POSSIBLE ANXIOLYTIC EFFECTS OF CANNABIDIOL

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I. INTRODUCTION

Cannabidiol (CBD) has anti seizure effects as demonstrated by several techniques, e.g., electroshock induced seizures (1), kindled seizures (2), pentylenetetrazol induced seizures (1), and in a small sample of human epileptics (3). In addition, CBD has sedative properties since it increases pentobarbital-induced sleeping time and decreases the ED50 of naloxone-induced withdrawal from morphine (4). While CBD seems to share some of these properties with delta-9-tetrahydrocannabinol (THC), differences between these marijuana constituents are also apparent. CBD depresses spontaneous motor activity much less than THC (4), and produces much less depression of lever-pressing behavior on a variable interval schedule of reinforcement (5). Finally, CBD does not produce a psychological "high" in human subjects (3,6-8). Likewise rats can discriminate THC from Tween-80/saline control solution, while they can discriminate CBD from the THC but cannot discriminate CBD from the same control solution (9).

In addition to these data, reviews of historical uses of cannabinoids have suggested that marijuana has been used for analgesic, antispasmodic, anti-hypertensive, and sleep-induction effects. Furthermore, on the basis of clinical observation the author has clinical reports that marijuana can block spasmodic-toritcolus and relieve situational anxiety. While none of these reports is clearly experimental nor do they separate the effects of one cannabinoid molecule from another, these reports are suggestive enough to warrant experimental investigation of cannabinoid effects in animal experiments which may reflect anxiolytic properties of such molecules. The present experiments were designed to test the effects of

CBD on body tremor and seizures during withdrawal from alcohol intoxication in mice, on active avoidance acquisition in mice, on lick-suppression in rats and on ulcerogenesis in mice. If CBD has effects which are similar to known anxiolytic drugs on these tests, such data would suggest further examination of CBD for anxiolytic effects.

II. EXPERIMENT I: Effects of CBD, THC and Clonidine on Alcohol Withdrawal Symptoms in Mice.

Since anti-anxiety-type compounds are often recommended for their calming effects during alcohol withdrawal, the present investigation was designed to test the effectiveness of THC and CBD during alcohol withdrawal in mice. In addition, recent human studies have suggested that clonidine (an alpha-2-adrenergic blocker) has anti-anxiety properties (12). It is anti-hypertensive, in both animals (13-15) and man (16) and has calming effects during withdrawal from opiates in animals (17-18) and in man (19). Finally, clonidine is effective in reducing symptoms during withdrawal from alcohol in man (20). Thus the present study was also designed to test the relative efficacy of clonidine during alcohol withdrawal.

A. METHOD

1. Subjects. A total of 450 naive male C57BL/6J mice (23-268) (a high alcohol preference strain) were obtained from Jackson Laboratory, Bar Harbor, Maine. They were maintained on a 12-hour light-dark cycle at 21°C. Animals were given food and water ad libitum and were group housed until the experiment was started, at which time they were placed in single cages for the duration of the experiment.

2. Drugs. The following drugs and doses were used: 1) Delta-9-tetrahydrocannabinol (THC): of 0.5, 2.0 and 4.0 mg/kg; 2) Cannabidiol (CBD): 20, 40, and 80 mg/kg; 3) Clonidine, 4, 40 and 80 mg/kg; 4) Tween-80 + saline placebo (control); 5) No drug (sham injection control).

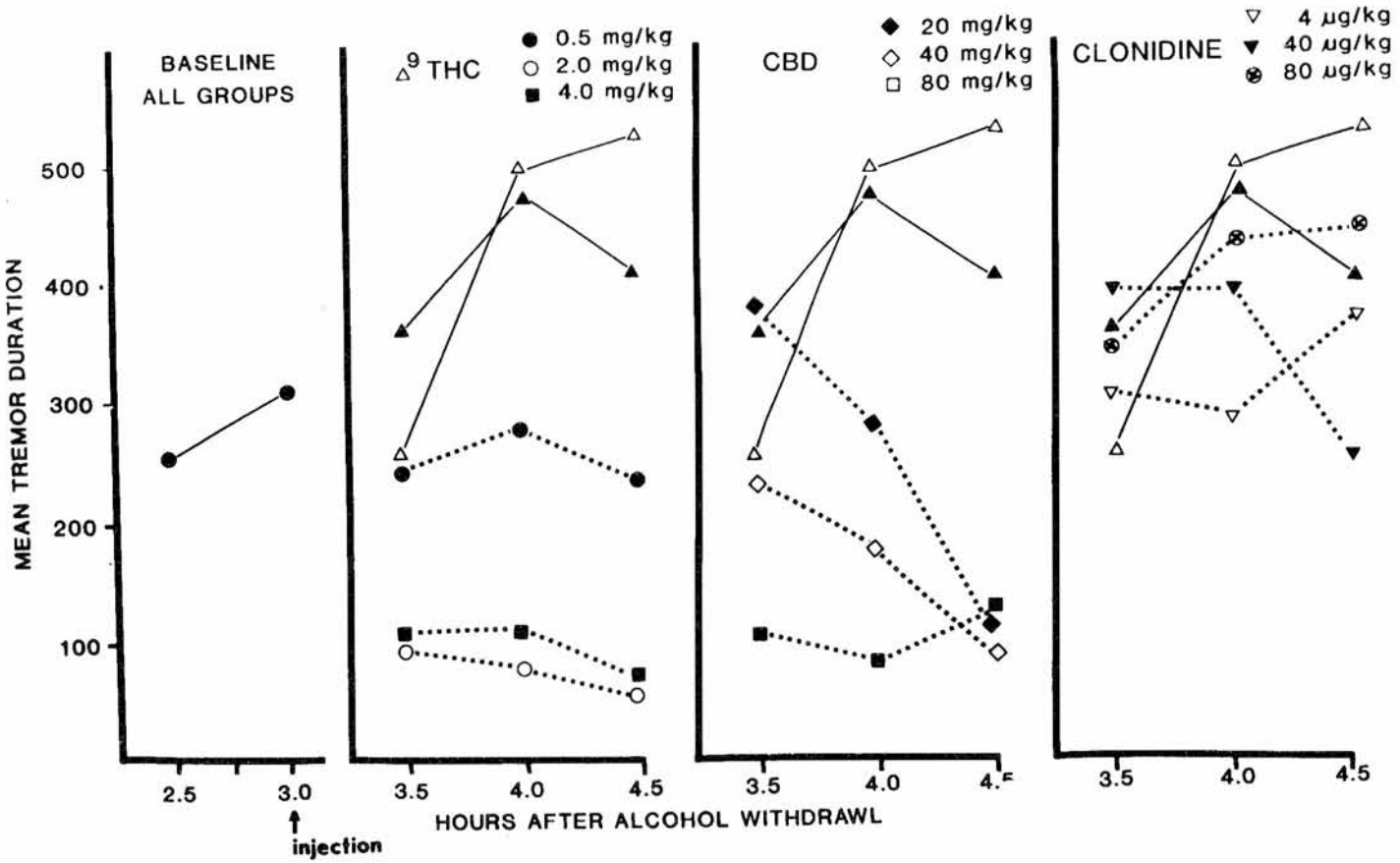
THC and CBD were obtained from the National Institute on Drug Abuse (NIDA). Clonidine was obtained fromBoehringer Ingleheim, Inc. All drugs were prepared in a 0.6% Tween-80 in 9% physiological saline solution and injected as a constant volume concentrations. Drugs were administered intraperitoneally (i.p.) at three consecutive times during with withdrawal period; since the duration of the effect of each drug may have

been short, due to rises and falls in blood levels of the drugs, we decided to inject each at 1.0, 2.5, and 4.0 h after the beginning of the measurement period.

3. Experimental Design. Twelve to 16 mice were withdrawn from alcohol and injected with one of the 9 drugs by dose combinations or 2 control injections in each of 20 replications across 40 weeks. In each replication, the injection of drug or control treatments were randomized. The final number of animals in each treatment group was 16.

4. Intoxication Procedure. The intoxication and withdrawal procedures were adapted from those of Freund (21,22); since our procedures are significantly different from his, they are described in detail. Mice were weighed and then placed on a feed deprivation schedule for 4 days. Each mouse was fed once daily for 1 hour. Water was available ad libitum. On the fifth day, each mouse was given a liquid diet dispensed in a 30 ml plastic syringe with a stainless steel drinking tube attached to it (23). The diet was a mixture of Sustacal, alcohol, and water and was the sole source of food and water during the phase of the experiment. Diet was prepared daily and given to the animals at 0900 h. Liquid diet consumption was measured at 0900, 1400 and 1900 h daily. The degree of intoxication was rated at the same times that diet consumption was recorded, as follows: Stage 1: ataxic, with rapid gait; Stage 2: grossly impaired gait or body movements, falling to the side, with the righting reflex intact; Stage 3: no righting reflex or comotose. After four days mice which had reached an intoxication criterion of Stage 2 or greater for in two-thirds of all daily ratings were used for withdrawal. Approximately 60% of the mice in each replication reached this criterion in 4 days, while about 20% died and 20% did not reach criterion.

5. Behavioral Measures of Withdrawal Data Recordings. Trained observers recorded the frequency and duration of two withdrawal signs: 1. tremors, which were defined as tremor of the hindquarters, or the whole body; 2. seizures, which were defined as tonic-clonic behavioral convulsions. Recording of these behaviors was accomplished by the observer depressing a switch at the beginning of each behavioral event and releasing the switch upon its termination. Switches were connected to an ABLE-40 computer (New England Digital Corporation), which recorded the frequency and durations of tremors and seizures in 5-min time blocks. The data were stored on diskettes, for subsequent reduction and analysis. Each observer recorded tremors and seizures for 4 mice simultaneously, in 3.5 h shifts. Interrater reliability was periodically


BASELINE
ALL GROUPS
Δ9 THC
0.5 mg/kg
2.0 mg/kg
4.0 mg/kg
CBD
20 mg/kg
40 mg/kg
80 mg/kg
CLONIDINE
4 µg/kg
40 µg/kg
80 µg/kg
MEAN TREMOR DURATION
500
400
300
200
100
2.5
3.0
injection
3.5
4.0
4.5
HOURS AFTER ALCOHOL WITHDRAWL

checked throughout the experiment and was considerably 0.90 or better.

6. Withdrawal Procedure. On the fifth day, the alcohol diet was withdrawn at 0800 h. A diet of Sustacal and water was provided during the subsequent 9 h withdrawal period. At 1100 h, observation of withdrawal symptoms began, for a baseline period of 1 h. At this point, the appropriate drug or control solution was injected, using a double blind procedure. Recording continued for 6 additional hours (9 hours after withdrawal of the alcohol diet).

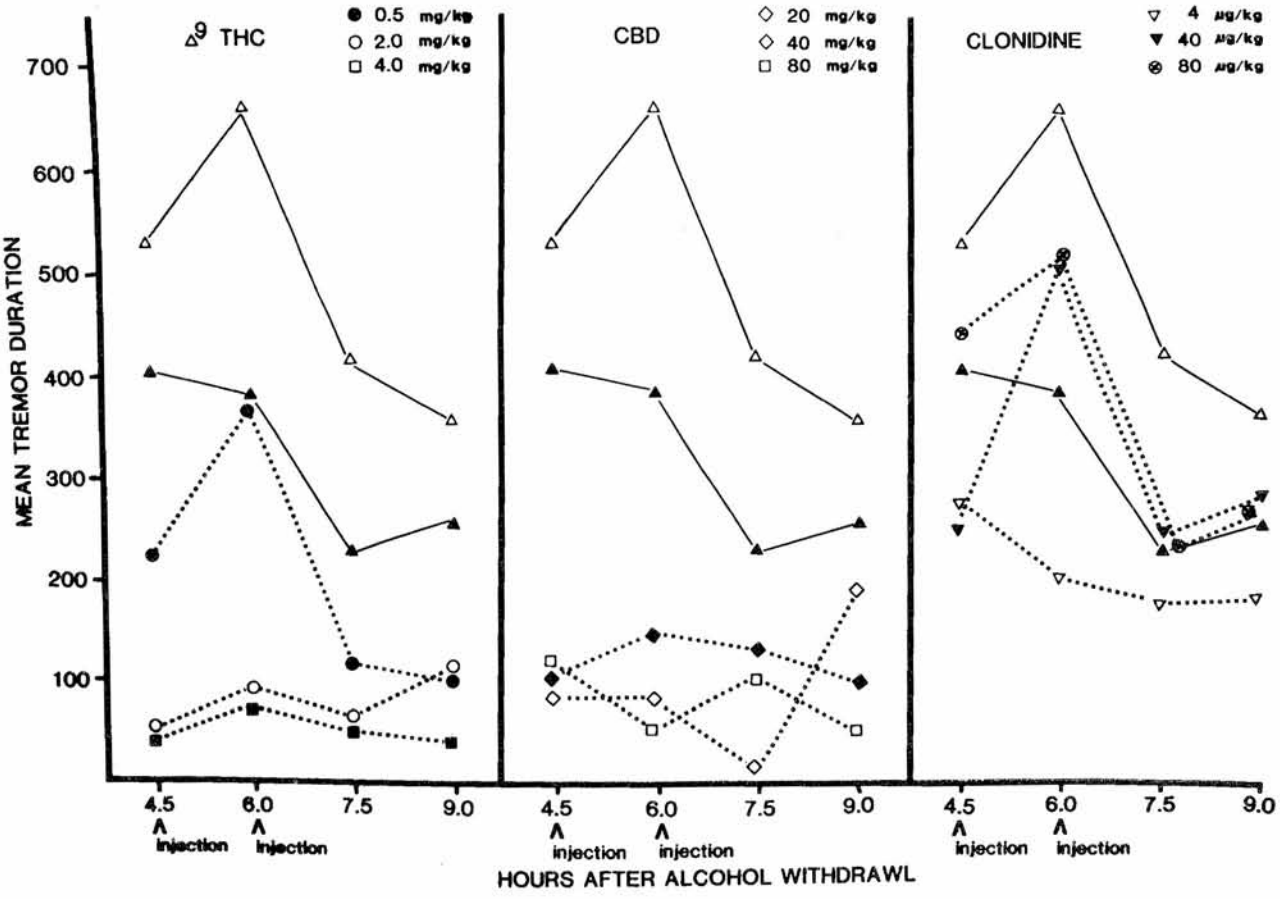
7. Audiogenic Seizure Tests. In addition to recording spontaneous seizures, tests for audiogenic seizures were conducted at 1.5, 3.0, 4.5 and 6.0 h after the beginning of the measurement period. These times correspond to 30 min after each drug injection and 2.0 h after the last drug injection. (Seizures were elicited by sounding a fire alarm bell for 90 sec at each of the above times.) The sound intensity was measured as 100 db at 2 cm above the cage floor.

8. Data Analysis. The data for each drug were analyzed against the control injection conditions using two-way analyses of variance with repeated measures on one factor, on all behavioral measures. Post hoc comparisons were conducted only if main effects or interactions were significant, as recommended by Winer (24). For these comparisons, Simple-effects analyses and Newman-Keuls analyses were used (24). Initial analyses were conducted using drug x 5 min time blocks, and subsequent analyses were made in 30 min time blocks. All data are presented in reduced 30 min form.

B. RESULTS

1. Tremors. Figure 1 shows the effects of THC, CBD and clonidine on the duration of tremors during baseline and the 1.5 h period following the first injection of each drug. THC produced significant decreases in tremor duration at all

FIGURE 1. Mean tremor duration plotted as a function of the first 4.5 hours after alcohol withdrawl. Baseline shows the mean of all groups prior to injection. Appropriate drug injections occurred at 3.0 hours after withdrawl. Control groups are no injection (open triangles) and Tween-80 and saline injection (closed triangles) in each panel. Each drug group is shown in panels to the right of the baseline plot.

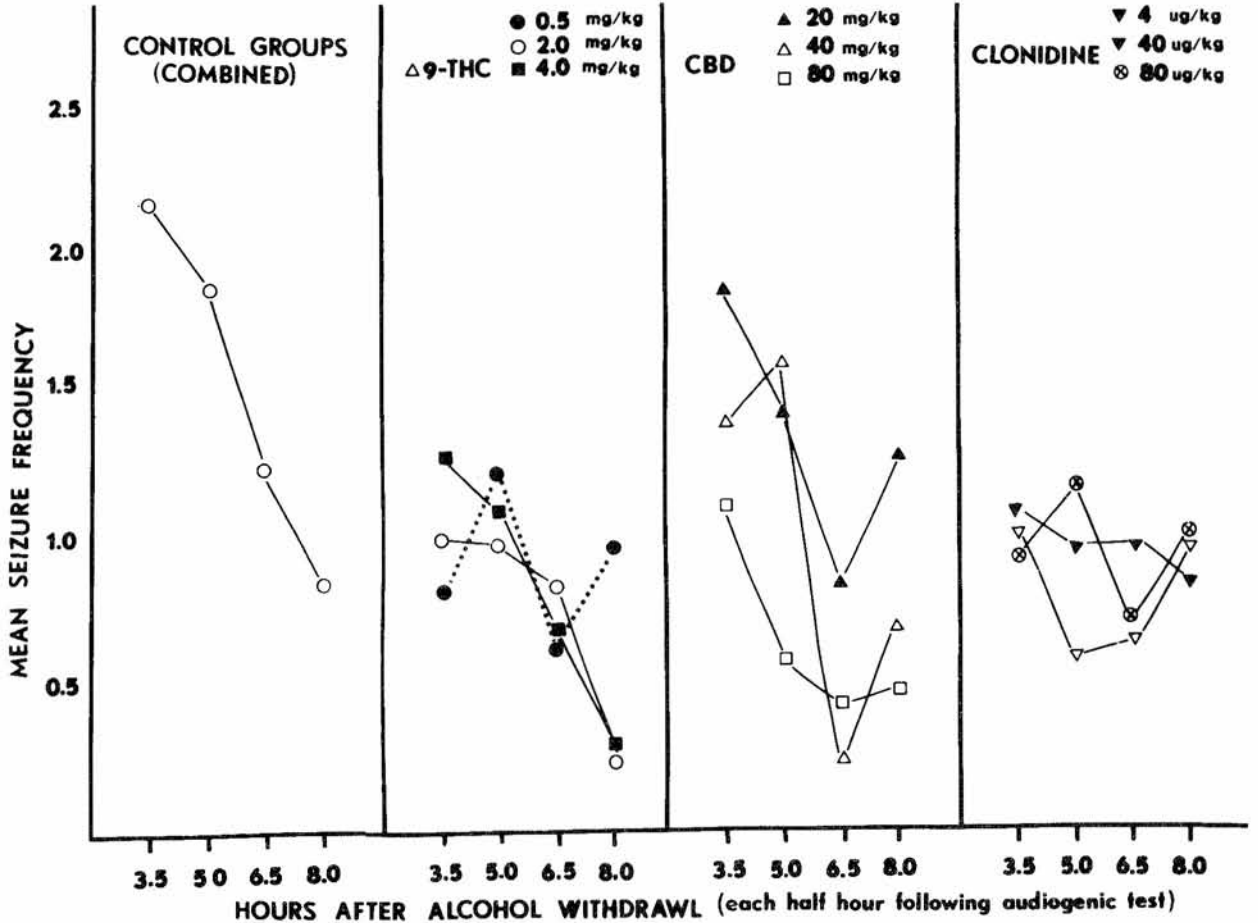

Δ9 THC
● 0.5 mg/kg
○ 2.0 mg/kg
□ 4.0 mg/kg
CBD
◇ 20 mg/kg
◇ 40 mg/kg
□ 80 mg/kg
CLONIDINE
▽ 4 µg/kg
▼ 40 µg/kg
⊗ 80 µg/kg
MEAN TREMOR DURATION
700
600
500
400
300
200
100
4.5
6.0
7.5
9.0
injection
injection
HOURS AFTER ALCOHOL WITHDRAWL

doses, when each dose was compared with the control groups (Newman-Keuls tests $p < 0.01$ for all comparisons). The two higher doses produced a significantly greater suppression of the tremors than the low dose (Newman-Keuls, $p < 0.01$). Control groups did not differ from each other. Simple-effects tests for each dose or control (condition) across that 1.5 h period (hours 3.0-4.5 after withdrawal) demonstrated that tremor durations increased significantly in both control groups and decreased in all THC groups ($p < 0.0001$ forall tests). CBD produced a significant decrease in tremor durations at all doses (Newman-Keuls, all tests, $p < 0.01$). CBD produced a dose related decrease in tremor at the 4.0 h point, but by 4.5 h, all doses were equally effective in reducing tremor durations. Simple-effects tests across the 1.5 h period indicate that the 20 and 40 mg/kg doses of CBD produced linear decreases in tremor duration, but the 80 mg/kg dose did not. (All tests, $p < 0.001$). With regard to clonidine, no significant changes in tremor duration were found at any dose (simple effects tests) during the 1.5 h period following injection.

Figure 2 illustrates the results of the second and third drug injections given during withdrawal. Simple-effects analyses demonstrated that subsequent injections of THC significantly suppressed tremor duration during the remainder of the test session. The two higher doses almost eliminated tremors entirely (Newman-Keuls, $p < 0.01$, all tests). The low dose of THC, however, produced significant decreases only in the last part of the test period (7.5 and 9.0 h after withdrawal). CBD, at all three doses, produced significant reductions in tremor durations (Simple effects, $p < 0.01$). No dose differences were found (Newman-Keuls). Clonidine significantly suppressed tremor durations only at the 6.0 and 7.5 h marks (Simple effects, $p < 0.01$). This effect was due solely to the 4.0 mg/kg dose. (Newman-Keuls, $p < 0.01$).

2. Seizures. Figure 3 illustrates the mean seizure frequencies for each half hour period following the presentations of the bell. Newman-Keuls analyses for each drug against control groups demonstrated that THC, CBD, and clonidine significantly suppressed seizure frequencies at the 3.5 and 6.0 h measurement periods. The frequency of seizures declined significantly in the control groups (Simple effects, $p < 0.01$).

FIGURE 2. Mean tremor duration plotted as a function of hours 4.5-9.0 after withdrawl from alcohol. Injections were made at 4.5 and 6.0 hours as indicated. Control groups are as in Fig. 1. Each drug group is shown in panels of the figure.


CONTROL GROUPS (COMBINED)
△9-THC
● 0.5 mg/kg
○ 2.0 mg/kg
■ 4.0 mg/kg
CBD
▲ 20 mg/kg
△ 40 mg/kg
□ 80 mg/kg
CLONIDINE
▼ 4 ug/kg
▼ 40 ug/kg
⊗ 80 ug/kg
MEAN SEIZURE FREQUENCY
2.5
2.0
1.5
1.0
0.5
3.5 5.0 6.5 8.0
HOURS AFTER ALCOHOL WITHDRAWL (each half hour following audiogenic test)

Thus, when drug groups were tested against the control groups at the 6.5 and 8.0 h measurement periods, no significant differences were found, for any of the drugs. No significant effects of drug doses were found for THC or clonidine. With CBD, a significantly lower frequency of seizures was found between the 80 mg/kg dose and the 20 and 40 mg/kg dose at the 4.5 and 6.0 h measurement periods. The two lower CBD doses were not significantly different from controls.

III. EXPERIMENTS II: Effects of CBD, THC, and Diazepam on Active Avoidance Acquisition in Mice.

Anti-anxiety drugs have been shown to improve active avoidance behavior (25). Similarly, THC improves the acquisition of two-way active avoidance in rats (26). Thus, the present experiment was designed to compare the effects of CBD, THC, and diazepam (DZP) on active avoidance and acquisition in mice.

A. Methods

1. Subjects. Subjects were 78 C57Bl6J mice bred in our colony at the University of Vermont, from breeding stock obtained from the Jackson Laboratory, Bar Harbor, Maine. They were maintained in group cages of six mice per cage.

2. Drugs and Experimental Design. Ten groups of mice were run in the following conditions (numbers in each group are shown in parentheses): Tween 80 (0.06%) + normal saline, control injection (8); CBD: 20 (5) and 40 (9) mg/kg; THC: 1 (8), 2 (8), and 4 (8) mg/kg; and diazepam: 2.5 (8), 5.0 (8) and 10.0 (8) mg/kg. All drugs were mixed daily and administered in constant volume injections of 1.0 mg/100 g of body weight.

3. Procedure. A plexiglass running wheel for mice was used. Shock was delivered at 0.25 mA by a Grason Stadler shock source. A mouse was placed in the running wheel. Five

FIGURE 3. Mean seizure frequency for each half hour following the presentation of bell-sound to elicit audiogenic seizures. See text for further explanation. Groups are plotted separately in panels as indicated.

seconds after being placed in the wheel a 0.25 mA shock was delivered continuously until the mouse ran and turned the wheel at least one-half revolution. Once this had occurred shock was terminated for 20 seconds. If the mouse continued to run, each half revolution delayed subsequent shock for 20 seconds. If, however, the mouse remained motionless for 20 seconds, shock reoccurred. Each mouse was run on this schedule for 30 minutes.

Data recorded were: The number of shocks received per 5 min interval and the number of half revolutions per 5 min interval. Since there is a very high correlation between these measures, the number of shocks received are reported here.

B. Results

Figure 4 displays the number of shocks received by each test group during the 30-minute session plotted as a function of the dose of drug administered. CBD produced a significant decrease in the number of shocks received ($F = 3.64$, $df = 2,19$; $p < 0.0001$), as did THC ($F = 4.21$, $df = 3,28$; $p < 0.01$), and DZP ($F = 2.85$, $df = 3,28$; $p < 0.05$).

IV. EXPERIMENT III: Effects of CBD and Diazepam (DZP) on Lick Suppression in Rats.

It has been demonstrated that benzodiazepines reduce the suppression of a learned response when that response is punished (27,28). Thus, the present experiment was designed to compare the effects of CBD and DZP in a punished response paradigm.

A. Method

1. Subjects. Seventy male, hooded rats weighing 200-225 g from Charles River Breeding Laboratories were used. They were maintained in cages in groups of two.

2. Drugs. Seven groups of rats were run in the following drug conditions (number of rats in each group are shown in parentheses): Tween 80 (0.6%) and normal saline, control injection (8); CBD: 15 (9), 30 (9), and 60 (9) mg/kg; DZP: 2.5 (10), 5 (10), and 10 (9) mg/kg. All drugs were mixed in

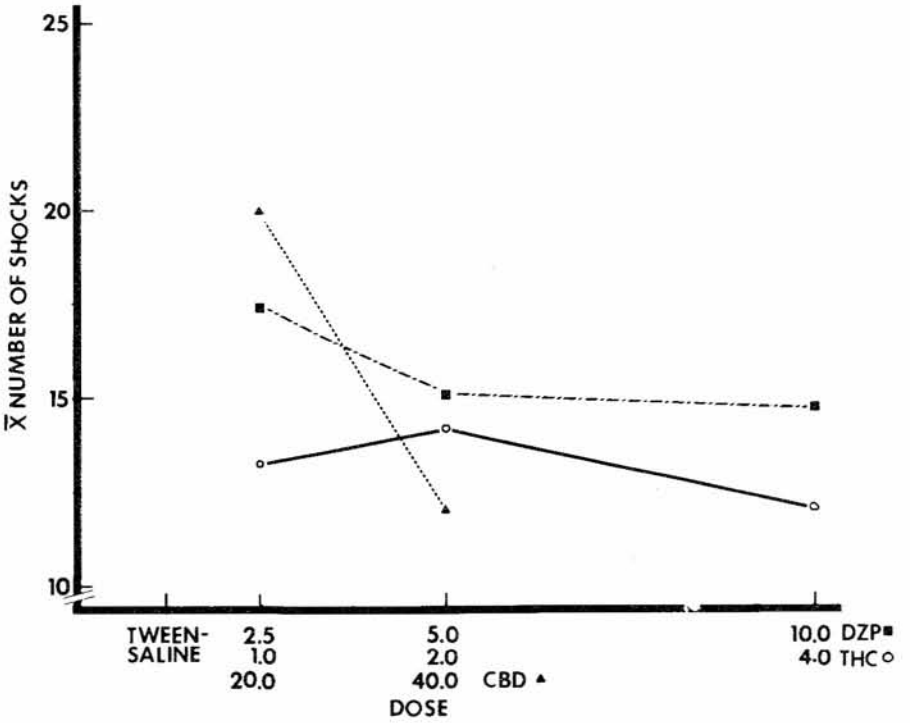
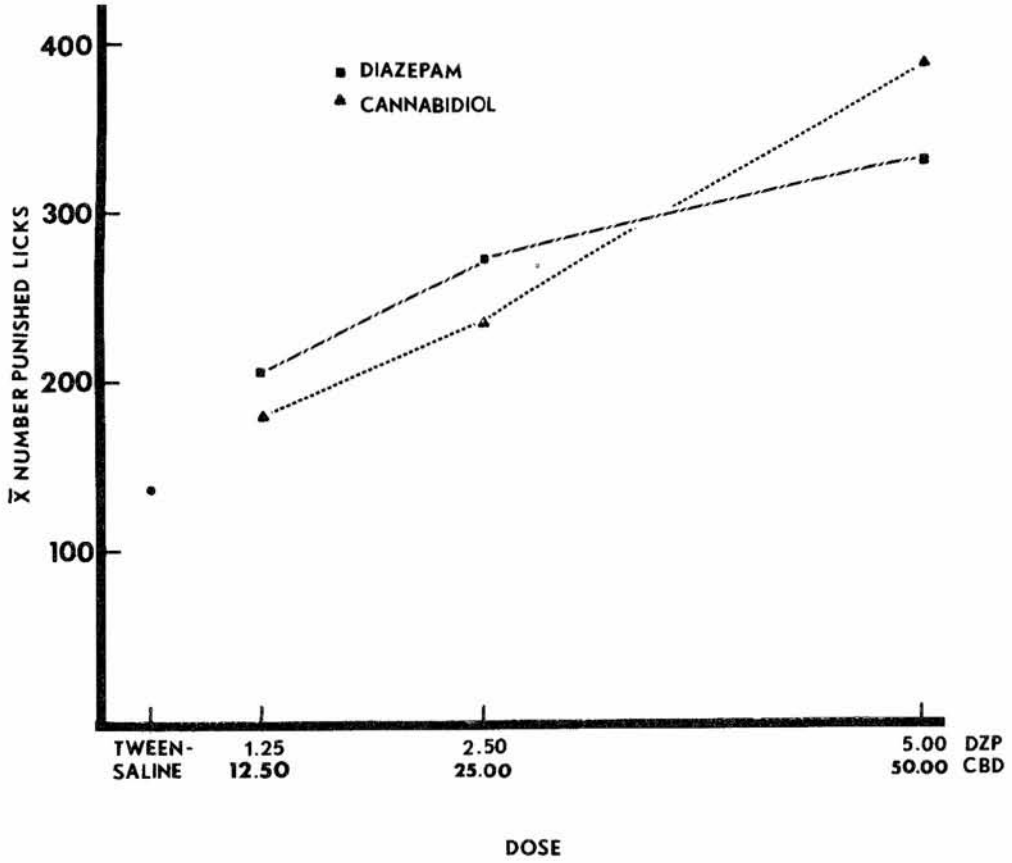


FIGURE 4. Mean number of shocks received in the running wheel avoidance task plotted as a function of control solution (Tween-80 + saline) and doses of diazepam (DZP), THC, and cannabidiol (CBD) as indicated on the abscissa.

Tween 80 - saline daily and were administered in constant volume injections of 1.0 ml/kg of body weight.

3. Procedure. A standard operant conditioning chamber with a stainless steel grid floor was used. On one wall a 20 ml syringe of water with a ball-point drinking tube projected into the chamber. Rats were first trained to drink from these tubes using the following procedure. On Day 1, the rats were deprived of water for 23 hours. Following this, they were allowed to drink in their home cage for one hour. This procedure was repeated for three consecutive days. On the fourth through the seventh days, each rat was allowed one hour to drink from the tube in the operant conditioning chamber. On


400
300
200
100
$\bar{X}$ NUMBER PUNISHED LICKS
DIAZEPAM
CANNABIDIOL
TWEEN-
SALINE
1.25
12.50
2.50
25.00
5.00 DZP
50.00 CBD
DOSE

the eighth day, each rat was placed in an operant conditioning chamber 20 min after the injection of CBD, DZP, or the Tween-80/saline control solution. The rat was allowed 1 min in which to freely drink water, during which time the number of licks was recorded using a Lehigh Valley lickometer circuit. From the end of the first minute for 9 additional minutes, the water spout was electrified with a mild shock (Grason-Stadler shock source 0.016 mA between the water spout and the grid floor). During this time, a lick was punished by electrical shock to the tongue of the rat. During this time, the number of punished licks (licks associated with shock) was recorded.

4. Data analysis. Data (number of punished licks) were analyzed by analysis of variance for each drug group.

B. Results

Figure 5 shows the results. Both CBD and DZP produce dose-related increases in the number of licks during the period in which drinking was punished (CBD: $F = 4.80$, $df = 3,37$, $p < 0.006$; and DZP: $F = 3.76$, $df = 3,39$, $p < 0.019$).

V. EXPERIMENT IV: Effects on CBD, THC and DZP on Stress Induced Ulcers in Mice.

The benzodiazapine chlordiazepoxide has been demonstrated to protect the rat against restraint induced ulcers (29). Glavin has presented evidence that experimentally induced ulcers is a valuable model for examining the relationship between stress and peptic ulcer disorder (30). Thus, the present experiments were designed to test the effects of CBD, THC, and DZP on the development of ulcers. In this experiment, we tested the effects of CBD and THC on ulcer development in mice.

FIGURE 5. Mean number of punished lick responses during the shock phase of a lick suppression test, plotted as a function of control solution (Tween-80 + saline) and doses of diazepam (DZP, squares) and cannabidiol (CBN, triangles) as indicated on the abscissa.

1. Subjects. Subjects were 70 male, CD-1 mice bred in our own colony at the University of Vermont from breeding stock obtained from Charles River Laboratories.

2. Drugs. Ten mice were tested in each of the following drug conditions: Tween-80 (0.6%) in saline, control injection; 2, 4, and 8 mg/kg THC; and 20, 40, or 80 mg/kg CBD. All drugs were mixed daily and administered in constant volume injections of 1.0 ml/100 g of body weight.

3. Procedure. In this paradigm, mice were administered the appropriate drug of control injection and placed in cylindrical chambers made of metal mesh 2 cm in diameter and 10 cm long and which were mounted on a board. An electrode was attached to the tail of the mouse after the technique of Weiss (31) and shocks of 0.24 mA were delivered to the feet of the mouse using a Grason-Stadler shock source. Shocks were automatically programmed to be delivered on a Variable-Interval, 60-second schedule for a period of 18 h. After this procedure, each mouse was returned to its home cage for 6 h without food and water being available. After this, each mouse was sacrificed by ether anesthesia and the stomach was removed, washed, and examined under a dissecting microscope for ulceration. Each ulcer was counted, the preparation photographed and later an independent observer recounted the ulcers to check the original observations.

B. Results

Figure 6 shows the results for this experiment. Both THC and CBD produced significant decreases in the frequency of ulcers (Chi square test, $p < 0.01$ for each drug).

VI. DISCUSSION

In the present series of experiments, we have demonstrated that cannabidiol: 1) reduces tremor and seizure activity associated with withdrawal from alcohol addiction, 2) facilitates the acquisition of an active avoidance behavior, 3) reduces the suppression of a punished response, and 4) reduces the development of stress induced ulcers. Several details about the comparative efficacy of cannabidiol require further discussion. First, in all of the tests used, CBD produced effects which were of equal or greater magnitude than various comparison drugs. In the alcohol withdrawal experiment, CBD and THC suppressed body tremor equally at the highest dose of

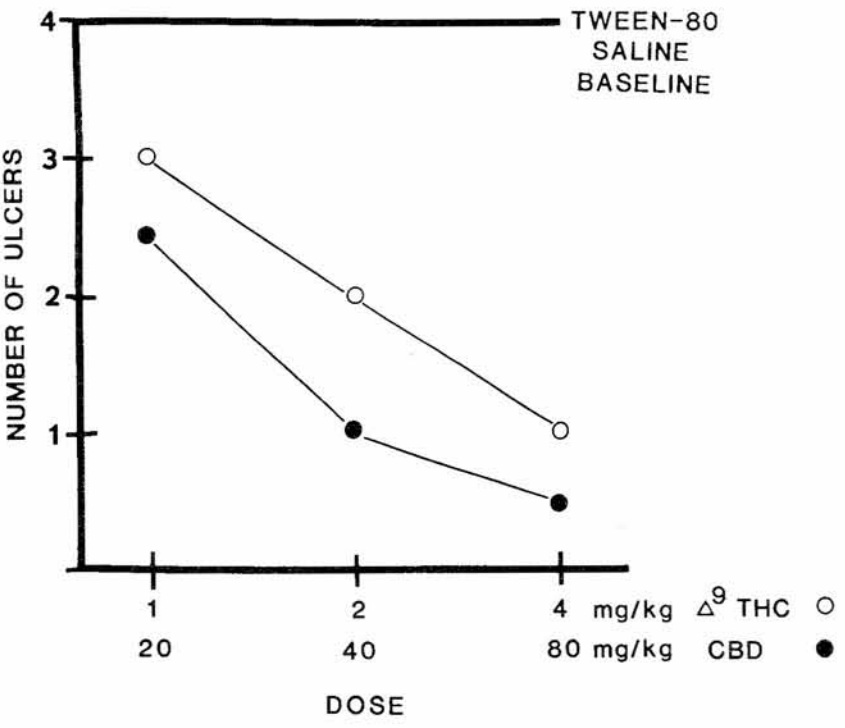


FIGURE 6. Mean number of ulcers which developed, plotted as a function of THC and CBD as shown on the abscissa. Mean number of ulcers in the control-injected group (Tween-80 + saline) is shown as a solid black line at the top of the plot.

each of the two drugs, and clonidine, the comparison drug, was also effective in reducing body tremor. With regard to seizures, drugs appeared to have seizure suppressing qualities and all were approximately equal in their efficacy in reducing the seizures, but dose response effects were not entirely clear. With regard to acquisition of active avoidance in the running wheel, CBD, THC, and DZP produced improved avoidance learning. Again, peak effects of each drug were not statistically different from each other. Similarly, in the lick-suppression test, both CBD and DZP produced a lack of response suppression and peak effects were not statistically different between the two drugs tested. Finally, both CBD and THC produced significant reductions in ulcerogenesis and were not different in the degree to which each drug reduced ulcers.

The consistency of these data strongly suggest that CBD can produce changes in anxiety-like responses which are at

least equal to the changes seen with the various comparison drugs. Secondly, CBD is not as potent as either THC, clonidine, or DZP in the various experiments. These CBD doses are similar to those which produce measurable effects in various behavioral pharmacological tests of CBD, e.g., suppression of electroshock induced seizures (6,32). Thirdly, CBD seems to produce dose response effects in all of the tests used suggesting that the observed effects are not attributed to nonspecific effects of CBD. Thus, it is safe to conclude that CBD produces pharmacological effects which are similar to drugs that have been established as having anxiolytic properties in both animals and man, e.g., clonidine (17-19) and DZP (33-35).

A short analysis of the behavioral paradigms used in this series of studies on CBD is appropriate. There are no direct tests of human anxiety in animal behavioral pharmacology because there are both cognitive and affective components to the anxiety state in the human. The anxiety state in humans, however, is often related to a recognizable different state, that of fear. Thus behavioral pharmacologists have often used fear conditioning or performance (avoidance conditioning or performance and the conditioned emotional response) as models to study fear states in animals and as tests oftranquillizers (36). In the experiments reported here, the running wheel avoidance task is a learning task in which the animal is learning to run to avoid shock. The benzodiazepine diazepam clearly facilitates the learning, presumably due to the anxiolytic properties of the drug, which presumably reduce arousal in the fear or anxiety situation. In the conditioned suppression paradigm, punishment (e.g., shock) is used to suppress an ongoing response (e.g., licking for water). Again diazepam seems to act via anxiolytic properties, as demonstrated by the fact that response suppression does not occur under the influence of the drug. In the alcohol withdrawal syndrome in man, anxiety and agitation are highly correlated symptoms. The remarkable calming effect of clonidine on agitation and anxiety in human alcoholics withdrawing from alcohol suggested that an animal model using body tremor (a component of motor agitation) and seizures might reveal anxiolytic properties of drugs. At least partial support for this approach was found with clonidine. Finally, we employed a stress-induced model in animals since stress-ulcers seem to reflect a symptom that is a well known consequence of stress and anxiety. Taken together, these four very divergent tests bear a logical relationship to anxiety states at both the human-behavioral and physiological level as a complex of multifactored consequences of anxiety-like effects. This approach seems to provide a valuable composite analysis of the anxiolytic properties of drugs.

Thus, we conclude that these behavioral pharmacological approaches provide a reasonable basis on which to posit anxiolytic properties of cannabidiol. Further research on cannabidiol analogs may reveal more potent or effective compounds which might later be tested in humans.

REFERENCES

1. Carlini, E. A., Lette, J. R., Tannhauser, M., and Berardi, A. C., Cannabidiol and cannabis sativa extract protect mice and rats against convulsive agents, Pharm. Pharmacol. 25:664-665 (1973).
2. Izuierdo, I., and Tannhauser, M., The effect of cannabidiol on maximal electroshock seizure in rats, J. Pharm. Pharmacol. 25: 916-917 (1973).
3. Cunha, J. M., Carlini, E. A., and Pereira, A. E., Chronic administration of cannabidiol to healthy volunteers and epileptic patients, Pharmacol. 21:175-185 (1980).
4. Carlini, E. A., Mechoulam, R., and Lander, N., Anticonvulsant activity of four oxygenated cannabidiol derivatives, Res. Comm. Chem. Pathol. Pharmacol. 12(1):1-15 (1975).
5. Musty, R. E., and Sands, R., Effects of marijuana extract distillate and cannabidiol on variable interval performance as a function of food deprivation, Pharmacol. 16:199-205 (1978).
6. Karniol, I. G., and Carlini, E. A., Pharmacological interaction between cannabidiol and delta-9-tetrahydrocannabinol, Psychopharmacol. (Berl.) 33:53-70 (1973).
7. Lemberger, L., Dalton, B., Martz, R., Rodda, B., and Forney, R., Clinical studies on the interaction of psychopharmacologic agents with marijuana, Ann. Acad. Sci. 281:219-228 (1976).
8. Hollister, L. E., Cannabidiol and cannabinol in man, Experientia 29:825-826 (1973).
9. Zuardi, A. W., Finkelfarb, E., Bunco, O. F. A., Musty, R. E., and Karniol, I. G., Cannabidiol effects on discriminative responses between delta-9-tetrahydrocannabinol and control solution in rats, Arch. Int. Pharmacodyn. Ther. 249:137-146 (1981).
10. Bhargave, H. N. Potential therapeutic applications of naturally occurring synthetic cannabinoids, Gen. Pharmacol. 9:195-213 (1978).
11. Mechoulam, R., and Carlini, E. A., Toward drugs derived from cannabis, Naturwissenschaften 65:174-179 (1978).
12. Redmond, 1977.

13. Chen, Y. H., and Chan, S. H. H., Clonidine-induced hypothesion and bradycardia in cats: The role of medial medullary reticular alpha-adrenoceptors and vagus nerve, Neurosci. Abstr. 4:18 (1978).
14. Chen, Y. H., and Chan, S. H. H., The involvement of medial medullary reticular formation in cardiovascular effects of clonidine in experimentally-induced hypertensive cats, Neurosci. Abstr. 5:39 (1979).
15. Reid, J. L., Clonidine and central noradrenaline turnover. In "Central Action of Drugs in Blood Pressure Regulation" (D. S. Davies and J. L. Reid, eds.), pp. 194-204. Pitman Medical, Tunbridge Wells, 1975.
16. Connolly, M. E., Clonidine in the treatment of hypertension, in :Central Action of Drugs in Blood Pressure Regulation" (D. S. Davies and J. L. Reid, eds.), pp. 268-276. Pitman Medical, Tunbridge Wells, 1975.
17. Tseng, L. Fu, Loh, H. H., and We, E. T., Effects of clonidine on morphine withdrawal signs in the rat, Eur. J. Pharmacol, 30:93-99 (1975).
18. Lipman, J. J., and Spencer, P. S. J., Clonidine and opiate withdrawal, The Lancet 2:521 (1978).
19. Gold, M. S., Redmond, Jr., D. E., Kleber, H. D., Clonidine blocks acute opiate-withdrawal symptoms, The Lancet, 599-602 (1978).
20. Bjorkquist, S. E., Clonidine in alcohol withdrawal, Acta. Psych. Scand. 52:256-263 (1975).
21. Freund, C., Alcohol withdrawal syndrome in mice, Arch. Neur. 21:315-320 (1969).
22. Freund, G., Animal models of ethanol withdrawal syndromes and their relevance to pharmacology, in "Biological and Behavioural Approaches to Drug Dependence" (H. D. Cappell and A. E. LeBlanc, eds.), pp. 13-25. Alcoholism and Drug Addiction Research Foundation of Ontario, Toronto, Canada, 1975.
23. Musty, R. E., Homeostatic drives and consummatory behavior: Hunger and thirst, in "Motivation, an Experimental Approach" (E. D. Ferguson, ed.). Holt, Rinehart and Winston, New York, 1976.
24. Winer, B. J., "Statistical Principles in Experimental Design." McGraw Hill, New York, 1971.
25. Heise, G. A., and Boff, E., Continuous avoidance as a base-line for measuring behavioral effects of drugs, Psychopharmacol. 3:264-282 (1962).
26. Pandina, R. J., and Musty, R. E., Effects of delta-9-tetrahydrocannabinol on active avoidance acquisition and passive avoidance retention in rats with amygdaloid lesions, Pharmacol. 13:297-308 (1974).
27. Geller, I., and Seifter, J., The effects of meprobamate, barbiturates, d-amphetamine and promazine on experimen-

tally induced conflict in the rat, Psychopharmacol. 1:482-492 (1960).
28. Stein, L. , Wise, C. D., and Berger, B. D., Antianxiety action of benzodiazepines: Decrease in activity of serotonin neurons in the punishment system, in "The Benzodiazepines" (S. Garattini, E. Mussini, and L. O. Randall, eds.), pp. 299-326. Raven Press, New York, 1973.
29. Hoat, J. B., Djahanguiri, Richelle, et M., Action protectrice du chloridazepoxide sur l'ulcere de contrainte chex le rat, Arch. int. Pharmacodyn. 148:557-559 (1964).
30. Glavin, G., Restraint ulcer: History, current research and future implications, Brain Res. Bull. 5:51-58 (1980).
31. Weiss, J. M., Effects of coping behavior in different warning signal conditions on stress pathology in rats, J. Comp. Physiol. Psychol. 77:1-13 (1971).
32. Consroe, P. F., and Wolkin, A. L., Cannabidiol-antiepileptic drugs. Comparisons and interactions in experimentally induced seizures in rats, J. Pharmacol. Exp. Ther. 201:26-32 (1977).
33. Miczek, K. A., Effects of scopolamine, amphetamine and benzodiazepines on punishment, Psychopharmacologia 28:373-389 (1973).
34. Miczek, K. A., Effects of scopolamine, amphetamine and benzodiazepines on conditioned suppression, Pharmacol. Biochem. Behav. 1:401-411 (1973).
35. Rickels, K., Benzodiazepines: Clinical use patterns, in "Benzodiazepines: A Review of Research Results, 1980" (S. Szara and J. P. Ludford, eds.), pp. 43-60. NIDA Research Monograph 33, DHHA Publication Number (ADM) 81-1052, Rockville, MD, 1981.