Δ^9-Tetrahydrocannabinol Increases Plasma Testosterone Concentrations in Mice

Abstract. *Oral administration of Δ^9-tetrahydrocannabinol had a biphasic effect on plasma testosterone concentrations in male mice, causing rapid sustained increases at low doses and subsequent decreases at higher doses. In hypophysectomized and intact mice receiving gonadotropins (human chorionic gonadotropin), treatment with Δ^9-tetrahydrocannabinol maintained higher plasma testosterone concentrations. Thus, this cannabinoid may interact with gonadotropin and directly influence testicular steroidogenesis in vivo.*

Marijuana and its purified constituents exert a variety of effects on male reproductive functions in laboratory animals and in men, including suppression of pituitary-testicular function, decreased sperm count, and sexual dysfunction (*1, 2*). The findings in humans have been questioned (*3*), and indeed may be considered controversial, because there is abundant, although mostly anecdotal, evidence that marijuana can act as an "aphrodisiac" (*4, 5*). There is evidence that cannabinoids suppress male sexual activity in laboratory animals (*6*), and that at the same time they often decrease plasma testosterone concentrations (*1, 7*). The present studies were conducted to determine the effects of a wide range of doses of Δ^9-tetrahydrocannabinol (THC) on plasma testosterone concentrations in adult male mice within 1 hour of oral administration. In some experiments hypophysectomized animals were used to determine whether the effects of THC on plasma testosterone in mice are mediated by the pituitary.

Adult male mice (60 to 80 days of age) were obtained from our colony of randomly bred animals. They were exposed to a schedule of 14 hours of light and 10 hours of darkness and given free access to Wayne Breeder Blox and tap water. Hypophysectomized CD-1 male mice were obtained from Charles River Breeding Laboratories, provided with 5 percent glucose as a drinking solution, and allowed 5 to 6 weeks of recovery after arrival. The THC (0.5, 5, or 50 mg per kilogram of body weight) or vehicle (sesame oil, 20 μl) was administered by oral feeding as described previously (*7*). Hypophysectomized mice received an intraperitoneal injection of 500 mIU of human chorionic gonadotropin (hCG, Follutein, Squibb) at 2 and at 4 days before THC administration and blood sampling. The third hCG injection was given concomitantly with the oral feeding of THC (50 mg/kg) or sesame oil. An additional group of intact mice received hCG (1 IU) at the time of THC (5 mg/kg) administration.

The animals were bled for a single sampling by cardiac puncture under ether anesthesia at the times indicated in Fig. 1. Plasma was stored frozen for radioimmunoassay determinations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone, as described previously (*6*). Hormone levels were not normally distributed and therefore the nonparametric Mann-Whitney *U* test was used in the data analysis (*8*).

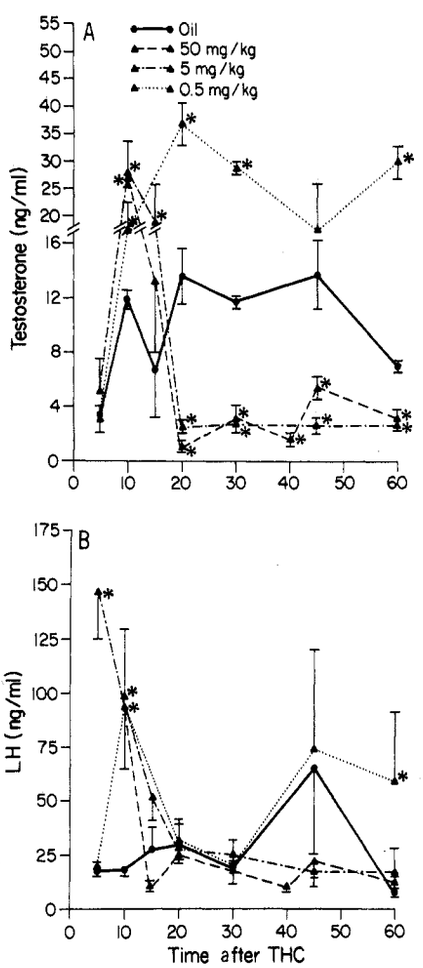

Fig. 1. Concentrations of (A) testosterone and (B) luteinizing hormone (*LH*) in the plasma of adult male mice at the times indicated after oral administration of THC at doses of 0.5, 5, and 50 mg per kilogram of body weight. Data are expressed as means ± standard error (*N* = three to six mice per point). Asterisks indicate data significantly different from controls, $P < .05$.

Figure 1A shows that all the THC doses increased the plasma testosterone concentrations within 10 minutes of administration. However, with the two higher doses of THC, these increases were followed by a reduction in plasma testosterone. In contrast, the lowest THC dose produced a rapid and sustained increase in testosterone during the entire sampling period (Fig. 1A). Figure 1B shows that there was a striking parallelism between the changes in the concentration of LH and testosterone after administration of the two higher doses of THC. That is, LH increased simultaneously with the increase in plasma testosterone and the decline in the concentrations of both hormones followed a very similar time course. This was not true for the low dose of THC (0.5 mg/kg), which produced a sustained increase in testosterone and fluctuations in the concentration of LH during the 1-hour sampling period.

Control mice showed no changes in either testosterone or LH concentrations during the sampling period. Plasma follicle-stimulating hormone concentrations were not significantly affected by THC administration (data not shown).

In hypophysectomized mice, administration of both THC (50 mg/kg) and hCG (500 mIU) resulted in significantly higher levels of plasma testosterone than did treatment with hCG alone. This was particularly evident during the last half of the sampling period (Fig. 2A).

In intact mice, administration of both THC (5 mg/kg) and hCG also produced higher concentrations of testosterone in plasma than did hCG alone (Fig. 2B).

Comparison of the testosterone concentrations in mice given both the THC and hCG and in animals given the same dose of THC alone (Fig. 1A) suggests that hCG may have delayed the increase and prevented the decrease in plasma testosterone after THC administration. Indeed, intact mice that received both hCG and THC showed increased concentrations of plasma testosterone during the entire sampling period.

These studies indicate that THC, in addition to its ability to suppress LH release and testosterone production, can also stimulate the release of both these hormones. The doses that can probably be considered as moderate to high (5 and 50 mg/kg) exerted a clearly biphasic effect, that is, stimulation followed by suppression, whereas the low dose (0.5 mg/kg) produced a more sustained increase in peripheral LH and testosterone with no suppression being evident within the 1-hour period of sampling.

The results obtained in hypophysecto-

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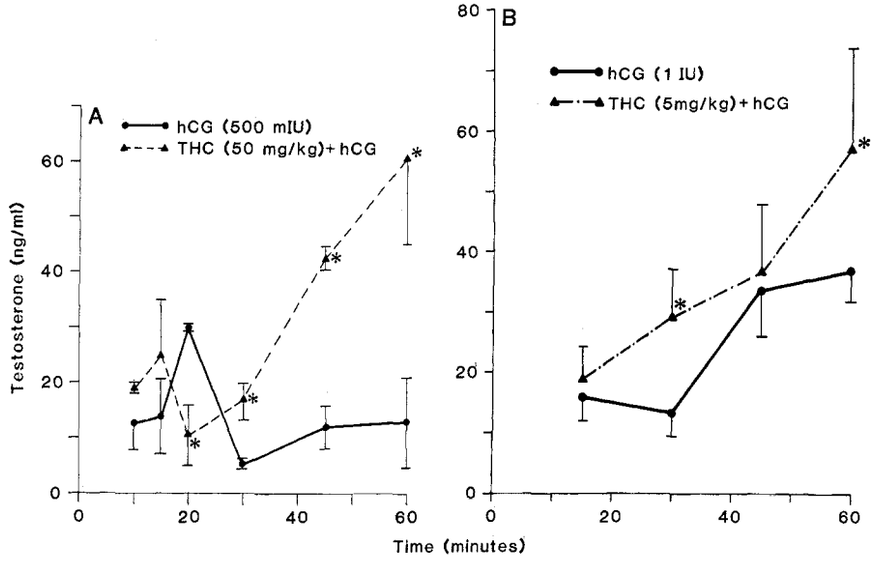

Fig. 2. (A) Plasma testosterone in hypophysectomized male mice receiving hCG (500 mIU, intraperitoneally) 2 and 4 days prior to blood sampling. The third hCG injection was given with THC (50 mg per kilogram of body weight) at the times indicated before sampling (N = 3 to 6 animals per point). (B) The effect of concomitant administration of hCG (1 mIU) and THC (5 mg per kilogram body weight) on plasma testosterone in adult intact male mice (N = 4 to 7 animals per point). Asterisks indicate data significantly different from controls, $P < .05$.

mized mice demonstrate that the stimulatory action of THC on plasma testosterone does not require the presence of the pituitary. The rapid increases in testosterone after THC administration in intact animals are difficult to explain on the basis of changes in LH release, since an increase in LH did not precede the increase in testosterone. Instead, there was a marked correlation between THC effects on plasma LH and testosterone at specific time periods. Such a correlation suggests that the increased testosterone failed to suppress pituitary LH release in THC-treated animals. Thus, in addition to its effects on LH release and on testicular steroidogenesis, THC may disrupt feedback regulation of the pituitary-gonadal axis. This may be related to action on the hypothalamus (*9*), alterations in steroid metabolism (*10*), or synergistic activity of THC and gonadotropins in stimulating testicular steroid production, as was observed with hCG in hypophysectomized and intact mice in the studies described here. It is also conceivable that THC prolongs the action of gonadotropins on their testicular receptors. This might explain the finding in hypophysectomized mice that hCG injection alone produced a rapid increase in plasma testosterone, with a subsequent decline that was prevented by concomitant THC administration.

Several studies have indicated that cannabinoids can have a direct effect on testicular function in vitro. Suppression of lipid, protein, and nucleic acid synthesis, as well as microsomal function, were obtained in rat testicular slices (*11*). In mice, inhibition of testosterone production and cholesterol esterase activity was observed in isolated Leydig cells and whole testes (*12*). The increased accumulation of esterified cholesterol in testes of mice given a single dose of THC in vivo may have been related to the concomitant suppression of plasma gonadotropin (*7*) with a resulting decrease in cholesterol ester hydrolase activity and cholesterol utilization. However, the evidence that cannabinoids inhibit cholesterol esterase activity in the testis, ovary, and adrenal in vitro (*13*) may indicate that the effects of these compounds on steroidogenic activity may result from direct actions of cannabinoids on the enzymes controlling steroidogenesis, and thus may not be due primarily or exclusively to suppression of trophic hormone release from the hypothalamus or pituitary (*14*).

The finding that THC significantly increases plasma LH and testosterone in adult mice may explain some of the reported behavioral consequences of marijuana use in humans. In one study, occasional users reported that exposure to marijuana produced a period of intense sexual arousal for about 40 minutes, after which the effect seemed to diminish. In the same study it was noted that, at lower doses, marijuana exposure seemed to increase sexual desire, while higher doses did not (*5*). Although the relation between plasma testosterone and libido and potency in men remains to be clarified, increases in testicular steroidogenesis, as well as administration of testosterone or estradiol, can be followed by copulatory behavior in mice and rats (*15*). Therefore, it seems reasonable to suspect that, if rapid increases in plasma LH and testosterone also occurs in human marijuana users, it could stimulate sexual interest and activity. Thus, the anecdotal reports on the aphrodisiac effects of marijuana and the evidence for suppression of testicular activity after heavy use of this substance are not contradictory, but represent effects of different doses or different phases in the temporal sequence of physiological changes induced by marijuana. In support of these speculations, we previously reported that THC administration causes a pronounced suppression of copulatory behavior in male mice (*7*), but have recently observed that animals of the same strain given the same dose of THC can normally complete the full copulatory sequence if the female is introduced immediately (*16*) rather than 1 to 4 hours after drug administration (*7*).

Thus, it is evident that the action of THC on the hypothalamic-pituitary-gonadal axis is biphasic, involving stimulation as well as inhibition.

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Tolerance and Cross-Tolerance in Chronic Alcoholics: Reduced Membrane Binding of Ethanol and Other Drugs

Abstract. *Membrane binding of ethanol, anesthetics, and hydrophobic molecules in brain synaptosomes and liver mitochondria from rats is conspicuously reduced after long-term consumption of ethanol. The membranes are resistant to structural disordering by both ethanol and halothane. Tolerance, cross-tolerance, and dependence in chronic alcoholics may in part result from membrane alterations that inhibit the binding of ethanol and other drugs.*

In chronic alcoholics ethanol tolerance and dependence probably arise from alterations in the membrane properties of the nervous system (*1*). This is consistent with the fact that the acute effects of ethanol, similar to other anesthetics, result from membrane interactions (*2*). We recently reported that liver mitochondrial membranes from rats chronically fed ethanol are resistant to the uncoupling effect of ethanol, which is associated with a resistance to the disordering of the membrane lipid structure by ethanol (*3*). The resistance is caused by altered lipid composition of the mitochondrial membranes. We now report that this resistance is associated with a drastic reduction in the partitioning of ethanol into liver mitochondrial membranes. We also report that brain synaptosomal plasma membranes in ethanol-fed rats are resistant to disordering by ethanol, a finding similar to previous observations in ethanol-fed mice (*1*). While the cause of the latter resistance may arise, in part, from increased cholesterol content (*4*), the end result of ethanol feeding is the same for both mitochondrial and synaptosomal membranes, namely, a reduction in the binding of ethanol. Synaptosomal and mitochondrial membranes from ethanol-fed rats are also resistant to structural disordering by the inhalation anesthetic halothane, and this resistance is also associated with reduced binding of halothane by these membranes. The binding of the sedative phenobarbital is also reduced in membranes from ethanol-fed rats.

To study the effect of ethanol and halothane on membrane structure, we employed two spin-labeled membrane probes. We calculate the order parameter S from the hyperfine splitting of the electron paramagnetic resonance (EPR) spectra of 5-doxyl stearate, a fatty acid analog incorporated into the lipid domain in cell membranes. The order parameter provides a measure of the freedom of motion of the probe in the membrane and is decreased when the membrane becomes more fluid (*5*, *6*). The nitroxide-labeled decane 4-butyl-2,2-dimethyl-1, 4-pentyloxazolidine -*N*- oxyloxazolidine (5N10) is a partition probe. The partition coefficient of the spin probe between the hydrophobic membrane and the medium is calculated from the contribution of the bound and free species to the composite EPR spectrum (*7*). The partitioning into the membrane increases as the membrane becomes more fluid.

Figure 1 shows the results of these measurements in liver mitochondria and brain synaptosomes from ethanol-fed rats and their pair-fed controls. Each curve represents membranes from a different rat. Both the order parameters and the probe partitioning show that membranes from ethanol-fed rats are more rigid than those from the controls. When titrated with ethanol (25 mM to 1.0M), control membranes become much more fluid, as indicated by a decrease in the order parameter and an increase in the partition coefficient. Membranes from ethanol-fed rats are much less affected by the addition of ethanol and remain relatively rigid even at high concentrations of ethanol. In the presence of moderate concentrations of ethanol, membranes from ethanol-fed animals are as fluid as membranes from the control animals in the absence of ethanol. The latter presumably are in a state that is optimal for membrane function.

There are similar differences in the

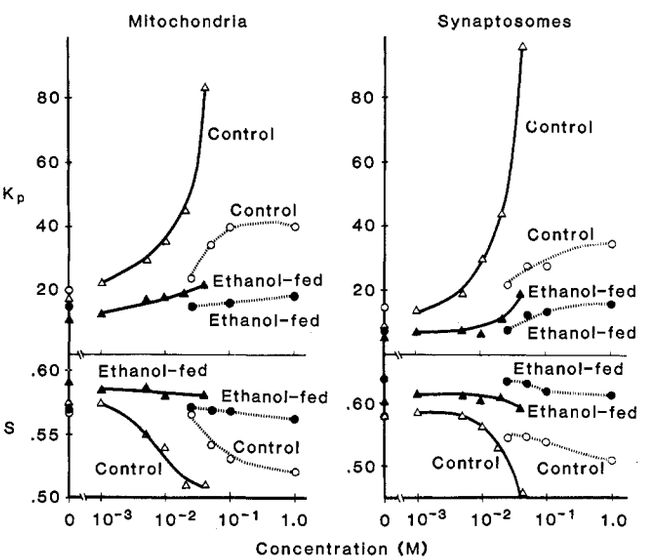

Fig. 1. Effect of ethanol (○, ●) and halothane (△, ▲) on the 5N10 partition coefficient K_p (upper panels) and on the 5-doxyl stearate order parameter S (lower panels) in liver mitochondrial membranes and brain synaptosomal membranes from ethanol-fed rats and their pair-fed controls. EPR spectra were obtained at 35° ± 0.2°C with a Varian E-109 spectrophotometer. The nitroxide-decane derivative 5N10 was added to membrane suspensions (10 mg of protein per milliliter) to give an apparent concentration of 0.1 mM. Partition coefficients were calculated by estimating the amount of bound and free probes from the EPR spectra (*7*). Sufficient 5-doxyl stearic acid was added to the membrane suspension to obtain a probe-to-lipid ratio of 1:200. The order parameter was estimated by the method described by Gaffney (*6*). Rats were given ethanol (14 g per kilogram of body weight per day) for 35 days; pair-fed controls received isocaloric carbohydrate instead of ethanol (*3*). Liver mitochondria (*3*) and brain synaptosomal (*9*) membranes were prepared by conventinal procedures. For EPR measurements the membranes were suspended in 0.25M sucrose, 20 mM tris-Cl (*p*H 7.4), 2 μM rotenone, and 10 μM ferrocyanide. At least three pairs were tested for each of the effects shown. The results are typical examples of these experiments.

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