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Conditioned Taste Aversions Produced by Active and Inactive Cannabinoids[1,2]

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CORCORAN, M. E., I. BOLOTOW, Z. AMIT AND J. A. MCCAUGHRAN, JR. *Conditioned taste aversions produced by active and inactive cannabinoids.* PHARMAC. BIOCHEM. BEHAV. 2(6) 725–728, 1974. – Single intraperitoneal injections of subtoxic doses of Δ^8-tetrahydrocannabinol (THC), Δ^9-THC, cannabidiol (CBD), or cannabigerol (CBG) induced a conditioned aversion to a saccharin solution in rats, while only a behaviorally toxic dose of cannabichromene (CBC) was capable of motivating an aversion. In view of evidence suggesting that the taste aversions were not due to local irritation effects, we conclude that CBD and CBG are pharmacologically active in rats, and could be responsible for some of the behavioral effects of cannabis in animal studies.

Cannabis Δ^8-THC Δ^9-THC Cannabidiol Cannabigerol Conditioned taste aversion Cannabichromene

BASED on the observation that Δ^8-tetrahydrocannabinol (THC) and Δ^9-THC but not other cannabinoid drugs examined produced detectable effects in the ongoing behavior (e.g., active avoidance, locomotor activity) of several infrahuman species [8,22], Mechoulam [20] concluded that the two isomers of THC are the principal psychoactive constituents of cannabis, and that the other cannabinoids (e.g., cannabinol, cannabidiol, cannabigerol, cannabichromene) are behaviorally inactive (see also [21]). Yet evidence from other laboratories suggests that some of the inactive cannabinoids may produce behavioral effects in animals. For example, pretreatment of mice with cannabinol can reduce the potentiation of barbital-induced sleep produced by Δ^9-THC [16]. Cannabidiol (CBD) and cannabinol both produce marked behavioral effects when injected intracerebrally in mice [4]. Peripherally-administered CBD can alter the behavior of rats in the open-field situation and can potentiate some behavioral effects of Δ^9-THC and block others [15], perhaps due to its effects on metabolism of Δ^9-THC [13]. Cannabinol and especially CBD exert antiepileptic effects in animals [11, 12, 14], and CBD has been shown to disrupt acquisition of an avoidance response as well as to affect certain neurophysiological events in rats [10].

The present experiment was intended to provide further information about the behavioral effects of active and inactive cannabinoids. We examined the activity of Δ^8-THC, Δ^9-THC, CBD, cannabigerol (CBG), and cannabichromene (CBC) in the conditioned taste aversion paradigm (CTA). In the CTA situation rats are able to demonstrate their ability to associate novel gustatory cues with noxious internal states produced by known emetic agents such as lithium chloride, x-irradiation, or apomorphine (reviewed in [7]). Drug-induced aversive or punishing effects are indicated by a learned avoidance of the taste paired with the drug when the subject is examined in a retest session subse-

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quent to the original conditioning session. Recent experiments indicate that taste aversions can be produced in pharmacologically-naive rats by a variety of drugs, including some that are self-administered at similar doses in different situations. These drugs include d-amphetamine [2], methamphetamine [18], morphine (e.g. [3]), and ethanol [3]. A number of other psychoactive drugs widely used in behavioral research can produce CTAs: e.g., scopolamine [1], parachlorophenylalanine [23], and, of special relevance to the present study, Δ^9-THC [6] and hashish extract [5]. Thus the CTA paradigm is a sensitive measure of the punishing effects that seem to be common to a large number of psychoactive drugs, and it seemed reasonable to us to use the CTA to determine whether such effects are produced by cannabinoids other than Δ^9-THC.

METHOD

Animals

Two hundred twenty-eight male Wistar rats 200–300 g were used. They were housed individually and had free access to food and water except where noted otherwise. The technique used to study the ability of the cannabinoids to produce a CTA was similar to that described by Nachman *et al.* [23] and Corcoran [5]. Three days after arriving in the laboratory the rats were placed on a 23-hr and 50-min water deprivation schedule. A single bottle of tap water was available to each rat in the home cage for 10 min each day. The baseline intake of fluids stabilized by Day 8, at which time the rats were randomly assigned to experimental or control groups. A 0.1% (w/v) sodium saccharin solution was available instead of water for the usual 10-min drinking period on Day 9. Within 1 min of the end of the drinking period each rat received an intraperitoneal injection of one of the cannabinoids or of the propylene glycol-ethanol vehicle. In order to verify the efficacy of our CTA procedure in case negative results were obtained with the cannabinoids, we decided to test some rats with one of the well established emetic agents. An additional group of rats therefore received an i.p. injection of lithium chloride. The food and fluid intake of some rats receiving cannabinoid injections was depressed for several days after the injection; so retest with saccharin did not occur until the fifth session postinjection, by which time the food and fluid intake of all rats had recovered to the preinjection baseline as verified by *t*-tests for related samples. The strength of any aversion obtained was calculated as percentage change from baseline according to the following formula:

$$\frac{\text{retest day intake minus injection day intake}}{\text{injection day intake}} \times 100$$

Groups of 12 rats each received a single dose of a particular cannabinoid: Delta^8-THC and Δ^9-THC were tested at doses of 1, 5 and 10 mg/kg; CBD and CBG at doses of 1, 5, 10, and 30 mg/kg; and CBC at 10 and 30 mg/kg. All cannabinoids were injected at a constant volume of 1 ml/kg regardless of concentration. Control groups of 12 rats each received the vehicle (19 parts propylene glycol to 1 part 95% ethanol) at a volume of 1 ml/kg, 0.9% saline at 1 ml/kg, or 0.15 M lithium chloride at 20 ml/kg.

Drugs

The Department of National Health and Welfare of Canada supplied the cannabinoids. Delta^9-THC was received dissolved in 100% ethanol and was reported to be 95% pure, while Δ^8-THC was dissolved in 95% ethanol and was reported to be 95% pure. The stock solution of each THC isomer was added to the vehicle of propylene glycol-ethanol (PG-E) to produce solutions of the desired concentration. CBC was received dissolved in CCl_4; the CCl_4 was evaporated in a vacuum and the cannabichromene redissolved in the PG-E vehicle. CBD and CBG, which were received in crystalline form, were also dissolved in the vehicle.

RESULTS

As expected, the 2 control solutions (saline and PG-E) failed to produce a CTA. Upon retest the rats treated with these solutions increased their intake of saccharin slightly above baseline levels, although the increase was not significant. The injection of lithium chloride produced a strong CTA, also as expected, with saccharin intake decreasing to a mean of 22% of baseline (Wilcoxon matched pairs test, $p<0.005$).

As can be seen from Fig. 1, all the cannabinoids were capable of producing a CTA. However, their efficacy in producing a CTA varied considerably. At 1 mg/kg only Δ^8-THC and Δ^9-THC produced significant deviations from baseline (Wilcoxon test, $p<0.005$ and $p<0.025$, respectively). It is interesting that Δ^8-THC produced a stronger CTA than Δ^9-THC at this dose (Mann-Whitney U, $p<0.01$). All the cannabinoids except CBC produced a significant CTA at the 5 mg/kg dose (Δ^8-THC, Δ^9-THC, CBG, and CBD: each $p<0.005$), and all but CBC were effective at the 10 mg/kg dose. Since Δ^8-THC and Δ^9-THC were effective at all 3 dose levels, we decided to terminate further testing of these 2 cannabinoids, and to compare only the other 3 inactive cannabinoids at a dose of 30 mg/kg. All 3 compounds produced a significant CTA at this level (each $p<0.005$).

General Observations

Although no formal attempt was made to quantify the gross behavioral effects of the drugs, the rats' behavior was observed after the injections and some general observations can be reported. In agreement with our previous findings [19], there were no obvious general behavioral effects produced by the 1 mg/kg doses of Δ^8-THC and Δ^9-THC. However, signs of behavioral toxicity, as defined in the commonly used neurotoxicity battery developed by Swinyard and colleagues [24], appeared at the 2 higher doses of the THC isomers, including: abnormal locomotion; catalepsy; hypoactivity; urination, defecation, and vocalization in response to handling; and hyperreactivity to noise or movement. No overt toxic symptoms appeared when CBD, CBG, or CBC were administered in doses of 10 mg/kg or below. At the 30 mg/kg dose of the three cannabinoids, however, several symptoms of toxicity were observed, including hypoactivity, abnormal stance, and hyperreactivity to noise or movement.

DISCUSSION

The present experiment demonstrates that three inactive cannabinoids, as well as the two active isomers of THC, produce detectable effects in the conditioned taste aversion paradigm, which suggests that the inactive cannabinoids do

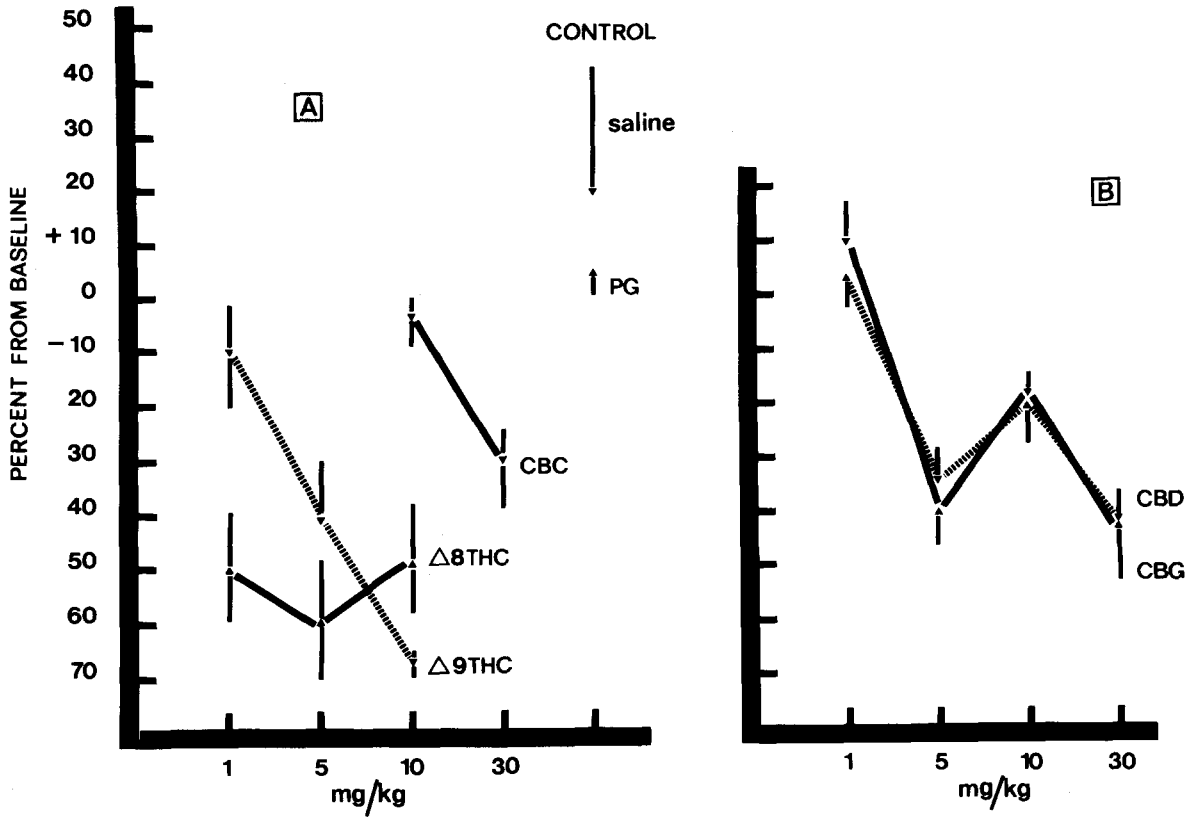

FIG. 1. Dose-response relations of 5 cannabinoids and controls in the conditioned taste aversion paradigm. The response measure is expressed as mean percentage change from baseline intake of the saccharin solution paired with drug injection. S.E.M.s are represented by the vertical bars. Figure 1A: Δ^8-THC, Δ^9-THC, cannabichromene (CBC), saline control, and propylene glycol-ethanol control (PG). Figure 1B: cannabidiol (CBD) and cannabigerol (CBG).

in fact produce pharmacological effects. Before this conclusion can be accepted, however, at least one alternative explanation of these results must be considered. It is possible that the findings are due solely to painful tissue irritation localized to the peritoneal cavity, the site of drug injection (e.g. [17]). According to this hypothesis, the rats learned to avoid saccharin because they associated its taste with painful local tissue irritation produced by the cannabinoid injections. In contrast to this hypothesis we think it likely that the cannabinoid-induced taste aversions are due to pharmacological effects of the drugs. There are a number of arguments in support of this conclusion: First, Elsmore and Fletcher [7] reported that Δ^9-THC produced significant aversions when injected intragastrically, as well as when injected intraperitoneally, suggesting that local irritation due to the somewhat acidic pH of cannabinoid solutions cannot account for the ability of these drugs to produce CTA. Second, Corcoran [5] reported that pretreatment with SKF 525-A, an hepatic enzyme inhibitor, reduced the strength of a CTA produced by an i.p. injection of hashish extract. It is difficult to see how an hepatic-active compound like SKF 525-A could affect the strength of a CTA if the aversion were caused only by local peritoneal irritation at the site of the injection. Third, we have found that a strong CTA can be produced by injecting a small quantity (15 μg) of Δ^9-THC bilaterally into the dorsal hippocampus of rats, whereas a larger dose injected into the cerebral ventricles has no effect (Bolotow, Amit, and Corcoran, in preparation). This is strong evidence that, at least in the case of Δ^9-THC, a CTA can be produced by the central pharmacological properties of the drug.

Given that the aversions resulted from the pharmacological properties of the cannabinoids, the significance of the finding is not immediately obvious. As Cappell and Le Blanc [2] have pointed out, it would not be surprising if a CTA were obtained with a high, toxic dose of a psychoactive drug. Although toxic behavioral manifestations were observed at the highest doses of each cannabinoid, toxicity alone cannot explain the present results, since significant aversions were also obtained at apparently nontoxic doses of all drugs but CBC (Δ^8-THC and Δ^9-THC: 1 mg/kg; CBD and CBG: 5 and 10 mg/kg). Further examination of the dose-response relations is necessary to determine whether evidence of gross behavioral toxicity necessarily accompanies the CTA produced by any dose of CBC, but our results taken at face value suggest that subtoxic doses of CBC have little pharmacological activity in this test. In order to explain these results, therefore, we suggest that the effective drugs produce a discriminable pharmacological state which the animals associate with the taste of saccharin; since this state is aversive or punishing, the rats subsequently avoid saccharin. Consistent with this idea, Δ^8-THC and Δ^9-THC can acquire discriminative control over behavior (e.g., [10]). It is not known whether CBD

and CBG can also acquire discriminative control in nontoxic doses, but the present results suggest that they might. Although the locus (peripheral vs. central) or the mechanism of these punishing drug effects cannot be specified, the fact that they occur with CBD and CBG as well as with the THC isomers indicates that the former cannabinoids are not behaviorally inactive, a point which is supported by other recent work with CBD [10, 11, 12, 14, 15]. Thus the possibility remains open that some behavioral effects of cannabis in animal studies may be due to activity of cannabinoids other than or in addition to THC.

REFERENCES

1. Berger, B. Conditioning of food aversions by injections of psychoactive drugs. *J. comp. physiol. Psychol.* **81:** 21–26, 1972.
2. Cappell, H. and A. E. Le Blanc. Punishment of saccharin drinking by amphetamine in rats and its reversal by chlordiazepoxide. *J. comp. physiol. Psychol.* **85:** 97–104, 1973.
3. Cappell, H., A. E. Le Blanc and L. Endrenyi. Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol, and chlordiazepoxide. *Psychopharmacologia* **29:** 239–246, 1973.
4. Christensen, H. D., R. I. Freudenthal, J. T. Gidley, R. Rosenfeld, G. Boegli, L. Testino, D. R. Brine, C. G. Pitt and M. E. Wall. Activity of delta 8- and delta 9-tetrahydrocannabinol and related compounds in the mouse. *Science* **172:** 165–167, 1971.
5. Corcoran, M. E. Role of drug novelty and metabolism in the aversive effects of hashish injections in rats. *Life Sci.* **12:** 63–72, 1973.
6. Elsmore, T. F. and G. Fletcher. Delta 9-tetrahydrocannabinol: aversive effects in rat at high doses. *Science* **175:** 911–912, 1972.
7. Garcia, J. and F. R. Ervin. Gustatory-visceral and telereceptor-cutaneous conditioning – adaptation in internal and external milieus. *Communs. Behav. Biol.* **1:** 389–415, 1968.
8. Grunfeld, Y. and H. Edery. Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. *Psychopharmacologia* **14:** 200–210, 1969.
9. Henriksson, B. G. and T. Jarbe. The effect of two tetrahydrocannabinols, (Δ^9-THC and Δ^8-THC) on conditioned avoidance learning in rats and its transfer to normal state conditions. *Psychopharmacologia* **22:** 23–30, 1971.
10. Izquierdo, I. and A. G. Nasello. Effects of cannabidiol and diphenylhydantoin on the hippocampus and on learning. *Psychopharmacologia* **31:** 167–175, 1973.
11. Izquierdo, I., O. A. Orsingher and A. C. Berardi. Effect of Cannabidiol and of other Cannabis sativa compounds on hippocampal seizures. *Psychopharmacologia* **28:** 59–102, 1973.
12. Izquierdo, I. and Tannhauser, M. The effect of cannabidiol on maximal electroshock seizures in rats. *J. Pharm. Pharmac.* **25:** 916–917, 1973.
13. Jones, G. and R. G. Pertwee. A metabolic interaction *in vivo* between cannabidiol and Δ^1-tetrahydrocannabinol. *Br. J. Pharmac.* **45:** 375–377, 1972.
14. Karler, R., W. Cely and S. A. Turkanis. The anticonvulsant activity of cannabidiol and cannabinol. *Life Sci.* **13:** 1527–1531, 1973.
15. Karniol, I. G. and E. A. Carlini. Pharmacological interaction between cannabidiol and Δ^9-tetrahydrocannabinol. *Psychopharmacologia* **33:** 53–70, 1973.
16. Krantz, J. C., H. J. Berger and B. L. Welch. Blockade of (-)-trans-Δ^9-tetrahydrocannabinol depressant effect by cannabinol in mice. *Am. J. Pharm.* **143:** 149–152, 1971.
17. Manning, F. J., J. H. McDonough, Jr., T. F. Elsmore, C. Saller and F. J. Sodetz. Inhibition of normal growth by chronic administration of Δ^9-tetrahydrocannabinol. *Science* **174:** 424–426, 1971.
18. Martin, J. C. and E. H. Ellinwood, Jr. Conditioned aversion to a preferred solution following methamphetamine injections. *Psychopharmacologia* **29:** 253–261, 1973.
19. McCaughran, Jr., J. A., M. E. Corcoran and J. A. Wada. Anticonvulsant activity of delta 8- and delta 9-tetrahydrocannabinol in rats. *Pharmac. Biochem. Behav.* **2:** 227–233, 1974.
20. Mechoulam, R. Marihuana chemistry. *Science* **168:** 1159–1166, 1970.
21. Mechoulam, R. and H. Edery. Structure-activity relationships in the cannabinoid series. In: *Marijuana. Chemistry, Pharmacology, Metabolism, and Clinical Effects,* edited by R. Mechoulam, New York: Academic Press, 1972, pp. 101–136.
22. Mechoulam, R., A. Shani, H. Edery and Y. Grunfeld. Chemical basis of hashish activity. *Science* **169:** 611–612, 1970.
23. Nachman, M., D. Lester and J. LeMagnen. Alcohol aversion in the rat: behavioral assessment of noxious drug effects. *Science* **168:** 1244–1246, 1970.
24. Swinyard, E. A., W. C. Brown and L. S. Goodman. Comparative assays of antiepileptic drugs in mice and rats. *J. Pharmac. exp. Ther.* **106:** 319–330, 1952.