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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 Δ^9-tetrahydrocannabinol, however, did not interact with the estrogen receptor. Ten Δ^9-tetrahydrocannabinol metabolites also failed to compete with estradiol 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). Δ^9-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*. Δ^9-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*, Δ^9-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 Δ^9-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, Δ^9-THC, 10 Δ^9-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

Δ^9-THC (lot no. ADL-16792-98), 98% pure, was obtained from the NIDA (Rockville, MD). Its purity was reconfirmed by repeat analysis at NIDA after these experiments had been completed.

An ethanol extract of crude marijuana (lot no. 1326-107-A) containing 31% Δ^9-THC and less than 2.6% cannabidiol was obtained from NIDA. A smoked marijuana condensate (batch no. 2380-29) containing 0.4% Δ^9-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-^{3}H]Estradiol with a specific activity of 92 Ci/mmol was obtained from New England Nuclear (Boston, MA) for use in estrogen receptor studies.

Estrogen receptor cytosol preparation. Uteri from six to seven adult female Sprague-Dawley rats, ovariectomized 24 hr previously, were homogenized at 0°C in 40-ml 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.

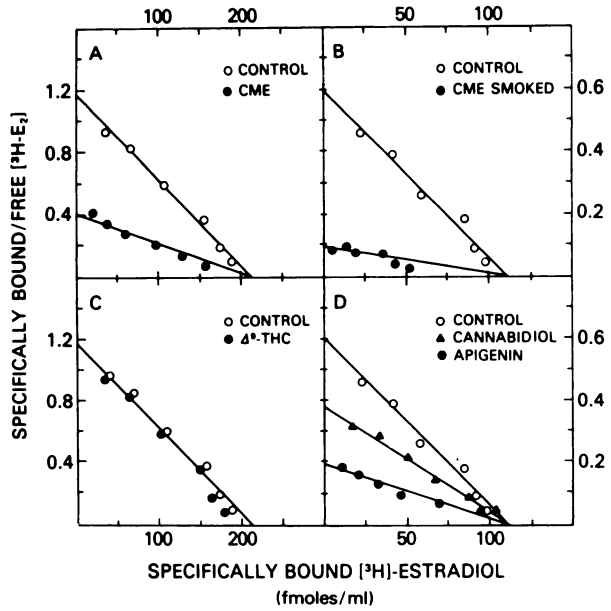

Fig. 1. Scatchard plots from representative experiments indicating the effect of cannabis and various cannabinoids on specific [^{3}H]estradiol (E_2) binding to the cytoplasmic estrogen receptor of rat uterus. See "Materials and Methods" for details of the receptor assay procedure. The final apigenin concentration in the experiment shown in panel D was 6.7×10^{-7} M. Each point represents the mean of duplicate incubates.

cooling periods of 30 sec between each burst. After centrifugation of the homogenate for 1 hr at 105,000 × *g*, uterine cytosol was collected and stored frozen at −80°C.

Competition of cannabinoids for 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 (fig. 1)] were incubated in darkness for 16 hr at 0°C with [^{3}H]estradiol at concentrations of 0.07 to 2.2 nM in the presence and the absence of cannabinoid. Bound and free [^{3}H]estradiol were separated using dextran-coated charcoal as previously described (Cutler *et al.*, 1978). Receptor affinity for cannabinoids (K_i) was estimated from the change in the apparent affinity for [^{3}H]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 Δ^9-THC concentration of 2.4×10^{-5} M and cannabidiol concentration of less than 2×10^{-6} M. Condensed marijuana smoke was added to the incubates at a final Δ^9-THC concentration of 6.7×10^{-5} M and cannabidiol concentration of 4.7×10^{-6} M. Pure Δ^9-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 Δ^9-THC metabolites for the estrogen receptor. Aliquots of 0.3 ml of uterine cytosol having an estrogen binding capacity of 0.10 to 0.15 nM (determined from the Scatchard plot of specific [^{3}H] estradiol binding) were incubated at 4°C for 16 hr with 2 nM [^{3}H] estradiol alone and with 200 nM unlabeled estradiol or 20 μM concentrations of each THC metabolite. THC metabolites were dissolved in ethanol and added to the incubates at a final ethanol concentration of 1%. Bound and free [^{3}H]estradiol were separated with a dextran-charcoal assay as previously described (Cutler *et al.*, 1978). Metabolites were stored in darkness and receptor experiments were performed in dim lighting.

Effect of cannabis *in vivo* on uterine growth. Female Sprague-Dawley rats, 21 days old, were injected s.c. once daily for 6 days with 0.2 ng to 10.0 μg 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 injection. The CME tar was suspended directly in the sesame oil using a motor-driven Teflon-glass homogenizer.

In a second experiment animals injected with estradiol as in the previous experiment were given 50 mg/day of CME in 0.5 ml of sesame oil directly into the stomach *via* a polyethylene tube. The control group received 0.5 ml/day of sesame oil by stomach tube and the same s.c. estradiol injections.

On the 7th day animals were weighed and asphyxiated with CO_2. Their uteri were dissected out and weighed, and the uterus/body weight ratio was used to estimate total estrogen effect.

Results

CME, in quantities containing 2.4×10^{-5} M Δ^9-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 Δ^9-THC concentration ($n = 3$) (fig. 1B). Three different preparations of Δ^9-THC (each greater than 98% pure) failed to inhibit [^{3}H] 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 ($n = 2$) ($K_i = 1.0 \times 10^{-5}$ M) (fig. 1D). Apigenin was a potent inhibitor of [^{3}H] estradiol binding at concentrations ranging from 5 to 50×10^{-7} M ($n = 6$) ($K_i = 3\text{–}15 \times 10^{-7}$ M) (fig. 1D).

Ten metabolites of Δ^9-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 Δ^9-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 Δ^9-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

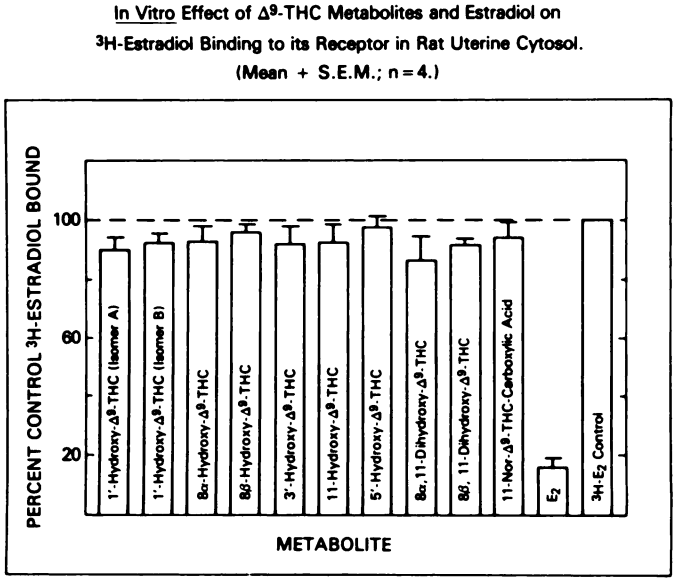

Fig. 2. Effect of Δ^9-THC metabolites and estradiol (E_2) on [^{3}H]E_2 binding to its receptor in rat uterine cytosol. See "Materials and Methods" for details of receptor binding procedure. Each value represents the mean ± S.E. of four duplicate experiments.

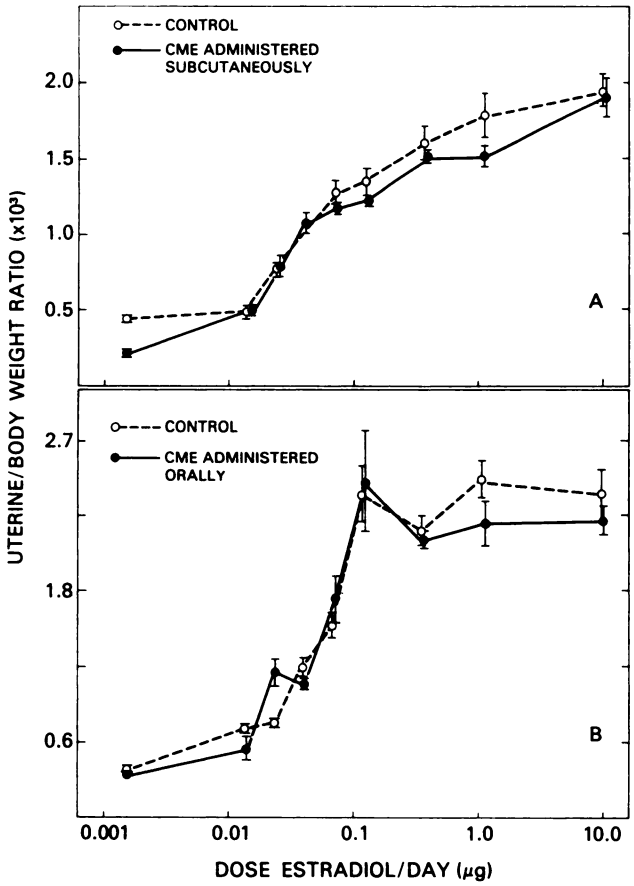

Fig. 3. The effect of cannabis on the dose-response curve to estradiol of the uterus/body weight ratio in immature rats. See "Materials and Methods" for details of the assay procedure. Each value in panel A represents the mean ± S.E. of 10 animals. Each value in panel B represents the mean ± S.E. of three to six animals.

attributed to Δ^9-THC or most of its important metabolites. It cannot be attributed to cannabidiol 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 uterotropic 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,

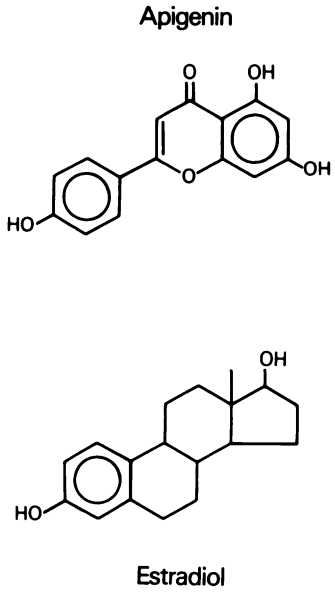

Fig. 4. Structures of apigenin and estradiol.

a situation analogous to that recently demonstrated for the catechol estrogen, 2-hydroxyestrone (Merriam *et al.*, 1980).

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