Br. J. Pharmac. (1979), **67**, 93–101

DEVELOPMENT OF TOLERANCE TO THE PROLONGATION OF HEXOBARBITONE SLEEPING TIME CAUSED BY CANNABIDIOL

HENRY K. BORYS, GLYNNIS B. INGALL & RALPH KARLER

Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah 84132, U.S.A.

1 The effects of acute and subacute cannabidiol (CBD) administration on hexobarbitone sleeping time and on some constituents of the hepatic microsomal drug-metabolizing system were assessed in the mouse.

2 Acutely administered CBD prolonged sleeping time; but with subacute treatment, tolerance to the effect rapidly developed.

3 Brain hexobarbitone concentration upon awakening was unchanged by either acute or subacute CBD treatment, which suggests that neither the prolongation of sleeping time nor the tolerance is the result of a change in sensitivity of the central nervous system to the barbiturate.

4 Acute CBD treatment increased the half-time of hexobarbitone in the brain, which returned toward normal with the development of tolerance.

5 Acutely, CBD caused a 30% decrease in hepatic cytochrome P-450 level; with tolerance, the cytochrome concentration returned to normal.

6 The evidence suggests that the CBD-induced prolongation of barbiturate sleeping time and the tolerance to this effect are the result of changes in the rate of drug metabolism, which are related to changes in the amount of cytochrome P-450.

7 The effects of CBD on the hepatic microsomal drug-metabolizing enzyme system are different from those attributed to SKF 525-A and piperonyl butoxide because the cannabinoid does not decrease cytochrome P-450 quantitatively by complex formation, it does not produce a recovery overshoot in the cytochrome concentration and, finally, it does not cause an induction of the hexobarbitone-metabolizing enzymes.

Introduction

The toxicology of cannabidiol (CBD) is important for at least two reasons: first, it is a major constituent of marihuana (Mechoulam, McCallum & Burstein, 1976); secondly, the preclinical evaluation of its anticonvulsant properties suggests that the drug may be a useful anti-epileptic agent (Karler & Turkanis, 1976). The clinical potential of the drug is enhanced by its apparent lack of toxicity; for example, in man, massive intravenous doses of CBD do not produce the characteristic psychotoxic and cardiovascular effects of marihuana or Δ^9-tetrahydrocannabinol (Δ^9-THC) (Perez-Reyes, Timmons, Davis & Wall, 1973). However, animal studies have demonstrated that CBD can prolong barbiturate sleeping time (Loewe, 1944), which was subsequently shown to be the result of the ability of the cannabinoid to inhibit barbiturate metabolism by the liver (Paton & Pertwee, 1972; Fernandes, Kluwe & Coper, 1974; Seimens, Kalant, Khanna, Marshman & Ho, 1974). Because CBD is an inhibitor of hepatic drug metabolism, a potential exists for toxic drug interactions with use of the drug. The present study was designed to determine the effects in mice of both acute and subacute CBD treatment on hexobarbitone sleep time and on some constituents of the oxidative drug-metabolizing enzymes in the liver.

Methods

Male Charles River mice (ICR) weighing 20 to 30 g were used in all the experiments. CBD was suspended in isotonic saline containing 3% Tween 80, as described by Karler, Cely & Turkanis (1974), and hexobarbitone was dissolved in isotonic saline immediately before injection. All drugs were administered intraperitoneally. The dose of CBD was 120 mg/kg which represents the anticonvulsant ED_{50} in mice in a maximal electroshock test (Karler, Cely & Turkanis, 1973).

Hepatic microsomes were prepared by a modification of the procedure of Franklin & Estabrook (1971).

0007-1188/79/090093-09 $01.00

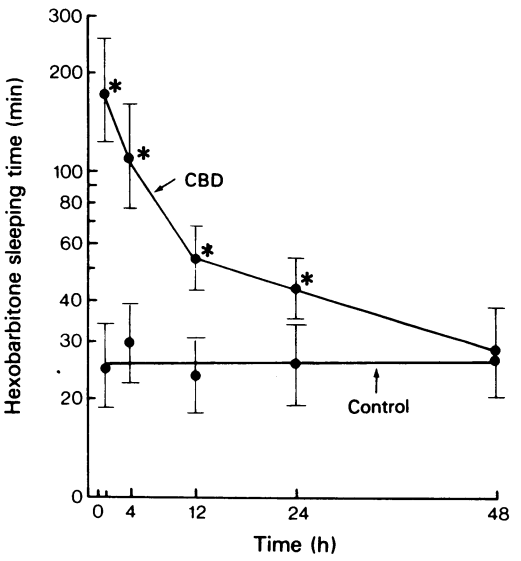

Figure 1 Time course of the effect of cannabidiol (CBD) on hexobarbitone sleeping time. Hexobarbitone (100 mg/kg) sleeping times were measured 1, 4, 12, 24 and 48 h after a single dose of CBD (120 mg/kg). Each value is expressed as the mean and standard deviation of a group of 20 animals, and no animals were used more than once. The asterisks indicate the values that are significantly different from their corresponding controls, as determined by a t test ($P < 0.05$).

Livers were homogenized in 0.25 M sucrose solution (1:5, w/v), and the resulting homogenates centrifuged at 20,000 g for 20 min in a Beckman L-2 ultracentrifuge. The supernatant fraction was then centrifuged at 100,000 g for 30 min. The recovered pellet was suspended in 1.15% KCl and the suspension was centrifuged at 100,000 g for 30 min. The pellet was finally resuspended in 0.25 M sucrose solution containing 50 mM Tris-chloride buffer, pH 7.4, to obtain a final protein concentration of approximately 10 mg/ml. For the metabolic complex detection experiments, only freshly prepared microsomes were used; for all other studies, microsomal suspensions were obtained from a frozen stock (−20°C), which was stored for no longer than 4 days. All spectral work was performed at room temperature in a Cary 118 spectrophotometer. For these studies, microsomes were suspended in 50 mM Tris-chloride buffer, pH 7.4, containing 1.15% KCl and 10 mM $MgCl_2$, to obtain a final protein concentration of 1 mg/ml. Cytochrome P-450 was measured using an extinction coefficient of 91 mM^{-1} cm^{-1}, as described by Omura & Sato (1964).

For the spectral studies, drugs were prepared as follows: an alcoholic solution of Pluronic F68 (BASF Wyandotte Corp.) was mixed with CBD, Δ^9-THC or piperonyl butoxide. Nitrogen was used to evaporate the ethanol; water was then added to the residue and the resulting mixture was sonicated in a Branson Sonifier W-350. The concentration of Pluronic in the drug stock preparation was 5 mg/ml and in the microsomal suspensions, 0.1 mg/ml. Drug concentrations in the microsomal suspensions were: CBD, 0.06 mM; Δ^9-THC, 0.13 mM; piperonyl butoxide, 2.4 mM. The microsome-drug suspensions were divided into the sample and reference cuvettes, and drug metabolizing reactions were started by the addition of NADPH (0.24 mM) to the sample cuvette only. During a 20 min reaction period, samples were monitored periodically for complex formation by scanning the 500 to 400 nm region.

Microsomes isolated from CBD-treated mice were examined for drug-cytochrome P-450 complexes formed *in vivo*. Ferricyanide oxidation, as described by Buening & Franklin (1976), was used as a test for complexes of the SKF 525-A type. In order to detect complexes of the piperonyl butoxide type, the microsomes were divided into two cuvettes and the sample cell was reduced by the addition of a few crystals of dithionite (Franklin, 1976).

Cytochrome c reductase activity was determined by the method of Williams & Kamin (1962), and protein concentrations by the method of Lowry, Rosebrough, Farr & Randall (1951). Hexobarbitone sleeping times were measured from the loss to the recovery of the righting reflex, and brain hexobarbitone concentrations were determined by the method of Vessel (1968).

Results

The time course of the prolongation of hexobarbitone sleeping time was determined following a single dose of CBD (Figure 1). Significant increases in sleeping times were observed for as long as 24 h after CBD administration; the response at 48 h was essentially normal. In subsequent experiments, measurements of sleeping time were made 4 h after CBD treatment.

The mean sleeping time induced by 100 mg/kg hexobarbitone, measured 4 h after a fixed dose of CBD, was found to vary during the course of these experiments, as illustrated by a sleeping time of 110 min shown in Figure 1 compared with 68 min in Figure 2. These two particular experiments were conducted several months apart, and the disparity in the results suggests the presence of an uncontrolled variable in our inbred mouse colony. The variable appears limited to the CBD effect because no such variation in control sleeping times was observed. Nevertheless, the results obtained within a period of at least several days were reproducible. For example, the data shown in Figure 1 represent only one set

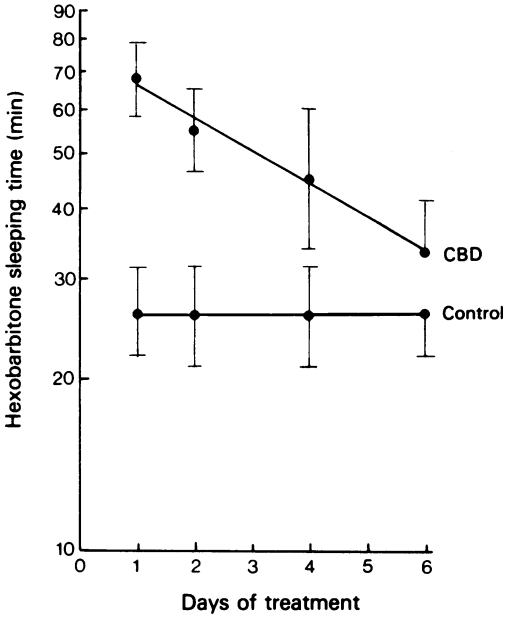

Figure 2 Effect of subacute cannabidiol (CBD) treatment on hexobarbitone sleeping time. Animals were treated every 24 h with CBD (120 mg/kg) for the indicated number of days. Hexobarbitone (100 mg/kg) sleeping times were measured 4 h after the final treatment; each treatment group consisted of 13 to 17 animals and the mean and standard deviation for each group are shown. The data were subjected to a regression analysis; the slope of the line is −0.058 and the correlation coefficient is 0.63. The slope is significantly different from zero, as determined by a t test ($P < 0.01$).

of duplicate experiments, which were conducted on about the same date; and the duplicate results were essentially identical. The same was found to be true for the data in Figure 2.

The effect of subacute CBD treatment on sleeping time is shown in Figure 2. Mice were given daily doses of CBD for the indicated number of days, and hexobarbitone sleeping times were measured 4 h after the last dose. Animals were used only once for a sleeping time determination; therefore, each test time represented a different group of animals. The results of the repeated treatment suggest that tolerance develops to the sleeping time effect of CBD. For example, acutely administered drug prolongs sleeping time about 2.5 times the control value, as shown on day 1 in Figure 2; but after 6 days of treatment, the sleeping time is only slightly longer than that of the control. Furthermore, an analysis of the data indicates that the slope of the line in Figure 2 is negative and significantly different from zero ($P < 0.01$), thus confirming the interpretation that tolerance develops rapidly to the effect of CBD on hexobarbitone sleeping time. In subsequent studies of tolerance, animals were given CBD daily for 4 days, because this treatment period was adequate to produce consistently a measurable degree of tolerance.

The data in Table 1 were obtained from experiments that were designed, in part, to find out whether the CBD-enhanced sleeping time was the result of an increased sensitivity of the central nervous system to hexobarbitone. The results in Table 1 show that 4 h pretreatment with CBD increased sleeping time; however, the brain concentration of hexobarbitone upon awakening was unchanged, which suggests that

Table 1 Effect of acute and subacute cannabidiol (CBD) treatment on the awakening brain concentration of hexobarbitone

	Hexobarbitone	
*Treatment**	*Sleeping time†* (*min*)	*Brain concentration‡* (*μg/g*)
Control	39 ± 7*	37 ± 6
Acute	111 ± 14*	35 ± 8
Subacute	62 ± 20*	38 ± 7

* Control animals received vehicle for 4 days; acute, vehicle for 3 days and CBD (120 mg/kg) on the fourth day; subacute, CBD (120 mg/kg) for 4 days. All measurements were made 4 h after the last treatment.

† Sleeping times were measured following a single dose of hexobarbitone (100 mg/kg i.p.). Each value represents a mean and standard deviation of 15 animals, and all values are significantly different from each other, as determined by the multiple range test ($P < 0.05$).

‡ Brain hexobarbitone concentration upon awakening was determined following a single dose of hexobarbitone (100 mg/kg). Each value represents a mean and standard deviation of 12 of the animals used for the sleeping time determination. There are no significant differences between any of the values as determined by the multiple range test ($P < 0.05$).

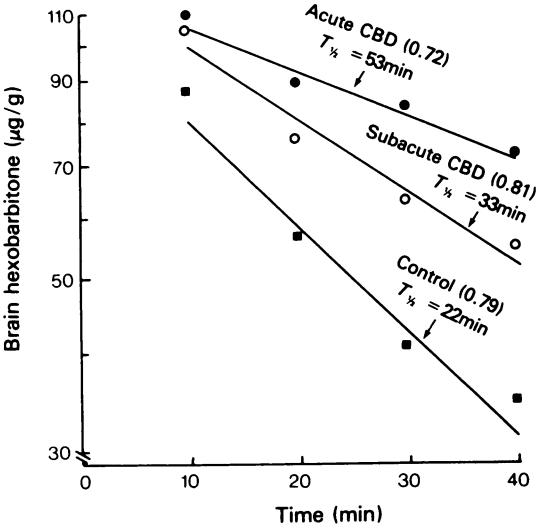

Figure 3 The half-times of hexobarbitone in the brain following acute and subacute treatment with cannabidiol (CBD). The acute group received vehicle daily for 3 days, CBD the fourth day; the subacute, CBD daily for 4 days; and the control, vehicle daily for 4 days. The dose of CBD was 120 mg/kg. Four h after the final treatment, each animal received a single dose of hexobarbitone (130 mg/kg). Each point represents the mean brain hexobarbitone concentrations of 5 animals. The slopes of the lines, determined by linear regression analysis, are: −0.013 for the acute, −0.022 for the subacute, and −0.031 for the control treatment; the correlation coefficients are in parentheses. The slopes are significantly different from each other, as determined by a t test ($P < 0.05$). The half-times of hexobarbitone in the brain were calculated from the first-order decay equation.

the drug, acutely administered, did not interact centrally with the barbiturate. Furthermore, the subacutely treated group in this study clearly showed the development of tolerance to CBD, as indicated by the decrease in sleeping time from 111 min in the acutely treated to 62 min; however, the tolerance was not accompanied by a decrease in the sensitivity of the central nervous system to hexobarbitone, because, upon awakening, the brain concentration of the barbiturate in the subacutely treated mice was not significantly different from that in the acutely treated group.

The results shown in Figure 3 illustrate that acute CBD treatment increases the half-time of the barbiturate in the brain from a control value of 22 min to 53 min; such an effect is indirect evidence that the rate of hexobarbitone metabolism is decreased by CBD. In the subacutely treated animals the half-time is 33 min, which is a significant reduction from the 53 min half-time in the acutely treated group; that is, the barbiturate half-time tends to return to normal in tolerant animals.

The effect of CBD on hepatic microsomal cytochrome P-450 concentration is shown in Table 2. A single dose of CBD caused a significant decline in cytochrome P-450 concentration, an effect which lasted for 12 h after drug administration; the duration of this decline approximates that of the effect on sleep time (Figure 1). However, such depression in cytochrome P-450 content was not followed in the recovery period by a greater-than-normal cytochrome P-450 level; that is, no rebound phenomenon was recorded either at 24 or 48 h after treatment, suggesting that the rapid development of tolerance does not appear to involve cytochrome P-450 induction.

The *in vitro* microsomal metabolism of CBD was studied in order to determine whether the decline in cytochrome P-450 was due to a CBD metabolite-cytochrome P-450 complex. In these studies, CBD was compared to Δ^9-THC (a cannabinoid which is not as potent an inhibitor of drug metabolism; Paton & Pertwee, 1972; Fernandes *et al.*, 1974; Seimens *et al.*, 1974) and to piperonyl butoxide (a compound which is known to produce metabolic-intermediate complexes with cytochrome P-450; Franklin, 1976). CBD, Δ^9-THC and piperonyl butoxide were metabolized in the presence of hepatic microsomes and NADPH, and each system was monitored spectrophotometrically (Figure 4). Two concentrations of CBD and Δ^9-THC were used in these experiments: one approximately equal to the apparent K_m, and the other, 10 times the apparent K_m for the metabolism of the drugs. The spectral data from only the higher drug-concentration experiments are shown in Figure 4, but the results from the lower drug concentrations were identical. Under these conditions, piperonyl butoxide produced its characteristic metabolic intermediate complex absorption spectrum with a maximum at 455 nm; however, neither CBD nor Δ^9-THC formed a spectrophotometrically detectable complex (Figure 4a).

The dithionite reduction of the microsomes following *in vitro* metabolism resulted in the appearance of an absorption spectrum with a maximum at 450 nm (Figure 4b). This result was uniquely associated with the CBD system and not with either Δ^9-THC or piperonyl butoxide. The appearance of the cytochrome P-450 spectrum suggested that CBD metabolism endogenously generated carbon monoxide; and, in fact, the P-450 complex was dissociated by bubbling the cuvette with oxygen. The generation of carbon monoxide was also shown to require CBD metabolism; that is, a non-metabolizing system containing only CBD and microsomes did not cause the appearance of the cytochrome P-450 absorbance spectrum. Moreover, the data indicate that the source of the carbon monoxide is not the enzymatic destruc-

tion of cytochrome P-450, because the quantity of P-450 did not change during the *in vitro* metabolism of CBD; both initial and final concentrations of cytochrome P-450 (before and after 10 min incubation) were 0.6 nmol/mg protein.

Table 2 shows that in acutely treated animals CBD caused a decrease in cytochrome P-450; in order to determine if such decrease *in vivo* was due to a reduction in the available cytochrome P-450 by the formation of a metabolic complex, the following experiment was performed: animals were given a single dose of CBD and their livers were removed 4 h later, at which time a significant *in vivo* depression in the amount of cytochrome P-450 was recorded (Table 2). Microsomes prepared from these livers were divided into two cuvettes and one cell was either oxidized with ferricyanide or reduced with dithionite. The cells were spectrophotometrically scanned in the 500 to 400 nm range, and no complex formation was revealed. The observed decrease in cytochrome P-450 cannot, therefore, be attributed to the presence of complexes of the SKF 525-A and piperonyl butoxide types.

The data on the half-time of brain hexobarbitone in Figure 3 suggest that the tolerance observed in subacutely treated animals involves either a reversal of the inhibitory effect, or a compensatory increase in the activity (induction) of the microsomal enzyme system. In order to test the latter possibility, a determination of the influence of acute and subacute CBD treatment on some hepatic constituents was essayed (Table 3). In these experiments, mice were treated with CBD once (acute: or daily for 4 days (subacute). Twenty-four hours after the last dose of CBD, liver weight increased significantly by 21%; however, the concentrations of liver protein and microsomal protein were unaffected, which suggests that the increase in liver weight is a consequence of an increase in the number of hepatic cells and not a change in the composition of the existing cells. The hepatic microsomal content of cytochrome P-450 and cytochrome *c* reductase was measured in another group of animals 4 h after the final dose of CBD: acute CBD treatment produced a significant 22% decline in cytochrome P-450 concentration, but in the subacute group there was no effect on the cytochrome, pointing to a development of tolerance. Cytochrome *c* reductase results were the reverse of those for cytochrome P-450; that is, in acutely treated animals there was no change in the activity of the reductase; however, in the subacutely treated group the activity was elevated significantly by 17%.

The above measurements in tolerant animals revealed only two possible signs of microsomal induction: an increase in liver weight and in cytochrome *c* reductase activity. The functional significance of these changes in terms of hexobarbitone sleeping time was measured in tolerant animals. The data shown in Table 4 were obtained from animals treated daily with CBD for the indicated number of days, and hexobarbitone sleeping times were determined either at 24 or 48 h after the last dose of CBD. For as long as 12 days of daily CBD treatment, there was no reduction in hexobarbitone sleeping time; therefore, the CBD tolerance cannot be ascribed to a functionally significant induction of the hexobarbitone-metabolizing enzymes.

Discussion

The ability of CBD to inhibit hepatic drug metabolism poses a toxic potential in terms of drug interactions, whether the drug is used for licit or illicit purposes. The dose of CBD in illicit usage is several orders of magnitude lower than that of the present study, and the work of Dalton, Martz, Rodda, Lemberger & Forney (1976) suggests that the relatively low doses derived from marihuana do not affect seco-

Table 2 Cannabidiol (CBD)-induced cytochrome P-450 changes in mouse hepatic microsomes

Time (*h*)	*Cytochrome P-450* % *control* (*range*)	P *value*
1	78 (67–92)	<0.005
4	73 (52–88)	<0.005
12	69 (40–92)	<0.01
24	89 (52–111)	>0.05
48	88 (61–119)	>0.05

Each animal was given a single dose of vehicle or CBD (120 mg/kg) and the concentration of cytochrome P-450 was determined after treatment at the various times indicated. The values represent means of groups consisting of 12 animals. The mean absolute concentration of cytochrome P-450 in the controls was 0.72 nmol/mg microsomal protein (range, 0.86–0.64). The drug data were compared to their vehicle controls by the Mann-Whitney U test.

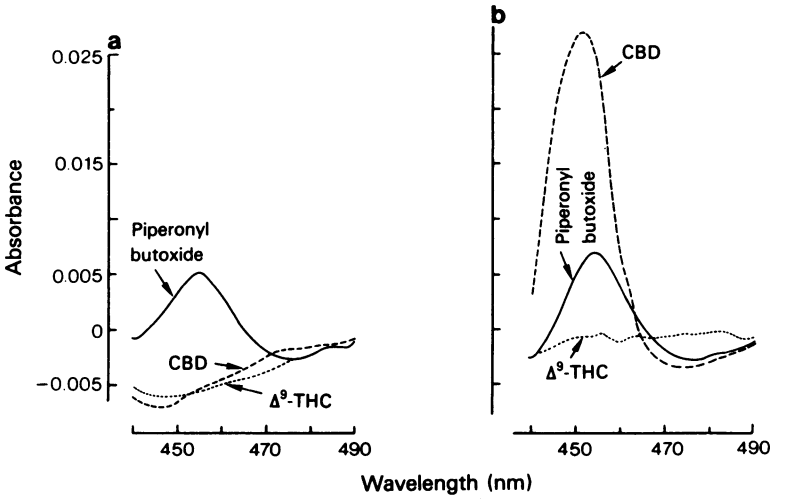

Figure 4 Spectral analyses of microsomal systems metabolizing cannabidiol (CBD), Δ^9-tetrahydrocannabinol (Δ^9-THC) and piperonyl butoxide. (a) Analysis for metabolic-intermediate complexes with cytochrome P-450. Hepatic microsomes were prepared from an untreated mouse; microsomal suspensions contained 1 mg protein/ml. The concentration of CBD (0.06 mM) and Δ^9-THC (0.13 mM) were approximately 10 times the apparent K_m values for these drugs. The concentration of piperonyl butoxide was 2.4 mM. NADPH (0.24 mM) was added only to the sample cuvette. After a reaction time of 9 min at room temperature, a difference spectrum was obtained (500–400 nm). (b) Effect of dithionite reduction on the spectral analysis of the reaction systems in (a). After a reaction time of 10 min, microsomal systems in (a) were reduced by the addition of dithionite and a difference spectrum was obtained.

Table 3 Effect of acute and subacute cannabidiol (CBD) treatment on the liver*

	% control	
	Acute	*Subacute*
Liver weight†	—	121‡ (107–139)
Liver protein/g liver†	—	102 (86–115)
Microsomal protein/g liver†	—	113 (91–146)
Cytochrome P-450/g microsomal protein§	78‡ (57–86)	99 (81–135)
Cytochrome *c* reductase/g microsomal protein§	99 (85–114)	117‡ (98–157)

* Control group received vehicle daily for 4 days; acute, vehicle daily for 3 days and CBD on the fourth day; subacute, CBD daily for 4 days. Each value is expressed as a percentage of the control and represents a mean of 8 to 12 animals; the range of each value is in parentheses. The absolute mean values for the control group are: liver, 1.7 g; liver protein, 263 mg/g liver; microsomal protein, 13 mg/g liver; cytochrome P-450, 0.77 nmol/mg microsomal protein; and cytochrome *c* reductase activity, 77 nmol min^{-1} mg^{-1} microsomal protein.
† Determined 24 h after the last treatment.
‡ Value is significantly different from control, as determined by the Mann-Whitney U test ($P < 0.05$).
§ Determined 4 h after the last treatment.

barbitone metabolism in man. Because of the interest in CBD as an anti-epileptic agent, the principal goal of the present study was to determine and define some of the characteristics of the interaction between anticonvulsant doses of CBD and the hepatic microsomal drug-metabolizing enzymes. The dose of CBD selected for the investigation, 120 mg/kg, is the anticonvulsant ED_{50} for mice in a maximal electroshock test (Karler *et al.*, 1973). The *in vivo* drug-metabolizing activity was assessed in terms of hexobarbitone sleeping time because hexobarbitone is primarily metabolized by the hepatic microsomal enzymes (Cooper & Brodie, 1955) and the sleeping time is proportional to the activity of these enzymes (Vesell, 1968).

Acutely administered CBD was shown to prolong significantly hexobarbitone sleeping time in mice for 24 h, an effect similar to that described by Paton & Pertwee (1972). Previous studies with lower CBD doses (10 mg/kg) demonstrated that the prolongation of barbiturate sleeping time is not the result of a central interaction between CBD and the barbiturate, but rather the consequence of the ability of CBD to inhibit barbiturate metabolism by the liver (Loewe, 1944; Paton & Pertwee, 1972; Fernandes *et al.*, 1974). These conclusions were also found to be valid for the relatively high dose of CBD used in the present study, because the concentration of hexobarbitone in the brain upon awakening was unchanged following acute CBD treatment. Furthermore, the treatment increased the half-time of hexobarbitone in the brain, which is indirect evidence that CBD decreased the rate of hexobarbitone metabolism.

In the subacute experiments, tolerance developed rapidly to the CBD effect on hexobarbitone sleeping time, and the tolerance appears to be of the dispositional rather than of the cellular type. This conclusion is based on the apparent restoration of the rate of hexobarbitone metabolism, as evidenced by the return of the brain hexobarbitone half-time toward the control value, and on the failure of the subacute treatment to change the brain concentration of the barbiturate upon awakening.

The above findings imply that both the acute and subacute effects of CBD involve the hepatic drug-metabolizing system. An investigation of the effect of CBD on this system revealed that a single dose of CBD caused a significant depression in cytochrome P-450 concentration, an observation of particular interest because other inhibitors of drug metabolism, such as SKF 525-A and piperonyl butoxide, exert their effect by reducing the amount of cytochrome P-450 (Buening & Franklin, 1976; Franklin, 1976). The decrease in cytochrome P-450 caused by CBD may, therefore, account for its inhibitory effect on hexobarbitone metabolism. This conclusion is supported by the evidence that the duration of the cytochrome P-450 decline following acute CBD treatment approximates to the duration of the sleep-time prolongation, about 24 h. Furthermore, following subacute CBD treatment, the cytochrome P-450 level returns to normal; therefore, animals that are tolerant to the effect of CBD on sleep time are also tolerant to the drug's action on cytochrome P-450. There is, therefore, some evidence to support a cause–effect relationship between the CBD-induced changes in cytochrome P-450 and in sleeping time.

The mechanism by which CBD decreases cytochrome P-450 was also investigated. Other inhibitors, like SKF 525-A and piperonyl butoxide, form metabolic intermediate complexes with cytochrome P-450, which prevent carbon monoxide binding and, thereby reduce the amount of spectrally measurable and functional cytochrome P-450 (Buening & Franklin, 1976; Franklin, 1976). The complexes themselves have characteristic absorption maxima that can be detected

Table 4 Influence of daily cannabidiol (CBD) administration on hexobarbitone sleeping time

	*Sleeping time**	
Days of treatment	*Vehicle*	*CBD*
1	26 ± 8	44 × 10†
2	20 ± 5	31 ± 10
4	19 ± 7	20 ± 6
8	21 ± 6	30 ± 8
12	21 ± 5	32 ± 7
12‡	29 ± 9	32 ± 6

* Hexobarbitone (100 mg/kg i.p.) sleeping times in groups 1 through 12 were measured 24 h after the final CBD treatment (120 mg/kg daily). All sleeping-time values represent means and their standard deviations of groups of 20 mice. Each group was tested for sleeping time once only.

† Value is significantly different from control as determined by a t test ($P < 0.05$).

‡ Sleeping time assessed 48 h after final CBD treatment.

in microsomes following both *in vivo* and *in vitro* metabolism of these drugs. In contrast, the *in vivo* administration of CBD did not yield a spectrally detectable complex, although there was a significant *in vivo* decline in cytochrome P-450 at the time of the measurement. Moreover, no complex absorption maxima and no decline in spectrally measurable cytochrome P-450 were seen after the *in vitro* metabolism of either relatively low (apparent K_m) or high (10 times apparent K_m) concentrations of CBD, even though the high concentration (0.06 mM) was probably sufficient to inhibit non-competitively hexobarbitone metabolism *in vitro* (Fernandes *et al.*, 1974).

Even though the *in vivo* decrease in cytochrome P-450 cannot be accounted for by conventional complex formation, the possibility still exists that a spectrally non-detectable complex is formed. Other explanations for the decrease in the cytochrome P-450 are also conceivable: for example, CBD may decrease the synthesis of cytochrome P-450 (Levin, Ryan, Kuntzman & Conney, 1975); however, the turnover of this cytochrome in the mouse is not known. The decrease in cytochrome P-450 might also be a consequence of a destruction of the cytochrome, as has been attributed to certain other drugs (de Matteis, 1973; Levin, Jacobson, Sernatinger & Kuntzman, 1973).

As described, repeated CBD treatment is apparently accompanied by a restoration of the normal rate of hexobarbitone metabolism, which is indicative of a development of tolerance. Such tolerance may be due either to a reversal of the inhibitory action of CBD or to a compensatory increase in the activity of the microsomal enzyme system. For example, SKF 525-A has been shown to induce the activity of the hepatic microsomal system (Serrone & Fujimoto, 1962; Kato, Chiesara & Vassanelli, 1964), and Buening & Franklin (1976) demonstrated that repetitive treatment produced a marked increase in the concentration of cytochrome P-450. In order to determine whether CBD tolerance is the result of induction, the influence of subacute CBD on some constituents of the drug-metabolizing system was assessed. Unlike SKF 525-A, subacute CBD treatment does not increase the cytochrome P-450 levels; in addition, microsomal protein per g liver and liver protein per g liver remained unchanged. The only signs of induction were an increase in cytochrome *c* reductase activity and in total liver weight. However, following CBD withdrawal after 12 days of daily treatment sleep time was normal; therefore, CBD tolerance cannot be ascribed to a functionally significant induction of the hexobarbitone-metabolizing enzymes.

Generally speaking, the results of the present study are consistent with other investigators' findings that CBD prolongs sleeping time by inhibiting the hepatic drug-metabolizing system. Although the precise mechanism of the inhibitory action of CBD is still unknown, it does appear that the depression of cytochrome P-450 may account, at least in part, for the effect of CBD on drug metabolism; and the tolerance to the CBD effect relates to a restoration of the cytochrome P-450 concentration.

The authors are indebted to Dr Monique C. Braude, Division of Research, NIDA, for supplying the cannabis derivatives and for her advice and encouragement. The work was supported by USPHS Research Grant DA-00346 and Pharmacology Training Grant GM-00153.

References

BUENING, M.K. & FRANKLIN, M.R. (1976). SKF 525-A inhibition, induction, and 452-nm complex formation. *Drug Metab. Dispos.*, **4**, 244–255.

COOPER, J.R. & BRODIE, B.B. (1955). The enzymatic metabolism of hexobarbital (Evipal). *J. Pharmac. exp. Ther.*, **114**, 409–417.

DALTON, W.S., MARTZ, R., RODDA, B.E., LEMBERGER, L. & FORNEY, R.B. (1976). Influence of cannabidiol on secobarbial effects and plasma kinetics. *Clin. Pharmac. Ther.*, **20**, 695–700.

DE MATTEIS, F. (1973). Drug-induced destruction of cytochrome P-450. *Drug Metab. Dispos.*, **1**, 267–274.

FERNANDES, M., KLUWE, S. & COPER, H. (1974). Cannabinoids and hexobarbital induced loss of righting reflexes. *Naunyn-Schmiedebergs Arch. Pharmac.*, **283**, 431–435.

FRANKLIN, M.R. (1976). Methylenedioxyphenyl insecticide synergists as potential human health hazards. *Envir. Hlth. Perspect.*, **14**, 29–37.

FRANKLIN, M.R. & ESTABROOK, R.W. (1971). On the inhibitory action of mersalyl on microsomal drug oxidation: A rigid organization of the electron transport chain. *Archs Biochem. Biophys.*, **143**, 318–329.

KARLER, R., CELY, W. & TURKANIS, S.A. (1973). The anticonvulsant activity of cannabidiol and cannabinol. *Life Sci., Oxford*, **13**, 1527–1531.

KARLER, R., CELY, W. & TURKANIS, S.A. (1974). A study of the development of tolerance to an anticonvulsant effect of Δ^9-tetrahydrocannabinol and cannabidiol. *Res. Commun. chem. Pathol. Pharmac.*, **9**, 23–39.

KARLER, R. & TURKANIS, S.A. (1976). The antiepileptic potential of the cannabinoids. In *The Therapeutic Potential of Marihuana*. ed. Cohen, S. & Stillman, R.C. pp. 383–397. New York: Plenum Press.

KATO, R., CHIESARA E. & VASSANELLI, P. (1964). Further studies on the induction and stimulation of microsomal enzymes of rat liver by various compounds. *Biochem. Pharmac.*, **13**, 69–83.

LEVIN, W., JACOBSON, M., SERNATINGER, E. & KUNTZMAN, R. (1973). Breakdown of cytochrome P-450 heme by secobarbital and other allyl-containing barbiturates. *Drug Metab. Dispos.*, **1**, 275–285.

LEVIN, W., RYAN, D., KUNTZMAN, R. & CONNEY, A.H. (1975). Neonatal imprinting and the turnover of microsomal cytochrome P-450 in rat liver. *Molec. Pharmac.*, **11**, 190–200.

LOEWE, S. (1944). Studies in the pharmacology of marihuana. In *The Marihuana Problems in the City of New York*, The Mayor's Committee on Marihuana. pp. 149–212. Lancaster, Pa: The Jacques Cattell Press.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–274.

MECHOULAM, R., MCCALLUM, N.K. & BURSTEIN, S. (1976). Recent advances in the chemistry and biochemistry of cannabis. *Chem. Rev.*, **76**, 75–112.

OMURA, T. & SATO, R. (1964). The carbon monoxide-binding pigment of liver microsomes. *J. biol. Chem.*, **239**, 2370–2378.

PATON, W.D.M. & PERTWEE, R.G. (1972). Effects of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. *Br. J. Pharmac.*, **44**, 250–261.

PEREZ-REYES, M., TIMMONS, M.D., DAVIS, K.H. & WALL, M.E. (1973). A comparison of the pharmacological activity in man of intravenously administered Δ^9-tetrahydrocannabinol, cannabinol, and cannabidiol. *Experientia*, **29**, 1368–1369.

SEIMENS, A.J., KALANT, H., KHANNA, J.M., MARSHMAN, J. & HO, G. (1974). Effect of cannabis on pentobarbital-induced sleeping time and pentobarbital metabolism in the rat. *Biochem. Pharmac.*, **23**, 477–488.

SERRONE, D.M. & FUJIMOTO, J.M. (1962). The effect of certain inhibitors in producing shortening of hexobarbital action. *Biochem. Pharmac.*, **11**, 609–615.

VESELL, E.S. (1968). Factors altering the responsiveness of mice to hexobarbital. *Pharmacology*, **1**, 81–97.

WILLIAMS, C.H., JR. & KAMIN, H. (1962). Microsomal triphosphopyridine nucleotide-cytochrome *c* reductase of liver. *J. biol. Chem.*, **237**, 587–595.

(Received October 31, 1978.
Revised February 5, 1979.)