Pharmacology Biochemistry & Behavior, Vol. 30, pp. 675–678. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Cannabinol and Cannabidiol in Combination: Temperature, Open-Field Activity, and Vocalization[1]

ARTO J. HILTUNEN,*†[2] TORBJÖRN U. C. JÄRBE† AND KERSTIN WÄNGDAHL*

**University of Uppsala, Department of Clinical Psychology, Uppsala, Sweden*
†University of Uppsala, Department of Psychology, Uppsala, Sweden

Received 10 February 1987

HILTUNEN, A. J., T. U. C. JÄRBE AND K. WÄNGDAHL. *Cannabinol and cannabidiol in combination: Temperature, open-field activity, and vocalization.* PHARMACOL BIOCHEM BEHAV 30(3) 675–678, 1988.—Rectal temperature as well as unconditioned activity in an open-field (O-F) arena, and palpation-induced vocalization were examined in rats treated intraperitoneally with cannabinol (CBN, 17.5 or 56 mg/kg) and cannabidiol (CBD, 10 or 30 mg/kg), either singly or in combination. CBN singly resulted in hypothermia which was not attenuated by the addition of CBD. CBN reduced ambulation and rearing activities as compared to vehicle-treated rats. CBD in combination with CBN did not attenuate these effects; the CBD doses in themselves appeared inactive. Vocalization occurred to a significantly greater extent in the CBN singly-treated rats as compared to the controls and the CBD singly-treated rats. Thus, CBD did not counteract the temperature and open-field effects induced by CBN. This is discussed in relation to previous results from drug discrimination experiments.

CBN CBD Temperature Open-field Vocalization Rats

THE interest concerning constituents of cannabis preparations (hashish, marijuana), such as Δ^1-tetrahydrocannabinol (Δ^1-THC), cannabidiol (CBD) and cannabinol (CBN) has focused on THC (Δ^6-THC and Δ^1-THC), either alone or in combination with other cannabinoids [14, 17, 20], and information regarding possible interactions between cannabinoids such as CBN and CBD is very limited.

We have examined the effects of Δ^1-THC and CBN in a drug discrimination learning (DDL) paradigm, and found that CBN produced THC-like stimulus effects in rats, albeit CBN was weaker than Δ^1-THC; combinations of these substances tended to be additive [11] (see also [2, 3, 10]). In an open-field (O-F) test, the unconditioned effects of CBN appeared qualitatively similar to, though weaker than those of Δ^1-THC [11]. Both THC and CBN produced dose-related decreases in the colonic temperature of rats [11] and elicited vocalizations in response to touch [11].

When CBD was administered together with THC in rats trained to discriminate between Δ^1-THC and vehicle, the Δ^1-THC discriminative stimulus effects were prolonged [5]. However, when CBD (10 and 30 mg/kg) and CBN (10 and 17.5 mg/kg) were combined in the DDL procedure, the THC-like stimulus effects of CBN were attenuated [6].

To determine if the attenuation of the THC-like discriminative effects of CBN together with CBD [6] would be accompanied by a normalization of overt behaviors, we examined the effects of combinations of CBN and CBD on unconditioned, "spontaneous" activity in an O-F procedure as well as by measuring the rectal temperature. Palpation-induced vocalization behavior was also assessed [4,9].

METHOD

Animals

Seventy-two experimentally naive male Sprague-Dawley rats (ALAB AB, Sollentuna, Sweden) were used for the rectal temperature recordings and O-F tests. The animals were housed in groups of 4 to 6 rats in macrolone cages under standard laboratory conditions (temperature 20–22°C; relative humidity of about 50–60%; and 12 hr light/dark cycle). Water and food pellets (type R3; Ewos AB, Södertälje, Sweden) were freely available in the home cages. At the time of testing the average free-feeding weights were 402 g.

Apparatus

Temperature. Rectal temperature recordings were performed with a thermocouple (Type TE3, Ellab, Copenhagen, Denmark). The thermistor probe was inserted a constant

[1]Brief accounts of these data were communicated at the Third International Meeting on Drug Discrimination and State Dependency, July 2–7, Antwerp and Beerse, Belgium, 1986 [7].

[2]Requests for reprints should be addressed to Arto J. Hiltunen, Department of Psychology, University of Uppsala, Box 227, S-751 04 Uppsala, Sweden.

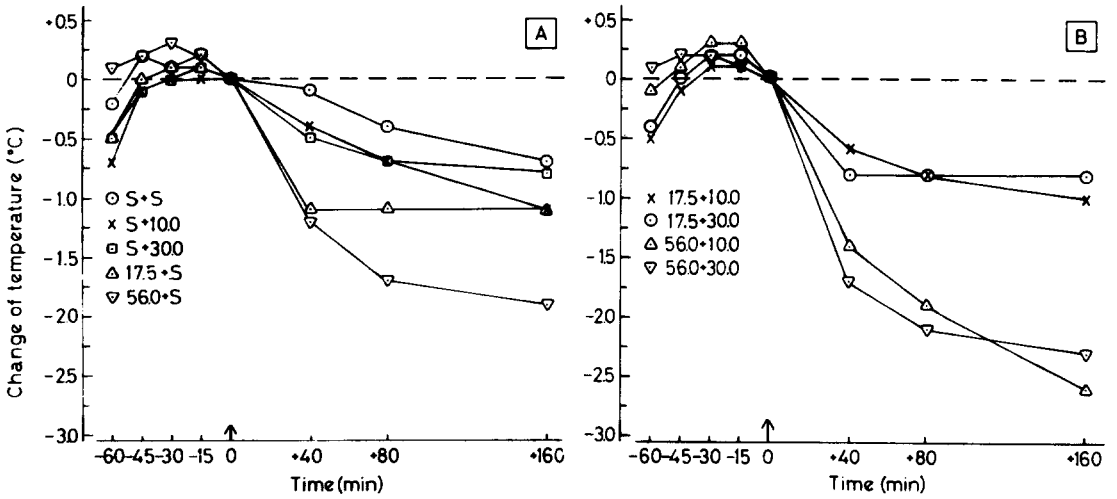

FIG. 1. Effects of CBN and CBD singly (section A) or in combination (section B) on rectal temperature of rats. Y-axis, average deviations from the score recorded at the fifth measurement (time point zero). X-axis, elapsed time during the recording period (−60 to 0 min referring to recordings prior to drug administrations and +40 to +160 min referring to recording intervals after drug administration). Injections (IP) were given immediately after the fifth measurement as indicated by an arrow at time point zero; the reference (zero) is also indicated by the dotted line. Variability measures are omitted for the sake of clarity in data presentation. S+S=suspension plus suspension, i.e., two vehicle injections of 4 ml/kg (5% of propylene glycol, tween-80 each, and 90% isotonic saline, respectively); S+10.0=suspension plus 10 mg/kg CBD; S+30.0=suspension plus 30 mg/kg CBD; 17.5+S=CBN 17.5 mg/kg plus suspension; 56.0+S=CBN 56 mg/kg plus suspension; 17.5+10.0=CBN 17.5 mg/kg plus CBD 10 mg/kg; 17.5+30.0=CBN 17.5 mg/kg plus CBD 30 mg/kg; 56.0+10.0=CBN 56 mg/kg plus CBD 10 mg/kg; 56.0+30.0=CBN 56 mg/kg plus CBD 30 mg/kg.

depth of 6 cm into the rectum [16] for a total of 90 sec. The room temperature ranged between 20.4 to 23.5°C, the average being 21.9°C on the experimental days.

Open-field. The O-F arena was a wooden box (60×60×50 cm) with an open top and the floor divided into 16 squares (15×15 cm). A circle was marked in the center of the field. The squared floor was covered with an acrylate plate (60×60 cm). Illumination was provided by the normal room lighting (69 lux at the floor level of the O-F box according to measurements by Spectra Photometer, model 301).

Procedure

Temperature, open-field and vocalization. On the day of examination, the rats were placed in individual macrolone cages. Each experimental day two randomly selected drug conditions were compared, two rats in each condition, i.e., 4 rats per test day. The rectal temperature was recorded intermittently at 15 min intervals for 60 min, i.e., 5 recordings were collected before injection; subsequently three recordings generally were performed viz. 40, 80 and 160 min after administration. Thirty min after the intraperitoneal (IP) administration, the presence/absence of vocalization was assessed according to a palpation procedure described elsewhere [9]. Immediately afterwards the animal was placed in the center of the O-F arena where the rat was allowed to explore the field during 5 min. Records were kept on the following behaviors: *Ambulation*=the number of squares crossed with all four feet; *Rearing*=the number of times the animal stood on its hind feet; *Defecation*=the number of fecal boli deposited; *Urination*=the number of urination spots deposited; *Latency*=time in sec before leaving the center circle with all four feet; and *Grooming*=the number of cleaning bouts, including washing of the face with front paws and trimming of the fur. The acrylate plate was rinsed with water between trials to minimize odors from previously tested animals. Immediately after the O-F testing, the presence or absence of vocalization behavior was again noted; thereafter, the postinjection temperatures were recorded.

Data Analysis

Analyses of variance (ANOVA) were performed on the temperature recordings (two-way, split-plot design) and the O-F data (one-way, completely randomized design) followed by post hoc (Tukey's HSD, $p \leq 0.05$) statistical procedures [8,15]. Presence/absence of vocalization was evaluated with the Fischer exact probability test [19].

Drugs

Freshly prepared suspensions of CBN and CBD contained propylene glycol (5%, v/v), tween-80 (5%, v/v) and physiological (0.9%) saline (90%, v/v), and all administrations were IP in the volume of 4 ml/kg. The cannabinoids were first dissolved in propylene glycol, after which the solution was mixed with tween-80 by ultrasonification, and finally saline was slowly added while shaking the mixture [12]. Crystalline (−)-CBD and CBN (purity GLC ≥98%) were used. The compounds (CBD, UNC 393, and CBN, UNC 479) were obtained through the courtesy of Drs. O. Braenden and E. Lumsden (U.N. Narcotics Lab., Geneva, Switzerland), Makor Chemicals, Jerusalem, Israel (CBN), or were prepared (CBN and CBD) by Dr. R. Mechoulam (Department of Natural Products, Hebrew University of Jerusalem, Israel). No THC was detected (by GLC) in any of these samples of CBD and CBN (Ewa Johansson, personal communication, Dept. of Pharmacognosy, University of Uppsala, Sweden).

RESULTS

Temperature Effects

Figure 1 shows the mean effects of IP administrations of CBN and CBD either separately (together with vehicle) or in combination on rectal temperature. The groups consisted of eight rats which were administered vehicle (4 ml/kg), CBN (17.5 and 56 mg/kg), or CBD (10 and 30 mg/kg) singly (Fig. 1A), or in combination (Fig. 1B).

ANOVA indicated a significant Groups × Time interaction, $F(56,441)=6.55$, $p<0.01$. Subsequent analyses suggested that a difference between means of the groups being equal to or exceeding 0.75°C be declared significant. No differences between groups occurred during the preinjection intervals (−60 to 0 min), but with increasing CBN doses a reduction in temperature was noted yielding significantly lower temperature in the high dose CBN groups (56 mg/kg of CBN singly and together with 10 or 30 mg/kg of CBD) as compared to all the other groups at 80 and 160 min after administrations. No further decrease in rectal temperature was recorded in the high dose CBN groups at 220 min after administrations (data not shown in the figure). In the low dose CBN (17.5 mg/kg) group no significant ($p>0.05$) differences occurred as compared to the controls.

Open-Field Activity

Figure 2 shows the results of administering CBN (17.5 and 30 mg/kg) singly as well as in combination with CBD (10 and 30 mg/kg) on ambulatory (Fig. 2A) and rearing (Fig. 2B) activities 30 min after injections, during the 5 min observation period. For both the ambulation, $F(8,63)=3.20$, $p<0.01$, and rearing, $F(8,63)=10.79$, $p<0.01$, measures, as well as for defecation, $F(8,63)=3.52$, $p<0.01$, ANOVA indicated significant overall effects.

For the ambulation activity, Tukey's HSD revealed that the group treated with 56 mg/kg of CBN together with 30 mg/kg of CBD ambulated less than either of the control or the CBD 30 mg/kg singly groups, or the group treated with CBN (17.5 mg/kg) together with CBD (30 mg/kg). The critical HSD value with respect to ambulation is 47.8. Other comparisons were not significant ($p>0.05$).

For rearing, Tukey's HSD revealed that any differences between groups exceeding 15.7 (critical HSD value) were significant. The high dose of CBN (56 mg/kg) lowered rearing and this reduction appeared enhanced when CBD (10 and 30 mg/kg) was administered simultaneously; results from tests with 17.5 mg/kg of CBN were not significant. Details of the pair-wise comparisons are indicated in the legend of Fig. 2.

For defecation, pair-wise comparisons indicated that the controls as well as the CBN (17.5 mg/kg) singly group differed significantly from the groups treated with 17.5 mg/kg of CBN together with CBD (10 and 30 mg/kg), the means being 1.50, 1.50, 0.13, and 0.13 boli, respectively. Other comparisons were not significant ($p>0.05$).

No significant differences ($p>0.05$) were observed with regard to the remaining O-F parameters (latency, grooming and urination).

Vocalization

As no attempts were made to quantify this behavior, only the presence/absence was utilized for statistical evaluation. The Fischer exact probability test [19] suggested no statistically reliable differences between the groups at the first registration ($p>0.05$). Subsequent to O-F testing, the rats treated with CBN singly (17.5 and 56 mg/kg) differed ($p=0.04$ in all comparisons) from the controls and from the CBD singly-(10 and 30 mg/kg) treated rats. Vocalization was completely absent in the control rats as well as in the CBD-treated rats during both registrations. The animals treated with CBN and CBD in combination did not differ significantly ($p>0.05$) in vocalization behavior from the other groups.

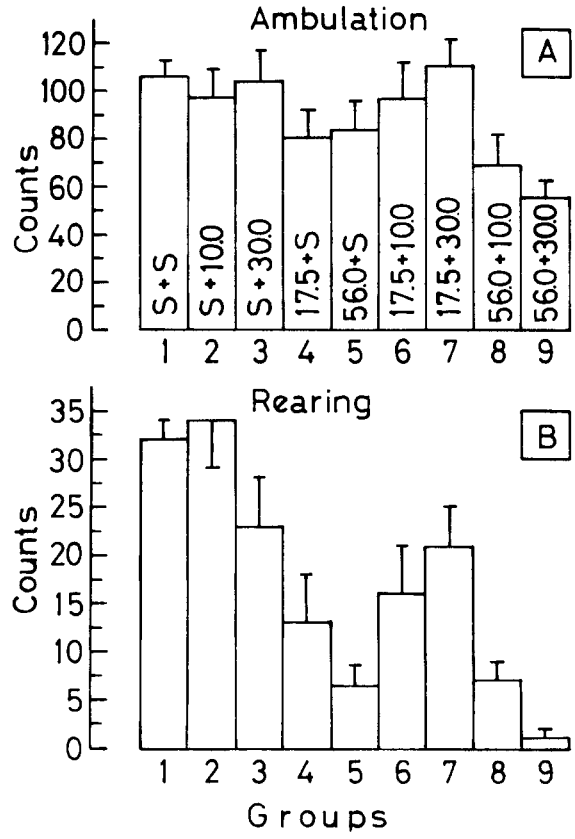

FIG. 2. Effects of CBN and CBD singly or in combination on ambulation and rearing activities of rats subjected to an open-field (O-F) test. Y-axis, total (mean) counts of ambulation (section A) and rearing (section B) activities noted during 5 min observation period. Vertical lines indicate 1 S.E.M. X-axis, the different treatment groups examined (abbreviations as in Fig. 1). Recordings were initiated 30 min after the IP administrations. Significant differences ($p\leq0.05$, Tukey's HSD) were obtained between the following groups: 9≠1,3,7 (A); 1≠4,5,6,8,9; 2≠4,5,6,8,9; 3≠5,9; 7≠9 (B).

DISCUSSION

In the present study combinations of two naturally occurring cannabinoids, CBN and CBD, were investigated in an O-F situation as well as by recording vocalization behavior and by measuring the rectal temperature of rats. The major impetus for the present efforts was the finding that the Δ^1-THC-like discriminative stimulus effects of CBN were attenuated by CBD [6].

Assuming competition for the same binding site(s), a normalization by CBD of the CBN-induced vocalization, O-F and temperature effects would have been in concert with the DDL experiment and possibly supported competitive antagonism. Apparently this was not the case since CBD did not counteract the CBN-induced alterations of behavior. With regard to the high dose combination (30 mg/kg of CBD and 56 mg/kg of CBN) the ambulation and rearing behaviors

seemed to be more disturbed as compared to the group treated with 56 mg/kg of CBN singly. Also the temperature measurements disclosed a tendency towards a lowering of the temperature in the high dose combination as compared to CBN (56 mg/kg) singly, indicating lack of antagonism.

With the lower dose of CBN (17.5 mg/kg) very marginal changes were observed when the drug was given singly and when tested in combination with CBD (10 and 30 mg/kg). Using still a lower dose of CBN (10 mg/kg), evaluated singly and in combination with CBD (the above doses), examining the presently employed parameters no tendencies at all were detected; in high doses (100 mg/kg) CBD singly depresses O-F activity and decreases rectal temperature (personal observations). Hence, the CBN and CBD doses presently used for the interaction studies are not too limited. Doses higher than those used here are, apart from toxicological considerations, of limited interest for understanding the pharmacology of hashish/marijuana intoxication.

An ancillary finding was the difference in defecation among the groups, rats treated with CBD evincing lower scores. Rats (N=16) treated with 100 mg/kg of CBD also evince reduced defecation (mean 0.25) as compared to controls (mean 1.13) (unpublished). This would seem consistent with demonstrations of a blockade by CBD of (1) Δ^1-THC-induced increase in defecation [13], and (2) suppression of diarrhea in morphine abstinent rats [1], possibly pointing towards a reduced intestinal motility by CBD.

In conclusion, unlike the DDL situation [6], a tendency towards augmentation rather than attenuation by CBD of behavioral effects induced by CBN occurred in the present study. One way of reconciling the present results with those of the DDL procedure [6] is to propose that CBD might have masked the presumably weak THC-like discriminative stimulus properties of CBN in much the same way as a strong stimulus might mask the effects of a less intense or significant sensory stimulus in experimental psychology research [18].

ACKNOWLEDGEMENTS

We thank M. R. Kamkar and G. Ohlin both for taking care of the animals and for their assistance during the course of the experiments. We also thank O. Braenden, E. Johansson, E. Lumsden and R. Mechoulam for their contributions. This work was supported by grants from the Swedish Medical Research Council (5717-05; 5717-06; 5717-07), the Magnus Bergvall's Foundation (1985; 1986), as well as by a grant from the Swedish Social Welfare Board (1986) awarded to T.U.C.J. A grant from the Sydney Alrutz's Foundation (1986) was awarded to A.J.H.

REFERENCES

1. Bhargava, H. N. Effects of some cannabinoids on naloxone-precipitated abstinence in morphine-dependent mice. Psychopharmacology (Berlin) 49:267–270; 1976.
2. Browne, R. G.; Weissman, A. Discriminative stimulus properties of Δ^9-tetrahydrocannabinol: mechanistic studies. J. Clin. Pharmacol. 21:227S–234S; 1981.
3. Bueno, O. F. A.; Carlini, E. A.; Finkelfarb, E.; Suzuki, J. S. Δ^9-tetrahydrocannabinol, ethanol and amphetamine as discriminative stimuli-generalization tests with other drugs. Psychopharmacologia 46:235–243; 1976.
4. Henriksson, B. G.; Järbe, T. U. C. Cannabis-induced vocalization in the rat. J. Pharm. Pharmacol. 23:457–458; 1971.
5. Hiltunen, A. J.; Järbe, T. U. C. Interactions between Δ^9-tetrahydrocannabinol and cannabidiol as evaluated by drug discrimination procedures in rats and pigeons. Neuropharmacology 25:133–142; 1986.
6. Hiltunen, A. J.; Järbe, T. U. C. Cannabidiol attenuates Δ^9-tetrahydrocannabinol-like discriminative stimulus effects of cannabinol. Eur. J. Pharmacol. 125:301–304; 1986.
7. Hiltunen, A. J.; Järbe, T. U. C.; Wängdahl, K. Interactions between cannabinol and cannabidiol. Psychopharmacology (Berlin) 89(abstract 146):S1–S57; 1986.
8. Jaccard, J.; Becker, M. A.; Wood, G. Pairwise multiple comparison procedures: a review. Psychol. Bull. 94:589–596; 1984.
9. Järbe, T. U. C.; Henriksson, B. G. Vocalization: a characteristic cannabis-induced behavior in rat? Physiol. Psychol. 1:351–353; 1973.
10. Järbe, T. U. C.; Henriksson, B. G.; Ohlin, G. C. Δ^9-THC as a discriminative cue in pigeons: effects of Δ^8-THC, CBD, and CBN. Arch. Int. Pharmacodyn. Ther. 228:68–72; 1977.
11. Järbe, T. U. C.; Hiltunen, A. J. Cannabimimetic activity of cannabinol in rats and pigeons. Neuropharmacology 26:219–228; 1987.
12. Järbe, T. U. C.; Swedberg, M. D. B.; Mechoulam, R. A repeated tests procedure to assess onset and duration of the cue properties of (−)-Δ^9-THC, (−)-Δ^8-THC-DMH and (+)-Δ^8-THC. Psychopharmacology (Berlin) 75:152–157; 1981.
13. Karniol, I. G.; Carlini, E. A. Pharmacological interaction between cannabidiol and Δ^9-tetrahydrocannabinol. Psychopharmacologia 33:53–70; 1973.
14. Kettenes-Van Den Bosch, J. J.; Salemink, C. A.; Van Nordwijk, J.; Khan, I. Biological activity of the tetrahydrocannabinols. J. Ethnopharmacol. 2:197–231; 1980.
15. Kirk, R. E. Experimental design: Procedures for the behavioral sciences. Belmont, CA: Brooks Cole; 1968.
16. Lomax, P. Measurement of 'core' temperature in the rat. Nature 210:854–855; 1966.
17. Mechoulam, R.; McCallum, N. K.; Burstein, S. Recent advances in the chemistry and biochemistry of cannabis. Chem. Rev. 76:75–112; 1976.
18. Overton, D. A. Test for a neurochemically specific mechanism mediating drug discriminations and for stimulus masking. Psychopharmacology (Berlin) 81:340–344; 1983.
19. Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill; 1956.
20. Sofia, R. D. Cannabis: structure-activity relationships. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology. New York: Plenum Press, 1978:319–371.