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Further studies on the inhibition of Leydig cell testosterone production by cannabinoids

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Earlier reports from our laboratory have shown that Δ^1-tetrahydrocannabinol (Δ^1-THC), the psychoactive principle of marihuana, can inhibit the synthesis of testosterone by isolated Leydig cells [1, 2] and of progesterone by isolated luteal cells [3]. A detailed study of this effect revealed that the site of inhibition was the cholesterol esterase which provides precursor for steroid hormone biosynthesis [4]. A number of substances that are structurally related to Δ^1-THC and that do not exhibit psychoactivity occur in cannabis preparations to which subjects would be exposed when using marihuana, hashish, etc. [5]. Our previous reports [1–3] suggested that the non-psychoactive cannabinoids may be physiologically active, since one of these, namely cannabinol (CBN), showed a potency similar to that of Δ^1-THC in lowering testosterone levels. We now present the results of a more extensive study that included the major naturally occurring cannabinoids (Fig. 1).

The Leydig cells were prepared from testes of 60- to 90-day-old mice (Charles River, CD-1) as described previously [2]. Aliquots containing 2×10^6 cells each in Krebs–Ringer bicarbonate buffer (2 ml, pH 7.4) were then incubated for 2 hr with 25 mIU hCG (Organon "Pregnyl") at 32° under 95%/O_2 : 5% CO_2. Testosterone production averaged 20.3 ng/10^6 cells for non-drug-treated controls. The can-

Table 1. Inhibition of testosterone production in isolated mouse Leydig cells*

	Inhibition of testosterone production						
	Cannabinoid concentration (μM)						
	0.032	0.16	0.32	1.6	3.2	9.0	16
CBG	0.5 ± 0.01	15.6 ± 0.18†	25.3 ± 1.6‡	47.7 ± 0.87§			
CBD			16.1 ± 0.75‡	62.1 ± 2.83§	74.5 ± 2.88§	82.9 ± 2.59§	85.4 ± 1.69§
CBCy	3.0 ± 0.06	9.2 ± 0.32†	22.6 ± 0.53§	53.5 ± 1.28§			
CBN		5.5 ± 0.25	19.1 ± 0.31§	51.3 ± 1.25§	68.8 ± 1.62§		
Δ^1-THC			35.4 ± 3.5†	36.7 ± 0.71†	49.6 ± 4.0‡	65.4 ± 12.4§	84.5 ± 30.0§
CBC			17.5 ± 0.24§	13.7 ± 0.46‡	25.5 ± 0.74§	46.4 ± 2.8§	63.7 ± 1.13§
Olivetol	4.6 ± 0.06	1.5 ± 0.05	3.3 ± 0.10	15.4 ± 0.28§			

* Values are per cent inhibition ± S.E. (N = 5). Individual controls were provided for each cannabinoid. Abbreviations: CBG, cannabigerol; CBD, cannabidiol; CBCy, cannabicyclol; CBN, cannabinol; Δ^1-THC, Δ^1-tetrahydrocannabinol; and CBC, cannabichromene.
† $P < 0.05$.
‡ $P < 0.005$.
§ $P < 0.001$.

Table 2. Comparison of the inhibitory activities of the cannabinoids

Cannabinoid	ID_{50} (μg/ml) or (ng/mg)	ID_{50} (μM)
Cannabigerol	0.28	0.90
Cannabidiol	0.32	1.0
Cannabicyclol	0.35	1.1
Cannabinol	0.53	1.7
Δ^1-Tetrahydrocannabinol	1.0	3.2
Cannabichromene	3.1	9.9

nabinoids (NIDA) in 20 μl ethanol were added in doses ranging from 0.032 to 16 μM; control incubations of 20 μl ethanol alone had no effect on testosterone levels. At the end of 2 hr, the cells were removed by centrifugation at 1500 g, and the supernatant fraction was analyzed for testosterone by radioimmunoassay (NEN "RIA Pak"). Cannabinoids at the highest level used did not interfere with the assay and had no observable effect on cell viability as determined by Trypan blue exclusion.

Table 1 gives the extent of inhibition of testosterone synthesis by the various cannabinoids and olivetol, which is structurally related to the cannabinoids. Table 2 lists the dose of each cannabinoid required to reduce testosterone production by 50 per cent (ID_{50}) under the above conditions; the dose was determined by extrapolation of the data in Table 1. Cannabigerol showed the greatest potency (ID_{50} = 0.90 μM), while cannabichromene was the least active with only about one-tenth the potency. Interestingly, Δ^1-THC was among the least active substances tested.

No obvious structure–activity relationship can be seen from the series reported here. Olivetol represents the structural denominator of the cannabinoids and, although it showed slight activity, it was clearly less potent than any of the cannabinoids. It does seem, however, that the terpenoid portions of the various cannabinoids generally enhance activity when compared with olivetol. This differs from our previous observations on the relative potencies of a similar series of cannabinoids in inhibiting the synthesis of prostaglandin E_2 by bovine seminal vesicle microsomes [6]. In that system, olivetol showed a potency greater than that of most of the cannabinoids tested and CBN was the most active cannabinoid. This suggests that the two effects operate by different mechanisms. Most probably all of the cannabinoids inhibit testosterone production by the same mechanism as Δ^1-THC, does, i.e. by inhibition of cholesterol esterase [4].

Our findings indicate that clinical studies involving steroid hormone levels should take into account the abundance of each of the cannabinoids in the drug samples used. Assuming that the distribution and metabolism of each of the cannabinoids are similar [7], chronic exposure to cannabis could lead to appreciable levels of non-psychoactive cannabinoids at steroidogenic sites *in vivo*. The attainment *in vivo* of levels similar to those used in this study has been reported for Δ^1-THC in dogs [8], suggesting that our findings could be of toxicological importance in humans. A further conclusion that may be drawn from our results is that some of the non-psychoactive cannabinoids may be useful in therapeutic applications where a reduction in the synthesis of hormonally active steroids is desired, e.g. in the treatment of steroid-dependent tumors.*

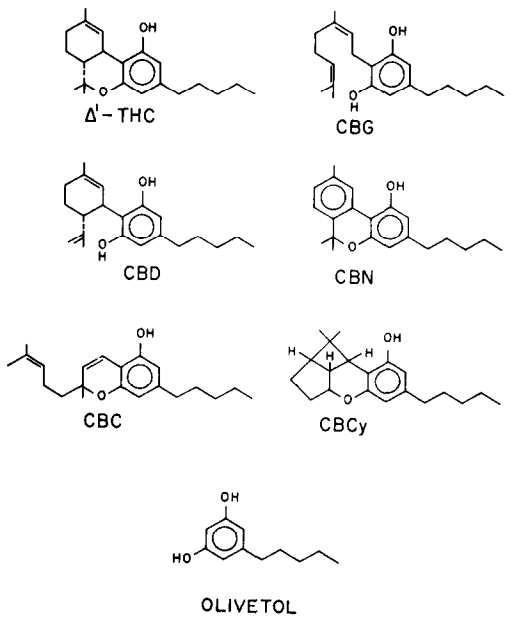

Fig. 1. Structures of substances tested. Abbreviations: Δ^1-tetrahydrocannabinol (Δ^1-THC), cannabigerol (CBG), cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), and cannabicyclol (CBCy).

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