterus that was rapidly and transiently up-regulated by THC, but not by CBD, in ovariectomized mice in the absence of ovarian steroids. This effect, unlike that of  $17\beta$ -estradiol ( $E_2$ ), was not influenced by a pure antiestrogen, ICI 182780, suggesting that the THC-induced uterine lactoferrin gene expression does not involve estrogen receptors. We propose that the uterus is a new target for cannabinoid ligand–receptor signaling.

Marijuana has been used for centuries as a psychoactive drug.  $\Delta^9$ -Tetrahydrocannabinol (THC) is the major psychoactive component of marijuana. Marijuana and its cannabinoid derivatives induce a wide spectrum of effects including analgesia, antiemetic, anticonvulsion, attenuation of intraocular pressure in glaucoma, antiinflammation, and immunosuppression (1). One of the major concerns of habitual marijuana smoking or exposure to cannabinoid derivatives is their reported adverse effects on reproductive functions, including fetal loss, pregnancy failure, retarded embryonic development, and reduced fertilizing capacity of sperm (reviewed in refs. 2–5). The mechanisms by which these effects of cannabinoids are mediated are poorly defined. Furthermore, controversies still prevail surrounding the “estrogenic–antiestrogenic” effects of cannabinoids in uterine biology (2, 6, 7) and their interactions with uterine estrogen receptors (8, 9). Previous identification of guanine nucleotide-binding protein (G-protein)-coupled cannabinoid receptors together with the recent cloning of these cannabinoid receptors in the brain (CB1-R) and spleen (CB2-R) (10–12) have begun to provide evidence that many of the central and peripheral effects of cannabinoids are likely to be mediated via these receptors. Furthermore, the identification of a putative endogenous cannabinoid ligand, anandamide (arachidonyl ethanolamide), in the brain suggests that canna-

binoid ligand–receptor signaling may be normally operative in the central nervous system (13, 14), although the physiological significance of this signaling pathway is yet to be defined.

The CB2-R gene has been shown to be expressed only in the spleen, while the CB1-R gene is primarily expressed in the brain (11, 12). The other tissues that have been shown to express CB1-R mRNA are the testis, spleen, and peripheral blood leukocytes (15–17). The expression of cannabinoid receptors in the spleen and leukocyte has been associated with the antiinflammatory and immunosuppressive roles of cannabinoids (16, 17), while the observation of reduced fertilizing capacity of sperm exposed to cannabinoid ligands is consistent with the detection of CB1-R mRNA in the testis and cannabinoid binding sites in the sperm (4, 5, 15). On the other hand, the effects and mode of action of cannabinoids in uterine biology and pregnancy remain largely undefined and controversial in spite of the numerous reports published in this field during the last two decades (2, 3). In the present investigation, we sought to examine whether functional cannabinoid receptors are expressed in the uterus and whether this organ has the capacity to synthesize anandamide. We further examined whether THC can influence uterine functions at the molecular level by examining the expression of the gene encoding lactoferrin (LF), an estrogen-responsive gene in the mouse uterus.

### MATERIALS AND METHODS

**Northern Blot Hybridization of CB1-R mRNA.** Uteri from CD-1 mice on days 1, 4, and 7 of pregnancy (day 1 = vaginal plug) were used in this study to determine whether the changing hormonal status of the mother and corresponding changes in uterine physiology alter the expression of CB1-R mRNA (18). Total RNA from the mouse brain or uteri was extracted by using a modified guanidine thiocyanate procedure (19). Poly(A)<sup>+</sup> RNA was isolated by oligo(dT)-cellulose column chromatography (19). A 1.17-kb fragment encoding 97–1271 nucleotides of the rat CB1-R cDNA clone SKR6 was subcloned into a pGEM vector (11). A cDNA clone of human fibroblast cytoplasmic  $\beta$ -actin was subcloned into a pGEM vector (20). For Northern hybridization, antisense  $^{32}\text{P}$ -labeled complementary RNA probes were generated. Probes had specific activities of about  $2 \times 10^9$  dpm/ $\mu\text{g}$ . Total RNA or poly(A)<sup>+</sup> RNA was denatured, separated by formaldehyde/agarose gel electrophoresis, transferred to nylon membranes, and UV-cross-linked. Northern blots were prehybridized, hybridized, and washed as described (19). Transcripts were detected by autoradiography. Each blot was reprobated with  $\beta$ -actin to ensure RNA integrity.

**Reverse Transcription (RT)–PCR Analysis of CB1-R and CB2-R mRNAs.** For RT–PCR analysis, the following primers were used: 5′-GGAGAACATCCAGTGTGGGG-3′ (sense) and 5′-CATTGGGGCTGTCTTTACGG-3′ (antisense) for

Abbreviations: CB1-R, brain-type cannabinoid receptor; CB2-R, spleen-type cannabinoid receptor; THC,  $\Delta^9$ -tetrahydrocannabinol; CBD, cannabidiol; LF, lactoferrin; RT–PCR, reverse transcription–PCR; G protein, guanine nucleotide-binding protein; G<sub>i</sub> protein, inhibitory G protein; E<sub>2</sub>,  $17\beta$ -estradiol.

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the CB1-R transcript (11) and 5'-CCTGTTGAAGATCGGC-AGCG-3' (sense) and 5'-GGTAGGAGATCAACGCCG-AG-3' (antisense) for the CB2-R transcript (12). The internal oligonucleotides 5'-GGTTCTGGAGAACCTACTGG-3' and 5'-TGGGCAGCCTGCTGACT-3' were used for Southern hybridization of the amplified products for CB1-R and CB2-R, respectively. The  $\beta$ -actin sense and antisense primers designed from the mouse  $\beta$ -actin cDNA were 5'-GTGGGCCGCTCTAGGCACCAA-3' and 5'-CTCTTTGATGTCACGCA-CGATTTC-3', respectively (21). An internal oligonucleotide, 5'-CCACGGGCATTGTGATGGAC-3', was used for Southern analysis. RNA from brain, spleen, or uterus was isolated (19, 22). Total RNA (1  $\mu$ g) from each sample was reverse-transcribed by using specific antisense primers. A portion (one-third) of the RT products were subjected to PCR amplification by using the sense and antisense primers in the buffer as described by us (22). PCR cycle parameters were as follows: 94°C for 4 min, 55°C for 1.5 min, and 72°C for 2.5 min for the first cycle followed by 94°C for 1 min, 55°C for 1.5 min, and 72°C for 2.5 min for a total of 35 cycles. One-tenth of the total amplified product was run on 1.5% agarose gels, stained with ethidium bromide, and blotted for Southern hybridization. Experimental and negative control reactions were run simultaneously.

**Analysis of Forskolin-Stimulated cAMP Accumulation.** Day 1 pregnant uteri were homogenized in 1 ml of 50 mM Tris (pH 7.5) containing 1 mM EGTA and 0.5 mM 3-isobutyl-1-methylxanthine and centrifuged at 2000  $\times$  *g* for 15 min. The supernatant was centrifuged at 100,000  $\times$  *g* for 1 h to obtain membranes. Membranes were resuspended, and an aliquot (350  $\mu$ g of protein) was incubated in 1 ml of the same buffer containing 10  $\mu$ M GTP, 1 mM ATP, and 3 mM MgSO<sub>4</sub> with or without forskolin (1–5  $\mu$ M) at 25°C for 10 min to determine its maximal stimulatory concentration. To assess the effects of agonists, THC (0.1–10  $\mu$ M), cannabidiol (CBD; 0.1–10  $\mu$ M), or anandamide (0.1–10  $\mu$ M) was added to the incubation mixture with 5  $\mu$ M forskolin. To determine that the effects of the agonists were coupled to the inhibitory G protein (G<sub>i</sub>), preincubation with pertussis toxin at 5 ng/ml for 5 min was performed before adding forskolin and THC. Reactions were terminated by boiling (5 min) followed by centrifugation. The cAMP contents of the supernatants were determined by protein-binding assay (23, 24). Results are means  $\pm$  SEM of four or five experiments except for the experiments with CBD or pertussis toxin pretreatment, which were carried out twice.

**Autoradiographic Localization of Ligand-Binding Sites.** Autoradiographic detection was performed with [<sup>3</sup>H]anandamide (22). Frozen uterine sections (10  $\mu$ m) mounted onto poly(L-lysine)-coated slides were incubated with 4.5 nM [<sup>3</sup>H]anandamide (specific activity, 221 Ci/mmol; DuPont/NEN; 1 Ci = 37 GBq) in 50 mM Tris-HCl buffer (pH 7.4) containing 5% bovine serum albumin (BSA) in the absence or presence of 1000-fold molar excess of unlabeled anandamide for 4 h at 37°C. After incubation, sections were washed five times in the same buffer containing 1% BSA at 4°C, air-dried, and subjected to autoradiography (22). Autoradiographic exposure was for 7 days. Dark-field photomicrographs at  $\times$ 40 magnification are shown. White grains indicate the binding sites.

**Radioligand-Binding Studies.** Uterine epithelial-stromal cell mixtures were obtained from day 4 pregnant mice by gently scraping the endometrium from the myometrium. Membrane preparations and binding studies were performed as described (15). In brief, membranes (150  $\mu$ g of protein) were incubated for 1 h at 30°C in 1 ml of binding buffer (15) containing tritiated WIN 55212-2 (45.5 Ci/mmol; DuPont/NEN) ranging from 0.5 to 5.0 nM. Nonspecific binding was determined by performing parallel binding reactions in the presence of 1  $\mu$ M unlabeled WIN 55212-2. After incubation, membranes were filtered on glass fiber filters (G6; Fisher Scientific) and washed

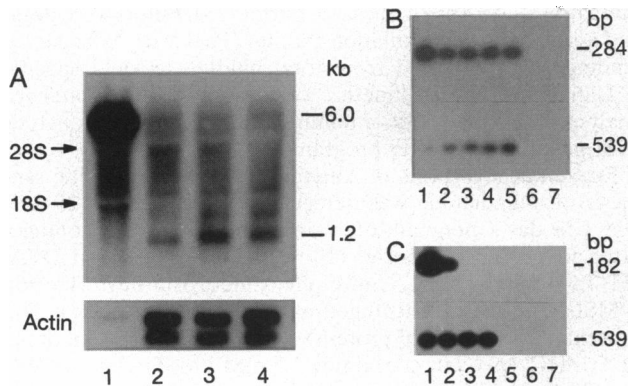
three times with 5 ml of the same buffer (15). Filters were dried and assayed in a  $\beta$ -scintillation counter (Packard). Nonspecific binding was subtracted from total binding to yield specific binding. The binding kinetics were calculated by Scatchard analysis with ENZFITTER, a nonlinear regression data analysis IBM personal computer program for ligand binding.

**Enzymatic Synthesis of Anandamide.** The enzymatic synthesis of anandamide was determined as described (25–27). Day 1 or day 4 pregnant uteri or brain tissues were homogenized in ice-cold TE buffer (10 mM Tris-HCl/1 mM EDTA, pH 7.6) containing 1.5 mM phenylmethylsulfonyl fluoride (PMSF) and were centrifuged at 2000  $\times$  *g* for 15 min. The supernatants (500  $\mu$ g of protein) were incubated in 1 ml of 0.1 M Tris-HCl (pH 9.0) containing 1.5 mM PMSF with 1  $\mu$ Ci of [5,6,8,9,11,12,14,15-<sup>3</sup>H(N)]arachidonic acid (specific activity, 221 Ci/mmol; DuPont/NEN) in the presence or absence of 7 mM ethanolamine at 37°C for 1 h. The reaction mixture was extracted with 2 ml of chloroform/methanol, 1:1 (vol/vol). The organic phase was removed, dried under N<sub>2</sub>, and redissolved in 10  $\mu$ l of 1:1 chloroform:methanol. The samples were spotted onto silica gel-coated plates and run parallel with <sup>3</sup>H-labeled standards. TLC was performed in a solvent system consisting of the organic phase derived from ethyl acetate/hexane/acetic acid/water, 100:50:20:100 (vol/vol), or diethylether/methanol/water, 100:3:0.5 (vol/vol). The plate was sprayed with autoradiographic enhancer (EN<sup>3</sup>HANCE; DuPont/NEN) and exposed to an x-ray film at –70°C for 1–2 days. Reactions run with heat-treated tissue extracts showed no conversion of arachidonic acid to anandamide in the presence of ethanolamine (data not shown).

**Analysis of LF Gene Expression.** Mice were ovariectomized without regard to their estrous cycle and rested for 2 weeks. To determine the acute effects of THC, they were treated with an injection of various doses of THC and killed at the indicated times. To determine whether the THC effects were mediated via uterine estrogen receptors, ovariectomized mice were given an injection of 17 $\beta$ -estradiol (E<sub>2</sub>; 250 ng per mouse) or THC (2.5 mg/kg of body weight) with or without a prior (30 min) injection of a pure antiestrogen, ICI 182780 (25  $\mu$ g per mouse), and were killed at the indicated times. THC, E<sub>2</sub>, or ICI 182780 was dissolved in sesame oil and injected (0.1 ml per mouse) subcutaneously. Ovariectomized mice treated with oil (0.1 ml per mouse) served as controls. Total uterine RNA (2  $\mu$ g) was subjected to Northern blot hybridization with a mouse LF complementary RNA probe as described (28). The same blot was reprobbed with  $\beta$ -actin probe, and duplicate gels were stained with acridine orange to ensure RNA integrity and equal loading. After hybridization, the blots were subjected to autoradiography and radioimage quantitation by using the radioanalytic image system (AMBIS).

## RESULTS AND DISCUSSION

**Analysis of Cannabinoid Receptor mRNAs in the Uterus.** As reported (11), Northern blot analysis detected a 6.0-kb transcript in total brain or pregnant uterine poly(A)<sup>+</sup> RNA samples. The abundance of CB1-R mRNA was markedly lower in the uterus. However, a predominant 1.2-kb transcript was detected in the uterus and showed a higher accumulation on days 4 and 7 of pregnancy than that on day 1 (Fig. 1A). Whether this smaller transcript is the result of alternate splicing or represents a truncated form of the receptor is not known. RT-PCR also detected CB1-R mRNA in the uterus, confirming the results of Northern blot hybridization (Fig. 1B). This mRNA was detected in the uterus during early pregnancy and in ovariectomized uteri before and after THC treatment (Fig. 1B). In contrast, RT-PCR could not detect CB2-R mRNA in the uterus, although this mRNA was detected in the rat or mouse spleen (Fig. 1C).



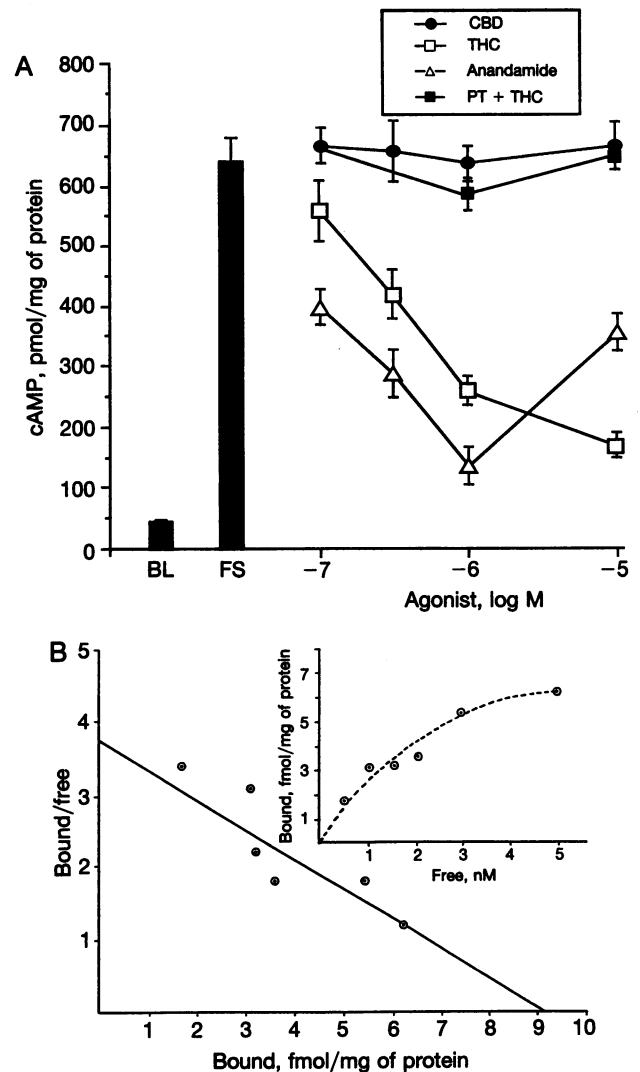
**FIG. 1.** Analysis of cannabinoid receptor transcripts in the uterus. (A) Northern blot analysis of CB1-R. Lanes: 1, total RNA (6.0  $\mu$ g) from the whole brain; 2–4, poly(A)<sup>+</sup> uterine RNA (10  $\mu$ g) from days 1, 4, and 7 of pregnancy, respectively. The blot was reprobed with  $\beta$ -actin. Autoradiographic exposures were 2 days for CB1-R and 2 h for  $\beta$ -actin. (B) Southern analysis of RT-PCR-amplified products of CB1-R (284 bp) and  $\beta$ -actin (539 bp). Lanes: 1, brain; 2, day 1 pregnant uterus; 3, day 4 pregnant uterus; 4, ovariectomized uterus; 5, THC-treated ovariectomized uterus; 6, brain RNA without the RT reaction, and 7, primer control. After 2 weeks of rest, adult ovariectomized mice were given a subcutaneous injection of sesame oil (0.1 ml) or THC (2.5 mg/kg of body weight in oil) and killed 6 h later. (C) Southern analysis of RT-PCR-amplified products of CB2-R (182 bp) and  $\beta$ -actin (539 bp). Lanes: 1, rat spleen; 2, mouse spleen; 3, day 1 pregnant uterus; 4, day 4 pregnant uterus; 5 and 6, rat and mouse spleen RNA without the RT reaction, respectively; 7, primer control.

**Effects of Cannabinoid Agonists on Forskolin-Stimulated cAMP Accumulation in the Uterus.** To examine whether the cannabinoid receptor expressed in the uterus, like that in the brain, was coupled to a G protein ( $G_i$ ) (11) and biologically active, the effects of cannabinoid ligands (THC or anandamide) on forskolin-stimulated cAMP accumulation in uterine membrane preparations were monitored with or without pertussis toxin pretreatment. The results show that these ligands inhibited forskolin-stimulated cAMP accumulation in a dose-dependent manner, and this inhibition was prevented by pertussis toxin pretreatment (Fig. 2A). Anandamide was more potent than THC in this response. However, as observed for other cannabinoid ligands (29), anandamide was less inhibitory at higher concentrations. These effects of THC or anandamide appear to be specific, since an inactive and nonpsychoactive cannabinoid, CBD, did not alter this response (Fig. 2A).

**Analysis of WIN 55212-2 Binding Kinetics in the Endometrium.** Although 60% of the binding in the endometrium, like that observed in other systems (15), was nonspecific, analysis of the equilibrium binding data (Fig. 2B) indicates a single class of high-affinity binding sites with an apparent  $K_d$  of 2.4 nM and  $B_{max}$  of  $5.4 \times 10^9$  molecules per mg of protein. The Hill coefficient was calculated to be 1, suggesting a single class of receptor sites and an absence of cooperative interactions. Although the binding affinity of Win 55212-2 appears to be higher in the endometrium, the binding capacity is considerably lower than that obtained with cerebellar sections (30).

**Analysis of Autoradiographic Binding Sites in the Uterus.** The possible sites of cannabinoid action in the uterus were determined by examining the [<sup>3</sup>H]anandamide-binding sites by autoradiography in frozen uterine sections from day 1 and day 4 pregnant mice. Specific binding sites were primarily noted in uterine epithelial and stromal cells (Fig. 3).

**Analysis of Anandamide Synthesis in the Uterus.** The results demonstrate that the uterus, like the brain, has the capacity to synthesize anandamide in the presence of ethanolamine (Fig. 4); about 7% of the [<sup>3</sup>H]arachidonic acid was converted to anandamide by the brain or uterine homogenates. Similar results were obtained with the use of two different solvent



**FIG. 2.** Characterization of cannabinoid receptors in the uterus. (A) Effects of cannabinoid agonists on forskolin-stimulated cAMP accumulation in the day 1 pregnant uterus. BL, basal; FS, forskolin-stimulation; PT, pertussis toxin pretreatment. (B) Scatchard analysis of tritiated WIN 55212-2 binding in day 4 pregnant endometrial membranes. (Inset) Equilibrium binding kinetics. Results shown are the average of duplicate experiments.

systems in TLC analysis as described in *Materials and Methods*. Furthermore, as observed for the brain (25), uterine microsomal preparations exhibited a markedly enhanced synthesizing capacity (data not shown). Of the tissues previously examined (25–27), the brain has been shown to exhibit the highest anandamide-synthesizing capacity. However, our data provide evidence that the anandamide-synthesizing capacity of the uterus is comparable to that of the brain. Although the uterus has the capacity to synthesize anandamide, it is not yet established whether this synthesis occurs *in vivo*. Arachidonic acid and ethanolamine are likely to have cooperative effects on anandamide synthesis, suggesting coactivation of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and phospholipase D (PLD) (25, 27). Although PLA<sub>2</sub> activity is present in the rodent uterus and is regulated by steroid hormones (31), the presence of PLD has not yet been examined. It is to be noted, however, that an alternative pathway for the formation of anandamide through phosphodiesterase-mediated cleavage of *N*-arachidonoyl-phosphatidylethanolamine has recently been described in cultured rat neurons (32).

**Effects of THC on Uterine LF Gene Expression.** To determine whether THC can affect uterine functions at the molec-



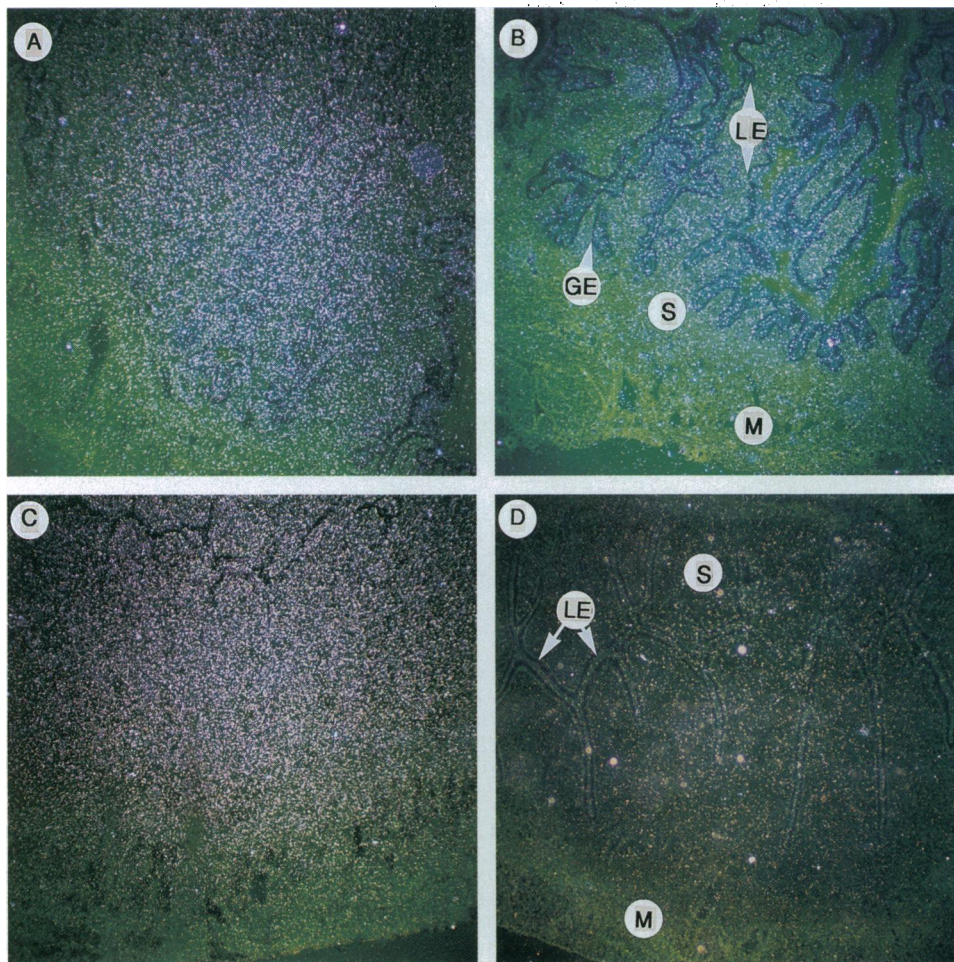


FIG. 3. Autoradiographic binding sites of [<sup>3</sup>H]anandamide in the pregnant uterus. (A and B) Day 1 pregnant uterus. (C and D) Day 4 pregnant uterus. (B and D) Non-specific binding in the presence of unlabeled anandamide. LE, luminal epithelium; GE, glandular epithelium; S, stroma; M, myometrium. ( $\times 25$ .)

ular level, the expression of the gene encoding LF, an estrogen-responsive gene in the mouse uterus (28, 33), was examined by Northern blot hybridization before and after an injection of THC in adult ovariectomized mice. LF is an iron-binding glycoprotein that has been proposed to be involved in immunomodulation and growth promotion (33, 34). THC rapidly and transiently upregulated the levels of uterine LF mRNA (Fig. 5A). This drug at 0.5, 2.5, or 10 mg/kg of body weight increased the mRNA levels 2- to 4-fold within 2 h, whereas it did not influence the mRNA levels at 0.1 mg/kg until 6 h (3.5-fold) after the injection. The maximal induction of LF

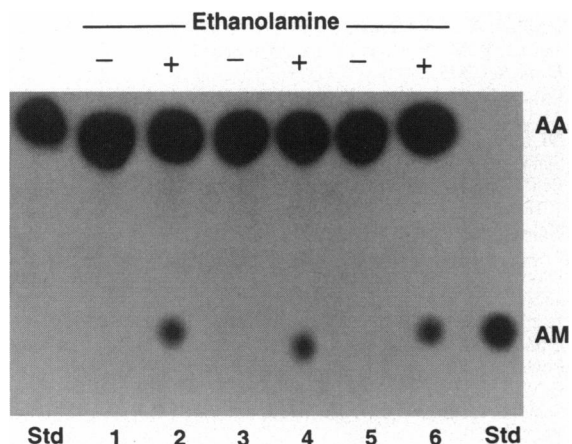


FIG. 4. The synthesis of anandamide catalyzed by the brain or uterine homogenates. Lanes: 1 and 2, day 1 pregnant uterus; 3 and 4, day 4 pregnant uterus; 5 and 6, brain homogenates. Standards of [<sup>3</sup>H]arachidonic acid (AA) and [<sup>3</sup>H]anandamide (AM) are shown.

mRNA levels ( $\approx 5$ -fold) by 0.5 or 2.5 mg/kg occurred at 6 h. However, the levels obtained 2 h after an injection of THC at 10 mg/kg declined by 4 h. These results suggest that the up-regulation of LF mRNA levels in the uterus by THC is time- and dose-dependent. This THC response was specific, since an inactive cannabinoid, CBD (at 0.5 or 2.5 mg/kg), did not induce the uterine LF gene (data not shown). The responses of the uterine LF gene to THC are different from those observed for E<sub>2</sub>. As reported previously (28), the major accumulation of uterine LF mRNA began 12 h after an injection of E<sub>2</sub>. As expected, this E<sub>2</sub>-mediated response was attenuated by pretreatment with a pure antiestrogen, ICI 182780 (35). In contrast, the THC-mediated effect was not influenced by this antiestrogen (Fig. 5B). This suggests that THC influenced this uterine gene by a mechanism not involving the estrogen receptor. In addition, THC did not compete with E<sub>2</sub> for uterine nuclear estrogen receptors (data not shown), suggesting a different mode of action. The more rapid and transient response of the uterine LF gene to THC treatment as compared with E<sub>2</sub> treatment suggests that THC exerted this effect perhaps via its interaction with cannabinoid receptors. The presence of CB1-R mRNA in the ovariectomized or THC-treated uterus (Fig. 1B) suggests that cannabinoid receptors were available for interaction with the ligands.

## CONCLUSION

Since cannabinoid ligand-receptor signaling can modulate adenylyl cyclase and Ca<sup>2+</sup> channel activities (14), the two important members of the second-messenger system, it could be proposed that this signaling may be important for modulating uterine

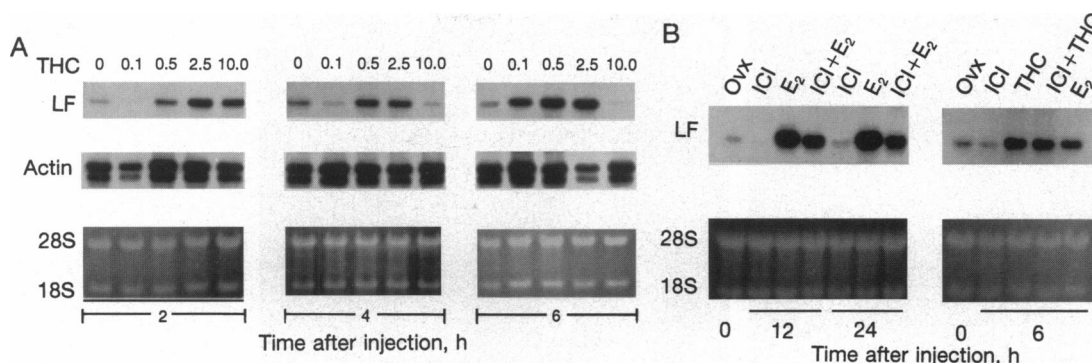


FIG. 5. Northern blot analysis of uterine LF mRNA expression. (A) Dose- and time-dependent effects of THC on the ovariectomized uterus. The dose of THC in mg/kg of body weight is at the top of each lane. (B) Effects of the antiestrogen ICI 182780 on THC-induced (Right) or E<sub>2</sub>-induced (Left) responses on the ovariectomized uterus. Ovariectomized (Ovx) mice killed 6 h after oil injection served as controls (0 h). Autoradiographic exposures were 2 h for both LF and  $\beta$ -actin.

functions. This is consistent with the recent observation of modulation of steroid receptor functions by cAMP (36, 37). Furthermore, intracellular levels of cAMP and Ca<sup>2+</sup> can serve as second messengers for immunomodulation (17, 38). Thus, the local immunosuppression considered to be required for acceptance and implantation of the "semiallogenic" embryo in the uterus (reviewed in ref. 39) may be mediated in part by this signaling pathway. On the other hand, aberrant synthesis of anandamide and/or expression of the cannabinoid receptors in the uterus may contribute to early pregnancy failure. Thus, the pregnancy failures and fetal losses that have been reported with exposure to cannabinoids (2, 3) suggest that the adverse effects are mediated via interactions with uterine cannabinoid receptors. Furthermore, aberrant uterine expression of the endogenous ligand and/or the receptors may be contributing factors in many incidences of uterine disorders and infertility (2, 3). Although non-receptor-mediated cannabinoid effects cannot be ruled out (14), our results suggest that a receptor-mediated signaling pathway is present in the mouse uterus to respond to cannabinoid ligands. This study also establishes that cannabinoids can alter the expression of a uterine gene (LF) that is considered to be regulated only by steroid hormones. The present findings are exciting and present us with a new avenue for further exploration of cannabinoid ligand-receptor signaling in uterine biology.

We thank Drs. T. Bonner (National Institute of Mental Health), L. Murphy (Southern Illinois University, Carbondale), and the National Institute of Drug Abuse (NIDA) for providing us with the rat brain cannabinoid receptor cDNA clone, cannabidiol, and  $\Delta^9$ -THC, respectively. This work was supported by grants from NIDA (DA 06668) and the National Institute of Child Health and Human Development (HD 12304) to S.K.D.

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