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Aversive Effects of the Synthetic Cannabinoid CP 55,940 in Rats

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McGREGOR, I. S., C. N. ISSAKIDIS AND G. PRIOR. *Aversive effects of the synthetic cannabinoid CP 55,940 in rats.* PHARMACOL BIOCHEM BEHAV **53**(3) 657–664, 1996.—A series of experiments investigated the behavioral and hedonic effects of the synthetic cannabinoid CP 55,940 in male Wistar rats. CP 55,940 had a biphasic effect on locomotor activity, with a 10 μg/kg dose causing locomotor stimulation and a 100 μg/kg dose causing profound hypoactivity. CP 55,940 (100 μg/kg) also caused a marked hypothermia for at least 3 h following administration, while lower doses (2.5 and 10 μg/kg) had no effect. CP 55,940 (100 μg/kg) had anorexic and hyperdipsic effects for up to 24 h following administration and caused significant reductions in body weight. CP 55,940 (100 μg/kg) also caused significant avoidance to a flavoured fluid (saccharin) with which it was paired. In the conditioned place preference paradigm both the 10 μg/kg and 100 μg/kg doses of CP 55,940 produced significant place avoidance. It is concluded that CP 55,940 is aversive to rats. The possible mechanisms underlying this aversion are discussed.

Body temperature Body weight Conditioned place preference Conditioned taste aversion
Conditioned taste avoidance CP 55,940 Cannabis Cannabinoid Drug abuse Hypothermia
Ingestive behavior Rat Reinforcement Reward

THE PAST few years have seen significant advances in our understanding of the mode of action of cannabis and cannabis-like drugs on the brain and behavior. It is now clear that cannabinoids exert their effects through specific receptors, with both a central (23) and a peripheral (28) type of cannabinoid receptor having recently been discovered. An endogenous ligand for the cannabinoid receptor—termed anandamide—has been isolated from porcine brain and its chemical structure identified (8) and behavioral effects elucidated (7,34). In addition, many new synthetic cannabinoids have been developed that have high affinity at the cannabinoid receptor, and interest has been aroused in the possible therapeutic potential of such drugs (4).

One such synthetic cannabinoid is CP 55,940, which has been used to label, characterize, and localize cannabinoid receptors in the brain (15). CP 55,940 binds to the brain cannabinoid receptor with high affinity, and this binding is not altered by a large range of noncannabinoid agonists and antagonists, suggesting a high specificity of action (15). In addition, the behavioral potency of CP 55,940 is some 30 times that of Δ^9-THC in rodents, although its behavioral effects appear similar in profile and time course (12,20). Further, the stimulus properties of CP 55,940 appear to be very similar to those of Δ^9-THC because CP 55,940 substitutes fully for Δ^9-THC in the drug discrimination paradigm in rats and primates (12).

The behavioral effects of CP 55,940 have been most extensively investigated in mice, where analgesic, cataleptic, and hypothermic effects, typical of cannabinoids, have been demonstrated (20). The present study initially aimed to test the generality of these effects across species by assessing the effects of CP 55,940 on locomotor activity and body temperature in rats. Because cannabinoids have been reported to alter energy balance and appetite in humans (5,16) the effects of CP 55,940 on ingestive behavior and body weight in rats were also determined.

Nearly every drug that humans find pleasurable can be shown to have positively reinforcing properties in rats as determined by the self-administration, self-stimulation, and conditioned place preference paradigms. However, the evidence for a positively reinforcing effect of cannabinoids in laboratory animals is, at best, equivocal. Early studies failed to find self-administration of cannabis or Δ^9-THC in rodents or primates (6,13,19). However, more recently a reinforcing effect

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of Δ^9-THC has been reported in the self-stimulation paradigm, although only with the Lewis strain of rat (11). While the in vivo reinforcing effects of CP 55,940 have not to our knowledge been investigated, there is in vitro evidence of a reinforcing effect. In a rat hippocampal slice preparation, neurons have been shown to increase their firing rate when CP 55,940 administration is made contingent upon this response (37).

The present study used the conditioned place preference paradigm to investigate the possible reinforcing properties of CP 55,940 in vivo. Many drugs that give rise to a conditioned place preference also produce paradoxical conditioned taste avoidance (17). That is, rats will avoid a novel flavor, the consumption of which is followed by injection of a drug. Previous studies suggest that high doses of Δ^9-THC can produce conditioned taste avoidance (9,18,31). However, it is also known that cannabinoids possess antiemetic properties when given to humans undergoing chemotherapy (33) and can attenuate cyclophosphamide-induced taste avoidance in rats (18). It was, therefore, of interest to determine the effects of CP 55,940 in the conditioned taste avoidance paradigm.

GENERAL METHOD

Subjects

The subjects were adult male Wistar rats weighing 300–550 g, obtained from the SPF Breeding Centre at the University of Sydney. Unless otherwise stated, the rats were group housed in large plastic tubs (eight rats per tub) and maintained on a 14 L : 10 D cycle in a temperature-controlled vivarium (22 ± 2°C) with ad lib access to water and food (Allied Rat and Mouse Kubes, Sydney). All testing was performed during the light cycle.

Drug Preparation and Doses

(−)-CP 55,940, the active enantiomer of CP 55,940, was exclusively used in this study. Three doses were initially employed, namely 2.5, 10, and 100 μg/kg. The 2.5 μg/kg dose was selected because pilot data suggested that a 1 μg/kg dose had no observable effects. It has been estimated that an IP dose of about 0.5–2.0 mg/kg of Δ^9-THC to a rat produces an effect at the cannabinoid receptor similar to a human smoking two cannabis cigarettes (11). Assuming that CP 55,940 is approximately 30 times as potent as Δ^9-THC in the rat (12), we surmised that a dose of 10 μg/kg CP 55,940 may have an effect similar to one cannabis cigarette.

In the first three experiments, the CP 55,940 was dissolved in a vehicle of 5% pure ethanol (Rhone-Poulenc), 5% Tween 80 (Sigma), and 90% saline. The resulting solutions were then sonicated prior to use. The drug was always injected IP at a volume of 1 ml/kg. Two control treatments were used, namely vehicle and saline (0.9%), which were both injected IP at a volume of 1 ml/kg. The use of these two controls ensured that any behavioral effects of the ethanol and Tween 80 vehicle could be determined.

In the conditioned taste avoidance and conditioned place preference experiments a vehicle of 2.5% pure ethanol, 2.5% Tween 80, and 95% saline was used to minimize the amount of ethanol that the rats were exposed to when the reinforcing effects of CP 55,940 were assessed. In addition, because the initial experiments failed to establish clear effects of the 2.5 μg/kg does of CP 55,940, only the 10 and 100 μg/kg doses were used in these later experiments.

In the conditioned place preference experiment, one group received an injection of morphine sulphate (3 mg/kg, dissolved in saline and injected (IP) as a procedure designed to verify that a conditioned place preference could be obtained with the apparatus being used.

Locomotor Activity

The subjects were 36 experimentally naive male Wistar rats. Locomotor activity was measured in four operant chambers (29.5 × 25.5 × 35.5 cm) as described previously (24). The boxes were constructed with front and back walls of stainless steel and left and right walls of clear perspex. The floor of each cage consisted of 16 metal bars (1 cm diameter, spaced 1 cm apart) connected to a high impedance amplifier. Contact or breaking of contact between any of four evenly spaced bars and the other 12 caused a count to be recorded by computer. Each box was placed inside a wooden sound attenuation chamber and a low level of masking noise coming from the fan inside each box was applied during testing.

The rats were randomly allocated to one of the five groups, saline, vehicle, and CP 55,940 (2.5, 10, or 100 μg/kg). All groups had an n of 7 except the 100 μg/kg group, which had an n of 8. Rats were removed from their home cages and brought to the testing room. Each rat was then injected with its assigned dose, placed in the apparatus, and the computer recording of activity was initiated. This was approximately 3 min following the first injection. Testing continued for 90 min.

Body Temperature

The subjects were 40 experimentally naive male Wistar rats. On the test day rats were removed from the their home cages one at a time and taken to the testing room. Each rat was injected with its allocated dose and then core body temperature was taken by means of a rectal probe attached to a Bailey BAT 8 digital thermometer, which was left in situ for 45 s. This time frame allowed a stable temperature reading. After this procedure, the rat was returned to its home cage, and the next one taken. This procedure continued until all 40 rats had been injected and measured. Temperature was similarly tested at 60, 120, 180, and 270 min postinjection. The order of testing, both across and within home cages, remained constant throughout the experiment.

Ingestive Behavior

The subjects were 39 experimentally naive male Wistar rats. All groups had an n of 8 except for the 2.5 μg/kg CP 55,940 group, which had an n of 7. All rats were individually housed with ad lib access to food and water. On the first day of testing, each rat, plus its food and water, were weighed. Immediately following this initial measurement, rats were injected with the appropriate treatment. Food and water measurements were retaken at 4, 24, 48, and 72 h postinjection in the same testing order across subjects. Body weight was also measured at 24, 48, and 72 h postinjection.

Conditioned Taste Avoidance

The subjects were 40 experimentally naive male Wistar rats housed individually with ad lib access to food. Drinking was restricted to 30 min daily when bottles were placed in the home cages. Each rat drank from the same bottles in each session.

The experiment lasted a total of 13 days. During a 6-day baseline period, water was available in two bottles during the drinking session. For each rat, one bottle was designated as

the saccharin bottle (i.e., to contain saccharin on the test day) and the position (left or right) of this bottle was counterbalanced across rats and alternated over consecutive experimental days. On the last baseline day subjects were randomly allocated to one of the four treatment groups with minimal adjustment to ensure that groups were balanced for bottle preference.

On the conditioning day, rats were given a 30-min drinking session where a single bottle containing 0.1% sodium saccharin solution was placed in the centre of the cage, equidistant from the two normal bottle positions. After this session, rats were immediately removed from their cages, taken to a separate room, and injected with their given treatment (saline, vehicle, or CP 55,940−10 or 100 μg/kg). Following conditioning, a 2-day recovery period followed with two bottle water drinking sessions identical to those given during baseline.

After the 2 recovery days, 4 consecutive test days followed. In each test rats were given two bottles, one containing water and the other containing 0.1% saccharin. The position of the saccharin bottle was alternated daily over the four tests. Preference for the saccharin solution over water was compared across groups.

Conditioned Place Preference

The subjects were 49 experimentally naive male Wistar rats. The place preference apparatus consisted of four rectangular wooden boxes partitioned into two large compartments (30 cm long × 30 wide × 30 high) and one smaller middle compartment (15 cm long × 30 wide × 30 high). The partitions were made of plywood and contained rectangular doorways through which rats could move from one compartment to the other. These doorways could be blocked off by two plywood doors. A wire mesh lid (1 × 1 cm grid) was placed over the top of the boxes during testing and conditioning to prevent rats from escaping.

One large compartment (the light compartment) consisted of white Plexiglas walls and a light-colored plywood floor with a wire grid (1 × 1 cm) secured to it. Two strips of household vinegar were dabbed along the rear wall of the compartment. The other large compartment (the dark compartment) consisted of black Plexiglas walls and a black textured plastic floor. The smaller middle compartment consisted of blue-grey Plexiglas walls and floor. Extensive pilot studies demonstrated that there was no overall preference among groups of rats for either of the large compartments, thus allowing an unbiased design to be used.

Movement of the rat from the middle into either of the large compartments caused the box to tilt slightly, and this tilting was detected by microswitches on the base of the apparatus. The output of these microswitches was fed into a PC compatible computer running Workbench PC software [see (25) for description]. The software allowed the total time spent in each compartment to be recorded.

In the single baseline session, rats were placed in the boxes with the doors removed and allowed to freely explore the apparatus for 20 min. Rats were randomly allocated to one of five treatment groups (saline, vehicle, morphine, or CP 55,940−10 or 100 μg/kg) and within these groups were allocated, in a counterbalanced fashion, to the light or the dark compartment for conditioning.

For the following 4 days, two conditioning trials (one drug and one vehicle) per day were given to each rat, with a minimum of 5 h separating the two sessions. This 4-day twice-a-day conditioning procedure has been shown to have no impact on the expression of a place preference (3). It is also known that no place preference is seen with morphine injections 4.5 h after injection (32) and that the effects of 100 μg/kg of CP 55940 on body temperature dissipate after 270 min (see below). On the basis of these findings it was predicted that there would be minimal carry over from one daily conditioning trial to the other. On any given conditioning day, half the rats received drug in the first conditioning session and the other half vehicle, with the order reversed on alternate conditioning days.

Immediately prior to each conditioning session rats were taken in their home cages into a separate room for injection. They were then taken four at a time into the testing room and immediately placed in the relevant compartment with the door in place for a total of 30 min. All rats were tested and conditioned in the same box and in the same order each day. Between every trial boxes were thoroughly cleaned out, wiped with tap water, and dried. The vinegar in the light compartment was also washed off and reapplied.

The test day procedure was identical to that of the baseline day, with rats being allowed to freely explore the apparatus for 20 min with the doors removed and in the absence of any drug treatment. Change in preference for the drug-paired side from baseline to test was compared between groups.

Data Analysis

The data from all experiments were analyzed using planned contrasts with a Bonferroni procedure to control for the probability of a type 1 error. An initial contrast compared vehicle and saline treatments. If no significant difference was found between these treatments (as was the case in each experiment performed), then data for these two treatments were combined and compared with each CP 55,940 treatment.

RESULTS

Locomotor Activity

The effects of the various treatments on locomotor activity are illustrated in Fig. 1. Planned contrasts with Bonferroni adjustment showed no overall difference in activity between the vehicle and saline controls ($F < 1$), so data from these two groups were combined for subsequent contrasts. A significant difference was seen between the 10 μg/kg group and the combined control groups, $F(1, 31) = 10.37$, $p < 0.01$. A significant difference was also seen between the 100 μg/kg group and the combined controls, $F(1, 31) = 27.43$, $p < 0.01$. Figure 1 shows that these effects were in opposite directions, with a stimulation of locomotor activity in the 10 μg/kg group and an inhibition in the 100 μg/kg group. None of the contrasts for group × time interactions reached statistical significance.

Body Temperature

The effects of the various doses of CP 55,940 on body temperature are shown in Fig. 2. Planned contrasts with Bonferroni adjustment showed no difference between the saline and vehicle groups at any time interval (Fs < 1), so data for these two groups were combined. Subsequent contrasts determined that the 100 μg/kg CP 55,940 group were hypothermic relative to the combined control groups at 60, $F(1, 35) = 44.38$, $p < 0.01$; 120, $F(1, 35) = 50.61$, $p < 0.01$; 180, $F(1, 35) = 14.08$, $p < 0.01$; but not 270, $F(1, 35) = 3.10$, NS, mins following injection.

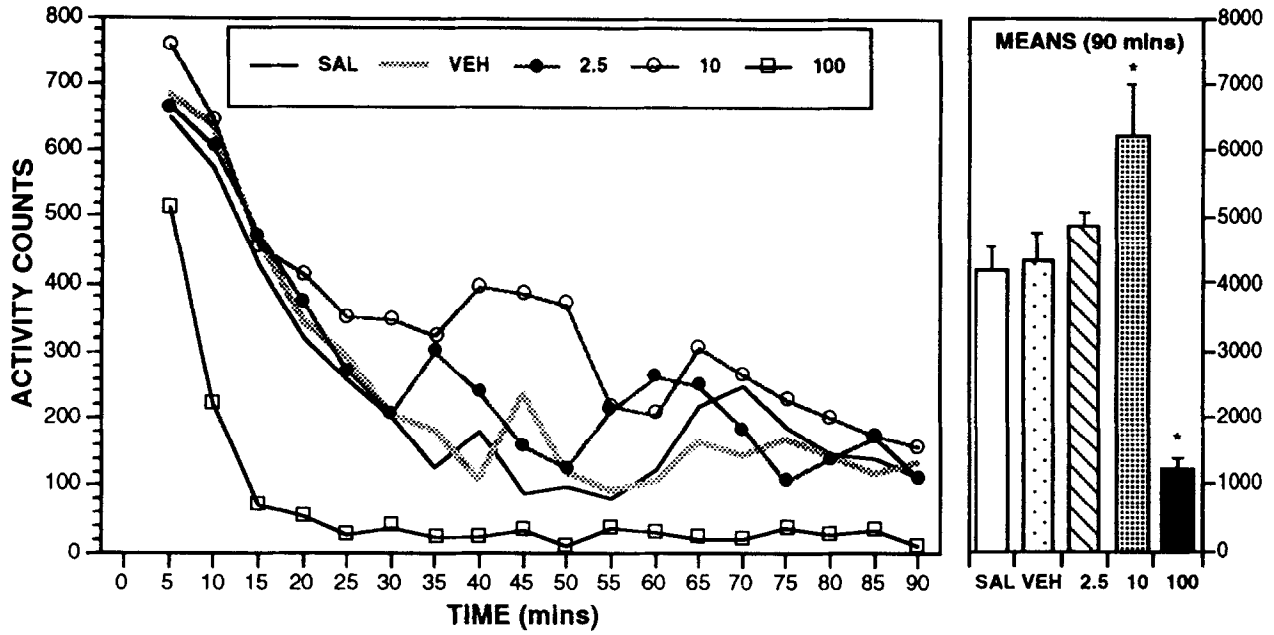

FIG. 1. Effects of saline, vehicle, and three doses of CP 55,940 (2.5, 10, and 100 μg/kg) on locomotor activity. The time course of activity changes over 5-min bins is shown on the main graph, while the right hand graph shows mean total activity for the 90-min test session across groups (*$p < 0.05$ for drug group relative to combined control groups).

Ingestive Behavior

The effects of treatment on food intake, water intake, and body weight are shown in Fig. 3. There was no significant difference between the vehicle and saline groups in food intake at any of the time intervals tested (Fs < 1.6). The 100 μg/kg CP 55,940 group showed a significant inhibition of food intake relative to the combined control groups in the 4–24-h postinjection period, $F(1, 34) = 18.03, p < 0.01$.

With water intake there was no significant difference between vehicle and saline groups at any of the time intervals tested, although there was a clear tendency for increased consumption in the vehicle group after 4 h, $F(1, 34) = 3.90, p > 0.05$. A significant increase in water intake was seen in the 100 μg/kg CP 55,940 group relative to the combined control groups for the 4–24-h period, $F(1, 34) = 18.30, p < 0.01$.

With body weight, there was again no significant difference between vehicle and saline groups at any of the time intervals tested, although an effects at 72 h approached significance, $F(1, 34) = 4.04, p > 0.05$. The 100 μg/kg group were found to have gained significantly less weight at both 48 h, $F(1, 34) = 7.70, p < 0.05$, and 72 h, $F(1, 34) = 12.11, p < 0.01$, postinjection relative to the combined control groups.

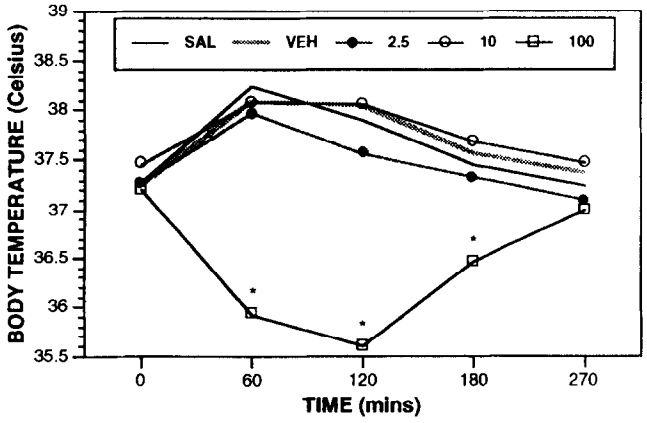

FIG. 2. Effects of saline, vehicle, and three doses of CP 55,940 (2.5, 10, and 100 μg/kg) on body temperature over a 270-min test period (*$p < 0.05$ for drug group relative to combined control groups).

Conditioned Taste Avoidance

Analysis of total fluid intake (in ml) across the 6-day baseline period showed no overall difference between any of the groups in water intake (Fs < 1.05). Similarly, there was no difference between groups in saccharin intake on the conditioning day (Fs < 1) or across the 2 recovery days (Fs < 1). Mean daily fluid intake during the 4 test days, however, was significantly decreased in the 100 μg/kg CP 55,940 group relative to the combined control groups, $F(1, 36) = 6.34, p < 0.05$, probably due to the lesser intake of saccharin in this group. Mean milliliter intake for each group during test are presented in the legend for Fig. 4.

No significant difference in bottle preference (saccharin vs. water bottle) was seen between groups over the baseline period or in the 2 recovery days (Fs < 1). Mean preference scores for these groups over baseline and recovery days are given in the legend for Fig. 4. Changes in saccharin bottle preference over the 4 test days period are presented in Fig. 4. There was no difference in the preference for saccharin between the saline and vehicle-treated groups on any of the 4 test days (Fs < 1.2). The 100 μg/kg CP 55,940 group showed clear conditioned avoidance of saccharin relative to the combined control groups for each of the 4 test days [test 1: $F(1, 36) = 284.66, p < 0.01$; test 2: $F(1, 36) = 446.40, p < 0.01$; test 3: $F(1, 36) = 100.80, p < 0.01$; test 4: $F(1, 36) = 62.41, p < 0.01$].

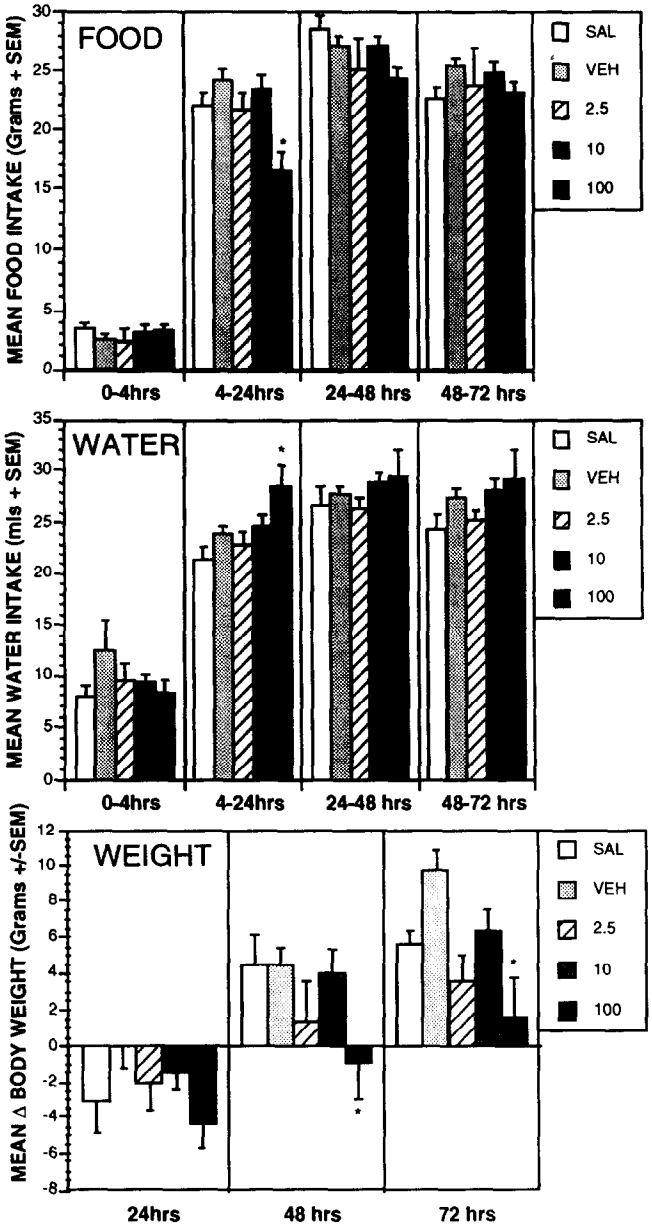

FIG. 3. Effects of saline, vehicle, and three doses of CP 55,940 (2.5, 10, and 100 μg/kg) on food intake (upper), water intake (middle), and body weight (lower) over a 3-day test period (*$p < 0.05$ for drug group relative to combined control groups).

Conditioned Place Preference

The results of the conditioned place preference experiment are presented in Fig. 5. Due to equipment malfunction during the test day, data for 6 of the 49 rats tested had to be discarded. This involved data from rats in the morphine ($n = 3$), saline ($n = 1$), 100 μg/kg CP 55,940 ($n = 1$), and vehicle ($n = 1$) groups.

No significant change in preference from baseline to test was found in the vehicle group relative to the saline group, $F(1, 38) = 2.79, p > 0.05$), so data for these two groups were combined. A significant increase in preference for the drug

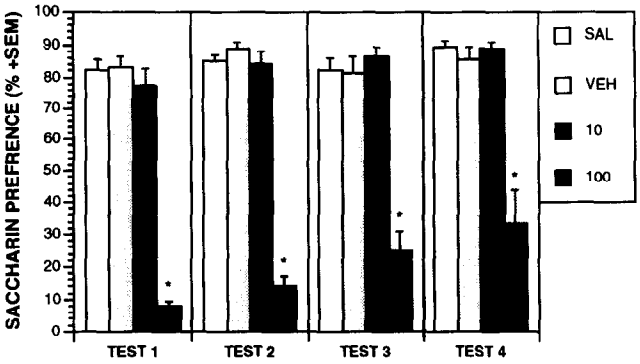

FIG. 4. Effects of saline, vehicle, and two doses of CP 55,940 (10 and 100 μg/kg) in the conditioned taste avoidance paradigm (*$p < 0.05$ for drug group relative to combined control groups). The mean daily milliliter intake across the 4 test days for each groups was as follows: saline 22.45 ml; vehicle 21.99 ml; 10 μg/kg 22.24 ml; 100 μg/kg 19.59 ml. Mean baseline and recovery day "saccharin" bottle preference for each group was as follows: saline 58.99%, 57.70%; vehicle 53.01, 56.69%; 10 μg/kg 54.55%, 57.32%; 100 μg/kg 54.28%, 53.25%.

paired side was found in the morphine group relative to the saline group, $F(1, 38) = 12.61, p < 0.01$. In contrast, a significant aversion towards the drug-paired side was found in the 10 μg/kg CP 55,940 group, $F(1, 38) = 13.95, p < 0.01$), and 100 μg/kg group, $F(1, 38) = 60.78, p < 0.01$, relative to the combined control groups.

GENERAL DISCUSSION

The main finding of this study is that CP 55,940 has aversive effects in Wistar rats, as determined by the conditioned place preference and conditioned taste avoidance paradigms. In this respect, our data agree with those of previous experiments, showing that cannabis and Δ^9-THC are not self-administered in laboratory animals (6,13,19) and that high doses of Δ^9-THC can produce conditioned taste avoidance

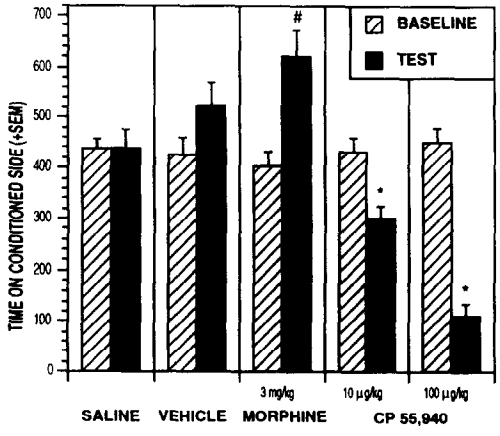

FIG. 5. Effects of saline, vehicle, morphine (3 mg/kg), and two doses of CP 55,940 (10 and 100 μg/kg) in the conditioned place preference paradigm. Amount of time in the drug paired compartment for baseline and test sessions are shown (*$p < 0.05$ for drug group relative to combined control groups, #$p < 0.05$ for morphine group relative to saline control).

(9,18,31) and conditioned place avoidance (31). Although it remains possible that lower doses of CP 55,940 than were tested here might produce a place preference, the balance of evidence suggests that this is unlikely. In the first two experiments here, a lower dose of CP 55,940 (2.5 μg/kg) produced no detectable effects on locomotor activity or body temperature. In addition, it has recently been shown that a low dose of Δ^9-THC (0.2 mg/kg) fails to induce a place preference (31).

The present results are somewhat at odds with those that suggest a positively reinforcing effect of Δ^9-THC in the self-stimulation paradigm (11). This reinforcing effect was only found in rats from the Lewis strain and not in rats from the Long-Evans, Sprague-Dawley, and Fischer 344 strains. In recent experiments we have also failed to find a reinforcing effect of CP 55,940 in Wistar rats using the self-stimulation paradigm (Prior and McGregor, unpublished). Thus, reinforcing effects of cannabinoids may only be obtained in certain strains of rat, suggesting an important genetic influence upon cannabinoid reinforcement. However, even then, Lewis rats have recently been shown to display conditioned place avoidance with Δ^9-THC (31), showing that the apparent reinforcing effects of cannabinoids depend upon the animal model being used.

The first experiment reported here showed clear biphasic effects of CP 55,940 on locomotor activity with a 10 g/kg dose producing locomotor stimulation and a 100 μg/kg dose producing hypoactivity. The latter effect is in agreement with the effects of CP 55,940 in the mouse where a 68% reduction of activity and pronounced catalepsy was noted following a 100 μg/kg dose. More recently, catalepsy was also reported in rats following 100 μg/kg of CP 55,940 (12).

The stimulatory effect of 10 μg/kg CP 55,940 on locomotor activity has not to our knowledge been hitherto reported, although a low dose stimulatory effect of some, but not all, cannabinoids has been seen in mice (20). This locomotor stimulation initially suggested to us that low doses of CP 55,940 may be positively reinforcing, because many reinforcing drugs stimulate locomotion in rodents. However, our later experiments, particularly the conditioned place preference experiment, refuted this hypothesis. It is notable, however, that drugs such as phencyclidine also have the property of producing marked behavioral activation as well as conditioned place avoidance in rats (1).

A biphasic effect of Δ^9-THC has been reported on cerebral metabolism using the 2-DG autoradiography method (22), with a low dose (0.2 mg/kg) causing a significant increase in 2-DG uptake in cortical and limbic sites and higher doses (2 and 10 mg/kg) causing decreased glucose uptake in these regions. This is an interesting parallel to the present locomotor activity data, although the link between changes in cerebral metabolism and changes in locomotor activity is perhaps rather tenuous.

The hypothermic effect of CP 55,940 in the present experiment is in agreement with previous studies using mice, where a 100 μg/kg dose of CP 55,940 produces a mean decrease in rectal temperature of 2.2°C at 60 min following administration (20). The well-documented hypothermic effect of cannabinoids is thought to reflect an action on thermoregulatory centers within the preoptic region of the anterior hypothalamus (10).

To date, there have been no published reports of the effect of CP 55,940 on ingestive behavior. Because evidence suggests that cannabinoids act as appetite stimulants in humans (4, 5,16), it was thought that CP 55,940 might have a similar effect in rats. However, an opposite pattern of results was obtained, with decreased food intake, reduced body weight gain, and increased water intake following 100 μg/kg CP 55,940. Previous studies have shown that chronic oral or intraperitoneal administration of Δ^9-THC inhibits food intake and body weight gain in rats (21,27). The current results complement these findings with evidence of similar acute effects of CP 55,940 on ingestive behavior. Moderate densities of cannabinoid receptors have been found in various hypothalamic nuclei in rats (15), and it is possible that the anorexic effects of CP 55,940 are mediated through an effect at these centers.

It is also possible that the 100 μg/kg dose of CP 55,940 induced a general feeling of malaise, which lead to an inhibition of food intake. Previous studies have shown that high doses of Δ^9-THC can cause nausea in humans (16) and vomiting and gastric changes in laboratory animals (35). The fact that CP 55,940 caused conditioned taste avoidance at the same dose that decreased food intake, supports this hypothesis. Furthermore, a recent study has shown that doses of Δ^9-THC, comparable in strength with the 100 μg/kg dose of CP 55,940 used here, have an aversive profile on the taste reactivity test, with rats reexposed to a flavor that has been paired with Δ^9-THC showing chin rubs, gapes, and paw pushes (31). This is striking because, while most psychoactive drugs produce conditioned taste avoidance, few, if any, produce aversive taste reactivity towards solutions with which they have been paired (17,30). This suggests a nausea-inducing property of cannabinoids in rats.

Such a nauseating effect might perhaps explain the place avoidance induced by CP 55,940. However, one problem with this is that the 10 μg/kg dose of CP 55,940, while giving rise to a clear conditioned place avoidance, failed to condition taste avoidance. This suggests that the mechanism that causes the conditioned place avoidance with this dose of CP 55,940 does not involve nausea or general malaise, either of which would be expected to lead to a strong conditioned taste avoidance. While the place preference protocol involved four drug conditioning trials, compared to only one drug conditioning trial in the taste avoidance paradigm, it is unlikely that this lesser number of pairings was responsible for the lack of taste avoidance seen. Rather, it seems that low doses of cannabinoids generally do not support taste avoidance learning (9, 18,31). In particular, there is no aversive taste reactivity seen in rats to tastes paired with low doses of Δ^9-THC (0.25 and 0.75 mg/kg) that are of similar or greater potency to the 10 μg/kg dose of CP 55,940 used here (31). Further, low doses of Δ^9-THC (0.3 mg/kg and 1.0 mg/kg) were found to attenuate the conditioned taste avoidance induced by the emetic agent cyclophosphamide in mice (18). So it appears that cannabinoids have the unusual quality of being antiemetic in low doses and emetic in higher doses.

Given the fact that nausea is an unlikely explanation of the CP 55,940 place aversion, an alternative hypothesis could invoke the anxiogenic effects of the drug. Previous studies have shown that cannabinoids have an anxiogenic profile on the elevated plus maze in mice (29). In addition, cannabis has well-documented effects of producing paranoia, panic, and anxiety in a proportion of human users (2,16). Various studies have shown that cannabinoids reliably activate the hypothalamic-pituitary-adrenal axis in a way that is reminiscent of external stressors such as foot shock (27,36). In addition, recent studies using c-*fos* immunohistochemistry have also demonstrated that cannabinoids activate brain regions involved in stress responses, including the paraventricular nucleus of the hypothalamus, central nucleus of the amygdala, and lateral septum (14,26). Whether these stressor-like effects are due to

the cannabinoids per se or are a reaction by the animal to some other effect that the cannabinoids produce (e.g., catalepsy) is difficult to say.

The finding of apparent in vitro reinforcing effects of CP 55,940 with hippocampal slices suggests that the action of this drug in some brain regions may be positively reinforcing (37). However, actions in other sites may engage aversive processes such as anxiety or nausea, which mask any rewarding effect of the drug. Future studies assessing the reinforcing, aversive, or anxiogenic effects of CP 55,940 directly injected into various brain sites would be most helpful in addressing this issue.

In conclusion, the present study adds to the growing literature suggesting that cannabinoids are often strongly aversive to rats, and as such, the value of rats as subjects may be limited in studies investigating the human predilection for cannabis use.

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