Behavioural Pharmacology (1996), 7, 276–284

The endogenous cannabinoid receptor agonist anandamide impairs memory in rats

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Anandamide was recently discovered to be an endogenous substance that acts as a partial agonist at cannabinoid receptors in the central nervous system. Because exogenous cannabinoids such as Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive ingredient of marijuana, have been found to impair memory, we undertook the present study to examine the mnemonic effects of anandamide. Memory was assessed in rats well-trained in a two-component instrumental discrimination task with a conditional discrimination to test reference memory and a delayed nonmatch-to-position to test working memory. Since anandamide has a short metabolic half-life, we examined the mnemonic effects of anandamide (0.0–2.0 mg/kg) in rats pretreated with the protease inhibitor phenylmethylsulfonyl fluoride (2.0 mg/kg), serving to increase the metabolic half-life of anandamide. Under these conditions, a dose-dependent impairment of the nonmatch-to-position, but not the conditional discrimination component, was found, closely resembling that observed following Δ^9-THC (0.0–4.0 mg/kg). This is the first report that anandamide impairs memory; results suggest that endogenous cannabinoids may be involved in cognitive processes influencing memory.

Keywords: Anandamide – Cannabinoids – Conditional discrimination – Δ^9-tetrahydrocannabinol – Nonmatch-to-position – Operant conditioning – Reference memory – Working memory

INTRODUCTION

Renewed interest in cannabinoid research has been fuelled by the recent isolation of anandamide (arachidonylethanolamide), the first of several naturally occurring ligands for the cannabinoid receptor (Devane *et al.*, 1992). This characterization was based on anandamide's ability to inhibit both the electrically-evoked twitch response of the mouse vas deferens and the specific binding of the radiolabelled cannabinoid probe [^{3}H]HU-243 to rat brain synaptosomal membranes (Devane *et al.*, 1992). Recent results suggest that anandamide may act as a partial agonist (Fride *et al.*, 1995). In vivo administration of anandamide mimics several of the behavioral and biological effects produced by Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive ingredient of marijuana, including analgesia, catalepsy, hypomotility, and hypothermia (Crawley *et al.*, 1993; Fride and Mechoulam, 1993; Smith *et al.*, 1994).

Our preliminary studies revealed that doses of anandamide as high as 8.0 mg/kg did not impair memory when administered alone (unpublished results). These results are consistent with recent findings from another laboratory demonstrating that anandamide does not impair choice accuracy in a delayed nonmatch-to-sample task using doses up to 10.0 mg/kg (Crawley *et al.*, 1993).

A recent study has shown that anandamide does not accumulate on receptors of cultured neuroblastoma and glioma cells since it is enzymatically degraded to arachidonic acid; this effect can be prevented by the protease inhibitor phenylmethylsulfonyl fluoride (PMSF) (Deutch and Chin, 1993). The same investigation identified anandamide amidase activity in brain homogenates, which may explain why anandamide alone does not impair memory.

It is well documented that the acute administration of Δ^9-THC or marijuana impairs memory in animals (Essman, 1984; Nakamura *et al.*, 1991; Heyser *et al.*, 1993) and in humans (for a review, see Miller and Branconnier, 1983); thus, the purpose of the present investigation was to assess the mnemonic properties of anandamide. It was expected that the administration of anandamide to rats pretreated with PMSF would impair working memory because the metabolic half-life of anandamide would be lengthened by PMSF. As a verification of the methods used here, the mnemonic effects of Δ^9-THC were also examined.

Memory was assessed using a two-component instrumental discrimination task that served to assess reference memory and working memory during the same session.

In the first component, a cued conditional discrimination, rats were required to press one of two levers, depending upon the presence of one of two stimuli. Once the rats had learned the relationship between the levers and the stimuli, that information could be used to make the correct choice on all subsequent trials; by definition, in trained animals this component required the use of reference memory (Honig, 1978). In the second component, a delayed nonmatch-to-position task (Dunnett, 1985), rats were required to press the lever opposite the one pressed in the first component. Even after learning this rule, a rat could only respond at a greater-than-chance level by remembering which of the two levers was pressed in the first component of that particular trial of the discrimination; by definition, this component required the use of working memory (Honig, 1978). The non-mnemonic demands of both components were identical; for example, each component required a lever press for food reward. Thus, a selective impairment in the second component could confidently be said to indicate impaired memory because an impairment of non-mnemonic abilities necessarily would reduce performance in both components (Mallet and Beninger, 1993).

METHOD

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

Subjects

Fifty-one experimentally naive male Wistar rats (20 for experiment 1 and 31 for experiment 2) weighing 200–250 g were housed individually in a temperature-controlled ($21 \pm 1°C$) room kept on a 12 h light–dark cycle (lights on at 07.00 h; experiment conducted 08.00 h–12.00 h). Rats were maintained at 85–90% of their free-feeding weights, adjusted for normal growth, by daily feedings with measured rations of dry laboratory chow (Purina Laboratory Rodent Chow #5001); water was available in the home cage at all times. Four rats (two from experiment 1 and two from experiment 2) were dropped from the study prior to any drug treatments due to poor performance.

Apparatus

Training and testing took place in four identical $20 \times 25 \times 30$ cm operant boxes. The front and rear walls of each box were constructed of stainless steel. The side walls and ceilings were constructed of clear plastic. Stainless steel bars, spaced 1 cm apart, made up the floors. Mounted on the center of each front panel, 3 cm above the floor, was a stainless steel food cup. A food dispenser (Ralph Gerbrands Company, model G5100) was connected to each food cup via a plastic hose and delivered 45 mg food pellets (Bioserv). Each food cup contained an infrared photo-emitter and an infrared photo-detector that could record when the rat's snout was placed in the feeder. Two retractable stainless steel levers (5 cm wide $\times$ 1 cm high) were mounted 3 cm to the left and right of each food cup and 6 cm above the floor. A light bulb (2 W) was mounted 13 cm above the floor, directly over the food cup. A speaker that could produce white noise (85 dB) was mounted in the center of the ceiling of each box. Operant chambers were housed in styrofoam-insulated sound-attenuating wooden boxes, in which a fan provided ventilation and masking noise (65 dB). A 7.5 W bulb was used to illuminate each chamber and remained lit at all times. Each box was controlled by a 6809 micro-controller using custom-made software (available upon request), written in ECBASIC version 2.06.

Drug preparation and administration

Δ^9-tetrahydrocannabinol (Health and Welfare Canada, > 98% purity), ethanol (95%) and Tween 80 were mixed, yielding a 3.0 ml etharol + 1.0 ml Tween 80 per 50.0 mg Δ^9-THC suspension. The suspension was stirred continuously under a stream of compressed nitrogen gas until all ethanol was evaporated. To promote evaporation, the suspension was bathed in warm water. Saline (0.9%) was then added resulting in a 4.0 mg Δ^9-THC/ml stock solution. All other concentrations were obtained by adding the appropriate amount of vehicle (1.0 ml Tween 80 per 11.5 ml saline) to a portion of stock Δ^9-THC solution. Solutions were frozen in aliquots at $-20°C$ until needed. Injections were administered i.p. in a volume of 1.0 ml/kg body weight 30 min prior to testing.

Phenylmethylsulfonyl fluoride (PMSF, Sigma) was dissolved in ethanol and diluted with distilled water, yielding a concentration of 2.0 mg/ml 30% ethanol. Due to its rapid breakdown in solution, PMSF was injected within 5 min of fresh preparation. Injections preceded anandamide administration by 35 min and were administered i.p. in a volume of 1.0 ml/kg body weight.

Anandamide (arachidonylethanolamide) was prepared and stored in a similar manner to Δ^9-THC, with the exception that the amounts used were 3.0 ml ethanol, 1.0 ml Tween 80, and 100.0 mg anandamide. Injections preceded testing by no more than 5 min and were administered i.p. in a volume of 1.0 ml/kg body weight.

Experiment 1

Each rat received 60 discrimination training trials per session, once per weekday. For the conditional discrimination component of each trial, either a visual (light) or auditory (white noise) stimulus was presented 1 s before both levers were extended. Pressing one lever, but not the other, resulted in the delivery of a food pellet. The correct lever depended upon which stimulus was present. For half the rats, pressing the right or left lever was correct when the visual or auditory stimulus was present, respectively; for the other half, the contingencies between the levers and the stimuli were reversed. The stimulus remained present, and the levers remained extended for a maximum of 30 s. If neither lever was pressed during this interval, both levers were retracted and the trial was scored as null. Once either the correct or incorrect lever was pressed, both levers were retracted. This signified the end of the conditional discrimination component and the beginning of the delayed nonmatch-to-position component of the trial. The nonmatch-to-position component was presented even if an incorrect response occurred in the conditional discrimination.

Following a 4 s delay, both levers were again extended, this time in the absence of the visual or auditory stimulus. Here, the lever opposite the one pressed during the conditional discrimination component resulted in the delivery of a food pellet, i.e. the rule for successful performance was nonmatch-to-position. Once either a correct or an incorrect lever was pressed, both were retracted, thus terminating the nonmatch-to-position component of the trial. A variable delay (range 8–12 s) elapsed before beginning the next trial, starting again with the conditional discrimination component. During each component, levers remained extended for a maximum of 30 s. If neither lever was pressed during this interval, both levers were retracted and the trial was scored as null. Null trials from the conditional discrimination and from the nonmatch-to-position were excluded from all analyses. Discrimination training continued until performance exceeded 85% correct on both components over three consecutive sessions.

Rats then received seven delay training sessions that were identical to training, with the addition of longer delays between the conditional discrimination and nonmatch-to-position components (4, 8, 12 and 16 s), serving to increase the mnemonic demands of the task. The order of delays was randomized such that each delay occurred once every four trials. Following the conclusion of delay training, drug testing began. Except for the drug injections, test sessions were identical to delay training sessions.

Drug testing consisted of six treatments in a counterbalanced order: no injection, vehicle alone, and 0.5, 1.0, 2.0 and 4.0 mg/kg Δ^9-THC. Drug test sessions were always separated by at least one non-drug session, identical to the delay training sessions.

Experiment 2

Two squads of rats were tested. The first squad ($n = 15$) was trained in the same manner as the rats used in experiment 1. The second squad ($n = 16$) was trained in the same manner as the first, with two small exceptions. To reduce the number of training sessions required to reach the acquisition criterion, each session consisted of 80 rather than 60 trials. In addition, nose-poke responses (breaking the beam in the food cup) were required before insertion of the levers, to prevent the animals from adopting a position habit during the retention intervals. Data from both squads were combined, since these slight differences in training and testing did not produce any significant differences in performance.

Following training, rats received 2.0 mg/kg PMSF before each of six treatments, given in a counterbalanced order prior to testing: no injection, vehicle alone, and 0.25, 0.5, 1.0 and 2.0 mg/kg anandamide. As a partial replication of experiment 1, rats from the second squad from experiment 2 also received 4.0 mg/kg Δ^9-THC, counterbalanced with anandamide treatments. As in experiment 1, drug test sessions were always separated by at least one non-drug session, identical to the delay training sessions.

RESULTS

Experiment 1

For the conditional discrimination, the percentage of correct choices following no injection or vehicle injection exceeded 90%. Following these two treatments, nonmatch-to-position performance was similar at each delay, but performance decreased as delays increased (Table I for no injection; Fig. 1C for vehicle). To assess the possibility that the injection procedure itself affected performance, a single-factor (treatment) repeated-measures

TABLE I. Percentage (±SEM) of correct choices in the conditional discrimination and nonmatch-to-position (at each delay interval) for the no-injection treatments from experiments 1 and 2.

	Experiment 1	Experiment 2
Conditional discrimination	93.78 ± 0.82	93.18 ± 0.78
Nonmatch-to-position		
4 s	84.00 ± 2.82	80.37 ± 2.42
8 s	80.00 ± 3.62	66.05 ± 3.28
12 s	74.22 ± 4.73	64.82 ± 2.54
16 s	66.67 ± 4.74	56.79 ± 2.63

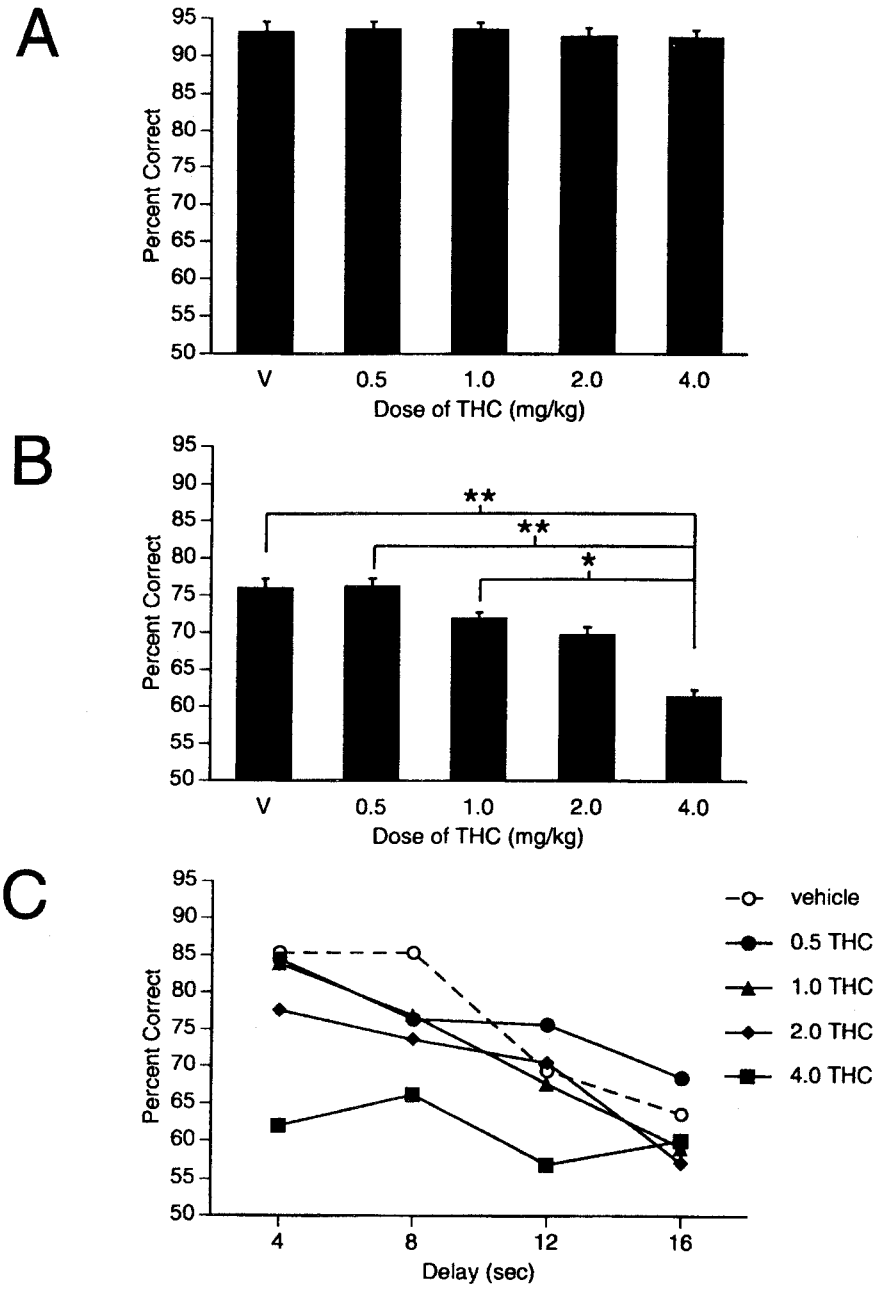

FIG. 1. A. Mean (+SEM) percentage of correct choices in the conditional discrimination task for vehicle injection (V) and four doses of Δ^9-THC. B. Mean (+SEM) percentage of correct choices in the nonmatch-to-position task for vehicle injection (V) and four doses of Δ^9-THC. Each bar represents the mean of all delay intervals at that particular dose: $^*p < 0.05$, $^{**}p < 0.01$. C. Mean percentage of correct choices in the nonmatch-to-position task at each of the four delay intervals (4, 8, 12 and 16 s) for vehicle and all doses of Δ^9-THC.

analysis of variance (ANOVA) was conducted on the percentage of correct choices in the conditional discrimination for no-injection and vehicle treatments. For the same two treatments, a two-factor (treatment by delay) ANOVA with both factors repeated was conducted on the percentage of correct choices in the nonmatch-to-position. For the conditional discrimination, the treatment main effect was not significant [$F(1,14) < 1.0$, NS]. For the nonmatch-to-position, the treatment main effect [$F(1,14) < 1.0$, NS] and the interaction [$F(3,42) = 1.59$, NS] were not significant. As expected, a significant main effect of delay was found [$F(3,42) = 20.44$, $p < 0.001$].

Since the treatment main effects were not significant for either component, and the interaction was not significant for the nonmatch-to-position component, and because the vehicle injections more closely resembled drug treatments, the no-injection condition was dropped from all subsequent analyses.

Performance in the conditional discrimination was not affected by Δ^9-THC, and was highly accurate at all doses tested (Fig. 1A). However, Δ^9-THC produced a dose-dependent impairment of choice accuracy in the nonmatch-to-position (Fig. 1B). At the highest dose tested (4.0 mg/kg), nonmatch-to-sample choice accuracy was severely impaired at all delays. Nonmatch-to-position choice accuracy decreased as delay increased following all other treatments (Fig. 1C). To evaluate the mnemonic effects of Δ^9-THC, a single-factor (treatment) repeated-measures ANOVA was conducted on the percentage of correct choices in the conditional discrimination for vehicle injection and all doses of Δ^9-THC. For the same treatments, a two-factor (treatment by delay) ANOVA with both factors repeated was conducted on the percentage of correct choices in the nonmatch-to-position at each of the four delays. For the conditional discrimination, the treatment main effect was not significant [$F(4,56) < 1.0$, NS] (Fig. 1A). For the nonmatch-to-position, the treatment main effect [$F(4,56) = 5.20$, $p < 0.005$], delay main effect [$F(3,42) = 24.46$, $p < 0.001$] and the interaction [$F(12,168) = 2.26$, $p < 0.05$] were significant (Fig. 1B and 1C). *Post-hoc* Tukey tests, comparing the percentage of correct choices in the nonmatch-to-position for vehicle injection and all doses of Δ^9-THC to one another, revealed that the 4.0 mg/kg dose was significantly different from vehicle injection ($p < 0.01$), 0.5 mg/kg Δ^9-THC ($p < 0.01$) and 1.0 mg/kg Δ^9-THC ($p < 0.05$). None of the other comparisons was significant ($p > 0.05$; Fig. 1B). Thus, the administration of Δ^9-THC produced a dose-dependent and selective impairment of performance in the nonmatch-to-position task. *Post-hoc* Tukey tests, comparing nonmatch-to-position performance at each delay to all other delays, indicated that the 16 s delay was significantly different from the 4 and 8 s delays ($p < 0.01$), the 12 s delay was significantly different from the 4 s delay ($p < 0.01$), and the 12 s delay was significantly different from the 8 and 16 s delays ($p < 0.05$). Tests of the simple effects on the treatment by delay interaction revealed a significant effect of treatment at the 4 s [$F(4,56) = 6.79$, $p < 0.001$], 8 s [$F(4,56) = 4.01$, $p < 0.01$] and 12 s [$F(4,56) = 3.23$, $p < 0.05$] delays. The treatment simple effect was not significant at the 16 s delay [$F(4,56) = 1.56$, NS] (Fig. 1C). Very few null trials occurred in experiment 1 (data not shown). Repeated measures ANOVAs comparing the number of null trials at each dose of Δ^9-THC yielded no significant effects ($p > 0.05$).

Experiment 2

The percentages of correct choices for the conditional discrimination and the nonmatch-to-position following no injection or vehicle injection were similar to those observed in experiment 1 (above). For the conditional discrimination, choice accuracy was highly accurate ($> 90\%$ correct) for no injection and vehicle injection. Nonmatch-to-position performance was similar at each delay for no injection and vehicle, but choice accuracy decreased as delays increased (Table I for no injection; Fig. 2C for vehicle). To assess the possibility that the injection procedure itself affected performance, a single-factor (treatment) repeated-measures ANOVA was conducted on the percentage of correct choices in the conditional discrimination for no injection and vehicle treatments. For the same two treatments, a two-factor (treatment by delay) ANOVA with both factors repeated was conducted on the percentage of correct choices in the nonmatch-to-position. The treatment main effect was not significant for the conditional discrimination [$F(1,26) < 1.0$, NS]. For the nonmatch-to-position, the treatment main effect [$F(1,26) = 3.45$] and the interaction [$F(3,78) = 1.24$] were not significant ($p > 0.05$); as expected, a significant main effect of delay was found [$F(3,78) = 32.79$, $p < 0.001$]. As in experiment 1, the no-injection condition was dropped from all subsequent analyses.

Performance in the conditional discrimination was highly accurate and was not affected by anandamide at any of the doses tested (Fig. 2A). However, anandamide produced a dose-dependent impairment of choice accuracy in the nonmatch-to-position (Fig. 2B). In addition, choice accuracy in the nonmatch-to-position decreased as delay increased following all treatments. To evaluate the mnemonic effects of anandamide, a single-factor (treatment) repeated-measures ANOVA was conducted on the percentage of correct conditional discrimination choices for vehicle injection and all doses of anandamide. For the same treatments, a two-factor (treatment by delay) ANOVA with both factors repeated was conducted on the percentage of correct choices in the nonmatch-to-position at each of the four delays. For the conditional discrimination, the treatment main effect was not significant [$F(4,104) < 1.0$, NS] (Fig. 2A). For the nonmatch-to-position, the treatment main effect [$F(4,104) = 5.17$, $p < 0.005$] (Fig. 2B) and the delay main effect [$F(3,78) = 37.89$, $p < 0.001$], were significant; however, the interaction was not significant [$F(12,312) = 1.00$, NS] (Fig. 2C). *Post-hoc* Tukey tests comparing the vehicle injection and all doses of anandamide to one another revealed that the vehicle injection differed significantly from the 1.0 and 2.0 mg/kg doses ($p < 0.05$). In addition, the 0.5 mg/kg dose differed significantly from the 2.0 mg/kg dose ($p < 0.01$). None of the other compari-

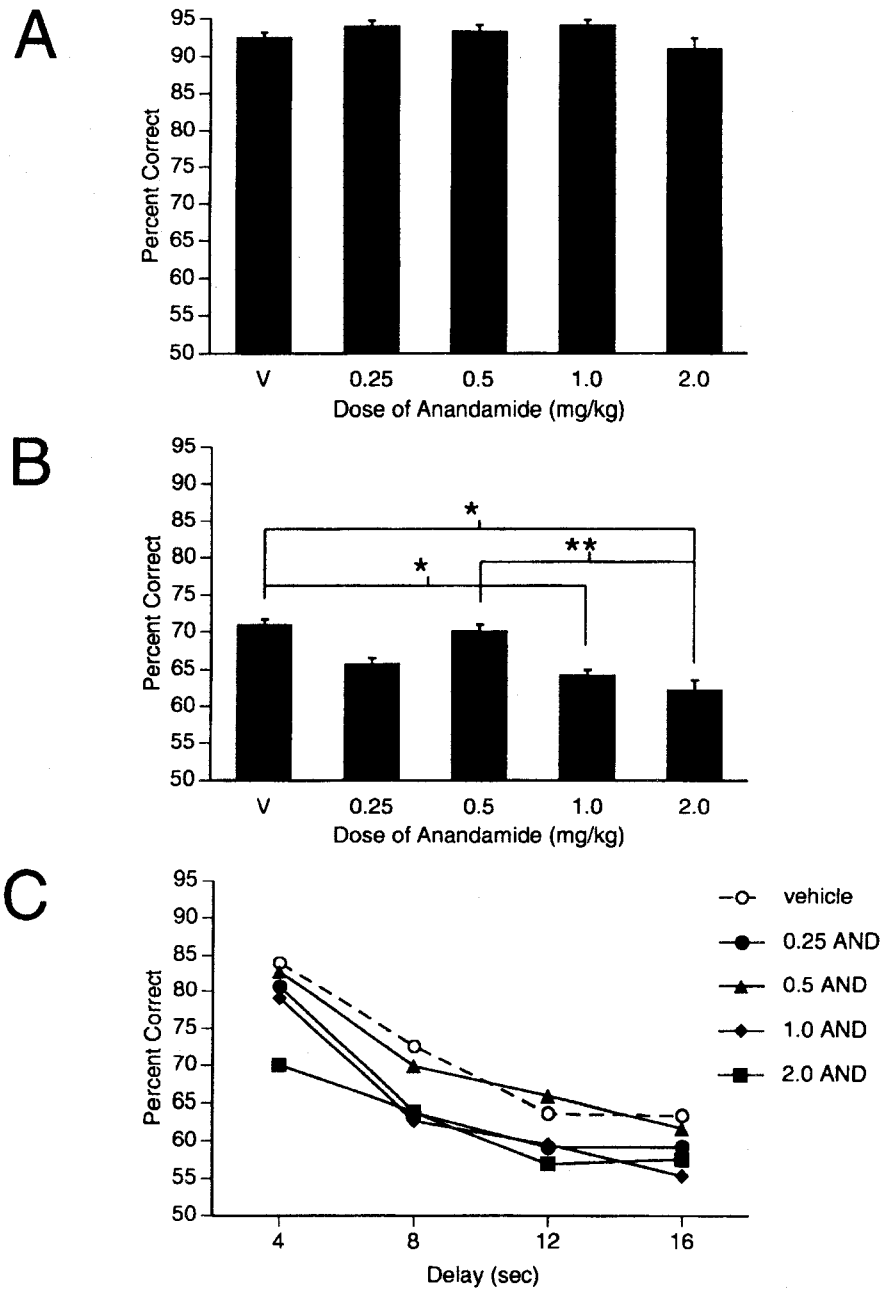

FIG. 2. A. Mean (+SEM) percentage of correct choices in the conditional discrimination task for vehicle injection (V) and four doses of anandamide. B. Mean (+SEM) percentage of correct choices in the nonmatch-to-position task for vehicle injection (V) and four doses of anandamide. Each bar represents the mean of all delay intervals at that particular dose: $^{*}p < 0.05$, $^{**}p < 0.01$. C. Mean percentage of correct choices in the nonmatch-to-position task at each of the four delay intervals (4, 8, 12 and 16 s) for vehicle and all doses of anandamide.

sons was significant ($p > 0.05$; Fig. 2B). Thus, the administration of anandamide produced a dose-dependent and selective impairment of performance in the nonmatch-to-position. *Post-hoc* Tukey tests comparing nonmatch-to-position performance at each delay to that at all other delays indicated that the 4 s delay was significantly different from the 8, 12, and 16 s delays ($p < 0.01$), the 8 s delay was significantly different from the 16 s delay ($p < 0.01$), and the 8 s delay was significantly different from the 12 s delay ($p < 0.05$).

Δ^9-THC (4.0 mg/kg) was included as a treatment in the second squad from experiment 2 to partially replicate

TABLE 2. Percentage (±SEM) of correct choices in the conditional discrimination and nonmatch-to-position (at each delay interval) for the vehicle injection and Δ^9-THC treatments from the second squad in experiment 2.

	Vehicle	Δ^9-THC
Conditional discrimination	95.73±0.95	93.85±1.67
Nonmatch-to-position		
4 s	90.42±2.08	75.83±6.39
8 s	73.75±3.65	67.08±4.62
12 s	64.17±3.93	55.83±5.25
16 s	62.92±3.23	55.42±3.56

experiment 1. As in experiment 1, this dose did not affect performance in the conditional discrimination, but impaired performance in the nonmatch-to-sample (Table II). To evaluate the mnemonic effects of the Δ^9-THC in experiment 2, a single-factor (treatment) repeated-measures ANOVA was conducted on the percentage of correct choices in the conditional discrimination for vehicle injection and 4.0 mg/kg Δ^9-THC. For the same treatments, a two-factor (treatment by delay) ANOVA with both factors repeated was conducted on the percentage of correct choices in the nonmatch-to-position at each of the four delays. Data from one rat were excluded from the analysis due to a lack of responding during the drug test. For the conditional discrimination, the treatment main effect was not significant [$F(1,11) < 1.0$, NS]. For the nonmatch-to-position, the treatment main effect [$F(1,11) = 5.05$, $p < 0.05$] and the delay main effect [$F(3,33) = 19.08$, $p < 0.001$] were significant; however, the interaction was not significant [$F(3,33) < 1.0$, NS]. Thus, performance in the nonmatch-to-position was selectively impaired by 4.0 mg/kg Δ^9-THC, partially replicating the results of experiment 1 (above). Tukey tests, comparing nonmatch-to-position performance at each delay to all other delays indicated that the 4 s delay was significantly different from the 8, 12 and 16 s delays ($p < 0.01$), and that the 8 s delay was significantly different from the 12 and 16 s delays ($p < 0.05$). Very few null trials occurred in experiment 2 (data not shown). Repeated-measures ANOVAs comparing the number of null trials at each dose of anandamide and Δ^9-THC yielded no significant effects ($p > 0.05$).

DISCUSSION

The present results suggest that endogenous cannabinoids may influence neuronal activity that mediates memory. Thus, systemically administered anandamide, like Δ^9-THC, significantly impaired performance in a nonmatch-to-position task at the highest dose tested. In contrast to these findings with nonmatch-to-position, anandamide and Δ^9-THC did not affect performance in a conditional discrimination task. This is the first report of impaired memory produced by a naturally occurring cannabinoid receptor ligand.

The finding that Δ^9-THC produced a mnemonic deficit in the present investigation is in agreement with other reports demonstrating that marijuana or Δ^9-THC impairs memory in animals (Essman, 1984; Heyser *et al.*, 1993) and in humans (for a review, see Miller and Branconnier, 1993). These results are also consistent with those of Nakamura *et al.* (1991), who demonstrated that Δ^9-THC produces an impairment of working memory, but not reference memory, in the radial maze.

The nonmatch-to-position component of the task employed here was similar to that used by Dunnett (1985) to evaluate working memory in rats, using an automated lever-press procedure. In that task, a number of treatments including Δ^9-THC (Heyser *et al.*, 1993), systemic scopolamine (Dunnett, 1985), intra-medial prefrontal cortex scopolamine (Broersen *et al.*, 1994), as well as fimbria-fornix transection (Dunnett, 1985), produce progressively greater impairments as the delay between the sample stage and test stage is increased. In our studies of Δ^9-THC and anandamide, we failed to observe comparable delay-dependent effects. It is possible that a delay-dependent impairment produced by Δ^9-THC, similar to that observed by Heyser *et al.* (1993), could have been found if a zero-second delay interval had been used. However, achieving a true zero delay is problematic (as discussed in Beninger *et al.*, 1989) because the movement of the levers is not instantaneous and a period of time inevitably must elapse between the presentation of the sample and the subject's response. Moreover, in traditional delayed matching and nonmatching tasks, a delay-dependent decrease in performance must be demonstrated to ensure that performance deficits are due to impaired memory. Rather than using a zero-delay condition, we modified the task to include a conditional discrimination, requiring the use of reference memory, at the sample stage. Both tasks required a discriminated choice between two levers and a lever-press response, and both tasks were motivated by food reward. If cannabinoid treatments affected sensory/perceptual abilities, motor capacity or the motivation to eat, performance of each component might have been expected to be affected. Using this procedure, we showed that cannabinoids at high but not low doses selectively impaired working memory, as indexed by impaired performance in the nonmatch-to-position component. The observation of a differential impairment of the second component leads us to conclude that Δ^9-THC and anandamide selectively affected memory.

Response latencies were very short, regardless of drug treatment, ranging on average between 0.4 and 1.1 s in

experiment 1, and 0.5 to 1.6 s in experiment 2 (data not shown). Inspection of Fig. 1C and 2C clearly shows that these short response latencies cannot account for the observed deficits in performance. In addition, one dose of anandamide (1.0 mg/kg) produced a significant impairment of performance in the delayed nonmatch-to-position task, but did not lengthen response latencies.

The observation that baseline performance of trained rats was better on the first (conditional discrimination) than the second (nonmatch-to-position) component, in spite of these components making similar demands on non-mnemonic capacities such as sensory/perceptual ability and motor performance, suggests that the second component was more mnemonically demanding. This conclusion finds further support in the observation of significant delay effects on the second component, and validates the second component as a working memory task (Honig, 1978). Nevertheless, one alternative interpretation of the present results is that they reflect the differential difficulty of the two components and not their differing mnemonic demands. This alternative cannot be ruled out from the present results, and its evaluation would require the development of a task with equal baseline performance on the two components that differ only in their mnemonic demands. We know of one report of such a task (Hepler *et al.*, 1985) in which the authors found that ibotenic acid lesions of the nucleus basalis magnocellularis impaired working memory, but not reference memory, in a T-maze task. This result was in good agreement with the findings of our studies (Biggan *et al.*, 1991; Ingles et al., 1993; Beninger *et al.*, 1994; Mallet *et al.*, 1995) using a double Y-maze in which the baseline performance on the reference memory component was better than performance on the working memory component. Results suggest that differential difficulty of working memory and reference memory components of tasks like the one used here probably do not account for the observation of selective drug effects on the working memory component (cf. Knowlton *et al.*, 1985).

It is of interest to note that the 0.25 mg/kg dose of anandamide from experiment 2 appeared to produce a greater impairment of performance in the nonmatch-to-position component than did the 0.5 mg/kg dose (Fig. 2B), although this result did not achieve statistical significance. As described earlier, subjects from experiment 2 were trained and tested in two squads, using a slightly different method for each run. The greater impairment produced by 0.25 mg/kg than by 0.5 mg/kg was observed in the first run and replicated in the second (data not shown). At present, possible mechanisms for this result remain unclear.

Although the neural substrates underlying cannabinoid ligand-induced memory impairments remain unknown, the large number of cannabinoid receptors that are found in various regions of the hippocampus (Herkenham *et al.*, 1990, 1991) suggests that this is a possible site where the mnemonic effects of anandamide are produced. Supporting this notion is the finding that hippocampal cell discharge during the sample phase of a delayed matching-to-sample task is reduced in rats following Δ^9-THC administration (Heyser *et al.*, 1993). Experiments are planned in our laboratory with the goal of identifying the mnemonic effects of intra-hippocampal micro-infusions of anandamide and other cannabinoid receptor ligands such as Δ^9-THC. The present findings raise the exciting possibility that specific antagonists acting at the cannabinoid receptor such as the recently developed compound SR141716A (Rinaldi-Carmona *et al.*, 1994) may lead to novel pharmacotherapeutics for the treatment of debilitating memory disorders.

Acknowledgements

We thank Health and Welfare Canada and Raphael Mechoulam for their generous gifts of Δ^9-THC and anandamide, respectively, as well as Stephen Fergusson and Bill Hilhorst for their excellent technical assistance in building the operant chambers. We also thank Barrie J. Frost, Khem Jhamandas, Raphael Mechoulam, Vernon L. Quinsey and Ronald G. Weisman for their helpful comments on an earlier draft of this manuscript. This research was supported by a grant from the Natural Science and Engineering Research Council of Canada to R.J. Beninger.

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(Received 19 June 1995; accepted as revised 9 January 1996)