Cannabidiol, a non-psychoactive component of cannabis and its synthetic dimethylheptyl homolog suppress nausea in an experimental model with rats

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Rats display conditioned rejection reactions during an oral infusion of a flavor previously paired with an emetic drug; considerable evidence indicates that these rejection reactions reflect nausea. Here we report that cannabidiol, a major non-psychoactive cannabinoid found in marijuana and its synthetic dimethylheptyl homolog interfere with nausea elicited by lithium chloride and with conditioned nausea elicited by a flavor paired with lithium chloride. These results suggest that cannabinoids without psychoactive side-effects may have therapeutic value in the treatment of chemotherapy-induced nausea. *NeuroReport* 13:1–4 © 2002 Lippincott Williams & Wilkins.

Key words: Cannabidiol; Cannabinoid; Emesis; Marijuana; Nausea; Taste avoidance; Taste reactivity

INTRODUCTION

The therapeutic potential of cannabinoids (CB) in the treatment of nausea resulting from chemotherapy has been the subject of considerable interest. Anecdotal accounts and early clinical trials indicate that marijuana reduces nausea in such treatment [1]. Marijuana contains ~80 cannabinoids [2], including the psychoactive component, Δ^9-tetrahydrocannabinol (THC) [3]. Nabilone (a synthetic THC) eliminates vomiting in cats [4] and THC eliminates vomiting in shrews [5]. These effects appear to be mediated by action at CB_1 receptors, because the CB_1 antagonist, SR-141716, blocks the antiemetic action of THC [5] and the CB agonist, WIN 55, 212-2 [6].

Both THC (generic name Dronabinol) and nabilone are approved anti-nausea drugs in human patients, but many users claim that marijuana is a better suppressor of nausea than THC [7]. Another major cannabinoid found in marijuana is cannabidiol (CBD); however, unlike THC, CBD does not produce psychoactive effects [8]. CBD, unlike THC, does not bind to cannabinoid receptors; it may act by blocking the reuptake of anandamide [9], an endogenous cannabinoid. In rats, CBD is a highly effective anti-inflammatory agent [10] as well as a neuroprotective antioxidant for the treatment of neurological disorders such as cerebral ischemia [11]. Here we evaluate the potential of CBD and its dimethylheptyl homolog (CBD-DMH) to interfere with nausea in rats.

Conditioned rejection reactions in the taste reactivity test reflect nausea in the rat [12–17]. These reactions are exclusively elicited by treatments that produce emesis in animals that are capable of vomiting [12–15]. Furthermore, anti-emetic drugs, such as the 5-HT_3 receptor antagonist ondansetron [17] and THC [18] prevent the establishment of conditioned rejection reactions in rats. Ondansetron [17] and THC [18] also suppress the expression of previously established conditioned rejection reactions when administered in a test of conditioned responding. In the present study, we demonstrate that CBD and its synthetic dimethylheptyl homolog attenuate unconditioned and conditioned nausea as measured by conditioned rejection reactions in the rat.

MATERIALS AND METHODS

Subjects: The subjects in Experiment 1 were 29 and in Experiment 2 were 24 male Sprague–Dawley rats (Charles River Labs, St. Constant, Quebec), which weighed 290–350 g on the conditioning day. They were individually housed in stainless steel hanging cages in a colony room kept at 21°C on a 12:12 h light:dark schedule with the lights on at 07.00 h. Throughout the experiment, the rats were maintained on *ad lib* Purina rat chow and water. The procedures were approved by the Wilfrid Laurier University Animal Care

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Committee according to the guidelines of the Canadian Council on Animal Care.

Surgery: The rats were surgically implanted with intra-oral cannulae as described by Parker [19]. The surgical anesthesia preparation included administration of 0.4 mg/kg atropine solution i.p. 15 min prior to ketamine (75 mg/kg, i.p.) combined with xylazine (10 mg/kg, i.p.) which was dissolved in sterile water and administered at a volume of 1 ml/kg. On each of three subsequent days during recovery from surgery, the cannulae were flushed with a chlorhexidine rinse (Novlosan; 0.1% chlorhexidine) to prevent infection.

Design: The design of the experiments evaluated the effect of CBD (Experiment 1) and CBD-DMH (Experiment 2) on the establishment of conditioned rejection reactions, on the expression of conditioned rejection reactions during testing and the potential role of state dependent learning decrements in responding. The rats were randomly assigned to independent groups on the basis of the pretreatment drug and the conditioning drug. In Experiment 1, the groups were as follows: CBD–lithium ($n = 8$), CBD–saline ($n = 6$), vehicle–lithium ($n = 8$), vehicle–saline ($n = 7$). In Experiment 2, the groups were as follows: CBD–DMH– lithium ($n = 6$), CBD–DMH–saline ($n = 6$), vehicle–lithium ($n = 6$), vehicle–saline ($n = 6$). All rats were administered two test trials, one following an injection of the drug (Experiment 1: CBD; Experiment 2: CBD–DMH) and the other following an injection of the vehicle. The order of the test trials was counterbalanced among the rats in each group.

Drugs: CBD and CBD–DMH were prepared in a mixture (2.5 mg/ml vehicle) of 1 ml alcohol/1 ml emulsifier/18 ml saline and were administered at a volume of 2 ml/kg. Lithium chloride was prepared in a 0.15 M (w/v) solution with sterile water and was administered at a volume of 20 ml/kg. All injections were administered i.p.

Procedure: One week following the surgery, the rats were adapted to the conditioning procedure. On the adaptation trial, each rat was transported into the room that contained the Plexiglass test chamber (25 × 25 × 12 cm). The room as illuminated by four 25 W light bulbs located 30 cm from either side of the chamber. Each rat was placed individually in the test chamber, and a 30 cm infusion hose was then connected to the cannula through the ceiling of the chamber. A syringe was connected to the hose and placed into the holder for the infusion pump (Model 22; Harvard Apparatus, South Natick, MA). After 60 s, the pump delivered water through the tube into the rat's mouth at the rate of 1 ml/min for 2 min. The rat was then returned to its home cage.

The conditioning trial occurred on the following day; it was identical to the adaptation trial, except that the rats were infused with 0.1% saccharin solution rather than water. Thirty minutes prior to the conditioning trial, the rats were injected i.p. with either 2 ml/kg of the drug (CBD: Experiment 1; CBD–DMH: Experiment 2) or with the vehicle in which the drug was mixed. Immediately following the infusion of saccharin solution, the rats were injected i.p. with 20 ml/kg lithium chloride or saline. During the intraoral infusion, the orofacial and somatic responses displayed by the rats were videotaped from a mirror mounted at a 45° angle beneath the test chamber. Immediately following the TR test, the rat was returned to its home cage.

The taste reactivity (TR) test trials were administered 4 and 6 days after the conditioning trial; on the day prior to the first test trial, the rats received an adaptation trial as described above. On each of two test trials, the rats were injected with either 5 mg/kg of the test drug (CBD: Experiment 1; CBD–DMH: Experiment 2) or with the vehicle, 30 min prior to receiving an infusion of saccharin solution for 2 min at the rate of 1 ml/min. The order of the tests was counterbalanced among the rats within each group. The orofacial and somatic reactions displayed by the rats were videotaped during the saccharin exposure.

In both experiments, on the day following the final TR test trial, the rats were administered a consumption test trial in a non-deprived state. On this trial, the water bottles were replaced with tubes containing the saccharin solution and the amounts consumed over a 6 h period of drinking were recorded.

Taste reactivity scoring: A rater blind to the experimental conditions scored the videotapes on two occasions in slow motion (1/5 speed) using the Observer (Noldus, NL) event-recording program on a PC computer. The frequency of the rejection reactions of gaping (rapid large amplitude opening of the mandible with retraction of the corners of the mouth), chin rubbing (mouth or chin in direct contact with the floor or wall of the chamber and body projected forward) and paw treads (sequential extension of one forelimb against the floor or wall of the chamber while the other forepaw is being retracted) were summated to provide a rejection reaction score (inter-rater reliability: Experiment 1: vehicle test $r(29) = 0.91$, CBD test $r(29) = 0.90$; Experiment 2: vehicle test $r(24) = 0.95$; CBD–DMH test $r(24) = 0.97$.

RESULTS

Taste reactivity test: Figure 1 and Fig. 2 present the mean frequency of rejection reactions displayed by the rats in the various groups during the vehicle test trial and during the drug (CBD: Experiment 1, CBD–DMH: Experiment 2) test trial. In both experiments, the pattern of responding indicates that the cannabinoid drug interfered with both the establishment of conditioned rejection and with the expression of previously established conditioned rejection reactions.

In Experiment 1 with CBD, the 2 × 2 × 2 mixed factor ANOVA revealed significant effects of pretreatment drug ($F(1,25) = 6.0$; $p = 0.022$), conditioning drug ($F(1,25) = 10.9$; $p = 0.003$), test drug ($F(1,25) = 7.4$; $p = 0.0120$), test drug × conditioning drug ($F(1,25) = 6.0$; $p = 0.021$) and a pretreatment × conditioning drug interaction that approached statistical significance ($F(1,25) = 3.6$; $p = 0.069$). Subsequent least significant difference (LSD) *post-hoc* pairwise comparison tests [20] revealed that the lithium-conditioned rats, but not the saline-conditioned rats, displayed significantly fewer conditioned rejection reactions during the CBD test trial than during the vehicle test trial

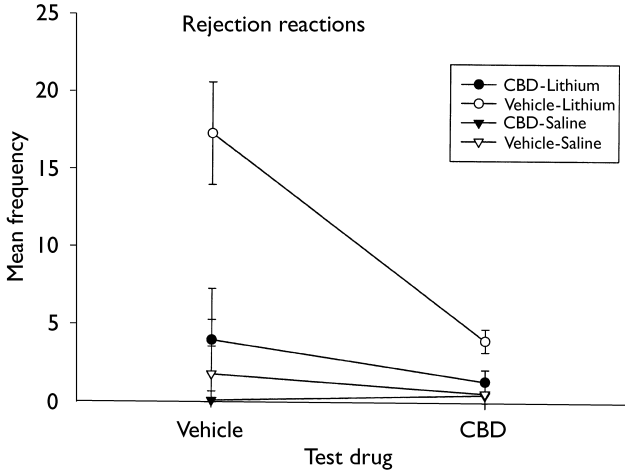

Fig. 1. Mean (± s.e.m.) frequency of conditioned rejection reactions elicited by a lithium- or saline-paired saccharin solution in Experiment 1 when rats were tested 30 min after an injection of vehicle or cannabidiol (CBD). The groups varied on the basis of the pretreatment drug (CBD or vehicle) administered 30 min prior to an intraoral infusion of saccharin solution during the conditioning trial and the conditioning drug (lithium or saline) administered following saccharin exposure.

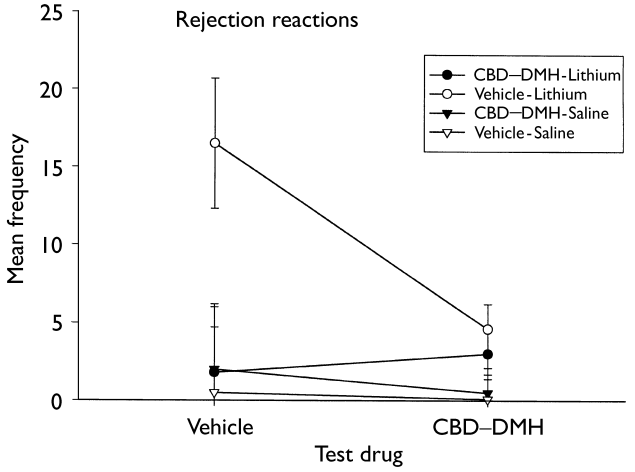

Fig. 2. Mean (± s.e.m.) frequency of conditioned rejection reactions elicited by a lithium- or saline paired saccharin solution in Experiment 2 when the pretreatment and test drug was cannabidiol dimethylheptyl (CBD–DMH).

($p < 0.05$). This indicates that CBD attenuated the expression of previously established conditioned rejection reactions. Additionally, across both test drug conditions, the lithium-conditioned rats pretreated with CBD displayed fewer rejection reactions than those pretreated with vehicle ($p < 0.05$) indicating that the CBD pretreatment during conditioning attenuated the establishment of conditioned rejection reactions, presumably by interfering with lithium-induced nausea.

In Experiment 2, with CBD–DMH, the 2 × 2 × 2 mixed factors ANOVA revealed a significant effect of test drug ($F(1,20) = 4.6$; $p = 0.044$) and a significant pretreatment drug × conditioning drug × test drug interaction ($F(1,20) = 5.6$; $p = 0.028$). Subsequent LSD *post-hoc* pair-wise comparison tests revealed that the vehicle–lithium group displayed significantly more rejection reactions during the vehicle test than any other group ($p < 0.01$) and that this group displayed more rejection reactions during the vehicle test than during the drug test ($p < 0.01$). CBD–DMH interfered with the establishment of conditioned rejection reactions when administered prior to a saccharin–lithium pairing and with the expression of these conditioning rejection reactions when administered prior to the subsequent test of conditioning.

The attenuation of lithium-induced conditioned rejection reactions during conditioning or testing cannot be interpreted as state-dependent learning decrement, because when rats were trained and tested in the same cannabinoid sate, they displayed fewer rejection reactions than when they were trained and tested in the same vehicle state.

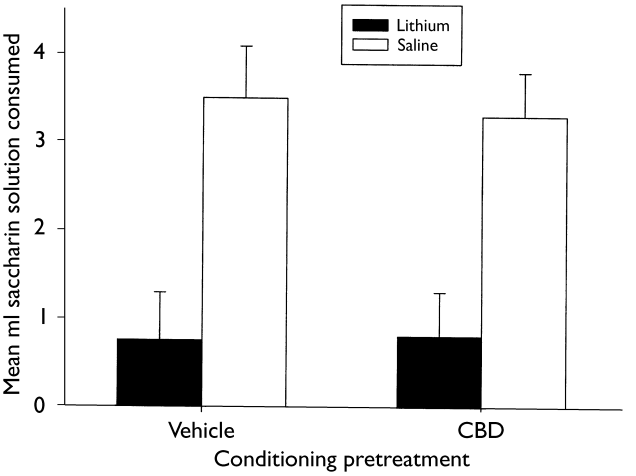

Fig. 3. Mean (± s.e.m.) volume of lithium-paired or saline-paired saccharin solution consumed during a 6 h consumption test on the day following the final TR test trial among rats pretreated with 5 mg/kg CBD or vehicle prior to the conditioning trial in Experiment 1.

Consumption test: Figure 3 and Fig. 4 show the mean volume of saccharin solution consumed by the various groups in Experiments 1 and 2 respectively. Rats suppressed their consumption of a lithium-paired saccharin solution, but pretreatment with CBD (Experiment 1) or CBD–DMH (Experiment 2) prior to conditioning did not modulate the strength of the avoidance response.

A 2 × 2 ANOVA for each experiment revealed only a significant effect of conditioning drug for Experiment 1 ($F(1,22) = 25.01$; $p < 0.001$) and a marginally significant effect of conditioning drug for Experiment 2 ($F(1,19) = 4.36$; $p = 0.051$). There were no other significant effects.

DISCUSSION

The non-psychoactive cannabinoids CBD and CBD–DMH interfered with the establishment of conditioned rejection reactions (presumably by reducing the lithium-induced nausea) and with the expression of previously established

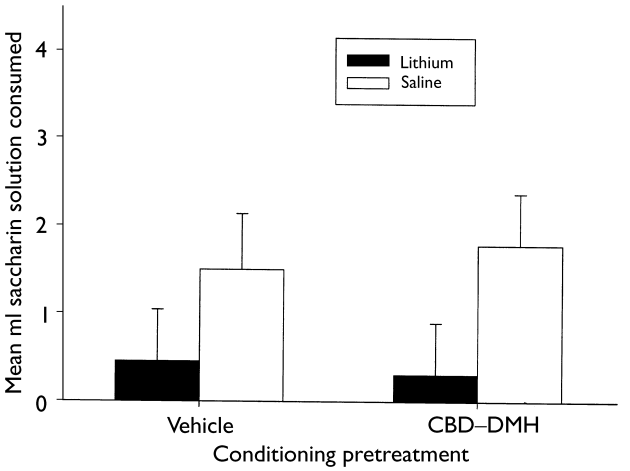

Fig. 4. Mean (± s.e.m.) volume of lithium-paired or saline-paired saccharin solution consumed during a 6 h consumption test on the day following the final TR test trial among rats pretreated with 5 mg/kg CBD–DMH or vehicle prior to the conditioning trial in Experiment 2.

conditioned rejection reactions (presumably by reducing conditioned nausea during the test). These results are the first to describe the anti-nausea properties of the naturally occurring cannabinoid, cannabidiol, found in marijuana and its dimethylheptyl homolog. We have previously reported similar effects produced by the 5HT 3 antagonist anti-emetic agent ondansetron [10] and thc [18]; that is, both agents interfered with the establishment and the expression of conditioned rejection reactions in rats.

As we have previously reported using the antiemetic agent ondansetron [17] as the pretreatment agent, CBD and CBD–DMH pretreatment did not interfere with the establishment of conditioned taste avoidance in a consumption test. Since treatments without emetic properties elicit taste avoidance, but not conditioned rejection reactions [12–16], taste avoidance does not reflect conditioned sickness. On the other hand, only treatments with emetic effects [12–16] produce conditioned rejection reactions in rats, suggesting that this affective change in taste palatability is mediated by nausea.

The anti-emetic effects of cannabinoid agonists such as THC and WIN 55-212 appear to be mediated by specific actions at the CB_1 receptor, because these effects are blocked by administration of the CB_1 receptor antagonist SR-141716. On the other hand, CBD and CBD–DMH have relatively weak affinity for the CB_1 receptor and may be act by preventing the uptake of the endogenous cannabinoid agonist anandamide [9]. Further research is necessary to determine the specific mechanism by which CBD and CBD–DMH prevent nausea in rats.

CONCLUSION

These results are the first to demonstrate that the non-psychoactive component of marijuana, cannabidiol, and its synthetic analog, cannabidiol dimethylheptyl, interfere with nausea and with conditioned nausea in rats.

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