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Δ^9-TETRAHYDROCANNABINOL, BUT NOT THE ENDOGENOUS CANNABINOID RECEPTOR LIGAND ANANDAMIDE, PRODUCES CONDITIONED PLACE AVOIDANCE

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Summary

Although exogenous cannabinoid ligands such as Δ^9-tetrahydrocannabinol (THC) have been implicated in reward-related learning and aversion, the hedonic effects of the endogenous cannabinoid agonist anandamide (arachidonylethanolamide) have never been assessed. Thus, the effects of anandamide were tested in a place conditioning task. Male Wistar rats received THC (0.0-8.0 mg/kg) or anandamide (0.0-16.0 mg/kg) during conditioning sessions. The half-life of anandamide was increased by pretreatment with the protease inhibitor phenylmethylsulfonyl fluoride (2.0 mg/kg). A significant place aversion was found at the 1.0 and 1.5 mg/kg doses of THC. No significant place conditioning effects were found with anandamide. Locomotor activity during conditioning was significantly decreased by the 1.0, 1.5, 2.0 and 4.0 mg/kg doses of THC as well as the 8.0 and 16.0 mg/kg doses of anandamide. These results fail to implicate the endogenous cannabinoid anandamide in reward-related learning or aversion.

Key Words: **cannabinoid, place conditioning, Δ^9-tetrahydrocannabinol, anandamide**

In most cases, drugs abused by humans yield rewarding effects in animals as assessed by the intracranial self-stimulation (ICSS), self-administration, and place conditioning paradigms. Although marijuana has been used widely by humans primarily for its euphoric effects, the results of animal studies suggest that it is not a typical drug of abuse. Thus, under most conditions, animals fail to self-administer marijuana (1,2) or Δ^9-tetrahydrocannabinol (THC), its principle psychoactive ingredient (3-5, but see 6). Results of experiments using the ICSS task have also been equivocal; Start and Dews (7) failed to see an enhancement of ICSS with THC and other cannabinoid drugs, but Gardner *et al.* (8) report an enhancement with THC.

The place conditioning paradigm has been used to assess the rewarding or aversive effects of many drugs (9). Cannabinoid drugs only recently have been studied in this task and have yielded mixed results. THC produced a conditioned place avoidance in two studies (10,11) and in another report produced a place preference or avoidance, depending upon the dose and conditioning schedule (12). These results, together with the finding that administration of the synthetic cannabinoid CP-55,940 produced a conditioned place avoidance (13), suggest that cannabinoids are anomalous drugs of abuse.

The arachidonic acid derivative anandamide (arachidonylethanolamide) is a putative endogenous cannabinoid receptor ligand. First isolated from porcine brain (14) and recently in human and rat brain and peripheral tissues (15), anandamide displaces binding of the radiolabelled cannabinoid probes [^{3}H]HU-243 and [^{3}H]CP-55,940 (14). Anandamide also inhibits N-type calcium channels

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(16) and adenylate cyclase (17). Anandamide produces many of the behavioral and physiological effects of other cannabinoids such as hypothermia, hypomotility, catalepsy, antinociception, and memory impairment (18-22). To date, the hedonic properties of anandamide have not been evaluated. Thus, the purpose of the present investigation was to examine the rewarding or aversive properties of anandamide using the place conditioning task. For comparison, the hedonic properties of THC were also evaluated.

Anandamide is highly susceptible to metabolic degradation (23). Phenylmethylsulfonyl fluoride (PMSF) has been shown to be a potent inhibitor of the hydrolysis of anandamide (24,25). In previous studies, anandamide did not impair memory in rats when administered alone (19,22), but produced a dose-dependent impairment of memory when rats were pretreated with PMSF (22). Thus, to slow anandamide's hydrolysis, PMSF was administered prior to anandamide treatment in the present study.

Method

Treatment of animals was approved by the Queen's University Animal Care Committee, and was in accordance with the guidelines of the Canadian Council on Animal Care and the Animals for Research Act.

Subjects

Male experimentally naive Wistar rats (Charles River Canada), weighing 200-250 g upon arrival to the colony, were housed three or four per cage in a temperature-controlled (21°C) room, kept on a 12 h light-dark cycle (lights on at 07.00 h). Dry laboratory chow (Purina Laboratory Rodent Chow #5001) and water was available in the home cage at all times.

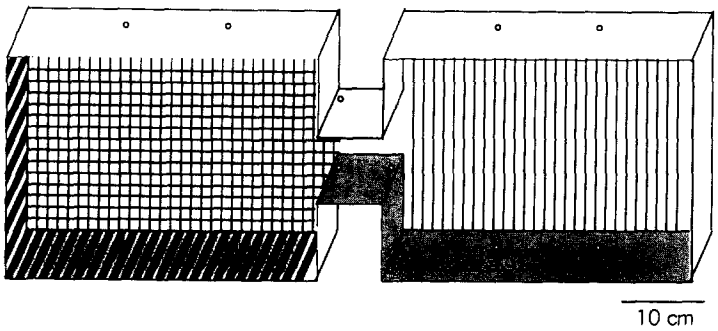

Fig. 1

Schematic drawing of the place conditioning apparatus showing two compartments joined by a tunnel. The floors and walls were of two types. Small circles represent the location of optical sensors used for monitoring locomotor activity and animal location.

Apparatus

Four wooden rectangular shuttleboxes (Fig. 1) consisted of two compartments (38 x 27 x 36 cm) connected by a tunnel (8 x 8 x 8 cm). The two compartments were visually distinct: one had unpainted urethane-sealed walls, and the other had walls consisting of black and white stripes (1 cm wide), covered with clear Plexiglas. The compartment floors were also of two types: one was constructed of 1 cm galvanized steel mesh and the other of parallel stainless steel rods, spaced 1 cm apart. The galvanized mesh floor was on the left in two shuttleboxes and on the right in the other two. Similarly, the striped walls were on the left in two shuttleboxes and on the right in the other two. The walls and floors were arranged in such a way that each shuttlebox used one of the four possible configurations. Shuttleboxes were covered by clear Plexiglas lids and the tunnels could be blocked by the insertion of two opaque Plexiglas guillotine doors. Six photocells were located in each shuttlebox: two in each compartment (height 5 cm) and one at each end of the tunnel (height 3 cm). A 80C188EB-based Experiment Control Board using custom-made software written in ECBASIC recorded the amount of time spent in each area of the shuttlebox.

Shuttleboxes were housed in individual wooden chambers which were insulated with sound-attenuating styrofoam, illuminated by one 7.5 W light bulb, and ventilated with a small fan. For a detailed description of the place conditioning apparatus, see Brockwell *et al.* (26).

Drug Preparation and Administration

Δ^9–tetrahydrocannabinol (Health and Welfare Canada, >98% purity), available as a 200 mg THC/ml ethanol solution, was mixed with a small amount of Tween 80 (polyoxyethylene-sorbitan monooleate, Sigma-Aldrich Canada, Oakville, Ontario). The suspension was stirred continuously under a stream of nitrogen gas until all ethanol was evaporated, which was determined by weight. Saline (0.9%) then was added and mixed until the Tween 80/THC suspension was well dispersed. Care was taken to mix the solution slowly to prevent foaming.

The final solution contained the desired amount of THC, suspended in a vehicle consisting of Tween 80:saline in a ratio of 1:19. Solutions were aliquotted and frozen at –20°C and thawed immediately prior to injection. Injections were administered 30 min prior to conditioning sessions.

Anandamide (Research Biochemicals Inc., Natick, MA) was prepared and stored in a similar manner to THC, with the exception that the initial solution consisted of 5 mg anandamide/ml ethanol. Injections preceded conditioning sessions by no more than 5 min. Phenylmethylsulfonyl fluoride (PMSF, Sigma-Aldrich Canada, Oakville, Ontario) was dissolved in absolute ethanol and then prepared and stored in a similar manner to anandamide and THC. PMSF injections preceded anandamide administration by 35 min. All drugs were administered i.p. in a volume of 1.0 ml/kg body weight.

Place Conditioning

Place conditioning consisted of 3 distinct phases: preconditioning, conditioning and test carried out over 14 consecutive days; the experiment was conducted between 09.00 h and 15.00 h. Each rat received three 15-min preconditioning sessions, one per day, during which they were placed in one of the two compartments (hereafter called the start compartment) and given access to the entire shuttlebox (guillotine door removed). No drugs were administered during preconditioning. The start compartment was counterbalanced across rats and was unchanged throughout the experiment. Following preconditioning, each rat received eight 30-min conditioning sessions with both guillotine doors in place. Drug injections were paired with one compartment on days 1, 3, 5, and 7 while vehicle injections were paired with the other compartment on days 2, 4, 6, and 8. Drug-paired compartments were counterbalanced across rats such that the start compartment was paired with drug for half of the rats and with vehicle for the other. Locomotor activity, as indexed by the number of sensor counts, was recorded during the conditioning phase. Following conditioning, each rat received three test sessions, identical in all respects to the preconditioning sessions. The amount of time spent in each compartment was recorded during the preconditioning and test phases.

The ability of THC and anandamide to produce place conditioning was examined. THC doses were 0.0 (n=8), 0.1 (n=8), 0.5 (n=8), 1.0 (n=21), 1.5 (n=12), 2.0 (n=10), 4.0 (n=8), and 8.0 (n=8) mg/kg. Anandamide doses were 0.0 (n=8), 0.031 (n=12), 0.125 (n=16), 0.313 (n=10), 0.5 (n=10), 2.0 (n=10), 8.0 (n=10) and 16.0 (n=11) mg/kg. To slow the hydrolysis of anandamide, rats received 2.0 mg/kg PMSF prior to anandamide injections. To control for the possible effects of PMSF or the injection procedure on place conditioning, rats in the anandamide groups also received PMSF during vehicle conditioning sessions.

Statistical Analyses

The establishment of place conditioning was assessed by comparing time spent in the drug-paired compartment from the preconditioning phase to the test phase. At each dose, a phase (preconditioning vs test) by session repeated measures ANOVA was conducted on the time (sec) spent in the drug-paired compartment. Previous research has shown that the first test session represents the strongest place conditioning effect (27,28); thus, in addition to the omnibus F-tests, one planned t-test comparing the mean of the three preconditioning sessions to the first test session was conducted at each dose.

The number of sensor counts during the conditioning sessions (an index of drug effects on locomotor activity) was collected and stored in bins of 5-min each. At each dose, the mean of the four drug conditioning sessions was compared to the mean of the four vehicle conditioning sessions with a two-factor (treatment by bin) repeated measures ANOVA.

Epsilon-corrected degrees of freedom were used in all ANOVAs to correct the positive bias that could result from violating the sphericity assumption in within-subject designs (29,30). For clarity, only the uncorrected degrees of freedom are shown. Unless stated otherwise, the outcome of the analysis using the epsilon correction was the same as that observed without the correction. Rats demonstrating a strong side preference during the preconditioning phase were dropped from the experiment prior to the administration of drugs. A side preference was defined as spending greater than 700 s or less than 200 s on one side during the mean of the three preconditioning sessions, or during the last preconditioning session.

Results

Of 170 rats, 7 rats were dropped from the experiment prior to the administration of drugs because a strong place preference or aversion was observed during the preconditioning phase. The amount of time spent in the drug-paired compartment for rats receiving 0.0 mg/kg THC or 0.0 mg/kg anandamide was compared using a three-factor (group by phase by session) ANOVA, with repeated measures on the last two factors. Because the ANOVA did not yield any significant effects, these two groups were combined into a single control group. Data for the control group are shown as the 0.0 mg/kg THC dose (below).

Δ^9-Tetrahydrocannabinol

THC appeared to produce a place aversion at several doses, with the largest effects occurring at the 1.0 and 1.5 mg/kg doses (Fig. 2). Repeated measures ANOVAs resulted in a significant main effect of phase at the 1.0 [$F(1,19)=8.13$, $p<0.05$] and 1.5 [$F(1,10)=4.98$, $p<0.05$] mg/kg doses. Additionally, the phase by session interaction was significant at the 1.5 mg/kg dose [$F(2,20)=4.03$, $p<0.05$], reflecting the weakening of the aversion on the third test session. Planned t-tests comparing the mean of the three preconditioning sessions to the first test session resulted in the same conclusion as the ANOVAs; that is, a significant effect was found at the 1.0 [$t(19)=3.25$, $p<0.005$] and 1.5 [$t(10)=2.87$, $p<0.05$] mg/kg doses.

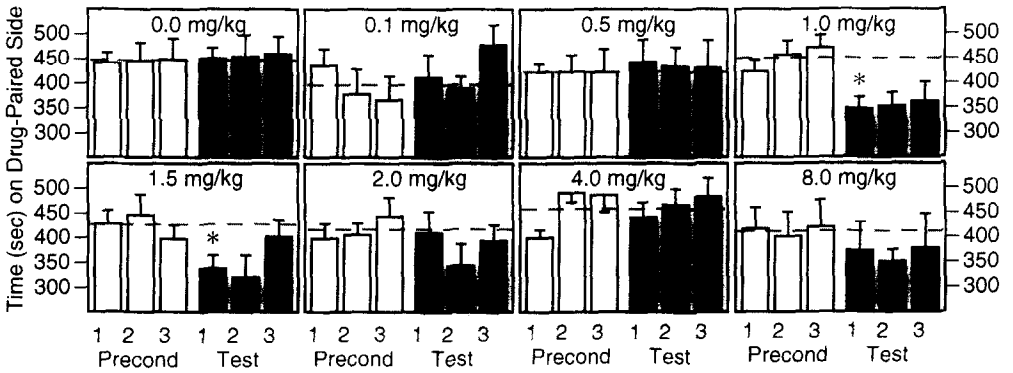

Fig. 2

Time (+SEM) in seconds spent on the drug-paired side for animals receiving THC (0.0-8.0 mg/kg). Data are shown for all three preconditioning and test sessions. The dashed line represents the mean of the three preconditioning sessions. *significantly different from the mean of the three preconditioning sessions, $p<0.05$.

In general, locomotor activity decreased as a function of time across all doses of THC (Fig. 3). Locomotor activity was not affected by the 0.0 or 0.1 mg/kg doses of THC, but all remaining doses produced a decrease lasting the entire 30-min session (Fig. 3). A treatment (drug or vehicle) by time (six 5-min bins) repeated measures ANOVA conducted at each dose resulted in a significant

main effect of time (bin) at all doses ($p<0.001$), confirming that locomotor activity decreased as a function of time regardless of treatment. The main effect of treatment was significant at the 1.0 [$F(1,19)=60.98$, $p<0.001$], 1.5 [$F(1,10)=13.24$, $p<0.005$], 2.0 [$F(1,9)=56.87$, $p<0.001$], and 4.0 [$F(1,7)=21.08$, $p<0.005$] mg/kg doses, demonstrating that these doses of THC decreased activity. The interaction was significant at the 1.0 [$F(5.95)=9.11$, $p<0.001$] and 1.5 [$F(5,50)=2.93$, $p<0.05$] mg/kg doses, reflecting the greater decrease in activity produced by THC early versus late in the session. For the 4.0 mg/kg dose, the interaction was significant without the epsilon correction, but not when it was applied.

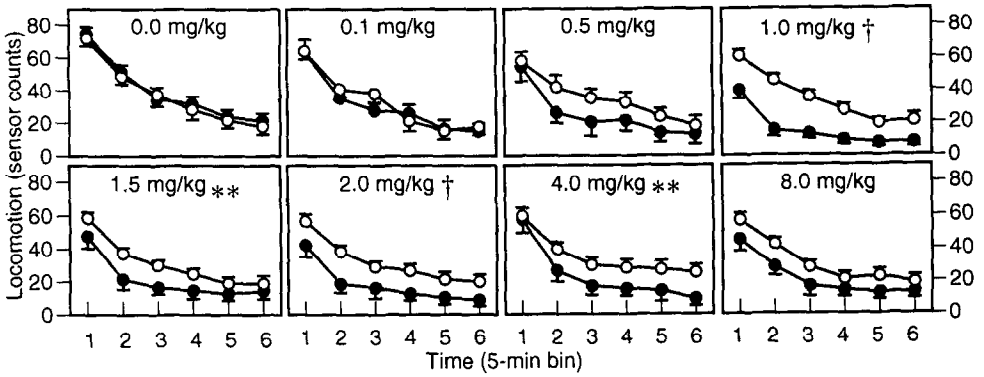

Fig. 3

Locomotor activity (number of sensor counts ± SEM) for rats receiving THC (0.0-8.0 mg/kg). Each graph represents the mean of the four drug-paired (filled circles) or vehicle-paired (open circles) conditioning sessions, plotted as a function of time (six blocks of 5-min each). The THC-induced decrease in locomotor activity was significant at the 1.0, 1.5, 2.0, and 4.0 mg/kg doses. †Significantly different from vehicle, $p<0.001$; **$p<0.01$.

Anandamide

The 0.031 and 16.0 mg/kg doses of anandamide appeared to produce a slight place aversion, while the 0.313 and 0.5 mg/kg doses appeared to produce a slight place preference (Fig. 4). However, none of the phase main effects, session main effects, or phase by session interactions were significant ($p>0.05$). In addition, none of the planned t-tests comparing the mean of the preconditioning sessions to the first test session were significant ($p>0.05$).

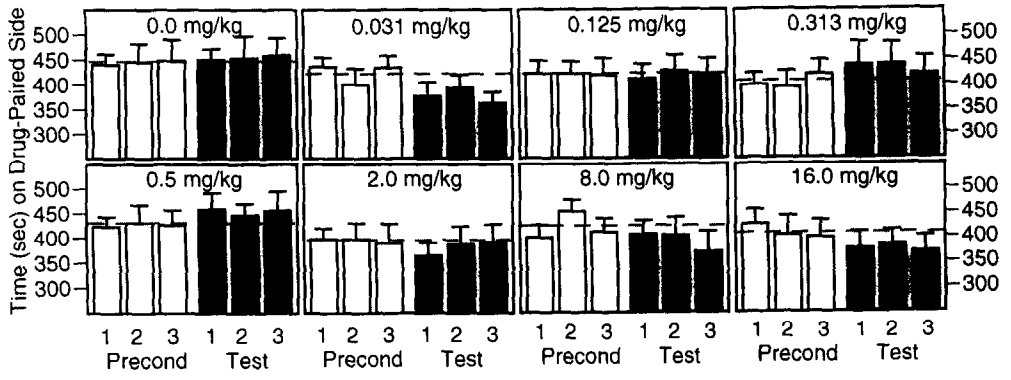

Fig. 4

Time (+SEM) in seconds spent on the drug-paired side for animals receiving anandamide (0.0-16.0 mg/kg). Data are shown for all three preconditioning and test sessions. The dashed line represents the mean of the three preconditioning sessions.

Locomotor activity decreased as a function of time, regardless of treatment (Fig. 5). The 0.031 mg/kg dose appeared to produce a slight increase, and 0.5 mg/kg appeared to produce a slight decrease in locomotor activity. Moreover, the 8.0 and 16.0 mg/kg doses appeared to produce a large depression of locomotor activity lasting the entire 30-min conditioning sessions (Fig. 5). A treatment (drug or vehicle) by time (six 5-min bins) repeated measures ANOVA conducted at each dose resulted in a significant main effect of time (bin) at all doses ($p<0.001$), confirming that activity decreased as a function of time regardless of treatment. The main effect of treatment and the treatment by bin interaction were significant at the 8.0 mg/kg dose [$F(1,9)=60.98$, $p<0.005$, and $F(5,45)=2.82$, $p<0.05$, respectively] and at the 16.0 mg/kg dose [$F(1,10)=56.44$, $p<0.001$, and $F(5,50)=5.98$, $p<0.005$, respectively], but not at any other dose. However, the interaction was not significant at the 8.0 mg/kg dose when the epsilon correction was applied.

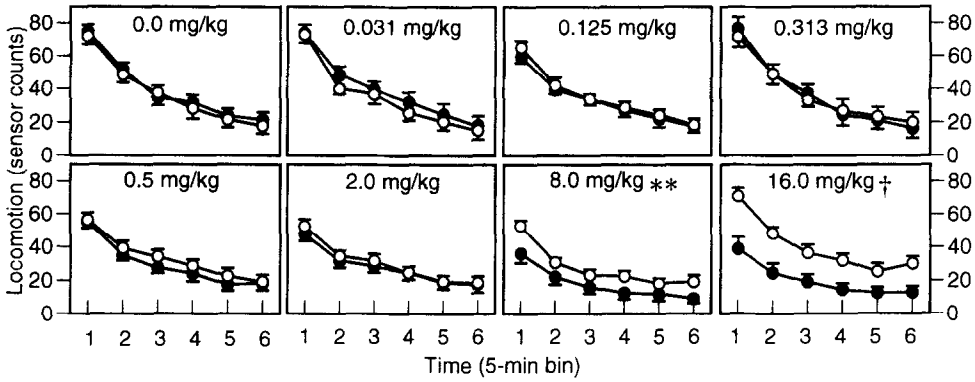

Fig. 5

Locomotor activity (number of sensor counts ± SEM) for rats receiving anandamide (0.0-16.0 mg/kg). Each graph represents the mean of the four drug-paired (filled cirlces) or vehicle-paired (open circles) conditioning sessions, plotted as a function of time (six blocks of 5-min each). The anandamide-induced decrease in locomotor activity was significant at the 8.0 and 16.0 mg/kg doses; †$p<0.005$, **$p<0.001$.

Discussion

The results can be summarized as follows: 1) THC produced a conditioned place avoidance, as indexed by a decrease in time spent in the drug-paired compartment from the preconditioning to test phases. 2) The administration of anandamide did not produce any significant effects on place conditioning. 3) Locomotor activity was significantly depressed by both THC and anandamide.

The present finding that THC produces place avoidance is in agreement with the THC results of Parker and Gillies (10) and Sañudo-Peña *et al.* (11), but not with those of Gardner *et al.* (12). One possible explanation for the discrepant results is that the establishment of cannabinoid-induced place conditioning is dependent on the strain or outbred stock of rat used. Thus, the only report of a cannabinoid-induced conditioned place preference to date used Long-Evans rats (12); conditioned place avoidance has been reported with Sprague-Dawley (10,11), Lewis (10) and Wistar rats (ref 13 and in the present investigation), suggesting that cannabinoid reinforcement may be genetically influenced. Although the place conditioning data suggest that Long-Evans rats may provide a better rat model of cannabinoid abuse, experiments using ICSS have found an enhancement suggesting a rewarding effect in Lewis rats (8,31), but not Long-Evans rats (7). Similarly, microdialysis experiments have found that accumbens dopamine release is enhanced by THC in Lewis (31) but not Long-Evans rats (32). Thus, genetic differences do not appear to account sufficiently for the conflicting results.

THC produced a place avoidance at the 1.0 and 1.5 mg/kg doses, but not at any others. Although it is unusual that lower, but not higher doses of THC produced significant place conditioning, it is possible that with the higher doses, rats were unable to form an association between the aversive

state (if they were in such a state) and the environmental stimuli of the conditioning chambers. This may have been due to either a drug-induced impairment of memory, or a drug-induced alteration in perception. Indeed, THC- or cannabis-induced memory impairment has been reported consistently with humans (see refs 33,34 for reviews) and animals (35-38). We have demonstrated that memory is impaired with doses of 2.0 mg/kg or higher, but not with lower doses of THC (22,39). Thus, the 2.0 mg/kg and higher doses of THC used here may have produced an aversive state, but because memory was impaired, a place avoidance may not have been found.

Locomotor activity decreased as a function of time, regardless of treatment, presumably due to a gradual decrease in exploratory behavior. In addition, the 0.5-4.0 mg/kg doses of THC, as well as the 8.0 and 16.0 mg/kg doses of anandamide produced a significant depression of locomotor activity, lasting the entire 30-min conditioning sessions. These results are in good agreement with previous reports of THC- (7,20,21) and anandamide-induced hypomotility (18-21,25), and demonstrate that THC and anandamide are similar in their effects on locomotion, though THC is more potent.

A recent study has shown that the administration of the cannabinoid CB_1 receptor antagonist SR141716A produces a place preference (11). This finding, together with the observation that THC produces place avoidance, has led to the speculation that endogenous cannabinoids serve to produce an aversive motivational or counter-reward state. The present finding that anandamide does not produce conditioned place avoidance does not support this suggestion.

The reasons that anandamide failed to produce place avoidance are unclear, but there are several possibilities. First, it has been shown that THC and anandamide are partial agonists (16,40,41) and this may result in their producing different effects. Thus, it is possible that anandamide and THC are dissimilar in their aversive and anxiogenic abilities. For example, THC produces anxiogenic effects in the elevated plus maze (42), but anandamide has no effect in the dark-light model of anxiety (19). Although it is unusual for an endogenous ligand to be a partial agonist, Mackie *et al.* (16) suggest that anandamide might serve to limit the actions of another endogenous cannabinoid that is a full agonist, or might be a more stable metabolite of this other endogenous ligand. Second, anandamide has been shown to be less potent than THC in its ability to produce many behavioral effects (*e.g.*, 20), a finding that was replicated with the observation of decreased locomotion following THC or anandamide in the present experiment. However, in our previous studies we have found that anandamide is more potent than THC in its effects on memory when rats are pretreated with 2.0 mg/kg PMSF, as was done in the present experiment. It is therefore possible that the minimum dose of anandamide required to produce place conditioning is sufficiently higher than the dose required to impair memory. Thus, an impairment of memory produced by anandamide may have overshadowed completely the establishment of place conditioning in the present investigation. Third, it is possible that anandamide was rapidly hydrolyzed and therefore was not available in a sufficient concentration to produce aversive effects. However, the locomotor activity data in the present study provide evidence against this possibility. That is, the present finding that the two highest doses of anandamide tested significantly depressed spontaneous locomotor activity over the entire 30-min conditioning sessions suggests that anandamide was not completely metabolized. Whether this was due to the attenuation of amidase activity by PMSF cannot be determined from these results.

Taken together, the present results suggest that endogenous cannabinoids do not serve as a natural counter-reward mechanism. The reasons underlying the differential effects of THC and anandamide in the place conditioning paradigm remain to be elucidated.

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