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Metabolic Interactions of Cannabinoids with Steroid Hormones

Kazuhito Watanabe

Organization for Frontier Research and Department of Hygienic Chemistry, Hokuriku University, Kanazawa 920-1181, Japan

ABSTRACT: Metabolic interactions of the three major cannabinoids, Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) with steroid hormones were investigated. These cannabioids concentration-dependently inhibited 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase in rat adrenal and testis microsomes. CBD and CBN were the most potent inhibitors of 3β-hydroxysteroid dehydrogenase and progesterone 17α-hydroxylase, respectively, in rat testis microsomes. Three cannabinoids highly attenuated hCG-stimulated testosterone production in rat testicular interstitial cells. These cannabinoids also decreased in levels of mRNA and protein of StAR in the rat testis cells. These results indicate that the cannabinoids could interact with steroid hormones, and exert their modulatory effects on endocrine and testicular functions.

Metabolic interaction of a THC metabolite, 7β-hydroxy-Δ^8-THC with steroids is also investigated. Monkey liver microsomes catalyzed the stereoselective oxidation of 7β-hydroxy-Δ^8-THC to 7-oxo-Δ^8-THC, so-called microsomal alcohol oxygenase (MALCO). The reaction is catalyzed by CYP3A8 in the monkey liver microsomes, and required NADH as well as NADPH as an efficient cofactor, and its activity is stimulated by some steroids such as testosterone and progesterone.

Kinetic analyses revealed that MALCO-catalyze reaction showed positive cooperativity. In order to explain the metabolic interaction between the cannabinoid metabolite and testosterone, we propose a novel kinetic model involving at least three binding sites for mechanism of the metabolic interactions.
KEY WORDS: cannabinoid, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, StAR protein, steroid, metabolic interaction

INTRODUCTION

Marijuana is known to be one of the most abused drugs in the world, and exerts a variety of pharmacological effects in animals and humans (Dewey, 1986). There have been an extensive number of studies on the effects of cannabinoids on the endocrine system. Δ^9-Tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), the three major cannabinoids, have been reported to exert some effects on endocrine and testicular function in mammals (Dalterio and Bartke, 1979; Brown and Dobs, 2002). However, there is relatively a little evidence that the cannabinoids have direct effects on biosynthesis and degradation of steroids through metabolic interaction. The metabolic interaction between the cannabinoids and steroids is suggested since the structures of cannabinoids are similar to those of steroids such as testosterone and progesterone. We previously reported that CBD inhibited testosterone oxidation in male rat liver microsomes (Narimatsu *et al.*, 1988). More recently, we have reported that progesterone 17α-hydroxylase in rat testis microsomes was inhibited by Δ^9-THC, CBD, and CBN (Watanabe *et al.*, 2005). The present study focused on the metabolic interactions of three major cannabinoids with steroid hormones. In addition, the metabolic interaction of a THC metabolite, 7β-hydroxy-Δ^8-THC with steroids is also investigated.

MATERIALS AND METHODS

Chemicals: Δ^9-THC, CBD, and CBN were isolated and purified from cannabis leaves by the method of Aramaki *et al.* (1968). Steroids were purchased from Sigma Chem. Co. All other chemicals were of the highest grade available from commercial sources.

Steroid metabolism: Male Sprague-Dawley rats (8 weeks old) were killed by decapitation and their adrenals and testes were dissected. Microsomal fractions were prepared by different centrifugation force. The activities of 3β-hydroxysteroid dehydrogenase (pregnenolone to progesterone) and 17α-hydroxylase (progesterone to 17α-hydroxyprogesterone and androstenedione) were assayed using radioactive substrates by measuring the conversion to corresponding metabolites.

Microsomal alcohol oxygenase (MALDO): Liver microsomes were prepared from a liver sample of male Japanese monkey. MALCO activity for 7β-hydroxy-Δ^8-THC was determined as described previously (Matsunaga *et al.*, 2000). Testosterone 6β-hydroxylation was assayed by the previous method (Narimatsu *et al.*, 1988).

Kinetic analysis: Kinetic analyses of MALCO activity and testosterone 6β-hydroxylation were performed using a sigmoidal *V*max model. Values of v at various substrate concentrations were fitted using a non-linear least-squares regression analysis program. The n (Hill coefficient) value was obtained from plots of log $[v/(V\text{max} - v)]$ vs log S (Hill plot) using Origin 6.1 J program.

RESULTS AND DISCUSSION

The three major cannabinoids inhibited 3β-hydroxysteroid dehydrogenase in rat testis microsomes and adrenal homogenate. In both cases, CBD was the most potent inhibitor of the dehydrogenase (IC_{50} = 17.9 – 115.9 μM) (Fig. 1). The three major cannabinoids also inhibited progesterone 17α-

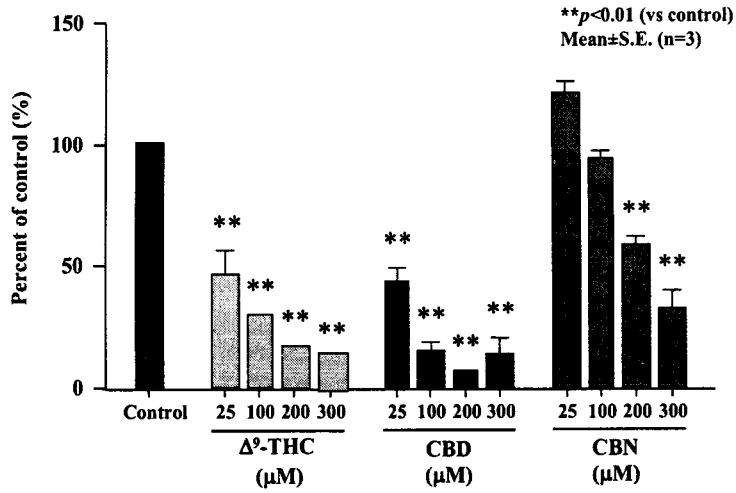


Fig. 1 Inhibitory Effects of Major Cannabinoids on 3β-Hydroxysteroid

Table 1 Effects of Cannabinoids on Kinetic Parameters of Microsomal 17α-hydroxylase in Rat Testis

	K_m (nM)	V_{max} (pmol/min/mg protein)	Ki (μM)
Study 1			
Control	51.3	142.9	
Δ^9-THC (50 μM)	119.2	119.2	15.9
Δ^9-THC (100 μM)	202.2	90.9	
Study 2			
Control	41.4	133.3	
CBD (50 μM)	41.7	105.3	124.4
CBD (100 μM)	51.3	92.6	
Study 3			
Control	53.2	129.9	
CBN (50 μM)	582.6	116.3	4.5
CBN (100 μM)	1657.6	172.4	

Dehydrogenase in Rat Adrenal Homogenate hydroxylase activity in rat testicular microsomes, and CBN is thought to act as the most potent inhibitor among cannabinoids tested (Table 1). Kinetic analyses indicate that the type of testis microsomal 17α-hydroxylase inhibition by CBN is a competitive manner, while that by Δ^9-THC and CBD is the mixed-type. The inhibition constants (*Ki*, μM) for Δ^9-THC, CBD, and CBN were 15.9, 124.4 and 4.5, respectively. While Δ^9-THC

did not directly bind to estrogen and androgen receptors (Chakravatry and Naik, 1983; Sauer *et al.*, 1983), it is well characterized that the structures of these cannabinoids are similar to those of steroids such as testosterone and progesterone (Martin, 1986), suggesting the interaction of cannabinoids with steroids. At present, it is not known whether 17α-hydroxylase catalyzes the hydroxylation of these cannabinoids or not, although the metabolic interaction of the cannabinoids with progesterone may be responsible for the inhibition of 17α-hydroxylase activity by the cannabinoids. Our previous study demonstrated that Δ^9-THC, CBD, and CBN inhibited testosterone 6β- and 16α-hydroxylase activities in rat liver microsomes. These findings and the present study demonstrate that the metabolic interactions of these cannabinoids with steroids may be responsible for the inhibition of steroid metabolism by the cannabinoids. Further study is, however, necessary to clarify whether these cannabinoids inhibit the steroid metabolism *in vivo* situation.

Effects of cannabinoids on hCG-stimulated testosterone production by rat testis interstitial cells are shown in Fig. 2. All cannabinoids tested concentration-dependently attenuated the hCG-stimulated testosterone production.

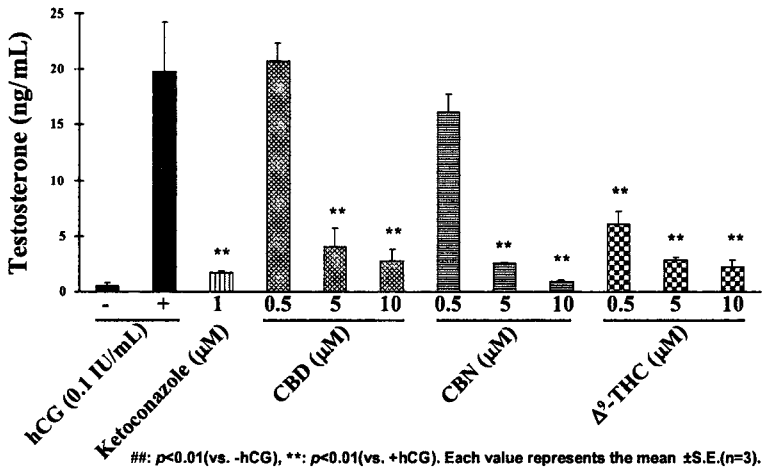
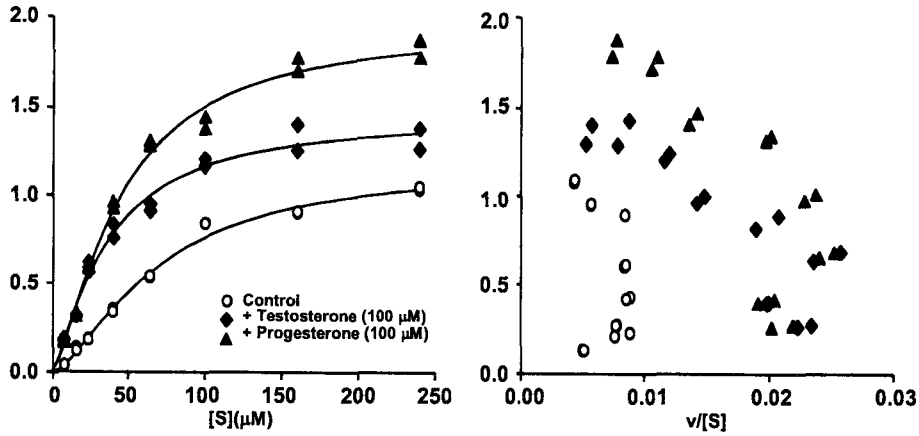


Fig. 2 Effects of Cannabinoids on hCG-Stimulated Testosterone Production in Rat Testicular Interstitial Cells

The testosterone production was significantly inhibited at concentrations greater than 5 μM comparable to inhibitory effect of ketoconazole. Treatments of testis cells with increasing concentrations (0.1–100 μM) of three cannabinoids suppressed mRNA and protein levels of StAR. These results suggest that the cannabinoids have modulatory effects on the endocrine and testicular functions through a suppression of StAR protein level as well as a direct inhibition of biosynthetic enzymes.

THC is extensively biotransformed to a great number of oxidized metabolites by cytochrome P450 in mammals (Yamamoto *et al.*, 1995). The major metabolic sites are allylic positions at the C-11, C-8 for Δ^9-THC, and C-7 for Δ^8-THC. We previously characterized the MALCO which catalyzes the stereoselective oxidation of 7α- and 7β-hydroxy-Δ^8-THC to 7-oxo-Δ^8-THC in liver microsomes of various animal species including monkeys and humans (Matsunaga *et al.*, 2000, 2002). CYPs belonging to the 3A subfamily are the major enzymes responsible for the MALCO activity. CYP3A enzymes are the most abundant CYPs in many mammals, and their catalytic activities have been shown to be stimulated by certain chemicals including steroids such as testosterone and progesterone. Main enzyme responsible for monkey liver MALCO is assumed to be CYP3A8. We previously demonstrated that NADH as well as NADPH required for the MALCO activity of 7-hydroxy-Δ^8-THC in monkey liver microsomes (Matsunaga *et al.*, 2005). The present study investigated the metabolic interaction of 7-hydroxy-Δ^8-THC with steroids using monkey liver microsomes. Some steroids stimulated the NADH- and NADPH-dependent MALCO activity in monkey liver. In particular, progesterone and testosterone (100 μM) highly stimulated NADPH-dependent activity by 4.1-fold and 3.0-fold, respectively. Kinetic analysis demonstrated that positive cooperativity was seen in the NADH-dependent MALCO activity (Fig. 3), indicating that multiple binding sites are involved in the catalytic reaction. Testosterone decreased S_{50}, while n value remained unchange. Our data could not

fit equations of a modified two-sites model (Korzekwa *et al.*, 1998; Hosea *et al.*, 2000). Based on kinetic analyses, we propose a model of at least three binding sites for the CYP3A8-catalyzed oxidation of 7β-hydroxy-Δ^8-THC to 7-oxo-Δ^8-THC in monkey liver microsomes.



NADH	S_{50} (μM)	V_{max} (nmol/min/mg protein)	n
Control	68.24 ± 5.44	1.18 ± 0.051	1.56 ± 0.11
+ Testosterone	35.70 ± 3.85	1.45 ± 0.072	1.34 ± 0.15
+ Progesterone	44.31 ± 3.42	1.96 ± 0.074	1.43 ± 0.11

Analyzed by Hill equation. Values are expressed as means± S.E. of duplicate.

Fig. 3 Substrate-Velocity Curves and Eadie-Hofstee Plots of NADH-Dependent MALCO Activities in Monkey Liver Microsomes

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