Biochemical Pharmacology, Vol. 22, pp. 2981–2987. Pergamon Press, 1973. Printed in Great Britain.

INTERACTIONS OF SEVERAL CANNABINOIDS WITH THE HEPATIC DRUG METABOLIZING SYSTEM*

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(*Received* 4 *May* 1973; *accepted* 10 *July* 1973)

Abstract—Spectral interactions of various cannabinoids with rat liver microsomes and their effects on several microsomal enzymes were studied. Δ^9-Tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabinol (CBN), and cannabidiol (CBD) produced type I spectral changes; the spectral dissociation constants K_s were 42, 37, 46 and 11·2 μM, respectively,. Aminopyrine demethylation was competitively inhibited by Δ^9-THC, Δ^8-THC, CBN and CBD, by the latter only in concentrations below 10 μM. The inhibitor constants were found to be 58, 60, 68 and 49 μM, respectively. In a similar way morphine demethylation was inhibited. Δ^8-THC, however, did not inhibit this reaction, and inhibition by CBD was of mixed type at all concentrations. There was no effect of cannabinoids on aniline hydroxylation. The inhibitory potencies of cannabis constituents on drug metabolism *in vitro* parallel the *in vivo* results obtained by interaction studies with hexobarbitone. It must be concluded that CBD, which is by far more potent in inhibiting drug metabolism than other cannabinoids, contributes significantly to the effects of crude cannabis preparations at least in rodents.

EVIDENCE has been presented that various resinous cannabis constituents, the so-called cannabinoids, are metabolized by mammalian liver preparations mainly to mono- and dihydroxylated compounds.[1–5] Δ^9-Tetrahydrocannabinol (Δ^9-THC) and recently Δ^8-tetrahydrocannabinol (Δ^8-THC) and cannabinol (CBN) have been shown to produce type I spectral changes with rat liver microsomes indicating the formation of an enzyme–substrate-complex with cytochrome P-450.[6–8] This leads to the suggestion that cannabinoids are metabolized by the microsomal mono-oxygenase system. Furthermore, Δ^9-THC has been shown to inhibit *in vitro* the metabolism of typical type I compounds, e.g. ethylmorphine, 6-aminopyrine,[9] and hexobarbitone.[8,9] Despite strong interactions with liver microsomes *in vitro* Δ^9-THC and its psychotomimetic analogue Δ^8-THC failed to inhibit the metabolism of hexobarbitone *in vivo*.[6,10] Recently we presented evidence that cannabidiol (CBN), another constituent of cannabis resin, exclusively prolongs the hexobarbitone "sleeping time" of rats by slowing the decline of blood and brain levels of the barbiturate.[8] Furthermore, Paton and Pertwee[11] reported that CBD is a stronger inhibitor of phenazone metabolism in mouse liver supernatant than THC. These observations lead to the suggestion that significant differences exist between various cannabinoids in affecting the hepatic drug metabolizing system.

* This paper was presented in part at the Joint Meeting of the German and French Pharmacological Societies, Paris, October 1972, and at The Bayer Symposium in Große Ledder 1972.

This study was supported by a grant from the Bundesministerium für Jugend, Familie und Gesundheit.

The present investigation examines Δ^8-THC, Δ^9-THC, CBN and CBD with respect to both: spectral interactions with male rat liver microsomes and effects on several drug metabolizing enzymes.

MATERIALS AND METHODS

Δ^9-THC, CBN and CBD were prepared from hashish by ethanol extraction and column chromatography on silica gel or alumina by a modification of the method of Aramaki.[12] Synthetic Δ^8-THC was kindly supplied by Dr. Petrzilka, ETH Zürich, and further purified on silica gel using benzene as eluent. Purities were confirmed qualitatively by t.l.c. (benzene–*n*-hexene–diethylamine, 25:10:1) and quantitatively by g.l.c. (1% SE-30 on chromosorb G, 80–100 mesh, inlet-column-detector temp.: 240–220–250°) assuming the relative responses according to Agurell and Leander.[13] Only cannabinoid samples with a purity better than 98·5 per cent were used. Main contaminants were cannabigerol and cannabichromene making up to 0·8 per cent of the samples.

Male Wistar rats (250–300 g) were killed by decapitation. The microsomes were prepared from 0·25 M sucrose liver homogenates (1:8, w/v) by differential centrifugation as described by Schenkmann.[14] The pellet was washed once with ice-cold 1·15% KCl + 0·02 M Tris–HCl, pH 7·4 and resuspended in 1·15% KCl + 0·05 M Tris, pH 7·4.

The difference spectra were recorded in 3 ml cuvettes containing microsomal suspension (1·5 mg protein/ml). The cannabinoids were added in small volumes of ethanol (up to 20 μl) and an equivalent amount of the vehicle was added to the reference cuvette. Measurements were made in a Beckman DK-2A Ratio Recording Spectrophotometer.

Enzyme activities were determined at 37° in a medium containing 50 mM Tris–HCl-buffer, pH 7·4, 5 mM $MgCl_2$, 0·4 mM NADP, 4 mM glucose-6-phosphate, 1 IE glucose-6-phosphate dehydrogenase (Boehringer Mannheim, pur. grad. II) in 3 ml. The cannabinoids in ethanol were mixed with the microsomes (1 μl ethanol/ml microsomal suspension) before they were added to incubation medium thus facilitating its solution. In controls aliquots of ethanol were added to the microsomes and in all cases the incubation was started by adding the microsomes (3–4·5 mg protein/3 ml). Aniline hydroxylation was determined by measuring *p*-aminophenol formation.[14] Demethylation of aminopyrine and morphine were determined by formaldehyde production using Nash's reagent B.[15] Reactions were linear within the time range used. Maximum velocity, apparent K_m and K_s values were calculated from double reciprocal data by the method of least square analysis on a Monroe 1165 table computer. Statistical analysis has been performed by one way analysis of variance, individual comparisons have been made by the F-test.

RESULTS

Difference spectra. The addition of the four cannabinoids to rat liver microsomes elicited a type I spectrum showing maxima between 388 and 392 nm and minima between 419 and 422 nm. This is in accordance with recent reports.[6,7] Whereas maximum Δ absorbance (390–420 nm) was identical for all compounds major differences existed in the affinities to microsomal cytochrome (Table 1). The spectral

TABLE 1. EFFECTS OF CANNABINOIDS ON THE HEPATIC DRUG METABOLIZING SYSTEM

	Δ^9-THC	Δ^8-THC	Cannabinol	Cannabidiol	Statistical analysis
Max. extinction difference (390–420 nm) = ΔE_{max}	0·028 ± 0·002 (5)	0·025 ± 0·006 (5)	0·029 ± 0·004 (4)	0·030 ± 0·005 (5)	d.f. 3, 15; F = 1·10 n.s.
Spectral dissociation constant K_s	41·6 ± 7·2 (5)	37·0 ± 4·2 (5)	46·1 ± 6·4 (5)	11·2 ± 1·4 (5)	d.f. 3, 15; F = 42·4 P < 0·001
Inhibitor constant in aminopyrine demethylation, K_i (μM)	58 ± 11* (6)	80 ± 14* (4)	68 ± 22* (6)	4·9 ± 0·8†	d.f. 3, 18; F = 29·5 P < 0·001
Inhibitor constant in morphine demethylation, K_i (μM)	68 ± 36* (6)	No inhibition at 80 μM	52 ± 8* (6)	4·7 ± 2·7†‡	d.f. 2, 15; F = 14·26 P < 0·001
Effect on aniline hydroxylation	No inhibition at 200 μM	No inhibition at 200 μM	No inhibition at 200 μM	No inhibition at 200 μM	

In brackets the number of experiments is given. Statistical analysis has been performed by one-way-analysis of variance between all 4 groups. The apparent K_m values (μM) of the uninhibited reactions were: aminopyrine demethylation 51 ± 8 (12), morphine demethylation 40 ± 7 (12), aniline hydroxylation 2·8 ± 0·3 (9).

Values calculated as mean ± S.E. d.f. = degrees of freedom.

* Inhibitor constant determined at 40 μM of the cannabinoid.

† Inhibitor constant determined at 5 μM of the cannabinoid.

‡ No true inhibitor constant, since V_{max} of the CBD inhibited reaction differed significantly (P > 0·05) from control.

dissociation constants K_s of CBN, Δ^9-THC and Δ^8-THC were found to be 46, 42 and 37 μM, respectively. These values are somewhat higher than those reported[6,7] and might represent strain differences. The binding affinity of CBD, however, was significantly higher as indicated by its lower K_s (11·2 μM). From this a strong interaction between CBD and the microsomal drug metabolizing system can be expected.

Aminopyrine demethylation. Δ^9-THC, Δ^8-THC and CBN inhibited the formaldehyde production from aminopyrine by microsomes in a competitive manner (Fig. 1). The inhibitor constants were found to be in the same order of magnitude as the spectral dissociation constants (Table 1) and did not differ significantly from each other (d.f. 2, 13, $F = 1{\cdot}97$, $P < 0{\cdot}05$). Accordingly, the differences between all groups revealed by analysis of variance must be attributed to CBD. With up to 10 μM CBD the inhibition was competitive. At a concentration of 40 μM, however, a mixed type occurred (Fig. 1). The inhibitor constant of CBD determined at 5 μM was 10 times lower than those of the other cannabis constituents (Table 1).

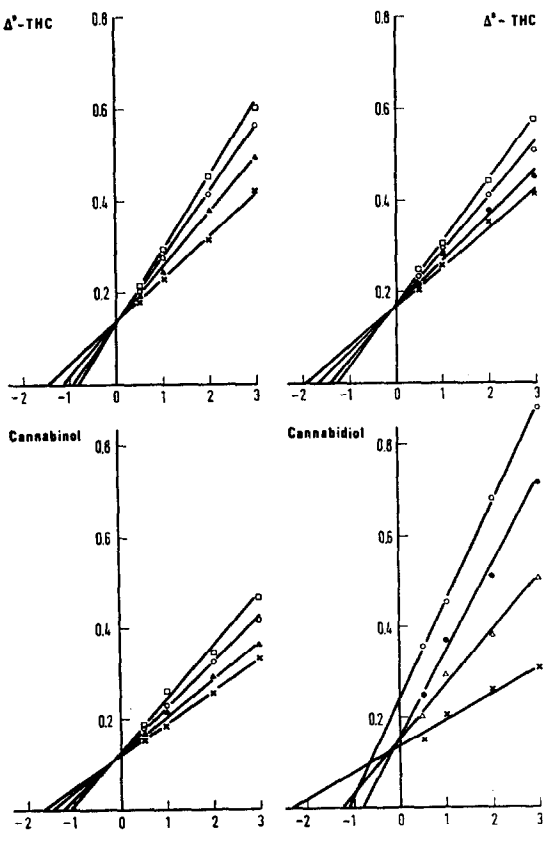

FIG. 1. Lineweaver–Burk plots showing inhibition of aminopyrine demethylation by Δ^9-THC, Δ^8-THC, CBN and CBD. Ordinate: 1/nmole HCHO formed in 1 min by 1 mg microsomal protein. Abscisse: 1/substrate 10^{-3} M. The concentrations of the cannabinoids were 0 μM (×), 5 μM (△), 10 μM (●), 40 μM (○), and 80 μM (□).

Morphine demethylation. Similar effects were exerted by the cannabinoids on morphine demethylation (Table 1). Surprisingly, however, Δ^8-THC did not inhibit formaldehyde production from this substrate in our experiments. The inhibition of CBN and low concentrations of Δ^9-THC were found to be competitive, too, whereas CBD even at low concentrations (5–10 μM) produced an inhibition of mixed type (Fig. 2). Since the maximum velocity of the cannabidiol treated microsomes differed significantly from control values, the K_i given in Table 1 for this compound is not a true inhibitor constant. Despite this, the K_i-values of Δ^9-THC, CBN and CBD in morphine demethylation agree fairly well with those for aminopyrine demethylation. CBD again proved to be the most potent inhibitor of the tested cannabinoids.

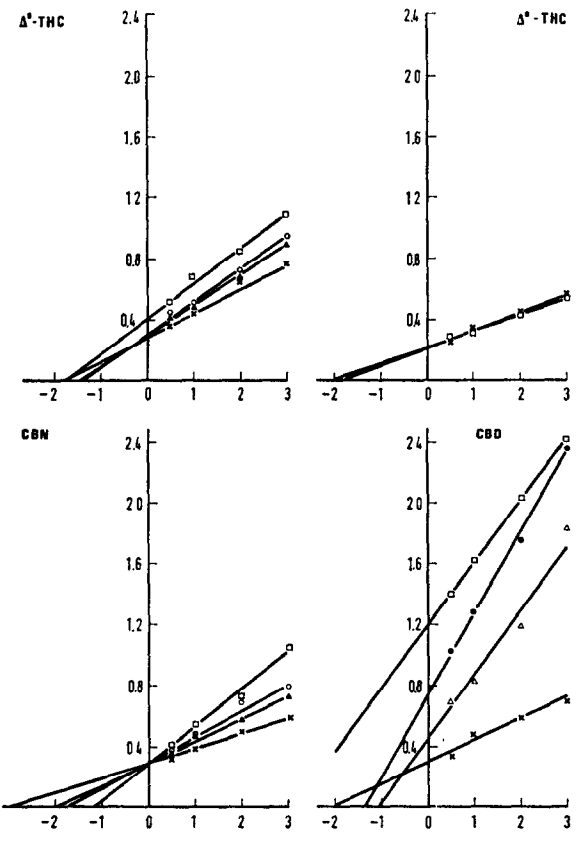

FIG. 2. Inhibition of morphine demethylation by cannabinoids. For explanation see legend of Fig. 1.

Aniline hydroxylation. Apparent K_m for aniline hydroxylation as measured by *p*-aminophenol formation agree well with those reported by others. The cannabinoids were added in concentrations of up to 200 μM. No effect on apparent K_m or maximum velocity could be detected in four separate experiments.

DISCUSSION

The present investigation shows that not only Δ^9-THC which is believed to be the major psychoactive principle of cannabis but also other cannabinoids interact with the hepatic drug metabolizing system *in vitro*. They are bound to rat liver microsomes producing type I difference spectra, which is in agreement with the recent report by Kupfer *et al.*[7] Furthermore, all tested cannabinoids are effective inhibitors of several microsomal drug metabolizing enzymes *in vitro*. Aminopyrine demethylation is competitively inhibited by CBN, Δ^9-THC and low concentrations of CBD. Recently, we reported almost identical effects on the side chain oxidation of hexobarbitone, another type I binder to microsomes.[8] Morphine demethylation was inhibited, likewise. Δ^8-THC, however, had no effect on this enzyme. Further differences to the inhibition of the other enzymes are substantiated by the fact that the inhibition of morphine demethylation by CBD was of mixed type at all concentrations used (5, 10, 40 μM). By contrast, no effect could be detected on the hydroxylation of aniline, a typical type II binder, by concentrations up to 200 μM of the cannabinoids, respectively. Failure in inhibition of the metabolism of another type II binder, *p*-chloro-*N*-methylaniline, has been reported also by Cohen.[6] This implies that the inhibition of drug metabolism by cannabinoids is somewhat selective.

The most important result of the present investigation, however, is that CBD, which has been supposed for a long time to be pharmacologically inactive, is a much stronger inhibitor of drug metabolism than other cannabinoids, the psychotomimetic tetrahydrocannabinols inclusive. The spectral dissociation constant, K_s of CBD is four-times lower than those of CBN, Δ^8-THC and Δ^9-THC. An even greater difference exists between the inhibitor constant K_i in aminopyrine and morphine demethylation. In these cases the inhibitor constants of CBD are 10 times lower than those of CBN, Δ^8-THC or Δ^9-THC. The finding that the K_s of CBD differs less from the spectral dissociation constants of Δ^8-THC, Δ^9-THC and CBN than the K_i of CBD differs from the inhibitor constants of the other cannabinoids (Table 1) indicates that the stronger inhibitor potency of CBD cannot be completely explained by the different affinity to cytochrome P-450. The exact mechanism of inhibition remains to be elucidated. In addition, the Lineweaver–Burk plots show that to some extent factors other than competition for a common receptor or intermediate contribute to the inhibitory effects of at least CBD. In particular this is the case in morphine demethylation.

Of greater practical interest than *in vitro* interactions is whether cannabinoids can inhibit drug metabolism also in the intact animal. Recently we presented experiments on hexobarbitone metabolism.[16] These investigations have shown that CBD, administered i.p. in 10% cremophor EL, strongly inhibits the elimination of the barbiturate *in vivo*, whereas the vehicle is without effect. One hr after a single application of 10 mg/kg/CBD the half life time of hexobarbitone in blood and brain of male rats was increased from about 20 to 40 min. The effect lasted for at least 48 hr. By contrast, the hexobarbitone metabolism *in vivo* was not affected by Δ^9-THC and CBN until 40 mg/kg of the cannabinoids were administered.

Obviously, these *in vitro* results parallel the *in vitro* inhibitory potencies of cannabinoids on the hepatic drug metabolizing system, demonstrated in the present investigation. It is somewhat surprising, however, that compounds like CBN and Δ^9-THC, having such low spectral dissociation constants and inhibitor constants

in vitro must be applicated *in vivo* in such high doses to cause a slight inhibition of drug metabolism. From experiments with radioactively-labelledΔ^9-THC it is known that radioactivity even accumulates in rat liver.[17–19] The rapid metabolism of these compounds, however, could mean that the original drugs do not remain in the liver a sufficient length of time to cause inhibition. The failure of 11-OH-Δ^8-THC (a metabolite of Δ^8-THC) in producing a difference spectrum with liver microsomes[7] might indicate the formation of non inhibitory metabolic products.

From its inhibitory action on drug metabolism it must be concluded that CBD contributes significantly to the effects of crude cannabis preparations at least in rodents. Interactions with other drugs are especially concerned. Moreover, it has been shown recently, that CBD also inhibits the metabolism of the psychotomimetic Δ^9-THC in mice *in vivo*.[20] By this mechanism the general acute potentiation of THC-effects in the rat by cannabidiol[16] can be explained in a simple way.

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