Δ^9-Tetrahydrocannabinol and 17β-Estradiol Bind to Different Macromolecules in Estrogen Target Tissues

Abstract. *Δ^9-Tetrahydrocannabinol (Δ^9-THC), added to the limit of its solubility, did not compete with tritiated 17β-estradiol for binding to estrogen receptor sites in mouse mammary or uterine cytosols. On sucrose density gradients of low-ionic strength, mammary cytosol labeled with [^{3}H]estradiol exhibited a binding peak near the "8S" region typical of estrogen receptor whereas in cytosol labeled with Δ^9-[^{3}H]THC binding was limited to the nonspecific 4- to 5S region. Differences in sedimentation properties and reciprocal competition studies strongly refute previous claims that Δ^9-THC binds to estrogen receptor and that by so doing it directly acts as an estrogen.*

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the psychoactive component of marihuana, and other cannabinoids have a variety of effects on reproductive tissues in rodents (*1, 2*) and possibly in man (*3*). Some of the effects are reminiscent of those caused by estrogenic hormones, that is, demasculinization and feminization; this has led some workers to the understandable belief that cannabinoids feminize or demasculinize by acting directly as estrogenic compounds. Two general approaches are available to test such a hypothesis: (i) bioassays can be conducted in whole animals to determine if cannabinoids have the same specific effects on target tissues (uterus, breast) as do known estrogens; (ii) competition experiments can be conducted for binding in vitro to specific receptor sites which mediate the growth-promoting activities of estrogens.

We reported previously that cannabinoids had no specific estrogenic effects either in vivo (*4*) or in vitro (*5*), but a recent report (*6*) claims that Δ^9-THC binds to estrogen receptor and thus has a direct primary estrogenic action. We have assessed this claim by directly measuring the binding of Δ^9-[^{3}H]THC and ^{3}H-labeled 17β-estradiol in mammary and uterine cytosols and by competition of unlabeled cannabinoids for estrogen receptor sites.

Two separate techniques were used to test cannabinoids for possible competition with [^{3}H]estradiol for estrogen receptor sites. In the first procedure mouse mammary cytosol was incubated with different concentrations of [^{3}H]estradiol in the presence or absence of 1 m*M* unlabeled Δ^9-THC (*7*). Scatchard plot analyses (*8*) (Fig. 1) show that Δ^9-THC did not reduce the concentration of high-affinity binding sites for [^{3}H]estradiol and had no significant effect on the affinity of the [^{3}H]estradiol-receptor interaction. Sucrose density gradient analysis of [^{3}H]estradiol binding in mouse mammary cytosol (Fig. 2A) shows that Δ^9-THC, added to the limit of its solubility, does not interfere with [^{3}H]estradiol binding to "8*S*" estrogen receptor. Known estrogens, such as diethylstilbestrol, totally abolish the specific "8*S*" peak at concentrations as low as 10 n*M* (*9*). We also tested Δ^8-THC and 11-hydroxy-Δ^9-THC (10 μ*M*) for possible competition in the same assay; binding peaks in the presence of these cannabinoids were identical to the noncompetitor control (data not shown).

The possibility that Δ^9-THC might sediment with macromolecules in the same region as [^{3}H]estradiol was tested by incubating Δ^9-[^{3}H]THC with mammary cytosol. No binding peaks appeared in the region of estrogen receptor (8*S*) (Fig. 2B); binding which occurred in the 4- to 5*S* region was not inhibited by excess unlabeled Δ^9-THC (10 μ*M*) nor by 1 m*M* unlabeled estrogen. Binding of Δ^9-[^{3}H]THC in the 4- to 5*S* region was stripped by repeated charcoal treatments (Fig. 2C); thus, Δ^9-[^{3}H]THC binding in this region is to a high-capacity, nonspecific, low-affinity macromolecule which does not meet the criteria for a receptor. Similar nonspecific, low-affinity binding of Δ^9-[^{3}H]THC was observed in experiments with uterine cytosol (data not shown).

Since the binding of ^{3}H-labeled Δ^9-THC coincided directly with the BSA marker (*10*), we tested the possibility that binding to albumin contaminants from plasma might be responsible for the "4- to 5*S*" peak. As shown in Fig. 3, BSA has a high capacity for Δ^9-[^{3}H]THC and this binding can be moderately inhibited by very high concentrations of unlabeled estradiol. From these and previous (*4, 5*) experiments we find that Δ^9-THC does not compete for estrogen receptor sites in uterine or mammary cytosol from rat or mouse, when examined by sucrose density gradient assay or by Scatchard plot analysis of charcoal assays; nor is there any competition for receptor by cannabis resin, Δ^8-THC or 11-hydroxy-Δ^9-THC. Although Δ^9-[^{3}H]THC binds to

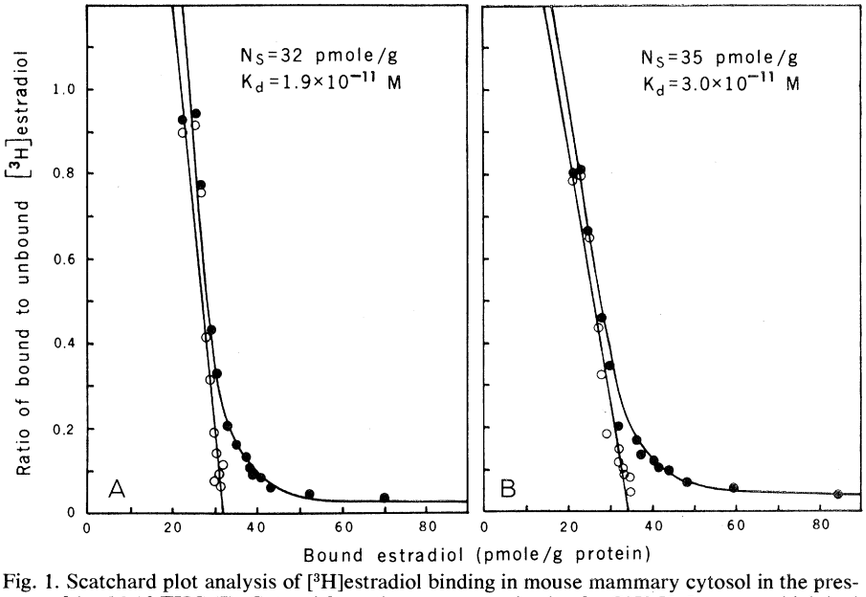

Fig. 1. Scatchard plot analysis of [^{3}H]estradiol binding in mouse mammary cytosol in the presence of 1 m*M* Δ^9-THC (*7*). Cytosol from the mammary glands of a C3H-HeJ mouse which had been lactating for 10 days was prepared in TDE buffer (0.01*M* tris-HCl, 0.001*M* dithiothreitol, 0.015*M* ethylenediaminetetraacetic acid). Portions of cytosol (1.9 mg of protein per milliliter) were incubated for 16 hours at 4°C with concentrations of [^{3}H]estradiol (138.1 Ci/mmole; New England Nuclear) between 10 n*M* and 70 p*M*. The Δ^9-THC was added in 10 μl of ethanol 15 minutes prior to the addition of [^{3}H]estradiol. Dextran-coated charcoal was used to remove "unbound" and loosely bound [^{3}H]estradiol. Specific high-affinity binding was computed by the method of Chamness and McGuire (*8*). (A) Binding in samples containing [^{3}H]estradiol without competitor. (B) Binding in the presence of 1 m*M* Δ^9-THC added prior to [^{3}H]estradiol. Symbols: (●), uncorrected data; (○), data corrected for nonspecific binding; N_s, number of high-affinity (receptor) sites per gram of cytosol protein; K_d = dissociation constant for the [^{3}H]estradiol-receptor interaction.

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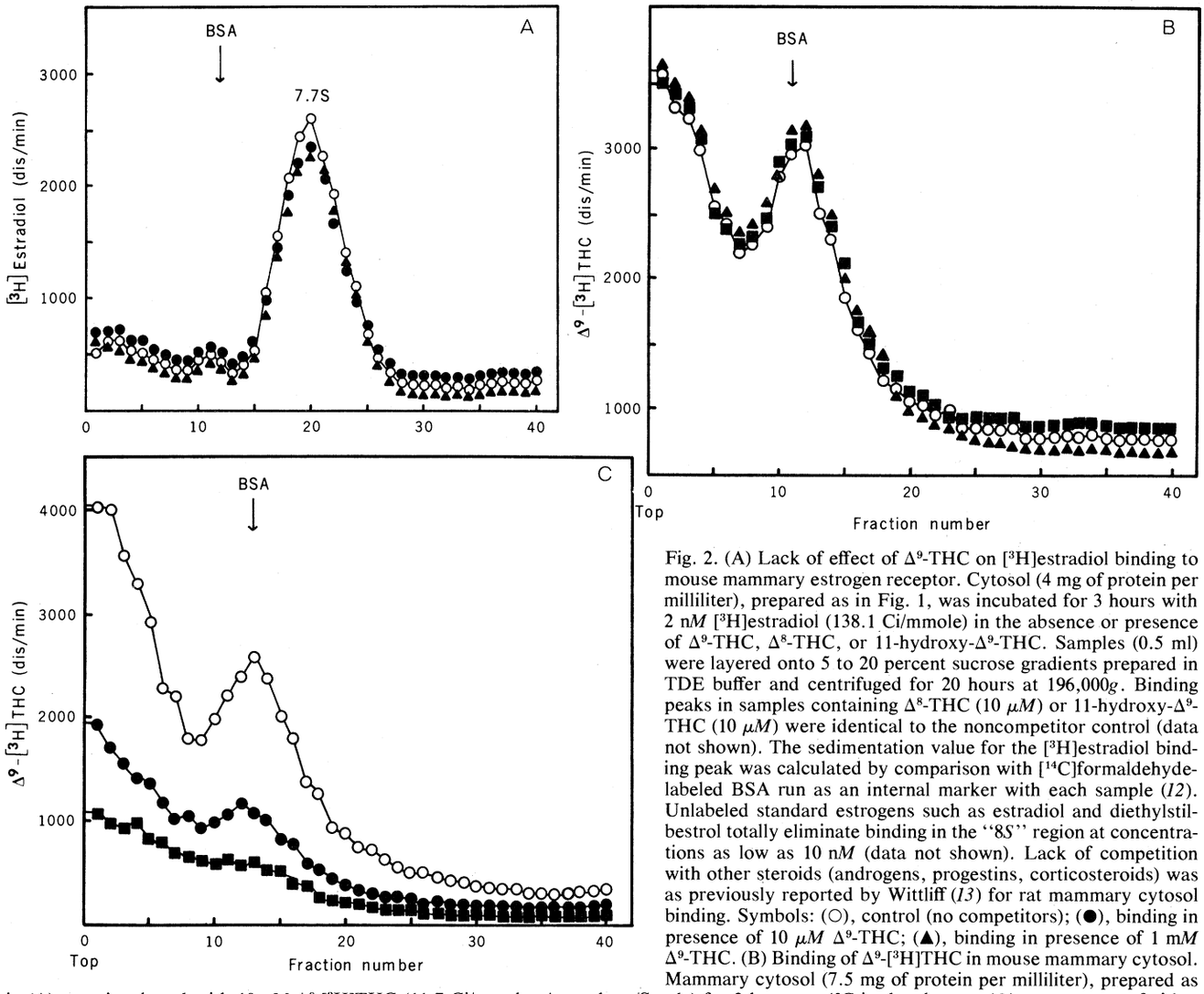

Fig. 2. (A) Lack of effect of Δ^9-THC on [^{3}H]estradiol binding to mouse mammary estrogen receptor. Cytosol (4 mg of protein per milliliter), prepared as in Fig. 1, was incubated for 3 hours with 2 n*M* [^{3}H]estradiol (138.1 Ci/mmole) in the absence or presence of Δ^9-THC, Δ^8-THC, or 11-hydroxy-Δ^9-THC. Samples (0.5 ml) were layered onto 5 to 20 percent sucrose gradients prepared in TDE buffer and centrifuged for 20 hours at 196,000*g*. Binding peaks in samples containing Δ^8-THC (10 μ*M*) or 11-hydroxy-Δ^9-THC (10 μ*M*) were identical to the noncompetitor control (data not shown). The sedimentation value for the [^{3}H]estradiol binding peak was calculated by comparison with [^{14}C]formaldehyde-labeled BSA run as an internal marker with each sample (*12*). Unlabeled standard estrogens such as estradiol and diethylstilbestrol totally eliminate binding in the "8*S*" region at concentrations as low as 10 n*M* (data not shown). Lack of competition with other steroids (androgens, progestins, corticosteroids) was as previously reported by Wittliff (*13*) for rat mammary cytosol binding. Symbols: (○), control (no competitors); (●), binding in presence of 10 μ*M* Δ^9-THC; (▲), binding in presence of 1 m*M* Δ^9-THC. (B) Binding of Δ^9-[^{3}H]THC in mouse mammary cytosol. Mammary cytosol (7.5 mg of protein per milliliter), prepared as in (A), was incubated with 10 n*M* Δ^9-[^{3}H]THC (11.7 Ci/mmole; Amersham/Searle) for 3 hours at 4°C in the absence (○) or presence of either 10 μ*M* unlabeled Δ^9-THC (■) or 1 m*M* unlabeled estradiol (▲). Gradients were centrifuged as in (A). (C) Removal of Δ^9-[^{3}H]THC from the "4*S*" binding peak by multiple dextran-coated charcoal (DCC) treatments. Cytosol (7.0 mg of protein per milliliter) was incubated with 10 n*M* Δ^9-[^{3}H]THC as described in (A). Symbols: (○), sample treated once with DCC before layering; (●), sample treated twice with DCC before layering; (■), sample treated three times with DCC before layering (samples in Figs. 2, A and B, and 3 were treated once with DCC before layering). Multiple charcoal treatments do not significantly reduce [^{3}H]estradiol binding to "8*S*" receptor from lactating mouse mammary gland if all procedures are done at 0° to 4°C (*9*).

components in mammary and uterine cytosols, it is clear from sedimentation profiles and reciprocal competition studies that this binding is not to estrogen receptor.

The claim by Rawitch *et al.* (*6*) that Δ^9-THC competes for estrogen receptor sites was based on a simple charcoal-separation assay at a single [^{3}H]estradiol concentration rather than full Scatchard analysis at several different concentrations. Additionally, on sucrose density gradient analysis, these authors demonstrated [^{3}H]estradiol binding only in the 4- to 5*S* region and failed to detect any binding in the expected specific "8*S*" region. They also claimed to have demonstrated "direct binding of Δ^9-THC with estrogen receptor . . . by sucrose density

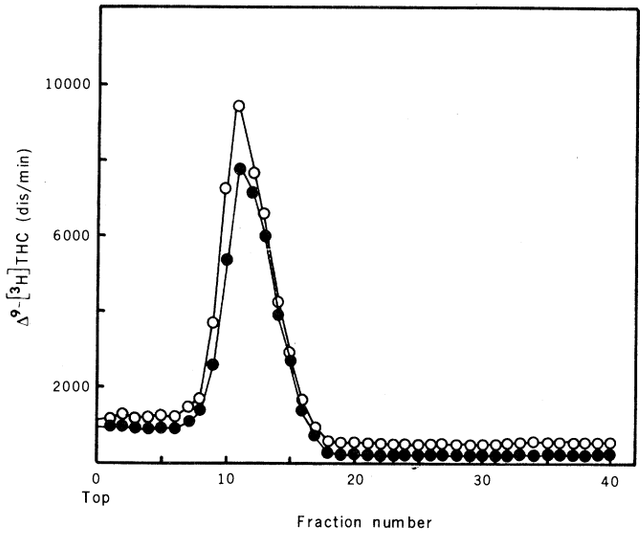

Fig. 3. Binding of Δ^9-[^{3}H]THC to bovine serum albumin. Portions (10 mg/ml) of BSA (Sigma) were incubated for 3 hours with 10 n*M* Δ^9-[^{3}H]THC either alone (○) or in the presence of 100 μ*M* unlabeled estradiol (●). Samples were treated once with DCC, then layered and centrifuged as with the mammary cytosol samples.

gradient centrifugation'' using ^{14}C-labeled Δ^9-THC (specific activity: 10.5 mCi/mmole). If the low specific activity Δ^9-[^{14}C]THC sedimenting at ''10.4*S*'' in their assay were indeed bound to estrogen receptor as claimed (*6*), the concentration of receptor sites would greatly exceed 100,000 fmole per milligram of cytosol protein (compared with the few hundred femtomoles per milligram usually detected in uterine cytosol).

Evidence from bioassays in vivo also is weighted strongly against any direct estrogenic action by cannabinoids. Several reports on various species have shown that Δ^9-THC and other cannabis preparations either exert antiestrogenic effects (*2, 4*) or are without effect on the uterus. The authors of the two reports (*11*) claiming that Δ^9-THC has estrogenic actions on the uterus and vagina used only 21 rats treated with Δ^9-THC and indicated that a very moderate ''estrogenic'' response appeared to occur only in seven rats injected with 2.5 mg/kg doses of Δ^9-THC.

The fact that high doses of cannabinoids can alter reproductive tissues in experimental animals is unquestioned. The reproductive effects in humans are unclear. In either case it is unlikely that any reproductive alterations are due to the direct estrogenic activity of Δ^9-THC.

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Activity Patterns of Human Skeletal Muscles: Relation to Muscle Fiber Type Composition

Abstract. *The muscle activity of normal ambulatory individuals was recorded continuously for 8-hour (working day) periods. Parameters of activity patterns were defined and numerical outcomes for these parameters were compared across a diverse population of muscles. Several pattern parameters, such as the average percentage of time active, were highly correlated with the percentage of type I fibers of a muscle.*

Mammalian skeletal muscles can be classified in a number of ways (*1*). Historically, classification has been based on gross muscle characteristics, such as vascularity, and on the average contraction time of the twitch response. More recent histochemical methods show that mammalian skeletal muscles are composed of metabolically diverse fibers (*2, 3*). Two main muscle fiber types (''archetypes'' I and II) have been defined as a function of both histochemical and physiological measures (*4*).

Metabolic profiles of mammalian skeletal muscles, based either on fiber type composition alone or on more complex classification schemes, differ. There is increasing evidence that profile differences reflect differences in normal functional muscle use. At least a limited adaptive range exists, since several enzyme systems have been shown to gradually adapt to changes in functional demand (*5, 6*). However, it remains to be established what specific physiological factors (such as average speed of movement) modulate what aspects of the metabolic profile (such as enzyme systems). In the study reported here we define a number of parameters of normal activity patterns. Several of these parameters are highly correlated with the muscle's fiber type composition. In contrast to most previous approaches, in which the activity pattern of a single muscle was manipulated experimentally and profile changes were observed in time by sequential sampling, our approach involves a comparison between defined parameters of naturally occurring patterns and profiles, for a diverse population of human muscles. This acknowledges that the intrinsic properties of a muscle's motoneuron pool, such as the synaptic organization of its input and the distribution of cell sizes, affect a muscle's activity pattern in a specific manner.

Muscle usage patterns of 12 normal males between the ages of 20 and 35 years were recorded continuously over 8-hour (working day) periods (9 a.m. to 5 p.m.). These individuals were either hospital employees (N = 7) or paid volunteers (N = 5). Muscle usage was based on the electrical activity of the muscle during contraction, recorded on an electromyogram (EMG) (*7*). Recordings were made simultaneously from two functionally linked (*8*) but histochemically different muscles. The analysis of pattern parameters, such as percentage of time active, could thus be normalized for variations in day-to-day usage by only considering relative differences in outcome between the two paired muscles. There were no restrictions on the types of activities that individuals could engage in, but some experimental variables were controlled in the recording of specific muscles [for example, heel height, which affects the relative use of ankle flexors and extensors during quiet standing (*9*)]. Activity patterns were recorded with a pocket-size tape recorder (*10*), which ensured unobtrusive data collection and did not encumber the individual. Tapes were reproduced at 60 times the recording speed. Parameters of each reproduced pattern were analyzed by computerized scanning of the rectified and smoothed electromyogram (|EMG|) (*11*).

Selection of the population of muscles was influenced by the availability of comparative data on metabolic profiles of human skeletal muscles. The available data apply to a variety of species and were obtained by a number of histochemical methods. For consistency, histochemical data were taken from a single study (*12, 13*) in which multiple muscle biopsies were obtained from 36 different muscles of six males in the age range 18 to 30 years. The metabolic profile parameter examined in (*12*) was the percentage of type I fibers, based on the staining density of myofibrillar adenosinetriphosphatase.

The selection of muscles also depend-

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