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Modification of Δ^9-THC-Actions by Cannabinol and Cannabidiol in the Rat

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Abstract. Cannabinol (CBN) and Cannabidiol (CBD) were tested in several test procedures known to be altered by Δ^9-tetrahydrocannabinol (THC) or crude cannabis preparations. They were inactive in doses up to 80 mg/kg in tests on animal motility, food and water intake, body temperature and catalepsy. In contrast, CBD enhanced the hexobarbitone "sleeping time" more pronounced than Δ^9-THC whereas CBN increased the "sleeping time" only slightly. When administered in combination CBD prolonged all actions of THC, whereas CBN selectively blocked the effect of THC on hexobarbitone "sleeping time". The enhancement by CBD is best explained by an inhibition of THC-metabolism.

Key words: Δ^9-Tetrahydrocannabinol — Cannabinol — Cannabidiol — Interaction — Hexobarbitone — Motility — Food Intake — Water Intake — Body Temperature — Catalepsy.

Introduction

Since the Δ^9- and Δ^8-tetrahydrocannabinols have been established as the psychotomimetic active principles of cannabis samples, the research during the last decade has mainly been focused on these compounds. In organic solvent extracts of hashish, however, there are many other constituents, in particular cannabinol and cannabidiol (Mechoulam, 1970). These compounds were found to be centrally less active in reducing the spontaneous activity in mice (Christensen *et al.*, 1971). They do not alter the behaviour of rhesus monkeys (Edery *et al.*, 1972) and seem to have no psychotomimetic potency in man (Valle, 1969). Some evidence exists, however, that these compounds produce own pharmacological effects and modify the actions of the psychotomimetic tetrahydrocannabinols. As early as 1950 Loewe (1950) stated that the barbiturate sleeping time prolongation caused by cannabis preparations in small rodents is more closely related to the cannabidiol content than to the THC-content. Recently, the prolongation by CBD has been established as a strong interaction with the hepatic drug metabolizing system *in vitro* (Coper and Fernandes, 1973). Even the metabolism of Δ^9-THC seems to be inhibited *in vivo* in mice since Δ^9-THC and his 11-hydroxi-

metabolite were significantly elevated by CBD in the brain (Jones and Pertwee, 1972). However, a potentiation of the THC-effect could not be demonstrated. Carlini *et al.* (1970) and Karniol and Carlini (1972) observed that the THC content of Brazilian marihuana did not explain all effects of the crude preparations. They suggested an antagonism between CBD and Δ^9-THC in spontaneous motor activity in mice. Finally, Krantz and co-workers (1971), using pure compounds, observed an antagonism between cannabinol and THC in mice with respect to barbiturate potentiation. They speculated that a possible antagonism might be clinically useful in antagonizing the cannabis intoxication.

In the present investigation we examined whether cannabinol and cannabidiol were capable of modifying the Δ^9-THC-actions in the rat in several test procedures known to be altered by THC or crude cannabis preparations. In all of these tests it was necessary to investigate possible own effects of CBN or CBD and to evaluate dose-response-relationships, likewise.

Materials and Methods

Male Wistar rats weighing 160—220 g were used throughout the experiment. Synthetic Δ^9-THC was obtained from Dr. Petrzilka, the purity as determined by GLC (1% SE 30 on chromosorb G, 80—100 mesh, inlet-column-detector temperature: 240—220—250°C) was 85% with additional 6% Δ^8-THC, 7% ortho-Δ^9-THC and 3% cannabinol. CBN and CBD were prepared from hashish of Lebanese or Turkish origin up to a purity of 98% by column chromatography. Main contaminants were cannabigerol, cannabigerol-methylester, and cannabichromene, each making up to 0.8%. The cannabinoids were injected using 10% cremophor EL as vehicle (0.2—0.4 ml per 100 g of body weight).

The body temperature was recorded by an electric Universal Thermometer the probe of which was inserted 6 cm deep into the rectum. According to Lomax (1971), the area between individual body temperature curves and the mean of the controls has been defined as hypothermic response.

The duration of the loss of righting reflexes following 100 mg/kg hexobarbitone i.p. was defined as hexobarbitone sleeping time. The catalepsy was determined in the following way: Both front paws of the rats were placed onto a plastic bar (height 7 cm, length 20 cm, depth 7 cm) and the time recorded until both paws touched the floor (= "holding time"). In animals climbing on the bar the test was immediately repeated. The cataleptic response was defined as the area under the "holding time"—time curve during 8 h. The animal motility has been recorded with an Animex motility apparatus. Groups of 3 animals were held in a day-night schedule (light from 5 a.m. to 5 p.m.) and tested in the home cage (42 × 24 cm). The animals were injected at 4.50 p.m. and the activity was recorded in hourly intervals for the following 6 h. This schedule has been chosen because the control animals are particularly active during the night phase and motility depressing effects like those of the cannabinoids should be more prominant than in the light phase.

In food and water intake experiments the rats were held in groups of 4 animals. The cannabinoids were injected between 10 and 12 a.m. The intake was determined by measuring drinking bottle and pellet weights immediately after injection and 24 h later.

Statistical analysis has been performed by individual comparison using student's *t*-test (two tailed). The cataleptic response has been compared by the U-test according to Mann and Whitney.

Results

Motility. The effect of Δ^9-THC, CBN and CBD on the motility of the animals is shown in the top of Fig. 1 (left) where the cumulative counts of 6 h following administration are plotted against the log of the dose. Each point represents the mean of at least 6 groups. Up to 80 mg/kg only Δ^9-THC significantly decreased the 6 h-cumulative motility in a dose-dependent manner.

In the combination experiments CBD further increased the THC-depression whereas CBN had no influence on the THC effect (Tab. 1). A timeresponse curve of the motility following CBD, THC and a combination of both shows that the enhancement of the THC-effect by CBD is mainly produced by a prolongation of action rather than by an increase in maximum inhibition (Fig. 2, middle). By contrast, higher THC doses increased the duration as well as the intensity of action.

Food Intake. Δ^9-THC decreased, dose-dependent, the food intake in agreement with other reports (Fig. 1, middle left). Each point of Fig. 1 represents the mean of 6 groups (4 animals per group). In interaction experiments 20 mg/kg of the cannabinoids, were administered. CBD enhanced the food intake lowering effect of Δ^9-THC, whereas CBN had no influence on this THC-action (Tab. 1).

Water Intake. Similar results were obtained when the 24 h-water intake was measured, with the exception that higher Δ^9-THC-doses are needed for the depression (Fig. 1, bottom left). CBD and CBN were ineffective. CBD, however, further increased the water intake inhibition by Δ^9-THC (Tab. 1).

Body Temperature. Δ^9-THC caused a marked dose dependent decrease in body temperature (Fig. 2, top) which means an increase in hypothermic response (Fig. 1, top right). CBN and CBD had no hypothermic potencies. CBD, however, markedly enhanced the THC-effect. The additional increase in hypothermic response was mainly produced by a prolongation of the THC induced hypothermia (Fig. 2, top). It should be mentioned, however, that the increase in hypothermic response caused by 40 mg/kg THC as compared to 20 mg/kg is mainly produced by an increase in duration of effect, likewise. CBN did not interfere with THC.

Catalepsy. Also in this test situation only Δ^9-THC produced a significant effect. CBN (10 mg/kg) and CBD (up to 80 mg/kg) were ineffective (Fig. 1, middle right). CBN did not alter the response to THC whereas CBD markedly enhanced it (Tab. 1). As in former tests this enhancement

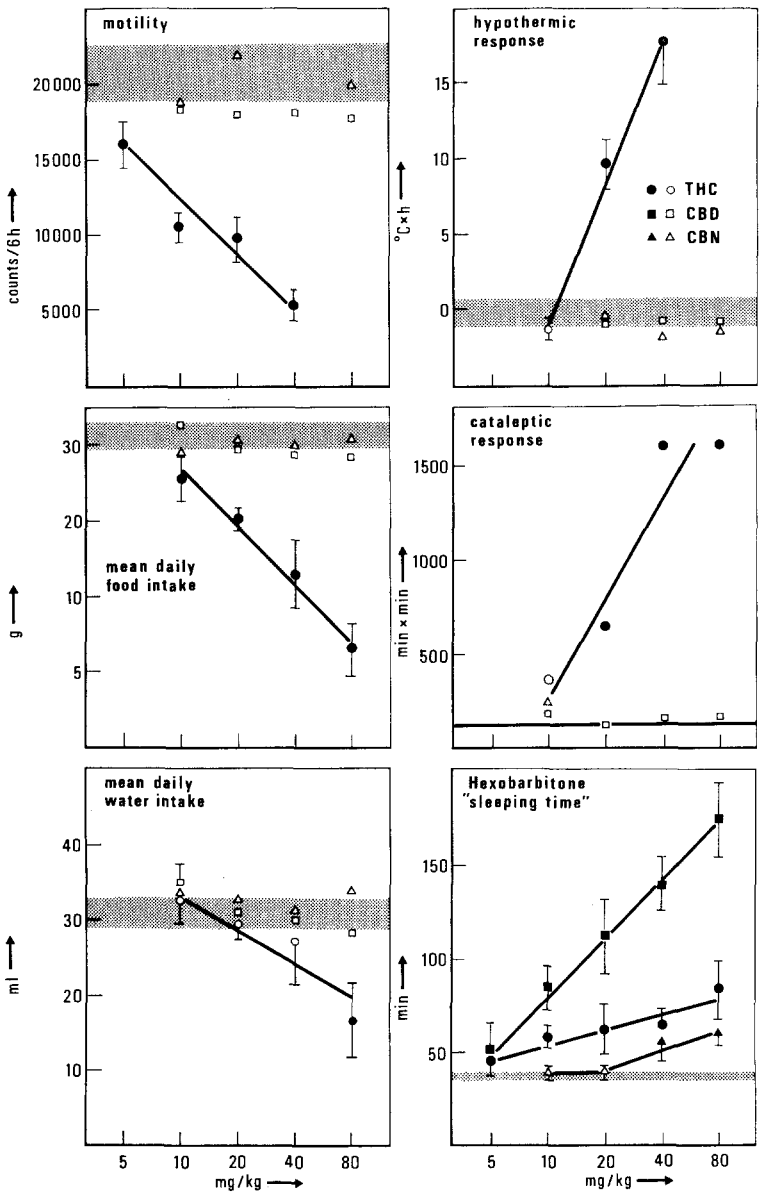

Fig. 1. Dose-response-relationships of THC, CBN, CBM in different test situations. The shadowed areas give the mean of the control ± SD. Filled symbols indicate a significant difference from control (at least $P < 0.05$). Further details are given under "Methods"

Table 1. Interactions between THC, CBN and CBD in several test situations

	Control	THC	CBN	CBD	THC+CBN	THC+CBD	CBN+CBD	Doses (mg/kg)		
								THC	CBN	CBD
hypothermic response, °C × h	0.0 ± 2.2 ($n = 17$)	11.4 ± 4.0[c] ($n = 9$)	1.1 ± 2.4 ($n = 8$)	− 1.5 ± 3.1 ($n = 8$)	11.2 ± 2.6[c] ($n = 12$)	19.9 ± 4.8[c f] ($n = 13$)	2.4 ± 1.9 ($n = 8$)	20	20	20
food intake, g/day	20.6 ± 1.3 ($n = 13$)	15.1 ± 1.1[c] ($n = 10$)	20.0 ± 1.0 ($n = 6$)	20.0 ± 1.6 ($n = 6$)	14.8 ± 3.5[c] ($n = 10$)	11.2 ± 1.2[c f] ($n = 8$)	19.9 ± 0.9 ($n = 5$)	20	20	20
water intake, g/day	31.6 ± 3.1 ($n = 14$)	29.5 ± 3.0 ($n = 10$)	33.3 ± 4.3 ($n = 6$)	31.2 ± 4.4 ($n = 6$)	29.0 ± 5.3 ($n = 9$)	20.4 ± 2.3[c f] ($n = 6$)	31.1 ± 2.0 ($n = 5$)	20	20	20
motility, 10 counts/6 h	20.8 ± 4.4 ($n = 14$)	16.1 ± 3.2[a] ($n = 9$)	18.4 ± 1.9 ($n = 5$)	18.2 ± 3.1 ($n = 6$)	14.7 ± 3.1[b] ($n = 6$)	11.8 ± 1.8[c e] ($n = 8$)		5	10	10
cataleptic response, min × min	123 ($n = 9$)	640[a] ($n = 8$)	255 ($n = 8$)	200 ($n = 8$)	755[a] ($n = 8$)	1510[b d] ($n = 8$)		20	10	10
hexobarbitone sleeping time, min. 1 h p.i.	34 ± 5 ($n = 14$)	53 ± 10[c] ($n = 11$)	35 ± 4 ($n = 10$)	86 ± 25[c] ($n = 12$)	35 ± 8[f] ($n = 10$)	100 ± 26[c f] ($n = 10$)	82 ± 16[c] ($n = 10$)	10	10	10
hexobarbitone sleeping time, min. 6 h p.i.	36 ± 6 ($n = 11$)	40 ± 6 ($n = 10$)		76 ± 25[c] ($n = 10$)		102 ± 19/ [c f g] ($n = 11$)		10	—	10

The values represent the mean ± SD. In brackets the number of animals or groups of animals is given.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. control.

[d] $P < 0.05$, [e] $P < 0.01$, [f] $P < 0.001$ vs. THC alone. [g] $P < 0.01$ vs. CBD.

The last column gives the doses used in the interaction experiments in the following sequence: THC, CBN and CBD.

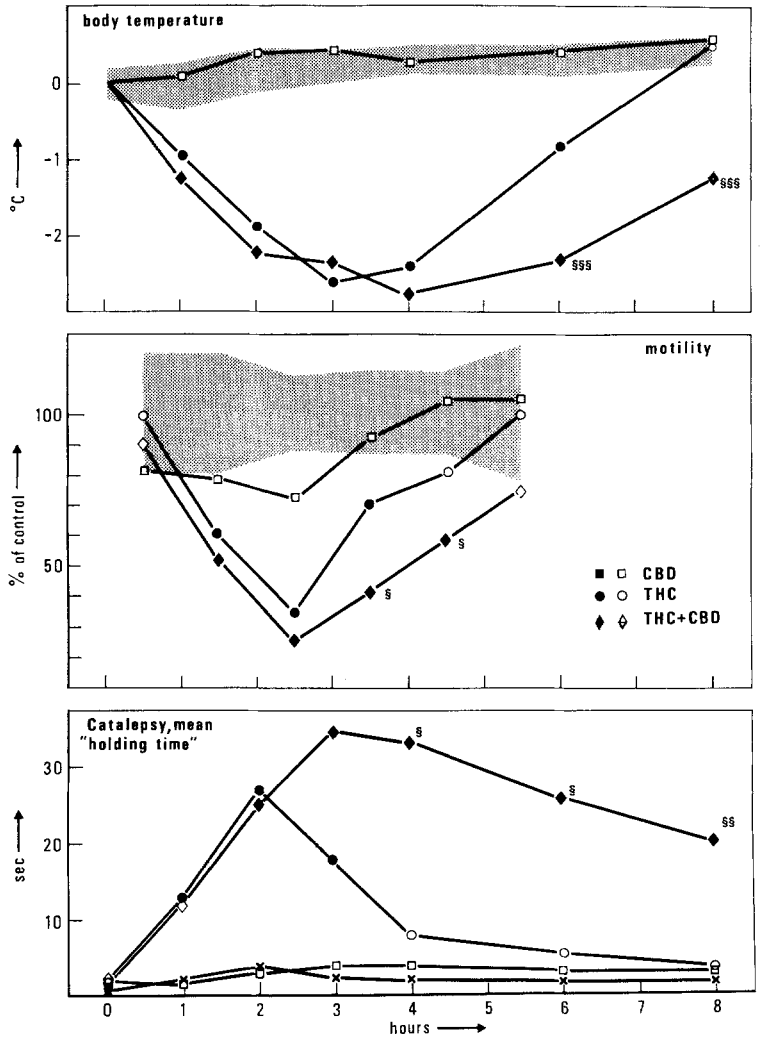

Fig. 2. Time-response-curves of THC, CBD and a combination of both. The shadowed area gives the mean of the control + SD. Filled symbols indicate a significant difference from control (at least $P < 0.05$). § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$ vs. THC alone. The doses used in the interaction experiments are given in Table 1.
×——× control

was predominantly caused by a prolongation of catalepsy (Fig.2, bottom), whereas the mean maximum "holding" time only slightly increased. As in the motility experiments, however, the higher cataleptic response to higher THC-doses is produced by an increase of the peak effect as well as a prolongation of catalepsy.

Hexobarbitone Sleeping Time. The hexobarbitone sleeping time was increased by all three cannabinoids (Fig. 1, bottom right). The figure shows the results when hexobarbitone (100 mg/kg) was injected 1 h after the cannabinoids. By contrast to the other tests CBD was more effective than THC. CBN increases the sleeping time only when given in doses greater or equal to 40 mg/kg. In interaction experiments, when the barbiturate was administered 1 h after the cannabinoids we observed a complete abolition of the THC-effect by CBN (Tab. 1) supporting the earlier findings of Krantz *et al.* (1971) in mice. The antagonism between CBN and THC, however, is limited to this test situation. CBD-prolongation was not antagonized. As expected THC and CBD produced a more intense prolongation than THC alone. There seems to be a small increase also relative to CBD alone. Statistical significance, however, was not reached in this experiment. If it really was the case it could mean a simple addition of THC- and CBD-effects. As can be seen from Fig. 2 the enhancement of THC-actions by CBD is mainly a prolongation and cannot be detected until 4 h after administration. We, therefore, repeated the combination experiment between CBD and THC by applicating the hexobarbitone 6 h after the injection of the cannabinoids (Tab. 1). No effect of 10 mg/kg Δ^9-tetrahydrocannabinol as compared to cremophor controls could be detected. CBD, however, produced almost the same prolongation as 1 h before hexobarbitone. The combination Δ^9-THC + CBD was significantly more potent than CBD alone. This result is more likely a consequence of a prolongation of the THC-effect than a potentiation of CBD-effect by THC.

Discussion

The present investigation has shown the following facts:

a) In most test procedures Δ^9-THC was the most potent cannabinoid tested. CBN and CBD were ineffective up to 80 mg/kg i.p. with exception of the barbiturate induced loss of righting reflexes. The "sleeping time" was potentiated in particular by CBD which was found to be more effective than THC.

b) In all test procedures cannabidiol was capable of enhancing the THC-effects. In those procedures in which the observation of the time course of actions was possible the enhancement was evaluated to be mainly produced by a prolongation of the THC-effects.

c) Cannabinol was capable of abolishing the barbiturate potentiation by Δ^9-THC but not that caused by CBD. The antagonism was limited to this particular test, in others the THC-actions were not modified at the doses used.

These results support earlier observations that CBD and CBN are less potent than THC in most of the test procedures used to show

cannabis like activity (Loewe, 1950; Edery, 1972). The strong effect of CBD on the barbiturate sleeping time, however, shows that also other cannabinoids might be more effective than Δ^9-THC in particular pharmacologic test situations. This has been supported recently by Izquierdo *et al.* (1973) and Izquierdo and Tannhauser (1973) who found CBD to possess a strong diphenylhydantoin like anticonvulsant activity in the rat.

A discussion on probable mechanisms by which CBD enhances the THC-effects includes central and metabolic interactions. The time course of interaction—a small increase in peak effect and a pronounced prolongation—favours a metabolic hypothesis for the following reason: The uptake of Δ^9-THC from peritoneal cavity is undoubtedly slow but relatively quick compared to its much slower excretion. A metabolic inhibitor should increase the peak THC-level in the body only slightly, the main effect should be seen during the excretion phase. Indeed, as mentioned above CBD has been shown in mice to inhibit the THC-metabolism *in vivo* (Jones and Pertwee, 1972). Also in the rat CBD is a strong inhibitor of the microsomal drug metabolism *in vitro* (Fernandes *et al.*, 1973) and it has been shown that the microsomal system is capable of metabolizing THC to metabolites found *in vivo* (Burstein and Kupfer, 1971; Kupfer *et al.*, 1973).

By determining blood and brain levels of hexobarbitone we found the increase in hexobarbitone "sleeping time" produced by CBD exclusively caused by an inhibition of the metabolism of the barbiturate (Coper and Fernandes, 1972). The hexobarbitone levels in the brain did not differ from controls on awakening. The THC effects, however, were produced by a central interaction with the barbiturate. To get a slight inhibition of the hexobarbitone metabolism, 40 mg/kg of either Δ^9-THC or CBN were necessary. These observations strongly suggest an inhibition of THC-metabolism by CBD also in the rat.

On the other hand the limited antagonism between CBN and THC regarding the potentiation of the hexobarbitone sleeping time is likely to be a central interaction. Several attempts to find possible effects of other cannabinoids than THC by comparing the effects of cannabisextracts and THC failed (Ferraro and Billings, 1972; Hollister, 1971 a and b) probably because the Marihuana Extract Destillate used contained almost no CBD. Jones and Pertwee (1972) tested the effects of CBD and THC in combination 15 min after application of the cannabinoids. In mice they could demonstrate an increase of brain Δ^9-THC- and more pronounced of 11-OH-Δ^9-THC-content, but not a potentiation in the "ring-test". As can be seen from our experiments the short time interval after injection used by these authors is not optimal to detect interactions.

A Brazilian group, however (Carlini, 1970; Karniol and Carlini, 1972), found differences in the activity of several cannabisextracts despite of the same THC content. The composition of these extracts, however, was not pure to justify a definite interaction. In a recent study (Karniol and Carlini, 1973) they reported about interaction experiments between pure CBD and THC in rabbits, rats, and mice. CBD blocked the following acute effects of THC: "catatonia" in mice, cornealareflexia in rabbits and the aggressiveness of rats previously stressed by REM sleep depravation.

It did not interfere with the decreased defecation in an open field situation. On the other hand, CBD potentiated the Δ^9-THC induced analgesia in mice and the Δ^9-THC-impairing effect on climbing rope performance of rats. These interactions were tentatively explained by postulating that CBD directly antagonizes the excitatory effects and/or indirectly potentiates the depressant effects of Δ^9-THC. The blocking effects of CBD seem to be contradictory to our results. It should be recognized, however, that the experiments by the Brazilian group were performed during the first 4 h after administration with exception of the climbing rope test (up to 6 h). All antagonistic effects were small, in part inconsistent (catatonia in mice) and doseresponse-relationships of THC were not given. Despite these limitations these experiments show that CBD might interfere with THC, also at a nonmetabolic level. Our present knowledge, however, about central CBD-actions and interactions seems us to be too limited to allow definite conclusions.

The present investigation shows that at least in rodents, other cannabis constituents than THC significantly modify the effects of the "active" tetrahydrocannabinol. Further preliminary observations (to be published) show that such interactions are not only of significance in acute but also in chronic experiments, e.g. CBD enhances the tolerance development to THC.

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