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ANTICONVULSANT PROPERTIES OF CANNABIDIOL

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Abstract

A series of laboratory tests were conducted in mice to evaluate the anticonvulsant properties of cannabidiol (CBD). The drug abolishes hind-limb extension induced by maximal electroshock (MES) and raises the MES (extensor) and 6-Hz-electroshock thresholds, but does not affect the 60-Hz-electroshock or minimal seizures caused by pentylenetetrazol (PTZ). As an anticonvulsant, CBD appears to be more closely related to diphenylhydantoin (DPH) than to its chemical congener, Δ^9-tetrahydrocannabinol (Δ^9-THC).

At an ambient temperature of 22°C, CBD in anticonvulsant doses lowers the rectal temperature of mice, whereas at 30°C the hypothermic

effect is greatly attenuated. Anticonvulsant tests conducted at both ambient temperatures indicate that the hypothermia produced by CBD does not appear to affect the anticonvulsant properties of the drug.

In another series of experiments, anticonvulsant activity against PTZ-induced maximal seizures was measured; in this study, CBD and DPH were more efficacious than Δ^9-THC. The results of a drug-interaction investigation indicate that pretreatment of mice with CBD enhances the anticonvulsant activity of DPH, phenobarbital and Δ^9-THC against MES.

Introduction

Some anticonvulsant properties of marijuana and its surrogates have been recognized for many years (Loewe and Goodman, 1947). Although various laboratories have reported that several pure cannabinoids exhibit anticonvulsant activity (for a review see Karler et al., 1973), an obvious limitation to the clinical usefulness of marijuana-like substances is their potential for central nervous system toxicity. We have, however, recently shown in laboratory tests that the anticonvulsant activity in a maximal electroshock (MES) test and motor toxicity of the cannabinoids are at least partially separable (Karler et al., 1974; Turkanis, 1974). Of the substances investigated, cannabidiol (CBD) warrants a detailed laboratory investigation because it abolishes MES-caused hind-limb extension in animals (Karler et al., 1973) and lacks marijuana-like psychic activity

in man (Mechoulam, 1970; Carlini et al., 1973; Hollister, 1973; Perez-Reyes et al., 1973). Thus, the following study was desiged to subject CBD to a series of standard anticonvulsant tests, so that its properties could be compared with those of other agents, such as Δ^9-tetrahydrocannabinol (Δ^9-THC) and diphenylhydantoin (DPH). In addition, since CBD is known to affect the actions of certain drugs (Paton and Pertwee, 1972), its interaction with Δ^9-THC and two well-established antiepileptics was investigated.

Methods

The experiments were conducted on 4- to 5-week-old male, Charles River mice (ICR). The drugs were prepared for administration in the following way: Pentylenetetrazol (PTZ) and sodium phenobarbital were dissolved in isotonic saline solution. To make fine suspensions of the other agents, Tween 80 was added to alcoholic solutions of CBD, DPH (sodium salt) and Δ^9-THC. After thorough mixing, the ethanol was evaporated by a stream of N_2; the resulting drug-Tween 80 residues were dispersed by ultrasound (Branson Sonifier S75) in an isotonic saline solution. The final drug mixtures contained about 3% Tween 80 and 97% saline solution. The cannabinoids, as prepared in the present study, were as effective against MES as were the previously described preparations containing sesame seed oil (Karler et al., 1973).

Anticonvulsant activity was measured against electrically caused convulsions in the following laboratory procedures: MES, MES threshold, 60-Hz-electroshock threshold (60-Hz-EST), and 6-Hz-electroshock threshold (6-Hz-EST). Stimuli were applied by means of conventional corneal electrodes. The end-points were abolition of hind-limb extension for the MES test, hind-limb extension (a maximal seizure) for the MES threshold test, and front limb and jaw clonus (a minimal seizure) for both EST tests. Anticonvulsant activity was also measured against minimal and maximal seizures caused by PTZ. The details of the above-described tests have been previously published (Swinyard, 1949). The time course of the anticonvulsant effect was determined by the use of the ratio of extensor-to-flexor time (E/F) (Karler et al., 1973); a decrease in the E/F ratio was interpreted as a manifestation of anticonvulsant activity. Studies were carried out at the peak-effect time for each drug, as denoted by the test time with the lowest E/F ratio and the greatest number of animals protected against MES.

In general, drugs were administered intraperitoneally; however, PTZ was given subcutaneously. All control animals received injections of the appropriate vehicle. Each dose-, current- and voltage-effect curve has three to six points; each point represents the results obtained from 15 to 20 mice. Median-effective values and their 95% confidence limits were determined by probit analysis (Finney, 1971), and relative potency tests

were carried out by the method of Litchfield and Wilcoxon (1949). The calculations were made by a Univac computer 1108. The curves shown in the figures were fitted by eye. Measurements of ambient and rectal temperatures were made with an electronic thermometer.

Results

As illustrated in Fig. 1, CBD lowers the rectal temperature.

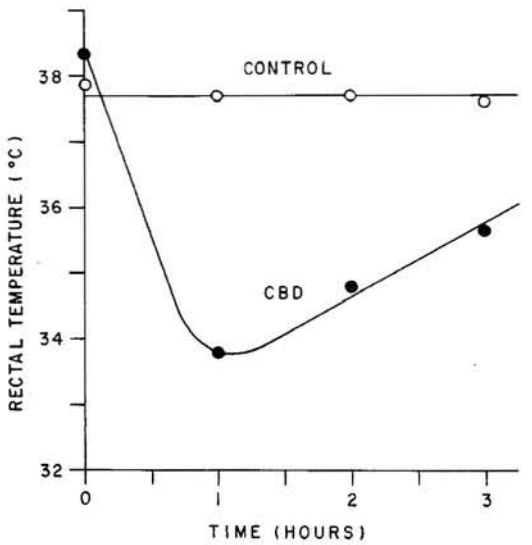

Fig. 1. Time course of the effect of CBD on rectal temperature. Two groups of 15 mice were given either vehicle or 120 mg/kg of CBD. Rectal temperature measurements were made with each group at hourly intervals; each point represents a mean value. Control data are illustrated by unfilled circles; treated, by filled circles. Ambient temp. was about 22°C.

Thus, the hypothermic property of the cannabinoids is separable from their psychic effects. The CBD-induced hypothermia appears to be similar to that produced by Δ^9-THC (Abel, 1972; Yagiela et al., 1973). Although hypothermia occurs when the ambient temperature is about 22°C, this response to CBD is greatly reduced when the temperature is 30°C (see Table 1). Therefore, raising the environmental temperature is a practical means of limiting the CBD-caused hypothermia. Other investigators have reported that the hypothermic effect of Δ^9-THC is also dependent upon the ambient temperature (Gill et al., 1970; Abel, 1972).

Table 1

Influence of ambient temperature on hypothermia caused by cannabidiol (CBD)

Ambient temperature (°C)	Mean rectal temperatures and their standard deviations (°C)*	
	Control	CBD treated
22	38.7 ±0.46	35.9 ± 0.84†
30	38.3 ±0.54	37.4 ± 0.65†

* Measurements were made 1 hr after treatment with either vehicle or 120 mg/kg of CBD; each group contained 20 mice.

† As indicated by two-way analysis of variance and Newman Keuls' multiple mean test, the value is significantly different from the other three mean values ($P<0.05$) (Snedecor and Cochran, 1967).

Since changes in body temperature can affect the results of laboratory tests for anticonvulsant activity (Swinyard and Toman, 1948; Brown, 1953), some of the studies described below were performed at ambient temperatures of 22 and 30°C to assess the influence of the CBD-induced hypothermia on the anticonvulsant properties. In the threshold experiments, the mice were given either vehicle or 120mg/kg of CBD; this dose represents a mean ED50 value determined in the present investigation for the MES test (three experiments). Previously, CBD was shown to block hind-limb extension in MES tests in both rats and mice (Izquierdo and Tannhauser, 1973; Karler et al., 1973). The effects of CBD on various threshold tests conducted at an ambient temperature of 30°C are summarized in Table 2. As depicted in Table 2, CBD raises the MES threshold and elevates the 6-Hz-EST; however, CBD does not alter either the 60-Hz-EST or the PTZ threshold.

Additional experiments were carried out at an ambient temperature of 22°C to determine the anticonvulsant effects of CBD in hypothermic mice. The results of studies at 22°C were similar to those obtained at 30°C. Therefore, hypothermia does not affect the anticonvulsant properties of CBD described in Table 2. In addition, hypothermia does not alter the ED50 value in the MES test. The complete lack of any temperature effect in the above tests is in partial contrast to the results obtained from identical experiments with Δ^9-THC (R. Karler, W. Cely and S. A. Turkanis,

unpublished). With the latter drug, the 60-Hz-EST value was lowered at an ambient temperature of 22°C, but all of the other tests were unaffected.

Table 2

Effects of cannabidiol (CBD) on various tests for anticonvulsant activity

	Median-effective values and their 95% confidence limits*	
Test	Control	CBD treated
MES threshold	8.5 mA (7.6-9.4)	13 mA (12-14)†
6-Hz-EST	11 V (10-12)	13 V (12-14)†
60-Hz-EST	5.5 mA (5.2-5.8)	5.2 mA (5.0-5.5)
PTZ threshold	52 mg/kg (48-55)	52 mg/kg (50-54)

* Tests were conducted 1 hr after administration of 120 mg/kg of CBD or vehicle (ambient temp., 30°C).

† Value is significantly different from control ($P \leq 0.05$) (Litchfield and Wilcoxon, 1949).

CBD appears to have some anticonvulsant properties that are similar to those of DPH. For example, it is well established that DPH in mice also abolishes hind-limb extension in the MES test and elevates the 6-Hz-EST, and does not change the 60-Hz-EST or raise the PTZ threshold (Woodbury and Esplin, 1959). For an additional comparison of the two agents, DPH was also evaluated in the MES threshold test. In order to

make the doses in the DPH and CBD experiments comparable, an ED50 for DPH, as measured in the MES test, was selected. As indicated in Table 3, DPH, like CBD, raises the MES threshold. Since DPH may even intensify PTZ-induced minimal seizures (Mitchell and Keasling, 1960; Toman, 1970), the effect of DPH on PTZ threshold was determined under the conditions of the present study. The results, as depicted in Table 3, indicate that an ED50 of DPH does not alter the PTZ threshold.

Table 3

Effects of diphenylhydantoin (DPH) on seizure thresholds

Threshold test	Median-effective values and their 95% confidence limits*	
	Control	DPH treated
PTZ†	62 mg/kg (55-68)	57 mg/kg (53-60)
MES	8.0 mA (7.1-9.0)	13 mA (11-14)‡

*Measurements were made 2 hr after treatment with either vehicle or 9 mg/kg of DPH (ambient temp., 22°C).

† End-point was a minimal seizure characterized by jaw and front-limb clonus.

‡ Value is significantly different from control ($P \leq 0.05$) (Litchfield and Wilcoxon, 1949).

In another series of experiments, Δ^9-THC, CBD and DPH were evaluated against PTZ-induced maximal seizures. The comparative results with these three anticonvulsant agents are shown in Fig. 2. It is apparent that both CBD and DPH are more efficacious than Δ^9-THC in blocking PTZ-caused maximal seizures.

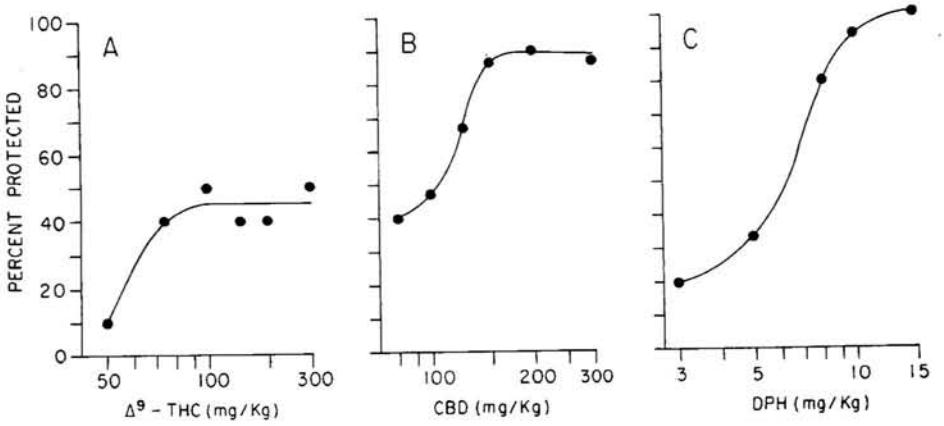

Fig. 2. Protective effect of Δ^9-THC, CBD or DPH against PTZ-caused maximal seizures (ambient temp., 22°C). Each point represents results obtained from 15 to 20 mice. Maximal seizures were produced by administration of 110 mg/kg of PTZ. The animals were tested at the peak-effect time for each drug in the PTZ test; the peak-effect times were as follows: Δ^9-THC, 1 hr; CBD, 0.5 hr; DPH, 1 hr.

Because CBD is known to alter the activity of other drugs (Paton and Pertwee, 1972), we studied the interaction of CBD with the effects of

DPH, phenobarbital or Δ^9-THC in the MES test. Such interaction experiments with CBD are complicated by the fact that CBD displays anticonvulsant activity. Therefore, the studies were conducted 20 hr after the administration of CBD, when no anticonvulsant effect of CBD was detectable (see Fig. 3A).

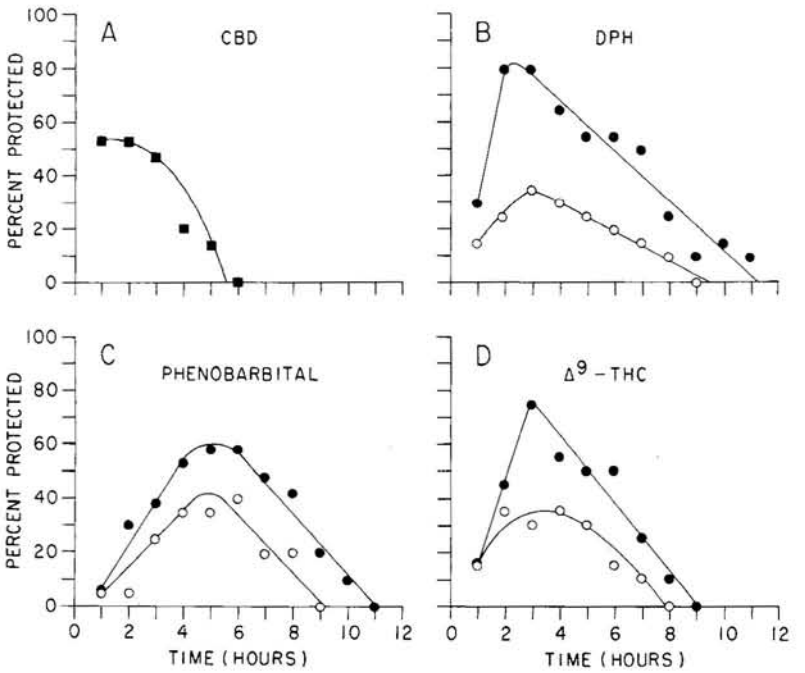

Fig. 3. Influence of CBD pretreatment on the anticonvulsant activity of several drugs in the MES test (ambient temp., 22°C). Each curve represents the results obtained from the repetitive testing of 20 mice. In A, the time course of the anticonvulsant activity of 150 mg/kg of CBD is illustrated. In B, C and D, filled circles represent data obtained from mice treated with CBD (150 mg/kg) 20 hr prior to the experiments. Open circles represent data from corresponding control mice. In B, both groups of animals received 7 mg/kg of DPH; in C, both received 12 mg/kg of phenobarbital; in D, both received 100 mg/kg of Δ^9-THC.

Typical results from the interaction experiments are illustrated in Figs. 3B, C and D. CBD pretreatment enhances and prolongs the anticonvulsant effect of all three pharmacological agents mentioned above. Such findings were observed in eight separate experiments (DPH, three; phenobarbital, two; Δ^9-THC, three).

The reason for the interaction of CBD with the other anticonvulsants is unclear. As shown in Fig. 3A, the anticonvulsant activity of CBD has ended in 6 hr; consequently, this property, by itself, is unlikely to contribute to the enhancement seen at 20 hr. The augmentation of anticonvulsant action may be related to inhibition of drug metabolism by CBD. Both marijuana and CBD prolong barbiturate sleep time, and this lengthened effect is probably attributable to inhibition of drug metabolism by CBD (Paton and Pertwee, 1972). However, there may be other explanations, such as an interaction at the receptor level within the central nervous system.

Discussion

In general, the spectrum of anticonvulsant properties for CBD and DPH is similar in the six laboratory tests described above. Both drugs abolish hind-limb extension caused by MES or PTZ and elevate the MES threshold and the 6-Hz-EST. In contrast, neither agent, in the doses employed, affects the 60-Hz-EST or the PTZ threshold. The

results of identical tests with Δ^9-THC illustrate that this drug appears to differ from both CBD and DPH only in the PTZ tests. First, Δ^9-THC is less efficacious than either CBD or DPH in blocking hind-limb extension produced by PTZ (Fig. 2). Secondly, an ED50 of Δ^9-THC, as determined against MES, significantly lowers the threshold for PTZ-induced minimal seizures (R. Karler, W. Cely and S. A. Turkanis, unpublished), which may be a manifestation of the excitatory effects of this drug (Hardman et al., 1971). Although it is difficult to extrapolate the implication of such laboratory findings to man, the possibility exists that Δ^9-THC may exacerbate some forms of epilepsy, as is the case with DPH.

As described above, there are differences in the anticonvulsant properties of CBD and Δ^9-THC; however, other important differences exist between these two drugs. For example, CBD is devoid of marijuana-like psychic activity in man (Mechoulam, 1970; Hollister, 1973). Furthermore, very high doses of intravenously infused CBD in man do not cause either cardiac acceleration or psychic effects that are characteristic of small doses of Δ^9-THC (Perez-Reyes et al., 1973). The absence of these toxic effects in man, in addition to its DPH-like anticonvulsant characteristics in mice, suggests that CBD may have clinical potential as an antiepileptic drug.

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