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Attenuation of Cyclophosphamide-Induced Taste Aversions in Mice by Prochlorperazine, Δ^9-Tetrahydrocannabinol, Nabilone and Levonantradol[1]

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LANDAUER, M. R., R. L. BALSTER AND L. S. HARRIS. *Attenuation of cyclophosphamide-induced taste aversions in mice by prochlorperazine, Δ^9-tetrahydrocannabinol, nabilone and levonantradol.* PHARMACOL BIOCHEM BEHAV **23**(2) 259–266, 1985.—A series of experiments were performed with adult CD-1 male mice to evaluate the antiemetic effects of several compounds using the conditioned taste aversion procedure. The antiemetics were administered IP immediately prior to a 30-min conditioning trial in which a novel tasting solution (0.3% saccharin) was presented to the subjects. The emetics, apomorphine and the cancer chemotherapeutic drug cyclophosphamide, were given IP immediately after the conditioning trial at doses that induced taste aversions. Three days later the mice received a two bottle preference test (saccharin vs. water) and the percent saccharin consumed of the total fluid intake was calculated. Doses of the phenothiazine antiemetic prochlorperazine (1 and 3 mg/kg) attenuated the aversions produced by 0.3 and 1.0 mg/kg apomorphine. Doses of drugs currently approved or under clinical investigation as antiemetics in conjunction with cancer chemotherapy, i.e., prochlorperazine (1.0 mg/kg), Δ^9-tetrahydrocannabinol (0.3 and 1.0 mg/kg) and nabilone (0.01 and 0.03 mg/kg), significantly attenuated the taste aversions induced by cyclophosphamide. Levonantradol at doses of 0.03 and 0.06 mg/kg, however, did not attenuate cyclophosphamide-induced taste aversions. Conditioned taste aversions produced by emetic drugs warrants investigation as a model for evaluating potential antiemetics.

Antiemetics Cancer chemotherapy Taste aversion Cyclophosphamide Tetrahydrocannabinol Nabilone Levonantradol Prochlorperazine Mice

NAUSEA and vomiting are common side effects of cancer chemotherapy. Consequently, antinauseant and antiemetic drugs are commonly co-administered with cancer chemotherapeutic agents. Chemotherapy-induced emesis has proven particularly refractory to treatment because of the multiple emetic inputs produced by these agents [7]. Recently, the active constituent of marihuana, Δ^9-tetrahydrocannabinol (THC), as well as other cannabinoids such as nabilone and levonantradol, have been shown to have some antiemetic activity in cancer chemotherapy (see reviews [2, 17, 32, 33, 40]). Animal models used for the evaluation of natural and synthetic cannabinoids as antiemetics have been limited to the dog and cat since these species exhibit emesis [25, 28, 29, 38]. These procedures are technically difficult, expensive, and suffer from the fact that severely toxic doses of the chemotherapeutic agents are needed to produce reliable emesis. Rodent models have not been developed because rats and mice are unable to vomit; however, they do show autonomic and behavioral signs consistent with the presence of nausea in response to emetic stimuli [7].

In order to study the effects of antiemetic compounds in rodents, we employed the conditioned taste aversion (CTA) paradigm. In this test, a novel tasting stimulus (e.g., saccharin) is paired with noxious effects produced by a drug or toxicant. Thus, the animal becomes conditioned and forms an association between the saccharin and the noxious chemical compound during the conditioning (training) trial and will show lower saccharin consumption relative to control subjects in a subsequent test trial. This avoidance response is conditioned in one trial [22].

The purpose of the present study was to establish a CTA for the cancer chemotherapeutic drug cyclophosphamide in mice as a model to test antiemetic or antinauseant properties of cannabinoids. Chemotherapeutic drugs have been shown to produce CTA's in both humans [3,5] and rodents [4,19]. The CTA model was first evaluated by testing the standard emetic-antiemetic pair, apomorphine and prochlorperazine.

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The approach we have taken is to give the potential antiemetic at the onset of the training trial, in an attempt to attenuate the direct effect of the illness-producing agent and thus modify the development of the aversion. This procedure, however, has the problem of producing aversions by the potential antiemetic. We have therefore also tested these drugs for CTA's when given alone.

EXPERIMENT 1

In order to determine whether antiemetic drugs would be effective in attenuating a taste aversion produced by an emetic that has been used clinically, the first experiment examined the effects of the phenothiazine prochlorperazine on taste aversions produced by apomorphine. Prochlorperazine was chosen as the antiemetic since it has been shown to antagonize the behavioral effects produced by apomorphine in rodents [34] and is commonly used clinically as an antiemetic.

METHOD

Animals

Adult CD-1 male mice (Charles River, Wilmington, MA) weighing between 21 and 32 g served as subjects. One week after arrival from the supplier, the animals were individually housed in clear plastic cages (28.5×17.5×12 cm) with wire mesh tops (Wahmann Manufacturing Co., Timonium, MD). Purina Laboratory Chow No. 5001 was freely available throughout the experiment. Animals were maintained on a 12:12 hour light-dark cycle in a temperature controlled (21°C) test room isolated from the rest of the animal colony. All limited access drinking sessions occurred during the light portion of the light-dark cycle. Drinking fluids were presented in modified 10-ml plastic syringes calibrated in 0.2 ml units for easy quantification of fluid intake which will be referred to as bottles [26,27].

Experimental Design

Three separate experiments were conducted in order to determine whether prochlorperazine would antagonize a CTA produced by apomorphine. The first two experiments established dose-response curves for the taste aversions produced by prochlorperazine and apomorphine alone. The third experiment presented doses of prochlorperazine in combination with doses of apomorphine. The two highest doses of prochlorperazine that did not produce taste aversions were paired with the two lowest doses of apomorphine that did produce aversions.

Conditioning Paradigm

Mice were acclimated to the test room for six days during which time they had 24-hr access to two bottles of deionized water. The bottles were positioned adjacent to one another on the cage top with 10 cm between the drinking spouts. For the next six days the animals were given limited access to these bottles for 60, 60, 30, 30, 30 and 30 min per day respectively. On day seven, the conditioning trial, the mice had limited access to a single bottle of 0.3% (w/v) sodium saccharin (Fisher Scientific) during the 30-min drinking period. This bottle was positioned in the middle of the cage top to control for the development of a position preference. For three consecutive days prior to the conditioning trial, all animals were handled immediately before and after their 30-min limited access drinking period to accustom the mice to the injection schedule on the conditioning day.

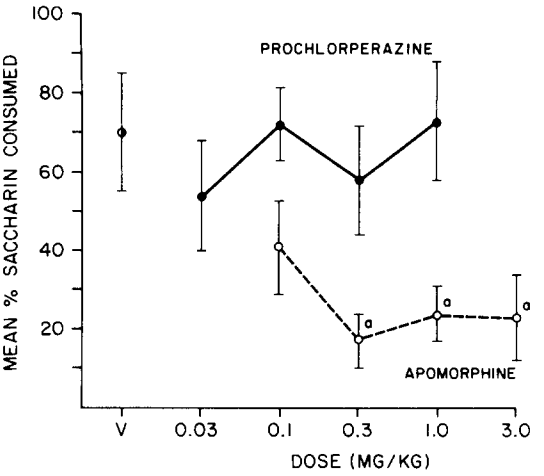

FIG. 1. Dose-response curves for conditioned taste aversions expressed as the mean percent saccharin consumed (±SEM) on two bottle preference tests given three days after the conditioning trial. Prochlorperazine, an antiemetic, was administered immediately before and apomorphine, an emetic, was given immediately after the conditioning trial. N=5/Group, a=significantly different from vehicle (V).

On the conditioning trial, two IP injections were given, one immediately before the saccharin presentation and one immediately after saccharin. When dose-response determinations were made for prochlorperazine alone, the first injection was the drug; the second, the saline vehicle. For apomorphine dose determinations, the saline vehicle was given before saccharin and apomorphine after the saccharin presentation. When the prochlorperazine-apomorphine interaction was examined, the first injection was prochlorperazine followed after the saccharin presentation by apomorphine. The 30-min injection interval between prochlorperazine and apomorphine was based on another study of this interaction in mice [34].

For two days after the conditioning trial the mice received two bottles of deionized water to allow them to recover from any direct effects of the drugs. On the third day after the initial saccharin presentation, all animals were given a single 30-min two bottle preference test [24] in which they had a choice between deionized water and 0.3% saccharin. The position of the saccharin solution was counterbalanced within each group. No injections were given on these test sessions. The percent saccharin consumed was calculated by dividing the volume (ml) of intake of saccharin by the total volume of fluid intake (saccharin + water) and multiplying by 100. A one-way analysis of variance was performed for each of the three tests conducted. The Dunnett's test was used to determine which doses differed significantly from the vehicle control group on the dose-response determinations for prochlorperazine and apomorphine. The Neuman-Keuls test was used to compare groups in the interaction study [41]. In all cases a p-value of 0.05 was used.

Selection and Presentation of Drugs

Prochlorperazine maleate (Compazine®, Smith, Kline & French, Philadelphia, PA) and apomorphine hydrochloride

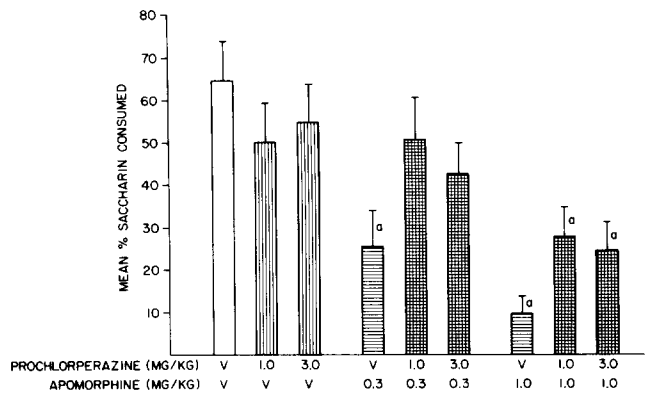

FIG. 2. Taste aversions produced by vehicle (V), prochlorperazine, apomorphine, and the interaction of prochlorperazine and apomorphine on two bottle preference tests. Prochlorperazine or V were given immediately before and apomorphine or V immediately after the conditioning trial which took place three days prior to the choice test. Vertical lines represent 1 SEM. N=10/Group. a=significantly different from vehicle (V-V) control group.

(Sigma, St. Louis, MO) were dissolved in a 0.9% saline solution. Both drugs were administered IP in an injection volume of 10 ml/kg. Doses are based on the salts.

Dose response determinations for prochlorperazine were made using the saline vehicle, 0.03, 0.1, 0.3 and 1.0 mg/kg of the drug (N=5/group). Apomorphine dose-response curves were constructed using the saline vehicle, 0.1, 0.3, 1.0 and 3.0 mg/kg of this drug (N=5/group).

Based on the dose-response data for the two drugs alone, two doses of prochlorperazine were chosen to be administered in combination with two doses of apomorphine, along with the appropriate control groups. This yielded nine treatment conditions (N=10–11/group). Since the highest dose of prochlorperazine (1.0 mg/kg) on the dose-response determination did not produce an aversion, this dose and the next higher half-log dose (3.0 mg/kg) were chosen for the interaction study. The two lowest doses of apomorphine that did produce an aversion (0.3 and 1.0 mg/kg) were selected to determine if this response could be blocked by prochlorperazine.

RESULTS AND DISCUSSION

The dose-response curves for prochlorperazine and apomorphine given alone are presented in Fig. 1. Since they were tested at the same time, the same vehicle control group (saline before and after the training trial) was used for both. Subjects receiving these saline injections averaged 70% of their fluid consumption on the test day from the saccharin bottle, demonstrating the normal preference mice show for 0.3% saccharin over water [26]. Doses between 0.03 and 1.0 mg/kg prochlorperazine failed to produce a taste aversion, and even when a higher dose (3.0 mg/kg) was used in the interaction study (Fig. 2), a taste aversion was not produced. Also, these doses did not significantly affect saccharin intake on the training trial conducted immediately after they were administered (data not shown). Apomorphine at doses between 0.3 and 3.0 mg/kg produced taste aversions when

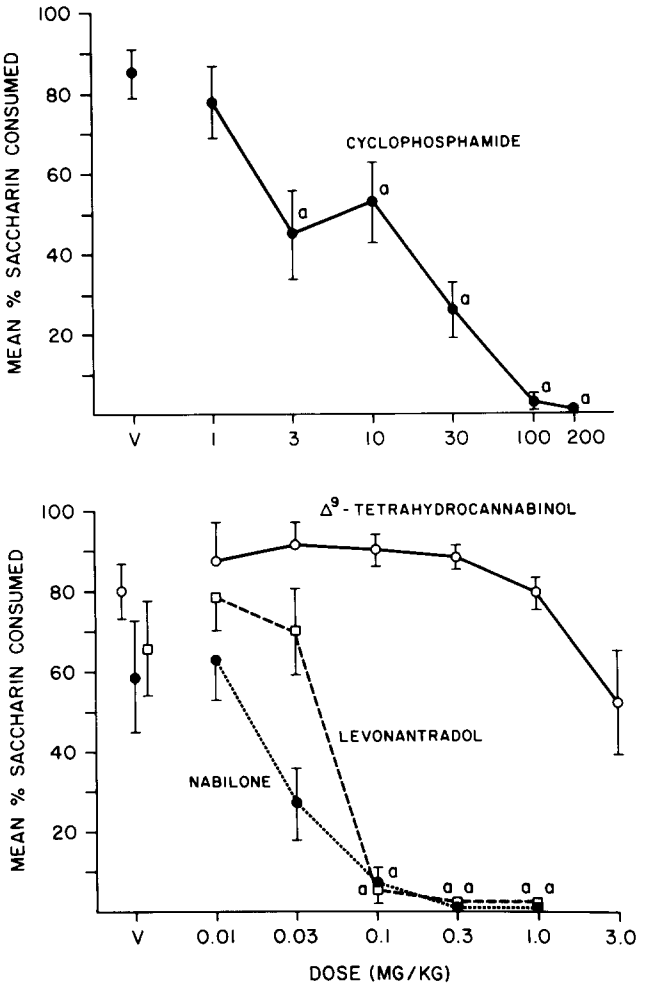

FIG. 3. (Upper Panel) Dose response curves for conditioned taste aversions produced by the cancer chemotherapeutic agent, cyclophosphamide. N=7/Group. Verticle lines represent ±1 SEM. a=significantly different from vehicle (V). (Lower Panel) Dose response curves for conditioned taste aversions produced by the cannabinoids, THC, nabilone and levonantradol. N=7–9/Group. a=significantly different from vehicle (V).

compared to the vehicle control group (Fig. 1). The effect was replicated to the interaction study with doses of 0.3 and 1.0 mg/kg (Fig. 2).

The interaction data presented in Fig. 2 revealed that doses of prochlorperazine that do not produce taste aversions themselves (1.0 and 3.0 mg/kg) are capable of attenuating the development of taste aversions produced by 0.3 and 1.0 mg/kg apomorphine. Thus, the aversion produced by 0.3 mg/kg apomorphine was reduced by both 1.0 and 3.0 mg/kg prochlorperazine such that the mean percent saccharin consumed with the combinations was not significantly different from vehicle control levels. While these doses of prochlorperazine in combination with 1.0 mg/kg apomorphine also resulted in higher saccharin intake than after this dose of apomorphine alone, saccharin consumption remained significantly lower than control levels. Total fluid intake did not differ in the prochlorperazine and apomorphine dose-response determinations or during the prochlorperazine-apomorphine interaction study. This was true during the ini-

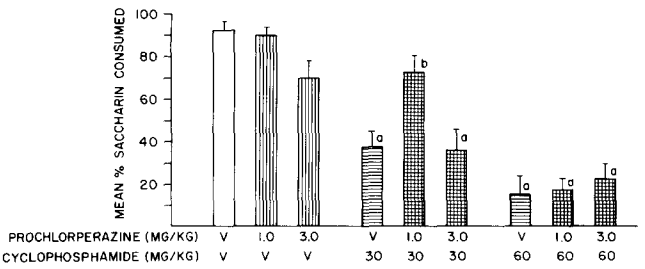

FIG. 4. Conditioned taste aversions produced by vehicle (V), prochlorperazine, cyclophosphamide and the interaction of prochlorperazine and cyclophosphamide. N=10–11/Group. a=significantly different from vehicle (V-V) control group, b=significantly different from V-30 mg/kg cyclophosphamide group.

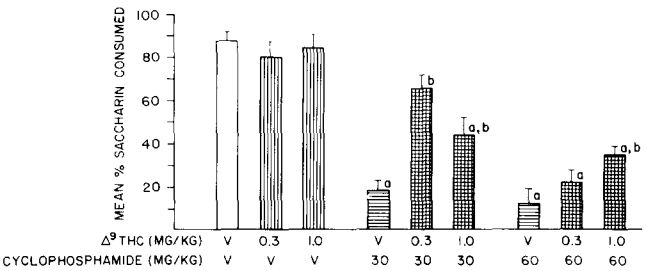

FIG. 5. Conditioned taste aversions produced by vehicle (V), THC, cyclophosphamide and the interaction of THC and cyclophosphamide. N=10–11/Group. a=significantly different from vehicle (V-V) control group, b=significantly different from respective V-cyclophosphamide group.

tial baseline determinations, the training trials and the choice tests.

The results of this study indicate that an antiemetic can attenuate the development of a CTA produced by the emetic, apomorphine. However, statistical comparisons between saccharin consumption for the combinations were in no cases significantly different from the corresponding apomorphine alone group. Nonetheless, attenuation was significant with the lower dose of apomorphine (0.3 mg/kg) since a significant aversion was only produced when it was given alone. These results show that a classic emetic-antiemetic pair of drugs produce expected results in this animal model and encouraged us to study the chemotherapeutic drug cyclophosphamide alone and in combination with potential antiemetics.

EXPERIMENT 2

Taste aversions to the cancer chemotherapeutic agent, cyclophosphamide, have been produced in both rats [4] and mice [19], although a dose-response determination has not been conducted. The purpose of the following experiment was to establish dose-response curves for cyclophosphamide as well as several cannabinoid drugs (THC, nabilone and levonantradol) that are being clinically evaluated for their antiemetic properties. Finally, prochlorperazine (Experiment 1), and each of the cannabinoids were tested in combination with cyclophosphamide, to determine whether they could attenuate the taste aversions produced by this agent.

METHOD

Subjects

The subjects were adult male CD-1 mice weighing between 20 and 32 grams. The animals were housed and maintained as in Experiment 1.

Procedure

Dose-response determinations for the antiemetic cannabinoids (THC, nabilone and levonantradol) and the cancer chemotherapeutic agent, cyclophosphamide, were conducted in a manner similar to that of Experiment 1. The antiemetic drugs were administered IP to the mice immediately prior to saccharin presentation on the conditioning trial and the animals received a vehicle injection immediately after the 30-min saccharin period. Since different vehicles were to be used for the antiemetics, the initial dose-response determination for cyclophosphamide consisted of a single injection immediately following the saccharin. All interaction studies between each of the four antiemetics (prochlorperazine, THC, nabilone, levonantradol) and cyclophosphamide consisted of two doses of the antiemetic and appropriate vehicle with two doses of cyclophosphamide with its vehicle control groups. As in Experiment 1, the antiemetic was administered immediately before and the cyclophosphamide immediately after the 30-min saccharin presentation.

Selection and Presentation of Drugs

Cyclophosphamide (Cytoxan®, Mead Johnson, Evansville, IN), and prochlorperazine maleate (Compazine®, Smith, Kline & French, Philadelphia, PA) were dissolved in 0.9% saline solution. The Δ^9-tetrahydrocannabinol (National Institute on Drug Abuse, Rockville, MD), nabilone (Lilly, Indianapolis, IN) and levonantradol (Pfizer, Groton, CT) were dissolved in 0.75% ethanol, 0.75% Emulphor-620 (GAF Corp., New York, NY) and 98.5% saline. All drugs were administered IP in an injection volume of 10 ml/kg.

The following doses were used to determine dose-response curves: cyclophosphamide: vehicle, 1, 3, 10, 30, 100, and 200 mg/kg (N=7/group); THC: vehicle, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0 mg/kg (N=7/group); nabilone: vehicle, 0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg (N=8–9/group); and levonantradol: vehicle, 0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg (N=8–9/group).

For each interaction study, two doses of each drug and the appropriate vehicle were paired with two doses (30 and 60 mg/kg) of cyclophosphamide. The doses used were: prochlorperazine (1.0 and 3.0 mg/kg), THC (0.3 and 1.0 mg/kg), nabilone (0.01 and 0.03 mg/kg) and levonantradol (0.03 and 0.06 mg/kg). These doses were selected on the basis of the dose-response determinations and either failed to produce a taste aversion themselves or produced mild ones. Doses chosen for cyclophosphamide were selected since they produced mild aversions which could potentially be attenuated by the antiemetic drugs that were evaluated.

RESULTS

The results of the two-bottle choice tests for cyclophosphamide and the three cannabinoids when given alone are presented in Fig. 3. Mice treated with vehicle and 1 mg/kg cyclophosphamide showed a clear preference for the saccharin solution. Doses of 3–200 mg/kg resulted in signifi-

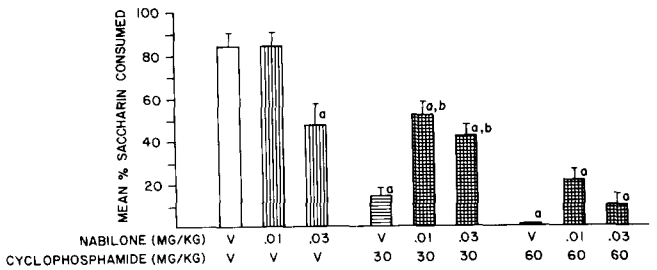

FIG. 6. Conditioned taste aversions produced by vehicle (V), nabilone, cyclophosphamide and the interaction of nabilone and cyclophosphamide. a=significantly different from vehicle (V-V) control group, b=significantly different from V-30 mg/kg cyclophosphamide group.

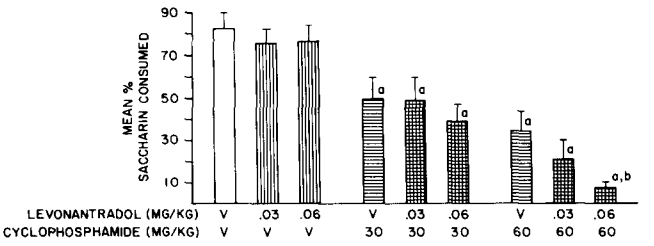

FIG. 7. Conditioned taste aversions produced by vehicle (V), levonantradol, cyclophosphamide, and the interaction of levonantradol and cyclophosphamide. N=10–11/Group. a=significantly different from vehicle (V-V) control group, b=significantly different from V-60 mg/kg cyclophosphamide group.

cantly decreased saccharin preference and doses of 30, 100 and 200 mg/kg produced clear saccharin aversions (less than 50% choice). Thus, doses of 30 and 60 mg/kg were chosen for the interaction studies. Based on the dose-response determinations for prochlorperazine from Experiment 1, 1.0 and 3.0 mg/kg were again chosen to be used in the interaction study with cyclophosphamide. The results of this experiment are shown in Fig. 4. A dose of 1.0 mg/kg prochlorperazine significantly attenuated the taste aversion produced by 30 mg/kg cyclophosphamide but was ineffective in blocking the taste aversion produced by 60 mg/kg. A dose of 3.0 mg/kg was ineffective in blocking the effects of either dose of the chemotherapeutic agent. In this test, however, this dose of prochlorperazine nonsignificantly decreased saccharin intake when given alone.

None of the doses of THC resulted in a significant decrease in saccharin consumption on the choice test (Fig. 3); however, 3.0 mg/kg resulted in lower values than control. A subsequent pilot study attempting to use 3.0 and 10.0 mg/kg THC resulted in significant saccharin aversions at both doses when given alone (data not shown); thus, doses of 0.3 and 1.0 mg/kg THC were chosen to test in combination with cyclophosphamide. Figure 5 presents the results of that study. The lack of effects of these doses of THC was replicated as well as the significant aversion produced by 30 and 60 mg/kg cyclophosphamide. Both doses of THC in combination with both doses of cyclophosphamide resulted in greater saccharin consumption than those doses of cyclophosphamide alone, but only 0.3 mg/kg THC with 30 mg/kg cyclophosphamide resulted in saccharin intake not significantly different from vehicle control. On the other hand, the greater intake of saccharin with the combinations than with cyclophosphamide alone was significant for both doses of THC with 30 mg/kg cyclophosphamide and for 1.0 mg/kg THC with 60 mg/kg cyclophosphamide.

Doses of 0.1, 0.3 and 1.0 mg/kg nabilone produced taste aversions that differed significantly from the vehicle control (Fig. 3). Doses of 0.01 and 0.03 were chosen for testing in combination with cyclophosphamide; and these results are shown in Fig. 6. During this test, 0.03 mg/kg nabilone given alone proved to have significant effects unlike in the initial dose-ranging study (Fig. 3), although saccharin intake was suppressed during that test as well. Both doses of nabilone significantly attenuated the aversion produced by 30 mg/kg cyclophosphamide. Although not statistically significant, more saccharin was consumed when 60 mg/kg cyclophosphamide was tested with nabilone than when it was given alone. None of the combinations, however, resulted in control levels of saccharin intake.

Mice administered 0.01 and 0.03 mg/kg levonantradol failed to exhibit a taste aversion while animals injected with 0.1, 0.3 and 1.0 mg/kg of this drug readily developed an aversion to the saccharin (Fig. 3). Because of the steep dose-effect function between 0.03 and 0.1 mg/kg, the doses selected for the interaction study were 0.03 and 0.06 mg/kg levonantradol. When these doses were presented in combination with 30 and 60 mg/kg cyclophosphamide (Fig. 7), they were not effective in blocking the aversions caused by either dose of the chemotherapeutic agent. When levonantradol was given with 60 mg/kg cyclophosphamide, even greater saccharin aversions were obtained than with this dose of cylcophosphamide alone, and in the case of 0.06 mg/kg this enhancement was significant.

Groups did not differ significantly in total fluid intake during baseline water consumption or during the training trial at which time only the saccharin solution was available. On the choice tests, during the initial dose-response determinations, the two highest doses of cyclophosphamide (100 and 200 mg/kg) and nabilone (0.3 and 1.0 mg/kg) produced significant decreases in total fluid intake. For the interaction studies, significant reductions in fluid intake only occurred when nabilone (0.01 and 0.03 mg/kg) and levonantradol (0.03 and 0.06 mg/kg) were administered in conjunction with the high dose (60 mg/kg) of cyclophosphamide.

DISCUSSION

Taken together, these results demonstrate that the taste aversion paradigm may be an effective model by which to evaluate potential antiemetic agents. Prochlorperazine, THC and nabilone were all effective in significantly attenuating the taste aversion produced by the low dose (30 mg/kg) of cyclophosphamide, but only THC attenuated the high dose of 60 mg/kg. Levonantradol was not effective in attenuating even the low dose of cyclophosphamide. Although the aversion produced by 30 mg/kg cyclophosphamide alone in the levonantradol study did differ significantly from the vehicle-vehicle control group, it was not as strong as that produced in the other interaction studies, thereby possibly making it more difficult to show attenuation. Nonetheless, the failure to find significant attenuation by levonantradol is not consistent with its demonstrated clinical antiemetic effects [15,16] and there is no evidence that levonantradol differs qualitatively in its pharmacology from the active can-

nabinoids in our tests. Thus, at least under these test conditions, levonantradol may be a false negative. We did not test drugs without known clinical antiemetic activity to determine if fase positives occur with this procedure. However, the relative ease of conducting taste aversion studies, the low cost due to the use of mice with no significant equipment requirements, and the ease of quantitation and statistical analysis of the data are factors which favor continued investigation of this approach.

Many parameters of our test conditions remain to be explored, and modifications would likely result in improvements. One of the difficulties is that in order to have the blocking actions of the antiemetic coincide with the actions of the emetic, it is necessary to consider carefully the onset and durations of action of both treatments. Because of the relative slow onset of effects for cannabinoids, we chose to administer them 30 min before cyclophosphamide. However, since we also wanted the onset of cyclophosphamide to occur as rapidly as possible after the saccharin drinking, we gave it immediately after the drinking session. This necessitated administering the antiemetics before the session, introducing the potential for them to interfere with drinking and thus preclude conditioning. This did not occur with the doses we used, in part due to the tendency of the subjects to consume most of the saccharin solution early in the session before the onset of drug effects. Nevertheless, these considerations limit doses and the temporal relationship of injections to the training session. Since taste aversions can also be produced when there is a considerable delay between the drinking session and the administration of the illness-producing agent, it may be possible to delay the administration of the chemotherapeutic drug to allow both drugs to be given after the conditioning session. Also, multiple administrations of the antiemetic during the period following the training trial could be used to evaluate a combination where the effects of the emetic were longer in duration than the effects of the antagonist. Clearly, the temporal relationship between administration of the drugs and the training session is an area for further exploration.

Although prochlorperazine given alone did not result in a taste aversion even at the highest dose tested (3.0 mg/kg), the cannabinoids all resulted in aversions at the higher doses. This was less clear for THC where 3.0 mg/kg produced a nonsignificant decrease in saccharin drinking in the test session. In other pilot studies, however, this dose was clearly active. The threshold dose for nabilone alone to produce an aversion was 0.03 mg/kg (Figs. 3 and 6) and for levonantradol 0.1 mg/kg was active. The synthetic cannabinoids are generally found to be more potent than THC, however the greater potency of nabilone than levonantradol is the reverse of most other studies (e.g., [8,42]). The considerable potency of the cannabinoids themselves in producing taste aversions precludes testing higher doses under these test conditions. Perhaps greater blocking effects would be present at larger doses. To administer higher doses of the potential antiemetic without aversions being produced can possibly be accomplished by slightly modifying the taste aversion paradigm. This would involve pre-exposing the mice to high doses of the antiemetic prior to the training trial. Pre-exposure to agents which produce taste aversions has been shown to reliably attenuate the aversion produced by these compounds (e.g., [9,10]), and this has been demonstrated for THC as well [20,39].

The mechanism by which the cannabinoids attenuated the taste aversion produced by cyclophosphamide is not known. In animals that are capable of emesis, it is known that nausea and vomiting are controlled by an emetic center located in the medulla [6], and lesions in the area postrema can disrupt the acquisition of a CTA in rats exposed to radiation or lithium chloride [37]. Stimuli from peripheral (vagal and sympathetic) and central sensors impinge on this center. The vomiting center receives input from chemical stimuli via the chemoreceptor trigger zone in the area postrema, while visceral afferent stimuli are relayed by the nucleus of the tractus solitarius (NTS). More than a dozen neurotransmitters have been identified in the area postrema and the nucleus of the tractus solitarius [6], but the mechanism by which the cannabinoids exert their antiemetic effects still remains unknown. Borison *et al.* [6] speculate that they may act through descending inhibitory connections to the emetic center in the lower brain stem. Interestingly, THC-induced taste aversions in rodents can be produced after both peripheral [1,14, 18] and central [1] administration.

It is also not clear that the taste aversion produced by cyclophosphamide or other cancer chemotherapeutic drugs is mediated by nauseant effects of these drugs. Without even considering the problem of whether or not nausea occurs in a species that cannot vomit, other evidence indicates that nausea may have little to do with the development of taste aversions. In the first place, the production of taste aversions is not specific to emetic or nauseant drugs. A wide array of psychoactive drugs can result in taste aversions, even, as shown here, the supposedly antiemetic cannabinoids. It is also inconclusive whether the administration of antiemetic drugs prior to test trials or during the conditioning trail will reverse the expression of a lithium chloride-induced taste aversion [13, 21, 23, 35]. Unlike these studies, in our study the antiemetics were administered prior to the conditioning trial, where we can presume some action of cyclophosphamide was blocked by the effective antiemetics. What this action of cyclophosphamide is remains unclear.

One possible mechanism for the failure of saccharin aversion to occur with many of the drug combinations is a possible state-dependent effect [36]. That is, a taste aversion acquired while subjects were intoxicated with cannabinoids or prochlorperazine could not be retrieved when the subjects were subsequently tested without any drug treatments. If this were the case, agonist-antagonist relationships between the emetic and antiemetics would be nonspecific, and would occur with any psychoactive drug. The fact that active doses of levonantradol did not elicit a state-dependent disruption of retention of the aversion is some evidence against a nonspecific, state-dependent effect. Other control procedures [31], including a 2×2 design where some animals are given the antiemetic drug before both training and testing sessions, could be used to evaluate the possibility of state-dependent effects in this procedure.

Interaction studies all utilized a single chemotherapeutic agent, cyclophosphamide. However, in oncology a single chemotherapeutic agent is rarely used. Cyclophosphamide is usually always given in combination with other agents and the majority of clinical studies investigating the antiemetic effects of cannabinoids have used patients with various combinations of chemotherapeutic drugs. Recent evidence suggests that some cannabinoids may be effective only when particular chemotherapeutic agents are used. For example, THC effectively reduced emesis in methotrexate treated patients [12] but was ineffective against patients treated with a combination of adriamycin and cyclophosphamide [11]. Therefore, future studies using the taste aversion paradigm

could attempt to attenuate aversions to various other chemotherapeutic agents (e.g., cisplatin or methotrexate) or combinations of agents.

In summary, it would appear that further investigation of taste aversions in rodents produced by cancer chemotherapeutic agents as a model of the nauseant and emetic side effects of these drugs is warranted. The model may serve as a useful procedure to evaluate antiemetic drugs to use in combination with these agents. We have discussed a number of parameters of the procedure which could be more systematically evaluated in an attempt to improve the magnitude and reliability of the reversal of the aversions. Studies of the specificity of the model for predicting clinically useful antiemetics are also needed, as well as studies of the mechanism for the antagonist effects.

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