Marijuana: Interaction with the Estrogen Receptor

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ABSTRACT

Crude marijuana extract competed with estradiol for binding to the estrogen receptor of rat uterine cytosol. Condensed marijuana smoke also competed with estradiol for its receptor. Pure Δ²-tetrahydrocannabinol, however, did not interact with the estrogen receptor. Ten Δ²-tetrahydrocannabinol metabolites also failed to compete for its receptor. Of several other common cannabinoids tested, only cannabidiol showed any estrogen receptor binding. This was evident only at very high concentrations of cannabidiol. Apigenin, the aglycone of a flavinoid phytoestrogen found in cannabis, displayed high affinity for the estrogen receptor. To assess the biological significance of these receptor data, estrogen activity was measured in vivo with the uterine growth bioassay, using immature rats. Cannabis extract in large doses exhibited neither estrogenic nor antiestrogenic effects. Thus, although estrogen receptor binding activity was observed in crude marijuana extract, marijuana smoke condensate and several known components of cannabis, direct estrogenic activity of cannabis extract could not be demonstrated in vivo.

Gynecomastia has been reported as a side effect of chronic, heavy marijuana use (Harmon and Aliapoulios, 1972, 1974). Impotence and decreased plasma testosterone levels in marijuana smokers, which were reversed by cessation of marijuana use, have also been described (Kolodny et al., 1974, 1976). All these findings are compatible with the hypothesis that cannabis contains a component(s) that acts systemically as an estrogen.

Animal experiments have demonstrated numerous effects of cannabis on the reproductive system, but the results have often been conflicting. Marijuana resin has been reported to decrease plasma testosterone levels in male rats (Okey and Truant, 1975). It has also been shown to act as an antiestrogen in adult female rats (Chakrabarty et al., 1975, 1976; Dixit et al., 1975). An interaction with the estrogen receptor, however, could not be identified (Okey and Truant, 1975). Δ²-THC, the major psychoactive component of marijuana, has been reported to have estrogenic (Solomon et al., 1976, 1977), antiestrogenic (Chakrabarty et al., 1975, 1976; Dixit et al., 1975) and antiandrogenic (Purohit et al., 1979, 1980) effects in vivo. Δ²-THC has also been shown to inhibit ovulation in the rhesus monkey (Asch et al., 1981), and to increase or decrease plasma testosterone concentration (depending on dose) and to impair spermatogenesis and fertility in male mice (Dalterio et al., 1981, 1982). In vitro, Δ²-THC has been shown to bind to the estrogen receptor in some studies (Rawitch et al., 1977; Solomon and Cocchia, 1977) but not in others (Smith et al., 1979; Okey and Bondy, 1977, 1978). One explanation for these divergent findings might be that a variable component of crude marijuana other than Δ²-THC is responsible for the estrogen-like effects of this drug. This could, for example, be a phytoestrogen that is an inconstant contaminant of marijuana and its partially purified components. The current study was undertaken to investigate this possibility by testing the interaction of CME, marijuana smoke condensate, Δ²-THC, 10 Δ²-THC metabolites and several known components of cannabis with the estrogen receptor of rat uterine cytosol. The biological significance of the estrogen receptor interaction of marijuana has been assessed with a uterine weight bioassay in immature rats.

Materials and Methods

Δ²-THC (lot no. ADL-16792-98) containing 31% Δ²-THC and less than 2.6% cannabidiol was obtained from NIDA. A smoked marijuana condensate (batch no. 2380-29) containing 0.4% Δ²-THC was obtained from NIDA. Cannabidiol was obtained from NIDA. A sample of apigenin, a phytoestrogen present in cannabis, was a gift from Dr. David Slatkin of the University of Pittsburgh (Pittsburgh, PA). [2,4,6,7-3H]Estradiol with a specific activity of 92 Ci/mmol was obtained from New England Nuclear (Boston, MA) for use in estrogen receptor cytosol preparation. Uteri from six to seven adult female Sprague-Dawley rats, ovariecetomized 24 hr previously, were homogenized at 0°C in 40-mM buffer, 10 mM Tris, 1.5 mM EDTA, and 0.5 mM dithiothreitol, pH 7.4. Tissue was homogenized in five bursts of 5 to 7 sec using a Brinkmann Polytron PT 10 20 3500 with

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ABBREVIATIONS: THC, tetrahydrocannabinol; CME, crude marijuana extract; NIDA, National Institute on Drug Abuse.
0.2 ng to 10.0 ng of estradiol in 0.2 ml of sesame oil containing 1.25% ethanol. To a second group, 21 mg of CME was added to each estradiol.

Dawley rats, 21 days old, were injected s.c. once daily for 6 days with ethanol and added to the incubates at a final ethanol concentration of 1%. Bound and free [3H]estradiol were separated using dextran-coated charcoal as previously described (Cutler et al., 1978). Receptor affinity for cannabinoids (K1) was estimated from the change in the apparent affinity for [3H]estradiol when cannabinoid was present, which was calculated by the change in slope of the Scatchard plot.

CME was added to the incubates at a final δ1-THC concentration of 2.4 × 10⁻⁶ M and cannabidiol concentration of less than 2 × 10⁻⁶ M. Condensed marijuana smoke was added to the incubates at a final δ1-THC concentration of 6.7 × 10⁻⁵ M and cannabidiol concentration of 4.7 × 10⁻⁶ M. Pure δ1-THC was added to incubates at a final concentration of 7.1 × 10⁻⁵ M. Pure cannabidiol and apigenin were added to the incubates at 5.6 × 10⁻⁶ M and 5 to 50 × 10⁻⁷ M, respectively. Marijuana extracts and components were dissolved in ethanol, which was added to the Tris-EDTA-dithiothreitol buffer described above. The final ethanol concentration in the incubations was 1%.

Competition of δ1-THC metabolites for the estrogen receptor. Aliquots of 0.3 ml of uterine cytosol having an estrogen binding capacity of 0.10 to 0.25 nM (determined by Scatchard analysis) were incubated in darkness for 1 hr at 0°C with [3H]estradiol at concentrations of 0.07 to 2.2 nM in the presence and the absence of cannabinoid. Bound and free [3H]estradiol were separated using dextran-coated charcoal as previously described (Cutler et al., 1978). Receptor affinity for cannabinoids (K1) was estimated from the change in the apparent affinity for [3H]estradiol when cannabinoid was present, which was calculated by the change in slope of the Scatchard plot.

CME, in quantities containing 2.4 × 10⁻⁵ M δ1-THC, showed significant competition with estradiol for the rat uterine cytosol receptor (n = 6) (fig. 1A). This interaction was also found in the condensate of marijuana smoke at an equivalent δ1-THC concentration (n = 3) (fig. 1B). Three different preparations of δ1-THC (each greater than 98% pure) failed to inhibit [3H]estradiol binding to uterine estrogen receptor at a concentration of 7.1 × 10⁻⁵ M (n = 6). A typical preparation is shown in figure 1C. Cannabidiol exhibited a weak interaction with estrogen receptor at a concentration of 5.6 × 10⁻⁶ M (n = 2) (K1 = 1.0 × 10⁻⁷ M) (fig. 1D). Apigenin was a potent inhibitor of [3H]estradiol binding at concentrations ranging from 5 to 50 × 10⁻⁷ M (n = 6) (K1 = 3-15 × 10⁻⁷ M) (fig. 1D).

Ten metabolites of δ1-THC at concentrations of 2 × 10⁻⁵ M also displayed little estrogen receptor interaction (fig. 2). CME, administered s.c. in a dose containing 6.3 mg/day of δ1-THC, did not shift the dose-response curve for estradiol-induced uterine growth in immature rats (fig. 3A). Oral administration of CME in a dose containing 15.2 mg/day of δ1-THC also did not shift the estradiol dose-response curve (fig. 3B).

**Discussion**

These studies demonstrate that components in CME, also present in condensed marijuana smoke, interact with the rat uterine cytosol estrogen receptor. This interaction cannot be

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**Fig. 1.** Scatchard plots from representative experiments indicating the effect of cannabis and various cannabinoids on specific [3H]estradiol (E2) binding to the cytoplasmic estrogen receptor of rat uterus. See "Materials and Methods" for details of the receptor assay procedure. The final apigenin concentration in the experiment shown in panel D was 6.7 × 10⁻⁷ M. Each point represents the mean of duplicate incubates.

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**Fig. 2.** Effect of δ1-THC metabolites and estradiol (E₂) on [3H]E2 binding to its receptor in rat uterine cytosol. See "Materials and Methods" for details of receptor binding procedure. Each value represents the mean ± S.E. of four duplicate experiments.
attributed to Δ9-THC or most of its important metabolites. It cannot be attributed to cannabinoid at the concentrations occurring in this marijuana preparation. Apigenin is one of several flavinoid phytoestrogens known to exist in marijuana leaves (fig. 4); it does interact with the estrogen receptor at concentrations that could account for the displacement of estradiol from its receptor seen with CME. The flavinoids vary not only in their distribution between subspecies of Cannabis sativa, but also in their distribution within the leaves, flowers, and stems of a single plant (Turner et al., 1976; Paris et al., 1976). Such variation may explain some of the contradictory experiments with cannabis.

Crude marijuana, however, does not exert a measurable estrogenic or antiestrogenic effect on the immature rat uterus. The use of immature animals ensured that a uterotrophic response could not be attributed to changes in the pituitary gonadal axis (Glass and Swerdloff, 1980). Sluggishness, ataxia and a substantial weight loss in animals given CME orally suggested that significant absorption of cannabis occurred. This dose of CME has produced neurotoxicity in rats in previous studies (Thompson et al., 1973; Luthra et al., 1975). The lack of demonstrable, estrogen-related effect in vivo, despite unequivocal estrogen receptor binding in vitro, probably reflects an insufficient plasma concentration of the estrogen-binding component. This could be due either to inadequate absorption of this component(s) or to a very rapid metabolic clearance rate, a situation analogous to that recently demonstrated for the catechol estrogen, 2-hydroxyestrone (Merriam et al., 1980).

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References


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