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ORIGINAL INVESTIGATION

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The cannabinoid CB_1 receptor antagonist SR141716A attenuates the memory impairment produced by Δ^9-tetrahydrocannabinol or anandamide

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Abstract The administration of Δ^9-tetrahydrocannabinol (THC), the principle psychoactive ingredient in marijuana, or the endogenous cannabinoid anandamide, has been shown to impair recent memory. The purpose of the present investigation was to determine if the cannabinoid CB_1 receptor antagonist SR141716A could attenuate THC- or anandamide-induced memory impairment, and to assess the effects on memory of SR141716A alone. Memory was assessed in rats well-trained in a two-component instrumental discrimination task, consisting of a conditional discrimination, and a non-match-to-position to assess recent or working memory. SR141716A (0.0–2.0 mg/kg) had no effect on either the conditional discrimination or the non-match-to-position. However, SR141716A (0.0–2.0 mg/kg) attenuated the memory impairment produced by THC (2.0 or 4.0 mg/kg) as indexed by an enhancement of performance in the non-match-to-position. When administered to rats pretreated with anandamide (2.0 mg/kg), SR141716A (0.0–2.5 mg/kg) impaired performance in the conditional discrimination at the highest dose. This was interpreted as a deficit in some capacity unrelated to memory (e.g., motor impairment). However, lower doses of SR141716A (0.1 and 0.5 mg/kg) attenuated the anandamide-induced impairment of performance in the non-match-to-position without affecting the conditional discrimination. This is the first report that the memory impairment produced by anandamide can be attenuated by a cannabinoid antagonist; results suggest that anandamide-induced memory disruption is mediated by CB_1 receptors.

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Introduction

It is well established that marijuana or its principle psychoactive ingredient, Δ^9-tetrahydrocannabinol (THC), impairs memory in animals (Essman 1984; Nakamura et al. 1991; Heyser et al. 1993) and in humans (for review, see Miller and Branconnier 1983). Similarly, the synthetic cannabinoids CP-55,940 or WIN-55,212-2 impair memory in rats (Lichtman et al. 1995).

Two subtypes of cannabinoid receptors have been identified. CB_1 receptors are found primarily in the central nervous system, with the highest concentrations in the cerebellum, hippocampus, and basal ganglia (Herkenham et al. 1990, 1991; Jansen et al. 1992; Mailleux and Vanderhaeghen 1992; Petitet et al. 1996). The CB_2 receptor is expressed in the periphery and appears to be involved in modulation of the immune system (Munro et al. 1993). SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] is a highly potent and selective CB_1 receptor antagonist (Rinaldi-Carmona et al. 1994). It reverses many of the biochemical, physiological and behavioral effects of cannabinoid receptor agonists (Rinaldi-Carmona et al. 1994; Compton et al. 1996; Mansbach et al. 1996; McGregor et al. 1996), including the inhibition of hippocampal long-term potentiation produced by WIN-55,212-2 (Terranova et al. 1995). In addition, SR141716A attenuates the memory disruptive effects of THC (Lichtman and Martin 1996), suggesting that the action of THC is receptor-mediated.

Although it was postulated that SR141716A would enhance memory, results have been equivocal. Thus, Terranova et al. (1996) demonstrated facilitation of memory with SR141716A, using a social recognition test in rats. However, SR141716A had no effect in pigeons trained

in an operant task reported to be sensitive to other memory-enhancing pharmacological agents (Mansbach et al. 1996), and failed to affect memory in a radial maze task (Lichtman et al. 1996). Thus, the first objective of the present investigation was to further examine the mnemonic effects of SR141716A.

Anandamide (arachidonylethanolamide) is a putative endogenous cannabinoid ligand (Devane et al. 1992) which binds to both CB_1 and CB_2 receptors (Howlett 1995). First isolated from porcine brain (Devane et al. 1992) and recently in human and rat brain and peripheral tissues (Felder et al. 1997), anandamide displaces binding of the radiolabeled cannabinoid probes [^{3}H]HU-243 and [^{3}H]CP-55,940 (Devane et al. 1992). Anandamide also inhibits N-type calcium channels (Mackie et al. 1993) and adenylate cyclase (Vogel et al. 1993). It produces many of the behavioral and physiological effects of other cannabinoids such as hypothermia, hypomotility, catalepsy and antinociception (Crawley et al. 1993; Fride and Mechoulam 1993; Smith et al. 1994; Romero et al. 1995). We recently have shown that both THC and anandamide impair memory in a delayed non-match-to-position task (Mallet and Beninger 1996). However, the contribution of cannabinoid receptors remained to be demonstrated. Thus, the second objective of the present study was to determine if the memory disruptive effect of anandamide can be attenuated by SR141716A. For comparison, the effects of SR141716A on THC-induced memory impairment were also examined.

Anandamide is highly susceptible to metabolic degradation (Deutch and Chin 1993). The protease inhibitor phenylmethylsulfonyl fluoride (PMSF) is a potent inhibitor of the hydrolysis of anandamide (Abadji et al. 1994; Pertwee et al. 1995). In previous studies, anandamide did not impair memory in rats when administered alone (Crawley et al. 1993; Mallet and Beninger 1996), but produced a dose-dependent impairment of memory when rats were pretreated with PMSF (Mallet and Beninger 1996). Thus, to prevent anandamide's rapid hydrolysis, PMSF was administered prior to anandamide treatment in the present study.

Memory was assessed in a two-component instrumental discrimination task with reference and working memory components in the same session. In the cued conditional discrimination, rats pressed one of two levers, depending upon the presence of one of two stimuli. Once the rats had learned this relationship, that information could be used to make the correct choice on all subsequent trials. By definition, in trained animals this component required reference memory (Honig 1978). In the delayed non-match-to-position task (Dunnett 1985), rats pressed the lever opposite the one pressed in the first component. Even after learning this rule, a rat could respond at a greater-than-chance level only by remembering which of the two levers was pressed in the first component of that particular trial. By definition, this required working memory (Honig 1978). The advantage of this task over others used to assess memory in rats is that it allows effects on working memory to be assessed independently from other mnemonic or non-mnemonic effects. Thus, a change in the ability to remember the rules of the task (i.e., reference memory) or an alteration of one or more non-mnemonic abilities (e.g., sensorimotor abilities, perception, motivation) would affect performance in both the conditional discrimination and the non-match-to-position tasks. On the other hand, an effect specific to recent or working memory would affect performance in the non-match-to-position only.

Hypotheses were as follows: THC and anandamide will replicate our earlier findings of impaired working memory (Mallet and Beninger 1996). SR141716A will attenuate the THC- and anandamide-induced impairments. SR141716A alone will produce a dose-dependent enhancement of working memory.

Materials and methods

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-six (16, 20 and 20 for experiments 1, 2, and 3, respectively) experimentally naive male albino Wistar rats (Charles River Canada), weighing 200–250 g upon arrival to the colony, were housed individually in a temperature-controlled (21°C) room, kept on a 12-h light-dark cycle (lights on at 0700 hours). 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.

Apparatus

Training and testing took place in four identical 20×25×30 cm operant boxes. The side walls and ceilings were constructed of clear Plexiglas and the front and rear walls of stainless steel. The floors were made of stainless steel bars spaced 1 cm apart. A food cup was mounted on the center of each front panel, 3 cm above the floor. 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 detector that could record when the rat's snout was in the feeder. Two retractable stainless steel levers (5 cm wide×1 cm high) extended 2 cm from the wall and were mounted 6 cm above the floor and 3 cm to the left and right of each food cup. 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 while the animals were in the operant boxes. Each box was controlled by a 6809 experiment controller (Walter and Palya 1984).

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). The suspension was stirred continuously under a stream of nitrogen gas until all ethanol was evaporated. 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 frozen at –20°C until needed. Injections were administered 30 min prior to testing.

Phenylmethylsulfonyl fluoride (PMSF, Sigma) was dissolved in absolute ethanol and diluted with distilled water yielding a concentration of 2.0 mg PMSF/ml 30% ethanol. Due to its rapid breakdown in solution, PMSF was injected within 3 min of fresh preparation. PMSF injections preceded anandamide administration by 35 min.

Anandamide (RBI) 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 testing by no more than 5 min. SR141716A (Sanofi Recherche, Montpellier, France) was dissolved in absolute ethanol and then prepared and stored in a similar manner to anandamide and THC. As in the preparation of THC, the ethanol was evaporated from both the anandamide and SR141716A solutions. In experiment 1, SR141716A was injected 30 min prior to testing. In experiment 2, SR141716A and THC were administered as a cocktail 30 min prior to testing. All drugs were administered IP in a volume of 1.0 ml/kg body weight.

General training procedure

Rats received 5 g Bioserv food pellets in the home cage for 2 consecutive days. On the following day, rats were placed in the operant chambers with the levers retracted and received 50 food pellets delivered automatically to the food cup from the food dispenser. Over the next 2 days, the lever press response was shaped. Formal training began the next day (experimental sessions conducted 0800–1600 hours). Each rat received 80 discrimination training trials per session. For the conditional discrimination, 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. 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 non-match-to-position component. The non-match-to-position component was presented even if an incorrect response occurred in the conditional discrimination.

Following a 4-s delay, both levers again were 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 non-match-to-position. Once either a correct or incorrect lever was pressed, both were retracted, thus terminating the non-match-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 non-match-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 five delay training sessions that were identical to discrimination training, with the addition of longer delays between the conditional discrimination and non-match-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. The order of drug treatments was counterbalanced. Between drug treatments, rats received one retraining session per day, identical to the initial training sessions, until performance exceeded 85% correct on both components of the task for 2 consecutive days.

Table 1 Drug treatment summary, and mean number of null trials for all three experiments. *SR* SR141716A; *AEA* arachidonylethanolamide (anandamide)

	Drug 1(mg/kg)	Drug 2(mg/kg)	Null trials
Experiment 1	0.0 SR	–	0.06
	0.5 SR	–	0.11
	0.1 SR	–	0.00
	1.0 SR	–	0.11
	2.0 SR	–	0.00
Experiment 2	0.0 SR	0.0 THC	0.00
	0.0 SR	2.0 THC	7.23
	0.1 SR	2.0 THC	3.69
	0.5 SR	2.0 THC	2.00
	1.0 SR	2.0 THC	2.62
	2.0 SR	2.0 THC	0.38
	0.0 SR	4.0 THC	24.46*
	0.1 SR	4.0 THC	21.85*
	0.5 SR	4.0 THC	17.31
	1.0 SR	4.0 THC	3.08
	2.0 SR	4.0 THC	2.23
Experiment 3	0.0 SR	0.0 AEA	0.00
	0.0 SR	2.0 AEA	1.75
	0.1 SR	2.0 AEA	1.05
	0.5 SR	2.0 AEA	2.10
	2.5 SR	2.0 AEA	9.60**

* Significantly different from vehicle alone (0.0 SR+0.0 THC)
** Significantly different from vehicle alone (0.0 SR+0.0 AEA)

Experiment 1

The effects of SR141716A alone on memory were investigated. Rats (n=20) were trained as described above, and then received five drug treatments: 0.0, 0.05, 0.1, 1.0 and 2.0 mg/kg SR141716A. Each rat received each treatment but the order was varied from rat to rat such that every treatment appeared in each ordinal position for at least one rat. SR141716A was postulated to enhance working memory, as indexed by a selective increase in response accuracy in the non-match-to-position.

Experiment 2

The purpose of experiment 2 was to examine the ability of SR141716A to attenuate the memory impairment produced by 2.0 and 4.0 mg/kg THC. Animals (n=16) were trained as described above, and then received vehicle alone, 2.0 mg/kg THC for five treatments, and 4.0 mg/kg THC for five treatments. Each of the five treatments of 2.0 and 4.0 mg/kg THC was co-administered

with one of five doses of SR141716A (0.0, 0.05, 0.1, 1.0 and 2.0 mg/kg). The order of SR141716A doses was as described for experiment 1 (see Table 1 for treatment summary).

THC was postulated to produce a selective impairment in the non-match-to-position but not the conditional discrimination component. Moreover, SR141716A was postulated to attenuate the THC-induced memory impairment.

Experiment 3

Experiment 3 examined the ability of SR141716A to attenuate the memory impairment produced by 2.0 mg/kg anandamide. Rats (n=20) were trained as described above, with one small exception. To reduce the number of days required to complete the experiment, each rat received two training sessions per day instead of one. Rats then received five drug treatments: (1) 0.0 mg/kg SR141716A+0.0 mg/kg anandamide, (2) 0.0 mg/kg SR141716A +2.0 mg/kg anandamide, (3) 0.1 mg/kg SR141716A+2.0 mg/kg anandamide, (4) 0.5 mg/kg SR141716A+2.0 mg/kg anandamide, (5) 2.5 mg/kg SR141716A+2.0 mg/kg anandamide (see Table 1 for treatment summary). To slow the hydrolysis of anandamide (Deutch and Chin 1993; Pertwee et al. 1995), all treatments were preceded by 2.0 mg/kg of the protease inhibitor phenylmethylsulfonyl fluoride (PMSF).

Retraining between drug sessions was slightly modified to reduce the number of days required to complete the experiment. Rats received one retraining session between drug treatments, identical to the initial training sessions. If the training criteria were met (85% correct in both components) on the session immediately following a drug treatment, the next drug treatment was administered on the following day. However, if the training criteria were not met on the first session, training continued until the criteria were met over two successive sessions. When a drug treatment occurred during the first session of the day, a second session was not given to that animal on that day. All drug treatments were separated by at least 48 h.

Anandamide was expected to impair non-match-to-position performance, as indexed by poorer performance in the 0.0 mg/kg SR141716A+2.0 mg/kg anandamide treatment relative to the 0.0 mg/kg SR141716A+0.0 mg/kg anandamide treatment. In addition, it was postulated that SR141716A would attenuate the anandamide-induced memory impairment.

Statistical analyses

The acquisition of the two components were evaluated separately so that they could be compared, using paired t-tests.

For the drug test sessions, three dependent measures were of interest: the percentage of correct responses in the conditional discrimination, the percentage of correct responses in the non-match-to-position, and the number of null trials. The percentage of correct responses in the conditional discrimination and at each delay in the non-match-to-position was determined by dividing the number of correct responses by the total number of responses completed; thus, the denominator would have a value of 20 minus the number of null trials. Performance in the conditional discrimination was analyzed by single-factor (treatment) repeated measures ANOVA. Performance in the non-match-to-position was analyzed by two-factor (treatment by delay) repeated measures ANOVA. The occurrence of null trials was analyzed by single-factor (treatment) repeated measures ANOVA.

Dunnett's tests (α=0.05) were used for *post hoc* analyses when a significant F-test was found. 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 (Keppel 1991). For clarity of interpretation, only the uncorrected degrees of freedom are shown when the epsilon correction did not change the outcome of the analysis.

Results

Five rats (three from experiment 1 and two from experiment 3) were dropped from the study prior to any drug treatments due to poor performance.

Experiment 1

The mean (±SEM) number of training sessions needed to reach the acquisition criteria was 19.44 (±1.22) for the conditional discrimination, and 21.94 (±1.67) for the non-match-to-position. A paired t-test comparing these means was not significant [$t(17)$=1.45, P>0.05], demon-

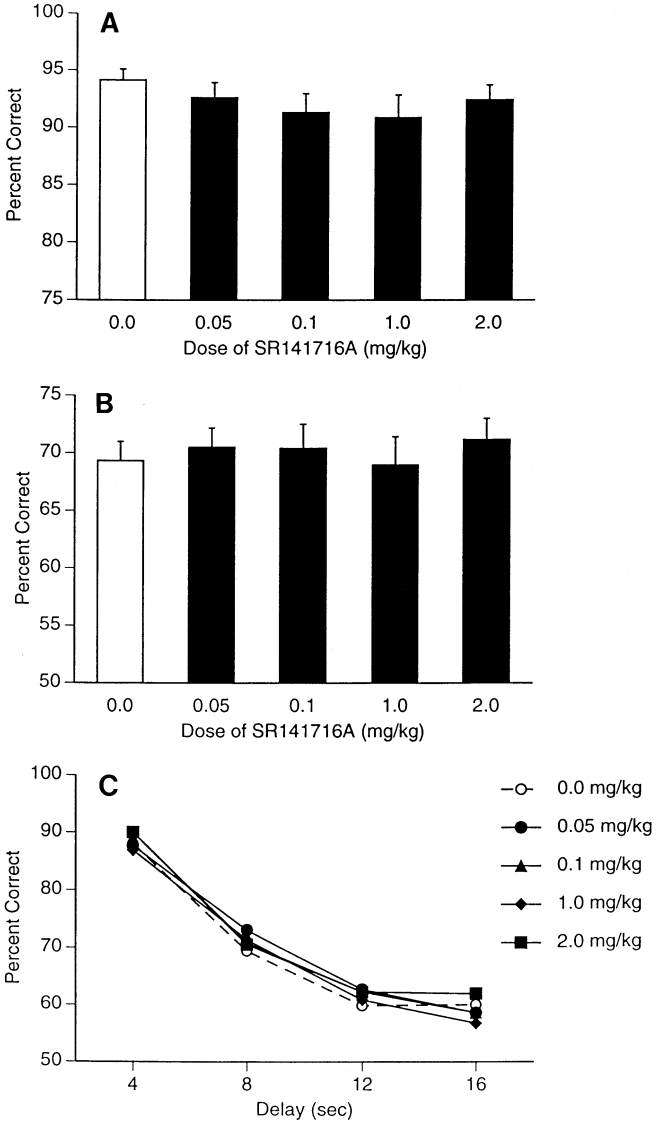

Fig. 1A–C Mean (+SEM) percentage of correct responses for both components of the task following five doses of SR141716A alone (0.0–2.0 mg/kg). **A** Choice accuracy for the conditional discrimination. **B** Choice accuracy for the non-match-to-position. **C** Choice accuracy for the non-match-to-position at each delay interval (4, 8, 12 and 16 s)

strating that the two components were acquired at a similar rate.

The percentage of correct responses in the conditional discrimination did not appear to be affected by SR141716A and was highly accurate (exceeding 90%) at all doses (Fig. 1A). Choice accuracy in the non-match-to-position decreased as a function of delay, but also did not appear to be affected by SR141716A (Fig. 1B, C). A single-factor (treatment) repeated measures ANOVA, conducted on the percentage of correct responses in the conditional discrimination, was not significant [$F(4,68)=1.74$, $P>0.05$]. For the non-match-to-position, a two-factor (treatment by delay) repeated measures ANOVA was conducted on the percentage of correct responses. The main effect of treatment [$F(4,68)<1.0$] and the treatment by delay interaction [$F(12,204)<1.0$] were not significant. As expected, the delay main effect was significant [$F(3,51)=77.00$, $P<0.001$].

Very few null trials occurred following all treatments in experiment 1 (Table 1). A single-factor repeated measures ANOVA conducted on the number of null trials was not significant [$F(4,68)<1.0$].

Experiment 2

The conditional discrimination and non-match-to-position tasks were acquired at similar rates in experiment 2. The mean (±SEM) number of sessions required to reach the acquisition criteria was 24.50 (±2.41) for the conditional discrimination, and 28.25 (±2.99) for the delayed non-match-to-position. A paired *t*-test comparing these values was not significant [$t(11)<1.0$].

The ability of SR141716A to attenuate the memory impairment produced by 2.0 and 4.0 mg/kg THC was evaluated separately for each dose. For 2.0 mg/kg THC, SR141716A did not to appear to produce any effects in the conditional discrimination (Fig. 2A). The 2.0 mg/kg dose of THC (0.0 mg/kg SR141716A in Fig. 2B) appeared to produce a large impairment in the non-match-to-position relative to the vehicle alone condition; this effect appeared to be attenuated to some extent by all doses of SR141716A. Moreover, non-match-to-position performance decreased as a function of delay over all treatments (Fig. 2C). A single-factor ANOVA conducted on the conditional discrimination results was not significant [$F(5,60)=1.78$, $P>0.05$]. For the non-match-to-position, a two-factor (treatment by delay) ANOVA was conducted on the percentage of correct responses. The treatment main effect [$F(5,60)=5.30$, $P<0.001$], delay main effect [$F(3,36)=64.238$, $P<0.001$], and treatment by delay interaction [$F(15,180)=2.03$, $P<0.05$] were significant. However, the treatment by delay interaction was not significant when the epsilon correction was applied [$F(6.3,75.6)=2.03$, $P>0.05$]. A Dunnett's test comparing vehicle alone to all other treatments revealed that the 0.0, 0.05 and 1.0 mg/kg doses, but not the 0.1 or 2.0 mg/kg doses of SR141716A, were significant. Thus, the impairment produced by 2.0 mg/kg THC was significantly attenuated by the 0.1 and 2.0 mg/kg doses of SR141716A.

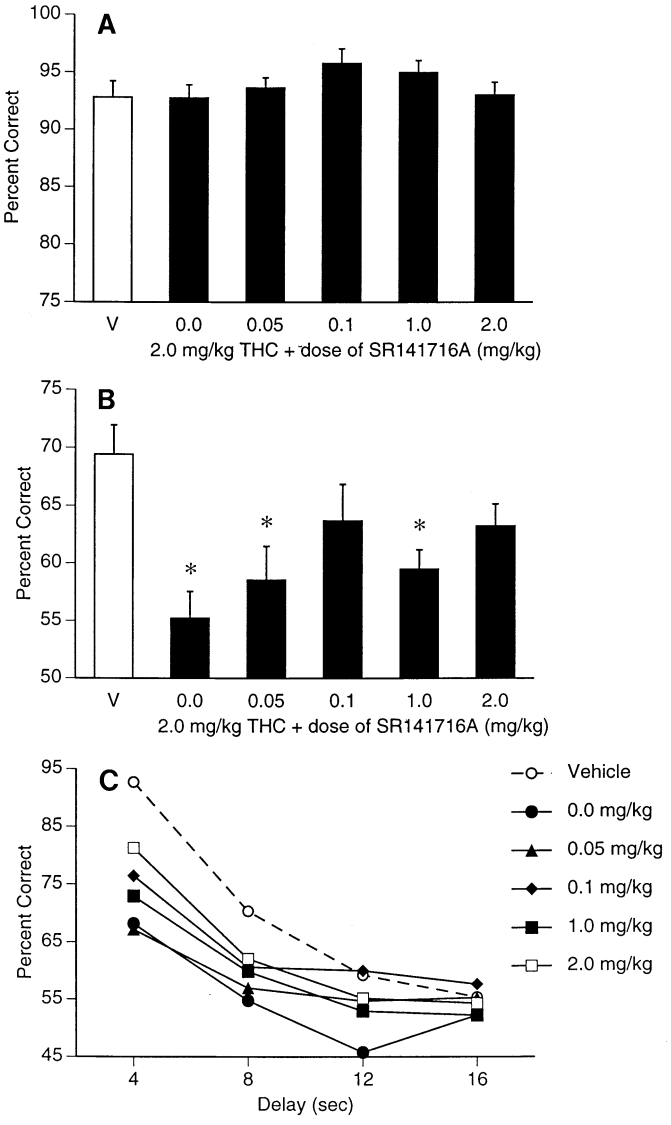

Fig. 2A–C Mean (+SEM) percentage of correct responses for both components of the task following injection of vehicle alone (*V*) or 2.0 mg/kg THC+five doses of SR141716A (0.0–2.0 mg/kg). **A** Choice accuracy for the conditional discrimination. **B** Choice accuracy for the non-match-to-position. Each *bar* represents the mean of all delay intervals at that particular dose. **C** Choice accuracy for the non-match-to-position at each delay interval (4, 8, 12 and 16 s). *Significantly different from vehicle alone using Dunnett's test following a significant ANOVA

THC (2.0 mg/kg) appeared to produce an increase in the frequency of null trials, an effect which seemed to be reversed by SR141716A (Table 1); however, this effect was not significant [$F(5,60)<1.0$].

For 4.0 mg/kg THC, the 0.05 dose of SR141716A appeared to produce a small impairment in the conditional discrimination (Fig. 3A). However, the ANOVA was not significant [$F(5,60)=1.14$, $P>0.05$]. For the non-match-to-position, 4.0 mg/kg THC alone appeared to produce a large impairment in the non-match-to-position relative to

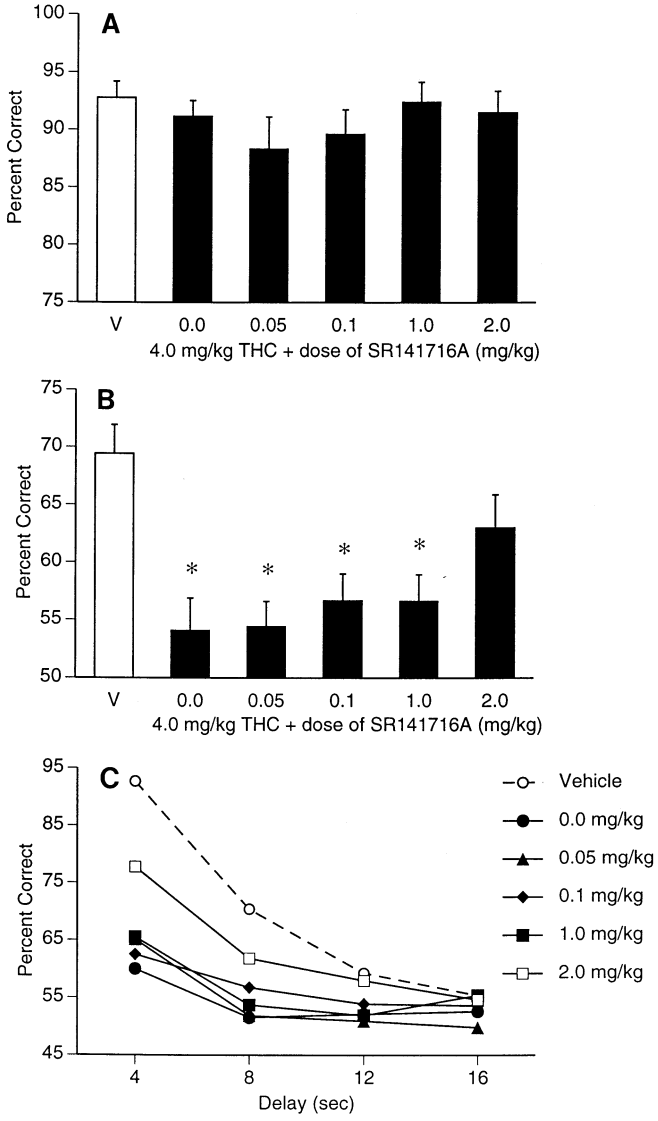

Fig. 3A–C Mean (+SEM) percentage of correct responses for both components of the task following injection of vehicle alone (*V*) or 4.0 mg/kg THC+five doses of SR141716A (0.0–2.0 mg/kg). **A** Choice accuracy for the conditional discrimination. **B** Choice accuracy for the non-match-to-position. Each *bar* represents the mean of all delay intervals at that particular dose. **C** Choice accuracy for the non-match-to-position at each delay interval (4, 8, 12 and 16 s). *Significantly different from vehicle alone using Dunnett's test following a significant ANOVA

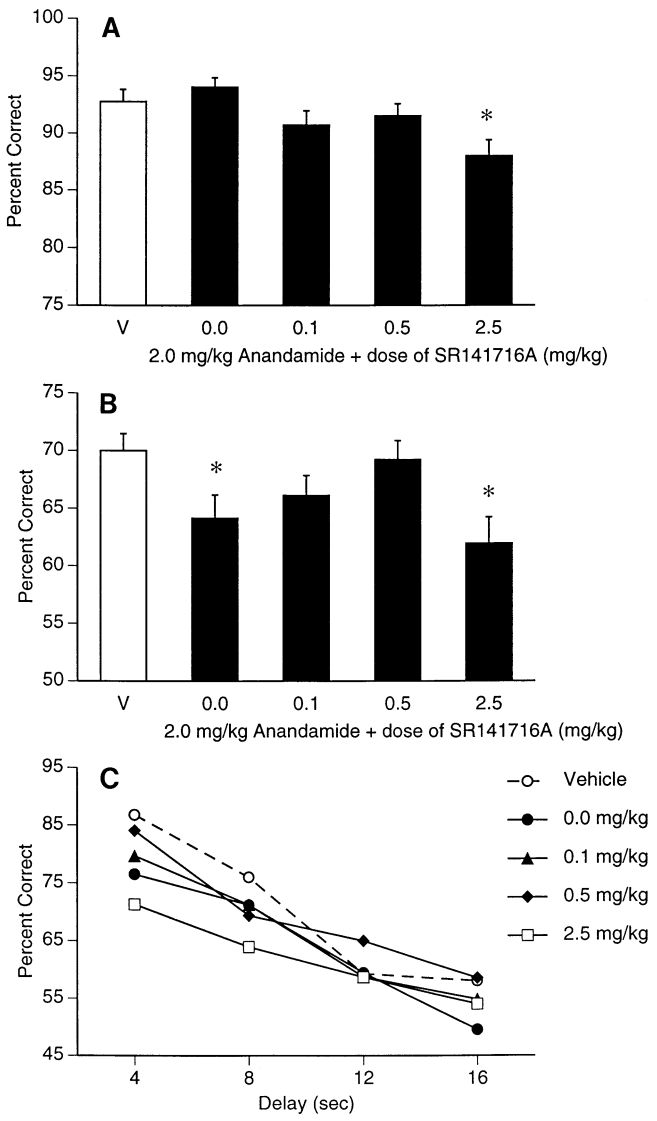

Fig. 4A–C Mean (+SEM) percentage of correct responses for both components of the task following injection of vehicle alone (*V*) or 2.0 mg/kg anandamide+four doses of SR14171A (0.0–2.5 mg/kg). **A** Choice accuracy for the conditional discrimination. **B** Choice accuracy for the non-match-to-position. Each *bar* represents the mean of all delay intervals at that particular dose. **C** Choice accuracy for the non-match-to-position at each delay interval (4, 8, 12 and 16 s). *Significantly different from vehicle alone using Dunnett's test following a significant ANOVA

the vehicle alone condition; this effect appeared to be attenuated slightly by the 0.1 and 1.0 mg/kg doses and largely by the 2.0 mg/kg dose of SR141716A (Fig. 3B). In addition, non-match-to-position performance decreased as a function of delay over all treatments (Fig. 3C). For the non-match-to-position, a two-factor (treatment by delay) repeated measures ANOVA was conducted on the percentage of correct choices. The treatment main effect [$F(5,60)=6.01$, $P<0.001$], delay main effect [$F(3,36)=32.55$, $P<0.001$], and treatment by delay interaction [$F(15,180)=2.79$, $P<0.001$] were significant. A Dunnett's test revealed that the 0.0, 0.05, 0.1, and 1.0 mg/kg doses, but not the 2.0 mg/kg dose of SR141716A, were significantly different from the vehicle alone condition. Thus, the non-match-to-position impairment produced by 4.0 mg/kg THC was significantly attenuated by the 2.0 mg/kg dose of SR141716A. Tests of the simple main effects of drug treatment conducted at each level of delay revealed a significant effect of drug at the 4- [$F(5,60)=8.40$, $P<0.001$] and 8-s delays [$F(5,60)=4.06$, $P<0.005$], but not at the 12- [$F(5,60)=1.11$, $P>0.05$] or 16- [$F(5,60)<1.0$] s delays.

THC (4.0 mg/kg) produced a large increase in the number of null trials. Moreover, as the dose of SR141716A

was increased, the frequency of THC-induced null trials was reduced (Table 1) [$F(5,60)=4.44$, $P<0.005$]. A Dunnett's test revealed that only the 0.0 and 0.1 mg/kg doses of SR141716A were significantly different from the vehicle alone condition.

Experiment 3

As in experiments 1 and 2, the conditional discrimination and non-match-to-position tasks were acquired at similar rates. The mean (±SEM) number of sessions required to reach the acquisition criteria was 22.50 (±1.63) and 23.20 (±1.79), respectively. A paired *t*-test comparing these values was not significant [$t(19)<1.0$].

The administration of anandamide alone did not appear to affect performance in the conditional discrimination (Fig. 4A). However, the combination of anandamide and 2.5 mg/kg SR141716A appeared to impair performance in the conditional discrimination relative to the vehicle alone condition. A single-factor (treatment) repeated measures ANOVA conducted on the percentage of correct responses in the conditional discrimination was significant [$F(4,76)=5.27$, $P<0.001$]. A Dunnett's test revealed that only the 2.5 mg/kg dose of SR141716A was significantly different from the vehicle alone condition. Anandamide alone appeared to produce an impairment in the non-match-to-position (Fig. 4B). In addition, performance in the non-match-to-position decreased as a function of delay (Fig. 4C). SR141716A appeared to improve performance at the 0.1 and 0.5 mg/kg doses (Fig. 4B). A two-factor (treatment by delay) repeated measures ANOVA revealed that the treatment [$F(4,76)=4.21$, $P<0.005$] and delay main effects [$F(3,57)=64.97$, $P<0.001$] were significant; the treatment by delay interaction was not [$F(12,228)=1.48$, $P>0.05$]. A Dunnett's test revealed that the 0.0 and 2.5 mg/kg doses of SR141716A were significantly different from vehicle alone; thus, the impairment of performance in the non-match-to-position was significantly attenuated by the 0.1 and 0.5 mg/kg doses of SR141716A.

Anandamide produced a slight increase in the number of null trials. When combined with anandamide, SR141716A had no effect, except at the highest dose (2.5 mg/kg) tested which produced a moderate increase (Table 1). A single-factor repeated measures ANOVA comparing the number of null trials was significant [$F(4,48)=3.47$, $P<0.05$]. A Dunnett's test revealed that only the 2.5 mg/kg SR141716A treatment was significantly different from vehicle alone.

Discussion

The present results provide strong evidence that the memory disruptive effects of THC and anandamide are mediated by CB_1 cannabinoid receptors. THC and anandamide disrupted working memory, as indexed by a selective impairment in the non-match-to-position task, replicating our previous findings (Mallet and Beninger 1996). The CB_1 receptor antagonist SR141716A attenuated the memory disruptive effects of THC. This finding is in agreement with a previous study showing that THC-induced disruption of radial maze performance can be blocked by SR141716A (Lichtman and Martin 1996).

THC produced an increase in the occurrence of null trials at the 4.0 mg/kg dose, which was attenuated by the administration of SR141716A (Table 1). Because performance in the conditional discrimination was not impaired by 4.0 mg/kg THC (Fig. 3A), the increased number of null trials was likely due to a motor impairment. These results are in agreement with previous studies showing that SR141716A attenuated the motor suppressive effects of the cannabinoid agonists THC (Compton et al. 1996) and WIN-55,212-2 (Rinaldi-Carmona et al. 1994), and further supports the notion that cannabinoids suppress locomotor activity by their action at CB_1 receptors.

SR141716A was postulated to produce an enhancement of memory in the present experiment. However, performance was unaffected by SR141716A (Fig. 1). Although the reasons for the lack of SR141716A-induced memory enhancement are not clear, it is possible that the doses used here were inappropriate; however, these doses were well within the effective range reported to improve social recognition memory in rats (Terranova et al. 1996). Another possibility is that the delays used by Terranova et al. in their social recognition task were very long (up to 120 min) compared to the delays used here (4–16 s). Perhaps longer delays are more amenable to the cognitive enhancing effects of SR141716A. Support for this idea comes from the observation that SR141716A enhanced performance in an eight-arm radial maze when a delay (30 min to 4 h) was imposed between the second last and last arm entries, but had no effect when a delay was not used (A. Lichtman, personal communication, May 26 1997). Alternatively, different tasks may be differentially sensitive to the possible promnemonic effects of SR141716A. For example, social recognition is an unconditioned behavior, whereas the tasks used here and by Mansbach et al. (1996), which were unaffected by SR141716A, involve conditioning.

When SR141716A was administered with anandamide, performance in the conditional discrimination was impaired relative to injections of anandamide alone, but the anandamide-induced disruption of performance in the non-match-to-position was attenuated (Fig. 4). It is worth noting that two doses of SR141716A (0.1 and 0.5 mg/kg) attenuated significantly the anandamide-induced impairment of performance in the non-match-to-position, but had no significant effect in the conditional discrimination. This is the first report that the memory impairment produced by the endogenous cannabinoid ligand anandamide can be attenuated pharmacologically, and suggests that like THC, the memory disruptive effects of anandamide are mediated by CB_1 cannabinoid receptors.

The administration of 2.5 mg/kg SR141716A to rats treated with anandamide in experiment 3 produced an in-

crease in the number of null trials (Table 1). A slightly smaller, but similar dose (2.0 mg/kg) of SR141716A did not produce these effects when administered alone in experiment 1; thus, the increase in null trials and decrease in conditional discrimination choice accuracy may have been the result of a synergistic effect of anandamide, PMSF, and/or SR141716A. Another possibility is that the ethanol vehicle used for the administration of PMSF may have produced a synergistic effect with one or more of the drugs used. Support for this comes from studies demonstrating that ethanol potentiates some of the effects of THC (Pryor et al. 1977; Doty et al. 1992). To our knowledge, anandamide-ethanol interactions have not been studied. Nonetheless, the impairment of performance in the conditional discrimination by the combination of 2.5 mg/kg SR141716A and anandamide was probably due to an impairment of motor function or some other factor unrelated to memory and not likely to be the result of impaired reference memory, as this would be expected to impair performance in the non-match-to-position as well; that is, success at performing either task requires remembering the rules of the task which would be stored in reference memory.

Response latencies were very short (data not shown), regardless of drug treatment, ranging on average between 0.4 and 0.8 s in experiment 1, and 0.4 and 1.9 s in experiments 2 and 3. Inspection of Figs 1C, 2C and 3C clearly shows that these short response latencies cannot account for the observed effects on choice accuracy.

The high concentration of cannabinoid receptors in various regions of the hippocampus (Herkenham et al. 1990, 1991) makes this region a likely candidate for playing a role in cannabinoid modulation of learning and memory, either directly, or through modulation of glutamatergic synapses (Shen et al. 1996). This idea is supported by the finding that the intra-hippocampal administration of the synthetic cannabinoid CP-55,940 impairs performance in a radial maze, without increasing time to completion, and in the absence of other pharmacological effects such as catalepsy, hypothermia, or antinociception (Lichtman and Martin 1996). Whether or not the anandamide-induced disruption of memory reported here is also mediated by hippocampal cannabinoid receptors remains an open question.

In conclusion, the present results further support the notion that the memory disruptive effects of THC are mediated by CB_1 receptors. These results also extend this idea by demonstrating that the memory impairment produced by the exogenous administration of anandamide is mediated by CB_1 receptors, and suggest that endogenous cannabinoids influence the neuronal activity that mediates memory. However, the finding that a CB_1 antagonist alone did not enhance memory suggests that memory may not be mediated by tonic activation of cannabinoid receptors.

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