Biochemical Pharmacology, Vol. 32, No. 2, pp. 253–256, 1983.
Printed in Great Britain.

0006-2952/83/020253-04 $03.00/0

ACTION OF DELTA-9-TETRAHYDROCANNABINOL ON THE BINDING OF ESTRADIOL TO UTERINE AND OTHER TISSUES IN RATS

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(*Received* 10 *June* 1982; *accepted* 15 *July* 1982)

Abstract—In the present paper three binding studies have been done to evaluate any possible interaction of delta-9-tetrahydrocannabinol (delta-9-THC) with estrogen in adult female rats. (1) Treatment with THC for 10 consecutive days and eventual binding studies with the uterine cytosol receptors in the presence of estradiol indicate that the drug has almost no effect on the binding affinity of the hormone. (2) *In vitro* incubation of cytosol receptors in the presence of different concentrations of THC further shows that there is no change in binding affinity to estradiol as well. (3) Treatment of ovariectomised rats with delta-9-THC and subsequently with radioactive estradiol indicates that binding of estradiol in most organs (viz. the uterus, vagina, liver, hypothalamus, pituitary, fallopian tube, kidney and adrenal) remains unaffected. The present paper therefore shows that delta-9-THC does not appear to alter the binding of estradiol to its receptors in any way. Hence, the antiestrogenic action previously reported to be exerted by the drug may be due to its direct action on the hypothalamo–pituitary–gonadal axis and not due to any inhibitory action at the steroid receptor level.

Most investigations of cannabis involve either behavioral or psychological effects evinced by the drug. However, in the past few years there has been a cumulative increase in the literature indicating that cannabis, as well as its major active component, delta-9-tetrahydrocannabinol (delta-9-THC), has a host of other pharmacological effects on the ophthalamic [1], respiratory [2] and endocrinological systems [3]. Several reports from our laboratories as well as others have indicated that the drug has an antiestrogenic type of action in female rats [4–8], along with a prominent inhibitory effect on the circulating gonadotrophin levels [9–11] and hypothalamic releasing hormones [12, 13]. However, except for a paper by Okey and Bondy [14], information is still lacking regarding any possible effect of cannabis on the binding of estradiol to uterine as well as other tissues in animals. Previous reports showing cannabis to have an antiestrogenic action at the level of the uterine tissues necessitate clarification of whether this effect is due to a direct action of the drug on the gonads by interaction with the estradiol receptors and/or entirely due to hormonal disbalance as previously indicated by several groups of workers.

In the present paper therefore we report the effect of delta-9-THC on the binding of estradiol to uterine cytosol receptors under both *in vivo* and *in vitro* conditions along with an added study to evaluate the binding of estradiol in several other tissues, viz. the uterus, vagina, liver, hypothalamus, pituitary, fallopian tube, kidney and adrenal.

MATERIALS AND METHODS

In vivo *effect of delta-9-THC on the cytosol receptors.* Adult female rats of the Holtzman strain weighing about 120–150 g were used for this study. The rats were bred in the laboratory animal house under a controlled lighting of 12 hr light and 12 hr dark with the temperature set at 26 ± 1°. For the *in vivo* effect of delta-9-THC on the uterine cytosol receptors, the rats were treated with delta-9-THC for 10 consecutive days at doses of 5 and 10 mg THC per kg body weight per day. Injections were given at 10.00 hr sharp every morning.

As reported previously by Chakravarty and Ghosh [15] after 10 days of consecutive treatment with THC all the rats reached a constant phase of diestrus. Therefore, for the control experiments, uterine tissues of normal rats in the diestrus phase were used.

After killing the rats by instant decapitation the uteri were carefully dissected out, weighed and homogenised. The cytosol was prepared in Tris–HCl buffer at pH 7.5 and used for binding experiments using radioactive estradiol according to the method of Korenman [16].

In vitro *effect of delta-9-THC on estradiol binding to cytosol receptors.* The uterine cytosol receptors from control rats prepared in the way mentioned earlier [16] were incubated with delta-9-THC at different concentrations and the binding of the drug to the receptor was evaluated.

Binding of radioactive estradiol to different organs

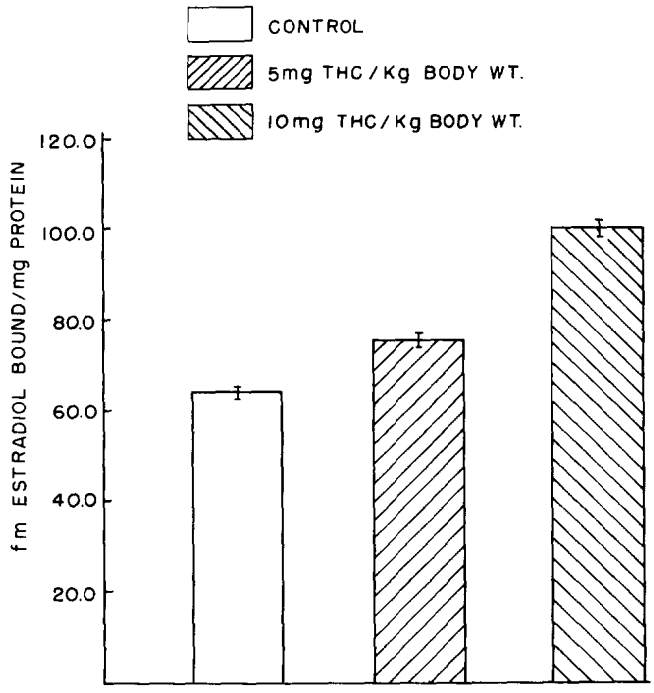

Fig. 1. Effect of *in vivo* treatment with delta-9-tetrahydrocannabinol on the binding of estradiol to uterine cytosol receptors.

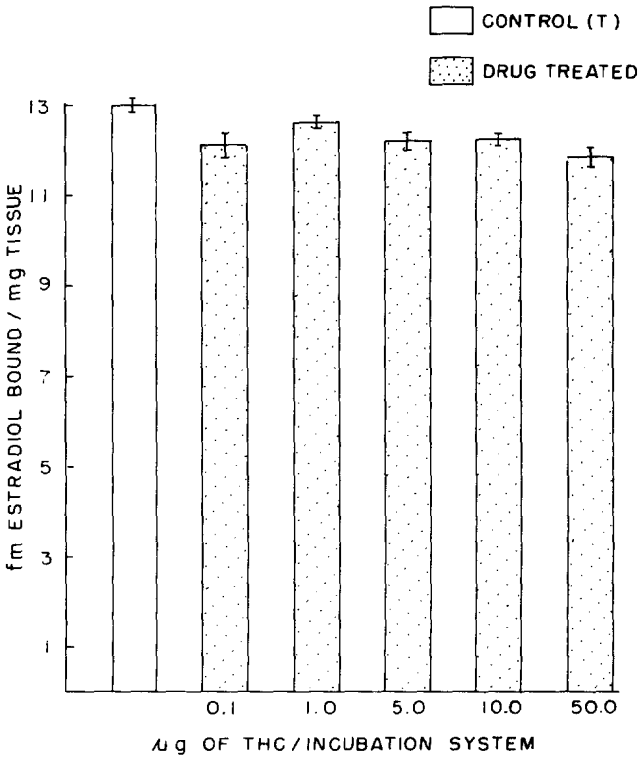

Fig. 2. *In vitro* effect of delta-9-tetrahydrocannabinol on the binding of estradiol to uterine cytosol receptors.

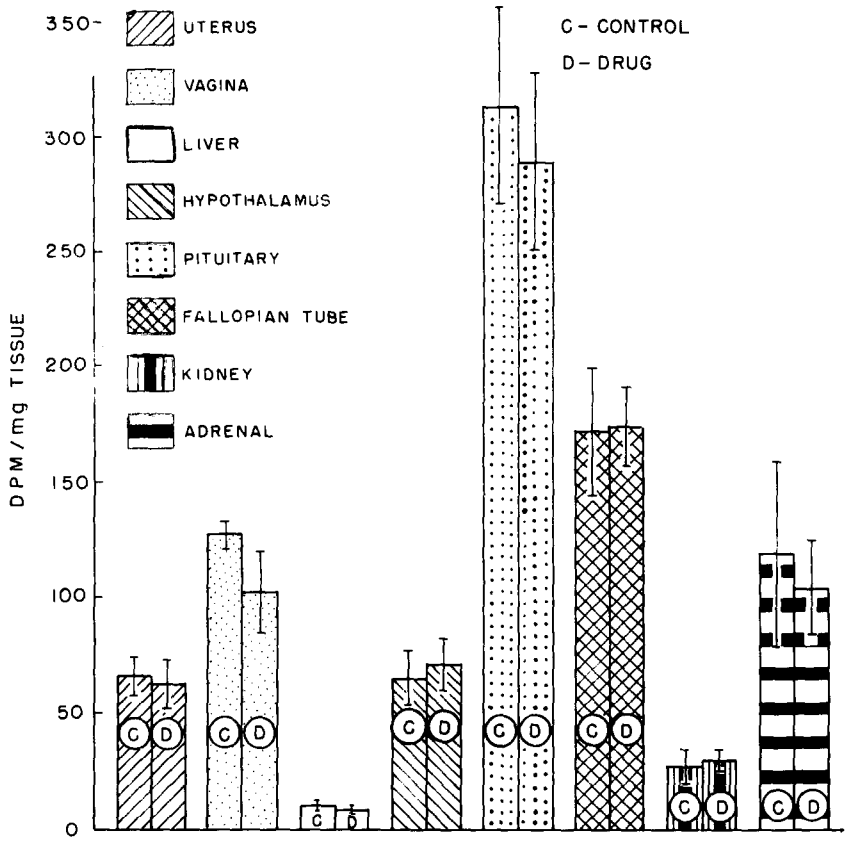

Fig. 3. Effect of delta-9-THC on the binding of estradiol to different tissues.

of the body. Regularly cycling normal female rats of the Holtzman strain weighing around 120–150 g were ovariectomised. To one group of rats a subcutaneous injection of delta-9-THC was given for two consecutive days at a dose of 10 mg delta-9-THC per kg body weight. The other group received the vehicle in an equal volume and served as controls. Forty-eight hours after the ovarietomy each animal of both groups was injected under light ether anesthesia, with 6,7-[^{3}H]estradiol (10 μCi) in 0.5 ml of a 10% ethanolic solution of saline through the jugular vein and was killed 1 hr later by decapitation [17]. The uteri, vagina, part of the liver, the hypothalamus, pituitary, fallopian tube, part of the kidney and the adrenal were carefully dissected out, blotted and weighed in an electric balance. Uptake of 6,7-[^{3}H]estradiol was estimated as per the standard procedure of DeHertogh [18].

The delta-9-THC used for the treatment was obtained from the United Nations Narcotics Laboratory (Geneva, Switzerland) and dissolved in normal saline containing 5% Tween-80. The original stock solution was maintained at 20 mg delta-9-THC per ml of saline–Tween-80.

RESULTS

Figure 1 show the effects of *in vivo* treatment with delta-9-THC on the binding of estradiol to the uterine cytosol receptors. It is evident from Fig. 1 that when compared to the control rats the uterine cytosol receptors of drug-treated rats are actually able to bind more estradiol in terms of fmoles estradiol bound per mg protein. This indicates that delta-9-THC treatment of rats does not appear to change the affinity between the hormone and its receptors in any way. The increased binding observed may be due to the fact that THC treatment leads to a lowering of estradiol levels within the body thereby rendering more receptor sites free to accept the radioactive estradiol added in the incubation system.

Figure 2 shows the *in vitro* effect of delta-9-THC on estradiol binding to uterine cytosol receptors. It is evident from Fig. 2 that, as in the previous study (Fig. 1), at doses varying from 0.1 to 50.0 μg delta-9-THC per incubation system the drug failed to have any effect on the binding of estradiol to the uterine cytosol receptors *in vitro*. This indicates that the drug not only has no effect on the affinity of the receptor or the hormone as such, but it also causes no physical or chemical damage to the receptors, even after direct incubation with the drug at a very high concentration (50.0 μg).

Figure 3 shows the effect of delta-9-THC on the binding of estradiol in different organs of the body in adult female rats. It is evident from Fig. 3 that binding of radioactive estradiol in all the organs studied remain unaltered after delta-9-THC treatment. Therefore, it may be suggested that even if delta-9-THC does bind to certain tissues in the body the sites of its attachment may be quite different from those of estradiol.

DISCUSSION

From the foregoing results it appears that neither delta-9-THC nor any of its metabolites compete with estradiol for its binding sites in the uterus or other organs of the body. Similar results have been reported by Okey and Bondy [14] who have estimated the possible competition of cannabinoids with [^{3}H]estradiol for receptor sites using both mammary gland and uterine cytosol receptors. They observed that delta-9-THC or its 11-hydroxy derivative added to the limit of solubility did not interfere with [^{3}H]estradiol binding to the 8S estrogen receptor in the mouse mammary gland. Moreover, Okey and Bondy [19] have further shown that the binding of delta-9-[^{3}H]THC, which is in the 4–5S region, could be removed by repeated charcoal treatment indicating that the binding agent in this region is simply a high-capacity, non-specific, low-affinity macromolecule which does not meet the criteria of a receptor.

From the present studies it is therefore concluded that delta-9-THC does not appear to have any competitive action with estradiol for its uterine cytosol receptors. Moreover the drug in general also does not appear to affect the binding of this hormone in any other organ of the body. Hence, the antiestrogenic action previously evinced by the drug is probably due to a lowering of the hormone level itself within the system and not due to any inhibition at the estradiol receptor interaction site.

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